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Reporting Summary

Nature Research 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 Research policies, see Authors & Referees and the Editorial Policy Checklist.

Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. 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
☐	☒	An indication of 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 statistics 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
☐	☒	Clearly defined error bars *State explicitly what error bars represent (e.g. SD, SE, CI)*

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection	FACS Diva Software version 8.0.1 (BD Biosciences)
Data analysis	FlowJo v10.2 (Tree Star Inc.), GraphPad Prism version 5.00 (GraphPad Software Inc.)

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 upon request. 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

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Field-specific reporting

Please select 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/authors/policies/ReportingSummary-flat.pdf

Life sciences study design

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

Sample size	For human studies, the sample size of 15 patients and 15 controls was chosen so that a two-tailed t-test comparing groups has 80% power to detect a difference that is 1.1 times the standard deviation of the outcome variable in each group. For animal studies sample sizes were chosen based on historical experience and were variable based on numbers of surviving mice available at experimental time-points or technical limitations.
Data exclusions	Female mice aged 6-12 weeks were included in studies, without additional exclusion criteria employed.
Replication	Data from all animal studies were replicated. Every figure states how many times each experiment had been repeated. To ensure experiments could be reliably reproduced, fully independent experiments were performed as defined by commonly accepted standards.
Randomization	Mice were pooled and then sequentially assigned to each pertinent group.
Blinding	Animal studies were blinded when applicable. Experiments comparing tumor-bearing to non-tumor bearing animals cannot be blinded to investigators due to obvious manifestations of the diseases. Blinded histopathological and immunohistochemical analyses were performed.

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a	Involved in the study	
☒	☐	Unique biological materials
☐	☒	Antibodies
☐	☒	Eukaryotic cell lines
☒	☐	Palaeontology
☐	☒	Animals and other organisms
☐	☒	Human research participants

Methods

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

Antibodies

Antibodies used	For human studies, fluorochrome-conjugated antibodies to CD3 (Cat#557705, Clone: SP34-2, Lot#5352959, 1:20; Cat#558117, Clone: UCHT1, Lot#3186876, 1:100; Cat#557851, Clone: SK7, Lot#3193549), CD4 (Cat#558116, Clone: RPA-T4, Lot#6224744, 1:100; Cat#557695, Clone: RPA-T4, 1:20), CD8 (Cat#565310, Clone: SK1, Lot#7003689, 1:20; Cat#557746, Clone: RPA-T8, Lot#79151, 1:20; Cat#558207, Clone: RPA-T8, 1:100), CD45RO (Cat#563722, Clone: UCHL1, Lot#7096923, 1:20), CD25 (Cat#562403, Clone: M-A251, Lot#7088762, 1:20), CD27 (Cat#558664, Clone: M-T271, Lot#7136657, 1:5), CD127 (Cat#563225, Clone: HIL-7R-M21, Lot#7012862, 1:20), CCR6 (Cat#559562, Clone: 11A9, Lot#7019800, 1:100), CCR7 (Cat#557648, Clone: 3D12, Lot#3186974, 1:20), and CXCR4 (Cat#560669, Clone: 12G5, 1:20) were obtained from BD Biosciences (San Diego, CA). Antibodies to human CD45RA (Cat#304128, Clone: HI100, 1:20) and CXCR3 (Cat#353738, Clone: G025H7, Lot#B228065, 1:100) were obtained from BioLegend (San Diego, CA). Antibodies to human S1P1 (Cat#50-3639-42, Clone: SW4GYPP, Lot#4299074, 1:20) were obtained from eBioscience (San Diego, CA). For murine studies, fluorochrome-conjugated antibodies to CD3 (Cat#557666, Clone: 145-2C11, Lot#7096805, 1:100; Cat#553066, Clone: 145-2C11, Lot#7150784, 1:100), CD4 (Cat#553049, Clone: RM4-5, Lot#4189673, 1:100; Cat#558107, Clone: RM4-5, 1:100), CD8 (Cat#551162, Clone: 53-6.7, Lot#4275549, 1:100; Cat#563234, Clone: 53-6.7, Lot#7047617, 1:100), CD44 (Cat#562464, Clone: IM7, Lot#6205542, 1:100; Cat#559250, Clone: IM7, Lot#25892, 1:100), CD62L (Cat#553152, Clone: MEL-14, Lot#40865, 1:100), NK1.1 (Cat#553164, Clone: PK136, Lot#80219, 1:100), B220 (Cat#558108, Clone: RA3-6B2, Lot#6175996, 1:100), and GR-1 (Cat#553128, Clone: RB6-8C5, Lot#09439, 1:100) were obtained from BD Biosciences (San Diego, CA). Antibodies to murine S1P1 (Cat#FAB7089A, Clone: 713412, Lot#ACNG0216051, 1:10) were obtained from R&D systems (Minneapolis, MN). In vivo therapeutic antibodies (anti-mouse PD-1 (Cat#BE0146, clone: RMP 1-14, Lot#640517M2) and 4-1BB agonist antibody (Cat#BE0169, clone: LOB12.3, Lot#647417M1)) were obtained from Bio-X-cell (West Lebanon, NH).

Validation

Certificates of analysis and validation statements are provided on manufacturer's websites

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

Cell lines studied included murine SMA-560 malignant glioma, CT-2A malignant glioma, E0771 breast medullary adenocarcinoma, B16F10 melanoma, and Lewis Lung Carcinoma (LLC). SMA-560 cells are syngeneic on the VM/Dk mouse background, while all others are syngeneic in C57BL/6 mice.

Authentication

All cell lines have been authenticated by using NIST published 9 species-specific STR markers to establish genetic profiles. Interspecies contamination check for human, mouse, rat, African green monkey and Chinese hamster was also performed for each cell line. The CellCheck Mouse PlusTM cell line authentication and Mycoplasma spp. testing services were provided by IDEXX Laboratories (Westbrook, ME).

Mycoplasma contamination

All cell lines have been tested negative for Mycoplasma spp.

Commonly misidentified lines
(See ICLAC register)

None are among the ICLAC database of commonly misidentified cell lines.

Animals and other organisms

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

Laboratory animals

Female C57BL/6, VM/Dk, and B6.129P2-S1pr1tm1.2Cys/J S1P1-KI mice were used at 6-12 weeks of age. The generation of B6.129P2-S1pr1tm1.2Cys/J (S1P1-KI) mice has been described22. S1P1-KI mice carry a Thr-Ser-Ser (TSS) to Ala-Ala-Ala (AAA) mutation in the C-terminus (the last 12 amino acids) of the sphingosine-1-phosphate receptor 1 (S1P1). This mutation leads to a loss in sensitivity for ligand-mediated receptor down-modulation, leading to the partial block in the desensitization process, resulting in resistance to S1P-mediated S1P1 internalization in naive T-cells. Parental transgenic mice are acquired from the Jackson Laboratory (Bar Harbor, ME) with in-house breeding colony expansion. C57BL/6 mice purchased from Charles River Laboratories (Wilmington, MA) were used as wild-type controls. S1P1 conditional knockout mice were created by crossing B6.129S6(FVB)-S1pr1tm2.1Rlp/J, which contains loxP sites flanking exon 2 of S1pr1 gene (JAX Stock #019141), with B6.Cg-Tg(UBC-cre/ERT2)1Ejb/1J (JAX Stock #007001), which contains tamoxifen-inducible Cre. These two mice were obtained from the Jackson Laboratory (Bar Harbor, ME) and crossed and then back-crossed to obtain mice with the genotype flox/flox Cre (+/-). The mice were then treated with tamoxifen to induce recombination. VM/Dk mice are bred and maintained as a colony at Duke University. Animals were maintained under specific pathogen-free conditions at Cancer Center Isolation Facility (CCIF) of Duke University Medical Center. All experimental procedures were approved by the Institutional Animal Care and Use Committee.

Wild animals

This study did not involve wild animals.

Field-collected samples

This study did not involve samples collected from the field.

Human research participants

Policy information about studies involving human research participants

Population characteristics

In the retrospective study, the median age of GBM patients is 66 yrs (ranging from 21-91 yrs) as compared to 62.5 yrs (ranging from 38-83 yrs) in controls. Female and Male GBM patients account for 46% and 54% of all patients in this cohort, respectively. Female and Male controls account for 39% and 61% of all controls in this cohort, respectively. In the prospective study, the median age of GBM patients is 56 yrs (ranging from 30-75 yrs) as compared to 68 yrs (ranging from 41-86 yrs) in controls. Female and Male GBM patients account for 27% and 73% of all patients in this cohort, respectively. Female and Male controls account for 46% and 54% of all controls in this cohort, respectively. Further details on patient characteristics are found in Supplementary Table 1 and 2.

Recruitment

For retrospective studies, records for all patients with a diagnosis of GBM over a ten-year period at Massachusetts General Hospital were reviewed. Criteria for inclusion and exclusion are as outlined in the results section. For prospective studies, 15 treatment-naïve GBM patients and 15 healthy age-matched controls undergoing spinal fusion were included in the prospective collection of whole blood and bone marrow aspirates. Informed consent was obtained from all subjects.

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	Spleen, thymus, cervical lymph nodes, and long bones of the legs (femur and tibia) were collected at defined and/or humane endpoints, in accordance with protocol. For intracranial tumor-bearing animals, humane endpoints include inability to ambulate two steps forward with prompting. For subcutaneous tumor-bearing animals, humane endpoints include tumor size greater than 20 mm in one dimension, 2000 mm3 in total volume, or tumor ulceration or necrosis. Spleens and thymuses were weighed prior to processing. Briefly, tissues were processed in RPMI, minced into single cell suspensions, cell-strained, counted, stained with antibodies, and analyzed via flow cytometry. Bone marrow cells were flushed out from one femur and one tibia. Blood samples were directly labeled with antibodies and red blood cells subsequently lysed using eBioscience RBC lysis buffer (eBioscience, San Diego, CA) or BD Pharm Lyse (BD Biosciences). Spleen and bone marrow were subjected to RBC lysis prior to antibody-labeling, while lymph nodes and thymus were labeled once single cell suspensions were created.
Instrument	BD LSR Fortessa Cell analyzer (BD Biosciences)
Software	FACS Diva Software version 8.0.1 (BD Biosciences), FlowJo v10.2 (Tree Star Inc.)
Cell population abundance	Flow-Count Fluorospheres from Beckman Coulter (Brea, CA) were used to determine absolute counts on the flow cytometer.
Gating strategy	FSC-A/FSC-H plots were used to determine singlet gates. FSC-A/SSC-A plots were used to determine cell population gates. Isotype controls were used to indicate the boundaries between positive and negative populations.

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