

Reporting Summary

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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	All scRNA-seq, scTCR-seq and single nucleus multiome libraries were sequenced on DNBSEQ-G400 (MGI). Mass cytometry data were collected using Helios (Standard BioTools). Imaging mass cytometry data were collected using Hyperion (Standard BioTools). Multiplex immunohistochemical staining was performed using the OPAL™ Multiplex IHC kit (Akoya Biosciences), and images were acquired using the Mantra Snap imaging system (Akoya Biosciences).A SH800Z cell sorter (SONY) was used for lymphocyte sorting.
Data analysis	Raw FASTQ files were processed using Cell Ranger (6.1.1 and 7.1.0, 10x Genomics) for scRNA-seq and scTCR-seq analysis, and Cell Ranger ARC (2.0.2, 10x Genomics) for multiome (single nucleus RNA-seq and ATAC-seq) data analysis. The downstream analysis was performed in Loupe browser (8.1.1, 10x Genomics) and in R (4.4.1) using Seurat (5.2.1), Signac (1.14), MAST (1.32.0), e1071 (1.7.16), caret (7.0.1), ggplot2 (3.5.1), EnhancedVolcano (1.24.0) and ComplexHeatmap (2.22.0) R packages. RNA velocity analysis was conducted in Python (3.6.13) using velocyto (0.17.17) and scVelo (0.2.5). Mass flowcytometry data were analyzed using FlowJo (10.8.1) and software on Cytobank. Multiplex immunohistochemical staining data were analyzed using inForm software (2.5.1, Akoya Biosciences). Imaging mass cytometry data were analyzed using MCD™ Viewer (1.0.560.6, Standard BioTools) and MCD™ SmartViewer (Standard BioTools Inc.). Additional statistical tests and data visualization were performed using GraphPad Prism 10.

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](#)

The datasets supporting the findings of this study have been deposited in NCBI's Gene Expression Omnibus (GEO) under the following accession numbers:

GSE267026
GSE267027
GSE295601
GSE295977
GSE308745
GSE312336

These entries are currently private but are available to reviewers via secure token-based links:

GSE267026 reviewer's link:

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GSE267027 reviewer's link:

<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE267027&token=oxmfioespfqnfqt>

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<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE295601&token=ubazsiiktgtbqn>

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GSE312336 reviewer's link:

<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE312336&token=cpwnqcgobqnmvmz>

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

This study included lung cancer patients at Saitama Medical University International Medical Center, regardless of gender. Patients are described as per genetically determined biological sex at birth.

Reporting on race, ethnicity, or other socially relevant groupings

All participants in this study were Japanese and racially Asian.

Population characteristics

Patient characteristics are shown in supplementary Table 1

Recruitment

The patients with stage I-II non-small cell lung cancer (NSCLC) who underwent radical surgery between October 2017 and October 2019 at Saitama Medical University International Medical Center (Hidaka city, Saitama pref., Japan) were recruited (n = 56).

The consecutive patients (n = 20) with stage II-IIIa NSCLC who underwent surgery after 3 courses of neoadjuvant therapy, including nivolumab and chemotherapy, between August 2023 and October 2024 at Saitama Medical University International Medical Center were also recruited.

Ethics oversight

All the samples were collected after written informed consent was obtained from the patients. All cohorts were conducted in a blinded manner. The Internal Review Board of Saitama Medical University International Medical Center approved the study protocol, in accordance with the Declaration of Helsinki (ethical approval Nos. 17-084, 15-221, and 2023-011).

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

Field-specific reporting

Life sciences study design

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

Sample size	This study is an observational study and does not set the number of cases based on treatment efficacy as an endpoint. Sample size was determined by number of available patients who consented to the study.
Data exclusions	No data exclusion was performed from the participating cases.
Replication	scRNAseq analysis has also been performed on advanced lung cancer and locally advanced lung cancer (n = 34) and on public healthy individual data (n = 135), yielding consistent results. Mouse adoptive transfer experiments were repeated three times (each group contains 4 mice or more), yielding consistent results.
Randomization	N/A
Blinding	N/A

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		Methods	
n/a	Involved in the study	n/a	Involved in the study
☐	☒ Antibodies	☒	☐ ChIP-seq
☐	☒ Eukaryotic cell lines	☐	☒ Flow cytometry
☒	☐ Palaeontology and archaeology	☒	☐ MRI-based neuroimaging
☐	☒ Animals and other organisms		
☐	☒ Clinical data		
☒	☐ Dual use research of concern		
☒	☐ Plants		

Antibodies

Antibodies used	Antibody information was also listed in supplementary Table 6, and 7. Antibodies for CyTOF in the human study: Antigen Conjugate Clone Lot # Manufacturer CD3 141Pr UCHT1 23020259-28, 23041292-12, 23051996-18, 23093940-12, 23115440-21, 23126009-27 Standard BioTools CD19 142Nd HIB19 23030892-24, 23062451-13, 23104393-06 Standard BioTools CD45RA 143Nd HI100 2301979-19, 23072803-06 Standard BioTools CD4 145Nd RPA-T4 23031054-31, 23062475-14, 23125886-19 Standard BioTools CD8a 146Nd RPA-T8 2206193-27, 23083537-21, 23093943-12 Standard BioTools CD194 (CCR4) 149Sm L291H4 23041153-05, 23093909-11, 23125802-14 Standard BioTools CD223 (LAG-3) 150Nd 11C3C65 23030550-13, 23062578-20, 23094048-19 Standard BioTools CD278 (ICOS) 151Eu C398.4A 2201570-11, 23073073-24, 23115569-28 Standard BioTools CD62L 153Eu DREG-56 23041137-04, 23083559-22, 23093834-06 Standard BioTools CD279 (PD-1) 155Gd EH12.2H7 23031031-30, 23072831-07, 23094076-19 Standard BioTools CD27 158Gd L128 2206891-08, 23093901-11 Standard BioTools CD26 161Dy BA5b 2861706, 23094041-18, 23125830-15 Standard BioTools CD80 (B7-1) 162Dy 2D10.4 23030643-14, 23062709-28, 23093929-12 Standard BioTools CD183 (CXCR3) 163Dy G025H7 23030978-28, 23062349-07, 24016112-09 Standard BioTools CD127 (IL-7Ra) 165Ho A019D5 2112314-09, 23083529-21, 23093825-06 Standard BioTools CD197 (CCR7) 167Er G043H7 23041098-03, 23062208-01, 23094124-20 Standard BioTools CD154 (CD40L) 168Er 24-31 2203390-02, 23093916-11, 23125921-20 Standard BioTools CD185/CXCR5 171Yb RF8B2 2204318-28, 23094043-18 Standard BioTools CD184 (CXCR4) 173Yb 12G5 23020013-15, 23062353-07 Standard BioTools CD274 (PD-L1) 175Lu 29E.2A3 2209212-13, 2203490-09 Standard BioTools CD196 (CCR6) 176Yb G034E3 2205704-26, 23041331-13, 23094024-18, 23115274-10 Standard BioTools FoxP3 159Tb PCH101 23041150-05, 23072983-17 Standard BioTools Tbet 160Gd D6N8B 2205778-31(Clone:4B10), 23073011-18(Clone:D6N8B), 23094028-18(Clone:D6N8B) Standard BioTools
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CD366 (Tim-3) 169Tm F38-2E2 3511910 Standard BioTools
 CD152 (CTLA-4) 170Er 14D3 2203367-01, 23062536-19, 23094261-27 Standard BioTools
 GZmK 144Nd GM26E7 B322082, B392457 Bio-Rad Laboratories
 GZmB 156Gd GB11 161827, 163522 BioLegend

Antibodies for CyTOF in animal experiments:

Antigen Conjugate Clone # Manufacturer
 CD45 89Y 30-F11 Standard BioTools
 CD4 145Nd RM4-5 Standard BioTools
 CD8a 146Nd 53-6.7 Standard BioTools
 CD194 (CCR4) 149Sm 2G12 BioLegend
 CD3e 152Sm 145-2C11 Standard BioTools
 CD196 (CCR6) 156Gd 29-2L17 Standard BioTools
 CD279 (PD-1) 159Tb 29F.1A12 Standard BioTools
 CD366 (Tim-3) 162Dy RMT3-23 Standard BioTools
 CD62L 164Dy MEL-14 Standard BioTools
 CD19 166Er 6D5 Standard BioTools
 CD183 (CXCR3) 173Yb CXCR3-173 BioLegend
 CD127 (IL-7Ra) 175Lu A7R34 Standard BioTools
 TCF1 151Eu C63D9 Cell Signaling Technology
 FoxP3 158Gd FJK-16s Standard BioTools
 Tbet 160Gd 4B10 BioLegend
 GZmB 171Yb GB11 BioLegend

Sorting in the human study:

Antigen Color Clone Lot # Manufacturer
 CXCR3 BV421 G025H7 B303861, B314468, B314468 BioLegend
 CCR6 PE-CF594 G034E3 B315651, B315651, B315651 BioLegend
 CCR4 PE-Cy7 L291H4 41513577 BioLegend

Sorting in animal experiments:

Antigen Color Clone # Manufacturer
 CXCR3 APC CXCR3-173 126512 BioLegend
 CCR6 BV421 29-2L17 129818 BioLegend
 CCR4 PE 2G12 131204 BioLegend

Hyperion

Antigen Conjugate Clone Lot # Manufacturer
 Smooth Muscle Actin 141Pr 1A4 23062566-20 Standard BioTools
 Collagen type I 169Tm Polyclonal 23072776-05 Standard BioTools
 E-cadherin 158Gd 24E10 23062546-20 Standard BioTools
 Histone H3 176Yb D1H2 23062686-28 Standard BioTools
 Vimentin 143Nd D21H3 23072881-11 Standard BioTools
 CD20 161Dy H1 23062479-14 Standard BioTools
 CD3 170Er Polyclonal, C-terminal 23051949-16 Standard BioTools
 CD4 156Gd EPR6855 23062206-01 Standard BioTools
 CD45RO 173Yb UCHL1 23093831-06 Standard BioTools
 CD68 159Tb KP1 2351671-02 Standard BioTools
 CD8a 162Dy C8/144B 23051981-17 Standard BioTools
 FoxP3 155Gd PCH101 23041446-20 Standard BioTools
 Pan-keratin 148Nd C11 23052171-31 Standard BioTools
 CD31 145Nd JC70A 1035700-3 Standard BioTools
 PNA1 152Sm MECA79-R C Standard BioTools

IHC

Antigen Clone Lot # Manufacturer
 CD4 4B12 6088902 Leica Biosystems
 CD8 C8/144B 20066516 DAKO
 CD31 JC70A 41513577 Abcam
 CCR6 EPR22259 GR3379673-8 Abcam
 CXCR3 1C6 B253149 BioLegend
 CD20 L26 200115013 Akoya
 CK AE1/AE3 GR3377091-6 Abcam
 PNAD MECA79 D110604-1 Novus

Validation

As the manufacturers' description in web sites.

<https://www.standardbio.com/products/antibody-and-panel-kit-selector/cytof-antibodies-and-panel-kits>

https://www.bio-rad-antibodies.com/flow-cytometry-antibodies.html?_gl=1*pxcth3*_gcl_au*MTM3Njc4ODcxMC4xNzQ4OTQzMjkw

<https://www.biolegend.com/en-gb/innate-signaling/products/antibodies>

<https://shop.leicabiosystems.com/ja-jp/ihc-ish/ihc-primary-antibodies/>
<https://www.agilent.com/ja-jp/dako-products>
<https://www.akoyabio.com/phenocycler/assays/antibodies/>
https://www.novusbio.com/?srsltid=AfmBOopVO84bVmowfrHeznj-Ba3jmgIBn1eQsQqli25hQ64_Yy7kuvpr
<https://www.bdbiosciences.com/enus/products/reagents/flow-cytometry-reagents/research-reagents/quality-and-reproducibility>
<https://www.cellsignal.jp/about-us/cst-antibody-validation-principles>

Eukaryotic cell lines

Policy information about [cell lines and Sex and Gender in Research](#)

Cell line source(s)	MCA205 murine fibrosarcoma cell line was originally created by Dr. Steve A Rosenberg at NCI and supplied through academic transfer. The B16 murine melanoma cell line was derived from a spontaneous melanoma in C57BL/6 mice and supplied through academic transfer.
Authentication	Cell line authentication was performed by verifying MHC haplotype (H-2b) and tumorigenic capacity in syngeneic mice.
Mycoplasma contamination	Cells were regularly tested and found negative for Mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	N/A

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	C57BL/6J (B6) mice were purchased from CLEA Japan (Tokyo, Japan).
Wild animals	N/A
Reporting on sex	Female
Field-collected samples	N/A
Ethics oversight	All animal experiments were approved by the Institutional Animal Care and Use Committee of Saitama Medical University (approval number 4081 and 4191), and conducted in accordance with institutional and national guidelines.

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	N/A
Study protocol	N/A
Data collection	Patient related data were collated from electronic health care records in accordance with ethical approval.
Outcomes	Disease free survival was determined according to clinicians and radiologists based on CT scans.

Plants

Seed stocks	N/A
Novel plant genotypes	N/A
Authentication	N/A

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

For human specimens:

To extract TILs, the collected tumors were sectioned into 2–3-mm pieces and incubated with Dulbecco's modified Eagle's medium supplemented with Dri Tissue & Tumor Dissociation Reagent (BD horizon: 661563; BD Biosciences, Franklin Lakes, NJ, USA) or with RPMI 1640 medium supplemented with Liberase TL Research Grade (05401020001; Roche, Basel, Switzerland) following the manufacturer's instructions. Erythrocytes were removed using a biotin anti-human CD235ab antibody (306617; BioLegend, San Diego, CA, USA) and streptavidin nanobeads (480016; BioLegend, San Diego, CA, USA) according to the manufacturer's instructions. Tissue dissociation and cell preparation were initiated immediately after surgical resection, and the procedure was advanced to the point where processing could be safely paused within the same day.

For mouse specimens:

Tumor tissues were digested in RPMI containing Liberase TL (25 µg/mL for MCA205; 100 µg/mL for B16) at 37 °C for 30 min with gentle inversion. Cell suspensions were passed through a 40-µm cell strainer, enriched by 40/80% Percoll density-gradient centrifugation, and used for mass cytometry analysis (Supplementary Fig. 11b). The monoclonal antibodies used for mass cytometry analysis are listed in Supplementary Table 7.

Instrument

SH800Z (for sorting) from SONY, Helios (for analyzing) from Standard BioTools were used for data collection.

Software

FlowJo (10.8.1) and software on Cytobank was used for analysis.

Cell population abundance

A sufficient number of cells was obtained for downstream analyses. The purity of the cell population after cell sorting was greater than 90%.

Gating strategy

mass cytometry for human specimens

First, cell events were identified based on 140Ce and 193Ir signals to exclude calibration beads and debris. Doublets were excluded based on DNA content using 193Ir. On the singlet population, live cells were identified by excluding 198Pt-positive cells. CD8+ T cells as CD45+CD19-CD3+CD8+, CD4+ T cells as CD45+CD19-CD3+CD4+, Tex cells as CD45+CD19-CD3+CD8+GZMB+GZMK+, Tpex cells as CD45+CD19-CD3+CD8+GZMB+GZMK+, CTL cells as CD45+CD19-CD3+CD8+GZMB+GZMK-, Th1 cells as CD45+CD19-CD3+CD4+CD62LlowCXCR3+CCR4-CCR6-, Th7R cells as CD45+CD19-CD3+CD4+CD62LlowCXCR3±CCR4-CCR6+, Th17 cells as CD45+CD19-CD3+CD4+CD62LlowCXCR3-CCR4+CCR6+.

mass cytometry for mouse specimens

First, cell events were identified based on 140Ce and 193Ir signals to exclude calibration beads and debris. Doublets were excluded based on DNA content using 193Ir. On the singlet population, live cells were identified by excluding 198Pt-positive cells. CD8+ T cells as CD45+CD19-CD3+CD8+, CD4+ T cells as CD45+CD19-CD3+CD4+, Tex cells as CD45+CD19-CD3+CD8+PD-1+TIM3+, Tpex cells as CD45+CD19-CD3+CD8+PD-1+IL7R+ or CD45+CD19-CD3+CD8+PD-1+TCF1+, CTL cells as CD45+CD19-CD3+CD8+PD-1-GZMB+, Th1 cells as CD45+CD19-CD3+CD4+CXCR3+CCR4-CCR6-, Th7R cells as CD45+CD19-CD3+CD4+CXCR3±CCR4-CCR6+, Th17 cells as CD45+CD19-CD3+CD4+CXCR3-CCR4+CCR6+.

sorting

For cell sorting, we gated based on physical parameters (FSC-A vs SSC-A). Th1 cells were sorted as CXCR3+CCR4-CCR6- cells, and Th7R cells were sorted as CCR4-CCR6+ cells. Sorted populations were collected for downstream analyses and ACT.

All representative gating strategies are shown in Supplementary Fig. 10 and 11.

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