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Last updated by author(s):	Jan 15. 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.

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

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	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.
x	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. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted <i>Give P values as exact values whenever suitable.</i>
x	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

Single-cell RNA sequencing were performed on the Illumina NovaSeq6000 platform. FACSCelestaTM (BD Biosciences) for flow cytometry and FACS. Biotek SynergyNeo2 for ELISA.

Data analysis

Single-cell RNA-seq analysis, statistical analysis and graphing were performed using Cell ranger v6.1.2, FlowJo v10, GraphPad PRISM v8,Adobe Illustrator 2023 (Adobe), R software v4.1.2 and the following R packages: Seurat v4.0.5, Harmony v0.1.1, AUCell v3.17, ClusterProfiler v4.2.0, Monocle 3 v1.0.0, iTalk v0.1.0 and ggplot2 v3.3.5. No new algorithms were developed for this manuscript. All code generated for single-cell RNA-seq data analysis is available from the corresponding authors upon reasonable request.

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

Sequence data generated in this work have been deposited in the Genome Sequence Archive in BIG Data Center, Beijing Institute of Genomics (BIG), Chinese

Academy of Sciences, under the accession code HRA004500 (https://ngdc.cncb.ac.cn/gsa-human/browse/HRA004500). Published single-cell expression datasets analyzed in this work are available from Genome Sequence Archive in BIG Data Center, Beijing Institute of Genomics (BIG), Chinese Academy of Sciences, under the accession code HRA000155 (https://ngdc.cncb.ac.cn/gsa-human/browse/HRA000155). Source data are provided with this paper.

Research involving human participants, their data, or biological material

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

Reporting on sex and gender

The detailed information on the sex of participants is provided in Supplementary Table 1 and 2. In summary, 4 female IA patients, 1 male IA patients, 7 female RA patients and 1 male RA patient participated in this study.

Reporting on race, ethnicity, or other socially relevant groupings

All the recruited patients are asians. No socially relevant categorization variables was used in this study.

Population characteristics

Recruitment

We prospectively recruited adult patients with new-onset IA induced by PD-1 inhibitors (PD-1-IA). Active PD-1-IA (IA_act) was defined as: 1) presence of joint inflammation diagnosed by a rheumatologist based on the comprehensive assessment of the history, physical examination, inflammatory markers and imaging findings; 2) joint inflammation developed after anti-PD-1 administration. IA patients were excluded if they had pre-existing autoimmune disease.

Patients who fulfilled the 2010 American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) classification criteria for RA were also recruited and further classified into seropositive or seronegative RA depending on the presence or absence of anticitrullinated-peptide antibodies (ACPA) and rheumatoid factor (RF).

Additionally, healthy donors with matched age and sex were recruited as control.

Ethics oversight

This study was performed following the ethical guidelines of the Declaration of Helsinki and was approved by the Peking Union Medical College Hospital Ethics Committee (no. JS-1940). Informed consent was obtained from all the participants before sample collection and clinical information.

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

See Above.

Field-specific reporting

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🗴 Life sciences 🔲 Behavioural & social sciences 🔲 Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see $\underline{\mathsf{nature.com/documents/nr-reporting-summary-flat.pdf}}$

Life sciences study design

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

Sample size

No standard methods were used to predetermine sample size. We recruited 5 patients who were diagnosed with newly developed inflammatory arthritis and 8 patients who fulfilled the 2010 ACR/EULAR Rheumatoid Arthritis classification criteria. The sample sizes were sufficient to provide stable single cell clustering results and to perform statistical analysis.

Data exclusions

For scRNA-seq, doublets detected by Scrublet and doublet clusters expressing markers of more than two major cell lineages were removed from analysis. In addition, low quality cells meeting the following criteria were also removed: 1) number of genes detected per cell less than 200 or more than 5000; 2) UMIs detected per cell over 200; 3) counts of mitochondrial genes constituting over 12% of all gene counts; and 4) counts of red blood cell genes constituting over 0.3% of all gene counts.

Replication

We have repeated each experiment at least three time to ensure consistent results. All repeats performed showed similar trends.

Randomization

Randomization was not included in the study, as this study did not involve clinical trials/clinical trial associated data.

Blinding

Blinding of participants was not relevant to our study, for the main group of the study population was patients diagnosed with arthritis. For scRNA-seq, flow cytometry, ELISA and transwell experiments, the experimenters were blinded to the sample information.

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 & experime	ental systems Methods		
n/a Involved in the study	n/a Involved in the study		
Antibodies	ChIP-seq		
Eukaryotic cell lines	Flow cytometry		
Palaeontology and a			
Animals and other of			
Clinical data			
Dual use research o	f.concorn		
Plants	redicem		
Fidilits			
Antibodies			
Antibodies used	Antibodies were applied for flow cytometry and FACS, listed as following: anti-CD14-BV605 (#367126, clone 63D3, 1/20 dilution,		
	BioLegend), anti-CD11b-BV421 (#301323, clone ICRF44, 1/20 dilution, BioLegend), anti-CD4-APC/Cy7 (#344615, clone SK3,		
	BioLegend), anti-CD8-BV510 (#344731, clone SK1, 1/20 dilution, BioLegend), anti-IL-1β-FITC (#508206, clone JK1B-1, 1/20 dilution, BioLegend), anti-CCL3-PE (#12-9706-42, clone CR3M, 1/20 dilution, Thermo Fisher), anti-CCR1-APC/Cy7 (#362917, clone 5F10B29,		
	1/20 dilution, BioLegend), anti-CXCL10-APC (#519505, clone J034D6, 1/20 dilution, BioLegend), and anti-CXCR3-APC (#353707, clone		
	G025H7, 1/20 dilution, BioLegend).		
Validation	All antibodies were obtained commercially validated by the respective company. All antibodies had validation statement provided on		
	the website of the manufacturer. Related technical data sheets can be obtained from the manufacturer's website using the catalog number provided above.		
	Indiffuer provided above.		
Clinical data			
Policy information about cl	inical studies		
,	with the ICMJEguidelines for publication of clinical research and a completedCONSORT checklist must be included with all submissions.		
Clinical trial registration	This is not a clinical trial.		
Cillical trial registration	This is not a chilical trial.		
Study protocol	Study design and recruitment of participants can be found in Methods section. Briefly, we prospectively recruited patients with newly		
	developed inflammatory arthritis after immune checkpoint inhibitor administration and rheumatoid arthritis. Fresh synovial fluid samples were obtained from patients with active IA or active RA through arthrocentesis. Peripheral blood samples were collected		
	from the patients through venepuncture. Mononuclear cells were isolated from synovial fluid and peripheral blood samples and were		
	further used for sequencing and flow cytometry. Supernatant of synovial fluid and serum were used for ELISA tests.		
Data collection	Clinical information were collected from medical records.		
Outcomes	Not applicable.		
Flow Cytometry			
Plots			
Confirm that:			
	he 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 p	plots with outliers or pseudocolor plots.		
🗶 A numerical value for	number of cells or percentage (with statistics) is provided.		
Methodology			
Sample preparation	SFMCs and PBMCs from recruited patients and healthy controls were collected. Cells were washed twice with FACS staining		
	buffer (PBS, 5% FBS, and 0.1% sodium azide) and stained with antibodies for 30 minutes on ice. For the detection of		
	intracellular IL-1B, CCL3, CXCL10 and CXCR3 expression, cells were first stimulated with phorbol myristate acetate and ionomycin for 4 h with GolgiStop in complete RPMI-1640 medium in an incubator at 37 °C with 5% CO2. The stimulated cells		
	were then fixed and permeabilized with a fixation/permeabilization kit (eBioscience) before intracellular staining.		
Instrument	FACS Celesta.		

Analysis was performed in FlowJo™ v10.8 Software (BD Life Sciences).

At least, 2x10^6/ml PBMCs or SFMCs were isolated from 20 ml of the patients' whole blood, and aliquots were used

Software

Cell population abundance

according to the purpose of the experiments.

Gating strategy

The gating strategies are shown in Supplementary Fig. 12.

SFMCs: Cells (FSC/SSC) -> Singlets -> CD11b+ PBMCs: Cells (FSC/SSC) -> Singlets -> CD14+ PBMCs: Cells (FSC/SSC) -> Singlets -> CD4+

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