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	o Flow cytometric analysis and cell sorting- Beckman Coulter CytoFlex LX, BD FACS SORP Aria Fusion, and Cytek Aurora o Metabolomics- Agilent MassHunter Workstation Software LC/MS Data Acquisition for 6400 Series Triple Quadrupole MS- Version B.08.02, UHPLC-QqQ; Agilent 1290 Infinity II / 6495C o qPCR – QuantStudio 7 Pro applied biosystems o Luminescence plate reader- GloMax Discover, Promega
Data analysis	o Flow cytometry data – FlowJo (Version 10.10.0) – Operating System: Mac OS X – Java Version 17.0.8+7-LTS – Build number 3b20e798 o Metabolomic data- Agilent MassHunter Workstation Quantitative Analysis- QQQ Versions 10.1 and 12.1, Morpheus o Statistical analyses – Prism 9 for macOS - Version 9.5.1 o Statistical analyses – Python version- Version 3.11.5 for MacOS o Statistical analyses – R- Version 4.2.1

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

Source data were provided. There are no restrictions.

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 as a biological variable was accounted for in this study and data were reported separately for individual sex. Gender information is not available.
Reporting on race, ethnicity, or other socially relevant groupings	Race and ethnicity information is not available. No groupings based on race, ethnicity or other socially relevant criteria were performed.
Population characteristics	Patients diagnosed with high-grade glioma, and healthy blood donors
Recruitment	Patients with glioma were recruited by Scott et al., and Yu et al., for their respective studies. For the PBMC analysis, healthy individuals eligible for blood donation were recruited by the SunCoast Blood Centers.
Ethics oversight	For the donor PBMC data, informed consent is available from the SunCoast Blood Centers. For the reanalysis of metabolic data from Scott et al., Nature, 2025, informed consent information is available on the original manuscript. For the reanalysis of GSE117891, the consent information is not reported.

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	o For animal studies, the number per arm is based upon the following calculation: n= (1+2C)(s/d)^2 n = Number of animals per experimental group C = 9.18 when α = 0.05 and 1 – β = 0.85 (Significance level of 5% with a power of 85%) s = Standard deviation (≈ 7 days) d = Difference to be detected (≥ 10 days) Thus, n = 10 animal/group was used for most of the in vivo studies. o For all other studies, sample size was determined based on previous experience. Exact sample sizes were provided in the figure legends.
Data exclusions	o For in vitro experiments: data were excluded based on an outlier test o For in vivo experiments: mice that died due to non-tumor reasons were excluded
Replication	in most cases in vivo experiments were replicated by using 2-3 independent cohorts. minimum 3 biological replicates were used for in vitro studies.
Randomization	all mice were randomized into drug and control treatment arms and were co-housed. Wells were randomly assigned to drug vs vehicle groups.
Blinding	experiments were blinded when possible. tumor collection from immune profiling experiments were conducted by a blinded lab member. It was not possible to blind drug treatment experiments, due to the nature of the study.

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

All antibodies were used at a 1:100 dilution.

In vitro mouse antibodies (Purchased from Biolegend)

o CD11b M1/70 (cat# 101212, Lot# B384396; cat#101206, Lot# B349919)
o Ly6C HK1.4 (cat# 128008, Lot# B358688; 128036, Lot# B357153)
o Ly6G 1A8 (cat# 127645, Lot# B402586; cat# 127624, Lot#B388205)
o NOS2 W16030C (cat# 696806; Lot# B356090)
o Arg-1 W210471 (cat# 165804; Lot# B399360)
o CD80 16-10A1 (cat# 104732; Lot# B383714)
o CD86 GL-1 (cat# 105040; Lot# B382775)
o CD163 S150491 (cat# 155308; Lot# B342458)
o CD204 1F8C33 (cat# 154718; Lot# B334063)
o IA/IE M5/114.15.2 (cat# 107628; Lot# B370049)

In vivo mouse antibodies (Purchased from Biolegend)

o CD11b M1/70 (cat# 101243; Lot# B402588)
o Ly6C HK1.4 9 (cat# 128036, Lot# B408433)
o Ly6G 1A8 (cat# 127618; Lot# B4099545)
o CD68 FA-11 (cat# 137026; Lot# B354896)
o F4/80 BM8 (cat# 123130; Lot# B402716)
o CD45 I3/2.3 (cat# 147706; Lot# B391648)
o CD11c N418 (cat# 117339; Lot# B400655)
o P2Ry12 S16007D (cat# 848004; Lot# B397099)
o IA/IE M5/114.15.2 (cat# 107628; Lot# B401702)
o PD-L1 10F.9G2 (cat# 124315; Lot# B417844)
o CD204 1F8C33 (cat# 154718; Lot# B334063)
o CD3 17A2 (cat# 100237; Lot# B401776)
o CD4 GK1.5 (cat# 100469; Lot# B409410)
o CD8a 53-6.7 (cat# 100750; Lot# B420449)
o B220 RA3-6B2 (cat# 103212; Lot# B379792)
o CD69 H1.2F3 (cat# 104526; Lot# B376513)
o CD152 UC10-4B9 (cat# 106305; Lot# B367716)
o PD1 29f.1A12 (cat# 135216; Lot# B401701)
o NK1.1 S17016D (cat# 156512; Lot# B383886)
o TIGIT 1G9 (cat# 142111; Lot# B422393)
o NOS2 W16030C (cat# 696808; Lot# B407290)
o Ki-67 11Fb (Cat# 151215)
o CD45 30-F11 (Cat# 103132)
o CD4 GK1.5 (Cat# 100422)
o CD8 6206.7 (Cat# 100712)
o granzyme B QA18A28 (Cat# 396413)
o TNF MP6-XT22 (Cat# 506329)

Human antibodies (Purchased from Biolegend)

o CD45 HI30 (cat# 304024; Lot# B393968)
o CD3 OKT3 (cat# 317336; Lot# B417078)
o CD19 HIB19 (cat# 302230, Lot# B402462; cat# 302218; Lot# B361547)

o CD56 5.1H11 (cat# 362506, Lot# B384641; cat# 362544, Lot# B358300)
o CD11c 3.9 (cat# 301636, Lot# B412803)
o CD123 6H6 (cat# 306006, Lot# B402932)
o CD11b ICRF44 (cat# 301324; Lot# B399075)
o Lox-1 15C4 (cat# 358606, Lot# B375318; cat# 358610, Lot# B392357)
o CD14 M5E2 (cat# 301804, Lot# B390672)
o CD33 WM53 (cat# 303430; Lot# B411906)
o HLA/DR L243 (cat3 307618; Lot# B396556)
o CD66b G10F5 (cat# 305122; Lot# B392324)
o CD68 Y1/82A (cat# 333826; Lot# B372283)
o Arg1 14D2C43 (cat# 369708; Lot# B409140)
o CD4 SK3 (cat# 344646; Lot# B369281)
o CD8 SK1 (cat# 344704; Lot# B367025)
o CD45RA HI100 (cat# 304130; Lot# B357476)
o CD62L DREG-56 (cat# 304806, Lot# B364312)
o CD69 FN50 (cat# 310934, Lot# B410619)
o CD95 DX2 (cat# 305646; Lot# B360356)

Other antibodies
o CD3 145-2C11 (Cat# 564379, BD Biosciences)
o human/ mouse/ rat iNOS antibody 2D2-B2 (cat # MAB9502, batch CRF0623061; Biotechne)
o InVivoMAb anti-mouse anti-Ly6G (BE0075-1, BioXCell)
o InVivoMAb rat IgG2a isotype control, anti-trinitrophenol (BE0089, BioxCell)

Validation

Validation statements on manufacturer websites

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)

SB28 (Dr. Hideho Okada, University of California, San Francisco), KR158 (Dr. Karlyne Reilly, NCI) , L1 cells (Dr. Loic Deleyrolle, University of Florida), DI318 (Dr. Justin Lathia, Cleveland Clinic) and GL261 (National Cancer Institute Division of Cancer Treatment and Diagnos) cells were acquired through material transfer agreements.

Authentication

These cell models were not authenticated independently. Sex of the cell lines are not available.

Mycoplasma contamination

Cell lines were tested for mycoplasma contamination

Commonly misidentified lines
(See ICLAC register)

Commonly misidentified lines were not 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

o C57BL/6 Jackson Laboratory, #000664) ; 4-8 weeks
o NCG (Charles River Laboratories, #572NCG); 4 weeks
o CAT2 knockout (Dr. Danielle Dean, Vanderbilt University); 4-8 weeks

Wild animals

Provide details on animals observed in or captured in the field; report species and age where possible. Describe how animals were caught and transported and what happened to captive animals after the study (if killed, explain why and describe method; if released, say where and when) OR state that the study did not involve wild animals.

Reporting on sex

Sex was used as a biological variable in the study and both male and female mice were used. Animal sex is clearly defined in the source data and figures.

Field-collected samples

For laboratory work with field-collected samples, describe all relevant parameters such as housing, maintenance, temperature, photoperiod and end-of-experiment protocol OR state that the study did not involve samples collected from the field.

Ethics oversight

All studies were performed in compliance with Institutional Animal Care and Use Committee (IACUC, 23-0142 and 23-057), University of Miami. To track intracranial tumor formation, we used the following criteria as a surrogate of tumor burden as approved by the IACUC protocol: 1- behavioral changes (aggression, guarding, hiding)
2- hair-coat (ruffled fur, lack of grooming)
3- neurological symptoms (head-tilt, seizure)
4- posture or ambulatory changes (tense, stiff gait, ataxia)
5- activity level changes (restlessness, pacing, lethargy)
6-difficulty in breathing or other sign of distress

Surrogate measures were not exceeded.

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

Plants

Seed stocks

Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.

Novel plant genotypes

Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.

Authentication

Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosiacism, off-target gene editing) were examined.

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

o Mouse bone marrow derived cells (250,000 cells/ well) were seeded in round bottom 96-well U-bottom plate in RPMI media and treated accordingly overnight. Post-treatment, cells were stained for flow cytometry

o Human blood was obtained from Suncoast Blood Center, Florida and PBMCs were isolated. 3-5 million PBMCs were cultured in 6-well plate in RPMI media and treated accordingly overnight. Post-treatment, cells were stained for flow cytometry

o Tumor, blood, and spleen were harvested from untreated and treated mice. Tissues were processed for single cell suspensions and stained for flow cytometry. For the analysis of mediator production from T cells, cells were stimulated with Cell Stimulation Cocktail (eBioscience).

o T cells isolated from mouse spleen and labelled with CFSE were co-cultured with sorted myeloid derived suppressor cell populations (monocytic and granulocytic) in a 96 well U-bottom plate and treated accordingly for 4 days and stained for flow cytometry

Instrument

Beckman Coulter Cytoflex LX o Cytek Aurora

Software

FlowJo (Version 10.10.0)

Cell population abundance

Sample purity was determined by internal, technical controls for each and every experiment, thereby providing a continuous surveillance of sample fluorescence and quality. The same cells used for controls and experimental groups were simultaneously seeded to provide single fluorescence samples. These samples were used to control for fluorescent cross-contamination and for compensation under analysis. Corresponding WT cells were used as negative controls to gauge autofluorescence and provide a non-fluorescent comparison for compensation, gating and analysis purposes

Gating strategy

Gating strategy for all relevant experiments – Preliminary FSC/SSC gates of the starting cell population – Boundaries between “positive” and “negative” populations. For single cells and live cells, boundaries between “positive” and “negative” cells were defined using standard gating in an FSC-A/FSC-H plot.

o The gating strategy was developed to quantify NOS2 expression in human monocytic myeloid derived suppressor cells and granulocytic myeloid derived suppressor cells as defined by CD45+ CD3/C19/56- CD11b+ CD68- CD11C- CD33+ HLADR- events. From these events monocytic myeloid derived suppressor cells were selected as CD14+ and granulocytic myeloid derived suppressor cells were selected as CD66b+ Lox-1+. Initially, technical controls were used for making a fluorescent-specific matrix to compensate for possible spillover which could affect results. Gates were then created by identifying live cells, single cells, above mentioned events followed by NOS2+ events in each subpopulation using the control sample. These gates were applied to the group as a whole, thereby creating a common gating tree for all experimental and control samples. Said gating strategy was applied to all experiments under no exception.

o For tumors, the gating strategy was developed to quantify differences in immune populations along with NOS2 expression in mouse monocytic myeloid derived suppressor cells and granulocytic myeloid derived suppressor cells. Since tumors had a

GFP tag, gates were created to identify GFP+ events. From live cells, CD45+ events were used to examine different immune populations as defined by CD11b+ myeloid and CD11b- non myeloid. From CD11b+ events, CD68-CD11c- Ly6G+ Ly6C- were granulocytic myeloid derived suppressor cells and CD68-CD11c- Ly6G- Ly6C+ were monocytic which were further differentiated as IA/IE+ monocytes and IA/IE- monocytic myeloid derived suppressor cells. Initially, technical controls were used for making a fluorescent-specific matrix to compensate for possible spillover which could affect results. Gates were then created by identifying live cells, single cells, above mentioned events followed by NOS2+ events in each subpopulation using the untreated samples. To define T cell populations, CD45+ cells were determined from the live fraction. T cell populations were determined based on co-expression of CD3 with CD4 or CD8. These gates were applied to the group as a whole, thereby creating a common gating tree for all experimental and control samples. Said gating strategy was applied to all experiments under no exception.

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