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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.

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	No software was used
Data analysis	ABSOLUTE v.1.0.6. CNVkit v.0.9.5. Cytoscape v.3.7.2. Combat sva package v.3.36.0. Comfocal v.1. DoubletDecon v.1.1.6. htseq-count v.0.11.1. GATK v.3.7-0. GISTIC2 v.2.0.22. GraphPad Prism v. 6.0. inferCNV v.0.99.7. Ingenuity Pathways Knowledge Base QIAGEN IPA 2020 Release. MAGIC v.1.5.7. Minfi v.1.24.0. MiRwalk v.2.0. MWW-GST v.2017.08.25. Partitioning Around Medoids (PAM) clustering algorithm. PhyC v.0.2.0. R v.3.4.4. randomForest R package. RGBM v.1.0.8. Rtsne v.0.15. Set cover pathway redundancy.

STAR v.2.0.5.
STREAM v.0.3.8.
ssGSEA.
TCGAbiolinks R/Bioconductor package v.2.14.0.
TRANSFAC v.7.0.
UNCOVER v.1.0.0.
yaGST v.1.

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. 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

Single cell RNAseq dataset 1, 2 and 3 are available from Gene Expression Omnibus (GEO, accession numbers GSE117891, GSE103224 and GSE131928, respectively). Single cell RNAseq read count data for the additional samples in dataset 2 (PJ053, PJ069 and PW032.706) and sequencing data for PDC samples (gene expression read counts, genetic mutations and copy number segmentation) have been submitted to Synapse (http://synapse.org accession number syn22314624). TCGA GBM data (Agilent chip G4502A and RNA-Seq gene expression, somatic mutation calls, Affymetrix SNP 6.0 array copy number segmentation, Illumina Infinium Human Methylation 450 K DNA methylation, Agilent miRNA and reverse phase protein array (RPPA) quantification data) are available from the GDC Data Portal (https://portal.gdc.cancer.gov/). CGGA GBM dataset is available from the CGGA data portal (http://cgga.org.cn/index.jsp). Data from Ref.69 are available from GEO (accession number GSE13041). Data from Ref.17 are available at the Sequence Read Archive (SRA, accession number SRP074425), European Genome-phenome Archive (EGA, accession numbers EGAS00001001033, EGAS00001000579, EGAS00001001044, EGAS00001001041, EGAS00001001800), Japanese Genotype-phenotype Archive (accession number JGAS00000000004). GBM longitudinal GLASS dataset is available from Synapse (http://synapse.org/glass). Further information is available from the corresponding authors upon reasonable request.
The list of figures that have associated biological raw data are:
Figure 5 a, b, c, d, e, f, i;
Figure 7 a, c, e, g, i;
Figure 8 a, b, c, d, f, g, i, j, k;
Extended Data Figure 9 b, c

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	We collected as many dataset as possible within our timeframe for evaluation by multimodal pipelines. No statistical methods were used to predetermine sample size. Sample sizes were chosen based on data availability and on previous studies that showed robust statistical power. All available samples passing the quality control were included.
Data exclusions	No data were excluded.
Replication	At least three technical replicates were performed and experiments were repeated at least two times with similar results. All attempts at replication were successful.
Randomization	Our work does not include clinical or biospecimen-based studies and therefore there was no requirement for randomization in any of the experiments performed.
Blinding	Molecular classification was performed independent of and blinded to the clinical features. Investigators were blinded to the clinical and molecular features during experiments and outcome assessments.

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
☒	☐ Animals and other organisms
☐	☒ Human research participants
☒	☐ Clinical data

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used

FLAG (Sigma, F1804, M2, monoclonal, 1:1000).
V5 (Invitrogen, #R960-25, 1:1000).
beta-actin (Sigma, A5441, AC-15, monoclonal, 1:4000).
HRP-conjugated goat-anti-mouse secondary (Invitrogen, 31438, 1:10000).

Validation

Antibodies used were commercially available and were validated in multiple previous studies. The following are the Research Resource Identifiers (RRIDs) from the Resource Identification Portal, supporting guidelines for Rigor and Transparency in scientific publications.
FLAG, RRID:AB_262044.
V5, RRID:AB_2556564.
beta-actin, RRID:AB_476744.
HRP-conjugated goat-anti-mouse secondary, RRID:AB_228217.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

U87 (ATCC HTB-14).
HEK293T (ATCC CRL-11268).
H502 and H423 (generous gift from Darrel Bigner, Duke University Medicak Center, Durham).
Patient-derived cells (PDCs) were obtained using excess material collected for clinical purposes from de-identified brain tumor specimens. Donors (patients diagnosed with glioblastoma) were anonymous. Progressive numbers were used to label specimens coded in order to preserve the confidentiality of the subjects. Work with these materials was designated as IRB exempt under paragraph 4 and it is covered under IRB protocol #IRB-AAAI7305 and Onconeurotek tumor bank certification (NF S96 900) and authorization from Ethics committee (CPP Ile de France VI, ref A39II), and the French Ministry for research (AC 2013-1962).

Authentication

Cell authentication was performed using short tandem repeats (STR) at the ATCC facility.

Mycoplasma contamination

Cells were routinely tested for mycoplasma contamination using the Mycoplasma Plus PCR Primer Set (Agilent Technologies) and were found to be negative.

Commonly misidentified lines
(See ICLAC register)

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

Human research participants

Policy information about studies involving human research participants

Population characteristics

Patient-derived cells (PDCs) were obtained using excess material collected for clinical purposes from de-identified brain tumor specimens and are obtained under exempt IRB protocols. Specimens were de-identified before reaching the research lab and link to personal data are not available to the research lab.

Recruitment

Study under protocol #IRB- AAAI7305 does not involve recruitment procedures; this is exempt research.

Ethics oversight

Work with these materials was designated as IRB exempt under paragraph 4 and it is covered under IRB protocol #IRB- AAAI7305 and Onconeurotek tumor bank certification (NF S96 900) and authorization from Ethics committee (CPP Ile de France VI, ref A39II), and the French Ministry for research (AC 2013-1962).

Note that full information on the approval of the study protocol must also be provided in the manuscript.