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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	The following software were used in data collection: Agilent MassHunter Profinder (version 10.0); Agilent MassHunter Quantitative Analysis (version B.08.02); Agilent Masshunter Workstation Software LC/MS Data Acquisition for 6400 Series Triple Quadrupole MS (version B.08.02); Bruker Daltonics SciLS Lab 2023b with an in-house script utilizing the SCiLS REST API (version 6.2.114), written in R (version 4.2.2), using RStudio (2022.12.0 Build 353); Bruker Daltonics SCiLS Lab 2023a with an in-house R (version 4.1.1) pipeline using rMSIproc (verson 0.3.1) and enviPat (version 2.7).
Data analysis	The following data analysis software were used: GraphPad Prism (version 10) R (versions 4.4.2 and 4.2.2) MATLAB R2021b with Artelys Knitro toolbox (version 12.4) MetaboScape (2023 version) TASQ (2022 version) FastQC (version 0.12.1) samtools (version 1.13) htseq-count (version 2.0.9) biomaRt library (version 2.54.0) Seurat (version 4.2.0) AUCell (version 1.28.0) MetaboAnalyst (versions 5.0 and 6.0) Maven (version 8.1.27.11) QuPath (version 0.5.1)

GenePattern ssGSEA (version 5)
GE02R (no version information found, accessed on Dec 27, 2024)
GlioVis (version 0.20)
AccuCor (version 0.3.1)
For flux studies using LC-MS data, custom code was used and deposited on GitHub: https://github.com/baharm1/iMFA/

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 data generated in this study are accessible through GEO Series accession number GSE299102 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE299102). The two RNA-seq datasets used in this study are available through GEO with accession numbers of GSE59612 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE59612) and GSE165595 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE165595). For molecular subtype classification based on RNA-seq data, TCGA-GBM dataset was accessed from GDC portal (https://portal.gdc.cancer.gov/; Dec 13, 2024). All other data supporting the findings of this study are available within the paper and its supplementary contents.

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	Sexes of the 8 participants were determined using the "Legal Sex" data element in the medical record. The small number of patients (6 men and 2 women) prevents well-powered analysis by sex.
Reporting on race, ethnicity, or other socially relevant groupings	We did not consider socially constructed or socially relevant categorization variables due to the small number of patients.
Population characteristics	Covariate-relevant population characteristics (sex, diagnosis, IDH status, 1p/19q deletion, MGMT promoter methylation, Ki-67 index) are shown in Extended Data Table 1.
Recruitment	We recruited patients undergoing standard-of-care craniotomies for resection of newly diagnosed brain tumors at the University of Michigan. Our study was limited to brain tumor patients undergoing tumor resection that would yield enough excess tissue for metabolomic analysis, introducing potential self-selection bias toward larger tumors. Thus, our findings may not be applicable to patients whose presentation and tumor burden require diagnostic biopsy alone rather than larger tumor resection.
Ethics oversight	This study was approved by the Institutional Review Board of the University of Michigan.

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 for animal experiments were determined by preliminary studies and the level of observed effect. Sample size for humans was determined by maximal enrollment.
Data exclusions	Mice failing to form intracranial tumors post-orthotopic implantation (<5%) or dying 0-5 days after implantation (<5%) or jugular/carotid catheter placement (<10%), likely due to surgical complications, were excluded. In LC-MS experiments, metabolites below detection thresholds were excluded. One mouse was excluded due to an unrelated condition requiring its humane euthanasia. The tumor sample from one control HF2303-bearing mouse with isotopic glucose infusion was excluded due to diffuse growth inseparable from cortical tissue. In developing our hypothesis-generating metabolic flux models of nucleotide synthesis, the need for multiple timepoints constrained sample numbers at each timepoint, and exclusions were necessary to minimize variance within groups and ensure data robustness. Corresponding

exclusion criteria are described at https://gitfront.io/r/baham1/31F7SiKgPJAw/iMFA/, and model-based predictions were validated by multiple orthogonal tracers.

Replication

Replication was limited by complexity of patient infusion studies and restricted tissue quantities; data from 8 human infusions are reported. In mice, labeled glucose infusion showed consistent tumor vs. cortex metabolic labeling in 3 orthotopic models in this study (GBM38 [≥3 times], HF2303, GBM12), matching human tracing. Similar consistency was observed in ≥5 serial labeled glucose injection experiments across HF2303, GBM38, and 2 additional models not presented. Isotopic serine infusion uptake was consistent across 3 models in this study (HF2303, GBM38, GBM12) and 1 additional model not shown, with GBM38 uptake replicated via tracer injection (not presented). Studies with amide-labeled glutamine infusions showed consistent GBM vs. cortex label patterns in ≥3 independent experiments. In vitro gliomasphere experiments were performed 3 times. All attempts at replicating the experiments were successful.

Randomization

Patients were not allocated into groups. Animal allocation was performed to achieve similar starting median tumor luminescence across groups.

Blinding

Blinding was not possible due to the complexity of multi-step procedures: group allocation, animal treatments, animal monitoring, data collection, and analyses were performed independently by separate investigators, including technicians unaware of hypotheses and without stake in outcome, minimizing bias.

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

Anti-Ki-67 antibody was purchased from BD Biosciences (catalog 550609). Secondary antibody was included in the Vectastain Elite ABC Kit (PK-6102).

Validation

The anti-Ki-67 antibody (BD Biosciences 550609) used for immunohistochemistry was validated based on manufacturer testing (https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/purified-mouse-anti-ki-67.550609?tab=product_details), our results, and its extensive use in peer-reviewed literature (cited over 700 times with at least 10 published images) such as Cell Death Dis 11,19(2020). In our experiments, presence of Ki-67 in tumor and not host brain further indicates specificity and agrees with in vivo tumor models and clinical practice.
This antibody was developed for the immunohistochemistry application. Applications below have been tested by the manufacturer.
Source: Mouse IgG1, κ
Species Reactivity: Human (QC Testing), Mouse (Tested in Development), Rat, Rhesus (Reported)
Application: Flow cytometry (Routinely Tested), Immunohistochemistry-frozen, Immunohistochemistry-formalin (antigen retrieval required) (Tested During Development)

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)

GBM374gs gliomaspheres were generated from a male patient at UCLA (Los Angeles, CA) by the laboratory of Dr. Harley Kornblum. GBM12 PDX tissue (from a male patient) and GBM38 PDX tissue (from a female patient) were generated at Mayo Clinic (Rochester, MN) by the laboratory of Dr. Jann Sarkaria. The HF2303 model was generated from a male patient at Henry Ford Hospital (Detroit, MI) and was provided by Dr. Alnawaz Rehemtulla, Dr. Ana deCarvalho, and the Henry Ford Health System.

Authentication

The GBM374gs gliomasphere model was authenticated regularly via short tandem repeat (STR) fingerprinting using the GenePrint 10 system (Laragen). All formal experiments using GBM12, GBM38, and HF2303 patient-derived models were conducted in vivo with the exception of in vitro culturing for lentiviral GFP/luciferase transduction, and were not authenticated beyond primary isolation. In vivo passage and marker retention ensured identity of these orthotopic models.

Mycoplasma contamination

All models cultured in vitro were tested regularly for mycoplasma using the MycoAlert Mycoplasma Detection Kit (Lonza) and confirmed negative.

Commonly misidentified lines (See ICLAC register)	No commonly misidentified lines were used.

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	Mice of age 4-12 weeks at experiment start, strains B6.129S7-Rag1tm1Mom (Jackson 002216 or bred in-house) and C57BL/6J (Jackson 000664), were used in this study.
Wild animals	This study did not involve wild animals.
Reporting on sex	Sex-based analyses could not be performed in humans due to low sample number and was therefore not extensively performed in mice. All studies were carried out with a mix of male and female mice, except for the time-course glucose tracing experiment that comprised only female mice and was validated using non-glucose tracers in both male and female mice.
Field-collected samples	This study did not involve samples collected from the field.
Ethics oversight	All animal studies were performed at the University of Michigan according to protocols approved by the University of Michigan Institutional Animal Care and Use Committee (IACUC).

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