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Last updated by author(s): Feb 17, 2021

Reporting Summary

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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

Flow cytometry data was acquired using the BD FACSDIVA software (version 9) or Attune NxT Software (version 2.5). Microscopy data was collected using the ZEISS ZEN software (version 2.3). MRI data was acquired with Osrix imaging software (version 4.12). bwa version 0.6.2-r126 for sequence alignment. http://bio-bwa.sourceforge.net/. A custom Perl script for the extraction of mRNA counts based on unique molecular identifiers.

Data analysis

Flow cytometry data was analyzed using FlowJo V9 or V10. Quantification of microscopy images was done using ImageJ v1.48. Tumor volumes from MRI data were calculated after manual segmentation of tumor areas using Osirix imaging software version 4.12. Metabolomics analysis with ESI-MS/MS data was done with the Micromass MassLynx MS Software. For UPLC-ESI-HRMS/MS data Bruker TargetAnalysis Version 4.3 was used to determine Trp concentrations. Full codes of all scripts are available on request. All other statistics were done using GraphPad prism 7.0.

Custom code for the transcriptomic and proteomic analyses can be found under: https://github.com/rsankowski/friedrich-et-al-IDH1wt-mut-micr.git.

Data analysis and visualization was conducted using R: RaceID3 for Single-cell RNA sequencing data analysis (https://github.com/dgrun/RaceID3_StemID2) and the tidyverse packages for further data processing and visualization. Versions of the used packages:

R version 3.6.1 (2019-07-05)
Platform: x86_64-pc-linux-gnu (64-bit)
Running under: Ubuntu 18.04.3 LTS

Matrix products: default
BLAS: /usr/lib/x86_64-linux-gnu/blas/libblas.so.3.7.1
LAPACK: /usr/lib/x86_64-linux-gnu/lapack/liblapack.so.3.7.1

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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/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research 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 list of figures that have associated raw data
- A description of any restrictions on data availability

Bulk and single-cell RNA-seq data that support the findings of this study have been deposited in the Gene Expression Omnibus (GEO) under the superSeries accession code GSE166420. It consists of the following subSeries: GSE166218 (mouse 10x data); GSE166418 (human CEL-Seq2 data); GSE166521 (GL261 bulk RNA-Seq data). Mass cytometry data have been deposited in the FlowRepository: https://flowrepository.org/id/FR-FCM-Z3G7. Source data have been provided for this study. TCGA dataset was downloaded from gliovis.bioinfo.cnio.es. Imaging source data of this manuscript can be found under this DOI: 10.6084/m9.figshare.14166983. All other data supporting the findings of this study are available from the corresponding author on reasonable request: m.platten@dkfz.de.

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 calculated with the help of a biostatistician using R version 3.4.0. Assumptions for power analysis were as follows: alpha error: 5%; beta error: 20%. Values for standard deviation and differences between experimental groups were based on previous experiments (whenever a similar data type was available). In all other cases a pilot group size was used.
Data exclusions	Low quality and non-myeloid cells (e.g. T cells, monocytes, oligodendrocytes) were excluded from the scRNA-seq data analysis. In case animals had to be sacrificed prior to the pre-defined endpoint (due to weight loss or other termination criteria), they were excluded from any downstream analysis.
Replication	Primary healthy tissue and glioma samples for scRNA-Seq were acquired over 6 independent experiments. It was ensured that data had similar characteristics, all replication attempts were successful. CyTOF experiments were conducted with appropriate positive and negative controls. All the CyTOF measurements in this study were performed in the Charité | BIH Cytometry Core (headed by Dr. Desiree Kunkel). The core facility ensures the possibility of generating reproducible quality data by implementing best practices, providing expert knowledge, and keeping the necessary instrumentation in good shape (Warth and Kunkel 2019, doi: 10.1007/978-1-4939-9454-0_1). Furthermore, we have regularly validated our CyTOF workflow (including sample collection, storage, processing and analysis) and antibody panels for microglia analysis throughout our studies (see also published papers including Böttcher et al., Nat. Neurosci. 2019; Böttcher et al., Acta Neuropathol Commun). In addition, to ensure the consistency of the overall read out across batches, in this study, we have designed the antibody panel A and B with an overlapping of five antibodies, namely P2RY12, CD45, CD11c, HLADR and CCL2. Results obtained from the antibody panel A were as comparable to those obtained from the antibody panel B, as shown in the results section. Key experiments (TAM phenotyping, AHR reporter assays) were all performed at least threefold and unless otherwise mentioned, data from one representative experiment are shown in this manuscript. All other experiments were performed once with biological replicates or technical replicates (as specified in figure legends).
Randomization	Mice were ranzdomized into treatment groups stratified for tumor size (measured by MRI) at the time of treatment start. Group assignment of human subjects was based on the presence of a glioblastoma and wildtype or R132H mutated IDH1 alleles. A randomization was not possible under these circumstances.
Blinding	Intracranial tumor experiments were performed in a blinded manner (MRI, flow cytometric analyses). For the human analyses, blinding of the patient diagnoses was not performed. However, data analysis was conducted in an unsupervised manner using the output of the Seurat R software. Thus, the drawn conclusions should not have been influenced by the lack of blinding.

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
☐	☒	Animals and other organisms
☐	☒	Human research participants
☒	☐	Clinical data

Methods

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

Antibodies

Antibodies used	Antibody used in vivo antigen clone manufacturer LOT # mPD-L1 n.a. 10F.9G2 BioXCell LOT: 66571701 Antibodies used in vitro antigen conjugate clone manufacturer LOT # CAT # m-PD-L1 10F.9G2 BioXCell LOT: 6657101 10F.9G2

mIL-10 ab9969 Abcam LOT: GR409-33-50 ab9969
mCD3e 145-2C11 eBioscience LOT: 4310230 16-0031-82
mCD28 37.51 eBioscience LOT: 4279532 16-0281-82
mCD11b APC M1/70 BioLegend LOT: B226978 101212
mCD11b PE-Dazzle M1/70 BioLegend LOT: B235643 101256
mCD16/32 n.a. (FC block) 93 eBioscience LOT: 4333612 14-0161- 82
mCD206 PE MR6F3 eBioscience LOT: 1995849 12-2061-80
mCD206 AlexaFluor700 C068C2 BioLegend LOT: B253654 141734
mCD25 PE PC61 BioLegend LOT: B19152 102008
mCD3 BV711 17A2 BioLegend LOT: B245637 100241
mCD3 FITC 17A2 BioLegend LOT: B221808 100204
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mCD4 APC RM4-5 BioLegend LOT: B218256 100516
mCD4 PB RM4-5 BioLegend LOT: B207497 100531
mCD4 PE-Texas Red RM4-5 Invitrogen LOT: 1837385 MCD0417
mCD45 BV510 clone BioLegend LOT: B260403 / B240739 100752
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mCD8 PE-Cy7 53-6.7 eBioscience LOT: E07510-1635 25-0081-82
mCD8 PerCP-Cy5.5 53-6.7 eBioscience LOT: 4291993 45-0081-82
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mCD80 PerCP-eFluor710 16-10A1 eBioscience LOT: 1928918 46 -0801-82
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mF4/80 BV421 BM8 BioLegend LOT: B251578 BM8
mF4/80 BV510 BM8 BioLegend LOT: B271685 123135
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mFOXP3 FITC FJK-16s Invitrogen LOT: 430671 11-5773-80
mGr-1 FITC RB6-8C5 BioLegend LOT: B210647 108405
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mI-A/I-E PE-Cy7 M5/114.15.2 eBioscience LOT: E14766-105 25-5321-82
mIFNγ PE-Cy7 XMG1.2 eBioscience LOT: 4332567 25-7311-82
mLAP (TGF-b1) PE TW7-16B4 BioLegend LOT: B248257 141404
mMHC II (I-A/I-E) AF700 M5/144.15.2 BioLegend LOT: 4310394 107622
mPD-1 BV785 29F.1A12 BioLegend LOT: B251641 135225
mPD-L1 BV605 10F.9G2 BioLegend LOT: B245034 124321
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mPD-L1 PE MIH5 eBioscience LOT: 4276911 12-5982-82
mTNFa BV421 MP6-XT22 eBioscience LOT: B252150 BDB563387
fixable viability dye eFluor780 n.a. eBioscience LOT: 1977883 65-0865-14

Antibodies used for flow cytometry – human cells
antigen conjugate clone manufacturer LOT # CAT #
human CD11b APC M1/70 BioLegend LOT: B243489 101212
human CD14 Pacific Blue M5E2 BioLegend LOT: B178012 301815
human CD16 APC-Cy7 3G8 BioLegend LOT: B170470 302018
human CD19 PE-Cy7 SJ25C1 eBioscience LOT: E13454-102 25-0198- 42
human CD274 (PD-L1) APC MIH1 eBioscience LOT: E12159-1634 17-5983-42
human CD45 eFluor450 2D1 eBioscience LOT: 4335205 48-9459-42
human CD80 PerCP-eFluor710 2D10.4 eBioscience LOT: 4325039 46-0809-42
human CD86 Pacific Blue IT2.2 BioLegend LOT: B225655 305423
human HLA-DR PE-Cy7 L243 eBioscience LOT: 4275098 25-9952-42

Secondary antibodies used
antigen conjugate clone manufacturer LOT #
rabbit IgG AlexaFluor 633 sc-2004 Invitrogen LOT: SF253701 CAT: A-21070.

flow cytometry antibodies used for sorting
anti-CD45 (clone HI30, BD Bioscience, Heidelberg, Germany; Cat# 555485; Lot# 8012762; dilution: 1:100)
anti-CD11b (clone M1/70, eBioscience, San Diego, USA; Cat# 101237; Lot# B245639; dilution: 1:800)
anti-CD3 (clone SP34-2, BD Bioscience, Heidelberg, Germany; Cat# 551916; Lot# B208643; dilution1:100)
anti-CD19 (clone SJ25C1, BioLegend, San Diego, USA; Cat# 363003; Lot# B275700; dilution: 1:100)
and anti-CD20 (clone 2H7, BioLegend, San Diego, USA; Cat# 302311; Lot# B257731; dilution: 1:400)

CyTOF antibodies:
CD45 (1:100, HI30 / Cat#: 3141009B / Fluidigm); CD19 (1:100, HIB19 / Cat#: 3142001B / Fluidigm); HLA-DR (1:100, L243 / Cat#: 3143013B / Fluidigm); CD11b (1:100, ICRF44 / Cat#: 3209003B / Fluidigm); CD64 (1:100, 10.1 / Cat#: 3146006B / Fluidigm); CD11c (1:100, Bu15 / Cat#: 3147008B / Fluidigm); CD16 (1:100, 3G8 / Cat#: 3148004B & 3165001B / Fluidigm); CCl2 (1:200, 5D3-F7 / Cat#: 502601 / Biolegend); CD68 (1:100, Y1/82A / Cat#: 333801 / Biolegend); TNF-a (1:100, Mab11 / Cat#: 3152002B / Fluidigm); Cyclin B1 (1:100, GNS-1 / Cat#: 3153009A / Fluidigm); CD3 (1:100, UCHT1 / Cat#: 3154003B / Fluidigm); CD56 (1:100, B159 / Cat#: 3155008B / Fluidigm); CCR5 (1:100, NP-6G4 / Cat#: 3156015A / Fluidigm); IRF4 (1:100, IRF4.3E4 / Cat#: 646402 / Biolegend); CD163 (1:100, GHI/61 / Cat#: 333602 / Biolegend); EMR1 (F4/80, A10 / Cat#: MCA2674GA / Bio-Rad); Ki-67 (1:100, B56 / Cat#: 3162012B / Fluidigm); TGF-b(1:100, TW4-2F8 / Cat#: 349602 / Biolegend); CD115 (1:100, 9-4D2-1E4 / Cat#: 347302 / Biolegend); P2Y12 (biotin) (1:100, Polyclonal / Cat#: HPA014518 / Sigma-Aldrich); IL-10 (1:100, JES3-9D7 / Cat#: 3166008B / Fluidigm); IRF8 (1:100, 7G11A45 / Cat#: 656502 / Biolegend); CD206 (1:100, 15-2 / Cat#: 3168008B / Fluidigm); CD33 (1:100,

WM53 / Cat#: 3169010B / Fluidigm); CD86 (1:100, IT2.2 / Cat#: 305402 / Biolegend); CCR2 (1:100, K036C2 / Cat#: 357202 / Biolegend); CX3CR1 (1:100, 12A9-1 / Cat#: 3172017B / Fluidigm); CD14 (1:100, RMO52 / Cat#: 3160006B / Fluidigm); TREM2 (1:100, 237920 / Cat#: MAB17291-100 / R&D Systems); CD116 (1:100, 4HI / Cat#: 305902 / Biolegend); CD44 (1:50, BJ18 / Cat#: 338802 / Biolegend); CD18 (1:100, TS1/18 / Cat#: 302102 / Biolegend); IL-6 (1:100, MQ2-13A5 / Cat#: 501101 / Biolegend); CD172a (1:100, 15-414 / Cat#: 372102 / Biolegend); CD54 (ICAM1) (1:100, HA58 / Cat#: 353102 / Biolegend); PD-L1 (1:100, 29E.2A3 / Cat#: 3156026B / Fluidigm); GM-CSF (1:100, BVD2-21C11 / Cat#: 502301 / Biolegend); CD32 (FITC) (1:100, 6C4 (CD32) / Cat#: 11-0329-42 / eBioscience); CD91 (1:100, A2MR- a2 / Cat#: 550495 / BD Bioscience); PU.1 (PE) (1:100, 7C6B05 / Cat#: 681308 / Biolegend); CCR7 (1:100, G043H7 / Cat#: 3167009A / Fluidigm); PD-1 (1:100, EH12.2H7 / Cat#: 3174020B / Fluidigm); TMEM119 (1:100, polyclonal / Cat#: HPA052650 / Sigma-Aldrich); CD4 (1:100, RPA-T4 / Cat#: 3145001B / Fluidigm); Galanin (1:100, 581403 / Cat#: 3148016B / Fluidigm); MIPb (1:100, D21-1351 / Cat#: 3150004B / Fluidigm); CD101 (1:100, BB27 / Cat#: 3158020B / Fluidigm); CD95 (1:100, DX2 / Cat#: 3152017B / Fluidigm); Glut5 (1:100, 195205 / Cat#: MAB1349 / R&D Systems); CD74 (1:100, LN2 / Cat#: 3166018B / Fluidigm); CD47 (1:200, CC2C6 / Cat#: 3209004B / Fluidigm); CD141 (1:100, 1A4 / Cat#: 3173002B / Fluidigm); CD130 (1:100, 2E1B02 / Cat#: 3168016B / Fluidigm); GPR56 (1:100, CG4 / Cat#: 358202 / Biolegend); ApoE (1:100, WUE-4 / Cat#: NB110-60531 / Novus Biologicals); CD127 (1:100, A019D5 /Cat#: 3176004B / Fluidigm)

Validation

Magnetic sorting human CD14+ antibodies have been validated by flow cytometry of purified human CD14+ cell populations. In vitro stimulation antibodies (aCD3/aCD28) have been titrated and established in previous experiments by our group (Bunse et al. Nat. Med. 2018). In vivo antibodies have been validated repeatedly in previous experiments by our group (Aslan et al. Nat. Commun. 2020). Validation data of flow cytometry antibodies can be found on the suppliers' website. Flow cytometry antibodies purchased from BioLegend were stained on 1-3 target cell types with either single- or multi-color analysis detailed in the QC specification (including positive and negative controls). Flow cytometry antibodies purchased by eBioscience were tested for specificity and sensitivity in the development stage. This is done by staining multiple target cells with either single- or multi-color analysis or by other testing approaches. The QC specifications and testing SOPs and gold standard for each product are then developed. The tested cells can be primary cells and/or cell lines known to be positive or negative for the target antigen. Anti-mIDH1 antibody used for immunofluorescence staining was validated by our group (Schumacher et al. Nature 2014) and used for diagnostic purposes worldwide.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

Murine glioma cell line GL261 was obtained from the Division of Cancer Treatment and Diagnosis (DCTD), National Cancer Institute, MD, USA. Embryonic kidney cell line HEK293 was obtained from ATCC and sold by LGC Standards.

Authentication

highthroughput Multiplex human Cell Authentification test (MCA), Castro et al.
2012 for HEK293 cell line. No authentication of GL261 cell line was performed. IDH1-S/MAR insertion was validated by antibiotic resistance, immunofluorescence staining and R-2-HG production.

Mycoplasma contamination

Cell lines were tesed negative for mycoplasma contamination regularly and before in vivo use. Highthroughput Multiplex Cell contamination (McCT), Schmitt M. et al. 2009

Commonly misidentified lines
(See ICLAC register)

no commonly misidentified lines from the ICLAC register were used in this study

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals

C57BL/6J wild-type (WT) mice were purchased from Charles River or Janvier Labs. Sex- and age-matched mice were used for further experiments. If not stated otherwise, female mice were used for the experiments. All mice were 7-12 weeks of age at use. Mice were kept under SPF conditions at the animal facility of the DKFZ Heidelberg. A 14-hour light/10-hour dark cycle is used in the animal facility. Lights are not used and researchers and technicians do not enter the mouse room during the dark cycle. Temperatures is regulated at 21°C with 50% humidity.

Wild animals

the study did not involve wild animals

Field-collected samples

the study did not involve samples collected from the field

Ethics oversight

Animal experiments were performed according to the rules of the German Animal Welfare Act and were licensed by the regional authority Karlsruhe.

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

Human research participants

Policy information about studies involving human research participants

Population characteristics

Human control (n=7, mean age=50±19.07 (21-74), gender: F:M(4:3)), IDHwt (n=7, mean age=65.9±14.9 (32-81), gender: F:M(4:5)) and IDH R132Hmut GBM (n=4, mean age=48.5±6.81 (39-55), gender: F:M(1:3))

Recruitment

Patient samples were prospectively collected from adult patients undergoing brain surgery after informed consent as described before (Sankowski et al. Nat. Neuroscience 2019). Prospective patient recruitment was conducted by staff that were not involved in this study. The final decision about the inclusion into the study was dependent on histological and molecular

	pathological diagnosis. Due to this two-step process and the prospective sample collection bias should have been minimized. However, limited availability of samples may have led to under-representation of female glioblatoma patients in the present study.
Ethics oversight	Ethical approval for the isolation of peripheral blood mononuclear cells (PBMCs) under consent from patients of the Neurology Clinic Heidelberg and the National Center for Tumor Diseases (NCT) Heidelberg was obtained from the Heidelberg Medical Faculty Ethics Committee (Reference number S-359/2016, 21.11.2016). Ethical approval for the isolation of tumor-infiltrating leukocytes (TILs) and the analysis of human gliomas and glioblastomas was obtained from the Heidelberg Medical Faculty Ethics Committee (Reference number S-064/2008, 31.03.2008). Research-only buffy coat formulations from healthy donors were purchased from the Institute of Clinical Transfusion Medicine and Cell Therapy (IKTZ) Heidelberg. Patient sample collection at the Freiburg site was regulated under ethics protocol 472/15.

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	Human brain samples were homogenized to cell suspensions. Myelin was removed using 37% Percoll centrifugation. To reduce batch effects cells were cryopreserved using FCS:SMSO (9:1). Sorting and processing for scRNA-seq was done in batches. Cells were stained and washed for FACS sorting using sterile filtered FACS buffer (PBS w/o Ca/Mg, 2% FCS, 2mM EDTA) Murine Gl261-containing brain hemispheres were excised, washed in HBSS (Sigma-Aldrich) and cut into small pieces before tissue disruption in HBSS supplemented with 50 µg/ml Liberase D for 0,5 h under slow rotation at 37 °C. Dispersed tissue was mashed through a 100µm and 70 µm cell strainer and lymphocytes. For Gl261 samples, myelin removal was performed using myelin removal beads II (Miltenyi Biotec; 130-096) or by percoll density gradient as described in online methods. Murine splenocytes were isolated by homogenization using a cell strainer and ACK lysis. CD3+ and CD11b+ cells were purified with the MagniSort™ Mouse T cell Enrichment Kit (eBioscience; 8802-6820), MagniSort™ Mouse CD3 Positive Selection Kit (eBioscience; 8802-6840) or by MagniSort™ Mouse CD11b Positive Selection Kit (eBioscience; 8802-6860-74) on isolated cells using MACS according to manufacturer's instructions. In some cases, as described in the online methods, cells were treated with Brefeldin A to prevent secretion of cytokines, chemokines, and other secretory proteins before analysis. If required, single cell suspension were labeled with CellTrace Far Red according to the manufacturer's instructions (see online methods). Human macrophages were generated by magnetic cell separation of CD14+ monocytes from human peripheral blood mononuclear cells (PBMCs) using CD14+ magnetic beads and LS positive selection columns (Miltenyi Biotech) according to the manufacturer's instructions. Cells were counted and resuspended in Iscove's Modified Dulbecco's Media (IMDM, Sigma-Aldrich), containing 10% human serum AB (Sigma-Aldrich), 100 U/ml Penicillin and 100 µg/ml Streptomycin (Invitrogen) and 2 mM L-Glutamine (Invitrogen). 5 ng/ml recombinant human macrophage colony-stimulating factor (M-CSF, Peprotech) was added to the medium and 50% of M-CSF-containing medium was exchanged every 3 days for 9 days in total. If not mentioned otherwise naïve macrophages were stimulated with 100 ng/ml LPS (Sigma-Aldrich) and 100 ng/ml recombinant human IFNγ (Peprotech) for 24 h. If treated with (R)-2-HG in vitro,cells were treated on day 9 overnight and stimulated for 24 h with LPS and IFNγ. Murine macrophages were generated from bone marrow-derived monocytes (BMDMs) by culturing BMDMs in Iscove's Modified Dulbecco's Media (IMDM, Sigma-Aldrich), containing 10% fetal bovine serum (FBS, Sigma-Aldrich), 100 U/ml Penicillin and 100 µg/ml Streptomycin (Invitrogen) and 2 mM L-Glutamine (Invitrogen), supplemented with 10 ng/ml recombinant murine M-CSF (Peprotech).
Instrument	FACS Canto II (BD Biosciences, V96300305), Attune NxT (Thermo Fisher Scientific, 2AAS232591116) or MoFlo Astrios (Beckman Coulter, Krefeld, Germany)
Software	Data was collected with FACS Diva Software (version 9, BD Biosciences) or Attune NxT Software version 2.5. Data was analysed with Flow Jo Version 9 or 10
Cell population abundance	Purity of isolated myeloid cells from human brain tumor samples or murine Gl261 tumor samples was >95% post sort.
Gating strategy	Lymphocytes were defined by size and granularity in FSC-A vs. SSC-A plots. Subsequently, duplets were excluded in FSC-W vs FSC-H plots and dead cells were excluded by means of fixable viability dye positivity. Subsequent gating strategies of multi-parameter flow cytometry analyses for each experiment are shown in Extended Data Figures. Boundaries between positive and negative cells were defined by use of fluorescence minus one (FMO) controls.

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

Magnetic resonance imaging

Experimental design

Design type	Block design. All animals within one experiment were subjected to MRI on the same day.
Design specifications	Animals were subjected to MRI 2-3 times during one experiment at intervals between 6 and 7 days., i.e. MRI was performed on days 8, 15, and 23 post surgery if not otherwise stated in methods or figure legends.
Behavioral performance measures	As only tumor volume was measured in sedated mice behavioral performance measures were not applicable for this study.

Acquisition

Imaging type(s)	structural T2 weighted structural magnetic resonance imaging
Field strength	9.4 Tesla small animal MRI
Sequence & imaging parameters	Turbo spin echo, coronal aquisition , matrix size 256x256, TE 33ms, TR 2500ms, two averages, flip angle 90°, resolution 78µm x 78µm, slice thickness 0.7mm
Area of acquisition	whole brain scan

Diffusion MRI ☐ Used ☒ Not used

Preprocessing

Preprocessing software	image files were exported as dicom files and segmentation of tumor volumes was performed in Osirix imaging software (version 4.12; Pixmeo) by manual segmentation.
Normalization	normalization was not performed
Normalization template	normalization was not performed
Noise and artifact removal	artifact or noise removal was not performed
Volume censoring	Osirix imaging software (version 4.12; Pixmeo). Volumes were exported to microsoft excel. No censoring was performed

Statistical modeling & inference

Model type and settings	Statistical modeling and inference for the above-mentionend manual volumetric analyses was not performed
Effect(s) tested	Effect testing e.g. modeling of treatment failure or predicted tumor growth was not performed from volumetric analysis data

Specify type of analysis: ☒ Whole brain ☐ ROI-based ☐ Both

Statistic type for inference (See Eklund et al. 2016)	Statistical modeling and inference for the above-mentionend manual volumetric analyses was not performed
Correction	Statistical modeling and inference for the above-mentionend manual volumetric analyses was not performed

Models & analysis

n/a	Involved in the study
☒	☐ Functional and/or effective connectivity
☒	☐ Graph analysis
☒	☐ Multivariate modeling or predictive analysis