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Reporting Summary

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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 new software nor code were used for data collection
Lipidomic data were acquired by mass spectrometry using Agilent MassHunter Acquisition software (Version B.08.00, <https://www.agilent.com/en/product/software-informatics/mass-spectrometry-software>)
EVOS M7000 Imaging system (<https://www.thermofisher.com/order/catalog/product/AMF7000?SID=srch-srp-AMF7000>)
Responses of neurons are recorded at a holding potential of -70 mV with the MultiClamp 700B amplifier, Axon Digidata 1550B acquisition system and Clampfit 11.1 (Molecular Devices).

Data analysis

>>No new software nor code were used to analyse data
Transcriptomic Analysis: The cell ranger pipeline (version 2.1.1) was used to pre-process the 10X sequencing data generated by the 10X Genomics Chromium Controller. Illumina BCL files were converted to FASTQ files using the cellranger "mkfastq" command. Each read from the FASTQ files were then aligned to a human reference genome (GRCh38-1.2.0) using the "Cellranger count" command in order to generate count matrix.
Using Seurat R package v3.2.2 (<https://satijalab.org/seurat/>), the data matrices were turned into Seurat object format, combined, log-normalised, and then passed through the FindIntegrationAnchors function in Seurat in order to identify the set of anchors (Anchorset) between the two datasets. The AnchorSet was then used to integrate the datasets using the IntegrateData function.
density score of iMicro transcriptome compared to microglia transcriptome from the Allen Brain Atlas was done by scoring genes as per the AddModuleScore function in Seurat. Single-cell transcriptomic data were also used to construct global gene regulatory networks (regulons) using the single-cell regulatory network inference and clustering (SCENIC) R package (Version 1.1.2, <https://scenic.aertslab.org/>).
Pathway Analysis: The pathways network to unravel biological and metabolic functions of cells using differentially-expressed proteins (DEP) was established using ClueGO (Version 2.5.7), a plug-in application in Cytoscape (Version 1689 3.8.0). (Available at <https://>

cytoscape.org/)

Gene Ontology: Analysis performed using the PANTHER classification system for GO analysis according to the developers' instructions (<http://www.pantherdb.org>)

Proteomic Data: Data were analysed using EdgeR (Version 4.2, <https://bioconductor.org/packages/release/bioc/html/edgeR.html>) in RStudio (Version 4.1.2, <https://www.rstudio.com/>). Identification was performed using Proteome Discoverer (Version 2.5) associated with Mascot algorithm (Version 2.5). Validation of identification and quantification was performed using Software Proline Web 2.1.

Lipidomic analysis: Lipidomic data acquired by mass spectrometry were analyzed using Agilent MassHunter Quant software (Version B.08.00, <https://www.agilent.com/en/product/software-informatics/mass-spectrometry-software/data-analysis/quantitative-analysis>).

Electrophysiology: Data were analyzed using AxoGraph Ver 1.7.0 (AxoGraph Company, <https://axograph.com/>)

Western Blot: Band intensity were quantified by imageJ (free software download: <https://imagej.en.softonic.com/?ex=DINS-635.2>).

Microscopy analysis: Data were analysed using the Imaris (Version 9.7.0, <https://imaris.oxinst.com/>) and ImageJ (Version 2.9.0, <https://imagej.nih.gov/ij/>) softwares.

Data are represented using Graphpad Prism 9 (Version 9.5.1 (733), subscription software, <https://www.graphpad.com/>)

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](#)

Data availability statement is provided in the manuscript. Data have been uploaded as follow: The raw RNA-seq and bulk-seq sequencing data of this study have been deposited in Gene Expression Omnibus with accession no. GSE241127 and (Data uploaded; Pending attribution of accession number) respectively. Proteomic data on purified cell populations have been deposited on JPOST (JPST001822) and Proteome Exchange (PXD042344). Neural rosette proteomic data are available via ProteomeXchange with identifier PXD044406. All lipidomic data have been deposited in Metabolights with accession number (Data uploaded; Pending attribution of accession number)

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

Our patient-derived iPSCs were generated from 3 boys (patient 515, 519 and 534) and 1 girl (patient 523) with H3K27M diffuse midline glioma. This information has been collected from the anonymized case report form (CRF) filled by the patient's medical doctor after having obtained patient/family's consent. No sex-based analysis was performed for the purpose of this paper due to the low number of patients.

Reporting on race, ethnicity, or other socially relevant groupings

No data on socially constructed or socially relevant categorization variable(s) was collected for these patients.

Population characteristics

Our patient-derived iPSCs were generated from pediatric patients with H3K27M diffuse midline glioma. They had a skin biopsy at diagnosis which was used to generate iPSC from fibroblasts for the purpose of this research. They received local radiotherapy and targeted treatment selected based on the biopsy results.

Recruitment

Four consecutive pediatric patients with a H3K27M diffuse midline glioma for whom we could obtain a fibroblast culture were included in the study.

Ethics oversight

Gustave Roussy Ethic Committee

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 methods were used to predetermine sample sizes. Minimum sample sizes were determined based on previously published studies. At

Sample size	least 3 independently generated samples were used for data collection. The sample numbers used for analysis is specified in the figure legends. Information is reported in the manuscript where appropriate
Data exclusions	No Data were excluded
Replication	Findings were confirmed with replicate experiments as noted in the figure legends and methodology where appropriate Multiple iPSC cell lines were used to confirm the organoid size reduction after co-culture with iMacs, this is extensively described throughout the manuscript
Randomization	Brain organoids were randomly assigned for co-culture with iMacs
Blinding	Image analysis was automated and blinded such as the measurement of neurite numbers and lengths; or electrophysiological measurements. Where appropriate, control and co-cultured groups were routinely processed in parallel using the same experimental conditions to reduce 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

Rabbit anti-Iba1 WakoCat # 019-19741
 Chicken anti-Iba1 Synaptic System Cat# 234009
 Goat anti-Iba1 Abcam Cat# ab5076
 Mouse anti-ABCA1 Abcam Cat# ab18180
 Goat anti-Sox2 RnD Cat # AF2018
 Rabbit anti-Sox2 Abcam Cat # AB97959
 Mouse anti-Tuj1 AbcamCat # AB78078
 Mouse anti-Tuj1 Promega Cat # G712A
 Mouse anti-Nestin MerckCat # MAB5326
 Mouse anti-Ki67 BiolegendCat # 350514
 Mouse anti-Ki67 DAKO Cat # M7240
 Rabbit anti-Ki67 Abcam Cat # AB15580
 Mouse anti-Plin2 ProteintechCat # ABIN1724917
 Rabbit anti-Plin2 Antibodies-onlineCat # 15294-1-AP
 Mouse anti-CD45 BiolegendCat # 368516
 Mouse anti-CD14 BiolegendCat # 325604; 301834
 Mouse anti-CD11b BD BiosciencesCat # 557918
 Mouse anti-CD11b eBioscience Cat # 25-0118-42
 Mouse anti-CD163 BD BiosciencesCat # 562670
 Mouse anti-CXCR4 BiolegendCat # 306516
 Mouse anti-CD44 BiolegendCat # 338806
 Mouse anti-CD271 BD BiosciencesCat # 743358
 Mouse anti-CD24 BD BiosciencesCat # 561646
 Mouse anti-CD15 BiolegendCat # 323034
 Mouse anti-P2RY12 BiolegendCat # 392104
 Rat anti-CX3CR1 BiolegendCat # 341626; 341608
 Rabbit anti-Tmem119 Sigma-AldrichCat # HPA051870-100UL
 Mouse anti-CD80 BD Pharmingen Cat# 557227
 Mouse anti-CD86 BD Pharmingen Cat# 555665
 Mouse anti-CD68 Biolegend Cat# 333808
 Mouse anti-HLA-DR Biolegend Cat# 327014
 Mouse anti-b3tubulin PromegaCat # G712A
 Mouse anti-NeuN SigmaCat # MAB377
 Alexa-Fluor 488-conjugated donkey anti-mouse Jackson ImmunoResearch Cat# 715545150
 Cy3-conjugated donkey anti-rabbit Jackson ImmunoResearch Cat# 711165152
 Cy5-conjugated donkey anti-chicken Jackson ImmunoResearch Cat# 703175155

Donkey anti-Goat IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 488 Invitrogen Cat # A-11055
APC Conjugation Kit (LNK03)- BioRad Cat# LNK032APC

Validation

The majority of antibodies used in this study were selected from published literature or which use has been optimized accordingly in the lab or by collaborators. Where possible they were validated and optimized using human primary cells as well as titration to ensure the optimal final concentration and cell number.

Eukaryotic cell lines

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

Cell line source(s)

Human iPSC KYOU1 (KYOU-DXR0109B, 1217 #ACS-1023) and IMR-90 (#CCL-186) were obtained from ATCC. CTRL iPSC line was obtained from Dr Ragnhildur Thora Karadottir. CAU iPSC was obtained from Atlantis Bioscience (#PCi-KER_KIT), HD33i from the NINDS iPSC Repository at Coriell Institute (#ND36997), PCi-ASI from Phenocell (#PCi-ASI_0.5M) and XCL-1 from XCell Science (#IP-001-1V).

Authentication

iPSC lines were confirmed by fluorescence / IF staining, RNAseq, and karyotyping.

Mycoplasma contamination

All cell Lines were routinely tested and found negative for mycoplasma

Commonly misidentified lines (See [ICLAC](#) register)

No misidentified lines were used in the study

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

C57BL/6J mice were sacrificed at E13.5, E14.5, P0 and 15 weeks old, and the brains were harvested and imaged.

Wild animals

No wild animals were used in this study

Reporting on sex

N/A

Field-collected samples

No field-collected samples were used in this study

Ethics oversight

C57BL/6 mice (CD45.1 and CD45.2) were from the Biological Resource Center, Agency for Science, Technology and Research, Singapore. All experiments and procedures were approved by the Institutional Animal Care and Use Committee of the Biological Resource Center (Agency for Science, Technology and Research, Singapore) in accordance with the guidelines of the Agri-Food and Veterinary Authority and the National Advisory Committee for Laboratory Animal Research of Singapore (ICUAC No. 151071).

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

Brain organoids were washed 5 times in PBS and incubated in digestion buffer containing Stempro Accutase, Collagenase (Merck, 1480 C5138) and DNase (Merck, #10104159001) for 30 minutes at 37°C. The organoids were then gently pipetted up and down 10 times using a 1ml pipette tip in order to release the cells from the organoids. Remaining cells were released from organoids by agitation at 1,400 rpm on an Eppendorf Thermomixer C, at 37°C for 10 mins before gently pipetting up and down 10 times using a 1 ml tip. Once the cell debris had settled at the bottom of the tube, the supernatant was gently collected, and filtered through 70 µm filter paper. The cell suspension was then washed in FACS buffer, centrifuged, and the cell pellet collected. The pellet was suspended and incubated in blocking buffer (PBS containing 5% FCS) containing the specified antibodies for 30 mins at 4°C. Cells were then incubated with DAPI for nuclear staining and analysed by flow cytometry. iMacs were washed 3 times in PBS and stained as described. All sample preparations are extensively described in the methodology section.

Instrument

BD FACSAria II/III or BD LSR II (BD Bioscience)

Software	Flowjo was used to collect and analyze the data
Cell population abundance	The cells were sorted, seeded and cultured on a culture dish. Immunostaining was performed using markers to confirm the identity and purity of the cells. Cell population statistics are reported in terms of absolute number or percentage (%) as described in the figure keys.
Gating strategy	Gating strategy is shown where appropriate. Iotype controls were used to determine positivity / negativity.

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