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Life Sciences Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form is intended for publication with all accepted life science papers and provides structure for consistency and transparency in reporting. Every life science submission will use this form; some list items might not apply to an individual manuscript, but all fields must be completed for clarity.

For further information on the points included in this form, see Reporting Life Sciences Research. For further information on Nature Research policies, including our data availability policy, see Authors & Referees and the Editorial Policy Checklist.

Experimental design

1. Sample size

Describe how sample size was determined.

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

2. Data exclusions

Describe any data exclusions.

No data were excluded from the analyses.

3. Replication

Describe whether the experimental findings were reliably reproduced.

All attempts at replication were successful.

4. Randomization

Describe how samples/organisms/participants were allocated into experimental groups.

For patient-derived xenograft studies, animals were IVIS imaged at baseline and randomized based on tumor size by a blinded investigator so that experimental groups contained an equivalent range of tumor sizes.

5. Blinding

Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

The experimenter performing IVIS imaging and histological quantifications was blinded to group allocation.

Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.

6. Statistical parameters

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

n/a	Confirmed
☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
☐	☒ A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ A statement indicating how many times each experiment was replicated
☐	☒ The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section)
☐	☒ A description of any assumptions or corrections, such as an adjustment for multiple comparisons
☐	☒ The test results (e.g. *P* values) given as exact values whenever possible and with confidence intervals noted
☐	☒ A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
☐	☒ Clearly defined error bars

See the web collection on statistics for biologists for further resources and guidance.

Software

Policy information about availability of computer code

7. Software

Describe the software used to analyze the data in this study.

Statistical tests were conducted using Prism (GraphPad) software for most analyses. The limiting dilution assay to test for neurosphere forming capacity was analyzed with a chi-squared test using the Extreme Limiting Dilution Analysis (ELDA) web-based tool (http://bioinf.wehi.edu.au/software/elda/). For RNA-seq analyses, reads were mapped to hg19 annotation using Tophat2 (version 2.0.13) and transcript expression was quantified against RefSeq gene annotations using featureCounts. Differential testing and log2 fold change calculation was performed using DESeq213. Gene Ontology analyses were performed using DAVID. For mass spectrometry, data acquisition and analysis were performed using the Analyst 1.6.1 software (AB SCIEX). Western blots were quantified and analyzed using ImageJ 1.48v. Pharmacokinetics were analyzed using WinNonlin6.3

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). *Nature Methods* guidance for providing algorithms and software for publication provides further information on this topic.

Materials and reagents

Policy information about availability of materials

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.

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.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

Antibodies used in Western blots: Anti-Neuroligin-3 (NovusBio; 1:250; #NBP1-90090 Lot A00876), Anti-phospho FAK pTyr861 (Thermo Fisher Scientific; 1:500; #44-626G Lot QJ221024), and anti-FAK (Cell Signaling Technologies; 1:500; #3285S Lot 9), anti-ADAM10 (Abcam; 1:500; #Ab1997 Lot GR282958-1), anti-rabbit IgG HRP-linked Antibody (Cell Signaling Technologies; 1:2000; #7074S Lot 26)

Antibodies used in immunohistochemistry: Chicken anti-GFP (Abcam; 1:500; #Ab13970 Lot GR236651-11), Rat anti-MBP (Abcam; 1:300; #Ab7349 Lot GR267330-2), Mouse anti-human nuclei clone 235-1 (Millipore; 1:100; #MAB1281 Lot 2886689), rabbit anti-Ki67 (Abcam; 1:500; #Ab15580 Lot GR292681-1), rabbit anti-cleaved caspase 3 (Cell Signaling; 1:200; #9661S Lot 43), mouse anti-NeuN (Millipore;1:2000; #MAB377), Alexa Fluor® 488 AffiniPure Donkey Anti-Chicken IgY (IgG) (H+L) (Jackson Immuno Research; 1:500; #703-545-155 Lot 129193), Goat anti-Mouse IgG Secondary Antibody Alexa Fluor 594 (Life Technologies; 1:500; #R37121 Lot 1572551), Goat anti-Rat IgG Secondary Antibody Alexa Fluor 594 (Life Technologies; 1:1000; #A21209 Lot 1547508), Goat anti-Rabbit IgG Secondary Antibody Alexa Fluor 647 (Life Technologies; 1:500; #A31573 Lot 1693297)

Antibody used in Phospho-tyrosine pull down assay: Anti-phosphotyrosine pY-1000 antibody (Cell Signaling Technologies; #8803)

All antibodies have been validated in the literature and/or in Antibodypedia for use in mouse immunohistochemistry or human cell Western blot analyses. 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) and at the correct mobility (for Westerns). For the case of cleaved caspase-3 staining, we confirmed antibody staining in mouse brain tissue of a disease model as a positive control. For NLGN3 Westerns, we confirmed staining of recombinant NLGN3 protein.

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.

The eukaryotic cell cultures used are patient-derived cultures of high-grade gliomas generated in the Monje lab from biopsy (SU-pcGBM2, SU-GBM035) or autopsy tissue (SU-DIPG-VI, SU-DIPG-XIII-FL, SU-DIPG-XIX).

b. Describe the method of cell line authentication used.

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

c. Report whether the cell lines were tested for mycoplasma contamination.

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

d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.

N/A

Animals and human research participants

Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals

Provide details on animals and/or animal-derived materials used in the study.

Mice of the following genotypes were used:
NOD-SCID-IL2R gamma chain-deficient (NSG); monitored over 6 months
Nlgn3y/-;NSG; monitored over 6 months
Nlgn3fl/fl;Pdgfra::CreER; used at p40
Nlgn3fl/fl;CAMKII::CreER; used at p40
Thy1::ChR2; used between 4-6weeks of age
MMP9-/-; used between 4-6weeks of age
ADAM10fl/fl;CamKII::CreER; used at p40
ADAM10fl/fl;Pdgfra::CreER; used at p40

Because Nlgn3 is a gene on the X-chromosome, all Nlgn3 mouse model experiments were performed with male mice. For all other experiments mice of both sexes were used equally.

Policy information about studies involving human research participants

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.

Patient-derived cell cultures and xenograft models were used and the clinical characteristics of the subject described in the methods or in previous reports. No living human research participants were involved in this study.