nature portfolio

Corresponding author(s): J. Silvio Gutkind (NI-A32832D)

Last updated by author(s): 5/2/2023

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 *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	No software was used to collect data in this study. All data analyzed in this study were publicly available, and analyzed with software described in the next section
Data analysis	For single cell RNA-seq integration, we used the Seurat scRNA-seq integration method. To account for technical differences between datasets, we used the standard Seurat v4.0.l integration workflow for batch-correction. Visualizations for data were performed through circlize v0.4.12, ComplexHeatmap v2.6.2, ggplot2 3.3.3, and Seurat 4.0.1. R version 4.0.5 was used for all analysis. Batch correction analysis of bulk RNA-seq analyses was through ComBat (v3). For differential expression analysis, DESeq2 version 1.32 was used. For gene set enrichment analysis, ClusterProfiler (v.4.6.2) was used. For data analysis and plot generation, GraphPad Prism version 9.2.0.

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 analyzed during the current study are available in the GEO repository with the following identifiers: GSE116256, GSE114727, GSE110686, GSE14018, GSE123813,GSE22898,GSE123139,GSE115978,GSE146771,GSE134520,GSE140228,GSE125449,GSE117570,GSE127465,GSE99254,GSE123813,GSE156728, GSE120575, GSE84820, GSE123235, GSE122969, GSE88987, GSE141299; the EGA archive with the following identifiers: EGAS00001002171, EGAS00001002486, EGAS00001002325 and EGAS00001002553.
This information is also included in Supplementary Table 1, and referenced in the Data Availability Statement in the manuscript.

Human research participants

Policy information about studies involving human research participants and Sex and Gender in Research.

Reporting on sex and gender	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 sizes were chosen based on the historical data of the variability of tumor growth and treatment response observed and determined to be adequate based on the consistency of measurable differences within and between groups. Additionally, our previous publication was used as a reference: DOI: 10.1038/s41467-019-13471-0
Data exclusions	No data were excluded from these analyses.
Replication	Every experiment was replicated at least twice with near-identical results. All attempts at replication were successful.
Randomization	Based on the tumor volumes on the first day of treatment, tumor bearing mice were randomly assigned to treatment groups such that each treatment group or time point/treatment group had the same average tumor volume. For all other experiments, treatment groups or samples were randomly assigned.
Blinding	Data reported for mouse experiments and all other experiments were not subjective but rather based on quantitative analyses.

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

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☐	☒ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used

The following flow cytometry antibodies (Mouse) were purchased from BioLegend, San Diego, CA:
CD45 (30-Fll) (1:100) (cat: 103128, lot: B348053)
CD3 (145-2Cll) (1:200) (cat: 100229, lot: B350667)
CD8a (53-6.7) (1:200) (cat: 100714, lot: B348531)
PD-1 (29F.1A12) (1:100) (cat: 135214, lot: B360160)
Tim-3 (RMT3-23) (1:100) (cat: 119727, lot: B362528)
IFN-gamma (XMGl.2) (1:100) (cat: 505810, lot: B335088)
Granzyme B (GBll) (1:100) (cat: 372216, lot: B349533)
TNF-alpha (MP6-XT22) (1:100) (cat: 506318, lot: B348963)
CTLA-4 (UC10-4B9) (1:100) (cat: 369604, lot: B269210)
LAG-3 (C9B7W) (1:100) (cat: 125212, lot: B290991)
Ki-67 (16A8) (1:100) (cat: 652420, lot: B343834)

Anti-CREB (pS133) / ATF-1 (pS63) (1:20) (cat: BD558436, lot: 1088580) was purchased from BD Biosciences, Franklin Lakes, NJ.

PD-1 antibody (clone J43, catalog #BE0033-2; clone RMPl-14, catalog #BE0146), CTLA-4 antibody (clone 9H10, catalog #BP0131), isotype antibody (Armenian hamster lgG isotype control, catalog #BE0091; Syrian hamster lgG isotype control, catalog #BE0087), and CD8 depletion antibody (Clone YTS 169.4, catalog #BE0117) were obtained from Bio X Cell (West Lebanon, NH, USA).

Validation

All antibodies were validated by the supplier and were checked in the lab by comparing to the manufacturer's or in-house results. From BioLegend: 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. Those listed under "verified" reactivity indicate species that have been tested and confirmed to react in-house by BioLegend. Species listed under "Reported" reactivity are those noted by external sources (e.g. literature publications, original antibody developer claims, etc.) but have not been confirmed in-house.

From BD Biosciences: The production process underwent stringent testing and validation to assure that it generates a high-quality conjugate with consistent performance and specific binding activity. However, verification testing has not been performed on all conjugate lots. The specificity is confirmed using multiple methodologies that may include a combination of flow cytometry, immunofluorescence, immunohistochemistry or western blot to test staining on a combination of primary cells, cell lines or transfectant models.

From BioXCell: Our lnVivoMAb™ antibodies are specifically formulated for in vivo use. They feature greater than 95% purity, ultra-low endotoxin levels, and are preservative, stabilizer, and carrier protein-free. Many of our lnVivoMAb™ antibodies can also be used for in vitro applications including Western blotting, ELISA, flow cytometric analysis, immunofluorescence, immunohistochemistry, and immunoprecipitation. All lnVivoMAb™ products are screened for purity and integrity via SDS-PAGE and guaranteed to contain less than 2 endotoxin units per milligram. Summarized from BioXCell website: Binding validation is determined by immunoblot, flow cytometry, or ELISA. Purity is determined by SDS-PAGE.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)

4MOSC1 cell lines were generated in-house as previously described (DOI: 10.1038/s41467-019-13471-0). MC38 cells were generously provided by Dr. Andrew Sharabi (doi: 10.1158/2326-6066.CIR-14-0196), the original commercial source was not verified, but DNA authentification was confirmed by STR profiling. 4MOSC-1OVA and MC38-OVA were generated in-house, as described in the methods section.

Authentication

DNA authentication of MC38 was confirmed by multiplex STR profiling (Genetica DNA Laboratories, Inc. Burlington, NC) to ensure the consistency of cell identity.

Mycoplasma contamination

All cell lines are frequently tested for mycoplasma contamination. No presence of mycoplasma was found according to Mycoplasma Detection Kit-QuickTest from Biomake (Houston, TX, USA).

Commonly misidentified lines (See ICLAC register)	No commonly misidentified cells 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	Female C57B1/6 mice (4-6 weeks of age and weighing 16-18g) were purchased from Charles River Laboratories (Worcester, MA, USA). OT-1 mice (C57BL/6-Tg(TcraTcrb)1100Mjb/J, stock no: 003831) were purchased from the Jackson Laboratory and bred in house. E8i-CreERT2 mice were obtained from Dr. Dario Vignali, University of Pittsburgh. Gnas-exon 1fl/fl and Tet-GFP-PKI mice were obtained from Dr. Ramiro Iglesias-Bartolome, National Institutes of Health. The ROSA26-rtTA-IRES-EGFP mice were obtained from Dr. Andras Nagy, Samuel Lunenfeld Research Institute. The ROSA26-Gs-DREADD-LSL mice were obtained from Dr. Rebecca Berdeaux, The University of Texas, Houston. Transgenic mice described were bred and maintained in house. Both male and female transgenic mice were used, and were approximately 6-10 weeks of age and weigh 16-20g. All the animal studies were approved by the Institutional Animal Care and Use Committee (IACUC) of University of California, San Diego, with protocol ASP #515195. Animal housing conditions are described in the manuscript. Animals were housed in temperature and humidity controlled rooms with 12:12 light/dark cycles.
Wild animals	Study did not involve wild animals.
Reporting on sex	Study did not involve sex-based analyses.
Field-collected samples	Study did not involve samples from the field.
Ethics oversight	All studies were approved by the Institutional Animal Care and Use Committee (IACUC) of University of California, San Diego, and mouse procedures were performed following ACP guidelines.

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

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 in vitro studies, cells were washed twice with PBS before proceeding with flow cytometric staining. For in vivo studies, tumors were isolated, minced, and resuspended into the Tumor Dissociation Kit (Miltenyi Biotec, San Diego, CA) and were processed with the gentleMACS Octa Dissociator, according to the manufacturer's protocol for tumor dissociation. Digested tumors were then passed through 70 µm strainers to generate single-cell suspensions, and processed further for flow cytometric analysis.
Instrument	NovoCyte Advanteon (Agilent Technologies)
Software	FlowJo
Cell population abundance	Cells were not sorted in this study.
Gating strategy	Single cells were gated from FSC/SSC (height/width) and live/dead cells were discriminated using Biolegend Zombie Aqua Fixable Viability Kit. For in vivo studies, CD8 T cells were distinguished by CD45+, CD3+, CD8+. For in vitro studies, purified CD8 T cell populations were distinguished by CD8+.

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