

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 (<i>n</i>) 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 <i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>
☒	☐ 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. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted <i>Give P values as exact values whenever suitable.</i>
☒	☐ 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 <i>d</i> , Pearson's <i>r</i>), 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	All libraries for scRNAseq were sequenced on Illumina NextSeq 500 according to manufacturer's instructions. Spatial transcriptomics data was generated using 10X Xenium platform according to manufacturer's instructions. MA900 sorter (Sony) and FACSARIA Fusion 2 (BD Biosciences) were used for single-cell sorting Leica Thunder Imager 3D Cell Culture, widefield Nikon Ti-HCS fluorescence microscope, and Zeiss Celldiscoverer7 with LSM 980 were used for live-cell imaging Zeiss 980 Confocal microscope was used for all confocal image acquisition
Data analysis	ImageJ/Fiji (v2.14.0) for imaging analysis FlowJo (v10) for flow cytometry data GraphPad Prism (v10) for data visualization and statistical tests 10X Xenium explorer (v3.0) for alignment of immunofluorescence images and transcripts R packages: hisat2 (v2.1.0), RSEM (v1.3.0), R (v4.3.3), Python (v3.10.11), Seurat (v5.0.2), SingleR (v1.6.1), InferCNV(v1.8.0), pagoda2 (v0.1.4), NMF (v0.28), stats(v4.4.2), Speckle (v1.8.0), SingleCellExperiment (v1.28.1), edgeR (v0.27), ClusterProfiler (v4.6.2), org.Hs.eg.db (v3.18.0), Seurat (v5.0.2), UCell (v2.8.0), car (v3.1.3), CellCharter (v0.3.4), ggplot2 (v3.5.0). All custom scripts for data processing and figure creation are uploaded on https://github.com/FilbinLab/Multidimensional_STEPN.git

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](#)

All transcriptomics data are available in Gene Expression Omnibus (GEO), with scRNAseq data under accession code GSE300150 and spatial data under GSE300146. Raw scRNAseq data are deposited in EGA (EGAS50000001513). We also downloaded the following single-cell gene expression datasets: human cortex development (<https://cells.ucsc.edu/?ds=cortex-dev>) and early human brain (<https://cells-test.gi.ucsc.edu/?ds=early-brain>). Human reads were aligned to the GRCh37 (hg19) reference genome (Genome Reference Consortium, NCBI, RefSeq GCF_000001405.13)

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	Study was conducted on patients diagnosed with supratentorial ependymomas. There are no reporting on sex and gender effects in this study.
Reporting on race, ethnicity, or other socially relevant groupings	No race or ethnicity information were included in this study.
Population characteristics	Population characteristics of the patient dataset are comprehensively described in Table S1.
Recruitment	All deidentified samples used in this study were obtained after informed consent of patients and/or their legal representatives who did not receive compensation treated at Boston Children's Hospital and collaborating institutions.
Ethics oversight	The study was approved by the institutional review board in agreement with local institutional ethics guidelines DFCI 10-417 (Boston Children's Hospital and Dana-Farber Cancer Institute), S-531/2020 (Heidelberg University), and EK Nr. 1244/2016 (Medical University of Vienna).

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 size was determined by the availability of patient-derived material.
Data exclusions	All of the data acquired was utilized for analysis, while quality control filters for scRNAseq are specified in the Methods.
Replication	We performed >4 independent biological replicates for each molecular subgroup via scRNAseq profiling. We successfully replicated our findings across different tumors of each molecular subgroup. For all other experiments, we performed and validated in at least 3 independent replicates.
Randomization	Patient tumor samples were allocated into groups based on respective molecular subgroups in an unbiased fashion. Xenograft mouse models were grouped by the engrafted patient-derived cell line (EP1NS, BT165 or VBT242).
Blinding	Blinding was not applicable as no effects of treatments were assessed.

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-Beta-III-tubulin (1:100; Novus Biologicals, NB100-1612, lot TUJ88977978), anti-S100B (1:500, Synaptic Systems, 287 044, lot 1-16), anti-Nestin (1:2500, Invitrogen, PA5-143578, lot ZD4294645), anti-Vimentin (1:1000, CST, 5741T, lot 8), anti-DCX (1:50, CST, 40619S, lot 1), anti-CCDC40 (1:100, ThermoFisher, PA5-54653, lot YI40449391B), anti-GFAP (1:500, Aves Lab, GFAP, lot GFAP857977, and anti-tdTomato (1:80, CST, 20163S, lot 1). Fluorophore-conjugated secondary antibodies at 1:1000 dilutions (Goat anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 488, A-11008; Goat anti-Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 568; A-11004; Goat anti-Chicken IgY (H+L) Secondary Antibody, Alexa Fluor™ 647; A-21449; Goat Anti-Guinea pig IgG H&L Alexa Fluor® 750, ab175758).

Validation

anti-Beta-III-tubulin: Validated for human, mouse and rat. Each lot was validated for use in ICC/IF, IHC, WB and flow cytometry
 anti-S100B: Validated for human, mouse and rat. Each lot was validated for use in ICC and IHC.
 anti-Nestin: Validated for human, mouse and rat. Each lot was validated for use in ICC/IF, IHC, and WB.
 anti-DCX: Validated for human, mouse and rat. Each lot was validated for use in IHC and WB.
 anti-CCDC40: Validated for human. Each lot was validated for use in IHC and ICC/IF.
 anti-GFAP: Validated for human, mouse and rat. Each lot was validated for use in ICC/IF, IHC, and WB.
 anti-tdTomato: Validated for human, mouse and rat, all species expected. Each lot was validated for use in IHC and WB.
 Goat anti-Rabbit Alexa Fluor™ 488: Validated for rabbit. Each lot was validated for use in IHC, ICC/IF, and flow cytometry.
 Goat anti-Mouse Alexa Fluor™ 568: Validated for mouse. Each lot was validated for use in IHC, ICC/IF, and flow cytometry.
 Goat anti-Chicken Alexa Fluor™ 647: Validated for chicken. Each lot was validated for use in IHC, ICC/IF, and WB.
 Goat anti-Guinea pig Alexa Fluor® 750: Validated for guinea pig. Each lot was validated for use in IHC, ICC/IF, ELISA, WB and flow cytometry.

Eukaryotic cell lines

Policy information about [cell lines and Sex and Gender in Research](#)

Cell line source(s)

EP1NS and BT165 patient-derived cell lines were kind gifts from Dr. Kristian Pajtler (KITZ Hopp Children's Cancer Center, Germany). EP1NS was derived from female patient with metastasizing ZFTA-RELA ST-EPN, while BT165 was derived from male patient with ZFTA-RELA fusion ST-EPN. VBT242 was a kind gift from Dr. Johannes Gojo (Medical University of Vienna, Austria), derived from male patient with ZFTA-RELA fusion ST-EPN.

Authentication

Cell lines were validated by genome-wide DNA methylation profiling and bulk RNA-sequencing.

Mycoplasma contamination

All cell lines were regularly checked as mycoplasma 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

6-week-old female NSG mice (NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ, The Jackson Laboratory, RRID: IMSR JAX:005557) were used for patient-derived ZFTA-RELA xenografts. For all in vivo imaging experiments, male NMRI nude mice (8-12 weeks) were used. Cortical cells were isolated from both male and female rat E19 embryos (late fetal stage). Mice and rats were housed in standard housing conditions of 20-24°C ambient temperature, with humidity around 40-60% and 12 hrs light/ 12 hrs dark schedule.

Wild animals

Not applicable as no wild animals were used.

Reporting on sex

Both male and female sex were used.

Field-collected samples	Not applicable as no field-collected were used.
Ethics oversight	Experiments were performed according to Dana Farber Cancer Institute Institutional Animal Care and Use Committee (IACUC)–approved protocols (13-053), and European Directive on animal experimentation (2010/63/EU) and institutional laboratory animal research guidelines after approval of the Regierungspräsidium Karlsruhe, Germany.

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

Plants

Seed stocks	Not applicable as no plants were used in the study.
Novel plant genotypes	Not applicable as no plants were used in the study.
Authentication	Not applicable as no plants were used in the study.

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	Single cell suspensions of fresh patient tumors of 100 μL (kept in a FACS-tube in PBS/BSA at 4°C) was used for all sorting procedures as unstained control. For stained controls 0.75μL Calcein AM (Life technologies, C34851) and 0.5μL TO-PRO3 iodide (Life technologies, T3605) were added to resuspended cells in 100μL PBS/1%BSA for 10 min at room temperature (RT). Single viable tumor cells were selected by positive staining for Calcein AM as well as negative staining for TO-PRO3 and sorted into pre chilled (4°C) 96 well plates containing TCL buffer (Qiagen, 1031576) + 1% β-mercaptoethanol (Thermo Fisher, 21985023). Sorted plates were centrifuged at 1000 x g for 1 minute at 4°C and frozen on dry ice followed by transfer to a -80°C freezer for long term storage before whole transcriptome amplification, library preparation and sequencing. Dissociated frozen tumor tissue derived single-nuclei suspensions were resuspended in PBS 1% BSA and stained with 3.5mM Vybrant DyeCycle Ruby Stain (Life Technology, V10309). Intact nuclei were sorted into pre-chilled (4°C) 96-well plates containing TCL buffer + 1% β-mercaptoethanol. Sorted plates were centrifuged at 1000 x g for 1 minute at 4°C and frozen on dry ice followed by transfer to a -80°C freezer for long term storage.
Instrument	Nuclei sorting was performed on a SH700 SONY sorter with a 100 μ m nozzle.
Software	SONY SH700 software
Cell population abundance	Overall, we observed 10-60% viable cells (Calcein+, TO-PRO-3-) following fresh tumor dissociations. For nuclei dissociation from frozen tumors, Vybrant Ruby Stain positive event rate ranged from 10-90%.
Gating strategy	Live single cells were identified by size (forward scatter), granularity (back scatter), singlet gating (back scatter are vs back scatter width), positive staining for calcein and negative staining for TO-PRO-3. Single nuclei were identified by were identified by size (forward scatter), granularity (back scatter), singlet gating (back scatter are vs back scatter width), positive staining for Vybrant Ruby stain and singlet gating (Ruby Stain laser light area vs Ruby Stain laser light height).

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