

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	DIVA 8.0.1
Data analysis	GraphPad Prism 10.2.3; FlowJo version 10.8.1, "TockyAnalysis" package in R (v 3.6.3)

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](#)

Bulk RNA sequencing data and gene counts generated in this study have been deposited in the Gene Expression Omnibus (GEO) under accession number GSE267158 [<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE267158>]. Raw bulk TCR sequencing data generated in this study have been deposited in the NCBI sequence read archive (SRA) under accession code PRJNA1132229 [<http://www.ncbi.nlm.nih.gov/bioproject/1132229>]. Additionally, TCR beta repertoires and the corresponding sample sheet are available using <https://doi.org/10.6084/m9.figshare.25795993.v1>. Previously published human HNSCC single-cell RNAseq

datasets used in this study are available through GEO with accession numbers GSE164690 [<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE164690>] and GSE139324 [<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE139324>]. Normalised expression and clinical data for the TCGA Head and Neck squamous cell carcinoma (HNSC) cohort were downloaded from the Firebrowse website [<http://firebrowse.org/>] version 2016012800.0.0. 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	N/A
Reporting on race, ethnicity, or other socially relevant groupings	N/A
Population characteristics	N/A
Recruitment	N/A
Ethics oversight	N/A

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	Sample sizes used a minimum of 3 mice per group (only 3-4 mice for RNA and TCR sequencing). Sample sizes were based from previous work in the lab in order to observe statistical significance, Patin EC et al., Journal for Immunotherapy of Cancer, 2022; Dillon MT et al., Clinical Cancer Research, 2018.
Data exclusions	Significant outliers were excluded using the Grubb's test with a predetermined significance level of 0.05.
Replication	Due to the multiplicity of preclinical models, the majority of experiments were performed once. However a substantial amount of experiments in the MOC1 model are cumulative of two to three independent experiments except for figures 2A, RNA sequencing in figure 3, Figures 3J, 3K, 4C, 5B, 5C, 5D, 5E, 5F and TCR sequencing in figure 7.
Randomization	Mice were randomised according to tumour sizes to obtain approximately same average tumour sizes between groups before the beginning of treatment regimens.
Blinding	Tumour measurements were blinded whenever possible due to technician availability. RNA, TCR sequencing and flow cytometry experiments were not blinded due to technician skills and availability.

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

Catalogue and lot numbers can be found in the supplementary information

Anti-mouse B220 BUV395 (BD Biosciences, clone RA3-6B2, 1/200)
 Anti-mouse CD107a BUV395 (BD Biosciences, clone 1D4B, 1/100)
 Anti-mouse CD107a PE (Biolegend, clone 1D4B, 1/200)
 Anti-mouse CD44 BUV395 (BD Biosciences, clone IM7, 1/100)
 Anti-mouse CD44 Pacific Blue (Biolegend, clone IM7, 1/100)
 Anti-mouse CD25 BUV395 (BD Biosciences, clone PC61, 1/100)
 Anti-mouse CD25 BV650 (Biolegend, clone PC61, 1/100)
 Anti-mouse CD25 APC (Biolegend, clone PC61, 1/100)
 Anti-mouse CD4 BUV496 (BD Biosciences, clone RM4-4, 1C/100)
 Anti-mouse CD4 PerCp (Biolegend, clone RM4-5, 1/100)
 Anti-mouse MHCII BUV496 (BD Biosciences, clone M5/114.15.2, 1/200)
 Anti-mouse MHCII PE-Dazzle 594 (Biolegend, clone M5/114.15.2, 1/300)
 Anti-mouse MHCII AF700 (Biolegend, clone M5/114.15.2, 1/300)
 Anti-mouse CD3 BUV563 (BD Biosciences, clone 17A2, 1/100)
 Anti-mouse CD3 BUV737 (BD Biosciences, clone 17A2, 1/100)
 Anti-mouse CD3 BV650 (Biolegend, clone 17A2, 1/100)
 Anti-mouse CD3 AF700 (Biolegend, clone 17A2, 1/100)
 Anti-mouse CD3 PerCp Cy5.5 (Biolegend, clone 17A2, 1/100)
 Anti-mouse TCR δ BUV615 (BD Biosciences, clone GL3, 1/100)
 Anti-mouse CD11c BUV615 (BD Biosciences, clone N418, 1/100)
 Anti-mouse CD11c BV750 (Biolegend, clone N418, 1/100)
 Anti-mouse CD11c PerCp Cy5.5 (Biolegend, clone N418, 1/100)
 Anti-mouse PD-1 Pe-Cy7 (Biolegend, clone 29F.1A12, 1/200)
 Anti-mouse PD-1 BV605 (Biolegend, clone 29F.1A12, 1/200)
 Anti-mouse CD103 BUV615 (BD Biosciences, clone 2E7, 1/100)
 Anti-mouse CD103 BV711 (Biolegend, clone 2E7, 1/100)
 Anti-mouse CD103 BV785 (Biolegend, clone 2E7, 1/100)
 Anti-mouse CD103 BV510 (Biolegend, clone 2E7, 1/100)
 Anti-mouse CD69 BUV661 (BD Biosciences, clone H1.2F3, 1/100)
 Anti-mouse CD69 BV785 (Biolegend, clone H1.2F3, 1/100)
 Anti-mouse CD69 PE-Dazzle 594 (Biolegend, clone H1.2F3, 1/100)
 Anti-mouse CD19 BUV661 (BD Biosciences, 1D3, 1/200)
 Anti-mouse CD19 APC (Biolegend, clone 6D5, 1/200)
 Anti-mouse NK1.1 BUV737 (BD Biosciences, clone PK136, 1/100)
 Anti-mouse NK1.1 BV421 (Biolegend, clone PK136, 1/100)
 Anti-mouse TCR β BUV737 (BD Biosciences, clone H57-597, 1/100)
 Anti-mouse CD45 BUV805 (BD Biosciences, clone 30-F11, 1/300)
 Anti-mouse F4/80 Pacific Blue (Biolegend, clone BM8, 1/100)
 Anti-mouse FoxP3 eFluor 450 (ThermoFisher Scientific, clone FJK-16s, 1/100)
 Anti-mouse NKG2A Pacific Blue (BD Biosciences, clone 20D5, 1/100)
 Anti-mouse NKG2A PE (Biolegend, clone 16A11, 1/100)
 Anti-mouse NKG2A Pe-Cy7 (Biolegend, clone 16A11, 1/100)
 Anti-mouse PD-L1 BV421 (Biolegend, clone MIH5, 1/100)
 Anti-mouse PD-L1 PerCp Cy5.5 (Biolegend, clone 10F.9G2, 1/100)
 Anti-mouse Ly6G BV510 (Biolegend, clone 1A8, 1/200)
 Anti-mouse CD62L BV510 (Biolegend, clone MEL-14, 1/100)
 Anti-mouse CD62L Pe-Cy5 (Biolegend, clone MEL-14, 1/300)
 Anti-mouse CD62L PE-Dazzle 594 (Biolegend, clone MEL-14, 1/300)
 Anti-mouse CD62L PerCp Cy5.5 (Biolegend, clone MEL-14, 1/100)
 Anti-mouse CD62L AF700 (Biolegend, clone MEL-14, 1/100)
 Anti-mouse CD8 α BV570 (Biolegend, clone 53.-6.7, 1/200)
 Anti-mouse CD8 α BV711 (Biolegend, clone 53.-6.7, 1/200)
 Anti-mouse CD8 α BV785 (Biolegend, clone 53.-6.7, 1/200)
 Anti-mouse CD27 BV650 (Biolegend, clone LG.3410, 1/100)
 Anti-mouse CD27 PE-Dazzle 594 (Biolegend, clone LG.3410, 1/100)
 Anti-mouse CD27 PerCp Cy5.5 (Biolegend, clone LG.3410, 1/100)

Anti-mouse CD27 AF700 (Thermofisher Scientific, clone LG-7F9, 1/100)
 Anti-mouse TIM-3 BV711 (Biolegend, clone RMT3-23, 1/100)
 Anti-mouse TIM-3 APC (Biolegend, clone RMT3-23, 1/100)
 Anti-mouse ICOS BV711 (Biolegend, clone C398.4A, 1/100)
 Anti-mouse ICOS BV750 (Biolegend, clone C398.4A, 1/100)
 Anti-mouse CD11b BV750 (Biolegend, clone M1/70, 1/200)
 Anti-mouse Ly6C APC (Biolegend, clone HK1.4, 1/200)
 Anti-mouse Ly6C AF700 (Biolegend, clone HK1.4, 1/200)
 Anti-mouse Ly6C BV785 (Biolegend, clone HK1.4, 1/200)
 Anti-mouse Qa-1b PE (BD Biosciences, clone 6F10.1A6, 1/100)
 Anti-mouse Ki67 BV786 (BD Biosciences, clone B56, 1/100)
 Anti-mouse Ki67 AF488 (Biolegend, clone 16A8, 1/100)
 Anti-mouse Ki67 PE (Biolegend, clone 16A8, 1/100)
 Anti-mouse Ki67 PE-Dazzle 594 (Biolegend, clone 16A8, 1/100)
 Anti-mouse NKG2D PE (Biolegend, clone CX5, 1/100)
 Anti-mouse NKG2D PE-Dazzle 594 (Biolegend, clone CX5, 1/100)
 Anti-mouse CD28 PE (Biolegend, clone 37.51, 1/100)
 Anti-mouse CD28 PE-Dazzle 594 (Biolegend, clone 37.51, 1/100)
 Anti-mouse CD28 PerCp Cy5.5 (Biolegend, clone 37.51, 1/100)
 Anti-mouse Perforin PE (Biolegend, clone S16009B, 1/100)
 Anti-mouse Granzyme B AF647 (Biolegend, clone GB11, 1/100)
 Anti-mouse CD40L APC (Biolegend, clone SA047C3, 1/100)
 Anti-mouse CD40 Pe-Cy7 (Biolegend, clone 3/23, 1/100)
 Anti-mouse CD40 PE-Dazzle 594 (Biolegend, clone 3/23, 1/100)
 Anti-mouse T-bet Pe-Cy7 (Thermofisher Scientific, clone 4B10, 1/100)
 Anti-mouse EpCAM FITC (Biolegend, clone G8.8, 1/100)

Validation

Validation for all antibodies is available on provider websites. All antibodies were acquired from commercial sources.

Eukaryotic cell lines

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

Cell line source(s)

The C57BL/6 mouse cancer cell line MOC1 and MOC2 was kindly provided by Ravindra Uppaluri (Dana-Farber Cancer Institute). The C57BL/6 mouse cancer cell line mEER was kindly provided by Paola Vermeer (Sanford Research). The C57BL/6 mouse cancer cell line TC-1, a modified mouse lung epithelial cell line transformed with HPV-16 E6 and E7 and oncogenic HRAS, were a kind gift from Prof. Tzyy-Chou Wu (Johns Hopkins University, Baltimore, MD) and Prof. Eric Deutsch (Institut Gustave Roussy, Villejuif, France).

Authentication

Cell lines used were not authenticated beyond obtaining them from collaborators.

Mycoplasma contamination

Cell lines were checked for Mycoplasma contamination and confirmed as negative

Commonly misidentified lines
(See [ICLAC](#) register)

No commonly misidentified lines were used

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

WT C57BL/6 mice were purchased from Charles River, UK. Nr4a3- Tocky: Foxp3- EGFP mice were obtained from Masahiro Ono.

Wild animals

No wild animals were used in this study.

Reporting on sex

Only female mice were used in the study to adhere to the 3Rs guiding principles and also to ensure reproducibility of our tumour growth.

Field-collected samples

No field samples were collected

Ethics oversight

All animals were handled according to the Institute and U.K. Home Office guidelines.

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

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	Harvested tumours were excised, finely minced, and enzymatically digested for 30-45 min at 37 °C in PBS containing 1 mg/ml collagenase type VI (Sigma-Aldrich) 1 mg/ml DNase type I (Roche), 100 ng/ml Dispase (Sigma-Aldrich) and Trypsin. Digested tumours were smashed and filtered through 70 µm-pore cell strainers. Cells were centrifuged at 1400 rpm at 4 °C for 10 min and pellets washed in PBS/2% FCS/5mM EDTA. Harvested spleen were mechanically dissociated, smashed and filtered through 70 µm-pore cell strainers. Red blood cells were lysed by a 1 min incubation in ACK lysis buffer (ThermoFisher Scientific). Cells were centrifuged at 1400 rpm at 4 °C for 10 min and pellets washed in PBS/2% FCS. For some experiments, mice were bled 20 µL from the tail vein. PBMCs from human blood samples were isolated on a density gradient medium (Lymphoprep, STEMCELL Technologies). Cells were incubated 4 °C for 10 min with anti-mouse or anti-human CD16/CD32 (BD Pharmingen) prior to surface staining, 4 °C for 30 min. Dead cells were excluded using the Fixable Viability Dye eFluor™ 780 (ThermoFisher Scientific). The complete list of antibodies used in this study can be found in the supplementary information. Intracellular detection of Ki67, FoxP3 and T-BET was performed using The Foxp3 Transcription Factor Staining Buffer Set (ThermoFisher Scientific) following the manufacturer's instructions. For surface detection of CD107a, cells were incubated in complete IMDM containing Golgi Plug/Golgi Stop (BD Biosciences) in the presence of a stimulation cocktail containing PMA and Ionomycin (Invitrogen) and mouse CD107a antibody for 4 h at 37 °C. Following surface antibody staining, cells were fixed using IC Fixation Buffer (Invitrogen), permeabilized in Permeabilization Buffer (Invitrogen), and stained with mouse IFN-γ antibody. Cell cycle position analyses were performed using the BD Pharmingen™ BrdU Flow Kits (BD Biosciences) following the manufacturer's instructions. Samples were analysed on a LSR II or FACSymphony A5 (BD Biosciences) and FACS analyses were performed using the FlowJo version 10 software. Tocky timer data was analysed using the "TockyAnalysis" package in R (v 3.6.3).
Instrument	BD Symphony A5 and BD LSR II
Software	DIVA 8.0.1, FlowJo version 10.8.1, "TockyAnalysis" package in R (v 3.6.3)
Cell population abundance	Cell population abundance were determined using counting beads and normalised to tumour weight.
Gating strategy	Gating is included in Supplementary Figure S14.

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