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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 (*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	10x Genomics Cell Ranger (version 5.0.0) for single-cell sequencing. Agilent 2100 Bioanalyzer for FFPE RNA sequencing.
Data analysis	"DescTools"(version 0.99.47),"CopyKAT"(version 0.1.0),"Seurat"(version 3.2.0),"Cell Ranger"(version 5.0.0) and"CellChat "(version 1.1.3) were used. Detailed information is publically available and stated in the Supplementary Software.

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 scRNA-seq, scTCR-seq and bulk RNA-seq data of this study have been deposited to GSA for Human (https://ngdc.cncb.ac.cn/gsa-human/) with an accession number of HRA003591 (https://ngdc.cncb.ac.cn/gsa-human/browse/HRA003591) for single cell sequencing data and HRA007035 (https://ngdc.cncb.ac.cn/gsa-human/browse/HRA007035) for FFPE RNA sequencing data, respectively. The data is available under restricted access and will be provided for scientific research

upon request complying with the law due to human patient privacy concerns. Readers can obtain access to the data via initiating application requests through the OMIX system of GSA for Human. Other relevant data employed for data processing are available in Source Data. Requests for access to the patient-level data from this study can be submitted via email to the corresponding author with a detailed proposal for approval. And we will respond to requests for the data within seven days. Once access has been granted, the data can be download within two weeks.

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	Analyses performed in this study are based on therapeutic efficacy and treatment timepoints. Gender as a biological variable was found without prominent association with immunotherapy efficacy in previous studies, and thus sex- or gender-based analyses are not involved.
Reporting on race, ethnicity, or other socially relevant groupings	Analyses performed in this study are based on therapeutic efficacy and treatment timepoints. Race, ethnicity, or other socially relevant groupings are not involved.
Population characteristics	Detailed information of population characteristics is provided in Supplementary Data 1 and 5.
Recruitment	A total of 23 fresh tumor specimens (7 pre-treatment biopsy samples & 16 post-treatment surgery samples) from 18 locally advanced ESCC patients, who received neoadjuvant immunochemotherapy followed by esophagectomy at Cancer Hospital, Chinese Academy of Medical Sciences from November, 2020 to July, 2023, were collected and enrolled in subsequent scRNA-seq combined scTCR-seq analyses.
Ethics oversight	This study was approved by the Ethical Committee of the National Cancer Center/Cancer Hospital, Chinese Academy of Medical Sciences (NCC3916) and compiled with all relevant ethical regulations. All patients were provided written informed consent for collecting tissue samples for research.

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	There were 6 pre-treatment biopsy samples and 17 post-treatment surgical samples from 18 ESCC patients enrolled in single-cell analysis. Additionally, pre-treatment biopsy FFPE samples and post-treatment surgical FFPE samples from 96 ESCC patients were collected for validation analysis.
Data exclusions	Cells with low quality were removed for analysis if they met the following criteria: 1) < 200 genes; 2) expressed gene counts < 3; 3) > 10% UMIs derived from the mitochondrial genome.
Replication	Animal experiments were carried out to explore the role of CXCL13 in anti-PD-1 therapy. 5 repeats were conducted in each group, and all attempts at replication were successful.
Randomization	Mice were randomly divided into 4 groups, which were designed as distinct treatment strategy.
Blinding	Blinding was not applicable to this study.

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

For human tissue multiplex immunofluorescence, anti-CXCL13 (1:200, Abcam, Catalogue# ab246518, Clone: EPR23400-92, Rabbit monoclonal); anti-CD8 (1:500, Servicebio, Catalogue# GB12068, Clone: 5C11B7-2, Mouse monoclonal); anti-LRRC15 (1:200, Abcam, Catalogue# ab150376, Clone: EPR8188(2), Rabbit monoclonal); anti-α-SMA (1:2000, Abcam, Catalogue# ab124964, Clone: EPR5368, Rabbit monoclonal); anti-CD4 (1:500, Abcam, Catalogue# ab133616, Clone: EPR6855, Rabbit monoclonal); anti-FOXP3 (1:50, CST, Catalogue# 98377S, Clone: D2W8E, Rabbit monoclonal).
For flow cytometry, anti-PD-1 (1:100, Biolegend, Catalogue# 135210, Clone: 29F.1A12, Mouse monoclonal), anti-CD20 (1:100, Biolegend, Catalogue# 150422, Clone: SA275A11, Mouse monoclonal), anti-CD3 (1:100, Biolegend, Catalogue# 100203, Clone: 17A2, Mouse monoclonal), and anti-CD8 (1:100, Biolegend, Catalogue# 126608, Clone: YTS156.7.7, Mouse monoclonal).
For animal tissue multiplex immunofluorescence, anti-CD4 (1:1000, Abcam, Catalogue# ab288724, Clone: RM1013, Rabbit multiclonal) and anti-CD8 (1:2000, Abcam, Catalogue# ab217344, Clone: EPR21769, Rabbit monoclonal).

Validation

Validations of primary antibodies were determined by manufacturers. The antibodies have been verified to be used on human and mouse samples, and have been quality tested to be used on flow cytometry and immunohistochemistry. The relevant information was available on the manufacturer's websites: https://www.abcam.cn/, https://servicebio.com/, https://www.biolegend.com/, https://www.cellsignal.cn/.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)

The mouse ESCC cell line (mEC25) was obtained from the Guangdong Provincial Key Laboratory of Regional Immunity and Diseases, Department of Pharmacology, and the Shenzhen University International Cancer Center, Shenzhen University Health Science Center.

Authentication

The cell line we used in this study was originally constructed and provided by the the Guangdong Provincial Key Laboratory of Regional Immunity and Diseases, Department of Pharmacology, and the Shenzhen University International Cancer Center, Shenzhen University Health Science Center, and they have performed the authentication of this cell line.

Mycoplasma contamination

The cell line tested negative for mycoplasma contamination.

Commonly misidentified lines
(See ICLAC register)

Not applicable.

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

All animals were C57BL/6 male mice, aged 5-6 weeks obtained from Changchun YISI experimental animal technology company and acclimated for seven days in our animal facility before experimentation. Mice were maintained in pathogen-free cages with adequate food and water supply, as well as appropriate and sufficient nesting and bedding materials. The housing conditions for the mice are as the following: Lighting - rodent rooms is 12:12, light:dark (7 am – 7 pm)
Room Temp = 68 – 72*F Relative Humidity = 30-70

Wild animals

No wild animals were used in the study.

Reporting on sex

As this study focused on immune features of different treatment response to neoadjuvant immunochemotherapy in esophageal squamous cell carcinoma and the majority was male patients, sex as a biological variable was not considered in the study design and only male mice were used in experiments.

Field-collected samples

No field collected samples were used in the study.

Ethics oversight

All mice were maintained in pathogen-free facilities and used strictly in accordance with the protocols approved by the Institutional Animal Care and Use Committee of Cancer Hospital, Chinese Academy of Medical Sciences. After eight injections of treatment, the mice were euthanized by cervical dislocation. No tumor reached 2000 mm3 in volume (this animal license limit was not exceeded).

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

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, mosiacism, 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	Tumors were harvested as mice sacrificed, and then tumors were digested into single-cell suspensions for flow cytometry analysis.
Instrument	BD Accuri C6 (BD Biosciences).
Software	BD Accuri C6 Software and FlowJo V10.
Cell population abundance	Over 10000 cells were analyzed for fluorescent intensity in the defined gate.
Gating strategy	A gate was drawn around the cells. Single cells were determined with the area and the height of the side scatter (SSC). The analysis was carried out in this gate.

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