nature portfolio

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# 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 (*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<br>*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<br>*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 | Flow cytometry data was recorded using BDFACSDiva (v8.0) software<br>No new bioinformatics tools or algorithms were created |
| Data analysis | Flow cytometry data was analyzed using Flowjo (v10.4.2) or OMIQ (Dotmatics).<br>Single cell RNA sequencing data was analyzed using CellRanger multi (version 6.0.0), Seurat (v4.0.6), UCell (v.1.3), Monocle3 (v.1.0.0), SingleR, CellChat, decontX, SingleR. Bulk data was analysed with consensusTME, ssGSEA<br>Data was visualized using Graphpad (V8.4.1, Prism software), GGplot2(v3.3.5) and ggpubr(v0.5.0).<br>Imaging was analyzed with Imaris (Bitplane – version 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 mouse scRNAseq data generated in this study have been deposited in the GEO database under accession code GSE260972. Datasets retrieved from 10X Genomics are licensed under the Creative Commons Attribution license. All data are included in the Supplemental Information or available from the authors upon reasonable requests, 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 | 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

# Life sciences study design

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

| | |
|---|---|
| Sample size | Sample size was estimated based on prior experience and complexity. Whenever possible, we used g*power to calculate sample size. Sample size was computed to detect difference of 50% in a treated group with a standard deviation of 20%, false positive of 0.05, and power of 0.80. Sample size was therefore calculated to be between 7-8. Where smaller or bigger changes were expected, sample sizes were increased accordingly. |
| Data exclusions | no data exclusion |
| Replication | All findings were replicated in two to four separate experiments (as specified in legends), with the exception of the single cell RNA sequencing experiment, in which data was aggregated from multiple separate biological samples as indicated in the figure legends and methods section. All separate experiments yielded comparable trends and results. |
| Randomization | For mouse experiments involving multiple genotypes, we used littermates, and controlled that gender and age was similar between all groups, but other than this, allocation was random.<br>Whenever possible, comparison was done between populations within the same mouse, for which randomization is not required.<br>For re-analysis of human data, we used the assignments of clusters from the original study. |
| Blinding | No blinding was performed during mouse experiments when all mice within an experiment received identical treatments. No subjective scoring methods which would require blinding were used. Most experiments were performed and repeated independently by two researchers.<br>Flow cytometry data were collected in an automatic and unbiased manner. |

# 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

Staining

| Conjugate | Antigen | Clone | Comapny | Catalog # | dilution |
|---|---|---|---|---|---|
| Spark387 | CD11c | 53-6.7 | Biolegend | 100798 | 400 |
| BUV615 | B220 | RA3-6B2 | eBioscience | 366-0452-82 | 200 |
| BUV661 | CD4 | RM4-5 | eBioscience | 376-0042-82 | 200 |
| BUV737 | CD44 | IM7 | eBioscience | 367-0441-82 | 400 |
| BUV805 | CD45 | 30-F11 | eBioscience | 368-0451-82 | 400 |
| BUV395 | CD8a | 53-6.7 | eBioscience | 363-0081-82 | 400 |
| BV421 | CD206 | C068C2 | Biolegend | 141717 | 200 |
| PB | Ly6G | 1A8 | Biolegend | 127612 | 800 |
| BV605 | CD86 | GL-1 | Biolegend | 105037 | 200 |
| BV650 | PD-L1 | 10F.9G2 | Biolegend | 124333 | 400 |
| BV711 | CD62L | MEL-14 | Biolegend | 121439 | 200 |
| BV785 | F4/80 | BM8 | Biolegend | 123115 | 200 |
| PerCP-Cy5 | PD-L1 | GK1.5 | Biolegend | 124334 | 400 |
| PerCP-Vio770 | CD68 | FA-11 | Miltenyi Biotech | 130-102-926 | 20 |
| PE | TREM2 | 237920 | R&D Systems | FAB17291P | 200 |
| PE-Cy7 | CD163 | S15049I | Biolegend | 155320 | 200 |
| APC | Ly6C | HK1.4 | Biolegend | 128015 | 400 |
| AF647 | NKp46 | 29A1.4 | Biolegend | 137628 | 100 |
| AF700 | MHC-II | M5/114.15.2 | Biolegend | 107621 | 400 |
| APC/Cy7 | CX3CR1 | SA011F11 | Biolegend | 149048 | 200 |
| APC-Fire810 | CD11b | M1/70 | Biolegend | 101288 | 400 |
| AF647 | KI67 | 16A8 | Biolegend | 652408 | 200 |
| AF488 | GFP | polyclonal | Invitrogen | A-21311 | 200 |
| eF450 | H2Db | 28-14-8 | Invitrogen | 48-5999-80 | 400 |
| AF700 | H2Kb | AF6-88.5 | Biolegend | 116521 | 200 |

Alexa Fluor 647- or BV421-conjugated, N4-specific MHC I tetramers (National Institutes of Health Tetramer Core Facility [Emory University, Atlanta]) 100

in vivo blocking
BioXCell, anti-CD8β Cat. BE0223, and anti-NK1.1 Cat. BE0036
50 µg antibodies every 2 to 3 days
Biolegend, anti-Ly6G (1A8, Cat. 127649) and Thermo Fisher anti-rat Kappa immunoglobulin (MAR18.5, Cat. I-2026)
25ug and 50ug combined antibodies every 2 ays

Validation

All antibodies were obtained from commercial vendors and we based specificity on descriptions and information provided in corresponding Data Sheets available and provided by the Manufacturers.
Dilution optimization was performed on splenocytes or immune cells isolated from tumours.

Biolegend - Flow Cytometry Reagents:-Specificity testing of 1-3 target cell types with either single- or multi-color analysis (including positive and negative cell types).-Once specificity is confirmed, each new lot must perform with similar intensity to the in-date reference lot. Brightness (MFI) is evaluated from both positive and negative populations.-Each lot product is validated by QC testing with a series of titration dilutions. https://www.biolegend.com/en-us/quality/quality-control

ebioscience/invitrogen: Part 1—Target specificity verification
This helps ensure the antibody will bind to the correct target. Our antibodies are being tested using at least one of the following methods to ensure proper functionality in researcher's experiments:
Knockout—expression testing using CRISPR-Cas9 cell models

Knockdown—expression testing using RNAi to knockdown gene of interest
Independent antibody verification (IAV)—measurement of target expression is performed using two differentially raised antibodies recognizing the same protein target
Cell treatment—detecting downstream events following cell treatment
Relative expression—using naturally occurring variable expression to confirm specificity
Neutralization—functional blocking of protein activity by antibody binding
Peptide array—using arrays to test reactivity against known protein modifications
SNAP-ChIP™—using SNAP-ChIP to test reactivity against known protein modifications
Immunoprecipitation-Mass Spectrometry (IP-MS)—testing using immunoprecipitation followed by mass spectrometry to identify antibody targets
Part 2—Functional application validation
These tests help ensure the antibody works in a particular application(s) of interest, which may include
(but are not limited to):
Western blotting
Flow cytometry
ChIP
Immunofluorescence imaging
Immunohistochemistry

Miltenyi: To validate the specificity of an antibody, a suitable counterstaining is performed, which verifies the target population. For this approach, the target gene is knocked out in a suitable cell line using site-specific nucleases and the knockout is confirmed by sequencing of the target locus. The antibody is considered to bind specifically to the intended epitope, if no antibody binding to the knockout cells can be detected. The antibody staining is controlled by fluorescence microscopy as well as flow cytometry. Knockdown of target antigens can be used to validate antibody specificity and their use in flow cytometry applications. In this approach, the target antigen is knocked down using RNA interference or RNAi. The translation of the target RNA is inhibited by transfecting cells with small non-coding RNA oligonucleotides. A comparison between the transfected cells with the control cells reveals the specificity of the tested antibody to its antigen.

R&D: All antibodies are tested for cross-reactivity with closely related molecules using a variety of applications, including direct ELISA, to ensure specificity. These efforts are facilitated by our extensive library of in-house developed antigens.

BioXCell: Advanced Binding Validation utilizes a library of recombinant proteins and bioassay expertise to validate that each lot of applicable InVivoPlusTM antibody binds strongly and specifically to its target antigen.

## Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

| | |
|---|---|
| Cell line source(s) | B16 Tyr-/- expresssing mCherry and Ovalbumin (B16OVA) and B16 expressing minOVA were provided by Ed Roberts, Beatson Institute, University of Glasgow. Those were infected with lentivirus expressing mCherry or ZsGreen, and/or KO for the IFNGR1, H2Db or H2Kb using CRISPR. |
| Authentication | Cell lines used were not authenticated. OVA expression of B16 melanoma cells has been confirmed using in vitro activation assays with transgenic T cells (OT-I respectively). mCherry and ZsGreen expression in B16 melanoma cells has been confirmed by microscopy. Tyrosine KO (Tyr-/-) inhibits melanin formation and could be confirmed macroscopically by looking at the colour of the B16. |
| Mycoplasma contamination | Cell lines have been regularly tested for Mycoplasma contamination and tested negative |
| Commonly misidentified lines (See ICLAC register) | No commonly misidentified cell lines were used in this study. |

## 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 | species: mus musculus,<br>strains: C57bl/6, GREAT, CD8 KO, CCR2 KO, OTI<br>sex: male and female<br>age: 6-14 week-old for in vivo experiments and 8- to 10-week-old for in vitro experiments |
| Wild animals | wild animals were not used in this study |
| Reporting on sex | Both genders were used in experiments. Males and Females were equally distributed throughout conditions. We did not initially find any difference between males and females and therefore did not take gender into account for analysis. |
| Field-collected samples | n/a |
| Ethics oversight | All experiments involving mice were conducted in agreement with the United Kingdom Animal Scientific Procedures Act of 1986 and |

performed in accordance with approved experimental procedures by the Home Office and the Local Ethics Reviews Committee (University of Oxford) under UK project licenses P4BEAEBB5 and PP3609558.

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 | See method section of manuscript. In some experiments, T cells were isolated from the lymph nodes of 6 to 12 weeks-old mice. In other experiments, tumours were dilacerated using scalpels to obtain <1mm sized pieces and resuspended in R10 supplemented with 1 mg/mL Liberase TL (Roche, Cat. 5401020001) and 10 µg/mL DNase I (Roche, Cat. 11284932001) for enzymatic digestion. Tumour suspensions were incubated at 37°C for 30 minutes before physical dissociation of remaining fragments through 70µm cell strainers to obtain single cell suspensions. |
| Instrument | FACSAria™ II (BD) was used for sorting and Fortessa X-20 or Aurora for analysis |
| Software | Data collection: BDFACSDiva (v8.0) software<br>Data analysis: FlowJo V.10 (BD), or OMIQ (Dotmatics). |
| Cell population abundance | Populations were sorted at >95% purity, determined by flow cytometric analysis of post-sort samples |
| Gating strategy | For myeloid cells, cells were sorted based on expression of CD45, and Cd11b. Different myeloid populations were then dissected using the markers Ly6C, Ly6G and MHC-II<br>For lymphoid cells, cells were sorted based on expression of CD45. Different lymphoid populations were then dissected using the markers CD3, CD4, CD8 and N4-Tetramer |

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