

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	Flow-cytometry data were acquired on a FACSaria flow cytometer (BD Biosciences), and multispectral fluorescence images were acquired using a Vectra Polaris Automated Quantitative Pathology Imaging System (Akoya Biosciences).
Data analysis	Single-cell data was first processed using Cell Ranger 6.1.0 and then analysis using Scanpy 1.9.1 in python 3.9.13. WES data was processed using Fastp 0.23.0, BWA 0.7.15, GATK 4.2.2.0. Statistical was conducted in R 4.1.3. Other tools or packages we used: Samtools 1.8, vcf2maf 1.6.21, Maftools 2.10.5, CNVkit 0.9.10, Sequenza 2.1.2, PyClone 0.13.1, NetMHCpan 4.1, OptiType 1.3.5, ANNOVAR (2020-06-07 version), Scrublet 0.2.3, Harmony 0.0.6, DESeq2, inferCNV 1.10.1, CellChat 2.1.1, Seurat 4.1.1, Kraken 2.1.2, ClusterProfiler 4.2.2, COSMIC mutational signatures v3.0, VEP 104.3. Flow-cytometry data were analysed using FlowJo v10, and multiplex immunofluorescence images were spectrally unmixed and quantified using inForm v2.4. Custom code is publicly available on GitHub (https://github.com/songmingl/esc-codes), and archived on Zenodo at https://doi.org/10.5281/zenodo.21559297 .

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](#)

Processed scRNA-seq data are publicly available in the Gene Expression Omnibus under accession GSE314906. Raw transcriptomic sequencing data have been deposited in GSA-Human under accession HRA011005. Access to the raw FASTQ files is controlled to protect participant privacy and comply with Chinese regulations on human genetic resources. Qualified researchers may request access for non-commercial scientific research through GSA-Human or by contacting the corresponding author. 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

Sex information was available for 40 patients (36 male and 4 female). Sex was not considered as a design variable, and sex-stratified analyses were not performed because the study was not designed to evaluate sex-specific effects.

Reporting on race, ethnicity, or other socially relevant groupings

All the participants involved in this study were of the Chinese population. This study did not analyze race, ethnicity, or other socially relevant categorization variables.

Population characteristics

A total of 63 patients with ESCC were enrolled, and finally, we conducted scRNA-seq from 52 ESCC patients. Age was available for 39 patients, with a median age of 62 years (range, 47–72 years). Of these patients, 36 received neoadjuvant chemotherapy plus camrelizumab, 11 received neoadjuvant chemotherapy alone, and treatment information was unavailable for five patients. Patients were categorized based on surgical evaluations: non-responders (NR, n = 16), who were characterized post-treatment with Mandard grades 3, 4, and 5, and responders (R, n = 25), who achieved Mandard grades 1 and 2; 11 patients were not evaluable for pathological response.

Recruitment

63 treatment-naïve patients with pathologically confirmed ESCC were prospectively enrolled at the Cancer Hospital. Tumors were classified according to WHO criteria, and clinical stage was assigned using the UICC/AJCC TNM system (8th edition). All patients were treated with platinum-based doublet chemotherapy; in the chemo-immunotherapy arm this backbone was combined with camrelizumab, whereas the comparator arm received chemotherapy alone.

Ethics oversight

The study protocol, including all amendments, was approved by the Institutional Review Board and Independent Ethics Committee of the National Cancer Center, Cancer Hospital, Chinese Academy of Medical Sciences (NCC2021C-421). The study adhered to the Declaration of Helsinki and international standards of good clinical practice.

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

We prospectively screened treatment-naïve ESCC patients who underwent neoadjuvant therapy between May 2021 and July 2022 at the Cancer Hospital, Chinese Academy of Medical Sciences, Beijing, China. 63 ESCC patients were enrolled, and finally, we conducted scRNA-seq on cells isolated from 81 samples collected from 52 ESCC patients. Additionally, WES was performed on 53 samples from 45 patients, including 45 pre-treatment and 8 post-treatment tumor samples. Thirty-four of these patients also had scRNA-seq data, contributing 39 WES tumor-normal pairs.

Data exclusions

No participants were excluded after enrolment solely on the basis of study outcome. For individual analyses, samples were excluded according to prespecified data-availability and quality-control criteria, including missing clinical annotations, unavailable paired samples and minimum cell-count thresholds.

Replication

Human sequencing analyses were performed once on each independent biological sample. Flow-cytometry and in vitro functional experiments were independently repeated as specified in the corresponding figure legends. Animal experiments used independent mice as biological replicates; exact sample sizes are reported in the figure legends and Source Data.

Randomization	For murine in vivo tumor experiments, mice were randomly allocated to distinct treatment cohorts subsequent to the inoculation of tumor cell lines or the commencement of treatment to mitigate selection biases.
Blinding	Blinding was not applicable in our investigation since samples were procured from patients upon clinic admission. Investigators conducting data collection and analysis were not blinded to the experimental conditions.

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	Antibodies for our IHC validation experiments: CX3CR1 (1:200; Abcam, ab8020, rabbit polyclonal), CD8 (1:2000; Abcam, ab237709, clone CAL66), GZMB (1:300; Abcam, ab255598, clone EPR22645-206), CD107a (1:4000; Abcam, ab320851, clone RM1217) and CXCL13 (1:1000; Abcam, ab246518, clone EPR23400-92). For flow cytometry sorting, cells were stained with fluorochrome-conjugated monoclonal antibodies, including anti-CCR7 (1:100; Biolegend, 353216, clone G043H7), anti-CD45RA (1:100; Biolegend, 304108, clone HI100), anti-CX3CR1 (1:100; Biolegend, 341620, clone 2A9-1), and anti-CD8 (1:100; Biolegend, 344722, clone SK1).
Validation	All antibodies are commercially available and have previously been used for the respective purposes. Validation statements can be found on the manufacturer's websites.

Eukaryotic cell lines

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

Cell line source(s)	We have revised the Methods and Reporting Summary to specify the names and sources of all cell lines. KYSE150 cells were kindly provided by Dr Yutaka Shimada (Kyoto University, Japan), and AKR cells were kindly provided by Dr Anil Rustgi (Columbia University, USA). AKR-OVA cells were generated from AKR cells in this study.
Authentication	KYSE150 and AKR cells were authenticated by short tandem repeat profiling and were confirmed to be free of mycoplasma contamination.
Mycoplasma contamination	All the cell lines were negative for mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	No

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	Female 6-8 weeks C57BL/6J were obtained from Vital River Laboratory Animal Technology (Beijing, China). Rag1-knockout mice (C57BL/6J-Cya-Rag1em1/Cya; S-KO-03999) were purchased from Cyagen Biosciences (Taicang, China), and OT-I mice (C57BL/6Smoc-Igs2em1(CD2-TCRa(OT-I)-CD2-TCRb(OT-I))Smoc; NM-KI-241328) were purchased from Shanghai Model Organisms Center (Shanghai, China). Mice were maintained under specific-pathogen-free conditions in a temperature-controlled facility maintained at 22±2°C and 50–60% humidity, under a 12-h light and 12-h dark cycle, with ad libitum access to food and water. Animals were acclimatised for at least 5 days before experimentation.
Wild animals	No wild animals involved in this study.
Reporting on sex	As the study focuses on immune microenvironment changes during ICB treatment in ESCC, sex- and gender-based analyses were not performed.

Field-collected samples	No field-collected samples were used in this study.
Ethics oversight	All animal experiments were approved by the Institutional Animal Care and Use Committee of Chinese Academy of Medical Sciences Cancer Hospital (NCC2021A222).

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

Plants

Seed stocks	Not involved
Novel plant genotypes	Not involved
Authentication	Not involved

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	PBMC from healthy donors were purchased from Milestone® Biotechnologies and primary CD8+ T cells were purified by negative selection using Human CD8+ T cell Enrichment kit (STEMCELL Technologies) according to the manufacturer's protocol. Next, cells were washed with 1xPBS and resuspended in 100ul PBS buffer with eBioscience™ Fixable Viability Dye eFluor™ 506, after 15min of incubation cells were washed with FACs buffer consisted of PBS supplemented with 2% FBS with 2 mM EDTA. Mouse tumor tissue was dissociated into single-cell suspensions with the Mouse Tumor Dissociation Kit (Miltenyi Biotec, Catalog #130-096-730) combined with the gentleMACS™ Dissociator (Miltenyi Biotec, Catalog #130-093-235). Total mouse T cells were isolated from splenocyte single-cell suspensions using the immunomagnetic negative selection method with the EasySep™ Mouse T Cell Isolation Kit (Stemcell Technologies, Catalog #19851). Blood samples were treated with BD Pharm Lyse™ Lysing Buffer (Catalog #555899) for red blood cell removal.
Instrument	FACSAria (BD Biosciences)
Software	FlowJo software v10
Cell population abundance	Abundance of the cell population in the sorted samples are reported in Fig.6 and Supplementary Fig. 9a-e.
Gating strategy	Simplified key steps: total cells → debris/dead exclusion → doublet removal → viability gating → CD3+ CD8+ selection → final CX3CR1 and OVA tetramer co-expression analysis Detailed description: After acquiring tumor tissue single-cell suspension, cells are first visualized on an SSC/FSC plot, where the right population (intact cells) is gated to exclude debris/dead cells (left population). Following singlet discrimination via FSC-A/FSC-H analysis, viability staining distinguishes live (dye-negative) from dead (dye-positive) cells. The live population is then analyzed for CD8 expression, with CD8+ cells selected for subsequent two-dimensional analysis of CX3CR1 and OVA tetramer co-expression. All gates are defined using single-stained and negative controls to establish thresholds and ensure specificity.

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