200 µl of XF Assay Medium Modified DMEM supplemented with 2 mM glutamine (XF-Glyco-MEM) and incubated at 37 °C in the absence of CO_2 for 1 h before the assay in 180 µl per well of XF-Mito-MEM. The ports of the sensor cartridge were sequentially loaded with 20 µl per well of the appropriate compound: glucose (10 mM final concentration; Sigma, 8769), oligomycin (2 µM final concentration; Sigma, O4876) and 2-DG (100 mM final concentration; Sigma, 8375). OCR and ECAR were measured through 16 rates: 4 rates under basal conditions, 4 rates after oligomycin or glucose injection, 4 rates after FCCP or oligomycin injection, and 4 rates after rotenone or 2-DG injection, for OCR or ECAR evaluation, respectively. The protocol was: mix (2 min), wait (1 min) and measure (2 min). OCR and ECAR values were normalized to the number of cells per well. The ratio OCR:ECAR was determined by dividing the normalized value of rate 4 of the OCR (basal condition) with the normalized value of rate 8 of the ECAR (after glucose injection).

ATP assay. Cells were cultured in DMEM medium containing 25 mM glucose, 2 mM glutamine and 10% FBS. After 24 h, 5,000 cells per 130 µl were plated in opaque white 96-well plates in 5 mM glucose, 2 mM glutamine and 0.2% FBS DMEM medium. CellTiterGlo assay reagent (Promega, G7570) was added 12–16 h later, according to the manufacturer's instructions and luminescence was measured using a GloMax-Multi+ Microplate Multimode Reader (Promega). For experiments testing the effect of oligomycin, cells were cultured in glucose-free DMEM medium for 24 h and ATP levels were determined as described above.

Cell growth assay. Time-course analysis of cellular growth of human astrocytes expressing F3–T3 or the empty vector was performed by plating 12,500 cells per well in triplicate in 6-well plate in DMEM containing 10% FBS. After 24 h, cells were washed and cultured in glucose-free DMEM medium containing 0.2% FBS and supplemented with 25 mM glucose or 25 mM galactose in the presence or absence of 100 nM oligomycin. Viable cells were scored every two days by Trypan blue exclusion. Survival assays of human and mouse GSCs treated with mitochondrial inhibitors were performed by plating 25,000 cells per well in 12-well plates in triplicate. Cells were counted after 72 h.

Gene set enrichment analysis (GSEA) for ROS detoxification genes. We generated a gene set of 46 genes participating in ROS detoxification programs by combining genes extracted from specific references[20,58] and the detoxification of reactive oxygen species reactome pathway R-HSA-3299685 (http://reactome.org/pages/download-data/). The full list of genes is reported in Supplementary Table 7c. The GSEA analysis was performed comparing the gene expression of F3–T3-expressing and vector control-transduced human astrocytes using default settings[59].

Analysis of protein biosynthesis and ROS by high content microscopy. Human astrocytes transduced as indicated in the figure legends were plated at a density of 6,000 cells per well in 96-well clear-bottom black plates (Greiner) 18 h before the analysis in preparation for the evaluation of both protein biosynthesis and cellular ROS. Protein biosynthesis was detected by the Click-iT Plus OPP Alexa Fluor-594 protein synthesis assay kit (Molecular Probes, C10457). Cells were incubated in the dark with O-propargyl-puromycin (OPP) reagent at a concentration of 10 µM for 30 min. Identical samples were treated with CHX at a concentration of 30 µM for 30 min before addition of OPP reagent and used as negative controls. Samples were washed with Click-iT rinse buffer, fixed in 3.7% formaldehyde for 15 min followed by permeabilization in 0.5% Triton X-100 for 15 min. Click-iT OPP reaction cocktail was then added for 30 min followed by one wash in Click-iT rinse buffer and nuclear counterstaining with DAPI. Acquisition of fluorescence intensity was performed using an IN Cell Analyzer 2000 (GE Healthcare) equipped with a 2,048 × 2,048 CCD camera. Assay conditions were: two-colour assay (DAPI and Cy3), 20× objective, exposure time 0.5 ms for Cy3 and 0.1 ms for DAPI. Four fields around the centre of each well, including 2,000 to 6,000 cells, were imaged. Data were analysed using IN Cell Investigator software (GE Healthcare). Fluorescence intensity was normalized to the number of cells as determined by the number of DAPI-positive objects in each well.

For determination of cellular ROS, cells were incubated in the dark with CellROX Deep Red reagent at concentration of 2.5 µM for 30 min at 37 °C. Identical samples were treated with *N*-acetyl-L-cysteine at concentration of 5 µM for 2 h before addition of CellROX Deep Red reagent and used as negative controls. Samples were washed once with PBS, fixed in 3.7% formaldehyde for 15 min followed by two additional washes in PBS and nuclear counterstaining with DAPI. Acquisition of fluorescence intensity was performed as described above. Assay conditions were: two-colour assay (DAPI and Cy5), 20× objective, exposure time 0.8 ms for Cy5 and 0.1 ms for DAPI. Four fields around the centre of each well, including 2,000 to 6,000 cells, were imaged. Data were analysed using IN Cell Investigator software (GE Healthcare). Fluorescence intensity was normalized to the number of cells as determined by the number of DAPI-positive objects in each well.

Immunofluorescence of cultured cells and primary tissue. Cells were fixed with 4% paraformaldehyde containing 4% sucrose, permeabilized with 0.1–1% Triton X-100, 0.1% BSA in TBS for 4 min at 4 °C, and blocked with 3% BSA, 0.05% Triton X-100 in TBS. The primary antibodies used were as follows: phospho-PIN4 (1:100); PMP70 (Sigma, SAB4200181, 1:150); PEX1 (BD Bioscience, 611719, 1:100); FGFR3 (Santa Cruz, sc-13121 B9, 1:1,000). Secondary antibodies were anti-mouse Alexa Fluor-647, anti-rabbit Alexa Fluor-568, or Cy3-conjugated (Molecular Probes, Invitrogen). Nuclei were stained with DAPI (Sigma).

Fluorescence microscopy was performed on a Nikon A1R MP microscope using a 100×, 1.45 Plan Apo Lambda lens. Images were recorded with a *z*-optical spacing of 0.15 µm and analysed using the NIS Elements Advanced Research software (Nikon Instruments). The number of peroxisomes per cell was scored as the average of $PMP70^{+}$ in five *z* sections (one at the equatorial plane and two above and two below the equatorial plane). Quantification of PEX1 fluorescence intensity was performed on maximum intensity images of *z* sections. After calibration and thresholding, the integrated density (product of the area and the mean intensity value (IMFI)) was averaged between 30 cells in at least six representative pictures per sample.

Tissue preparation and immunostaining on mouse and human tissues were performed as previously described[7,50,60]. The human GBM samples analysed by immunostaining had been stored in the Onconeurotek Tumourbank (certified NF S96 900), and received the authorization for analysis from ethical comittee (CPP Ile de France VI, A39II), and French Ministry for research (AC 2013-1962). In brief, tumour sections were deparaffinized in xylene and rehydrated in a graded series of ethyl alcohol. Antigen retrieval was performed in citrate solution pH 6.0 using a decloaking chamber (10 min for phosho-PIN4 and 7 min for COXIV, VDAC, NDUFS4 and FGFR3). Primary antibodies were incubated at 4 °C overnight: COXIV (Cell Signaling, 4850, 1:1,500), VDAC1 (Abcam, ab14734, 1:700), NDUFS4 (Abcam, ab55540, 1:700), phosho-PIN4(Y122) (1:200) and FGFR3 (Santa Cruz, B9, sc-13121, 1:500). Sections were incubated in biotinylated secondary antibody for 1 h, followed by 30 min of streptavidin–HRP-conjugated (Vector Laboratories) for phosho-PIN4(Y122), FGFR3, VDAC1, and NDUFS4 or HRP-conjugated anti-rabbit secondary antibody (DAKO) for COXIV and TSA–Cy3 or TSA–Fluorescein (Perkin-Elmer). Nuclei were counterstained with DAPI (Sigma). Images were acquired using 20× magnification using an Olympus 1X70 microscope equipped with digital camera. Quantification of fluorescence intensity was performed using NIH ImageJ software. After calibrating and standardizing the 8-bit grayscale images, the integrated density (IMFI) was averaged between three 20× representative pictures per sample section.

***Drosophila* studies.** The *UAS-F3–T3* flies were generated by inserting the human F3–T3 fusion gene into the pACU2 plasmid followed by embryo injection of the plasmid and selection of the correct transgenic fly. All other genotypes were established through standard genetics. *repo-Gal4* was used to drive gene expression in the glial lineage. *UAS-eGFP* or *UAS-mRFP* were introduced to visualize and quantify tumour volume. *repo-Gal4;UAS-dEGFR$^{\lambda}$;UAS-Dp110^{CAAX}* (as previously described[24]) and *repo-Gal4;UAS-F3–T3* stocks were balanced over the *CyoWeeP* and *TM6B* balancers. *srl* RNAi lines were obtained from the Bloomington *Drosophila* Stock Center (BDSC) and the Vienna *Drosophila* Resource Center (VDRC): *P{KK100201}VIE-260B* (VDRC v103355), *y^{1} sc* v^{1}; P{TRiP.GL01019}attP40* (BDSC 57043), *y^{1} sc* v^{1}; P{TRiP.HMS00857}attP2* (BDSC 33914), and *y^{1} sc* v^{1}; P{TRiP.HMS00858}attP2* (BDSC 33915). The following *ERR* RNAi lines were used: *y^{1} v^{1}; P{TRiP.JF02431}attP2* (BDSC 27085), *y^{1} v^{1}; P{TRiP.HMC03087}attP2* (BDSC 50686) and *P{KK108422}VIE-260B* (VDRC v108349). *y^{1} v^{1}; P{UAS-GFP.VALIUM10}attP2* (BDSC 35786) was used as a control for RNAi experiments.

Fly culture, immunohistochemistry and imaging. Flies were mated and maintained at 29 °C. Fly larvae were retrieved at late third instar stage for brain dissections followed by fixation and immunohistochemical analysis. Larval brains were dissected, fixed and stained as previously described[61]. In brief, third instar larval brains were dissected in PBS, fixed in 4% paraformaldehyde solution for 20 min at room temperature, and incubated with primary antibodies, including: rat anti-phospho-histone H3 (Abcam, ab10543, 1:300) and mouse anti-Repo (Developmental Study Hybridoma Bank, 1:60) overnight at 4 °C and secondary antibody for 2 h at room temperature. Fluorescence images were acquired using a Leica SP8 confocal microscope.

Image analysis. To determine tumour volume, we acquired image stacks using a Leica SP8 confocal microscope with a *z*-step size of 5.0 µm per optical slice using a 20× objective throughout the entire thickness of the brain and ventral nerve cord. The confocal LIF files were converted into Imaris files using ImarisFileConverter 6.4.2. All subsequent image processing was conducted with Imaris 5.5 software. *z*-series stacks were used to make three-dimensional reconstructions. A smooth level of 1.0 was used on every measurement for consistency. Brain tumour volumes were quantified using three-dimensional reconstructions.

Statistical analysis. In general, two to four independent experiments were performed. Comparisons between groups were analysed by *t*-test with Welch correction (two-tailed, unequal variance) and/or the MWW non-parametric test when