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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	Electrophysiology data were collected and analyzed using IgorPro v.5.05A (see below). Calcium imaging data was collected on a two-photon microscope, but analyzed using a modified Python (on Jupyter 5.5) script. Confocal images were acquired using Zen 2011 v8.1.
Data analysis	Statistical tests were conducted using Prism v8.0 (GraphPad) software for most analyses. Bowtie 0.12.7, RSEM 1.2.19, Seurat R v2.0, and RStudio 1.0.136 was used for QC, analysis, and exploration of single cell RNA-seq data. Electrophysiology data were analyzed using a custom program written with Igor Pro v.5.05A software (Wavemetrics). Confocal microscopy image analysis was done using Fiji ImageJ v2.0. Calcium imaging was analyzed using a modified Python (on Jupyter 5.5) script (available on GitHub).

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

All unique materials such as patient-derived cell cultures are freely available and can be obtained by contacting the corresponding author and with a standard MTA with Stanford University. Single cell RNAseq data in Figure 1 was analyzed from publicly available datasets on GEO (Accession GSE89567 and GSE102130). Single cell RNAseq for Extended Data Figure 2 will be made accessible on GEO. Data for all figures can be found in manuscript, in accompanying source data, or from 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	Based on the variance of xenograft growth in control mice, power calculations indicated use of at least 3 mice per genotype to give 80% power to detect an effect size of 20% with a significance level of 0.05. For electrophysiological studies, all studies were replicated across multiple cohorts of mice to verify reproducibility.
Data exclusions	No data were excluded from the analyses.
Replication	All attempts at replication were successful with biological replicates performed on separate cohorts of animals/cells.
Randomization	All animals xenografted with individual cell lines were analyzed in the same way - no randomization was necessary in the design of this study.
Blinding	The experimenter performing histological quantifications was blinded to group allocation.

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

Unique biological materials

Policy information about availability of materials

Obtaining unique materials	All unique materials such as patient-derived cell cultures are freely available and can be obtained by contacting the corresponding author and with a standard MTA with Stanford University.

Antibodies

Antibodies used	Primary antibodies used in immunohistochemistry: chicken anti-neurofilament-H (1:1000; #NFH Aves Labs; Lot#7857983),

chicken anti-neurofilament-M (1:1000; #NFM Aves Labs; Lot#1757985), guinea pig anti-synpasin1/2 (1:500; #106-004 Synaptic Systems), chicken anti-GFP (1:500; #Ab13970 Abcam; Lot#GR3190550-10), mouse anti-human nuclei clone 235-1(1:100; #MAB1281 Millipore; Lot#3189191), rabbit anti-Ki67 (1:500; #Ab15580 Abcam; Lot#GR3198167-1), rabbit anti-cleaved caspase 3 (1:200; #9661S Cell Signaling; Lot#43), mouse anti-MAP2 (1:1000; #MAB3418 Millipore; Lot#1993775), rabbit anti-histone H3.3 K27M mutant (1:500; #190631 Abcam; Lot#GR239194-7), mouse anti-nestin (1:500, #Ab6320 Abcam), rabbit anti-RFP (1:500; #600-401-379 Rockland; Lot#39707), or Streptavidin Alex Fluor 594 conjugate (1:500; #S32356 Thermo Fisher; Lot#1902487)

For secondary antibodies: Alexa 488 donkey anti-chicken IgG (#703-545-155; Lot#142355); Alexa 594 donkey anti-rabbit IgG (#711-585-152; Lot#140019), Alexa 647 donkey anti-mouse IgG (#715-605-150; Lot#139301), Alexa 405 donkey anti-guinea pig IgG (#706-475-148; Lot#140331), Alexa 647 donkey anti-rabbit IgG (#711-605-152; Lot#140551) , or Alexa 594 donkey anti-mouse IgG (#715-585-150; Lot#140149) all used at 1:500 (Jackson Immuno Research).

Validation

All antibodies have been validated in the literature and/or in Antibodypedia for use in mouse immunohistochemistry. To further validate the antibodies on our hands, we confirmed that each antibody stained in the expected cellular patterns and brain-wide distributions for immunohistochemistry. For the case of cleaved caspase-3 staining, we confirmed antibody staining in mouse brain tissue of a disease model as a positive control.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

The eukaryotic cell cultures used are patient-derived cultures of high-grade gliomas generated in the Monje lab from biopsy (SU-pcGBM2) or autopsy tissue (SU-DIPG-VI, SU-DIPGXIII-FL, SU-DIPG25). 293T cells (ATCC) were used only for virus production.

Authentication

Sort Tandem Repeat (STR) fingerprinting is performed every 3 months on all cell cultures to ensure authenticity.

Mycoplasma contamination

All cell cultures are routinely tested for mycoplasma contamination and all cultures used tested negative.

Commonly misidentified lines
(See ICLAC register)

No commonly misidentified lines were used.

Animals and other organisms

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

Laboratory animals

Both male and female NOD-SCID-IL2R gamma chain-deficient (NSG) were used between 4-12 weeks of age.

Wild animals

No wild animals were used.

Field-collected samples

No field-collected samples were used.

Human research participants

Policy information about studies involving human research participants

Population characteristics

The three human subjects whose data was used in Figure 5 were all adult males diagnosed with IDH WT glioblastoma. Clinical information is available in Table 1.

Recruitment

All human electrocorticography data was obtained passively from adult patients undergoing intraoperative brain mapping for surgical resection by co-author S.H.J. Each individual was recruited from a prospective registry of adults aged 18–85 with newly diagnosed frontal, temporal, and parietal IDH-wild type WHO IV glioblastomas. Informed consent for this study was obtained in accordance with the University of California, San Francisco (UCSF) institutional review board for human research (UCSF CHR 17-23215). Subjects with tumors projecting to the cortical surface and at least three electrocorticography channels overlaying healthy-appearing brain (as determined by absence of FLAIR or T1 post gadolinium enhancement) were selected for analysis.