Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
SARS-CoV-2 mRNA vaccination elicits a robust and persistent T follicular helper cell response in humans

**Highlights**

- SARS-CoV-2 vaccines induce robust human T<sub>FH</sub> cell responses in draining lymph nodes
- HLA-DPB1*04 restricts the immunodominant SARS-CoV-2 S<sub>167–180</sub> epitope
- S<sub>167–180</sub> is recognized by T cell receptors with a public α-chain motif
- S-specific T<sub>FH</sub> cells are maintained in draining lymph nodes 6 months after vaccination

**In brief**

Analysis of draining lymph nodes of individuals vaccinated with BNT162b2 mRNA vaccine against SARS-CoV-2 identifies viral-spike-specific follicular helper CD4<sup>+</sup> T cells that persist for months and contribute to long-term immunity.

**Authors**

Philip A. Mudd, Anastasia A. Minervina, Mikhail V. Pogorelyy, ..., Jamie Rossjohn, Paul G. Thomas, Ali H. Ellebedy

**Correspondence**

pmudd@wustl.edu (P.A.M.), paul.thomas@stjude.org (P.G.T.), ellebedy@wustl.edu (A.H.E.)

**In brief**

Analysis of draining lymph nodes of individuals vaccinated with BNT162b2 mRNA vaccine against SARS-CoV-2 identifies viral-spike-specific follicular helper CD4<sup>+</sup> T cells that persist for months and contribute to long-term immunity.
SARS-CoV-2 mRNA vaccination elicits a robust and persistent T follicular helper cell response in humans

Philip A. Mudd,1,2,16,* Anastasia A. Minervina,3,16 Mikhail V. Pogorelyy,3,16 Jackson S. Turner,4 Wooseob Kim,4 Elizaveta Kalaidina,5 Jan Petersen,6 Aaron J. Schmitz,4 Tingting Lei,4 Alem Haile,7 Allison M. Kirk,3 Robert C. Mettelman,3 Jeremy Chase Crawford,3 Thi H.O. Nguyen,8 Louise C. Rowntree,9 Elisa Rosati,9 Katherine A. Richards,10 Andrea J. Sant,10 Michael K. Klebert,7 Teresa Suessen,11 William D. Middleton,11 SJTRC Study Team, Joshua Wolf,12 Sharlene A. Teeffy,11 Jane A. O’Halloran,13 Rachel M. Presti,2,7,13,15 Katherine Kedzierska,8 Jamie Rossjohn,6,14 Paul G. Thomas,3,* and Ali H. Ellebedy 2,4,15,17,*

1Department of Emergency Medicine, Washington University School of Medicine, Saint Louis, MO 63110, USA
2Center for Vaccines and Immunity to Microbial Pathogens, Washington University School of Medicine, Saint Louis, MO 63110, USA
3Department of Immunology, St. Jude Children’s Research Hospital, Memphis, TN 38105, USA
4Department of Pathology and Immunology, Washington University School of Medicine, Saint Louis, MO 63110, USA
5Division of Allergy and Immunology, Department of Internal Medicine, Washington University School of Medicine, Saint Louis, MO 63110, USA
6Infection and Immunity Program & Department of Biochemistry and Molecular Biology, Biomedicine Discovery Institute, Monash University, Clayton, Victoria 3800, Australia
7Clinical Trials Unit, Washington University School of Medicine, Saint Louis, MO 63110, USA
8Department of Microbiology and Immunology, University of Melbourne, at Peter Doherty Institute for Infection and Immunity, Parkville, Victoria 3052, Australia
9Institute of Clinical Molecular Biology, Christian-Albrecht University of Kiel, Kiel 24105, Germany
10David H. Smith Center for Vaccine Biology and Immunology, University of Rochester Medical Center, Rochester, NY 14642, USA
11Malinckrodt Institute of Radiology, Washington University School of Medicine, Saint Louis, MO 63110, USA
12Division of Infectious Diseases, St. Jude Children’s Research Hospital, Memphis, TN 38105, USA
13Division of Infectious Diseases, Department of Internal Medicine, Washington University School of Medicine, Saint Louis, MO 63110, USA
14Cardiff University School of Medicine, Heath Park, Cardiff CF14 4XN, UK
15Andrew M. and Jane M. Bursky Center for Human Immunology and Immunotherapy Programs, Washington University School of Medicine, Saint Louis, MO 63110, USA
16These authors contributed equally
17Lead contact
*Correspondence: pmudd@wustl.edu (P.A.M.), paul.thomas@stjude.org (P.G.T.), ellebedy@wustl.edu (A.H.E.)
https://doi.org/10.1016/j.cell.2021.12.026

SUMMARY

SARS-CoV-2 mRNA vaccines induce robust anti-spike (S) antibody and CD4+ T cell responses. It is not yet clear whether vaccine-induced follicular helper CD4+ T (T_FH) cell responses contribute to this outstanding immunogenicity. Using fine-needle aspiration of draining axillary lymph nodes from individuals who received the BNT162b2 mRNA vaccine, we evaluated the T cell receptor sequences and phenotype of lymph node T_FH. Mining of the responding T_FH T cell receptor repertoire revealed a strikingly immunodominant HLA-DPB1*04-restricted response to S_{167–180} in individuals with this allele, which is among the most common HLA alleles in humans. Paired blood and lymph node specimens show that while circulating S-specific T_FH cells peak one week after the second immunization, S-specific T_FH persist at nearly constant frequencies for at least six months. Collectively, our results underscore the key role that robust T_FH cell responses play in establishing long-term immunity by this efficacious human vaccine.

INTRODUCTION

The COVID-19 pandemic necessitated rapid late-stage clinical trials of mRNA vaccine technology (Anderson et al., 2020; Baden et al., 2021; Jackson et al., 2020; Polack et al., 2020; Verbeke et al., 2021; Walsh et al., 2020; Widge et al., 2021) that resulted in the first FDA-approved vaccine using this technology platform. The two mRNA vaccines developed by Pfizer/BioNTech (BNT162b2) (Polack et al., 2020) and Moderna (mRNA-1273) (Baden et al., 2021) have proven instrumental in the initiation of widespread vaccination campaigns in the United States and around the world. Both vaccines engender high-titer circulating
anti-SARS-CoV-2 spike-protein-specific antibodies that can neutralize the originally circulating SARS-CoV-2 strain (Jackson et al., 2020; Walsh et al., 2020) as well as other variants that have emerged since the vaccine design phase (Chen et al., 2021; Wang et al., 2021a, 2021b; Wu et al., 2021). Neutralizing antibodies induced by mRNA vaccines appear to be the key correlate of protection from COVID-19 in animal models (Corbett et al., 2021) and in humans (Khoury et al., 2021). COVID-19 mRNA vaccines exhibit the highest efficacy in phase 3 studies among widely utilized COVID-19 vaccines worldwide (Al Kaabi et al., 2021; Baden et al., 2021; Logunov et al., 2021; Polack et al., 2020; Sadoff et al., 2021; Voysey et al., 2021). Understanding exactly how mRNA vaccines elicit such robust and protective immune responses in humans is necessary for extending the application of this novel platform to vaccines against other important human pathogens.

Germinal center (GC) reactions that occur in draining lymph nodes following infection or vaccination are critical for developing long-lasting, high-affinity antibody responses (Ripperger and Bhattacharya, 2021; Victor and Nussenzweig, 2012). T follicular helper (TFH) cell responses in the lymph node are necessary for forming and sustaining GC reactions and for the development of both long-lived plasma cells and memory B cells (Crotty, 2011; Qi, 2016; Ueno et al., 2015). Detailed analysis of the specificity and dynamics of vaccination-induced GC reactions in humans is increasingly being explored through sampling draining lymph nodes using serial fine-needle aspiration (FNA) following intramuscular immunization (Turner et al., 2020, 2021; Kim et al., 2021). Importantly, it appears during an active immune response is critical to understanding the role of TFH cells in the development of long-lived plasma cells and memory B cells following vaccination.

RESULTS

Human TFH population size mirrors the GC B cell population following mRNA vaccination

We conducted a prospective observational study to follow vaccine-induced immune responses in a cohort of 41 healthy adults who had received the BNT162b2 mRNA vaccine (Turner et al., 2021). Demographics of the full cohort have previously been reported (Turner et al., 2021). Fifteen members of the cohort underwent axillary lymph node FNA. All subjects were vaccinated with two 30 μg doses of BNT162b2, approximately twenty-one days apart. Blood and/or FNA samples were obtained at day 0 (prior to the first vaccine dose), day 21 (immediately prior to the second vaccine dose), day 28, day 35, day 60, day 110, and day 200 according to the schedule listed in Figure 1A. This manuscript reports exclusively on the 15 subjects who underwent lymph node FNA. Demographics of the included individuals are listed in Table 1. None of the included subjects reported previous infection with SARS-CoV-2.

We first evaluated the size of the human TFH population in relation to the size of the GC B cell population in the lymph node. We analyzed the frequency of the GC B cell response (defined as CD19+IgDlowBcl-6+CD38int B cells) among all lymph-node-resident B cells and the frequency of total lymph-node-resident
CD4+ T cells that exhibited a T_{FH} cell phenotype (Bcl-6"CXCR5"PD1"FoxP3") in 95 separate lymph node samples taken from each of the 15 individuals over the course of the study (Figure S1A; Table S1). These FNA samples were obtained between 21 and 200 days following primary vaccination. Six of the fifteen subjects underwent repeated sampling of two separate axillary lymph nodes (Table S1). We found a significant correlation between the size of the GC B cell population in the lymph node and the total T_{FH} cell population frequency following mRNA vaccination (Figure S1B). We also noted a significant correlation between the size of the SARS-CoV-2 spike-specific GC B cell population in the lymph node and the total lymph node T_{FH} cell population frequency (Figure S1C).

**Discovery and characterization of an immunodominant DPB1*04:01-restricted CD4+ T cell population**

Next, we sought to illuminate the antigen specificity of the lymph node T_{FH} population. To do this, we sorted total T_{FH} cells from FNA samples obtained on day 60 from four separate subjects (Figure 1B) and reconstructed their T cell receptor (TCR) repertoires using unpaired sequencing of the TCRβ and TCRα chains (Figure 1C). Surprisingly, clonally expanded TCRs formed a prominent α-chain cluster that was shared among all 4 donors (Figure 1C), corresponding to 0.9%–7.7% of the total lymph node T_{FH} cells in each donor. We did not observe a similar shared cluster in the TCRβ chain repertoires. We observed the same α-motif in a previously published paper (Minervina et al., 2021a), where it was the largest signal and corresponded to 0.2% of total CD4+ T cells and 16.3% of estimated SARS-CoV-2-responding CD4+ T cells in the blood at the peak of the acute response. Large clusters of TCRs with sequence similarity are an indication of convergent selection of similar receptors to the same antigen (Dash et al., 2017; Glanville et al., 2017; Pogorelyy et al., 2019). As this motif was present among expanded clones in many donors, it likely recognizes an immunodominant epitope from SARS-CoV-2 presented in the context of a common HLA class II allele.

In order to decode the specificity of the heterodimer αβTCR, we first needed to determine what β-chains pair with the TCRα chain motif that we identified (Figure 2A). To do this, we queried publicly available CD4+ paired TCR datasets. We used two datasets that have paired αβTCR sequences from CD4+ T cells after antigen-reactive T cell enrichment following stimulation with SARS-CoV-2 peptides (Bacher et al., 2020; Meckiff et al., 2020). We searched for our CDR3α motif ("CA[GA/V] XNYGGSQGLNLF") in these datasets and found 1,329 out of 44,256 unique TCRs in Bacher et al., but only 53 out of 43,745 in Meckiff et al. with the matched CDR3α motif. Next, we used the identified β-chains to look for overlap in the MIRA dataset (Nolan et al., 2020)—a large dataset produced by Adaptive Biotech linking TCR sequences to SARS-CoV-2 epitopes. We identified 64 TCRs from Bacher et al. highly similar (up to one amino acid mismatch in CDR3, identical CDR1 and CDR2) to MIRA TCRs reactive to the overlapping peptide pool from SARS-CoV-2 spike (S) protein 160–218 positions (Figure 2B). Interestingly, this part of the S protein was not used for stimulation in Meckiff et al., explaining why we found only a small number of TCRs of interest in this dataset and indirectly supporting the predicted identification of the peptide region from the MIRA dataset.

Five of six subjects recognizing this peptide pool in the MIRA database had available HLA-typing. These five shared the DPB1*04:(01/02) and DQB1*06:(02/03) alleles. To establish HLA restriction of the response of interest and to narrow the search to a single peptide, we used NetMHCII2.3 (Jensen et al., 2018) to look for predicted epitopes from the S_{160–218} peptide pool that are presented by one or both of these shared alleles. We found that peptides containing the core sequence YVSPF/LMD were predicted to strongly bind the DPB1*04:01 and DPB1*04:02 alleles, while no strong binders were identified for the DQB1*06:(02/03) alleles. Interestingly, SARS-CoV-2 epitopes with this core sequence (YVSPF/LMD, S_{170–178}) have previously been described in prominent epitope discovery studies.

---

**Table 1. Cohort demographics**

| Study ID | Sex | Race | Ethnicity | Age | Number of lymph nodes sampled |
|----------|-----|------|-----------|-----|-------------------------------|
| 01a      | Male| White| Non-hispanic | 34  | 1                             |
| 02a      | Male| White| Hispanic    | 37  | 2                             |
| 04       | Female| White| Non-hispanic | 38  | 2                             |
| 07       | Female| White| Non-hispanic | 33  | 1                             |
| 08       | Female| White| Non-hispanic | 27  | 1                             |
| 10       | Female| White| Non-hispanic | 27  | 2                             |
| 13       | Male  | White| Non-hispanic | 34  | 1                             |
| 15       | Female| Black | Non-hispanic | 52  | 2                             |
| 16       | Male  | White| Non-hispanic | 37  | 2                             |
| 20       | Female| White| Non-hispanic | 48  | 2                             |
| 21       | Female| White| Non-hispanic | 31  | 1                             |
| 22       | Male  | White| Non-hispanic | 36  | 1                             |
| 26       | Female| White| Non-hispanic | 38  | 1                             |
| 28       | Female| Asian | Non-hispanic | 44  | 1                             |
| 43       | Male  | Asian | Non-hispanic | 40  | 1                             |
obtained post-vaccination peripheral blood from participants in previously been reported to be HLA-DPB1*04-restricted. Further, we noted that each DPB1*04 + donor had activated poly-
(C) Average fold change in CD4+CD69+ T cells (producing IL2, TNF from S protein.

We determined that participants with the HLA-DPB1*04 allele measured by intracellular cytokine staining and flow cytometry. peptide (CTFEYVSQPFLMDLE) and the responses were searched in the MIRA dataset allowing for up to one mismatch in CDR3 amino acid sequence. The y axis shows the number of TCRi chains from Bacher et al. matching to TCRj from different MIRA SARS-CoV-2 peptide pools. Largest hit (red dot) corresponded to the peptide pool spanning amino acid positions 160–218 from S protein.

As an initial investigation of this possible HLA restriction, we obtained post-vaccination peripheral blood from participants in the ongoing SJTRC study (SJTRC, NCT04362995). PBMCs from these participants were stimulated with purified S_{166-180} peptide (CTFEYSQPFLMDLE) and the responses were measured by intracellular cytokine staining and flow cytometry. We determined that participants with the HLA-DPB1*04 allele had increased cell counts per million PBMCs of monofunctional CD4+CD69+ T cells producing IL-2, TNF, or IFNγ compared with participants without this allele (Figures 2C, S2B, and S2C). Further, we noted that each DPB1*04+ donor had activated polyfunctional T cells producing two or three cytokines in response to peptide stimulation, in both vaccinated-naive and SARS-CoV-2-convalescent individuals (Figures S2D and S2E).

We then moved forward with more rigorous experimental validation of our paired TCR, peptide epitope, and restricting HLA combination (Figure 2A). To do this, we selected two paired TCRs from Bacher et al. that included the same TCRβ chains that we designated TCR4.1 and TCR6.3. We transduced these each into separate Jurkat TCR-negative cells from paired TCR following TCR engagement. The transduced paired TCR, peptide epitope, and restricting HLA combination of our paired TCR, peptide epitope, and restricting HLA allele were found in the GISAID database (CTFEYSQPFLMDLE) as well as

Figure 2. S_{167-180} epitope discovery and HLA class II tetramer validation

(A) Response identification process. The identified TCRβ motif of interest was used to query large public scRNA-seq datasets (Bacher et al., 2020; Meckiff et al., 2020) to identify potential partner TCRα chains and then matched to the large MIRA dataset that used TCRα sequencing (Nolan et al., 2020) to predict HLA-restriction and cognate epitopes. To validate our prediction, we generated a T cell line expressing the putative TCRβ and we generated HLA class II tetramers. (B) Identification of peptide pool for the motif TCRs using the MIRA dataset. TCRβ chains from paired TCRs with CDR3β motif (CA[G/A/V]XNYGGSQGDLF) were searched in the MIRA dataset allowing for up to one mismatch in CDR3 amino acid sequence. The y axis shows the number of TCRα chains from Bacher et al. matching to TCRβ from different MIRA SARS-CoV-2 peptide pools. Largest hit (red dot) corresponded to the peptide pool spanning amino acid positions 160–218 from S protein.

(C) Average fold change in CD4+/CD69+ T cells (producing IL2, TNF, or IFNγ) per 10^6 cells following CTFEYSQPFLMDLE peptide stimulation of DPB1*04-positive and -negative SJTRC PBMCs. PBMCs collected during SARS-CoV-2 convalescence or post-vaccination with BNT162b2 were used for intracellular cytokine staining assay. Average fold changes were compared using a Mann-Whitney U test; p = 0.004. Gating strategy is shown in Figure S2.

(D) Jurkat cell line expressing the predicted TCR after stimulation with the predicted epitope. Left column: negative control; middle column: TCR4.1 cell line co-cultured with PBMCs from healthy DPB1*04:01-positive donor pulsed with CTFEYSQPFLMDLE peptide (S_{167-180}); right column: positive control. Top row: NFAT-GFP reporter expression. Middle row: CD69 surface expression. Bottom row: downregulation of the TCR on cell surface.

(E) S_{167-180} tetramer staining identifies epitope-specific T cells with high specificity. Top row: staining of TCR4.1 and TCR6.3 Jurkat cell lines. Bottom left: staining of Jurkat cell line expressing TCR with other known specificity; bottom right: staining of PBMCs from SARS-CoV-2-naive individual.

(F) S_{167-180} tetramer+ cells have predominantly effector memory phenotype in SARS-CoV-2-convalescent patients. Each row represents an individual donor. Left column: CCR7 and CD45RA distribution in bulk CD3+CD4+ cells. Middle column: S_{167-180} tetramer staining of CD3+CD4+ cells. Right column: memory/naive phenotypes of CD3+CD4+ S_{167-180} tetramer+ cells. Gating strategies for (D), (E), and (F) are shown in Figure S3.
Tracking S167–180 antigen-specific CD4+ T cell responses in blood and draining lymph nodes following BNT162b2 vaccination

With the discovery of an immunodominant SARS-CoV-2-S epitope restricted by the HLA-DBP1*04:01 allele that is found at high frequency (>40%) in many populations around the world (allelefrequencies.net), we used the S167–180 HLA class II tetramer to evaluate 14 of the mRNA vaccine study subjects with available blood and lymph node samples to empirically determine which individuals were HLA-DBP1*04:01+ and thus had the S167–180-specific CD4+ T cell response. Nine of the 14 subjects had a detectable S167–180-specific response in peripheral blood following boost vaccination. Next, we tracked and characterized this response over time in frozen PBMC (N = 8 subjects) and frozen lymph node FNA samples (N = 6 unique lymph node samples from 5 subjects) from a convenience sample of the subjects who had sufficient sample remaining for analysis. The S167–180-specific CD4+ T cell response peaked in peripheral blood 28 days after primary vaccination, 7 days after vaccine boost, and remained present in the blood at detectable frequencies through the entire study interval (Figures 3A and 3B).

Most S167–180-specific CD4+ T cells circulating in peripheral blood exhibited a CD45RO+CCR7+ effector memory surface phenotype similar to what we observed in SARS-CoV-2-convalescent donors (Figure 3C). A subset of tetramer-positive CD4+ T cells in the first 35 days following primary vaccination exhibited an activated surface phenotype characterized by upregulation of both CD38 and HLA-DR (Figure 3D). This activated CD4+ T cell phenotype disappeared by day 60 post-primary vaccination.

Next, we generated an HLA class II tetramer to probe the antigen-specific T cell response that we had discovered. We tested our HLA-DBP1*04 S167–180 tetramer using the two transduced TCR4.1 and TCR6.3 Jurkat cell lines and showed high sensitivity and low background staining (Figure 2E). We then used the S167–180 tetramer to look for antigen-specific CD4+ T cells in PBMC from three HLA-DBP1*04+ SARS-CoV-2-convalescent donors and a control HLA-DBP1*04+ SARS-CoV-2-naive donor. We found a small number of tetramer-specific cells predominantly in the naive subpopulation (CCR7+CD45RA+) in the naive donor and a much larger number of tetramer-specific cells that were primarily effector memory (CCR7-CD45RA-) in the SARS-CoV-2-convalescent donors (Figure 2F). The frequency of tetramer-positive cells was comparable to the frequencies of the total S-specific cells observed using a separate activation-induced marker (AIM) assay with overlapping S-peptides (Figure S5), suggesting that tetramer staining provided a higher sensitivity to detect epitope-specific responses. We then sequenced tetramer-specific TCRs from these convalescent donors using our previously described scTCR-seq approach (Wang et al., 2012). The majority (64%) of sequenced cells had the same TRAV35-CA(G/A)VJXNYGSSQGN-LIF TCR motif that we had initially identified, and >80% of all sequences included TRAV35, suggesting that the discovered TCR motif is the most frequent mode of recognition for this epitope (Table S2). We also found the TCR4.1β (exactly matching amino acid sequence) and TCR6.3β (one mismatch) in the single-cell TCR sequencing of tetramer-specific T cells from convalescent individuals. This is a further independent validation that the αβTCRs selected for Jurkat cell line generation are S167–180 specific and occur in multiple patients.

Tracking S167–180 antigen-specific CD4+ T cell responses in blood and draining lymph nodes following BNT162b2 vaccination

In contrast to circulating populations of Tfh cells, the frequency of S167–180-specific CD4+ Tfh cells remained high in the draining axillary lymph node through at least day 60 following primary vaccination and persisted at high frequency in three of the five study subjects through day 200 following primary vaccination—more than 170 days following vaccine boost (Figure 4). The prolonged persistence of S-specific Tfh cells that we report here in the draining axillary lymph nodes corresponds well with the long-lived GC B cell responses recently reported in the same cohort of subjects (Turner et al., 2021). The vast majority of S167–180-specific CD4+ T cells in lymph node FNA samples co-expressed CXCR5...
and PD1, surface markers of T<sub>FH</sub> cells, throughout the study interval (Figure 4A). Furthermore, the frequency of S<sub>167–180</sub>-specific CD4<sup>+</sup> T cells in the FNA samples remained consistently high or even increased as the frequency of S<sub>167–180</sub>-specific CD4<sup>+</sup> T cells in the peripheral blood contracted. These lymph node T<sub>FH</sub> responses remained at a high frequency until the conclusion of the GC response at day 200 in 2 of the 5 subjects (Figure 4B).

Next, we examined the frequency of S<sub>167–180</sub>-specific CD4<sup>+</sup> T cells in the total CXCR5<sup>+</sup>PD1<sup>+</sup> T<sub>FH</sub> population in both the blood and the lymph nodes over time. We found that this population rapidly expanded in the blood—peaking at day 28 after primary vaccination, 7 days after vaccine boost—and then became challenging to detect by days 110 and 200 (Figure 4C) as we had previously noted when examining this population as a proportion of total CD4<sup>+</sup> T cells in Figure 3G. In contrast, the frequency of the S<sub>167–180</sub>-specific T<sub>FH</sub> population remained consistently elevated within the total T<sub>FH</sub> population over time in the lymph node—until the resolution of the lymph node GC response at day 200 in 2 of the 5 subjects (Figure 4C; Table S1). Together, these results demonstrate that a small subset of antigen-specific CD4<sup>+</sup> T cells circulating in peripheral blood following vaccination develop a surface phenotype consistent with circulating T<sub>FH</sub> cells. This coincides with the development of T<sub>FH</sub> cells in the draining lymph node with the same antigen specificity. Furthermore, while this population nearly disappears from circulating blood 110 days after vaccination, the response remains constant in the lymph node in the presence of an ongoing GC reaction. Overall, our findings are consistent with the development of diverse lineages of effector CD4<sup>+</sup> T cells—that express a surface phenotype consistent with T<sub>FH</sub> and those that do not—from a single population of naive CD4<sup>+</sup> T cells that share a common TCR<sub>\alpha</sub> chain motif. This is consistent with observations in mouse models where the specificity and duration of the TCR/peptide/MHC class II interaction correlated with the overall balance between Th1 and T<sub>FH</sub> cell frequency (Tubo et al., 2013).
Diverse clonal populations of T<sub>FH</sub> in the human GC persist at a consistent frequency over time

We subsequently quantified the contribution of the S<sub>167–180</sub> T<sub>FH</sub> population to the broader clonotypic diversity of the T<sub>FH</sub> population found in the lymph node from four of the subjects by analyzing the TCR sequencing data from sorted T<sub>FH</sub> cells generated for Figure 1C. The clonotypes that compose the S<sub>167–180</sub> response made up the largest percentage of total clonotypes present in the lymph node for three of the four subjects and composed the second highest percentage of clonotypes in the fourth subject (Figure 5A). This underscores the importance of the immunodominant HLA-DPB1*04-restricted S<sub>167–180</sub> response in the total SARS-CoV-2-specific T<sub>FH</sub> cell response of HLA-DPB1*04 + vaccinees, who make up approximately 40%–50% of the world’s population.

To elucidate the clonal composition of the T<sub>FH</sub> cell response over time, we sequenced samples from two time points that were available from these individuals. Three subjects were sequenced at day 60 and day 110 post-primary vaccination and one subject was sequenced at day 28 and day 60 following primary vaccination (Table S3). Three of the subjects exhibited evidence of ongoing antigen-specific T<sub>FH</sub> responses associated with GC responses in our earlier flow cytometry experiments at all tested time points (Figure 4), while there were insufficient remaining samples from subject #22 for this analysis. In support of our observations in the flow cytometry analysis of the S<sub>167–180</sub> population, we found a positive correlation between the frequency of a large number of the TCR<sub>a</sub> clonotype sequences at the two time points (Figure 5B), including the known S<sub>167–180</sub>-specific TCR clonotypes (Figure 5B, red data points). This was especially true of the clonotypes found at the highest frequency in each FNA sample, which are those that are most likely to represent antigen-specific clonotypes due to their increased presence in the lymph node following vaccination. This positive correlation means that many of these clonotypes were found at similar frequency at both tested time points. Therefore, the maintenance of consistently high-frequency antigen-specific T<sub>FH</sub> cell responses over time during an ongoing antigen-specific GC B cell response (Turner et al., 2021) that we observed in the context of the S<sub>167–180</sub>-specific CD4<sup>+</sup> T<sub>FH</sub> response (Figure 4C) is generalizable to other clonally related and presumably antigen-specific T<sub>FH</sub> populations in the human lymph node following vaccination.
BNT162b2 vaccination. Our data support a model whereby the antigen-specific human GC T FH cell response is maintained at a relatively consistent and high frequency in the setting of an active and ongoing GC reaction, rather than a response that peaks or dynamically changes in frequency over time.

DISCUSSION

In this report, we show that the BNT162b2 COVID-19 mRNA vaccine induces robust and persistent T FH responses in the draining lymph nodes of vaccinated individuals. Indirect evidence has existed for some time that robust CD4+ T cell responses are required for the generation of high-titer neutralizing antibody responses following COVID-19 infection or mRNA vaccination. This includes data showing a lack of seroconversion in individuals with uncontrolled HIV and extremely low CD4+ T cell counts during vaccination (Touizer et al., 2021) as well as several reports that have demonstrated a lack of seroconversion to the standard two-dose BNT162b2 regimen in individuals subjected to T-cell-focused immunosuppressive regimens following solid organ transplantation (Kamar et al., 2021). Our current results provide strong and direct evidence that a high-magnitude, antigen-specific CD4+ T cell response in the draining lymph nodes is present during the development of high-titer neutralizing antibody responses in the setting of COVID-19 mRNA vaccination.

The temporal relationship we observe between the early appearance and then disappearance of S167–180-specific CD4+ T cells exhibiting a circulating T FH phenotype in the blood at the same time that we observe T FH cells in the draining lymph node suggests a complex relationship between these two populations of cells. Our present data support a model of human T FH cell development whereby phenotypically heterogeneous, or even plastic, antigen-specific CD4+ T cell populations induced by primary vaccination are activated and expand in the lymph node and circulating compartments prior to the development and migration of more specialized subpopulations that co-express CXCR5 and PD1 to the lymph node GC (Crotty, 2018). In our S167–180 tetramer data, most S167–180-specific CD4+ T cells in the blood did not exhibit a circulating T FH phenotype even at the day 28 post-primary vaccination peak of circulating S167–180-specific T FH. Very few S167–180-specific memory CD4+ T cells maintained in blood more than 3 months after vaccination expressed both CXCR5 and PD1. Nevertheless, S167–180-specific T FH cells compose the largest or second largest S-specific T FH population in the lymph node of all evaluated subjects despite the near absence of these cells in the circulating blood at the same late time points. Together, our data support a model whereby clonal populations of circulating CD4+ T cells develop into many different lineages, including the T FH cell lineage. Furthermore, we were unable to find a strong, direct relationship between the cells known as circulating T FH (circulating antigen-specific CD4+CXCR5+PD1+ cells) and the presence of large populations of clonally matched antigen-specific T FH cells participating in an ongoing GC in the lymph node. This is in contrast to data from a study of matched tonsil and blood samples in subjects who were not recently vaccinated or infected where they found substantial clonal overlap between tonsil T FH populations and circulating T FH populations but little overlap between tonsil T FH populations and circulating non-

Figure 5. The S167–180 response composes a large fraction of the T FH repertoire and maintains a consistent frequency over time.

(A) Abundance of the S167–180-specific clones (red boxes) in the lymph node T FH repertoires of 4 donors on day 60 after mRNA vaccination. Listed frequency is the frequency of the examined clonal group (defined as a cluster from Figure 1B) out of the total clonal sequences in the sorted T FH sample. The S167–180 response is the largest T FH response in 3 of the 4 examined HLA-DPB1*04+ subjects lymph nodes.

(B) Clonotype frequencies of sequenced sorted CXCR5+PD1+ T FH repertoires from lymph nodes sampled at two separate time points. Each dot corresponds to an individual TCRα clonotype. Frequencies are shown in log scale. Red dots correspond to S167–180-specific clones based on the known α-chain motif.
TFH populations (Brenna et al., 2020). Further studies are required to determine the relationship between populations of circulating and lymph-node-resident TFH cells in both the steady state and following vaccination, as these sequences are quite distinct.

The discovered DPB1*04-restricted S<sub>167−180</sub> response is notable for the extraordinarily constrained TCR<sub>β</sub> sequence diversity. This single TCR<sub>β</sub> motif is immediately obvious with even cursory inspection of bulk CD4<sup>+</sup> TCR sequences from vaccinated or infected individuals. Surprisingly, no prominent TCR<sub>β</sub> motif is observed in any of our sequencing of this response, emphasizing the importance of the α-chain in certain instances of specific epitope recognition (Dash et al., 2017; Minervina et al., 2020; Shomuradova et al., 2020). The high prevalence of DPB1*04 in worldwide populations means that this response is likely immunodominant across multiple populations and contributes significantly to the measured responses in many studies, though its restriction has not been previously assigned. Thus far, none of the prevalent SARS-CoV-2 strains including the delta and omicron variants have acquired stable mutations in this peptide sequence.

In conclusion, we find that mRNA vaccine technology has an exceptional ability to induce high-frequency antigen-specific B cell (Turner et al., 2021) and antigen-specific CD4<sup>+</sup> TFH cell responses in the human lymph node following prime-boost administration. These characteristics underlie the development of high-titer neutralizing antibodies and protection from infection in vaccinated individuals. The selective enhancement of lymph node TFH responses induced by vaccine regimens represents a broad strategy for improving future vaccines.

**Limitations of the study**

Our study has several limitations, including the small number of included subjects, the relatively young age of the included participants, and the lack of comprehensive epitope mapping beyond the immunodominant response that we identified. Furthermore, the complex nature of both the vaccination rollout during the ongoing pandemic and the FNA sampling procedure itself eliminated our ability to sample lymph nodes prior to vaccination and at earlier time points following the primary vaccination. Furthermore, although we repeatedly sampled some axillary lymph nodes until the apparent conclusion of the GC response in those nodes, we were unable to sample non-draining control lymph nodes at distal sites. Limitations in the small number of available cells from the FNA procedure precluded total S-specific T cell response measurement in the LN samples using assays such as AIM or ICS. In addition, limitations to the convalescent patient sample study precluded longitudinal analysis of these responses in the previously infected patient cohort. There are several questions that we did not address that will be useful topics for future studies, including the extent of clonal overlap between the blood and lymph node CD4<sup>+</sup> T cell compartments, and the transcriptional profiles of the lymph node TFH response over the long period of clonal stability.

**CONSORTIA**

The members of the SJTRC Study Team are Jeremie H. Estepp, Stacey Schultz-Cherry, Maureen A. McGargill, Aditya Gaur, James Hoffman, Motomi Mori, Li Tang, Elaine Tuomanen, Richard Webby, Randall T. Hayden, Hana Hakim, Diego R. Hijano, Kim J. Allison, E. Kaitlynn Allen, Resha Bajracharya, Wald Awad, Lee-Ann Van de Velde, Brandi L. Clark, Taylor L. Wilson, Alisha Souquette, Ashley Castellaw, Ronald H. Dallas, Ashleigh Gowen, Thomas P. Fabrizio, Chun-Yang Lin, David C. Brice, Sean Cherry, Ericka Kirkpatrick Roubidoux, Valerie Cortez, Pamela Freiden, Nicholas Wohlgemuth, and Kendall Whitt.

**STAR METHODS**

Detailed methods are provided in the online version of this paper and include the following:

- **KEY RESOURCES TABLE**
- **RESOURCE AVAILABILITY**
  - Lead contact
  - Materials availability
  - Data and code availability
- **EXPERIMENTAL MODEL AND SUBJECT DETAILS**
  - Human subjects
- **METHOD DETAILS**
  - Cell sorting and flow cytometry
  - Jurkat cell line generation
  - Jurkat peptide stimulation
  - Peptide stimulation and intracellular cytokine staining of SJTRC samples
  - Activation-induced marker (AIM) assay
  - Monomer generation
  - Tetrramer generation and staining of Jurkat cells
  - Tetrramer staining of SJTRC samples and scTCR sequencing
  - Bulk repertoire generation
  - Public TCR repertoire datasets
- **QUANTIFICATION AND STATISTICAL ANALYSIS**
  - TCR repertoire analysis
  - Statistical analysis

**SUPPLEMENTAL INFORMATION**

Supplemental information can be found online at https://doi.org/10.1016/j.cell.2021.12.026.

**ACKNOWLEDGMENTS**

The authors would like to thank the study participants for their invaluable contribution to this work. We also thank Greg Lennon from St. Jude Immunology flow core for his help with FACS, Hartwell Center at St. Jude Children’s Research Hospital for high-throughput sequencing, and Carmen Liema for assistance in HLA-DP4 protein purification. This study was funded in part by the Washington University Institute for Clinical and Translational Sciences grant UL1TR002345 from the National Center for Advancing Translational Sciences (NCATS) of the National Institutes of Health (NIH) (P.A.M.). J.R. is supported by an Australian Research Council Laureate Fellowship. K.K. was supported by the NHMRC Leadership Investigator Fellowship (#1173871), and T.H.O.N. was supported by the NHMRC Emerging Leadership Level 1 Investigator Fellowship (#1194036). This work was funded by ALSAC at St. Jude; the Center for Influenza Vaccine Research for High-Risk Populations (CIVR-HRP) contract number 75N93019C00052 (P.G.T. and K.K.); the St. Jude Center of Excellence for Influenza Research and Surveillance (P.G.T.) contract number HHSN272201400006C; and the St. Jude Center of Excellence for Influenza Research and Response (P.G.T.) contract numbers 75N93021C00016,
U01AI150747, U01AI144616-0251, and R01AI136514 (P.G.T.). The Elbebedy laboratory was supported by NIAID grants U01AI141990 and U01AI150747, NIAID Centers of Excellence for Influenza Research and Surveillance contracts HHSN272201400006C and HHSN272201400008C, and by NIAID Collaborative Influenza Vaccine Innovation Centers contract 75N93019C00051. The contents of this publication are solely the responsibility of the authors and do not necessarily represent the official views of the NIAID or NIH.

AUTHOR CONTRIBUTIONS
Conceptualization: P.A.M., A.A.M., M.V.P., P.G.T., and A.H.E.; methodology: P.A.M., A.A.M., M.V.P., J.S.T., J.P., J.C.C., M.K.K., S.A.T., J.A.O., R.M.P., P.G.T., and A.H.E.; investigation: P.A.M., A.A.M., M.V.P., J.S.T., W.K., E.K., J.P., A.J. Schmitz, T.L., A.H., A.M.K., R.C.M., J.C.C., T.H.O.N., L.C.R., E.R., K.A.R., A.J. Sant, T.S., W.D.M., and S.A.T.; formal analysis: P.A.M., A.A.M., M.V.P., and J.C.C.; visualization: P.A.M., A.A.M., M.V.P., and R.C.M.; resources: SJTRC Study Team; data curation: J.C.C., J.W., and SJTRC Study Team; funding acquisition: P.A.M., P.G.T., and A.H.E.; supervision: P.A.M., S.A.T., J.A.O., R.M.P., K.K., P.G.T., and A.H.E.; writing – original draft: P.A.M., A.A.M., and M.V.P.; writing – review & editing: all co-authors.

DECLARATION OF INTERESTS
The Elbebedy laboratory received funding under sponsored research agreements that are unrelated to the data presented in the current study from Emergent BioSolutions and from AbbVie. A.H.E. has received consulting payments from Mubadala Investment Company, InBios International, and Fimbriion Therapeutics and is the founder of ImmuneBio Consulting. P.G.T. has consulted and/or received honoraria and travel support from Illumina, Johnson and Johnson, and 10X Genomics. P.G.T. serves on the Scientific Advisory Board of Immunoscape and Cytoagents. The authors have applied for patents covering some aspects of these studies.

INCLUSION AND DIVERSITY
We worked to ensure gender balance in the recruitment of human subjects. We worked to ensure ethnic or other types of diversity in the recruitment of human subjects.

Received: September 6, 2021
Revised: November 12, 2021
Accepted: December 17, 2021
Published: December 23, 2021

REFERENCES
Al Kaabi, N., Zhang, Y., Xia, S., Yang, Y., Al Qahtani, M.M., Abdulrazzaq, N., Al Nusair, M., Hassany, J.S., Abdalla, J., et al. (2021). Effect of 2 inactivated SARS-CoV-2 vaccines on symptomatic COVID-19 infection in adults: A randomized clinical trial. JAMA 326, 35–45.
Anderson, E.J., Rouphael, N.G., Widge, A.T., Jackson, L.A., Roberts, P.C., Makhene, M., Choudhury, D., Zhang, J., Aparicio, L., Bom, S., Rashid, R., et al. (2021). Functional characterization of CD4+ T cell receptors crossreactive for SARS-CoV-2 and endemic coronaviruses. J. Clin. Invest. 131, e146922.
Egorov, E.S., Merzlyak, E.M., Shelenkova, A.A., Britanova, O.V., Sharonov, G.V., Staroverov, D.B., Bolotin, D.A., Davydov, A.N., Barsova, E., Lebedev, Y.B., et al. (2015). Quantitative profiling of immune repertoires for minor lymphocyte counts using unique molecular identifiers. J. Immunol. 194, 6155–6163.
Glanville, J., Huang, H., Nau, A., Hatton, O., Wager, L.E., Rubelt, F., Ji, X., Han, A., Kram, S.M., Pettus, C., et al. (2017). Identifying specificity groups in the T cell receptor repertoire. Nature 547, 89–93.
Good-Jacobson, K.L., Chen, Y., Voss, A.K., Smyth, G.K., Thomas, T., and Tarin, D. (2014). Regulation of germinal center responses and B-cell memory by the chromatin modifier MOZ. Proc. Natl. Acad. Sci. USA 111, 9585–9590.
Jackson, L.A., Anderson, E.J., Rouphael, N.G., Roberts, P.C., Makhene, M., Coler, R.N., McCullough, M.P., Chappell, J.D., Denison, M.R., Stevens, L.J., et al. (2020). An mRNA vaccine against SARS-CoV-2 – preliminary report. N. Engl. J. Med. 383, 1920–1931.
Jensen, K.K., Andreatta, M., Marcattil, P., Buus, S., Greenbaum, J.A., Yan, Z., Sette, A., Peters, B., and Nielsen, M. (2018). Improved methods for predicting peptide binding affinity to MHC class II molecules. Immunity 54, 394–406.
Kamar, N., Abramyan, F., Marion, O., Couat, C., Izopet, J., and Del Bello, A. (2021). Three doses of an mRNA Covid-19 vaccine in solid-organ transplant recipients. N. Engl. J. Med. 385, 661–662. https://doi.org/10.1056/NEJMc2108861.
Khoury, D.S., Cromer, D., Reynolds, A., Schub, T.E., Wheatley, A.K., Juno, J.A., Subbarao, K., Kent, S.J., Triccas, J.A., and Davenport, M.P. (2021). Neutralizing antibody levels are highly predictive of immune protection from symptomatic SARS-CoV-2 infection. Nat. Med. 27, 1205–1211.
Kim, W., Zhou, J.Q., Sturtz, A.J., Horvath, S.C., Schmitz, A.J., Lei, T., Kalai-dina, E., Thapa, M., Alsoussi, W.B., Haile, A., et al. (2021). Germinal centre-driven maturation of B cell responses to SARS-CoV-2 vaccination. bioRxiv. https://doi.org/10.1101/2021.03.21.466651.
Logunov, D.Y., Dolzhikova, I.V., Schcheblyakov, D.V., Tukhvatulin, A.I., Zubkova, O.V., Dzharullaiwa, A.V., Kovyrshina, A.V., Lubenet, N.L., Grousova, D.M., Erokhova, A.S., et al. (2021). Safety and efficacy of an rAd26 and rAd5 vector-based heterologous prime-boost COVID-19 vaccine: an interim analysis of a randomised controlled phase 3 trial in Russia. Lancet 397, 671–681.
Loyal, L., Braun, J., Henze, L., Kruse, B., Dingeldey, M., Reimer, U., Kern, F., Schwarz, T., Mangold, M., Unger, C., et al. (2021). Cross-reactive CD4+ T cells enhance SARS-CoV-2 immune responses upon infection and vaccination. Science 374, eab18283.
Imbalance of regulatory and cytotoxic SARS-CoV-2-reactive CD4+ T cells in COVID-19. Minervina, A.A., Pogorelyy, M.V., Mamedov, I.Z., Lebedev, Y.B., et al. (2020). Primary and secondary anti-viral response captured by the dynamics and phenotype of individual T cell clones. eLife 9, e53704.

Minervina, A.A., Komech, E.A., Titov, A., Benousda Koraichi, M., Rosati, E., Mamedov, I.Z., Franke, A., Efimov, G.A., Chudakov, D.M., Mora, T., et al. (2021). Longitudinal high-throughput TCR repertoire profiling reveals the dynamics of T-cell memory formation after mild COVID-19 infection. eLife 10, e65502.

Minervina, A.A., Pogorelyy, M.V., Kirk, A.M., Allen, E.K., Allison, K.J., Lin, C.-Y., Brice, D.C., Zhu, X., Vesegana, K., Wu, G., et al. (2021b). Convergent epitope-specific T cell responses after SARS-CoV-2 infection and vaccination. medRxiv. https://doi.org/10.1101/2021.07.12.21260227.

Niehrs, A., Garcia-Beltran, W.F., Norman, P.J., Watson, G.M., Hölzemer, A., Chapel, A., Richert, L., Pommerening-Röser, A., Körner, C., Ozawa, M., et al. (2019). A subset of HLA-DP molecules serve as ligands for the natural and synthetic exposure to SARS-CoV-2. Res. Sq. https://doi.org/10.5446/51964/v1.

Peng, Y., Mentzer, A.J., Liu, G., Yao, X., Yin, Z., Dong, D., Dejinrattisai, W., Rostron, T., Supasa, P., Liu, C., et al. (2020). Broad and strong memory CD4+ and CD8+ T cells induced by SARS-CoV-2 in UK convalescent individuals following COVID-19. Nat. Immunol. 21, 1336–1345.

Pogorelyy, M.V., Minervina, A.A., Shugay, M., Chudakov, D.M., Lebedev, Y.B., Mora, T., and Walczak, A.M. (2019). Detecting T cell receptors involved in immune responses from single repertoire snapshots. PLoS Biol 17, e3000314.

Polack, F.P., Thomas, S.J., Kitchin, N., Absalon, J., Gustman, A., Lockhart, S., Perez, J.L., Pellegrino, P., Waters, L., Burns, F., Kinloch, S., et al. (2020). Failure to seroconvert after two doses of BNT162b2 SARS-CoV-2 vaccine in a patient with uncontrolled HIV. Lancet HIV 8, e317–e318.

Tubo, N.J., Pagán, A.J., Taylor, J.J., Nelson, R.W., Linehan, J.L., Ertelt, J.M., Huseby, E.S., Way, S.S., and Jenkins, M.K. (2013). Single naive CD4+ T cells from a diverse repertoire produce different effector cell types during infection. Cell 153, 785–796.

Turner, J.S., O’Halloran, J.A., Kallalidena, E., Kim, W., Schmitz, A.J., Zhou, J.O., Lei, T., Thapa, M., Chen, R.E., Case, J.B., et al. (2021). SARS-CoV-2 mRNA vaccines induce persistent receptor human germline centre responses. Nature 596, 109–113.

Turner, J.S., Zhou, J.O., Han, J., Schmitz, A.J., Rizk, A.A., Aloussi, W.B., Lei, T., Amor, M., McIntre, K.M., Meade, P., et al. (2020). Human germline centres engage memory and naive B cells after influenza vaccination. Nature 586, 127–132.

Ueno, H., Banchereau, J., and Vinuesa, C.G. (2015). Pathophysiology of T follicular helper cells in humans and mice. Nat. Immunol. 16, 142–152.

Verbeke, R., Lentacker, I., De Smedt, S.C., and Dewitte, H. (2021). The dawn of mRNA vaccines: the COVID-19 case. J. Control. Release 333, 511–520, https://doi.org/10.1016/j.jconrel.2021.05.015.

Wang, P., Nair, M.S., Liu, L., Iketani, S., Luo, Y., Guo, Y., Wang, M., Yu, J., Zhang, B., Kwong, P.D., et al. (2021a). Antibody resistance of SARS-CoV-2 variants B.1.351 and B.1.1.7. Nature 592, 130–135.

Wang, Z., Schmidt, F., Weisblum, Y., Muecksch, F., Barnes, C.O., Finkin, S., Schaefer-Babajew, D., Cipolla, M., Gaebler, C., Lieberman, J.A., et al. (2021b). mRNA vaccine-elicited antibodies to SARS-CoV-2 and circulating variants. Nature 592, 616–622.

Weisel, F.J., Zuccarino-Catania, G.V., Chikina, M., and Shlomchik, M.J. (2016). A temporal switch in the germinal center determines differential output of memory B and plasma cells. Immunity 44, 116–130.

Widge, A.T., Rouphael, N.G., Jackson, L.A., Anderson, E.J., Roberts, P.C., Makhenke, M., Chappell, J.D., Denison, M.R., Stevens, L.J., Pruissieres, A.J., et al. (2021). Durability of responses after SARS-CoV-2 mRNA-1273 vaccination. N. Engl. J. Med. 384, 80–82.

Wu, K., Werner, A.P., Koch, M., Choi, A., Narayanan, E., Stewart-Jones, G.B.E., Colpitts, T., Bennett, H., Boyoglu-Barnum, S., Shi, W., et al. (2021). Serum neutralizing activity elicited by mRNA-1273 vaccine. N. Engl. J. Med. 384, 1486–1490.
### Key Resources Table

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| CD4 Alexa Fluor 700 (clone SK3) | BioLegend | cat#: 344622; RRID: AB_2563150 |
| CD19 PE (clone HIB19) | BioLegend | cat#: 302208; RRID: AB_314238 |
| CXCR5 PE-Dazzle 594 (clone J252D4) | BioLegend | cat#: 356928; RRID: AB_2563689 |
| PD1 BB515 (clone EH12.1) | BD Biosciences | cat#: 564494; RRID: AB_2738827 |
| IgG BV480 (goat polyclonal) | Jackson ImmunoResearch | cat#: 109-685-098; RRID: AB_2721846 |
| IgA FITC (clone M24A) | Millipore | cat#: CBL114F; RRID: AB_92852 |
| CD45 A532 (clone HI30) | Thermo | cat#: 58-0459-42; RRID: AB_11218673 |
| CD38 BB700 (clone HIT2) | BD Biosciences | cat#: 566445; RRID: AB_2744375 |
| CD20 Pacific Blue (clone 2H7) | BioLegend | cat#: 980204; RRID: AB_2632618 |
| CD27 BV510 (clone O323) | BioLegend | cat#: 302836; RRID: AB_2562086 |
| CD8 BV570 (clone RPA-T8) | BioLegend | cat#: 301038; RRID: AB_2563213 |
| IgM BV605 (clone MHM-88) | BioLegend | cat#: 314524; RRID: AB_2562374 |
| HLA-DR BV650 (clone L243) | BioLegend | cat#: 307650; RRID: AB_2563828 |
| CD19 BV750 (clone HIB19) | BioLegend | cat#: 302262; RRID: AB_2810434 |
| IgD PE-Cy5 (clone IA6-2) | BioLegend | cat#: 348250; RRID: AB_2876661 |
| CD14 PerCP (clone HCD14) | BioLegend | cat#: 325632; RRID: AB_2563328 |
| CD71 PE-Cy7 (clone CY1G4) | BioLegend | cat#: 334112; RRID: AB_2563119 |
| CD4 Spark 685 (clone SK3) | BioLegend | cat#: 344658; RRID: AB_2819981 |
| CD3 APC-Fire 810 (clone SK7) | BioLegend | cat#: 344858; RRID: AB_2860895 |
| FoxP3 BV421 (clone C398.4A) | BioLegend | cat#: 313524; RRID: AB_2563951 |
| Ki-67 BV711 (clone Ki-67) | BioLegend | cat#: 350516; RRID: AB_2563861 |
| Tbet BV785 (clone 4B10) | BioLegend | cat#: 644835; RRID: AB_2721566 |
| Bcl6 PE (clone K112-91) | BD Biosciences | cat#: 561522; RRID: AB_10717126 |
| BLIMP1 Alexa Fluor 700 (clone 646702) | R&D Systems | cat#: IC36081N |
| CD4 Alexa Fluor 700 (clone SK3) | BioLegend | cat#: 344622; RRID: AB_2563150 |
| CD45RO BV650 (clone UCHL1) | BioLegend | cat#: 304232; RRID: AB_2563462 |
| CCR7 BV785 (clone G043H7) | BioLegend | cat#: 353230; RRID: AB_2563630 |
| HLA-DR BV605 (clone L243) | BioLegend | cat#: 307640; RRID: AB_2561913 |
| ICOS BV421 (clone C398.4A) | BioLegend | cat#: 313524; RRID: AB_2562545 |
| IgD PE-Cy7 (clone IA6-2) | BioLegend | cat#: 348210; RRID: AB_10680462 |
| CD3 Alexa Fluor 700 (clone HIT3a) | BioLegend | cat#: 300324; RRID: AB_493739 |
| CD4 PerCP (clone SK3) | BioLegend | cat#: 344624; RRID: AB_2563326 |
| CD45RO APC-Fire 750 (clone UCHL1) | BioLegend | cat#: 304250; RRID: AB_2616717 |
| CXCR3 BV650 (clone G025H7) | BioLegend | cat#: 353730; RRID: AB_2563870 |
| TCR-beta chain APC-Fire 750 (clone H57-597) | BioLegend | cat#: 109246; RRID: AB_2629697 |
| CD3 BV421 (clone SK7) | BioLegend | cat#: 344834; RRID: AB_2565675 |
| anti-human CD28 purified (clone CD28.2) | BD Biosciences | cat#: 555725; RRID: AB_396088 |
| anti-human CD49d purified (clone 9F10) | BD Biosciences | cat#: 555501; RRID: AB_2130052 |
| human Fc block (cloneFc1.3216) | BD Biosciences | cat#: 564220; RRID: AB_2869554 |
| Human TrueStain FcX | BioLegend | cat#: 422302; RRID: AB_2818986 |
| CD19 BV510 (clone HIB19) | BioLegend | cat#: 302242; RRID: AB_2561668 |
| CD69 PerCP-eFluor 710 (clone FN50) | eBioscience | cat#: 460699-42; RRID: AB_2573694 |
| CXCR5 Super Bright 436 (clone MU5UBEE) | eBioscience | cat#: 62-9185-42; RRID: AB_2724064 |

(Continued on next page)
Continued

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| CD45RA eFluor 450 (clone HI100) | eBioscience | cat#: 48-0458-42; RRID: AB_1272059 |
| CD8 BV570 (clone RPA-T8) | BioLegend | cat#: 301038; RRID: AB_2563213 |
| CD3 BV750 (clone SK7) | BioLegend | cat#: 344846; RRID: AB_2800923 |
| CD4 BB515 (clone SK3) | BD Biosciences | cat#: 565996; RRID: AB_2739447 |
| PD-1 FITC (clone EH12.2H7) | BioLegend | cat#: 329904; RRID: AB_940479 |
| ICOS PerCP-Cy5.5 (clone C398.4A) | BioLegend | cat#: 313518; RRID: AB_10641280 |
| CD69 PE-Cy7 (clone FN50) | BioLegend | cat#: 310912; RRID: AB_314847 |
| CD40 (clone HB14) | Miltenyi Biotec | cat#: 304130; RRID: AB_10965547 |
| anti-human IFN-gamma BV480 (clone B27) | BD Biosciences | cat#: 566100; RRID: AB_2739503 |
| anti-human TNF-alpha BV605 (clone Mab11) | BioLegend | cat#: 502936; RRID: AB_2563884 |
| anti-human IL-17A BV785 (clone BL168) | BioLegend | cat#: 512338; RRID: AB_2566765 |
| anti-human IL-21 PE (clone 3A3-N2) | BioLegend | cat#: 513004; RRID: AB_2249025 |
| anti-human IL-2 APC (clone MQ1-17H12) | BioLegend | cat#: 316940; RRID: AB_469492 |
| CD40L BV605 (clone 24-31) | BioLegend | cat#: 304130; RRID: AB_2739503 |
| Co-stimulatory antibodies (CD28/CD49d, clones L293/L25)) | BD Biosciences | cat#: 347690; RRID: AB_647457 |
| CD40L BV605 (clone 24-31) | BioLegend | cat#: 304130; RRID: AB_2739503 |
| anti-human IFN-gamma BV480 (clone B27) | BD Biosciences | cat#: 566100; RRID: AB_2739503 |
| anti-human TNF-alpha BV605 (clone Mab11) | BioLegend | cat#: 502936; RRID: AB_2563884 |
| anti-human IL-17A BV785 (clone BL168) | BioLegend | cat#: 512338; RRID: AB_2566765 |
| anti-human IL-21 PE (clone 3A3-N2) | BioLegend | cat#: 513004; RRID: AB_2249025 |
| anti-human IL-2 APC (clone MQ1-17H12) | BioLegend | cat#: 347690; RRID: AB_647457 |

Co-stimulatory antibodies

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| Peripheral blood human samples after SARS-CoV-2 infection | St. Jude Tracking of Viral and Host Factors Associated with COVID-19 study (SJTRC, NCT04362995) | N/A |
| Peripheral blood human samples after BNT162b2 vaccination | St. Jude Tracking of Viral and Host Factors Associated with COVID-19 study (SJTRC, NCT04362995) | N/A |
| Peripheral blood and matched lymph node samples after BNT162b2 vaccination | WU-368 study (approval #2020-12-081) | N/A |

Chemicals, peptides, and recombinant proteins

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| Recombinant SARS-CoV-2 Spike protein – Biotin conjugated | Stadlbauer et al., 2020 | N/A |
| Recombinant SARS-CoV-2 Spike protein – Alexa Fluor 467 conjugated | Stadlbauer et al., 2020 | N/A |
| Recombinant SARS-CoV-2 Spike protein – Alexa Fluor 488 conjugated | Stadlbauer et al., 2020 | N/A |
| PE-labeled HLA-DPB1*04:01 S<sub>167-180</sub> tetramer | This paper | N/A |
| Brilliant Staining buffer | BD Biosciences | cat#: 566349 |
| streptavidin APC-Fire 750 | BioLegend | cat#: 405250 |
| True-Nuclear Transcription Factor Buffer Set | BioLegend | cat#: 423106 |
| Zombie Aqua | BioLegend | cat#: 424401 |
| Zombie NIR Fixable Viability Kit | BioLegend | cat#: 423106 |
| Lenti-X Concentrator | Clontech | cat#: 631232 |

(Continued on next page)
Continued

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| 1x Cell Stimulation cocktail | eBioscience | cat#: 00-4970-93 |
| Ghost Dye Violet 510 Viability Dye | Tonbo Biosciences | cat#: 13-0870-T100 |
| CTFEYVSQFPLMDLE peptide (>95% purity) | This paper | N/A |
| TFIEYSQFPLMDLE peptide | This paper | N/A |
| SARS-CoV-2 Prot_S Complete Peptivator | Miltenyi | cat#: 130-127-951 |
| NKLIANQF peptide (>95% purity) | Minervina et al., 2020 | N/A |
| GolgiPlug | BD Biosciences | cat#: 555029 |
| Fixation/Permeabilization Solution kit | BD Biosciences | cat#: 554715 |
| streptavidin PE | BioLegend | cat#: 405204 |

Critical commercial assays

- AllType NGS 11-Loci Amplification Kit | One Lambda | cat#: ALL-11LX |
- SuperScript VILO cDNA Synthesis kit | Invitrogen | cat#: 11754250 |
- SmartScribe Reverse Transcriptase | Takara | cat#: 639538 |
- Q5 Hot Start High-Fidelity DNA Polymerase | NEB | cat#: M0493 |

Deposited data

- Processed TCR repertoire sequencing data | This paper | GEO: GSE183393 |
- Raw TCR repertoire sequencing data | This paper | SRA: SRP335569 |

Experimental models: Cell lines

- 293T | ATCC | cat#: CRL-3216 |
- Jurkat 76.7 (variant of TCR-null Jurkat 76.7 cells that expresses human CD8 and an NFAT-GFP reporter) | gift from Wouter Scheper | N/A |

Oligonucleotides

- 5’ – template switch adapter (SmartNNNa): AAGCA GUGGTAAUACACGGAGUNNNUNNNUNUNNNU GCCU(TT)(G)4 | Egorov et al., 2015 | N/A |
- Primer for cDNA synthesis, human TCR alpha chain mRNA, C-region (ACR_st4): GTCTAGCA CAGTTTTGTC | Egorov et al., 2015 | N/A |
- Primer for cDNA synthesis, human TCR beta chain mRNA, C-region (BCR4short): GTATCTGGAGTCATTGA | Egorov et al., 2015 | N/A |
- Forward primer for PCR step 1, anneals on the switch adapter (M1ss): AAGCAGTGGTATC AACGCA | Egorov et al., 2015 | N/A |
- Nested reverse primer for PCR step 1, TCR alpha, C-region (ACR_st1):GTCACTGGATTAGAGTCA | Egorov et al., 2015 | N/A |
- Nested reverse primer for PCR step 1, TCR beta, C-region (BC2uniR):TGCTTCTGATGGCTCA AACAC | Egorov et al., 2015 | N/A |
- Barcoded forward PCR step 2 primer (M1s_i): [N]4(XXXXX)CAGTGGTATCAACGCAAG | Egorov et al., 2015 | N/A |
- Barcoded reverse PCR step 2 primer (TCR alpha): [N]4(XXXXX)GGTCTAGGGTTCTGGAGGAT | Egorov et al., 2015 | N/A |
- Barcoded reverse PCR step 2 primer (TCR beta): [N]4(XXXXX)ACACACCTTCTCAGGTCCTC | Egorov et al., 2015 | N/A |

huTRBV2ext: TCGATGATCAATTCTCAGTTG Wang et al., 2012 N/A
huTRBV3ext: CAAAATACCTGGTCACACAG Wang et al., 2012 N/A
huTRBV4ext: TCGCTTCTCACCTGAATG Wang et al., 2012 N/A
huTRBV5-1_4ext: GATTCTCAGGKCKCCAGTTC Wang et al., 2012 N/A
huTRBV5-5_8ext: GTACCAACAGGYCCTGGGT Wang et al., 2012 N/A

(Continued on next page)
| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| huTRBV6-1_3,5_9ext: | Wang et al., 2012 | N/A |
| huTRBV6-4ext: | Wang et al., 2012 | N/A |
| huTRBV7-1_3ext: | Wang et al., 2012 | N/A |
| huTRBV7-4_9extnew: | Wang et al., 2012 | N/A |
| huTRBV9ext: | Wang et al., 2012 | N/A |
| huTRBV10-1ext: | Wang et al., 2012 | N/A |
| huTRBV10-2ext: | Wang et al., 2012 | N/A |
| huTRBV10-3ext: | Wang et al., 2012 | N/A |
| huTRBV11ext: | Wang et al., 2012 | N/A |
| huTRBV12ext: | Wang et al., 2012 | N/A |
| huTRBV13ext: | Wang et al., 2012 | N/A |
| huTRBV14ext: | Wang et al., 2012 | N/A |
| huTRBV15extnew: | Wang et al., 2012 | N/A |
| huTRBV16ext1: | Wang et al., 2012 | N/A |
| huTRBV17ext_new: | Wang et al., 2012 | N/A |
| huTRBV18ext: | Wang et al., 2012 | N/A |
| huTRBV19ext: | Wang et al., 2012 | N/A |
| huTRBV20ext: | Wang et al., 2012 | N/A |
| huTRBV23ext: | Wang et al., 2012 | N/A |
| huTRBV24ext: | Wang et al., 2012 | N/A |
| huTRBV25ext: | Wang et al., 2012 | N/A |
| huTRBV27,28ext: | Wang et al., 2012 | N/A |
| huTRBV29ext: | Wang et al., 2012 | N/A |
| huTRBV30ext: | Wang et al., 2012 | N/A |
| huTRBCext: | Wang et al., 2012 | N/A |
| huTRAV1ext: | Wang et al., 2012 | N/A |
| huTRAV2ext_new: | Wang et al., 2012 | N/A |
| huTRAV3ext: | Wang et al., 2012 | N/A |
| huTRAV4ext: | Wang et al., 2012 | N/A |
| huTRAV5ext: | Wang et al., 2012 | N/A |
| huTRAV6ext: | Wang et al., 2012 | N/A |
| huTRAV7ext_new: | Wang et al., 2012 | N/A |
| huTRAV8-1ext: | Wang et al., 2012 | N/A |
| huTRAV8-3ext: | Wang et al., 2012 | N/A |
| huTRAV8-2,4ext: | Wang et al., 2012 | N/A |
| huTRAV8-6ext: | Wang et al., 2012 | N/A |
| huTRAV8-7ext_new2: | Wang et al., 2012 | N/A |
| huTRAV9-1_2ext: | Wang et al., 2012 | N/A |
| huTRAV10ext: | Wang et al., 2012 | N/A |
| huTRAV12-1_3ext: | Wang et al., 2012 | N/A |
| huTRAV13-1ext: | Wang et al., 2012 | N/A |
| huTRAV13-2ext_new: | Wang et al., 2012 | N/A |
| huTRAV14ext: | Wang et al., 2012 | N/A |
| huTRAV16ext: | Wang et al., 2012 | N/A |
| huTRAV17ext: | Wang et al., 2012 | N/A |
| huTRAV18ext_new3: | Wang et al., 2012 | N/A |
| huTRAV19ext: | Wang et al., 2012 | N/A |
| huTRAV20ext: | Wang et al., 2012 | N/A |

(Continued on next page)
Continued

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| huTRAV21ext: TTCCTGCAGCTCTGAGTG | Wang et al., 2012 | N/A |
| huTRAV22ext: GTCTCTCCAGACTTCTC | Wang et al., 2012 | N/A |
| huTRAV23ext_new: TGCTTATGAGAACACTGCG | Wang et al., 2012 | N/A |
| huTRAV24ext: TCTAGTCACTGCATGTTCAG | Wang et al., 2012 | N/A |
| huTRAV25ext_new: GGACTTCACCACGTACTGC | Wang et al., 2012 | N/A |
| huTRAV26-1ext: GCAAACCTGCTTGAATC | Wang et al., 2012 | N/A |
| huTRAV26-2ext: AGCCAAATTCAATGGAGAG | Wang et al., 2012 | N/A |
| huTRAV27ext: TCAGTTCTAGCAtCCAAGAG | Wang et al., 2012 | N/A |
| huTRAV29ext: GCAAGTAAAGCAAAATTCACC | Wang et al., 2012 | N/A |
| huTRAV30ext: CAAACACCAGTCAGAGTC | Wang et al., 2012 | N/A |
| huTRAV34ext: AGAAGAAGGAGGACTCAGTC | Wang et al., 2012 | N/A |
| huTRAV35ext: GGTCAACAGCTGAATCAGAG | Wang et al., 2012 | N/A |
| huTRAV36ext: GAAGACAAGGTGGTACAAAGC | Wang et al., 2012 | N/A |
| huTRAV38ext: GCACCATATGACCCACTGAG | Wang et al., 2012 | N/A |
| huTRAV39ext: CTGTTCCGTAGCATGAG | Wang et al., 2012 | N/A |
| huTRAV40ext_new: GCATCTGTGACTATGAACTGC | Wang et al., 2012 | N/A |
| huTRACext: GACCAGCTTGACATCACAG | Wang et al., 2012 | N/A |

Recombinant DNA

| pLVX-EF1α-IRES-Puro | Clontech | cat#: 631253 |
|----------------------|----------|-------------|
| TCR, 4.1-mCherry | This paper | N/A |
| TCR, 6.3-mCherry | This paper | N/A |
| psPAX2 packaging plasmid | gift from Didier Trono | Addgene plasmid #12260; RRID: Addgene_12260 |
| pMD2.G envelope plasmid | gift from Didier Trono | Addgene plasmid #12259; RRID: Addgene_12259 |

Software and algorithms

| Software | Developer | Link |
|----------|-----------|------|
| FlowJo v10.7.1 | BD Biosciences | https://www.flowjo.com/solutions/flowjo/downloads |
| SpectroFlo v2.2 | Cytek | https://cytekbio.com/pages/spectro-flo |
| R v. 4.0.1 | https://www.r-project.org |
| Prism v9.1.0 | GraphPad Software | https://graphpad.com |
| Biorender | | https://biorender.com |
| MiGEC v. 1.2.7 | Bolotin et al., 2015 | https://github.com/mikessh/migec |
| MiXCR v. 3.0.3 | Shugay et al., 2014 | https://github.com/milaboratory/mixcr |
| data.table R package v. 1.14.0 | | https://github.com/Rdatatable/data.table/wiki |
| stringdist R package v. 0.9.6.3 | | https://github.com/markvanderloo/stringdist |
| igraph R package v. 1.2.6 | | https://igraph.org/r/ |
| gephi v. 0.9.2 | | https://gephi.org |
| ggplot2 R package v. 3.3.3 | | https://cran.r-project.org/web/packages/ggplot2/index.html |

RESOURCE AVAILABILITY

**Lead contact**

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Ali H. Ellebedy (ellebedy@wustl.edu).
Materials availability
The HLA-DPB1*04 S_{167-180} HLA class II tetramer has been submitted to the NIH tetramer core facility (tetramer.yerkes.emory.edu). No other new unique reagents were generated in this study.

Data and code availability
- Processed TCR sequencing data have been submitted to the GEO database:GSE183393, and the raw sequencing data have been submitted to the SRA database: SRP355689. All sequencing data are publicly available as of the date of publication. Any raw flow cytometry data not available in the supplemental tables will be shared by the lead contact upon request.
- This paper does not report original code.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Human subjects
Human subjects who elected to receive the BNT162b2 mRNA vaccine were recruited into this prospective observational study. Written informed consent was obtained from each subject. The study was approved by the Washington University in St. Louis Institutional Review Board (approval # 2020-12-081). Details of the entire study cohort have been previously reported (Turner et al., 2021). The age and sex of the subjects included in the present study are listed in Table 1. Draining axillary lymph nodes ipsilateral to the deltoid vaccination site were located with ultrasound and sampled with multiple passes of 6 separate 25-gauge needles under real-time ultrasound guidance (Turner et al., 2020). Each needle was flushed with 3 mL of R10 (RPMI, 1640 supplemented with 10% FBS and 100 U/mL penicillin-streptomycin) and the 3 separate 1 mL rinses of R10. Red blood cells were lysed with 1xACK (Sacha and Watkins, 2010) and then washed with 2xPBS supplemented with 2% FBS and 2 mM EDTA. FNA samples were immediately stained for flow cytometry or cryopreserved in freezing media (10% dimethylsulfoxide and 90% FBS). Two subjects – 07 and 15 – received their BNT162b2 vaccine in the contralateral arm to the initial axillary lymph node FNA site. Subject 15 then had FNA performed on two lymph nodes, one ipsilateral and the other contralateral to the deltoid vaccination for all FNA samples completed starting on day 28. Matched blood samples from the same time-points were obtained by standard phlebotomy into EDTA anti-coagulated tubes and PBMC were prepared by density gradient centrifugation over Ficoll 1077 (GE). PBMC were treated with 1xACK for 5 minutes to lyse residual red blood cells before washing with R10 and immediate use in flow cytometry experiments or cryopreservation in freezing media.

For S_{167-180} tetramer validation and ICS experiments we used PBMC from SARS-CoV-2 convalescent and vaccinated donors obtained as a part of the St. Jude Tracking of Viral and Host Factors Associated with COVID-19 study (SJTRC, NCT04362995); a prospective, IRB-approved, longitudinal cohort study of St. Jude Children’s Research Hospital adult (≥ 18 years old) employees. Participants were screened for SARS-CoV-2 infection by PCR approximately weekly when on St. Jude campus. For this study, we utilized the convalescent blood draw for SARS-CoV-2 infected individuals (3-8 weeks post diagnosis) as well as post-vaccination blood draws for SARS-CoV-2 naive individuals. Blood samples were collected in 8 mL CPT tubes; and PBMC were isolated and frozen within 24 hours of collection. HLA typing of each included SJTRC participant was performed using the AllType NGS 11-Loci Amplification Kit (One Lambda; Lot 013) according to manufacturer’s instructions. Resulting libraries were sequenced on MiSeq lane at 150x150bp. HLA types were called using the TypeStream Visual Software from One Lambda.

METHOD DETAILS

Cell sorting and flow cytometry
Fresh or frozen PBMC and/or FNA samples were washed and re-suspended in P2. For sorting of T_{FH} populations from frozen FNA samples in Figures 1B and 5, cells were stained with CD4 Alexa Fluor 700 (SK3, BioLegend), CD19 PE (HB19, BioLegend), CXCR5 PE-Dazzle 594 (J252D4, BioLegend), PD1 BBS15 (EH12.1, BD Horizon), and Zombie Aqua (BioLegend) for a total of 30 minutes on ice. Cells were then washed twice with P2 and live, singlet, CD4-CD19 CXCR5-PD1+ cells were sorted on a FACSVector II into Trizol before being immediately frozen on dry ice.

To analyze antigen-specific B cell populations, we generated labeled recombinant soluble SARS-CoV-2 spike protein as previously described (Stadlbauer et al., 2020). A mammalian cell codon-optimized nucleotide sequence coding for the soluble ectodomain of the spike protein of SARS-CoV-2 (GenBank: MN908947.3, amino acids 1-1213) including a C-terminal thrombin cleavage site, T4 foldon trimerization domain, and hexahistidine tag was cloned into mammalian expression vector pCAGGS. The spike protein sequence was modified to remove the polybasic cleavage site (RRAR to A), and two pre-fusion stabilizing proline mutations were introduced (K986P and V987P, wild type numbering). Recombinant S was produced in Expi293F cells (ThermoFisher) by transfection with purified DNA using the ExpiFectamine 293 Transfection Kit (ThermoFisher). Supernatants from transfected cells were harvested 3 days post-transfection, and recombinant proteins were purified using Ni-NTA agarose (ThermoFisher), then buffer exchanged into phosphate buffered saline (PBS) and concentrated using Amicon Ultracel centrifugal filters (EMD Millipore). For flow cytometry staining, recombinant S was labeled with DyLight 488-NHS ester, Alexa Fluor 647-NHS ester or biotinylated using the EZ-Link Micro
Cells were incubated for 18 hours (37°C, CD49d) and positive control (CD28, CD49d, 1X Cell Stimulation Cocktail, PMA/ionomycin; eBioscience) were included in each assay.

BV711 (Ki-67, BioLegend), Tbet BV785 (4B10, BioLegend), Bcl6 PE (K112-91, BD Pharmingen) and BLIMP1 Alexa Fluor 700 were then washed twice with P2, fixed with the True Nuclear fixation kit (BioLegend) for 1 hour at room temperature, washed twice with True Nuclear wash buffer (BD Biosciences) and then stained for 1 hour at room temperature with FoxP3 BV421 (206D, BioLegend), Ki-67 BV711 (Ki-67, BioLegend), Tbet BV785 (4B10, BioLegend), Bcl6 PE (K112-91, BD Pharmingen) and BLIMP1 Alexa Fluor 700 (BD4702, R&D Systems). Cells were then washed twice with True Nuclear Permeabilization/Wash buffer before acquisition on a Cytek Aurora spectral flow cytometer using SpectroFlo v2.2 software (Cytek) and analyzed using FlowJo software (BD).

In tetramer staining experiments cells were stained in P2 for 10 minutes on ice with PE-labeled HLA-DPB1*04:01 S167-180 tetramer. Then, without washing away the tetramer, a master mix was added to the cells that included pre-titrated volumes of the following reagents: CD8 BV570 (RPA-T8, BioLegend) CD3 APC-Fire 810 (SK7, BioLegend) CD4 Alexa Fluor 700 (SK3, BioLegend) CD45RO BV650 (UCHL1, BioLegend) CCR7 BV785 (G043H7, BioLegend) CXCR5 PE-Dazzle 594 (J252D4, BioLegend) CD14 BV515 (CD14, BD Horizon) CD25 BV515 (CD25, BD Horizon) CD27 BV515 (O323, BioLegend) CD19 BV750 (HIB19, BioLegend) CD20 Pacific Blue (2H7, BioLegend). Samples were then incubated on ice for an additional 30 minutes before they were washed twice with P2 and fixed in a final concentration of 1% paraformaldehyde for 15 minutes at room temperature. Samples were then run on a Cytek Aurora spectral flow cytometer using SpectroFlo v2.2 software (Cytek) and analyzed using FlowJo software (v10.8.0, BD). The alternative staining method used to incorporate CXCR3 evaluation in Figure S6 substituted the following antibodies into the panel listed above: CD3 Alexa Fluor 700 (HIT3a, BioLegend), CD4 PerCP (SK3, BioLegend), CD45RO APC-Fire 750 (UCHL1, BioLegend), and added CXCR3 BV650 (G025H7, BioLegend). Tetramer responses over time in Figures 3 and 4 were graphed in Prism (v9.1.0, GraphPad Software, LLC).

Jurkat cell line generation

For Jurkat cell line generation we selected a TCRα (TRAV35, CAGMNYGGSQNLIF, TRAJ42) and two different TCRβ chains (TRBV4-1, CASSQGTVYTF, TRBJ1-2; TRBV6-3, CASSYGAYTF, TRBJ1-2) from Bacher et al. Both TCRα and TCRβ chains were modified to use murine constant regions to facilitate surface expression (murine TRAC*01 and murine TRBC2*01). Two gBlock sequence fragments were synthesized by Genscript to encode the modified TCRα and TCRβ chains, one of the modified TCRα chains, and mCherry fluorescent protein, linked together by 2A sites. These sequences were cloned into the pLVX-EF1α-IRES-Puro lentiviral expression vector (Clontech). To generate the lentivirus we transfected 293T packaging cell line (ATCC CRL-3216) with the pLVX lentiviral vector containing TCR_4.1-mCherry or TCR_6.3-mCherry insert, psPAX2 packaging plasmid (Addgene plasmid #12260), and pMD2.G envelope plasmid (Addgene plasmid #12259). Viral supernatant was collected and concentrated using Lenti-X Concentrator 24- and 48-hours after the transfection (Clontech). Jurkat 76.7 cells (a gift from Wouter Scheper; variant of TCR-null Jurkat 76.7 cells that expresses human CD8 and an NFAT-GFP reporter) were transduced, then antibiotic selected for 1 week using 1 μg/mL puromycin in RPMI (Gibco) containing 10% FBS and 1% penicillin/streptomycin. Transduction of Jurkat cell line was confirmed by expression of mCherry, and surface TCR expression was confirmed via flow cytometry on a BD Fortessa using FACSDiva software using antibodies against mouse TCRβ constant region (APC-Fire750-conjugated, Biolegend, clone H57-597) and human CD3 (Brilliant Violet 421-conjugated, Biolegend, clone SK7). Flow data were analyzed in FlowJo software.

Jurkat peptide stimulation

Jurkat 76.7 cells expressing TCRs 4.1 and 6.3 (2.5x10^5) were co-cultured with PBMCs from SARS-CoV-2 naive DBP1*04:01-positive donor (6x10^5) pulsed with 1 μM of peptide, 1 μg/mL each of anti-human CD28 and CD49d (BD Biosciences). An unstimulated (CD28, CD49d) and positive control (CD28, CD49d, 1X Cell Stimulation Cocktail, PMA/ionomycin; eBioscience) were included in each assay. Cells were incubated for 18 hours (37°C, 5% CO2). After the incubation cells were washed twice with FACs buffer (PBS, 2% FBS, 1 mM EDTA), resuspended in 50 μL of FACs buffer, and then blocked using 1 μL human Fc-block (BD Biosciences). Cells were then stained with 1 μL of biotinylated and Alexa Fluor 647 conjugated recombinant soluble Spike proteins as well as PD-1 BV515 (EH12.1, BD Horizon). Cells were then washed twice with P2 and stained with IgG BV480 (goat polyclonal, Jackson ImmunoResearch), IgA FITC (M24A, Millipore), CD45 A532 (HI30, Thermo), CD38 BB700 (HIT2, BD Horizon), CD20 Pacific Blue (2H7, BioLegend, CD27 BV510 (O323, BioLegend), anti-human CD3 (Brilliant Violet 421-conjugated, Biolegend, clone SK7), anti-human CD69 (PerCP-eFluor710-conjugated, eBioscience, clone FN50), and anti-mouse TCRβ chain (APC/Fire750-conjugated, Biolegend, clone H57-597). Cells were incubated for 20 minutes at room temperature and then washed with a FACs buffer. Cells were analyzed by flow cytometry on a custom-configured BD Fortessa using FACSDiva software (Becton Dickinson). Flow cytometry data were analyzed using FlowJo software (BD Biosciences).

Responsiveness to peptide stimulation was determined by measuring frequency of NFAT-GFP, CD69 and β7 TCR expression.
Peptide stimulation and intracellular cytokine staining of SJTRC samples

Donor PBMCs were thawed, suspended in RPMI 1640 supplemented with 10% heat-inactivated human AB serum (Gemini Bio-Products), 1% non-essential amino acids (Gibco), 1 mM sodium pyruvate (Gibco), and 100 U/mL penicillin-streptomycin (hR10), and plated at 2.5-4.0x10^5 cells/well in a 96-well U-bottom plate. PBMCs were stimulated with 5 μg/mL CTYEYVSPFLMDLE peptide or left unstimulated and incubated at 37°C and 5% CO₂. After 12 h, 1x PMA/ionomycin (eBioscience) was added to positive control wells and GolgiPlug (BD Biosciences) was added at 1:1000 to all wells. Cells were incubated for an additional 6 h (for 18 h total), washed twice with FACS (PBS, 2% FBS, 1 mM EDTA), resuspended in 50 μL FACS containing 5 μL human Fc-block (Biolegend), and blocked for 15 min at RT. Cells were surface stained in an additional 50 μL FACS buffer containing 1 μL Ghost Dye Violet 510 Viability Dye (Tonbo Biosciences) and a cocktail of fluorescent anti-human antibodies: CXCR5 SuperBright 436 (Thermo, clone MUSUBEE), CD45RA eFluor 450 (Thermo, clone H100), CD19 BV510 (Biolegend, clone HIB19), CD8 BV570 (Biolegend, clone RPA-T8), CD3 BV750 (Biolegend, clone SK7), CD4 BB515 (BD, clone SK3), PD1 FITC (Biolegend, clone EH12.2H7), ICOS PerCP/Cy5.5 (Biolegend, clone C398.4A), CD69 PE/Cy7 (Biolegend, clone FN50), and γδ TCR AlexaFluor 647 (Biolegend, clone B1) for 30 min at 4°C. Cells were washed twice with FACS buffer, fixed in Fix/Perm Solution (BD Biosciences) for 20 min at 4°C, and washed twice in Wash/Perm buffer (BD Biosciences). For detection of intracellular cytokines, cells were resuspended in 50 μL Perm/Wash buffer containing a cocktail of anti-human antibodies including IFNγ BV480 (BD Biosciences, clone B27), TNFα BV605 (Biolegend, clone MAβ11), IL17 BV785 (Biolegend, clone BL168), IL21 PE (Biolegend, clone 3A3-N2), and IL2 APC (Thermo, clone MQ1-17H12) and were incubated for 30 min at 4°C. Cells were washed twice in FACS buffer and analyzed by flow cytometry on a Cytek Aurora spectral flow cytometer using SpectroFlo software (Cytek) and analyzed using FlowJo software (BD Biosciences). Responsiveness to peptide stimulation was determined by comparing the number of activated CD4+(CD69+) T cells positive for IL2, IFNγ, or TNFα production per 10⁶ PBMCs to matched unstimulated controls and presented as either fold change of stimulated over unstimulated, or number of stimulated cells after background subtraction of paired unstimulated controls.

Activation-induced marker (AIM) assay

Donor PBMCs were thawed, suspended in RPMI 1640 supplemented with 10% heat-inactivated human AB serum (Gemini Bio-Products), 1% non-essential amino acids (Gibco), 1 mM sodium pyruvate (Gibco), and 100 U/mL penicillin-streptomycin (hR10) and plated in replicate wells at 5.0x10⁵ cells/well in a 96-well U-bottom plate. Cells were allowed to rest overnight in a 37°C and 5% CO₂ incubator. After 16 h, cells were treated with an anti-CD40 blocking antibody (Miltenyi, clone HB14) at a final concentration of 0.5 μg/mL for 15 min incubations in replicate wells at 5.0x10⁵ cells/well in a 96-well U-bottom plate. Cells were washed twice in 200 μL FACS buffer, resuspended in 300 μL FACS buffer containing a cocktail of anti-human antibodies including IFNγ BV480 (BD Biosciences, clone B27), TNFα BV605 (Biolegend, clone MAβ11), IL17 BV785 (Biolegend, clone BL168), IL21 PE (Biolegend, clone 3A3-N2), and IL2 APC (Thermo, clone MQ1-17H12) and were incubated for 30 min at 4°C. Cells were washed twice in 100 μL FACS buffer and analyzed by flow cytometry on a Cytek Aurora spectral flow cytometer using SpectroFlo software (Cytek) and analyzed using FlowJo software (BD Biosciences). Responsiveness to peptide stimulation was determined by comparing the number of activated CD4+(CD69+) T cells positive for IL2, IFNγ, or TNFα production per 10⁶ PBMCs to matched unstimulated controls and presented as either fold change of stimulated over unstimulated, or number of stimulated cells after background subtraction of paired unstimulated controls.

Monomer generation

HLA-DP4 monomers with the S₁₆₇₋₁₈₀ epitope were produced from purified HLA-DP4 containing the class II-associated invariant chain peptide (CLIP) (Niehrs et al., 2019) via HLA-DR catalyzed peptide exchange as described previously for HLA-DR (Scally et al., 2013). Briefly, HLA-DP4 CLIP was expressed in Trichoplusia Ni (Hi5) insect cells via a pFastBac-Dual construct encoding HLA-DPA1*01:03 α- and HLA-DPB1*04:01 β-chains with C-terminal fos/jun zipper domain. The HLA-DP4 β-chain further contained an N-terminal factor Xa-cleavable CLIP sequence, and a C-terminal biotinylation signal and His₆ tag (Niehrs et al., 2019). Following expression for 3 days at 27°C, cell supernatant was concentrated and buffer exchanged in a Tangential Flow Filtration system into 500 mM NaCl, 10 mM Tris-HCl pH 8 and subsequently purified via immobilised metal affinity chromatography and Superdex S200 gel permeation chromatography (GPC) in 150 mM NaCl, 10 mM Tris-HCl pH 8. The linked CLIP peptide was cleaved with factor Xa for 6 h at 21°C prior to peptide exchange, and factor Xa cleaved HLA-DP4 was subsequently incubated in the presence of a 10-fold molar excess of peptide and a 1/5 molar ratio of HLA-DM for 16 h at 37°C in 100 mM sodium citrate pH 5.4. HLA-DP4 loaded with S₁₆₇₋₁₈₀ peptide was buffer exchanged into 50 mM NaCl, 20 mM Tris-HCl pH 8, purified via Hi-Trap Q ion exchange chromatography and biotinylated using BirA biotin ligase. Following a final Superdex S200 GPC step in PBS, biotinylated HLA-DP4-S₁₆₇₋₁₈₀ monomer was concentrated to approx. 1 mg/ml and stored at -80°C.

Tetramer generation and staining of Jurkat cells

Biotinylated HLA-DP4 monomers loaded with TFEYVSPFLMDLE peptide (S₁₆₇₋₁₈₀) were tetramerized using PE-Streptavidin (Biolegend). One volume PE-conjugated streptavidin was added to one volume of HLA-DP4 monomer (1 mg/ml). The volume of
PE-streptavidin (0.2 mg/ml) was divided in 4 parts and added in 4 consecutive steps with 10 minutes incubation between. After adding all needed amounts of PE-streptavidin the mixture was incubated for at least 1 hour on ice prior to staining. Jurkat 76.7 cells expressing TCR4.1, TCR6.3, Jurkat 76.7 cell line expressing irrelevant TCR (specific to NQKLIANQF epitope from the spike protein of SARS-CoV-2 (Minervina et al., 2021b), and SARS-CoV-2 naive HLA-DPB1*04:01 positive donors’ PBMCs were stained with 1 µL Ghost Dye Violet 510 Viability Dye (Tonbo Biosciences) and 1 µL of HLA-DPB1*04-S167-180-tetramer. Cells were analyzed by flow cytometry on a custom-configured BD Fortessa using FACSDiva software (Becton Dickinson). Flow cytometry data were analyzed using FlowJo software (BD Biosciences). The quality of the S167-180 HLA class II tetramer was judged by staining of the relevant T cell line and low background in irrelevant Jurkats and naive PBMCs.

**Tetramer staining of SJTRC samples and scTCR sequencing**

Donor PBMCs were thawed and resuspended in 100 µL FACS buffer (PBS, 0.5% BSA, 2 mM EDTA). Cells were stained with 5 µL Fc-block (Human TruStain FcX, Biolegend) and 1.5 µL of S167-180 HLA class II PE-conjugated tetramer for 30 minutes on ice. After the incubation a cocktail of fluorescently-labeled surface antibodies (2 µL of each: Ghost Dye Violet 510 Viability Dye, Tonbo Biosciences; anti-human CD3 PerCP Cy5.5-conjugated, Biolegend, clone OKT3; anti-human CD4 BV711-conjugated, Biolegend, clone OKT4; anti-human CD45RA BV421-conjugated, Biolegend, clone HI100; and anti-human CCR7 FITC-conjugated, Biolegend, clone G043H7) was added. Samples were incubated for an additional 20 minutes on ice. Single, Live, CD3-positive, CD4-positive, tetramer-positive cells were sorted on the Sony SY3200 into 384-well plates with premixed SuperScript VILO cDNA Synthesis mix (Invitrogen) for subsequent scTCR sequencing. scTCR library preparation and sequencing was performed as previously described (Wang et al., 2012). In brief, cDNA underwent two rounds of nested multiplex PCR amplification with a forward primer mix specific for V-segments and reverse primers for C-segments of TCRalpha and TCRbeta and sequenced on Illumina MiSeq platform (2x150 read length). TCR sequences with undefined alpha-chain were excluded from the analysis. Resulting TCR sequences can be found in Table S2.

**Bulk repertoire generation**

TCRalpha and TCRbeta bulk repertoires were generated with the 5’RACE protocol described in (Egorov et al., 2015). RNA was isolated using Trizol reagent (Invitrogen) according to the manufacturer’s protocol. All RNA was used for cDNA synthesis with Smart-Scribe kit (Takara), using template switch oligonucleotide and primers specific for TCRalpha and TCRbeta constant segments. cDNA was amplified in two rounds of PCR using Q5 high-fidelity polymerase (NEB). Adapters necessary for sequencing on the Illumina platform were introduced with the KAPA HyperPrep kit (Roche). Libraries were sequenced on Illumina MiSeq platform (2x150). Sample cell counts along with sequencing quality metrics are listed in Table S3.

**Public TCR repertoire datasets**

TCRbeta dataset for MIRA class II peptide stimulation (ImmuneCODE MIRA release 002.1) was accessed via ImmuneACCESS database (Nolan et al., 2020). Processed single cell paired chain TCR datasets from ARTE assays after 6 and 24 hour stimulation with SARS-CoV-2 peptides were used as supplied by authors in original publications: Table S3 from (Bacher et al., 2020) and Table S4A from (Meckiff et al., 2020).

**QUANTIFICATION AND STATISTICAL ANALYSIS**

**TCR repertoire analysis**

Bulk TCR repertoire data was demultiplexed and assembled into the UMI consensuses with migec (v. 1.2.7; with collision filter and force-overseq parameters set to 1) (Shugay et al., 2014). V and J-segment alignment, CDR3 identification and assembly of reads into clonotypes were performed with MiXCR (v. 3.0.3) with default parameters (Bolotin et al., 2015). Resulting processed repertoire datasets and reference to raw TCR repertoire sequencing data are available at GEO database (acc. GSE183393). Analysis of bulk repertoire data was performed using R language for statistical computing, with merging and subsetting of data performed using data.table package. stringdist and igraph R packages were used to build TCR similarity network, gephi software was used for TCR similarity networks layout and visualization and ggplot2 library for other visualizations.

**Statistical analysis**

Descriptive and comparative statistics were employed in the manuscript as described in the figure legends with the number of replicates indicated.
Figure S1. Human lymph node T_{FH} population frequency correlates with the GC B cell population frequency, related to Figure 1; Tables 1 and S1
(A) Gating strategy for the lymph node T_{FH} (CD3+CD4+CXCR5+PD1+Bcl-6+FoxP3-) and GC B cell (CD19+IgDlowBcl-6+CD38int) populations. Spike+ GC B cells are gated on cells that stain positive for two individual SARS-CoV-2 spike-protein probes.
(B) The T_{FH} population measured as the frequency of total lymph node CD4+ T cells were compared with the total frequency of lymph node GC B cells using linear regression.
(C) Total T_{FH} population frequency compared with the total frequency of spike-specific GC B cells. n = 95 individual lymph node samples obtained from all 15 study subjects between and including study days 21 and 200.
Figure S2. Intracellular cytokine staining of PBMCs stimulated with S_{166-180} peptide, related to Figure 2C

(A) Gating strategy employed to resolve CD4+/CD69+ T cells producing IL2, TNFα, or IFNγ. Activated CD4+ T cells were defined as live/B cell lineage (CD19)^neg/ TFH lineage (CD45RA^-/CXCR5^neg/) gd TCRneg/ CD3+/CD4+/CD69+ and Boolean gated on IL2+, TNFα+, or IFNγ+ single-positive lymphocytes.

(B) The number of CD4+/CD69+ T cells producing IL2, TNFα, or IFNγ per 10^6 PBMCs following CTFEYVSQPFLMDLE peptide (black) or media (white) stimulation.

(C) CD4+/CD69+ T cells producing IL2, TNFα, or IFNγ per 10^6 PBMCs from DPB1*04:01/02-positive (dark teal bars; bold-italicized Sample ID) and -negative (light blue bars) participants presented as the Log_2 fold change of peptide-stimulated over unstimulated (left) and after background subtraction of unstimulated (right).

(D) The number of CD4+/CD69+ T cells (unstimulated portion subtracted) producing combinations of IL2, TNFα, and/or IFNγ per 10^6 PBMCs.

(E) Percentage of single, dual, and triple cytokine-producing CD4+/CD69+ T cells among total cytokine-producing CD4+/CD69+ T cells. Values calculated from cells per 10^6 PBMCs after background (unstimulated) subtraction. Differential Boolean gating on IFNγ, IL2, and TNFα was used to distinguish cytokine-producers; values in (D; color-coded bars at bottom) comprise the percentages in (E).
**Figure S3.** S\textsubscript{167–180} epitope discovery and validation, related to Figure 2

(A) Jurkat cell line expressing the predicted TCR after stimulation with the predicted epitope. Left column: negative control; middle column: TCR6.3 cell line co-cultured with PBMCs from healthy DPB1*04:01-positive donor pulsed with CTFEYVSQPFLMDLE peptide (S\textsubscript{166–180}); right column: positive control. Top row: NFAT-GFP reporter expression. Middle row: CD69 surface expression. Bottom row: downregulation of the TCR on cell surface.

(B) Gating strategy for (A), Figures 2D and S4.

(C) Gating strategy for Figure 2E.

(D) Gating strategy for Figure 2F.
Figure S4. Peptide stimulation of Jurkat cell lines expressing the predicted S\textsubscript{167-180} specific TCRs, related to Figure 2
(A and B) NFAT-GFP reporter expression.
(C and D) CD69 surface expression. Incubation without peptide (unstimulated) and with irrelevant SARS-CoV-2 derived DPB1*04-restricted peptide (RSFIEDLLFNKVTLA described in Dykema et al. 2021 and Loyal et al. 2021) as well as stimulation of line expressing irrelevant TCR (specific to NQKLIANQF epitope from the spike protein of SARS-CoV-2, described in Minervina et al., 2021b) with CTFEYVSQPFFMDLE peptide were used as negative controls.
Figure S5. Frequency of S\textsubscript{167-180} tetramer\textsuperscript{+} cells in comparison to the frequency of total spike AIM\textsuperscript{+} cells, related to Figure 2

(A) Gating strategy. Tetramer-positive cells were gated as cell-sized single live CD3\textsuperscript{+}CD4\textsuperscript{+}tetramer\textsuperscript{+}, AIM\textsuperscript{+} cells were defined as cell-sized single live CD3\textsuperscript{+}CD4\textsuperscript{+}CD45RA\textsuperscript{−}CD154\textsuperscript{+}CD200\textsuperscript{+}.

(B) S\textsubscript{167-180} tetramer\textsuperscript{+} cells (top row) and AIM\textsuperscript{+} (bottom row) for SARS-CoV-2 naive donor (left column) and SARS-CoV-2 convalescent donors.
Figure S6. \( \text{S}_{167-180} \)-specific CD4\(^+\) T cell response in peripheral blood of subject 16 following BNT162b2 vaccination is principally biased toward CXCR3 and not CXCR5 expression, related to Figure 3.

(A) \( \text{S}_{167-180} \)-CD4\(^+\) T cell responses over time in subject #16.
(B) CXCR3 and CXCR5 surface expression on tetramer-positive cells (red) and total CD4\(^+\) T cells (black) are visualized with overlaid contour plots. Provided frequencies are the frequency of \( \text{S}_{167-180} \)-tetramer-positive cells in the indicated quadrant.