

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 (<i>n</i>) 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 <i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>
☒	☐ 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>
☒	☐ 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 <i>d</i> , Pearson's <i>r</i>), 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	Data were acquired and processed using the following instruments and software: Mindray BC-6800 automated hematology analyzer for leukocyte differential counts. Mindray CL-900i chemiluminescence immunoanalyzer for serum KL-6 quantification. Beckman Coulter CytoFLEX flow cytometer for cytometric bead array cytokine measurement. Agilent Novocyte advanceon flow cytometer systems for immunophenotyping of PBMCs. Thermo Fisher Scientific QuanStudio 3 Real-Time PCR System for qRT-PCR. 3DHISTECH panoramic scan system for digital slide imaging of IHC and multiplex immunofluorescence. 10x Genomics Chromium Single-Cell 5' Kit and Singleron GEXSCOPE® Single Cell RNA Library Kits for single-cell library preparation. Illumina NovaSeq 6000 sequencer for all sequencing. Thermo Fisher Scientific NanoDrop One for RNA quality assessment.
Data analysis	Data were processed, analyzed, and visualized using the following software tools and packages: Cell Ranger (v7.0.0) for demultiplexing, alignment, and feature counting of scRNA-seq, TCR-seq, and BCR-seq data. Seurat (v4.3.0) in R (v4.2.2) for single-cell data quality control, normalization, integration, clustering, and UMAP visualization. DoubletFinder (v2.0.3) for identifying and removing potential doublets in single-cell data. SingleR (v2.0.0) for unbiased cell type annotation. presto (v1.0.0) for differential gene expression analysis. scRepertoire for integrating BCR/TCR clonality data with scRNA-seq data. Change-O repertoire clonal assignment toolkit for BCR clonal assignment.

Shazam (v1.1.0) for analyzing BCR somatic hypermutations.
 AUCCell (v1.22.0) for calculating gene set activity scores.
 CellChat (v1.6.1) for inferring and analyzing ligand-receptor interactions.
 clusterProfiler (v4.6.2) for Gene Ontology (GO) and KEGG pathway enrichment analysis.
 fgSEA (v1.11.1) for fast gene set enrichment analysis.
 FastQC (v0.11.9) for bulk RNA-seq read quality control.
 Trim Galore (v0.6.7) for adapter trimming of bulk RNA-seq reads.
 Hisat2 (v2.2.1) for aligning bulk RNA-seq reads to the GRCh38 genome.
 Stringtie (v2.1.7) for transcript assembly and quantification (TPM) of bulk RNA-seq data.
 Cytoscape (v3.10.0) for visualizing clonotype sharing networks.
 Slide viewer(v2.5.0) was employed for image browsing.
 Fiji (ImageJ v 1.53t) for quantitative analysis of immunohistochemistry and multiplex immunofluorescence images.
 GraphPad Prism (v9) and SPSS for statistical analysis and graph generation.

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](#)

Single-cell transcriptomic data generated in this study have been deposited in the National Genomics Data Center of China and are publicly available under accession code HRA006607 (<https://ngdc.cnbc.ac.cn/gsa-human/browse/HRA006607>). This research reanalyzes existing, publicly available genomic datasets, including data from the ArrayExpress under accession numbers E-MTAB-6149 (<https://www.ebi.ac.uk/biostudies/arrayexpress/studies/E-MTAB-6149>) and E-MTAB-6653 (<https://www.ebi.ac.uk/biostudies/arrayexpress/studies/E-MTAB-6653>), the GEO database under accession number GSE216329 (<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE216329>), Mendeley Data under accession number 8c3x28r5hk (<https://data.mendeley.com/datasets/8c3x28r5hk/1>), and the EGA European Genome-Phenome Archive under accession number EGAS00001005013 (<https://ega-archive.org/studies/EGAS00001005013>). Flow cytometry and immunofluorescence imaging data have been deposited in Zenodo (<https://doi.org/10.5281/zenodo.17318429>). All data are included in the Supplementary Information or available from the authors, as are unique reagents used in this Article. The raw numbers for charts and graphs are available in the Source Data file whenever possible. Source data are provided with this paper.

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	No analysis related to sex or gender. Patient sex information was collected in the study and the relevant information is reported in Supplementary table 1,2,3,4. Data were collected and reported disaggregated for sex and gender. The biological sex of participants was determined by clinicians based on medical records, and all participants self-reported their sex/gender, which was consistent with clinical identification.
Reporting on race, ethnicity, or other socially relevant groupings	The lung tissue and blood samples used in this study were obtained exclusively from Chinese individuals, including both Asian male and Asian female. All samples were collected from the First Affiliated Hospital of Guangzhou Medical University, located at Guangzhou, Guangdong, China. The race, ethnicity, or other socially relevant groupings were not used as covariates in this study.
Population characteristics	Patient characteristics are provided in Supplementary Table 1. The study was designed to include lung cancer patients who received immune checkpoint inhibitor (ICI) therapy. The experimental group comprised patients who developed checkpoint inhibitor-related pneumonitis (CIP) following ICI treatment, while the control group included patients without CIP. Donor ages ranged from 50 to 73 years across both groups. Results were cross-validated using multiple assays, including flow cytometry (19 patients), qPCR (14 patients), ELISA (26 patients), and cytometric bead assay (188 patients). In addition, bronchoalveolar lavage fluid (BALF) samples from 19 patients were analyzed by flow cytometry and ELISA.
Recruitment	Participants were prospectively recruited under strict clinical criteria: the CIP group comprised lung cancer patients who developed checkpoint inhibitor pneumonitis after immunotherapy, with samples obtained via pneumonitis tissue biopsy, while the control group consisted of patients undergoing surgical resection after neoadjuvant therapy without CIP, from whom noncancerous adjacent tissues were collected. The study protocol was reviewed and authorized by the Ethics Committee of the First Affiliated Hospital of Guangzhou Medical University (study code: ES2023-239-03). All patients provided written informed consent for obtaining the study samples. All sample collections and clinical procedures were integral to standard diagnostic and therapeutic care. No participants received financial compensation for their involvement, and all expenses related to the clinical study were covered by the project's research funding.
Ethics oversight	The study protocol was approved by the Ethics Committee of the First Affiliated Hospital of Guangzhou Medical University (approval code: ES2023-239-03). Written informed consent was obtained from all participants, and all procedures were conducted in accordance with the principles of the Declaration of Helsinki.

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](https://www.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	scRNA/TCR/BCR sequencing was performed on 7 CIP and 7 non-irAE patients, with scRNA sequencing conducted on 2 CIP patients, all patients were diagnosed with lung cancer.
Data exclusions	CIP tissue samples were screened by histology to confirm the disease diagnosis. The paired noncancerous adjacent tissues were pathologically evaluated via postoperative biopsy, confirming the absence of viable tumor cells.
Replication	FACS, multiplex IHC staining, elisa and qRT-PCR were confirmed in at least three biological replicates.
Randomization	Randomization are not applicable in our study.
Blinding	The nature of our study made full blinding of investigators to group allocation (CIP vs. CTL) during sample collection impractical, as the group identity was inherently known due to the distinct clinical context and sample origin (e.g., pneumonitis tissue from CIP patients versus adjacent normal tissue from control patients during surgery). However, we implemented blinding procedures to minimize potential bias during key stages of data analysis and quantification, particularly during the computational analysis of sequencing data and the quantitative assessment of histology images.

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	All antibodies used in this study are commercially sourced. Their specific applications and details are as follows: 1. Immunohistochemistry (IHC) and Multiplex Immunofluorescence (MIF) Anti-human IgG antibody: 1:500 dilution (Supplier: Abcam; Catalog #: ab109489; Clone: EPR4421) - Application: IHC Anti-human CD8 antibody: 1:100 dilution (Supplier: Cell Signaling Technology; Catalog #: 98941; Clone: D4W2Z) - Application: MIF Anti-human IFN-γ antibody: 1:500 dilution (Supplier: Abcam; Catalog #: ab231036; Clone: EPR21704) - Application: MIF Anti-human CD103 antibody: 1:200 dilution (Supplier: Abcam; Catalog #: ab224202; Clone: EPR22590-27) - Application: MIF Anti-human CD45 antibody: 1:2000 dilution (Supplier: Abcam; Catalog #: ab208022; Clone: EPR20033) - Application: MIF Anti-human CD14 antibody: 1:2000 dilution (Supplier: Abcam; Catalog #: ab133335; Clone: EPR3653) - Application: MIF Anti-human CCL3 antibody: 1:1000 dilution (Supplier: Abcam; Catalog #: ab259372; Clone: EPR23751-54) - Application: MIF Anti-human CCL4 antibody: 1:200 dilution (Supplier: Bioss; Catalog #: bs-2475R; Clonality: Polyclonal) - Application: MIF Anti-human CD68 antibody: 1:2000 dilution (Supplier: Abclonal; Catalog #: A23205; Clonality: Monoclonal) - Application: MIF Anti-human GSDME antibody: 1:100 dilution (Supplier: Abcam; Catalog #: ab230482; Clonality: Polyclonal) - Application: MIF Anti-human FR-β (FOLR2) antibody: 1:100 dilution (Supplier: Abcam; Catalog #: ab302532; Clone: EPR25731-70) - Application: MIF 2. Flow Cytometry Analysis of PBMCs PE/Cyanine7 anti-human CD3 antibody: 5 uL per million cells in 100 uL staining volume (Supplier: BioLegend; Catalog #: 300420; Clone: UCHT1) Pacific Blue™ anti-human CD4 antibody: 5 uL per million cells in 100 uL staining volume (Supplier: BioLegend; Catalog #: 344620; Clone: SK3) FITC anti-human CD25 antibody: 5 uL per million cells in 100 uL staining volume (Supplier: BioLegend; Catalog #: 356105; Clone: M-
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APC anti-human CD127 (IL-7R α) antibody: 5 μ L per million cells in 100 μ L staining volume (Supplier: BioLegend; Catalog #: 351315; Clone: A019D5)

PE anti-human IL-17A Recombinant antibody: 5 μ L per million cells in 100 μ L staining volume (Supplier: BioLegend; Catalog #: 385905; Clone: QA18A46)

APC anti-human CD8 antibody: 5 μ L per million cells in 100 μ L staining volume (Supplier: BioLegend; Catalog #: 344721; Clone: SK1)

FITC anti-human IFN- γ antibody: 5 μ L per million cells in 100 μ L staining volume (Supplier: BioLegend; Catalog #: 502505; Clone: 4S.B3)

PE anti-human TNF- α antibody: 5 μ L per million cells in 100 μ L staining volume (Supplier: BioLegend; Catalog #: 502908; Clone: MAb11)

PE/Cyanine7 anti-human CD68 antibody: 1 μ L per million cells in 100 μ L staining volume (Supplier: BioLegend; Catalog #: 333816; Clone: Y1/82A)

Anti-cleaved N-terminal DFNA5 / GSDME antibody: 1 μ L per million cells in 100 μ L staining volume (Supplier: Abcam; Catalog #: ab222408; Clone: EPR20867-248)

PE anti-human Folate Receptor β (FR- β) antibody: 1 μ L per million cells in 100 μ L staining volume (Supplier: BioLegend; Catalog #: 391704; Clone: 94b/FOLR2)

Validation

The antibody used in IHC staining:

Anti-human IgG antibody(ab109489, abcam), validation stated on supplier's website: <https://www.abcam.cn/products/primary-antibodies/human-igg-antibody-epr4421-ab109489.html>

The antibodies used in immunofluorescence staining:

Anti-human CD8 antibody(98941, Cell Signaling Technology), validation stated on supplier's website: <https://www.cellsignal.cn/products/primary-antibodies/cd8a-d4w2z-xp-rabbit-mab-mouse-specific/98941>

Anti-human IFN- γ antibody(ab231036, abcam), validation stated on supplier's website:

<https://www.abcam.cn/products/primary-antibodies/interferon-gamma-antibody-epr21704-ab231036.html>

Anti-human CD103 antibody(ab224202, abcam), validation stated on supplier's website:

<https://www.abcam.cn/products/primary-antibodies/cd103-antibody-epr22590-27-ab224202.html>

Anti-human CD45 antibody(ab208022, abcam), validation stated on supplier's website:

<https://www.abcam.cn/products/primary-antibodies/cd45-antibody-epr20033-ab208022.html>

Anti-human CD14 antibody(ab133335, abcam), validation stated on supplier's website:

<https://www.abcam.cn/products/primary-antibodies/cd14-antibody-epr3653-ab133335.html>

Anti-human CCL3 antibody(ab259372, abcam), validation stated on supplier's website:

<https://www.abcam.cn/products/primary-antibodies/macrophage-inflammatory-protein-1-alpha--ccl3--ccl3l1-antibody-epr23751-54-ab259372.html>

Anti-human CCL4 antibody(bs-2475R, bioss), validation stated on supplier's website:

http://www.bioss.com.cn/prolook_03.asp?id=AF08169606002011&pro37=1

Anti-human CD68 antibody(A23205, ABclonal), validation stated on supplier's website:

<https://abclonal.com.cn/catalog/A23205>

Anti-human GSDME antibody(ab230482, abcam), validation stated on supplier's website:

<https://www.abcam.cn/products/primary-antibodies/dfna5-gsdme-antibody-ab230482>

Anti-human FR- β (FOLR2) antibody(ab302532, abcam), validation stated on supplier's website:

<https://www.abcam.cn/products/primary-antibodies/folr2-antibody-epr25731-70-ab302532.html>

The antibodies used in flow cytometry:

PE/Cyanine7 anti-human CD3 antibody(catalog #300420, BioLegend), validation stated on supplier's website:

<https://www.biolegend.com/en-us/products/pe-cyanine7-anti-human-cd3-antibody-3070>

Pacific Blue™ anti-human CD4 antibody(catalog #344620, BioLegend), validation stated on supplier's website:

<https://www.biolegend.com/en-us/products/pacific-blue-anti-human-cd4-antibody-6507>

FITC anti-human CD25 antibody (catalog #356105, BioLegend), validation stated on supplier's website:

<https://www.biolegend.com/en-us/products/fits-anti-human-cd25-antibody-8390>

APC anti-human CD127 (IL-7R α) antibody(catalog #351315, BioLegend), validation stated on supplier's website:

<https://www.biolegend.com/en-us/products/apc-anti-human-cd127-il-7ralpha-antibody-7172>

PE anti-human IL-17A Recombinant antibody (catalog #385905, BioLegend), validation stated on supplier's website:

<https://www.biolegend.com/en-us/products/pe-anti-human-il-17a-recombinant-antibody-23446>

APC anti-human CD8 antibody(catalog #344721, BioLegend), validation stated on supplier's website:

<https://www.biolegend.com/en-us/products/apc-anti-human-cd8-antibody-6531>

FITC anti-human IFN- γ antibody (catalog #502505, BioLegend), validation stated on supplier's website:

<https://www.biolegend.com/en-us/products/fits-anti-human-ifn-gamma-antibody-1013>

PE anti-human TNF- α antibody (catalog #502908, BioLegend), validation stated on supplier's website:

<https://www.biolegend.com/en-us/products/pe-anti-human-tnf-alpha-antibody-1346>

PE/Cyanine7 anti-human CD68 antibody (catalog #333816, BioLegend), validation stated on supplier's website:

<https://www.biolegend.com/en-gb/products/pe-cyanine7-anti-human-cd68-antibody-9123?GroupID=BLG10058>

Anti-cleaved N-terminal DFNA5 / GSDME antibody (catalog #ab222408, Abcam), validation stated on supplier's website:

<https://www.abcam.cn/products/primary-antibodies/cleaved-n-terminal-dfna5--gsdme-antibody-epr20867-248-ab222408.html>

PE anti-human Folate Receptor β (FR- β) antibody (catalog #391704, BioLegend)

<https://www.biolegend.com/fr-ch/products/pe-anti-human-folate-receptor-beta-fr-beta-antibody-15115?GroupID=GROUP389>

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, mosaicism, 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	Peripheral blood was collected using EDTA-K2 tubes. Subsequently, within 4 hours of blood sample collection, PBMCs were isolated via Ficoll discontinuous density gradient centrifugation (catalog #F4375, Sigma-Aldrich). The PBMCs were resuspended in 2% FBS. The sample preparation involves incubating PBMCs with FcR blocking reagent, staining with surface antibodies, fixation, permeabilization, and intracellular cytokine staining prior to flow cytometry analysis.
Instrument	NovoCyte Advanteon (Agilent Technologies, Santa Clara, CA, USA)
Software	FlowJo software.
Cell population abundance	The purity was verified by flow cytometry.
Gating strategy	FSC (Forward Scatter) is related to cell size, while SSC (Side Scatter) reflects cell granularity. FSC-SSC plots are used to identify the target cell population and exclude debris. An SSC-Height vs. SSC-Area density plot is employed for doublet exclusion (single-cell analysis). Single-parameter histograms are used to identify cells expressing specific markers, while two-parameter density plots are utilized for further analysis.

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