nature portfolio

Corresponding author(s): Alexander Misharin, Marc Sala

Last updated by author(s): Sep 1, 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	BD FACSDiva 8.0.3 software was used for flow data collection.
Data analysis	Open source and custom code used for data analysis is available at https://github.com/NUPulmonary/Bailey_Puritz_Senkow_RPRA_2024 FlowJo 10.7.1 was used for flow data analysis. We used Python 3.9.12 and R 4.2.3. Plotting in R was performed using ggplot2 3.5.0. Figures were assembled using either patchwork 1.2.0. Heatmaps were generated using ComplexHeatmap 2.14.0. Significance bars were added to boxplots using ggsignif 0.6.4. Figure 1b was generated using ggalluvial 0.12.5. Plotting in Python was primarily performed using matplotlib 3.7.0/3.8.4 and seaborn 0.12.2/0.13.2. Upset plots were generated using upsetplot 0.8.0. Wilcoxon rank-sum tests and permutation tests were performed in R using coin 1.4-3, and in Python using scipy 1.10.0. Linear models and associated confidence intervals were computed using the stat_smooth function with method = "lm" in ggplot2 3.5.0. Data was processed using the Cell Ranger 7.0.0 pipeline with exon-only processing mode (10x Genomics). Data were processed using Scanpy 1.9.2, and multisample integration was performed with scvi-tools 0.20.0. Differential expression analysis was performed in R 4.2.3 using DESeq2 1.34.0. Matrix factorization was performed using Spectra 0.1.0. For cytokine analysis standard curves for each cytokine were then fit for each assay run using self-starting 5-parameter logistic (5PL) models using drc 3.2-0.

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

Count matrices are available via GEO: BAL from healthy volunteers GSE232616, nasal curettage from healthy volunteers GSE232623, and BAL and nasal curettage from patients with RPRA GSE232627. We also reanalyzed data from GSE122960 and GSE158127, both available via GEO.

Human research participants

Policy information about studies involving human research participants and Sex and Gender in Research.

Reporting on sex and gender	Sex and gender are reported in the study and were determined based on self-reports. Sex and gender were considered in analysis.
Population characteristics	Population characteristics (age, sex, race, ethnicity, disease duration) relevant for this study were reported in the manuscript.
Recruitment	35 patients were enrolled after undergoing evaluation at Northwestern Memorial Hospital between November 2020 and April 2022. Two patients were evaluated as inpatients; the remaining 33 patients were seen in the outpatient setting for symptoms related to RPRA. All patients enrolled in this study had a history of acute COVID-19 infection (nasopharyngeal swab PCR positive), persistent respiratory symptoms, and abnormal CT lung imaging at least three months after COVID-19 diagnosis. Two patients in the cohort subsequently underwent lung transplantation for COVID-19-induced lung fibrosis. Patients underwent chest radiography, pulmonary function testing, laboratory assessment, and in person or telehealth visits at the discretion of the treating physician. Bronchoscopy was usually performed to exclude ongoing COVID-19 infection or superimposed respiratory infections as a cause of persistent pulmonary symptoms and radiographic abnormalities prior to initiation of glucocorticoids.
Ethics oversight	All human subjects research was approved by the Northwestern University Institutional Review Board. Patients with RPRA were enrolled in the study STU00213592. Healthy volunteers were enrolled in the study STU00206783 and STU00214826 at Northwestern University, or Pro00088966 and Pro00100375 at Duke University. Two patients with severe lung fibrosis necessitating consideration for lung transplant after COVID-19 were co-enrolled in STU00212120 and STU00213592. All study participants or their surrogates provided informed consent.

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 samples size calculations were performed. All subjects who have provided consent to participate in the study between November 2020 and May 2022 were included in the study.
Data exclusions	We prepared, sequenced, and analyzed single-cell RNA-seq libraries from nasal curettage samples from seven healthy volunteers. However, after initial analysis one library was excluded due to overall low quality (HV14), and only six libraries were included in the final analysis.
Replication	This is observational clinical study. No replication was performed.
Randomization	This is observational clinical study. No randomization was performed.
Blinding	This is observational clinical study. No blinding was performed.

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

Methods

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

Antibodies

Antibodies used

Antigen, clone, fluorochrome, supplier, catalog number, dilution.
CD4, RPA-T4, BUV395, BD, 564724, 1:40.
CD19, HIB19, BUV395, BD, 740287, 1:40.
CD25, 2A3, BUV737, BD, 564385, 1:20.
CD56, NCAM16.2, BUV737, BD, 612766, 1:20.
HLA-DR, L243, eFluor450, ThermoFisher, 48-9952-42, 1:40.
CD45, HI30, BV510, Biolegend, 304036, 1:20.
CD15, HI98, BV786, BD, 563838, 1:20.
CD3, SK7, PE, ThermoFisher, 12-0036-42, 1:20.
CD127, HIL-7R, PECF594, BD, 562397, 1:20.
CD206, 19.2, PECy7, ThermoFisher, 25-2069-42, 1:40.
CD8, SK1, APC, Biolegend, 344721, 1:40.
CD14, M5E2, APC, Biolegend, 301808, 1:40.
EpCAM, 9C4, APC, Biolegend, 324208, 1:40.

Validation

We used standard commercially available previously-validated antibodies.

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

Not applicable.

Study protocol

This is observational study, all eligible participants who provided consent were enrolled in the study.

Data collection

35 patients were enrolled after undergoing evaluation at Northwestern Memorial Hospital between November 2020 and April 2022. Two patients were evaluated as inpatients; the remaining 33 patients were seen in the outpatient setting for symptoms related to RPRA. Clinical data was collected by chart review.

Outcomes

Outcomes were not predefined and were assessed based on results of the follow up chest CT scan.

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

BAL fluid samples were filtered through a 70 µm cell strainer, pelleted by centrifugation at 400 rcf for 10 min at 4°C, followed

Sample preparation	by hypotonic lysis of red blood cells with 2 ml of PharmLyse (BD Biosciences) reagent for 2 minutes. Lysis was stopped by adding 13 ml of MACS buffer (Miltenyi Biotech). Cells were pelleted again and resuspended in 100 µl of 1:10 dilution of Human TruStain FcX (Biolegend) in MACS buffer, and a 10 µl aliquot was taken for counting using K2 Cellometer (Nexcelom) with AO/PI reagent.The cell suspension volume was adjusted so the concentration of cells was always less than 5x107 cells/ml and the fluorophore-conjugated antibody cocktail was added in 1:1 ratio (Supplemental Table 5). After incubation at 4°C for 30 minutes, cells were washed with 5 ml of MACS buffer, pelleted by centrifugation, and resuspended in 500 µl of MACS buffer with 2 µl of SYTOX Green viability dye (ThermoFisher). Cells were sorted on a FACS Aria III SORP instrument using a 100-µm nozzle at 20 psi. Cells were sorted into 300 µl of 2% BSA in DPBS and cryopreserved using the protocol by Linas Mazutis45. Briefly, cells pelleted by centrifugation at 400 rcf for 5 min at 4°C, resuspended in Bambanker freezing media to ~2000 cells/µl concentration. Concentration was confirmed using K2 Cellometer (Nexcelom) with AO/PI reagent using "Immune cells low RBC" program with default settings and ~40 µl aliquots were immediately frozen at -80°C.
Instrument	BD FACSAria III SOPR.
Software	Analysis of the flow cytometry data was performed using FlowJo 10.6.2. using uniform sequential gating strategy reported in our previous publication (Grant et al., Nature, 2021) and reviewed by two investigators (SS, AVM).
Cell population abundance	Relative cell type abundance was calculated as a percent out of all singlets/live/CD45+ cells.
Gating strategy	Gating strategy is provided in Extended Figure S2a.

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