

## Reporting Summary

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### Statistics

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

- |                          |  |
|--------------------------|--|
| n/a                      | Confirmed  |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> The exact sample size ( $n$ ) for each experimental group/condition, given as a discrete number and unit of measurement  |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly  |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> The statistical test(s) used AND whether they are one- or two-sided<br><i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>   |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> A description of all covariates tested   |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons  |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals) |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> For null hypothesis testing, the test statistic (e.g. $F$ , $t$ , $r$ ) with confidence intervals, effect sizes, degrees of freedom and $P$ value noted<br><i>Give <math>P</math> values as exact values whenever suitable.</i>                            |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings   |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes   |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> 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 | All software used to perform data collection are described in the methods section of the manuscript or the supportive information. Multiparametric Flow cytometry data was collected on FACSCanto II, LSRFortessa with FACSDiva software version 10.6.2 (BD, Germany) or CytoFLEX (Beckman Coulter) with CytExpert Software version 2.3.0.84 or Cytek Aurora (Cytek Biosciences) with SpectroFlo® Software version 2.2.0.3. ELISA data was collected by SparkControl magellan software version 2.2.   |
| Data analysis   | All codes used to perform bioinformatic analyses are described in the methods section of the manuscript or the supportive information. Multiparametric flow cytometry data was analyzed using FlowJo software version 10.6.2 (Treestar, Becton Dickinson). The visualization of multiparametric flow cytometry data was done with R version 4.0.2 using the Bioconductor (version: Release (3.11)) CATALYST package (Crowell H, Zanotelli V, Chevrier S, Robinson M (2020). CATALYST: Cytometry dATa anALYSIS Tools. R package version 1.12.2, <a href="https://github.com/HelenaLC/CATALYST">https://github.com/HelenaLC/CATALYST</a> ). R code to reproduce the analyses of multiparametric flow-cytometry data is available at <a href="https://github.com/sagar161286/SARSCoV2_specific_CD8_Tcells">https://github.com/sagar161286/SARSCoV2_specific_CD8_Tcells</a> . Visualization and statistical analysis was performed using GraphPad 9 software. Sequence homology analyses were performed in Geneious Prime 2020.0.3 ( <a href="https://www.geneious.com/">https://www.geneious.com/</a> ) using Clustal Omega 1.2.2 alignment with default settings. Reference viral sequences SARS-CoV-2 (MN908947.3) <a href="https://www.ncbi.nlm.nih.gov/nuccore/MN908947.3">https://www.ncbi.nlm.nih.gov/nuccore/MN908947.3</a> 229E (NC_002645) <a href="https://www.ncbi.nlm.nih.gov/nuccore/NC_002645">https://www.ncbi.nlm.nih.gov/nuccore/NC_002645</a> , HKU1 (NC_006577) <a href="https://www.ncbi.nlm.nih.gov/nuccore/NC_006577">https://www.ncbi.nlm.nih.gov/nuccore/NC_006577</a> , NL63 (NC_005831) <a href="https://www.ncbi.nlm.nih.gov/nuccore/NC_005831">https://www.ncbi.nlm.nih.gov/nuccore/NC_005831</a> , OC43 (NC_006213) <a href="https://www.ncbi.nlm.nih.gov/nuccore/NC_006213">https://www.ncbi.nlm.nih.gov/nuccore/NC_006213</a> , MERS (NC_019843) <a href="https://www.ncbi.nlm.nih.gov/nuccore/NC_019843">https://www.ncbi.nlm.nih.gov/nuccore/NC_019843</a> , SARS-CoV-1 (NC_004718) <a href="https://www.ncbi.nlm.nih.gov/nuccore/NC_004718">https://www.ncbi.nlm.nih.gov/nuccore/NC_004718</a> were downloaded from the NCBI database ( <a href="https://www.ncbi.nlm.nih.gov/">https://www.ncbi.nlm.nih.gov/</a> ). |

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

## Data

Policy information about [availability of data](#)

All manuscripts must include a [data availability statement](#). This statement should provide the following information, where applicable:

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

Raw data in this study are provided in the Source data. Additional supporting data are available from the corresponding authors upon reasonable request. All requests for raw and analyzed data and materials will be reviewed by the corresponding authors to verify if the request is subject to any intellectual property or confidentiality obligations. Patient-related data not included in the paper were generated as part of clinical examination and may be subject to patient confidentiality. Any data that can be shared will be released via a Material Transfer Agreement.

Reference viral sequences 229E (NC\_002645), HKU1 (NC\_006577), NL63 (NC\_005831), OC43 (NC\_006213), MERS (NC\_019843), SARS-CoV-1 (NC\_004718) and SARS-CoV-2 (MN908947.3) were downloaded from the NCBI database (<https://www.ncbi.nlm.nih.gov/>).

## Field-specific reporting

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

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

For a reference copy of the document with all sections, see [nature.com/documents/nr-reporting-summary-flat.pdf](https://www.nature.com/documents/nr-reporting-summary-flat.pdf)

## Life sciences study design

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

### Sample size

Patients were recruited and patient material was banked at the University Hospital Freiburg; inclusion criteria were: (1) 32 health care workers that received a prime and boost vaccination with the mRNA vaccine bnt162b2/Comirnaty, (2) 59 acutely infected and convalescent individuals following a mild course of SARS-CoV-2 infection, SARS-CoV-2 infection was confirmed by positive PCR testing from oropharyngeal swab and/or SARS-CoV-2 spike IgG positive antibody testing, (3) 2 convalescent health care workers following a mild course of SARS-CoV-2 infection that received a single dose of bnt162b2/Comirnaty and (4) 8 age and sex-matched historic controls. No sample size calculations were performed. 32 vaccinated health care workers gave informed consent and were available to donate blood samples. Therefore, similar numbers of COVID-19 convalescents were selected.

### Data exclusions

For flow cytometrical analysis, cell populations containing less than 5 cells were excluded. This data exclusion strategy has been applied and validated previously by our group to gain reproducible results in studies investigating virus-specific CD8+ T cells in human viral infections.

### Replication

Analyses were performed in independent experiments. Findings were reproducible. Flow cytometry analysis: 5 longitudinally analyzed vaccinees for A\*01/S865 (n= 5 at BL, 4 at 3-4 dpp, 5 at 6-8 dpp, 5 at 9-12 dpp, 5 at 13-15 dpp, 2 at 16-18 dpp, 1 at 19-21 dpp, 4 at 3-4 dpb, 3 at 5-6 dpb, 2 at 7-9 dpb, 4 at 13-16 dpb, 4 at 28-30 dpb, 4 at 55-58 dpb and 5 at 80-120 dpb), 5 longitudinally analyzed vaccinees for A\*02/S269 (n=5 at BL, 3 at 3-4 dpp, 4 at 6-8 dpp, 4 at 9-12 dpp, 5 at 13-15 dpp, 3 at 16-18 dpp, 2 at 19-21 dpp, 5 at 3-4 dpb, 2 at 5-6 dpb, 1 at 7-9 dpb, 5 at 13-16 dpb, 5 at 28-30 dpb, 4 at 55-58 dpb and 5 at 80-120 dpb) and 4 longitudinally analyzed vaccinees for A\*03/S378 (n= 4 at BL, 3 at 3-4 dpp, 4 at 6-8 dpp, 4 at 9-12 dpp, 4 at 13-15 dpp, 1 at 19-21 dpp, 2 at 3-4 dpb, 2 at 5-6 dpb, 3 at 7-9 dpb, 3 at 13-16 dpb, 3 at 28-30 dpb and 4 at 80-120 dpb) in independent experiments (Figure 1, 2, Extended Data Figure 2d, e, 3, 4d, e, f, 5c), 2 longitudinally analyzed convalescent health care workers following a mild course of SARS-CoV-2 infection that received a single dose of bnt162b2/Comirnaty for A\*02/S269 (n=2 at convalescent time point prior vaccination, n=2 at 3-4 dpp, n=2 at 8-9 dpp, n=2 at 13-15 dpp and n=2 at 22-23 dpp) in independent experiments (Extended Data Figure 4a, b, c), 8 longitudinally analyzed vaccinees for DRB1\*15:01/S236 (n= 8 at BL, 5 at 3-4 dpp, 6 at 6-8 dpp, 7 at 9-12 dpp, 6 at 13-15 dpp, 4 at 16-18 dpp, 6 at 3-4 dpb, 4 at 5-6 dpb, 3 at 7-9 dpb, 4 at 13-16 dpb, 7 at 28-30 dpb, 1 at 55-58 dpb and 1 at 84-86 dpb) in independent experiments (Figure 3a, b, c, Extended Data Figure 5e, f, g), 8 cross-sectionally analyzed historic controls (DRB1\*15:01/S236) in independent experiments (Extended Data Figure 6d), 8 longitudinally analyzed vaccinees for S1 (n=8 at BL, 8 at 13-15 dpp, 2 at 16-18 dpp, 3 at 19-21 dpp, 5 at 2-4 dpp, 3 at 5-6 dpp, 7 at 28-30 dpp) and RBD (n=8 at BL, 8 at 13-15 dpp, 2 at 16-18 dpp, 3 at 19-21 dpp, 5 at 2-4 dpp, 3 at 5-6 dpp, 7 at 28-30 dpp), in independent experiments (Figure 3b, f, Extended Data Figure 7c), and 10 donors with a history of natural SARS-CoV-2 infection cross-sectionally for S1 and 10 cross-sectionally for RBD in independent experiments (Figure 3f, g, Extended Data Figure 7c), 5 longitudinally analyzed vaccinees for A\*01/S865 (n=5 at 20-40dpb and 4 at 40-80 dpb), 5 longitudinally analyzed vaccinees for A\*02/S269 (n=5 at 20-40dpb and 4 at 40-80 dpb), 4 longitudinally analyzed vaccinees for A\*03/S378 (n=4 at 20-40dpb and 2 at 40-80 dpb), 9 donors with a history of natural SARS-CoV-2 infection cross-sectionally for A\*01/S865 (n=5 at 20-40dpb, 4 at 40-80 dpb), 8 donors with a history of natural SARS-CoV-2 infection cross-sectionally for A\*02/S269 (n=4 at 20-40dpb, 4 at 40-80 dpb), 7 donors with a history of natural SARS-CoV-2 infection cross-sectionally for A\*03/S378 (n=4 at 20-40dpb, 3 at 40-80 dpb) in independent experiments (Extended Data Figure 8a, b, 9b, c), 11 cross-sectionally analyzed vaccinees for A\*01/S865 at 80-120 dpb, 9 cross-sectionally analyzed vaccinees for A\*02/S269 at 80-120 dpb, 8 cross-sectionally analyzed vaccinees for A\*03/S378 at 80-120 dpb, 10 donors with a history of natural SARS-CoV-2 infection cross-sectionally for A\*01/S865 80-120 dpb, 10 donors with a history of natural SARS-CoV-2 infection cross-sectionally for A\*02/S269 80-120 dpb and 10 donors with a history of natural SARS-CoV-2 infection cross-sectionally for A\*03/S378 80-120 dpb in independent experiments (Figure 4a, b, c, d, Extended Data Figure 9c, 10). ELISA and NT analysis: 8 longitudinally analyzed vaccinees for anti-SARS-CoV-2-S1 IgG (n= 8 at BL, 5 at 3-4 dpp, 7 at 6-8 dpp, 7 at 9-12 dpp, 8 at 13-15 dpp, 4 at 16-18 dpp, 3 at 19-21 dpp, 7 at 3-4 dpb, 4 at 5-6 dpb, 2 at 7-9 dpb, 7 at 13-16 dpb, 7 at 28-30 dpb, 8 at 55-58 dpb and 4 at 84-86 dpb) in independent experiments (Figure 3d), 8 longitudinally analyzed vaccinees for B.1 neutralizing titer (n= 6 at BL, 5 at 3-4 dpp, 6 at 6-8 dpp, 7 at 13-15 dpp, 2 at 19-21 dpp, 6 at 3-4 dpb, 3 at 5-6 dpb, 7 at 13-16 dpb, 7 at 28-30 dpb, 7 at 55-58 dpb and 5 at 84-86 dpb) in independent experiments (Figure 3d, Extended Data Figure 3e), 7 longitudinally analyzed vaccinees for alpha neutralizing titer (n=6 at BL, 6 at 6-8 dpp, 7 at 13-16 dpb, 2 at 19-21 dpp, 6 at 3-4 dpb, 3 at 5-6 dpb, 7 at 13-16 dpb, 5 at 28-30 dpb, 7 at 55-58 dpb and 5 at 84-86 dpb) and 7 longitudinally analyzed vaccinees for beta neutralizing titer

(n=6 at BL, 6 at 6-8 dpp, 7 at 13-16 dpp, 2 at 19-21 dpp, 6 at 3-4 dpb, 3 at 5-6 dpb, 7 at 13-16 dpb, 5 at 28-30 dpb, 7 at 55-58 dpb and 5 at 84-86 dpb) in independent experiments (Extended Data Figure 3d), 16 donors with a history of natural SARS-CoV-2 infection cross-sectionally/longitudinally for B.1 neutralizing titer (n=4 at 20-40 dps, 5 at 40-80 dps and 7 at >80 dps) in independent experiments (Extended Data Figure 7e), 9 longitudinally analyzed vaccinees for anti-SARS-CoV-2 S1 IgM supernatant (n=9 at BL, 9 at 13-15 dpp, 2 at 16-18 dpp, 3 at 19-21 dpp, 5 at 2-4 dpb, 4 at 5-6 dpb and 9 at 28-30 dpb) and 4 donors with a history of natural SARS-CoV-2 infection cross-sectionally for anti-SARS-CoV-2 S1 IgM supernatant (n=9 at BL, 9 at 13-15 dpp, 2 at 16-18 dpp, 3 at 19-21 dpp, 5 at 2-4 dpb, 4 at 5-6 dpb and 9 at 28-30 dpb) and 8 longitudinally analyzed vaccinees for S1 binding IgG production after re-stimulation (n=8 at BL, 8 at 13-15 dpp, 2 at 16-18 dpp, 3 at 19-21 dpp, 5 at 2-4 dpb, 3 at 5-6 dpb and 7 at 28-30 dpb) and 8 donors with a history of natural SARS-CoV-2 infection cross-sectionally in independent experiments (Figure 3g). Overlapping peptides analyses: 16 analyzed vaccinees for 182 overlapping peptides spanning the SARS-CoV-2 Spike sequence (9-59 dpb) in independent experiments (Extended Data Figure 1d).

Randomization	Vaccinated donors and donors with a history of natural SARS-CoV-2 infection were selected based on availability and HLA-typing. To analyze A*01/S865, A*02/S269, A*03/S378 and DRB1*15:01/S236-specific T cells participants needed to be allocated into experimental groups based on their HLA-typing. The covariates age and gender are well-documented: Median age of vaccinated donors was 39,6 years, donors with a history of natural SARS-CoV-2 infection was 47,2 years, donors with a history of natural SARS-CoV-2 vaccination and a single vaccination was 56,5 years, of historic controls 37,6 years. The gender ratio of vaccinated donors was m/f: 19/13, donors with a history of natural SARS-CoV-2 infection was m/f: 31/28, donors with a history of natural SARS-CoV-2 vaccination and a single vaccination was m/f: 1/1, of historic controls m/f: 5/3.
Blinding	Blinding was not applied. Non-objective parameters were not included in the study design. Due to standardized analyses of the flow cytometric data set, biased analysis can be excluded.

## Reporting for specific materials, systems and methods

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

### Materials & experimental systems

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

### Methods

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

## Antibodies

### Antibodies used

T cell analysis  
BD Biosciences:  
anti-CCR7-PE-CF594 (150503, 1:50), Cat# 353232  
anti-CCR7-BUV395 (3D12, 1:25), Cat# 740267  
anti-CD4-BV786 (L200, 1:200), Cat# 563914  
anti-CD8-BUV395 (RPA-T8, 1:400), Cat# 563795  
anti-CD8-BUV510 (SK1, 1:100), Cat# 563914  
anti-CD8-APC (SK-1, 1:200), Cat# 345775  
anti-CD11a-BV510 (HI111, 1:25), Cat# 563480  
anti-CD28-BV421 (CD28.2, 1:100), Cat# 562613  
anti-CD28 pure (CD28.2, 1:1000), Cat# 555726  
anti-CD38-APC-R700 (HIT2, 1:400), Cat# 564980  
anti-CD38-BUV737 (HB7, 1:200), Cat# 564686  
anti-CD39-BV650 (TU66, 33:1), Cat# 563681  
anti-CD45RA-BUV496 (HI100, 1:800), Cat# 750258  
anti-CD45RA-BUV737 (HI100, 1:200), Cat# 564442  
anti-CD69-BUV395 (FN50, 1:50), Cat# 564364  
anti-CD107a-APC (H4A3, 1:100), Cat# 560664  
anti-CD127-BUV737 (HIL-7R-M21, 1:50), Cat# 612795  
anti-CD127-BV421 (HIL-7R-M21, 3:100), Cat# 562436  
anti-Granzyme B-PE-CF594 (GB11, 1:100), Cat# 562462  
anti-ICOS-BV711 (DX29, 1:100), Cat# 563833  
anti-IFN-γ-FITC (25723.11, 1:8), Cat# 340449  
anti-IL-21-PE (3A3-N2.1, 1:25), Cat# 560463  
anti-PD-1-BV605 (EH12.1, 1:50), Cat# 563245  
anti-PD-1-PE-Cy7 (EH12.2H7, 1:200), Cat# 561272  
anti-PD-1-BV786 (EH12.1, 1013122, 3:100), Cat# 563789  
anti-T-BET-PE-CF594 (O4-46,93533305, 3:100), Cat# 562467  
anti-TNF-PE-Cy7 (Mab11, 1:400), Cat# 557647  
ViaProbe (7-AAD, 1:33), Cat# 555816

## BioLegend

anti-BCL-2-BV421 (100, 1:200), Cat# 658709  
 anti-CCR7-BV785 (G043H7, 1:50), Cat# 353230  
 anti-CD4-AlexaFluor700 (RPA-T4, 300526, 1:200), Cat# 300526  
 anti-CD25-BV650 (BC96, 1:33), Cat# 302633  
 anti-CD57-BV605 (QA17A04, 1:100), Cat# 563895  
 anti-CD127-BV605 (A019D5, 3:100), Cat# 351334  
 anti-CXCR3-PerCP-Cy5.5 (G025H7, 1:33), Cat# 353714  
 anti-CXCR3-BV510 (G025H7, 3:100), Cat# 353726  
 anti-CXCR5-BV421 (J252D4, 1:100), Cat# 356920  
 anti-IL-2-PerCP-Cy5.5 (MQ1-17H12, 1:100), Cat# 500322  
 anti-Ki67-BV711 (Ki-67, 1:200), Cat# 350516  
 anti-Ki67-PE-Cy7 (Ki67, 1:200), Cat# 350504

## Cell Signaling,

anti-TCF1-AlexaFluor488 (C63D9, 1:100), Cat# 6444

## eBioscience

anti-CD14-APC-eFluor780 (61D3, 1:400), Cat# 47-0149-42  
 anti-CD19-APC-eFluor780 (H1B19, 1:400), Cat# 47-0199  
 anti-CD27-FITC (O323, 1:100), Cat# 11-0279  
 anti-KLRG1-BV711 (13F12F2, 1:50), Cat# 67-9488-42  
 anti-T-BET-PE-Cy7 (4B10, 1:200), Cat# 25-5825  
 anti-TOX-eFluor660 (TRX10, 1:100), Cat# 50-6502  
 anti-EOMES-PerCP-eF710 (WD1928, 1:50), Cat# 46-4877-42  
 Viability Dye (APC-eFluor780 1:200, 1:400) Cat# 65-0865

## Invitrogen

anti-CD45RA-PerCP-Cy5.5 (HI100, 3:100), Cat# 45-0458-42

## B cell analysis

## BioLegend

anti-CD20-BV510 (2H7, 1:80), Cat# 302340  
 anti-IgM-BV605 (MHM-88, 1:200), Cat# 314524  
 anti-CD24-FITC (ML5, 1:1000), Cat# 334103  
 anti-CD71-FITC (CY1G4, 1:1000), Cat# 334103  
 anti-CD95-PE-Dazzle594 (DX2, 1:50), Cat# 305634  
 anti-CD38-PE-Cy7 (HB-7), Cat# 356608  
 anti-BAFF-R-AF647 (11C1, 1:100), Cat# 316914  
 anti-CD19-APC-Cy7 (H1B19, 1:150), Cat# 302218  
 Zombie NIR Fixable Viability Kit (1:800), Cat# 423106

## BD Biosciences

anti-IgG-BV650 (G18-145, 1:600), Cat# 740596  
 anti-CD27-BV786 (L128, 1:100), Cat# 563327  
 anti-CD69-BV480 (FN50, 1:200), Cat# 747519

## Jackson ImmunoResearch

anti-IgA-PerCP (polyclonal, 1:200), Cat# 109-125-011

## Invitrogen

anti-CD3-SB436 (OKT3, 1:200), Cat# 62-0037-42  
 anti-CD33-SB436 (WM-53, 1:50) Cat# 62-0338-42  
 anti-IgD-PerCP-eFluor 710 (IA6-2, 1:200), Cat# 46-9868-42

## Validation

All antibodies were obtained from commercial vendors and we based specificity on descriptions and information provided in corresponding data sheets available and provided by the manufacturers. Standardized analysis in different cohorts, antibody titration on PBMCs including unstained controls, comparisons of different antibody clones and conjugates and validated by publications: CCR7, clone 3D12 and 150503: antibody titration on PBMCs; control clone G043H7; validated with respect to differential expression of naïve and non-naïve T cell subpopulations  
 CD4, clone L200 and RPA-T4: antibody titration on PBMCs; control clones SK3; using B cells as negative control  
 CD8, clone SK1 and RPA-T8: antibody titration on PBMCs; control clone GHI/75; using B cells as negative control  
 CD11, clone HI111: antibody titration on PBMCs, control clones TS2/4 and G43-25B; validated with respect to differential expression of activated and non-activated T cell subpopulations  
 CD27, clone O323: antibody titration on PBMCs; control clone L128; validated with respect to differential expression of naïve and non-naïve T cell subpopulations  
 CD28, clone CD28.2: antibody titration on PBMCs; control clone B-T3; validated with respect to differential expression of naïve and non-naïve T cell subpopulations  
 CD45RA, clone HI100: antibody titration on PBMCs; validated with respect to differential expression of naïve and non-naïve T cell subpopulations  
 CD69, clone FN50: antibody titration on PBMCs; validated with respect to differential expression of activated and non-activated T cell subpopulations  
 CD107a, clone H4A3: antibody titration on PBMCs; validated with respect to differential expression of activated and non-activated T

## cell subpopulations

CD127, clone HIL-7R-M21 and A019D5: antibody titration on PBMCs; control clone eBioRDR5; validated with respect to differential expression of naïve and non-naïve T cell subpopulations

Granzyme B, clone GB11: antibody titration on PBMCs; polyclonal antibody as control; validated with respect to differential expression of activated and non-activated T cell subpopulations

IFN $\gamma$ , clone 25723.11: antibody titration on PBMCs; control clone 4S.B3; validated with respect to differential expression of activated and non-activated T cell subpopulations

IL-21, clone 3A3-N2.1: antibody titration on PBMCs; validated with respect to differential expression of activated and non-activated T cell subpopulations

PD-1, clone EH12.2H7 and EH12.1: antibody titration on PBMCs; control clone eBioJ105; validated with respect to differential expression of naïve and non-naïve T cell subpopulations

TNF, clone MAB11: antibody titration on PBMCs; validated with respect to differential expression of activated and non-activated T cell subpopulations

Bcl-2, clone 100: antibody titration on PBMCs; validated with respect to differential expression of naïve and non-naïve T cell subpopulations

CD25, clone BC96: antibody titration on PBMCs; control clone M-A251; validated with respect to differential expression of activated and non-activated T cell subpopulations

CD38, clone HB7 and HIT2: antibody titration on PBMCs; control clone HIT2.1; validated with respect to differential expression of naïve and non-naïve T cell subpopulations

CD39, clone TU66: antibody titration on PBMCs; control clones eBioA1 and A1; validated with respect to differential expression of naïve and non-naïve T cell subpopulations

CD57, clone QA17A04: antibody titration on PBMCs; control clone NK-1; validated with respect to differential expression of naïve and non-naïve T cell subpopulations

CXCR3, clone G025H7: antibody titration on PBMCs; control clone 1C6/CXCR3; validated with respect to differential expression of activated and non-activated T cell subpopulations

IL-2, clone MQ1-17H12: antibody titration on PBMCs; validated with respect to differential expression of activated and non-activated T cell subpopulations

TCF-1, clone C63D9: antibody titration on PBMCs; control clone 7F11A10; validated with respect to differential expression of naïve and non-naïve T cell subpopulations

Eomes, clone WD1928: antibody titration on PBMCs; validated with respect to differential expression of naïve and non-naïve T cell subpopulations

CD14, clone 61D3: antibody titration on PBMCs; control clones M5E2 and M $\phi$ P9; using T cell populations as negative control

CD19, clone HIB19: antibody titration on PBMCs; control clone SJ25C1; using T cell populations as negative control

T-bet, clone O4-46: antibody titration on PBMCs; control clones 4B10; validated with respect to differential expression of naïve and non-naïve T cell subpopulations

KLRG1, clone 13F12F2: antibody titration on PBMCs; validated with respect to differential expression of naïve and non-naïve T cell subpopulations

TOX1, clone TRX10: antibody titration on PBMCs; control clone REA473; validated with respect to differential expression of naïve and non-naïve T cell subpopulations

CXCR5, clone J252D4: antibody titration on PBMCs; control clone MU5UBEE; validated with respect to differential expression of naïve and non-naïve T cell subpopulations

ICOS, clone DX29: antibody titration on PBMCs; control clone ISA-3; validated with respect to differential expression of naïve and non-naïve T cell subpopulations

Ki67, clone Ki67: antibody titration on PBMCs; control clone B56; validated with respect to differential expression of naïve and non-naïve T cell subpopulations

Viability Dye was titrated on PBMCs; validated with respect to differential staining of live and dead cell populations

CD20, clone 2H7: Titration on fresh and cryopreserved PBMCs, staining compared to clone JDC-10 and polyclonal anti-IgM Ab (Jackson ImmunoResearch)

IgM clone MHM-88: Titration on fresh and cryopreserved PBMCs, staining compared to clone JDC-10 and polyclonal anti-IgM Ab

CD71 clone CY1G4: Titration on fresh PBMCs and in vitro activated B cells

CD95 clone DX2: Titration on fresh PBMCs and in vitro activated B cells

CD38 clone HB-7: Titration on fresh and cryopreserved PBMCs; staining compared to clone HIT2

BAFF-R clone 11C1: Titration on cryopreserved PBMCs

CD19 clone HIB19: Titration on cryopreserved PBMCs

IgG clone G18-145: Titration on fresh and cryopreserved PBMCs, staining compared to clone JDC-10 and polyclonal anti-IgG Ab

CD27 clone L128: Titration on fresh and cryopreserved PBMCs, staining compared to clone LG.3A10

IgA (polyclonal): Titration on fresh and cryopreserved PBMCs

IgD clone IA6-2: Titration on fresh and cryopreserved PBMCs

CD24, clone ML5: Titration on fresh PBMCs and in vitro activated B cells

CD3, clone OKT3: Titration on cryopreserved PBMCs

CD33, clone WM-53: Titration on cryopreserved PBMCs

CD69, clone FN50: Titration on in vitro activated B cells

## Human research participants

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

### Population characteristics

32 health care workers that received a prime and boost vaccination with the mRNA vaccine bnt162b2/Comirnaty, 59 acutely infected and convalescent individuals following a mild course of SARS-CoV-2 infection, 2 convalescent health care workers following a mild course of SARS-CoV-2 infection that received a single dose of bnt162b2/Comirnaty and 8 historic controls, carrying either of the following HLA alleles: A\*01:01, -A\*02:01, -A\*03:01, DRB1\*15:01 were recruited at the University Hospital Freiburg. Median age of vaccinated donors was 39,6 years, donors with a history of natural SARS-CoV-2 infection was 47,2 years, donors with a history of natural SARS-CoV-2 vaccination and a single vaccination was 56.5 years, of historic

controls 37,6 years. The gender ratio of vaccinated donors was m/f: 19/13, donors with a history of natural SARS-CoV-2 infection was m/f: 31/28, donors with a history of natural SARS-CoV-2 vaccination and a single vaccination was m/f: 1/1, of historic controls m/f: 5/3.

#### Recruitment

Vaccinated donors as well as SARS-CoV-2-infected and SARS-CoV-2-convalescent patients were recruited at the University Hospital Freiburg (in- and outpatient section); self-selection bias or other biases can be excluded since several people were included in the recruitment. Samples were banked and retrospectively selected according to the following inclusion criteria: HLA-A\*01:01, -A\*02:01, -A\*03:01, DRB1\*15:01. Banked samples from sex-, age- and HLA-matched historic controls were retrospectively selected.

#### Ethics oversight

Written informed consent was obtained from all participants and the study was conducted according to federal guidelines, local ethics committee regulations (Albert-Ludwigs-Universität, Freiburg, Germany; vote #: 322/20, #21-1135 and 315/20) and the Declaration of Helsinki (1975).

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).
- ☒ All plots are contour plots with outliers or pseudocolor plots.
- ☒ A numerical value for number of cells or percentage (with statistics) is provided.

### Methodology

#### Sample preparation

Cryopreserved isolated human PBMCs were thawed and prepared for flow cytometry or in vitro expansion described in the methods section

#### Instrument

FACSCanto II, LSRFortessa (BD, Germany), Cytex Aurora (Cytex) or CytoFLEX (Beckman Coulter), Tecan (LifeScience)

#### Software

FlowJo\_v10.6.2 (Treestar), R version 4.0.2 using the Bioconductor (version: Release (3.11)) .

#### Cell population abundance

Abundance of SARS-CoV-2-specific T cells are low ( $<10^{-4}$  %)

#### Gating strategy

CD8+ T cells: Lymphocytes gated on FSC-A and SSC-A, Doublet exclusion on FSC-A and FSC-H and FSC-A and FSC-W, Exclusion of dead cells, B cells and monocytes, Gating on CD8+ cells, Exclusion of naive cells (CCR7+CD45RA+), Gating of SARS-CoV-2-specific CD8+ T cells via tetramers described in methods part.

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