nature portfolio

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# 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.

Please do not complete any field with "not applicable" or n/a. Refer to the help text for what text to use if an item is not relevant to your study.
For final submission: please carefully check your responses for accuracy; you will not be able to make changes later.

## 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 | For full spectrum cytometry data collection was performd using SpectroFlo Cytek Aurora Software (Version 3.1.0). For single-cell RNAseq data |
| Data analysis | All code used to perform the analysis can be found at: https://github.com/BALLESTARLAB/CVID-COVID [https://doi.org/10.5281/ |

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

All the single cell RNAseq data generated in this study can be accessed via European Genome-Phenome Archive (EGA) under accesion number EGAD50000000543 [https://ega-archive.org/datasets/EGAD50000000543]. Spectral flow cytometry files have been deposited in FlowRepository under accession code FR-FCM-Z8F8 [http://flowrepository.org/id/FR-FCM-Z8F8]. The Whole Exome Sequencing (WES) data are not publicly available due to ethical and privacy regulations. Access to the data can be granted upon request by contacting the corresponding authors. Requests for access will be reviewed and responded to within 30 days, and any approved

## 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 | Only sex and not gender information was included in the study. Females and males were equally represented in the different |
| Reporting on race, ethnicity, or other socially relevant groupings | NA |
| Population characteristics | All population information is available in Supp. Table 1a. |
| Recruitment | Human blood samples were collected from CVID patients as previously diagnosed according to the European society of immune |
| Ethics oversight | All donors received oral and written information about the possibility that their blood would be used for research purposes, and |

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

# Life sciences study design

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

| | |
|---|---|
| Sample size | No statistical methods were used to predetermine sample size. We followed standards in the field and Human Cell Atlas criteria. |
| Data exclusions | For the final count matrix, we excluded cells based on pre-established criteria for single-cells: we excluded low quality samples and contaminating |
| Replication | Some of the most relevant findings in the nonCVID 1 cohort were replicated in an additional cohort of nonCVID patients. No replication using the |
| Randomization | The balance in sex and age covariates were tested in the two groups of comparison and non significant differences were found (Supp. Table 1b |
| Blinding | Blinding is not relevant for this study as the aim is describing differences between healthy donors and CVID patients. |

# Behavioural & social sciences study design

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

| | |
|---|---|
| Study description | |
| Research sample | |
| Sampling strategy | |
| Data collection | |
| Timing | |
| Data exclusions | |
| Non-participation | |
| Randomization | |

# Ecological, evolutionary & environmental sciences study design

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

| | |
|---|---|
| Study description | |
| Research sample | |
| Sampling strategy | |
| Data collection | |
| Timing and spatial scale | |

Data exclusions

Reproducibility

Randomization

Blinding

Did the study involve field work? Yes No

## Field work, collection and transport

Field conditions

Location

Access & import/export

Disturbance

# 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: CD11b-BB515 (BD, clone: ICRF44, Cat. No. 564518) Flow cytometry 1.25 uL:1M cells

Validation: CD11b-BB515 Flow cytometry (Routinely Tested). Flow cytometric analysis of CD11b expression on human peripheral blood leucocytes

## Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)

Authentication

Mycoplasma contamination

Commonly misidentified lines (See ICLAC register)

## Palaeontology and Archaeology

Specimen provenance

Specimen deposition

Dating methods

Tick this box to confirm that the raw and calibrated dates are available in the paper or in Supplementary Information.

Ethics oversight

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

## Animals and other research organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research, and Sex and Gender in Research

| | |
|---|---|
| Laboratory animals | |
| Wild animals | |
| Reporting on sex | |
| Field-collected samples | |
| Ethics oversight | |

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

## Clinical data

Policy information about clinical studies

All manuscripts should comply with the ICMJE guidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

| | |
|---|---|
| Clinical trial registration | |
| Study protocol | |
| Data collection | |
| Outcomes | |

## Dual use research of concern

Policy information about dual use research of concern

### Hazards

Could the accidental, deliberate or reckless misuse of agents or technologies generated in the work, or the application of information presented in the manuscript, pose a threat to:

| No | Yes | |
|---|---|---|
| ○ | ○ | Public health |
| ○ | ○ | National security |
| ○ | ○ | Crops and/or livestock |
| ○ | ○ | Ecosystems |
| ○ | ○ | Any other significant area |

### Experiments of concern

Does the work involve any of these experiments of concern:

| No | Yes | |
|---|---|---|
| ○ | ○ | Demonstrate how to render a vaccine ineffective |
| ○ | ○ | Confer resistance to therapeutically useful antibiotics or antiviral agents |
| ○ | ○ | Enhance the virulence of a pathogen or render a nonpathogen virulent |
| ○ | ○ | Increase transmissibility of a pathogen |
| ○ | ○ | Alter the host range of a pathogen |
| ○ | ○ | Enable evasion of diagnostic/detection modalities |
| ○ | ○ | Enable the weaponization of a biological agent or toxin |
| ○ | ○ | Any other potentially harmful combination of experiments and agents |

## Plants

Seed stocks

Novel plant genotypes

Authentication

## ChIP-seq

### Data deposition

☐ Confirm that both raw and final processed data have been deposited in a public database such as GEO.

☐ Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

Data access links
*May remain private before publication.*

Files in database submission

Genome browser session
(e.g. UCSC )

### Methodology

Replicates

Sequencing depth

Antibodies

Peak calling parameters

Data quality

Software

## 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: PBMCs were obtained from peripheral blood by Ficoll gradient using Lymphocyte Isolation Solution. One million PBMCs were

Instrument: Cells were acquired on a Cytek Aurora 5-laser spectral flow cytometer.

Software: Spectral cytometry analysis were performed using FlowJo v.10.10. For the UMAP and Phenograph clustering (FlowJo plugins),

Cell population abundance: NA

Gating strategy: For initial gating viability, SSC-A/FSC-A doublets, and cell-lineage markers (CD3, CD4, CD8, CD56 in NK+T cell panel, and CD19,

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

## Magnetic resonance imaging

### Experimental design

Design type

Design specifications

Behavioral performance measures

## Acquisition

Imaging type(s)

Field strength

Sequence & imaging parameters

Area of acquisition

Diffusion MRI ○ Used ○ Not used

## Preprocessing

Preprocessing software

Normalization

Normalization template

Noise and artifact removal

Volume censoring

## Statistical modeling & inference

Model type and settings

Effect(s) tested

Specify type of analysis: ○ Whole brain ○ ROI-based ○ Both

Statistic type for inference

(See Eklund et al. 2016 )

Correction

## Models & analysis

n/a

Involved in the study

- ○ Functional and/or effective connectivity
- ○ Graph analysis
- ○ Multivariate modeling or predictive analysis

Functional and/or effective connectivity

Graph analysis

Multivariate modeling and predictive analysis