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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	Single cell RNA sequencing data was generated using the Illumina NextSeq 500/550 (38bp paired-end sequencing) instrument according to manufacturer's guidelines. Small RNA sequencing data using a library of genome-wide miRNAs was generated using the Illumina NextSeq 500 system (50bp single-end sequencing). eCLIP data were generated on an Illumina instrument using 150bp paired-end sequencing according to manufacturer's instructions.
Data analysis	To analyze the data, we used publicly available open-source software such as RSEM, Bowtie, Samtools, and GATK. Downstream analysis was performed in R and python using several publicly available packages and tools such as CellPhoneDB, SCENIC, and velocyto. For image analysis the following software was used: QuPath version 0.4.2, inForm version 2.4.

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

Expression matrices of high-quality cells (including malignant and non-malignant cells) of all samples generated within this study are deposited in the Gene Expression Omnibus (GEO) repository under accession code GSE231614. Raw scRNA-seq data will be made available upon reasonable request to the corresponding authors. All datasets that were used within this study are summarized in Supplementary Table 1b. Provided source data files include further raw data such as annotation, embedding coordinates, and cell scores.

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	Tumor samples from 6 female and 5 male participants were collected and subjected to single-cell RNA sequencing. We did not observe sex-related differences concerning biological tumor properties.
Reporting on race, ethnicity, or other socially relevant groupings	We did not collect data on race, ethnicity, or other socially relevant groupings on the subjects that were included in this study.
Population characteristics	A description of the population characteristics is included in the manuscript. Data on patient ages, tumor location, tumor specificities (primary or metastatic lesion) were collected. Patient ages ranged from 0.4-4.8 years.
Recruitment	Participants were recruited based on availability of fresh and fresh-frozen biopsy material (tumor) taken at the time of neurosurgery. Patients were recruited in the course of their presentation in the clinics. All available ETMR samples were included. There was no selection bias. Tumor tissue was isolated in the course of the patient's routine therapeutic and diagnostic surgeries. Samples from three international clinical institutions were included.
Ethics oversight	The local Institutional Review Boards of the respective institutions (Boston Children's Hospital DFCI IRB 10-417, Medical University of Vienna MUV IRB 1244/2016) approved the collection of tumor material. Patients and/or their legal representatives, who did not receive compensation, provided written consent to the use of tumor tissue for research purposes and publication of de-identified data preoperatively according to respective Institutional Review Board guidelines. All procedures on human participants were in accordance with the Helsinki declaration.

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 defined by sample availability. There was no sample-size calculation performed.
Data exclusions	Initial tumor diagnosis as ETMR was confirmed by board certified pathologists and DNA methylation-based classification (www.molecularneuropathology.org). No data were excluded from the analyses
Replication	Each experiment was performed in at least three biological replicates unless otherwise stated in the text. RNA-ISH and FISH experiments were performed in the same ETMR tissue samples that were part of our scRNAseq cohort as indicated.
Randomization	No human or animal trials requiring randomization are included in this study.
Blinding	No human or animal trials requiring blinding are included in this study.

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-PAX6 antibody [AD2.38] (Abcam, Ab78545), STMN2 Polyclonal Antibody (Thermo Fisher, 720178), Purified Mouse Anti-Ki-67 Clone B56 (BD, 550609), LIN28A Monoclonal Antibody [14E6-4E6] (Thermo Fisher, MA1-016), NEUROD1 Monoclonal Antibody [3H8] (Abnova, H00004760-M01), PEG3 Polyclonal Antibody (Thermo Fisher, PA5-55282), NSE Monoclonal Antibody [5G10] (Thermo Fisher, MA1-22730), Purified anti-Tubulin β 3 (Biolegend, 801201), PLTP Polyclonal Antibody (Thermo Fisher, PA5102820), PAX3 Monoclonal Antibody [16H22L10] (Thermo Fisher, 701147), FGFR1 Polyclonal Antibody (Thermo Fisher, PA5-97748), FGFR2 Polyclonal Antibody (Thermo Fisher, PA5-14651), FGFR3 Recombinant Rabbit Monoclonal Antibody [JM110-33] (Thermo Fisher, MA5-32620), FGFR4 Recombinant Polyclonal Antibody [14HCLC] (Thermo Fisher, 710210), Cleaved Caspase-3 (Asp175) (D3E9) Rabbit mAb (Cell Signaling, 9579), DLL4 (D7N3H) Rabbit mAb (Cell Signaling, 96406), Goat anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ Plus 488 (Thermo Fisher, A32731). All primary antibodies were used at a 1:100 dilution. For validation appropriate positive controls were used and specific staining patterns were evaluated.

Validation

All the antibodies used in this study are commercially available and extensively validated by the respective company. Validation data and links to publications using these antibodies are available in each of these company's website. All antibodies used in this study exhibited expected staining patterns in accordance with the existing literature.
Anti-PAX6 antibody [AD2.38] (Abcam, Ab78545): https://www.abcam.com/en-us/products/primary-antibodies/pax6-antibody-ad238-ab78545
STMN2 Polyclonal Antibody (Thermo Fisher, 720178): https://www.thermofisher.com/antibody/product/STMN2-Antibody-Polyclonal/720178
Purified Mouse Anti-Ki-67 Clone B56 (BD, 550609): https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/purified-mouse-anti-ki-67.550609
LIN28A Monoclonal Antibody [14E6-4E6] (Thermo Fisher, MA1-016): https://www.thermofisher.com/antibody/product/LIN28A-Antibody-clone-14E6-4E6-Monoclonal/MA1-016
NEUROD1 Monoclonal Antibody [3H8] (Abnova, H00004760-M01): https://www.abnova.com/en-global/product/detail/H00004760-M01?gad_source=1&gclid=Cj0KCQiAouG5BhDBARIsAOc08RT0hGzwHGemJP0wxMP4NYtUrQLmFS0t7sbCC2yHu7BIUWfqTHHIPQkaAm3DEALw_wcB
PEG3 Polyclonal Antibody (Thermo Fisher, PA5-55282): https://www.thermofisher.com/antibody/product/PEG3-Antibody-Polyclonal/PA5-55282
NSE Monoclonal Antibody [5G10] (Thermo Fisher, MA1-22730):https://www.thermofisher.com/antibody/product/NSE-Antibody-clone-5G10-Monoclonal/MA1-22730
Purified anti-Tubulin β 3 (Biolegend, 801201): https://www.biolegend.com/de-de/products/purified-anti-tubulin-beta-3-tubb3-antibody-11580
PLTP Polyclonal Antibody (Thermo Fisher, PA5102820): https://www.thermofisher.com/antibody/product/PLTP-Antibody-Polyclonal/PA5-102820
PAX3 Monoclonal Antibody [16H22L10] (Thermo Fisher, 701147): https://www.thermofisher.com/antibody/product/PAX3-Antibody-clone-16H22L10-Recombinant-Monoclonal/701147
FGFR1 Polyclonal Antibody (Thermo Fisher, PA5-97748): https://www.thermofisher.com/antibody/product/FGFR1-Antibody-Polyclonal/PA5-97748
FGFR2 Polyclonal Antibody (Thermo Fisher, PA5-14651): https://www.thermofisher.com/antibody/product/FGFR2-Antibody-Polyclonal/PA5-14651
FGFR3 Recombinant Rabbit Monoclonal Antibody [JM110-33] (Thermo Fisher, MA5-32620): https://www.thermofisher.com/antibody/product/FGFR3-Antibody-clone-JM110-33-Recombinant-Monoclonal/MA5-32620
FGFR4 Recombinant Polyclonal Antibody [14HCLC] (Thermo Fisher, 710210): https://www.thermofisher.com/antibody/product/FGFR4-Antibody-clone-14HCLC-Recombinant-Superclonal/710210
Cleaved Caspase-3 (Asp175) (D3E9) Rabbit mAb (Cell Signaling, 9579): https://www.cellsignal.com/products/9579/datasheet?images=1&protocol=0&size=A4
DLL4 (D7N3H) Rabbit mAb (Cell Signaling, 96406): https://www.cellsignal.com/products/96406/datasheet?images=1&protocol=0&size=A4
Goat anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ Plus 488 (Thermo Fisher, A32731)https://www.thermofisher.com/antibody/product/Goat-anti-Rabbit-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A32731

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	BT183 were provided by Marcel Kool (German Cancer Research Center (DKFZ), Heidelberg, Germany); SUIV, DIPG007 and DIPGXIIIP* were provided by Michelle Monje (Stanford University School of Medicine, Stanford, CA); BT690 were provided by Keith Ligon (Dana-Farber Cancer Institute, Boston, MA); MBT011, 014, 021, 026, 028, 030, 032 and 037 were generated from primary patient tumor tissue in Alexander Beck's lab, U87, DAOY, SUPB15, JURKAR, REH and IMR32 were purchased from ATCC; A431, PC9 and SF8628 were purchased from Sigma-Aldrich. Sex of the primary cell lines generated from human participants are as follows: Male: BT183, MBT011, 026, 028, 030 Female: MBT014, 021, 032 and 037
Authentication	All cells purchased from ATCC or Sigma-Aldrich came with STR-authentification. Cells from Monje, Kool and Ligon lab were not further authenticated. All MBT cell lines from the Beck lab were authenticated by DNA methylation-based profiling and classification.
Mycoplasma contamination	No cultures tested positive for mycoplasma.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell 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	Female NOD-scid IL2rgnull (NSG) mice were used for patient-derived xenograft experiments. Animals were 6-8 weeks-old at the time of tumor cell injection.
Wild animals	No wild animals were used.
Reporting on sex	We did not consider sex-related differences is tumor engraftment.
Field-collected samples	No field-collected samples were used in this study.
Ethics oversight	Animal experiments were performed according to German Laws for Animal Protection and approved by the regional authorities (Approval number G91/20).

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

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 obtained from fresh tumors in PBS with 1% BSA were stained with 0.5-1 µM calcein AM (Life Technologies, C3100MP) and 0.33 µM TO-PRO3 iodide (Life Technologies, T3605) for 15 min at RT and kept on ice. Single-cell sorting wasperformed on a SH800 (Sony) sorter using 488 nm (calcein AM, 530/30 emission filter) and 633 nm (TO-PRO-3, 665/30 emission filter) lasers. Viable cells were identified by positive staining for calcein AM and negative staining for TO-PRO-3. Doublets were discriminated based on back scatter area (BSC-A) versus back scatter width (BSC-W). Singlet viable cells were sorted into 96-well plates containing cold TCL buffer (Qiagen, 1031576), briefly spun down, snap frozen on dry ice, and stored at -80°C. Single-nuclei suspensions extracted from frozen tumors were stained with 0.5 µM Vybrant DyeCycleTM Ruby Stain (Invitrogen, V10309) immediately before FACS. Intact nuclei were selected by positive staining for Ruby Stain on the SH800 sorter (633 nm laser, 665/30 nm emission filter). Doublets were excluded in the Ruby Stain area versus Ruby Stain height setting. Singlet nuclei were sorted into 96-well plates containing TCL buffer and 1% beta-mercaptoethanol, briefly spun down, snap frozen on dry ice and stored at -80°C
Instrument	SH800 {SONY) fluorescence-activated cell sorter with a 100 µm nozzle

Software	SONY SH800 software
Cell population abundance	FACS was performed to select for viable tumor cells as described previously (Patel 2014, Tirosh 2016, Venteicher 2017, Filbin 2018, Hovestadt 2019). We did not include information on the tumor purity in this study.
Gating strategy	Live, single cells were identified by size (forward scatter), granularity (back scatter), singlet gating (back scatter area vs. back scatter width), positive staining for calcein and negative staining for TO-PRO-3. Single nuclei were identified by 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.