nature portfolio

Corresponding author(s): Jincun Zhao, Jingxian Zhao, Nanshan Zhong, Yonghao Xu, Xiaobo Chen

Last updated by author(s): Dec 13, 2024

Reporting Summary

Nature Portfolio 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 Portfolio 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 *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	All flow cytometric data were acquired on BD LSR Fortessa X20 or FACSymphony with FACS Diva software (version 9.0) , or BD FACSVerse with FACS Suite software (version 1.0.6). The copy numbers of SARS-CoV-2 in BALF samples were measured by QuantStudio 6 (ABI). Single cell sequencing data were collected by Illumina Novaseq.
Data analysis	Flow data were analyzed with FlowJo software (version 10.6.2, BD Biosciences). Raw data were analyzed using R (version 4.2.3) and GraphPad Prism (version 9.5.1). Statistical data analysis were performed with R (version 4.4.0). Raw single-cell transcriptome and TCR sequencing data were processed following cellranger (version 7.2.0) or mobivision (version 3.0) count protocol against GRCh38 reference genome. The cellranger or mobivision output was further analyzed in R (version 4.2.3) platform using the Seurat package (version 4.3.0). A filtered gene-barcode matrix of all samples was integrated with harmony (version 0.1.1). Packages used were MixCR (version 3.0.3), Alakazam (version 0.3.0), ClusterProfiler (version 4.6.2), Monocle (version 2.26.0), HISAT (version 2.1), SubReads package (version 1.5.3), ggplot2 (version 3.3.3), ggpubr (version 0.6.0), ggSCvis (version 0.0.2), ComplexHeatmap (version 2.14.0), and RSEM suit (version 1.3.3).

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

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 description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

RNA and TCR sequencing data first reported in this study are deposited and accessible in the NCBI (https://www.ncbi.nlm.nih.gov) under accession number PRJNA1099166. Public SARS-CoV-2-specific TCR repertoires are acquired from IEDB database (DOI: 10.1093/nar/gky1006). All data supporting the findings of this study are available in the article, supplementary Information, or from the corresponding author JC.Z. on reasonable request. All statistical source data would be provided with paper for publication.

Research involving human participants, their data, or biological material

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

Reporting on sex and gender	Participants were not excluded based on sex or gender.
Reporting on race, ethnicity, or other socially relevant groupings	No socially constructed or socially relevant categorization variables were used in this manuscript.
Population characteristics	Population characteristics are described in Figure 1a and Extended Data Table 1.
Recruitment	In this prospective observational cohort study, adult patients were recruited with PCR-confirmed COVID-19. Participants were recruited from a hospital located in Guangzhou, Guangdong, China, named the First Affiliated Hospital of Guangzhou Medical University from January 2020 to January 2023. After informed consents were obtained, bronchoalveolar lavage fluid (BALF) samples were obtained by trained clinicians from donors who had tested positive for SARS-CoV-2 and were diagnosed as COVID-19 patients and agreed to participated the study. All methods were performed according to the relevant guidelines and regulations.
Ethics oversight	All human subjects research was approved by the First Affiliated Hospital of Guangzhou Medical University (Ethics ID: ES-2020-65, ES-2023-015-01). All study participants have provided written informed consents and did not received compensation for taking part in this study.

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

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 statistical method was used to predetermine the sample size for our experiments, but our sample sizes are similar to or larger than those reported in previous publications in the field. At least four replicates were included in the subgroup comparison. And no statistical tool was used to predetermine sample sizes.
Data exclusions	No data has been excluded for statistical analysis.
Replication	Replications of experiments are specified in the according figure legends.
Randomization	This was an observational study. Randomization was not applicable as this was not an intervention study.
Blinding	Cell clustering was performed on all cells, resulting in a de facto blinded assignment of cells to distinct populations.

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
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

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

Antibodies

Antibodies used

Specificity Label Brand catalog# clone dilution
anti-CD103-PE-Dazzle594 (Biolegend, 350224, Ber-ACT8) 1/50
anti-CD127-APC-R700 (BD Horizon, 565185, HIL-7R-M21) 1/200
anti-CD14-BV570 (BD, 624298, MϕP9) 1/100
anti-CD14-BV711 (BD Horizon, 563372, MϕP9) 1/200
anti-CD16-APC (BD pharmingen, 561304, B73.1) 1/20
anti-CD163-BB700 (BD, 624381, GHI/61) 1/200
anti-CXCR3 -PE-Cy7 (BD, 560831, 1C6) 1/500
anti-CCR4 -BV750 (BD, 746980, 1G1) 1/100
anti-CCR5 -BB790 (BD, 624296, 3A9) 1/200
anti-CCR6 -BB660 (BD, 624295, 11A9) 1/200
anti-CCR7 -PE-Cy7 (Biolegend, 353226, G043H7) 1/100
anti-CD206 -PE-Cy5 (BD, 624294, 19.2) 1/25
anti-CD25 -BUV737 (BD Horizon, 612806, 2A3) 1/100
anti-PD-1 -BV786 (BD, 563789, EH12.1) 1/50
anti-PD-1-PE-Cy7 (BD pharmingen, 561272, EH12.1) 1/50
anti-CD3 -AF700 (BD, 624358, HIT3a) 1/100
anti-CD3 -BUV395 (BD Horizon, 563546, UCHT1) 1/400
anti-CD38 -BV711 (BD, 563965, HIT2) 1/100
anti-CD4 -BV605 (BD, 562658, RPA-T4) 1/20
anti-CD4 -BV421 (BD Horizon, 562842, L200) 1/200
anti-CD4 -BB515 (BD Horizon, 564419, RPA-T4) 1/100
anti-CD45 -BUV805 (BD, 612891, HI30) 1/500
anti-CD45 -BV510 (BD Horizon, 563204, HI30) 1/250
anti-CD45RA -BUV737 (BD Horizon, 612846, HI100) 1/200
anti-CD56 -BUV395 (BD, 563554, NCAM16.2) 1/100
anti-CD56 -BV650 (BD Horizon, 564057, NCAM16.2) 1/400
anti-CD69 -BV510 (BD, 747521, FN50) 1/100
anti-CD8 -BUV615 (BD, 612994, SK1) 1/200
anti-CD8 -BV786 (BD Horizon, 563823, RPA-T8) 1/250
anti-CD8 -Percp-cy5.5 (BD Horizon, 565310, SK1) 1/200
anti-CD86 -APC-R700 (BD Horizon, 565149, 2331 FUN-1) 1/100
anti-GM-CSF-BUV563 (BD, 624284, BVD2-21C11) 1/50
anti-GM-CSF-Percp-cy5.5 (Biolegend, 502312, BVD2-21C11) 1/50
anti-HLA-A,B,C-FITC (Biolegend, 311403, W6/32) 1/100
anti-HLA-DR-BUV496 (Biolegend, 569674, G46-6) 1/200
anti-HLA-DR-BV605 (BD Horizon, 562845, G46-6) 1/100
anti-IFN-γ-APC (BD pharmingen, 554702, B27) 1/200
anti-IFN-γ-BV421 (Biolegend, 506538, B27) 1/200
anti-IL-10-PE-Cy5 (BD pharmingen, 624350, JES3-19F1) 1/500
anti-IL-10-BV711 (BD Horizon, 564050, JES3-9D7) 1/50
anti-IL-1β-PE (R&D, IC201P, # 8516) 1/100
anti-IL-2-BB515 (BD, 624279, MQ2-13A5) 1/250
anti-IL-2 -BV605 (BD Horizon, 564165, MQ1-17H12) 1/50
anti-IL-6-BV480 (BD, 624278, MQ2-13A5) 1/200
anti-IL-8 -BV650 (BD, 624280, G265-8) 1/500
anti-LAG-3-APC-Cy7 (BD, 624355, T47-530) 1/200
anti-TIM-3-BUV661 (BD, 624285, 7D3) 1/200
anti-TIM-3-APC (R&D, 344823, FAB2365A) 1/25
anti-TNF -BUV737 (BD, 624286, MAb11) 1/200
anti-TNF -PE (Biolegend, 502909, MAb11) 1/200
Fixable Viability Stain 440UV (BD, 566332) 1/1000
Pro5 MHC Class I Pentamer B*40:01-MEVTPSGTWL-Pentamer-R-PE (ProImmune, F4328-2B-C) 1/20

Validation	All antibodies used are commercial antibodies reported by the manufacturer to be validated for use. Likewise, we titrated the antibodies based on the staining condition in our study.

Plants

Seed stocks	*Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.*
Novel plant genotypes	*Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.*
Authentication	*Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosiacism, off-target gene editing) were examined.*

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	BALF sample were centrifuged at 800 g, 4oC for 10 minus to separate supernatant and cell pellets. PBMC were isolated from blood using Leucosep tubes (Greiner) and Ficoll-Paque PLUS (GE Healthcare) according to the manufacturer's instructions. BALF cell pellets and PBMC were stored in liquid nitrogen and supernatant and plasma were stored at -80oC before analysis. To detect specific T cells, 1E5 to 1E6 cells were incubated in a well of 96-well round-bottom plates at 37 °C for 16-18 h in the presence of SARS-CoV-2 peptide pool (200nM for each peptide at final) and brefeldin A (BD Biosciences, 1:1000 at final). For surface markers, cells were stained with the indicated antibodies at 4°C for 20-30 minutes, and then labeled with cell viability staining dye (FVS440UV, BD Biosciences) at room temperature (20–25°C) in the dark for another 10 minutes. The cells were subsequently fixed and permeabilized using Cytofix/Cytoperm Solution (BD Biosciences) to enable intracellular marker labeling at 4°C for 30-40 minutes. Then cells were washed with 1X Perm/wash buffer twice and resuspended in FACS buffer for flow cytometer detection.
Instrument	LSR Fortessa, FACSymphony, FACSVerse and FACS-sort AriaIII
Software	BD Diva software v9.0, FACS Suite software v1.0.6, and Flowjo v10.6.2
Cell population abundance	Frequencies of cell populations are stated in dot plots and graphs.
Gating strategy	Gating strategies are stated in the according Extended figures 1 and 4. In brief, all cells were gated on SSC-A/FSC-A and FSC-H/FSC-A to identified as singlet, then as live (FVS440-), leukocytes (CD45+), T cells (CD3+CD56-), and further subdivided into CD4+ and CD8+ T cells. SARS-CoV-2-specific T cells were identified as CD3+CD4+IFN-γ+, CD3+CD8+IFN-γ+, or B40/n322 pentamer+CD3+CD8+ T cells.

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