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☐ Initial submission ☐ Revised version ☒ Final submission

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.	No statistical methods were used to predetermine sample size. Samples sizes were selected based on previous experience to obtain statistical significance and reproducibility.

2. Data exclusions

Describe any data exclusions.	No data were excluded from the analyses

3. Replication

Describe whether the experimental findings were reliably reproduced.	Experimental findings were reliably reproduced in multiple independent experiments as indicated throughout the manuscript.

4. Randomization

Describe how samples/organisms/participants were allocated into experimental groups.	No randomization method was applied. Male and female mice were included in treated and control groups.

5. Blinding

Describe whether the investigators were blinded to group allocation during data collection and/or analysis.	Investigators were not blinded during data collection and analysis.

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.

1. Raghvendra Mall et al. "RGBM: Regularized Gradient Boosting Machines For The Identification of Transcriptional Regulators Of Discrete Glioma Subtypes". bioRxiv (2017) doi: https://doi.org/10.1101/132670. The RGBM package is available on CRAN at https://cran.r-project.org/web/packages/RGBM/index.html
2. Smoot, Michael E., et al. "Cytoscape 2.8: new features for data integration and network visualization." Bioinformatics 27.3 (2010): 431-432.
3. Isserlin, Ruth, et al. "Enrichment Map–a Cytoscape app to visualize and explore OMICs pathway enrichment results." F1000Research 3 (2014).
4. A collection of the R procedures to perform MWW-GST is available at http://github.com/miccec/yaGST.
5. The topological network was built using the Ayasdi platform (http://www.ayasdi.com).
6. The GraphPad Prism software 6.0 used to perform statistical analyses is available at http://graphpad-prism.software.informer.com/6.0/
7. The X!Tandem/Trans-Proteomic Pipeline (TPP) software suite is available at http://tools.proteomecenter.org/wiki/index.php?title=Software:TPP

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.

No restriction. Data supporting the findings of this study are available from the corresponding authors upon a reasonable request .

9. Antibodies

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

Immunoblot and immunoprecipitation: Antibodies and concentrations are: anti-phosphotyrosine antibodies (4G10, Millipore; PT-66, Sigma; p-Tyr-100, Cell Signaling Technology). FGFR3 1:1000 (Santa Cruz, B-9, sc-13121), PIN4 1:1000 (Abcam, ab155283), PKM2 1:1000 (Cell Signaling, #3198), DLG3/SAP102 1:1000 (Cell Signaling, #3733), GOLGIN 84 1:2000 (Santa Cruz, H-283, sc-134704); C1ORF50 1:1000 (Novus Biologicals, NBP1-81053), HGS 1:1000 (Abcam, ab72053), FAK 1:1000 (Cell Signaling, 3285), Paxillin 1:1000 (BD Transduction, 610051), PGC1a 1:500 (Santa Cruz, H300, sc-13067), PGC1a 1:1000 (Novus Biological, NBP104676), ESRRG 1:500 (Abcam, ab128930), ESRRG 1:500 (R7D, PP-H6812000) p-FRS2 1:1000 (Cell Signaling, 3861), FRS2 1:1000 (Santa Cruz, sc-8318), p-STAT3 1:1000 (Cell Signaling, #9131), STAT3 1:1000 (Santa Cruz, C-20 sc-482,), p-AKT 1:1000 (Cell Signaling, #4060), AKT 1:1000 (Cell Signaling, #9272), p-ERK1/2 1:1000 (Cell Signaling, #4370), ERK1/2 1:1000 (Cell Signaling, #9102), β-actin 1:2000 (Sigma, A5441), PEX1 1:500 (BD Biosciences #611719), PEX6 1:500 (Stress Marq, #SMC-470), NUP214 1:500 (Abcam #ab70497), SEC16A 1:500 (Abcam #ab70722), DHX30 1:500 (Novus Biologicals, NBP1-26203), SUN-2 1:500 (Abcam #ab124916), FLAG 1:1000 (Abcam ab1162), Retinoblastoma 1:1000 (BD Pharmingen 554136), a-tubulin 1:2000 (Sigma, T5168), total OXPHOS 1:1000 (Abcam, #ab110411), MTCO1 1:1000 (Abcam, #ab14705).

Immunofluorescence: The primary antibodies used were as follows: phospho-PIN4 (1:100); PMP70 (Sigma, #SAB420018, 1:20); PEX1 (BD Bioscience, #611719, 1:100); FGFR3 (Santa Cruz, sc-13121 B9, 1:1000). Secondary antibodies were anti mouse Alexa Fluor-647, anti-rabbit Alexa Fluor-568, or Cy3-conjugated (Molecular Probes, Invitrogen).

Immunohistochemistry: Primary antibodies were: COXIV (Cell Signaling # 4850, 1:1,500), VDAC1 (Abcam ab14734, 1:700), NDUFS4 (Abcam ab55540, 1:700), phosho-Y122-PIN4 (1:200) and FGFR3 (Santa Cruz, B9, sc-13121, 1:500).

Drosophila immunofluorescence: Primary antibodies were: rat anti-phospho-Histone-H3 (Abcam ab10543, 1:300) and mouse anti-repo (Developmental Study Hybridoma Bank, 1:60).
All antibodies were validated by the manufacturers and by extensive use in published work.
The anti-phospho-PIN4 antibody was generated by immunizing rabbits with a short synthetic peptide containing the phosphorylated Y122 (PVKTKFG–pY–HIIMVE) (Yenzym Antibodies, LLC). The antibody was validated in cells in which the endogenous PIN4 had been silenced by specific shRNA. The extent of PIN4 silencing was confirmed by western blot (See Methods, page 70 and page 72).

10. Eukaryotic cell lines

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

Cell lines have been purchased from certified vendors (ATCC and SIGMA) (See Methods, pages 67-68). Human GBM-derived GSCs were isolated as described (Singh, D. et al. Transforming fusions of FGFR and TACC genes in human glioblastoma. Science 337, 1231-1235, 2012).

Murine glioma stem cells were obtained from malignant glioma generated by transduction of cells of the dentate gyrus using lentivirus containing expression cassettes for FGFR3-TACC3 or RAS12V oncogenes and shTP53. (See Methods, page 67).

b. Describe the method of cell line authentication used.

We authenticate stable and primary cell lines by STR using the ATCC service. (See Methods, page 68).

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

Cell lines are regularly tested for mycoplasma contamination (See Methods, page 68).

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.

We have not used cell lines listed in the database of commonly misidentified cell lines.

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.

All animal studies were approved by the IACUC committee at Columbia University (Protocols: #AAAQ-2459; AAAL7600) We reported strain (athymic nude), sex (both female and male mice) and age (4-6 week old) For sub-cutaneous tumor xenograft studies, male and female mice were included; mice were humanely euthanised when the larger diameter of the tumor reached 20 mm, or when mice displayed a loss of body weight equal to or greater than 20% of total body mass, or showed signs of compromised health according to IACUC approved protocols (see Methods pages 69-70).
Murine glioma stem cells were obtained from malignant glioma generated by transduction of cells of the dentate gyrus using lentivirus containing expression cassettes for FGFR3-TACC3 or RAS12V oncogenes and shTP53. (See Methods, page 67).
The UAS-F3-T3 flies were generated by inserting the human F3-T3 fusion gene into the pACU2 plasmid followed by embryo injection of the plasmid and selection of the correct transgenic fly. All other genotypes were established through standard genetics. repo-Gal4 was used to drive gene expression in the glial lineage. UAS-eGFP or UAS-mRFP were introduced to visualize and quantify tumor volume. repo-Gal4; UAS-dEGFRλ; UAS-Dp110CAAX (as previously described in Read, R. D., Cavenee, W. K., Furnari, F. B. & Thomas, J. B. A drosophila model for EGFR-Ras and PI3K-dependent human glioma. PLoS Genet 5, e1000374, doi:10.1371/journal.pgen.1000374, 2009) and repo-Gal4; UAS-F3-T3 stocks were balanced over the CyoWeeP and TM6B balancers. srl RNAi lines were obtained from the Bloomington Drosophila Stock Center (BDSC) and the Vienna Drosophila Resource Center (VDRC): P{KK100201}VIE-260B (VDRC v103355), y1 sc* v1; P{TRiP.GL01019}attP40 (BDSC 57043), y1 sc* v1; P{TRiP.HMS00857}attP2 (BDSC 33914), and y1 sc* v1; P{TRiP.HMS00858}attP2 (BDSC 33915). The following ERR RNAi lines were used: y1 v1; P{TRiP.JF02431}attP2 (BDSC 27085), y1 v1; P{TRiP.HMC03087}attP2 (BDSC 50686), and P{KK108422}VIE-260B (VDRC v108349). y1 v1; P{UAS-GFP.VALIUM10}attP2 (BDSC 35786) was used as a control from RNAi experiments.

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.

The study did not involve human research participants

Corresponding author(s): Antonio Iavarone

☐ Initial submission ☐ Revised version ☒ Final submission

Flow Cytometry Reporting Summary

Form fields will expand as needed. Please do not leave fields blank.

Data presentation

For all flow cytometry data, confirm that:

☒ 1. The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☒ 2. 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).

☒ 3. All plots are contour plots with outliers or pseudocolor plots.

☒ 4. A numerical value for number of cells or percentage (with statistics) is provided.

Methodological details

5. Describe the sample preparation.	Cell were stained in vivo with Mitotracker Red (Life Technologies, M7512) for 20-30 min at a final concentration of 20-40nM. Cells were then washed with PBS, trypsinized, collected in Phenol Red Free Medium, strained and analyzed by flow cytometry.
6. Identify the instrument used for data collection.	Acquisition was performed on LSR II Flow Cytometer (BD Biosciences, San Jose, CA) on the basis of forward and sideward scatter parameters and Texas red fluorescence.
7. Describe the software used to collect and analyze the flow cytometry data.	Acquisition: BD FACSDiva Software; analysis: FCS Express 6 Flow.
8. Describe the abundance of the relevant cell populations within post-sort fractions.	Eight o ten thousands events from each sample were evaluated.
9. Describe the gating strategy used.	The gating strategy includes all viable cells and singlets.

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