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# Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see our Editorial Policies and the Editorial Policy Checklist.

## 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<br>*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<br>*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 | Patient data was collected using RedCAP data capture software. Flow cytometry data was collected on a Cytek Aurora flow cytometer using Cytek SpectroFlo software. Luminex data (including antigen specific data) was analyzed using a Luminex FLEXMAP 3D® instrument (Luminex; Austin, TX, USA) running xPonent 4.3 software |
| Data analysis | Computational analysis was carried out in R, version 3.6.2 (release 12.12.2019). Heatmaps were generated using the pheatmap library (version 1.0.12), with data pre-normalized (log-transformed z-scores calculated per feature) prior to plotting. Clustering was carried out using Ward's method. Custom plotting such as Ag-specific response curves was performed using the ggplot2 library for base analysis, and then post-processed in Adobe Illustrator. Circos plotting was carried out using Circos software, version 0.69-9. Lineage trees were claculated using GLaMST (Grow Lineages along Minimum Spanning Tree) software36, run on matlab, and then visualized in R using the iGraph package, version 1.2.4.2. Exported trees were then post-processed in Adobe Illustrator. Statistical analyses were performed directly in R, or in Graphpad Prism, version 8.2.1. |

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

All flow cytometry and sequencing data presented here are publically available in alignment with current requirements for public disclosure prior to peer review. All flow cytometry data presented and analyzed in this manuscript (Figures 1-4) have been made public in the FlowRepository, and are available here: http://flowrepository.org/id/FR-FCM-Z2XF. Single cell VDJ sequencing (Figure 5) is available on the sequence read archive here: https://www.ncbi.nlm.nih.gov/bioproject/642962.

# 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 was pre-selected. Samples from patients with COVID-19 were collected on an ongoing basis until clear patterns emerged in flow cytometric data capable of statistically distinguishing patients with critical SARS-CoV-2 infection from healthy donors. |
| Data exclusions | No patients were excluded from the analysis. |
| Replication | Patient samples were collected, processed, and analyzed on different days and often by different personnel. The nature of this study does not lend itself to simple replication, however all analysis presented maintained consistency over time and within technical replicates. |
| Randomization | No randomization was performed. |
| Blinding | Blinding was not relevant to this study. |

# Reporting for specific materials, systems and methods

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

## Materials & experimental systems

| n/a | Involved in the study |
|---|---|
| ☐ | ☒ Antibodies |
| ☒ | ☐ Eukaryotic cell lines |
| ☒ | ☐ Palaeontology and archaeology |
| ☒ | ☐ Animals and other organisms |
| ☐ | ☒ Human research participants |
| ☒ | ☐ Clinical data |
| ☒ | ☐ Dual use research of concern |

## Methods

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

## Antibodies

Antibodies used

Target; Fluorophore; Panel; Clone; Vendor; Cat#; Dilution (ul/100ul)
CD62L BV480 v1, v2 DREG-56 BD 566174 5 ul
CD86 PerCP-Cy5.5 v1, v2 IT2.2 Biolegend 305419 5 ul
CD27 BV750 v1, v2, ICS O323 Biolegend 302849 2.5 ul
CD19 BV570 v1, v2, ICS HIB19 Biolegend 302235 2.5 ul
CD45 Spark NIR 685 v2 2D1 Biolegend 368552 1.25 ul
CD1c BV510 v2 L161 Biolegend 331534 1.25 ul
IgM BV711 v1, v2, ICS MHM-88 Biolegend 314539 1.25 ul
CXCR3 A647 v1, v2, ICS G025H7 Biolegend 353711 1.25 ul

CXCR4 PerCP-e710 v1, v2 12G5 eBioscience 46-9999-41 1.25 ul
CCR7 A488 v1 G043H7 Biolegend 353205 1.25 ul
CD24 PerCP v1, v2, ICS ML5 Biolegend 311113 1.25 ul
CD3 BUV 805 v1, v2, ICS UCHT1 BD 612896 0.6 ul
CD11c APC-Fire750 v1, v2, ICS S-HCL-3 Biolegend 371509 0.6 ul
CD138 APC-R700 v1, v2 MI15 BD 566051 0.6 ul
HLA-DR BV650 v1, v2 L243 Biolegend 307649 0.6 ul
CD95 BV785 v1, v2 DX2 Biolegend 305645 0.6 ul
CD14 BUV805 v1, v2 M5E2 BD 612902 0.6 ul
CD23 APC v2 EBVCS-5 Biolegend 338514 0.3 ul
CD69 BUV 737 v1, v2 FN50 BD 612817 0.3 ul
IgD BV605 v1, v2, ICS IA6-2 Biolegend 348231 0.3 ul
CD21 PE-Dazzle594 v1, v2, ICS Bu32 Biolegend 354921 0.3 ul
CD38 BB515 v1, v2, ICS HIT2 BD 564499 0.3 ul
CXCR5 PE v1, v2, ICS J252D4 Biolegend 356903 0.3 ul
CD40 A532 v1, v2 5C3 Novus NBP1-43416AF523 0.3 ul
PD-1 PE-Cy7 v1, v2 EH12.2H7 Biolegend 239917 0.3 ul
IgG BV421 v1, v2 M1310G05 Biolegend 410703 0.15 ul
CD10 PE-Cy5 v1, v2 HI10a Biolegend 312205 0.15 ul
CD25 e450 v1 BC96 eBioscience 48-0259-41 5 ul
CD1d BV510 v1 51.1 Biolegend 350313 2.5 ul
ICOS-L APC v1 2D3 Biolegend 309407 5 ul
B220 Spark NIR 685 v1 RA3-6B2 Biolegend 103268 2.5 ul
T-bet APC ICS 4B10 Biolegend 644814 1.25 ul
Viability Zombie NIR v1,2 NA Biolegend 423106 0.2 ul

Validation

All antibodies have been validated by the manufacturer for use in targeting human proteins as indicated above.

## Human research participants

Policy information about studies involving human research participants

| | |
|---|---|
| Population characteristics | Population characteristics are fully described in Supplementary table 2 of the manuscript. |
| Recruitment | Written informed consent was obtained from all participants or, if they were unable to provide informed consent, obtained from designated healthcare surrogates. Healthy donors (n = 36) were recruited using promotional materials approved by the Emory University Institutional Review Board. Subjects with COVID-19 (n = 19) were recruited from Emory University Hospital, Emory University Hospital Midtown and Emory St. Joseph's Hospital, all in Atlanta, GA, USA. All non-healthy donor subjects were diagnosed with COVID-19 by PCR amplification of SARS-CoV-2 viral RNA obtained from nasopharyngeal or oropharyngeal swabs. Subjects with COVID-19 were included in the study if they were 18 to 80 years of age, not immunocompromised, and had not been given oral or intravenous corticosteroids within the preceding 14 days. |
| Ethics oversight | All research was approved by the Emory University Institutional Review Board (Emory IRB numbers IRB00058507, IRB00057983, and IRB00058271) and was performed in accordance with all relevant guidelines and regulations. |

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 samples were collected in heparin sodium tubes and processed within 6 hours of collection. PBMCs were isolated by density gradient centrifugation at 1000 x g for 10 minutes. Aliquots from the plasma layer were collected and stored at -80C until use. PBMCs were washed 2 times with RPMI at 500 x g for 5 minutes. |
| Instrument | Cells were analyzed on a Cytek Aurora flow cytometer (V3; 16V-14B-10YG-8R) |
| Software | Cells were analyzed on a Cytek Aurora flow cytometer using Cytek SpectroFlo software. Up to 3 x 106 cells were analyzed using FlowJo v10 (Treestar) software. For UMAP projections, all samples stained with panel 2 were downsampled using the DownSample plugin (V3.3) available on the FlowJo Exchange. All samples were concatenated to create a single composite, and a UMAP algorithm for dimensionality reduction was applied using the UMAP plugin (V3.1) available on the FlowJo |

| | |
|---|---|
| | Exchange. |
| Cell population abundance | NA |
| Gating strategy | Gating strategy is provided in Figure 1a, rather than as a supplement, as it is a core component of the work. |

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