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	Cell Ranger v5.0.1 (10x Genomics): Used for primary processing of raw single-cell RNA-seq and CITE-seq sequencing data, including read alignment to the GRCh38 reference genome and generation of count matrices. Space Ranger v3.0 (10x Genomics)Used for alignment and processing of raw Visium spatial transcriptomics sequencing data, including spot-level gene expression quantification and image alignment. 10x Genomics Xenium instrument software: Used for image acquisition, transcript detection, and generation of raw cell-by-gene count matrices for in situ spatial transcriptomics experiments. Illumina HiSeq 2500 and NextSeq 550 control software: Used for sequencing data acquisition for single-cell, CITE-seq, and spatial transcriptomics libraries. NIS-Elements (Nikon): Used for acquisition and preprocessing of high-resolution brightfield H&E images.
Data analysis	R (v4.5.2) and associated packages including -- Seurat, scran, inferCNV, scMAGIC, clusterDE, DESeq2, survival, survminer, ComplexHeatmap, circlize, ggplot2, EnhancedVolcano, caret, Rediscover, SPATA2. Harmony used for batch correction of single-cell datasets. Python (v3.9.23) and associated packages including -- Scanpy, cellphonedb, stlearn. Cytoscape (v3.10.3) -- Used for visualization of spatial neighborhood interaction networks. BioRender -- Used for creating Figure 1 DINO-DETR -- Used for deep learning–based nuclei detection and cell density estimation from H&E images. Custom analysis scripts -- All scripts used for data processing, integration, statistical analysis, and figure generation are publicly available at: https://doi.org/10.5281/zenodo.17622242

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

Raw single-cell RNA-sequencing data generated in this study have been deposited in the NCBI Sequence Read Archive (SRA) under BioProject accession codes to be released upon publication (current submission IDs: SUB15673052). Raw Visium spatial transcriptomics data have been deposited in the SRA under accession codes to be released upon publication (current submission IDs: SUB15686159).
Processed spatial transcriptomics data, corresponding images, and associated metadata are available on Zenodo under the following DOIs: 10.5281/zenodo.17622242 (Xenium) [http://doi.org/10.5281/zenodo.17622242] and 10.5281/zenodo.17572905 (Visium) [http://doi.org/10.5281/zenodo.17572905].
Single-cell RNA-seq, CITE-seq, and spatial transcriptomics data generated in this study, together with associated cell type annotations, are available via the UCSC Cell Browser under the dataset identifier multiomic-gbm [https://cells.ucsc.edu/?ds=multiomic-gbm]. These data are publicly available and do not require restricted access.
Bulk RNA-sequencing datasets analysed in this study were obtained from the following public repositories:
TCGA glioma dataset [https://portal.gdc.cancer.gov/]
CGGA glioma dataset [http://www.cgga.org.cn/]
GLASS consortium glioma dataset [https://glass-consortium.org]
Previously published spatial transcriptomics datasets analysed in this study include:
GSE237183 [https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE237183] (Greenwald et al., 10x Visium glioblastoma dataset)
Ravi et al. 10x Visium glioblastoma dataset [https://zenodo.org/records/16505469]
Previously published single-cell and single-nucleus RNA-sequencing datasets analysed in this study include:
Allen Institute human motor cortex single-nucleus RNA-seq dataset [http://www.brain-map.org]
GSE162631 [https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE162631] (Xie et al.)
Winkler et al. vascular single-cell RNA-seq dataset [https://adult-brain-vasc.cells.ucsc.edu/]
GSE131928 [https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE131928] (Abdelfattah et al.)
GSE163120 [https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE163120] (Pombo Antunes et al., CITE-seq)
GSE163108 [https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE163108] (Mathewson et al.)
The Source Data files are provided on Zenodo (10.5281/zenodo.18093125).

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 was recorded for all human participants. The study cohort comprised 14 female and 14 male individuals. Sex was not considered as a variable in downstream analyses, and no sex-based analyses were performed due to limited sample size and the primary focus of the study on tumor-intrinsic transcriptional and spatial programs.
Reporting on race, ethnicity, or other socially relevant groupings	All participants self-identified as Korean. Race, ethnicity, or other socially relevant groupings were not included as variables in downstream analyses.
Population characteristics	Population characteristics, including age, sex, diagnosis, and clinical annotations, are summarized in Supplementary Table 1.
Recruitment	Consecutive patients scheduled for glioma surgery were recruited.
Ethics oversight	The study protocol was approved by the Institutional Review Board of Seoul National University Hospital (IRB Nos. H-0507-509-153 and H-2010-123-1166).

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	No formal sample size calculation was performed. Sample sizes were determined by tissue availability and the feasibility of high-dimensional single-cell and spatial profiling. Single-cell RNA sequencing was performed on samples from 16 patients (9 glioblastoma, 3 IDH-mutant

astrocytoma, 1 oligodendroglioma, and 3 non-neoplastic or miscellaneous brain samples). CITE-seq was performed on samples from 10 patients (9 glioblastoma and 1 non-neoplastic control). Visium spatial transcriptomics was performed on samples from 17 patients (13 glioblastoma, 3 IDH-mutant astrocytoma, and 1 non-neoplastic control). Xenium in situ spatial transcriptomics was performed on tissue sections from 4 glioblastoma patients, with tissue selection designed to capture intratumoral anatomic heterogeneity.

Data exclusions: No data was excluded

Replication: Key findings were replicated using independent technologies, including in situ spatial transcriptomics with the 10x Genomics Xenium platform and immunohistochemistry. All replication attempts yielded consistent results.

Randomization: This study did not involve experimental group assignment, and randomization was therefore not applicable.

Blinding: This study did not involve interventional experimental groups, and blinding was therefore not applicable.

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

Immunofluorescence (IF)
The following primary antibodies were used for immunofluorescence on frozen mouse brain sections:
Anti-Cre recombinase (mouse monoclonal; Millipore Sigma, MAB3120; 1:500)
Anti-Serpina3n (rabbit polyclonal; Thermo Fisher Scientific, MA5-41236; 1:200)
Anti-Opalin (rabbit polyclonal; Abcam, ab121425; 1:300)
Anti-S100a1 (rabbit polyclonal; Thermo Fisher Scientific, PA1-932; 1:300)
Anti-B2M (rabbit polyclonal; Thermo Fisher Scientific, PI701250; 1:500)
Anti-Sox10 (goat polyclonal; R&D Systems, AF2864; 1:100)
Secondary antibodies raised in donkey and conjugated to Alexa Fluor 488, 594, or 647 were obtained from Jackson ImmunoResearch Laboratories or Invitrogen and used at 1:200 dilution.
Antibody validation was performed according to manufacturer specifications and prior literature.
Immunohistochemistry (IHC)
The following antibodies were used for immunohistochemistry on formalin-fixed paraffin-embedded tumor sections using the Ventana BenchMark ULTRA system:
Anti-GSN (Invitrogen, PA5-29910)
Anti-MAG (Cell Signaling Technology, D4G3)
Anti-NGFR (Thermo Fisher Scientific, MA5-31968)
Antibodies were validated by the manufacturers for immunohistochemical applications.
CITE-seq
For CITE-seq experiments, cells were stained using a commercially available oligonucleotide-conjugated antibody panel: TotalSeq™-C Human Universal Cocktail, V1.0 (BioLegend, 399905)
Fc receptor blocking was performed using Human TruStain FcX™ (BioLegend, 422301). All procedures were carried out according to the manufacturer's instructions. Individual antibodies within the panel were validated by the manufacturer.

Validation: Primary antibodies used for immunofluorescence and immunohistochemistry were validated by the manufacturers for the indicated applications and species, and were used according to established protocols. For CITE-seq experiments, oligonucleotide-conjugated antibodies were obtained as part of a commercially available panel (TotalSeq™-C Human Universal Cocktail) and were validated by the manufacturer.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s): *State the source of each cell line used and the sex of all primary cell lines and cells derived from human participants or vertebrate models.*

Authentication	*Describe the authentication procedures for each cell line used OR declare that none of the cell lines used were authenticated.*
Mycoplasma contamination	*Confirm that all cell lines tested negative for mycoplasma contamination OR describe the results of the testing for mycoplasma contamination OR declare that the cell lines were not tested for mycoplasma contamination.*
Commonly misidentified lines (See ICLAC register)	*Name any commonly misidentified cell lines used in the study and provide a rationale for their use.*

Palaeontology and Archaeology

Specimen provenance	*Provide provenance information for specimens and describe permits that were obtained for the work (including the name of the issuing authority, the date of issue, and any identifying information). Permits should encompass collection and, where applicable, export.*
Specimen deposition	*Indicate where the specimens have been deposited to permit free access by other researchers.*
Dating methods	*If new dates are provided, describe how they were obtained (e.g. collection, storage, sample pretreatment and measurement), where they were obtained (i.e. lab name), the calibration program and the protocol for quality assurance OR state that no new dates are provided.*

☐ Tick this box to confirm that the raw and calibrated dates are available in the paper or in Supplementary Information.

Ethics oversight	*Identify the organization(s) that approved or provided guidance on the study protocol, OR state that no ethical approval or guidance was required and explain why not.*

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

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	Laboratory animals used in this study were mice on a C57BL/6 genetic background. Compound transgenic mice (BRAF CA/WT; Ink4a/Arf flox/flox) were generated as previously described and maintained under standard housing conditions.
Wild animals	This study did not involve wild animals.
Reporting on sex	Sex was not considered as a biological variable in the animal experiments and was not recorded, as the study involved histological validation and was not designed to assess sex-specific effects.
Field-collected samples	This study did not involve samples collected from the field.
Ethics oversight	All animal procedures were performed in accordance with institutional and federal guidelines and were approved by the Stanford University Administrative Panel on Laboratory Animal Care (APLAC protocol #33633).

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

Clinical data

Policy information about clinical studies

All manuscripts should comply with the ICMJE guidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

Clinical trial registration	*Provide the trial registration number from ClinicalTrials.gov or an equivalent agency.*
Study protocol	*Note where the full trial protocol can be accessed OR if not available, explain why.*
Data collection	*Describe the settings and locales of data collection, noting the time periods of recruitment and data collection.*
Outcomes	*Describe how you pre-defined primary and secondary outcome measures and how you assessed these measures.*

Dual use research of concern

Policy information about dual use research of concern

Hazards

Could the accidental, deliberate or reckless misuse of agents or technologies generated in the work, or the application of information presented in the manuscript, pose a threat to:

No	Yes	
☒	☐	Public health
☒	☐	National security
☒	☐	Crops and/or livestock
☒	☐	Ecosystems
☒	☐	Any other significant area

Experiments of concern

Does the work involve any of these experiments of concern:

No	Yes	
☒	☐	Demonstrate how to render a vaccine ineffective
☒	☐	Confer resistance to therapeutically useful antibiotics or antiviral agents
☒	☐	Enhance the virulence of a pathogen or render a nonpathogen virulent
☒	☐	Increase transmissibility of a pathogen
☒	☐	Alter the host range of a pathogen
☒	☐	Enable evasion of diagnostic/detection modalities
☒	☐	Enable the weaponization of a biological agent or toxin
☒	☐	Any other potentially harmful combination of experiments and agents

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.

For "Initial submission" or "Revised version" documents, provide reviewer access links. For your "Final submission" document, provide a link to the deposited data.

Files in database submission

Provide a list of all files available in the database submission.

Genome browser session
(e.g. UCSC)

Provide a link to an anonymized genome browser session for "Initial submission" and "Revised version" documents only, to enable peer review. Write "no longer applicable" for "Final submission" documents.

Methodology

Replicates

Describe the experimental replicates, specifying number, type and replicate agreement.

Sequencing depth

Describe the sequencing depth for each experiment, providing the total number of reads, uniquely mapped reads, length of reads and whether they were paired- or single-end.

Antibodies

Describe the antibodies used for the ChIP-seq experiments; as applicable, provide supplier name, catalog number, clone name, and lot number.

Peak calling parameters	*Specify the command line program and parameters used for read mapping and peak calling, including the ChIP, control and index files used.*
Data quality	*Describe the methods used to ensure data quality in full detail, including how many peaks are at FDR 5% and above 5-fold enrichment.*
Software	*Describe the software used to collect and analyze the ChIP-seq data. For custom code that has been deposited into a community repository, provide accession details.*

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	*Describe the sample preparation, detailing the biological source of the cells and any tissue processing steps used.*
Instrument	*Identify the instrument used for data collection, specifying make and model number.*
Software	*Describe the software used to collect and analyze the flow cytometry data. For custom code that has been deposited into a community repository, provide accession details.*
Cell population abundance	*Describe the abundance of the relevant cell populations within post-sort fractions, providing details on the purity of the samples and how it was determined.*
Gating strategy	*Describe the gating strategy used for all relevant experiments, specifying the preliminary FSC/SSC gates of the starting cell population, indicating where boundaries between "positive" and "negative" staining cell populations are defined.*

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

Magnetic resonance imaging

Experimental design

Design type	*Indicate task or resting state; event-related or block design.*
Design specifications	*Specify the number of blocks, trials or experimental units per session and/or subject, and specify the length of each trial or block (if trials are blocked) and interval between trials.*
Behavioral performance measures	*State number and/or type of variables recorded (e.g. correct button press, response time) and what statistics were used to establish that the subjects were performing the task as expected (e.g. mean, range, and/or standard deviation across subjects).*

Acquisition

Imaging type(s)	*Specify: functional, structural, diffusion, perfusion.*
Field strength	*Specify in Tesla*
Sequence & imaging parameters	*Specify the pulse sequence type (gradient echo, spin echo, etc.), imaging type (EPI, spiral, etc.), field of view, matrix size, slice thickness, orientation and TE/TR/flip angle.*
Area of acquisition	*State whether a whole brain scan was used OR define the area of acquisition, describing how the region was determined.*

Diffusion MRI ☐ Used ☐ Not used

Preprocessing

Preprocessing software	*Provide detail on software version and revision number and on specific parameters (model/functions, brain extraction, segmentation, smoothing kernel size, etc.).*

Normalization

If data were normalized/standardized, describe the approach(es): specify linear or non-linear and define image types used for transformation OR indicate that data were not normalized and explain rationale for lack of normalization.

Normalization template

Describe the template used for normalization/transformation, specifying subject space or group standardized space (e.g. original Talairach, MNI305, ICBM152) OR indicate that the data were not normalized.

Noise and artifact removal

Describe your procedure(s) for artifact and structured noise removal, specifying motion parameters, tissue signals and physiological signals (heart rate, respiration).

Volume censoring

Define your software and/or method and criteria for volume censoring, and state the extent of such censoring.

Statistical modeling & inference

Model type and settings

Specify type (mass univariate, multivariate, RSA, predictive, etc.) and describe essential details of the model at the first and second levels (e.g. fixed, random or mixed effects; drift or auto-correlation).

Effect(s) tested

Define precise effect in terms of the task or stimulus conditions instead of psychological concepts and indicate whether ANOVA or factorial designs were used.

Specify type of analysis: ☐ Whole brain ☐ ROI-based ☐ Both

Statistic type for inference

Specify voxel-wise or cluster-wise and report all relevant parameters for cluster-wise methods.

(See Eklund et al. 2016)

Correction

Describe the type of correction and how it is obtained for multiple comparisons (e.g. FWE, FDR, permutation or Monte Carlo).

Models & analysis

n/a	Involved in the study
☒	☐ Functional and/or effective connectivity
☒	☐ Graph analysis
☒	☐ Multivariate modeling or predictive analysis