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	All flow samples were run on Attune Flow Cytometers (Thermo Fisher Scientific) or LSR Fortessa (BD). Attune Nxt Software 3.1.2 or BD DIVA 6.1 software were used for data collection. Mass spectrometry data were obtained using Q Exactive HF-X (Thermo Fisher Scientific). The bioluminescence images were captured by an IVIS imaging system (PerkinElmer). Sequencing was done using Illumina NovaSeq 6000. Epoch (BioTek) plate reader was used for requiring absorbance and data was analyzed by Gen5 software. Quantitative real-time PCR was performed using SYBR Green Master mix (cat: 4309155; Thermo Fisher Scientific) on an Applied Biosystems 7900HT Real-Time PCR System. Western blot were imaged using ChemiDoc MP Imaging System (Bio-Rad). Immunofluorescence images were collected using Leica SP8 Laser Confocal Microscope. Date were also collected using standard software, such as Microsoft Excel 2020 and GraphPad Prism version 9.
Data analysis	GraphPad Prism version 9 was used to perform general statistical analyses. FACS data were analyzed with FlowJo v.10. Mass spectrometry was analyzed using Thermo Fish Xcalibur 2.7 and R 4.0.3. ChIP-seq data were analyzed using Bowtie2 (version 2.5.3), FastQC (version 0.12.0), Trimmomatic (version 0.39), deepTools (version 3.5.5), IGV software, MACS2 (version 2.1.2). RNA-seq data were analyzed using Trim galore 0.6.6, Bowtie2 2.4.2, Hisat2 2.2.1, Samtools 1.10, FeatureCounts 1.6.2, DESeq2 1.30.1, ClusterProfiler 3.18.1, FactoMineR 2.9, Python 3.9 and R 4.0.3. Single cell RNA sequencing analysis was performed using Seurat 4.3.0, AUCell 1.20.2, Caret 6.0-94 in R 4.0.3. Virtual screening docking and Surface Plasmon Resonance assay were analyzed using Discovery Studio 2.5 and BIA evaluation software.

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

RNA-seq dataset 1: GSE59612 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE59612)
RNA-seq dataset 2: GSE153746 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE153746)
RNA-seq dataset 3: GSE89623 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi)
RNA-seq dataset 4: GSE140441 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi)
RNA-seq dataset 5: GSE54791 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi)
RNA-seq dataset 6: GSE291583 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE291583)
scRNA-seq dataset 1: GSE182109 (https://www.ncbi.nlm.nih.gov/gds/?term=GSE182109)
scRNA-seq dataset 2: GSE131928 (https://www.ncbi.nlm.nih.gov/gds/?term=GSE131928)
ChIP-seq data: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE291362
Public glioma databases are from GlioVis (https://gliovis.bioinfo.cnio.es/)
Mass spectrometry proteomics dataset 1: PXD072725 (https://www.ebi.ac.uk/pride/archive/projects/PXD072725)
Mass spectrometry proteomics dataset 2: PXD072748 (https://www.ebi.ac.uk/pride/archive/projects/PXD072748)

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 and gender were identified as insignificant factor in analysis. Flow cytometry and IHC staining were performed on both the edge and core regions of tumor tissues from a total of 6 patients, including 4 males and 2 females. Immunofluorescence staining was conducted on 6 patients, including 3 males and 3 females. In Fig. 3, for IHC staining: Normal brain (n=8) included 5 males and 3 females; Low-grade glioma (n=19) included 8 males and 11 females; High-grade glioma (n=30) included 19 males and 11 females; Recurrent glioblastoma (n=10) included 7 males and 3 females. For patient-derived GSCs: GSC456, female; GSC3028, female; GSC387, female; GSC23, male; MES20, unknown.
Reporting on race, ethnicity, or other socially relevant groupings	There was no bias of recruitment in terms of race, ethnicity, or other socially relevant.
Population characteristics	See above.
Recruitment	None selection had been made. Recruitment and collection of human samples adhered to approved IRB protocols.
Ethics oversight	All pathological glioma samples, adjacent brain tissues, and non-tumorous brain tissues (from traumatic brain injury) used in this study were obtained from excess samples resected during neurosurgical procedures at the University of Pittsburgh, Tongji Medical College, Huazhong University of Science and Technology. Written informed consent was obtained from all participants. All patient studies were conducted in accordance with the Declaration of Helsinki.

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	Sample sizes were not statistically predetermined but were chosen based on previous work on GSCs functional analysis (PMID: 34615656, Cancer Discovery, 2022; PMID: 37198486, Nature, 2023). Group sizes for in vivo experiments were selected empirically based upon prior knowledge of the intragroup variation of tumor growth and drug treatment, in which group sizes of 5-10 mice/genotype or treatment are sufficient. Similarly, group sizes for in vitro experiments were selected on the basis of prior knowledge of variation. These sample sizes are sufficient to allow for the determination of statistical significance between groups and minimize the number of animals and replicates needed for each experiment.

Data exclusions	No data were excluded from the analyses.
Replication	Replicates were used in all experiments as indicated in text, figure legends and methods. Animal experiments have been repeated as indicated in the figure legends.
Randomization	All mice were randomized to in vivo treatment. For experiments not involving mice, cells were randomized into experimental groups.
Blinding	Mice were randomly selected for intracranial injection. Blinding was not done due to the requirements for case labeling and staffing needs, as knowledge of the grouping information was essential for the staff to conduct the studies.

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

For immunoblotting and Co-immunoprecipitation:

SOX2 Polyclonal antibody（11064-1-AP, Proteintech, 1:1000）, SOX2 Monoclonal Antibody (20G5)（MA1-014, Thermo Fisher Scientific, WB(1:1000)/IP(5 μg)）, OLIG2 Polyclonal antibody（13999-1-AP, Proteintech, 1:1000）, GFAP (E4L7M) XP® Rabbit mAb（#80788T, Cell Signaling Technology, 1:1000）, Piezo1 (extracellular domain) Polyclonal antibody（15939-1-AP, Proteintech, 1:1000）, PIEZO1 Monoclonal Antibody（MA5-32876, Thermo Fisher Scientific, 1:1000）, FAK Monoclonal antibody（66258-1-Ig, Proteintech, 1:1000）, Phospho-FAK (Tyr397)（8556T, Cell Signaling Technology, 1:1000）, Alpha Tubulin Polyclonal antibody（11224-1-AP, Proteintech, 1:5000）, IQGAP3 Antibody（sc-393451, Santa Cruz Biotechnology, WB(1:200)/IP(5 μg)）, IQGAP3 Polyclonal antibody（25930-1-AP, Proteintech, 1:1000）, TEAD1 (D9X2L) Rabbit mAb（12292T, Cell Signaling Technology, 1:1000）, TEAD2 Polyclonal antibody（21159-1-AP, Proteintech, 1:1000）, TEAD3 Polyclonal antibody（13120-1-AP, Proteintech, 1:1000）, N-Myc (D4B2Y) antibody（51705T, Cell Signaling Technology, 1:1000）, Cdk9 Antibody（sc-13130, Santa Cruz Biotechnology, 1:200）, Anti-KDM1/LSD1 antibody（ab129195, Abcam, 1:1000）, YAP1 Antibody（NBP2-22117SS, Novus Biologicals, WB(1:1000)/IP(5 μg)）, YAP1 Polyclonal antibody（13584-1-AP, Proteintech, 1:1000）, NCAPG2 Antibody（NB100-1813, Novus Biologicals, 1:1000）, WDR62 Polyclonal antibody（23404-1-AP, Proteintech, 1:1000）, Phospho-Histone H2A.X (Ser139) antibody（9718S, Cell Signaling Technology, 1:1000）, Histone H2A.X antibody（7631T, Cell Signaling Technology, 1:1000）, Cleaved PARP antibody（5625T, Cell Signaling Technology, 1:1000）, PARP antibody（9532S, Cell Signaling Technology, 1:1000）, Cleaved Caspase-3 antibody（9661T, Cell Signaling Technology, 1:1000）, Caspase 3/p17/p19 Polyclonal antibody（19677-1-AP, Proteintech, 1:1000）, COBL Polyclonal Antibody（PA5-53941, Thermo Fisher Scientific, 1:1000）, C9orf58/AIF1L Polyclonal Antibody（H00083543-B01P, Thermo Fisher Scientific, 1:1000）, Histone H3 Polyclonal antibody（17168-1-AP, Proteintech, 1:5000）, WWP2 Polyclonal antibody（12197-1-AP, Proteintech, 1:1000）, UBR5 Monoclonal antibody（66937-1-Ig, Proteintech, 1:1000）, CUL4A-Specific Polyclonal antibody（14851-1-AP, Proteintech, 1:1000）, TRIM26 Polyclonal antibody（27013-1-AP, Proteintech, 1:1000）, ANTI-FLAG® antibody（F7425, Sigma-Aldrich, 0.4 μg/ml）, mCherry Polyclonal antibody（26765-1-AP, Proteintech, 1:1000）, ubiquitin antibody（80992-1-RR, Proteintech, 1:1000）, HA tag Polyclonal antibody（51064-2-AP, Proteintech, 1:5000）, Anti-rabbit IgG, HRP-linked Antibody（7074S, Cell Signaling Technology, 1:3000）, Anti-mouse IgG, HRP-linked Antibody（7076S, Cell Signaling Technology, 1:3000）,ATM Polyclonal antibody (27156-1-AP, Proteintech, 1:1000), Phospho-ATM (Ser1981) (5883T, Cell Signaling Technology, 1:1000), ATR Polyclonal antibody (19787-1-AP, Proteintech, 1:1000), Phospho-ATR (Thr1989) (30632T, Cell Signaling Technology, 1:1000), BRCA1 Polyclonal antibody (22362-1-AP, Proteintech, 1:1000), Phospho-BRCA1 (Ser1524) Antibody (9009T, Cell Signaling Technology, 1:1000), Chk1 Polyclonal antibody (25887-1-AP, Proteintech, 1:1000), Phospho-Chk1 (Ser345) (2348T, Cell Signaling Technology, 1:1000), RAD51 (65653T, Cell Signaling Technology, 1:1000), DNA-PKcs Polyclonal antibody (19983-1-AP, Proteintech, 1:500), Phospho-DNA-PKcs (Ser2056) (68716T, Cell Signaling Technology, 1:1000)

For flow cytometry:

Pacific Blue™ Mouse IgG1, κ Isotype Ctrl Antibody（400151, Biolegend, 1:200）, Pacific Blue™ anti-SOX2 Antibody（656111, Biolegend, 1:200）, FITC anti-human CD147 Antibody（306204, biolegend, 1:200）, FITC Mouse IgG1, κ Isotype Ctrl Antibody（400107, biolegend, 1:200）, PE Mouse Anti-Human CD133（566594, BD Biosciences, 1:100）, PE Mouse IgG1, κ Isotype Control（559320, BD Biosciences, 1:100）, PIEZO1 Antibody（NBP1-78446SS, Novus Biologicals, 1:100）, Donkey anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 488（A-21206, Thermo Fisher Scientific, 1:200）

For Immunofluorescence and immunohistochemistry staining:

SOX2 Monoclonal Antibody (20G5)（MA1-014, Thermo Fisher Scientific, 1:200）, Human/Mouse/Rat SOX2 Antibody（AF2018-SP, R&D Systems, 10 µg/mL）, FAK [p Tyr397] Antibody（NBP3-12897, Novus Biologicals, 1:200）, Phospho-Myosin Light Chain 2 (Ser19)（3671T, Cell Signaling Technology, 1:50）, PIEZO1 Monoclonal Antibody（MA5-32876, Thermo Fisher Scientific, 1:100）, IQGAP3 Antibody（sc-393451, Santa Cruz Biotechnology, 1:40）, IQGAP3 Polyclonal antibody（25930-1-AP, Proteintech, IHC (1:50)）, TEAD1 (D9X2L) Rabbit mAb（12292T, Cell Signaling Technology, 1:100）, YAP Antibody（sc-376830, Santa Cruz Biotechnology, 1:50）, GFAP (E4L7M) XP® Rabbit mAb（80788T, Cell Signaling Technology, 1:200）, Anti-Ki67 antibody（ab238020, Abcam, 2 µg/mL）, Phospho-Histone H2A.X (Ser139) (20E3) Rabbit mAb（9718S, Cell Signaling Technology, 1:200）, Donkey anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 488（A-21202, Thermo Fisher Scientific, 1:200）, Donkey anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 488（A-21206, Thermo Fisher Scientific, 1:200）, Donkey anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluo 568（A10037, Thermo Fisher Scientific, 1:200）, Donkey anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 568（A10042, Thermo Fisher Scientific, 1:200）, Donkey anti-Goat IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 568（A-11057, Thermo Fisher Scientific, 1:200）, Donkey anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 647（A-31571, Thermo Fisher Scientific, 1:200）

Chromatin immunoprecipitation:
YAP1 Polyclonal antibody（13584-1-AP, Proteintech, 5 µg）, TEAD1 (D9X2L) Rabbit mAb（12292T, Cell Signaling Technology, 5 µg）, SOX2 Monoclonal Antibody (20G5)（MA1-014, Thermo Fisher Scientific, 5 µg）

Validation

All antibodies for IB, IP, IF, FACS and ChIP were well-recognized in the field and validated by the manufacturers. These antibodies are further validated and routinely used in our lab.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	GSC3028 was derived from a recurrent GBM from a 65-year-old female patient. GSC23 was derived from a recurrent GBM biopsy specimen from a 63-year-old male patient and was provided as a generous gift by Dr. Erik Sulman (NYU Langone Health). GSC456 was derived from a GBM biopsy from an 8-year-old female patient and was provided as a generous gift from Dr. Darell Bigner (Duke University). GSC387 was derived from a 76-year-old female patient. NSC11 was derived from human IPS cells (ALSTEM, Cat# hNSC11). ENSA (ENStem-A) is a human embryonic stem-derived neural progenitor cell (Millipore Sigma, Cat# SCC003). HNP1 (STEMEZ HNP1) is a human neural progenitor cell (Neuro-mics, Cat# HN60001). HEK293T cells, purchased from ATCC (Cat# CRL-3216).
Authentication	All cell lines were routinely subjected to STR testing to confirm their original identity.
Mycoplasma contamination	All cell lines were tested to confirm lack of mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	No cell line used in the paper is listed in ICLAC database.

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	NSG mice (NOD. Cg-Prkdc scid Il2rg tm1Wjl/SzJ, strain 005557, Jackson Laboratory), aged 4–6 weeks, were randomly selected for intracranial injections. All mouse experiments were conducted under the approval of the Institutional Animal Care and Use Committee (IACUC) at the University of Pittsburgh (Protocol #21049014). Mice were housed in specific-pathogen-free conditions, maintained at an ambient temperature of 20–26 °C with 30–70% humidity, and subjected to a 12-hour light–dark cycle. A maximal tumor size of 15 mm in any direction was not exceeded in any expriment. Both male and female mice were included in the studies.
Wild animals	We did not use any wild animals.
Reporting on sex	Both male and female mice were used in studies.
Field-collected samples	No field-collected samples were used in this study.
Ethics oversight	All mouse experiments were conducted under the approval of the Institutional Animal Care and Use Committee (IACUC) at the University of Pittsburgh (Protocol #21049014).

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.*

ChIP-seq

Data deposition

☒ Confirm that both raw and final processed data have been deposited in a public database such as GEO.

☒ Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

Data access links
May remain private before publication.

https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE291362

Files in database submission

raw files:
GSC1IP_1.fq.gz
GSC2IP_1.fq.gz
GSC4IP_1.fq.gz
GSC5IP_1.fq.gz
GSC1IP_2.fq.gz
GSC2IP_2.fq.gz
GSC4IP_2.fq.gz
GSC5IP_2.fq.gz

Processed files:
3028 stiff CONT.bw
3028 stiff shIQGAP3.bw
MES20 stiff CONT.bw
MES20 stiff shIQGAP3.bw

Genome browser session
(e.g. UCSC)

Bigwig files were generated using Deeptools 3.2.1. Signal tracks were visualized using Integrative Genomics Viewer (IGV 2.8.12).

Methodology

Replicates — 1 replicates of each sample in GSC3028 and GSC MES20: shCONT, shIQGAP3.

Sequencing depth — paired-end reads

Antibodies — SOX2 (Thermo Fisher Scientific, cat: MA1-014, 5ug)

Peak calling parameters — Peak calling was performed with MACS2 (version 2.1.2) using default parameters and a p-value cutoff of 1 × 10-4.

Data quality — Quality control was performed using FastQC (version 0.12.0), and adapter trimming was done with Trimmomatic (version 0.39). Read coverage was analyzed using deepTools (version 3.5.5) and visualized in the IGV software.

Software — Bowtie2 (version 2.5.3), FastQC (version 0.12.0), Trimmomatic (version 0.39), deepTools (version 3.5.5), IGV software, MACS2 (version 2.1.2).

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	Brain tumors or GSCs-derived tumors were stained directly using single-cell preparations from dissected tumors, as described in the Materials and Methods section.
Instrument	Attune Flow Cytometers (Thermo Fisher Scientific) and LSR Fortessa (BD)
Software	Attune Nxt Software 3.1.2 and BD DIVA 6.1 software were used for data collection. FlowJo v.10. and ModFit LT v.3.3 were used for data analysis.
Cell population abundance	The population abundance was analyzed with FlowJo v.10.
Gating strategy	All gates were set based on isotype control antibodies after appropriate compensation using single-stained compensation controls.

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