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Reporting Summary

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Statistics

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

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

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

Software and code

Policy information about availability of computer code

Data collection	Miltenyi MACSquantify software v2.13 was used for flow cytometry data collection.
Data analysis	All the softwares and there version information are shown her. FlowJo (v9.9.6) was used for all FACS analyses. Microsoft Excel (v.14.1.0) was used to collect and arrange data and patient / donor information. GraphPad Prism (v5.0b and v8.4.2. (464)) was used to analyze data and create plots. Sequence alignments have been performed using R (v3.6.1) including package ClustalX (Larkin,M.A., Blackshields, G., Brown, N.P., Chenna, R., McGettigan, P.A., McWilliam, H., Valentin, F., Wallace, I.M., Wilm, A., Lopez, R., Thompson, J.D., Gibson, T.J., Higgins, D.G. (2007) Clustal W and Clustal X version 2.0. Bioinformatics, 23:2947-2948.) and using the Needlemann-Wunsch algorithm.

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/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

All flow cytometry data are made available in the FlowRepository,org (experiment ID: FR-FCM-Z2K3). An additional Supplementary Figure displaying the individual gating strategy for all donors is available in the online version of the paper.

Field-specific reporting

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

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

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

Life sciences study design

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

Sample size	No sample size calculation was performed. Within COVID-19 patients, 83% exhibited T cell reactivity to the Spike glycoprotein of SARS-CoV2. Within the healthy donors recruited, 35% were identified as reactive healthy donors. With the proportions, the recruited numbers of subjects are sufficient.
Data exclusions	No data were excluded from the analysis.
Replication	We remeasured several donors (accompanied by anti-SARS-CoV-2 IgG antibody testing) at later timepoints and used another Spike glycoprotein peptide pool from Miltenyi to ensure reproducibility of T cell reactivity in SARS-CoV-2 naive donors.
Randomization	No randomization was performed since it was not applicable to the study.
Blinding	Blinding was not applicable to this study.

Reporting for specific materials, systems and methods

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

Materials & experimental systems

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

Methods

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

Antibodies

Antibodies used	CD69-APCCy7 BioLegend Cat# 310914, RRID:AB_314849 CD4-BV605 BioLegend Cat# 300556, RRID:AB_2564391 CD8-PerCp BioLegend Cat# 344708, RRID:AB_1967149 IFNg-AlexaFluor700 BioLegend Cat# 502520, RRID:AB_528921 TNFa-PB BioLegend Cat# 502920, RRID:AB_528965 CD38-PeVio770 Miltenyi Biotec Cat# 130-118-982, RRID:AB_2751601 HLADR-VG Miltenyi Biotec Cat# 130-111-795, RRID:AB_2652164 CD154-APC Miltenyi Biotec Cat# 130-113-603, RRID:AB_2726191 CD137-PE BD Biosciences Cat# 555956, RRID:AB_396252 CD154-BV421 BioLegend Cat# 310824, RRID:AB_2562721 IL-2-APC BD Biosciences Cat# 341116, RRID:AB_400574 IL-17A-APCCy7 BioLegend Cat# 512320, RRID:AB_10613103 CCR7-AlexaFluor488 BioLegend Cat# 353206, RRID:AB_10916389 CD45RA-PeCy7 BioLegend Cat# 304126, RRID:AB_10708879 CD3-V500 BD Biosciences Cat# 560770, RRID:AB_1937322
Validation	All antibodies are established, well described and published elsewhere. Informations are accessible on the manufacturers websites under Catalogue or RRID numbers.

Human research participants

Policy information about studies involving human research participants

Population characteristics	The study included: - 18 COVID-19 patients (Table 1):age mean 52.6, range: 21-81 yrs; gender: female ratio 27.8%; sampling day (post symptom onset): mean 14.9, range: 2-39 - 7 additional COVID-19 patients (Extended data Table 3): age mean 53, range: 10-79 yrs; gender: female ratio 14.3%; sampling day (post symptom onset): mean 31.6, range: 10-53 - 68 healthy donors (age mean 41.93, range: 20-64 yrs; gender: female ratio 59%) All healthy donors stated to be free of symptoms indicating an acute infection.
Recruitment	Patients were hospitalised in the Charité. The patients were selected based on the disease severiyt to achieve a balanced representation of the three disease serverity groups.
Ethics oversight	Institutional Review Board of the Charité.

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	Peripheral blood mononuclear cells (PBMC) were isolated from heparinized whole blood by gradient density centrifugation according to manufacturer's instructions (Leucosep tubes, Greiner; Biocoll, Bio&SELL). Stimulation was conducted with 5x10e6 PBMC in RPMI 1640 medium (Gibco) supplemented with 10% heat inactivated AB serum (Pan Biotech), 100 U/ml penicillin (Biochrom), 0.1 mg/ml streptomycin (Biochrom), and PepMixTM SARS-CoV-2 in the presence of 1 µg/ml purified anti-CD28 (clone CD28.2, BD Biosciences). PepMixTM SARS-CoV-2 (Spike Glycoprotein) subpool 1 covering the N-terminal aa 1-643 (abbreviated to "S-I (N-term)") containing 158 15-mers overlapping by 11 and PepMixTM SARS-CoV-2 (Spike Glycoprotein) subpool 2 covering the C-terminal aa 633-1273 (abbreviated to "S-II (C-term)") (JPT) containing 156 15-mers overlapping by 11 and one 17-mer at C-terminus were used at 1 µg/ml per peptide, respectively. Stimulation controls were performed with equal concentrations of DMSO in PBS (unstimulated) or 1.5 mg SEB/1.0 mg TSST1 (Sigma-Aldrich) and PepMixTM CMV pp65 (Miltenyi) as positive controls, respectively. Incubation was performed at 37˚C, 5% CO2 for 14h with 10 µg/ml brefeldin A (Sigma- Aldrich) added after 2 h. Stimulation was stopped by incubation in 20 mM EDTA for 5 min and surface staining conducted for 15 min with the following fluorochrome conjugated antibodies titrated to their optimal concentrations: CD38-PE-Vio770 (clone REA671, Miltenyi), CD69-APC-Cy7 (FN50, Biolegend), HLAD-DR-VioGreen (REA805, Miltenyi), CD4-BrilliantViolet605 (RPA-T4, Biolegend), CD8-PerCP (SK1, Biolegend) with 1 mg/ml Beriglobin (CSL Behring) added prior to the staining. For exclusion of dead cells, Zombie Yellow fixable viability staining (Biolegend) was added for the last 10 min of incubation. Fixation and permeabilization were performed with eBioscienceTM FoxP3 fixation and PermBuffer (Invitrogen) according to the manufacturer's protocol and intracellular staining carried out for 30 min in the dark at room temperature with Beriglobin added prior to intracellular staining with 4-1BB-PE (clone 4B4-1, BD), CD40L-APC (5C8, Miltenyi) and Ki-67-AlexaFluor488 (B56, BD). For intracellular cytokine staining, we employed different antibodies. Surface staining was performed with CD3-V500 (SP34-2, BD), CD8-PerCP (SK1, Biolegend), CD4-BrilliantViolet605 (RPA-T4, Biolegend), CCR7-AlexaFluor488 (G043H7, Biolegend), CD45RA-PE-Cy7 (HI100, Biolegend). IFN-γ-AlexaFluor700, CD40L-BrilliantViolet421 (24-31, Biolegend), IL-2-APC (5344.111, BD), 4-1BB-PE (4B4-1, BD) and IL-17A-APC-Cy7 (BL168, Biolegend) were utilized for intracellular staining after fixation and permeabilization using BD FACSLysing Buffer and BD Perm2 Buffer, according to manufacturer's instructions.
Instrument	Samples were measured on a MACSQuant® Analyzer 16 and the instrument performance monitored daily with Rainbow Calibration Particles (BD).
Software	Miltenyi MACSquantif software (v2.13) and FlowJo (v9.9.6)
Cell population abundance	Cells have not been enriched or sorted, except by the generation of SARS-CoV-2-S-II reactive CD4 T cell lines. Data are shown within ex vivo stimulated peripheral blood mononuclear cells.
Gating strategy	All recorded events were gated according to FSC and SSC as lymphocytes; single cells were further selected using FSC-H vs. FSC-W and again using SSC-H vs. SSC-W. Subsequently living cells were identified as ZombieYellow negative cells gated against CD4-BV605. An artefact population in some samples (probably induced by DMSO) was observed disturbing data analysis and was gated out using V500 vs V450. The subsequent gating scheme is depicted in Fig.2a and Fig. 3a.

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