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	Cellranger was used for fastq generation and alignment; Publicly available data was downloaded in TPM format.
Data analysis	Data analysis was performed in R v 4.1.0. Packages used: Seurat - single cell RNA-seq analysis , spatial analysis scalop - single cell RNA-seq analysis ggplot2 , ggpubr - creating plots

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

All single cell RNA-seq produced by this study is available through the GFene Expression Omnibus with GEO accession GSE281796.

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	GS143 is derived from a male patient. Other models have been developed through IRB protocol in which patient-information is unidentifiable.
Reporting on race, ethnicity, or other socially relevant groupings	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	We grew 20 GBM patient-derived gliomasphere models separately and pooled them for stereotaxic injection into the mouse brains (10 models per pool – [Pool #1, Pool #2] with 2 mice replicates) and profiled them by scRNAseq. In addition, 2 of these models were injected into mouse brains individually and profiled by scRNA seq, and 1 model was profiled individually by spatial transcriptomics. No statistical method was used to determine sample sizes. The number of cells injected and profiled was determined based on prior studies and availability of rare invading cancer cell samples.
Data exclusions	Cells that did not pass QC were excluded from further analysis, as described in the methods.
Replication	We used 2 replicates of each model to identify a signature of contralateral invasion (n=4 models X2) or ipsilateral invasion (n=2 models X2). These were further validated in external dataset of spatial transcriptomics (n= 7 models). All attempts at replication were successful.
Randomization	Impact of treatment was not studied and all samples were treatment-naive. Thus, randomization is not relevant.
Blinding	GBM spheroids were pooled for multiplexed in vivo assays without regard to spheroid identity, but experiments were not randomized. Mouse brain tissue collection, processing, and single-cell data generation were performed without the knowledge of model identity. During data analysis, the identities of GBM spheroids used to generate transcriptome data were not blinded.

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	mouse CD45, and an antibody panel for CODEX as described in Table S5.
Validation	We validated the CD45 antibody by performing scRNA-seq of isolated cells and confirmed that this antibody marked mouse immune populations. the CODEX antibody panel was validated previously in Greenwald et al 2024.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	we used the following early passage patient-derived glioma spheroids.:MGG65, MGG70, MGG72, MGG87, MGG97, MGG75, MGG101, GS143, GS152, MGG154, MGG123, L0104, L0125, L0315 , L0512, L0627, L1312, L0306, BT513, L0605, L117
Authentication	Morphology of cells and their forming spheres was used as a test to authenticate each model freshly derived-from patients. Additional authentication was not performed.
Mycoplasma contamination	All the cell lines used in this study were mycoplasma negative, which was confirmed using PCR-based test detecting micrlasma ribosomal DNA.
Commonly misidentified lines (See ICLAC register)	N/A

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	Mouse. Jackson laboratory: NOD.Cg-Prkdc < scid > IL2rg < tm1Wjl > /SzJ (NSG). All mice were housed in specific pathogen-free conditions. Housing rooms employ centrally controlled and monitored light cycles that utilize a 12-hour light / 12-hour dark photoperiod. Individual room has remote temperature setpoint adjustment and maintained within plus or minus 2 degrees throughout a range of 18 - 26 C. Relative humidity within the animal suite is maintained at 30 - 70%.
Wild animals	N/A
Reporting on sex	We employed only female mice for developing multiplexed xenograft models. because each assay only require one or two mice each time. We then confirmed that identified models have the capacity to establish invasion phenotypes both in male and female animals.
Field-collected samples	N/A
Ethics oversight	Institutional Care and Use Committee (IACUC) at Massachusetts General Hospital (2019N000229) and the University of Michigan (PRO00011658)

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	The dissociated cells were first stained with calcein Blue AM (BD Biosciences) and Zombie NIR (BioLegend) for 25 min at 4C, and with anti-mouse CD16/32 (BD Biosciences) for 5 min. After washing cells with ice-cold 1% BSA/PBS, the cells were then stained with PerCP anti-mouse CD45 (clone 30-F11, BD Biosciences) for 30 min at 4C.
Instrument	Becton Dickinson Influx cytometer (Becton Dickinson)
Software	BD FACS Diva and FlowJo
Cell population abundance	Abundance of isolated invading cells vary across samples: nvasive cells 0.1%-70%
Gating strategy	Among viable single cells identified as calcein blue AM positive and Zombine NIR negative to low cells, gating strategy for GFP + cells was established with normal tissue (without GFP+ tumor cells) and cells from primary and invasion regions.

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