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 *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	SpectroFlo v.3.3.0 (Cytek), FACSDiva v.9.-0 (BD Biosciences) were used for flow cytometry data collection. Incucyte Live-cell-Analysis system v2023a (Sartorius) was used to track cell count. Victor Nivo v.4.5.0 (Perkin Elmer) was used for reading ELISA plates. QTrap 5500 mass spectrometer (AB Sciex) was used for mass spectometry analysis. QuantStudio 5 (Applied Biosystems) was used for real-time PCR analysis. PhenoImager HT (Akoya Biosciences) was used for Image analysis. RNA-seq libraries were sequenced on Illumina NovaSeq 6000 (bulk and scRNA-seq) and NovaSeq X Plus (Spatial transcriptomics).
Data analysis	FlowJo v.10 (BD biosciences), Incucyte software v2020C (Sartorius), MultiQuant v.3.0.3 (AB Sciex), CIBERSORTx, GraphPad Prism v9 (GraphPad Prism Software), MultiQuant v.3.0.3. (AB Sciex), QuPath v.0.6.0. (QuPath), Ingenuity Pathway Analysis (Qiagen), Imaria v.10.2 (Imaris).

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 bulk RNA-seq, scRNA-seq, and spatial transcriptomics data have been deposited with links to BioProject in the NCBI BioProject database: PRJNA1254286 (RNA-seq on tumor), PRJNA1254297 (RNA-seq on hypothalamus), PRJNA1254695 (scRNA-seq on immune cells), PRJNA1372687 (spatial transcriptomics). For image-localized biopsy data analysis, data is publicly available at https://www.synapse.org/#!Synapse:syn52256644. Mouse reads were aligned to the GRCm38 (mm10) mouse reference genome and human reads were aligned to the GRCh37 human reference genome. Analysis details are provided in the Methods. All data generated in this study are available upon request from the corresponding author, Dr. Justin D. Lathia (lathiaj@ccf.org).

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	Extended Data Figure 1 shows analysis of previously published dataset including patients' biological sex (Hu, L.S., D'Angelo, F., Weiskittel, T.M. et al. Integrated molecular and multiparametric MRI mapping of high-grade glioma identifies regional biologic signatures. Nat Commun 14, 6066 (2023). https://doi.org/10.1038/s41467-023-41559-1).
Reporting on race, ethnicity, or other socially relevant groupings	In Supplemental information for SEER analysis (Figure 1h-i), demographics of male GBM patients are indicated (Table 3).
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 in excess to show a significant effect based on pilot experiments used to determine mean and spread.
Data exclusions	Data were examined using Grubbs' test and outliers were excluded with the criterion of alpha = 0.05.
Replication	All experiments were replicated as indicated in the corresponding figure legends.
Randomization	Mice were randomized for castration surgery or treatment, and for tumor implantation. For the in vitro experiments, samples with identical pretreatment conditions were randomly assigned to treatment groups.
Blinding	Flow experiments were not blinded during data collection or analysis, as tracking each group is critical for data interpretation. In some survival analysis, experiment groups were blinded to the investigators. Mass spectrometry analysis was blinded during data collection as the samples were labeled as group 1 and group 2.

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 immune cell subsets:
For flow cytometry analysis:
CD11b (M1/70), BD Biosciences (#563553), surface 1:250;
CD69 (H1.2F3), BD Biosciences (#741234), surface 1:250;
CD11c (HL3), BD Biosciences (#612796), surface 1:250;
CTLA4 (UC10-4B9), BioLegend (#106312), Intra 1:250;
Ly6G (1A8), BD Biosciences (#560603), surface 1:250;
PD1 (29F.1A12), BioLegend (#135241), surface 1:250;
TIM3 (RMT3-23), BioLegend (#119727), surface, 1:100;
CD45R/B220 (RA3-6B2), BioLegend (#103237), surface 1:250;
Ki67 (16A8), BioLegend (#652413), Intra 1:250
TIM3 (RMT3-23), BioLegend (#119727), Intra 1:100;
CD3 (145-2C11), BD Biosciences (#564379), surface 1:250;
I-A/I-E (M5/114.15.2), BioLegend (#107606), surface 1:250;
CD45 (30-F11), BioLegend (#103132), surface 1:250;
Foxp3 (FJK-16s), eBioscience (#2260608), Intra 1:250;
LAG3 (C9B7W), BioLegend (#125224), surface 1:250;
NK1.1 (PK136), BioLegend (#108716), surface 1:250;
CD4 (GK1.5), BioLegend (#100422), surface 1:250;
CD8 (52-6.7), BioLegend (#100712), surface 1:250;
CD206 (C068C2), BioLegend (#141712), Intra 1:250;
Ly6C (HK1.4), BioLegend (#128024), surface 1:250;
CD68 (FA-11), BioLegend (#137024), intra 1:250;
F4/80 (BM8), BioLegend (#123117), surface 1:250;
IFN-γ (XMG1.2), BioLegend (#505846), Intra 1:250;
TNFα (MP6-XT22), BioLegend (#506329), Intra 1:250;
Granzyme B (QA18A28), BioLegend (#396414), Intra 1:250;
GR (NR3CR1) (BuGR2), eBioscience (#53-6189-82), Intra, 1:250
TCF1 (C63D9), cell signaling (#6709), intra, 1:250

For histology analysis:
1) 1' antibodies - 1:1000 dilution
cleaved caspase-3 (Asp175), Cell Signaling (#9662)
GFP, Aves Labs (#GFP-1020)
phospho-Histone H3 (Ser10), Cell Signaling (#9701)
cFos, EnCor Biotechnology (#RPCA-c-FOS)
Iba-1, Abcam (#ab5076)
ASC (AL,177), Adipogen (#AG-25B-0006-C100)

2) 2' antibodies - 1:500 dilution
Donkey anti-rabbit Alexa Fluor 488, Invitrogen (#A-21206)
Donkey anti-chicken Alexa Fluor 488, Invitrogen (#A-78948)
Donkey anti-rabbit Alexa Fluor 555, Invitrogen (#A-31572)
Rabbit anti-goat Alexa Fluor 647, Invitrogen (#A-21446)

For immunoblotting:
caspase-1 (p20), Adipogen (#AG-20B-0042-C100), 1:1000
beta-actin (AC-15), Sigma (#A5441-100ul), 1:10,000
Goat anti-mouse IR-670 secondary antibody, LiCor Bio (#92668070), 1:10,000

Validation

All antibodies used in this study are commercially available and were validated for specificity and applications by the manufacturers.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)

Mouse syngeneic glioma cell line SB28: female-derived, obtained from Dr. Hideho Okada (UCSF)

Cell line source(s)	Mouse syngeneic glioma cell line GL261: unknown sex-derived, obtained from the Developmental Therapeutic Program (NCI) Mouse syngeneic glioma cell line CPA: male-derived, obtained from the Castro-Lowenstein laboratory (University of Michigan) Mouse syngeneic glioma cell line KR158: unknown sex-derived, obtained from Dr. Loic Deleyrolle (Mayo clinic) Mouse syngeneic bladder carcinoma cell line MB49: male-derived, purchased from Animal Tumor Core (Cleveland Clinic) Mouse syngeneic melanoma cell line B16-F10: male-derived, shared by Dr. Thaddeus Stappenbeck (Cleveland Clinic) soon after they purchased the cells from ATCC (#CRL-6475) Glioblastoma patient-derived xenograft cell line L1: female-derived, originally from the laboratory of Dr. Brent Reynolds (University of Florida) (originally from the laboratory of Dr. Angelo Vescovi) Glioblastoma patient-derived xenograft cell line GBM23: male-derived, originally from the laboratory of Dr. Erik Sulman (MD Anderson Cancer Center).
Authentication	B16-F10 cells and MB49 were authenticated by the source. GBM cell lines were not further authenticated.
Mycoplasma contamination	All cell lines in our laboratory are routinely checked for mycoplasma. All cell lines used were 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	All animals were kept in a specific pathogen-free facility of the Biological Resource Unit (BRU) at Lerner Research Institute, Cleveland Clinic., with a 12-hour light-dark cycle. 5-10 weeks old mice were used for this study. All mouse strains used in the study are listed in Supplementary Table 8.
Wild animals	This study did not involve wild animals.
Reporting on sex	Since this study focuses on elucidating the role of androgens in brain tumors, male mice were mostly used. In some experiments, female mice were used (Extended Data Figure 6).
Field-collected samples	This study did not involve samples collected from the field.
Ethics oversight	All animal procedures were performed in accordance with the guidelines and protocols approved by the Institutional Animal Care and Use Committee (IACUC) at the Cleveland Clinic.

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	At the indicated time, mice were euthanized as described above, and brain tumor, spleen, and lymph nodes (inguinal) were harvested. Brain tumor tissue was minced into small pieces with scalpels and subjected to enzymatic digestion in the

presence of collagenase D (1 mg/ml; Roche) and DNase I (0.1 mg/ml; Roche) at 37°C. Digested tissue was filtered through a 70 µm cell strainer. To enrich for immune cells, gradient centrifugation was performed using 30% percoll solution (Sigma). Red blood cells (RBCs) were lysed using RBC lysis buffer (BioLegend). For spleen and lymph nodes, tissue was ground onto a 40 µm cell strainer, followed by RBC lysis. All single-cell suspension samples were filtered once more with a 40 µm cell strainer before staining for flow anlaysis. For cell staining, after live/dead staining with LIVEDEAD Blue (Thermo Fisher Scientific) on ice for 15 min, cells were washed and incubated with FcR blocker (Miltenyi Biotech) diluted in PBS/2% BSA on ice for 10 minutes. For surface staining, cells were incubated in an antibody mixture diluted in brilliant buffer (BD Biosciences) at 1:100 to 1:250 on ice for 30 minutes. After washing with PBS/2% BSA buffer, cells were fixed with Foxp3/ Transcription factor fixation buffer (eBioscience) overnight. For intracellular staining, antibodies were diluted in Foxp3/ Transcription factor permeabilization buffer at a ratio of 1:250, and cells were incubated at room temperature for 45 minutes. For intracellular cytokine detection, cells were stimulated using Cell Stimulation Cocktail plus protein transport inhibitor (eBioscience) in complete RPMI for 4 hours, followed by the cell staining procedures described above. Stained cells were acquired with an Aurora (Cytek Biosciences) and analyzed using FlowJo software (v10, BD Biosciences).

Instrument	Cytek Aurora, BDFortessa
Software	SpectroFlow, FACSDiva
Cell population abundance	N/A
Gating strategy	Gating strategy is provided in the supplementary information (supplementary figure 1). After clean-up process, T cells were defined as CD3+CD45+ and each T cell subset was further identified using CD4, Foxp3, and CD8. Cytokine expression was defined using negative control without re-stimulation.

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