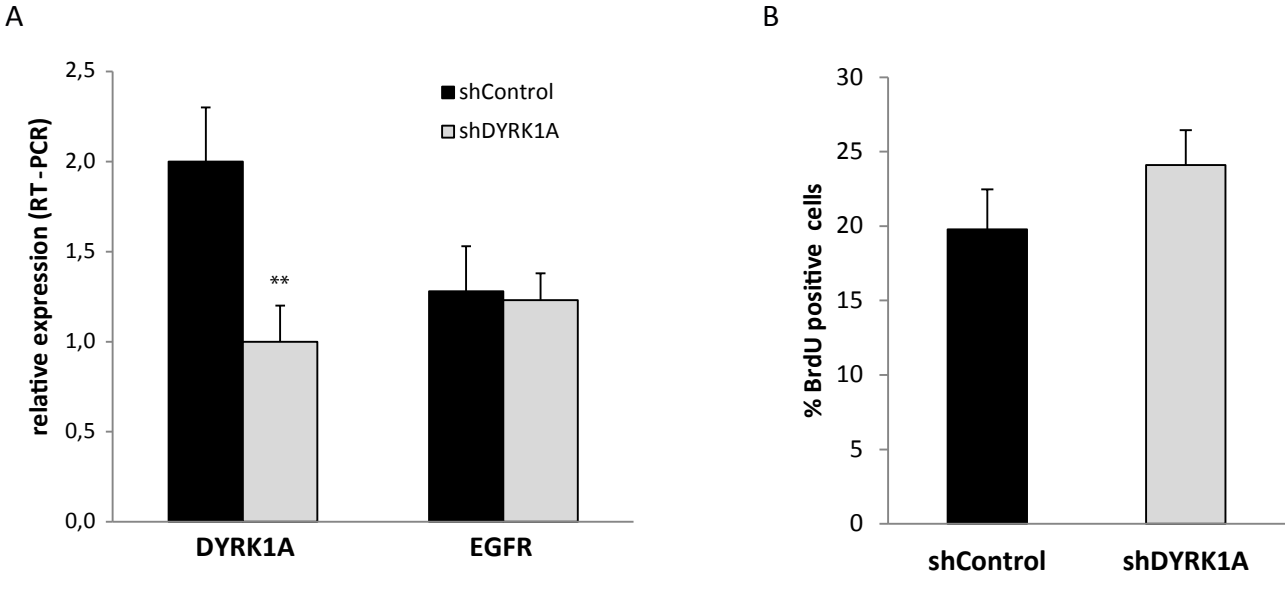
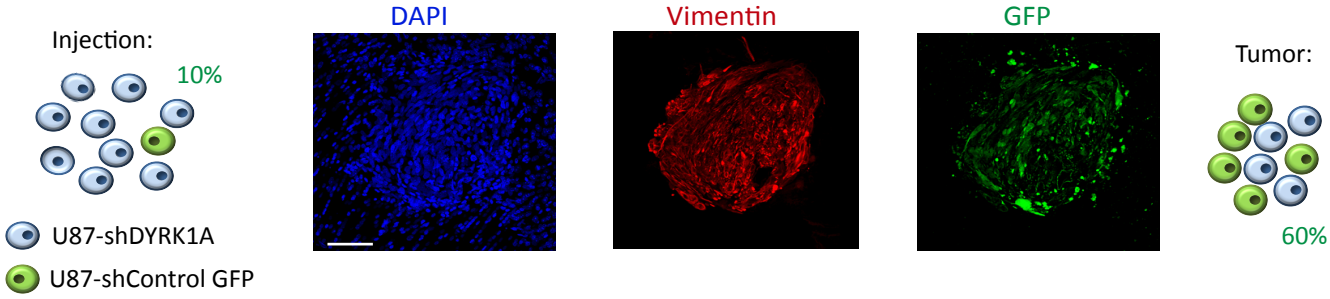
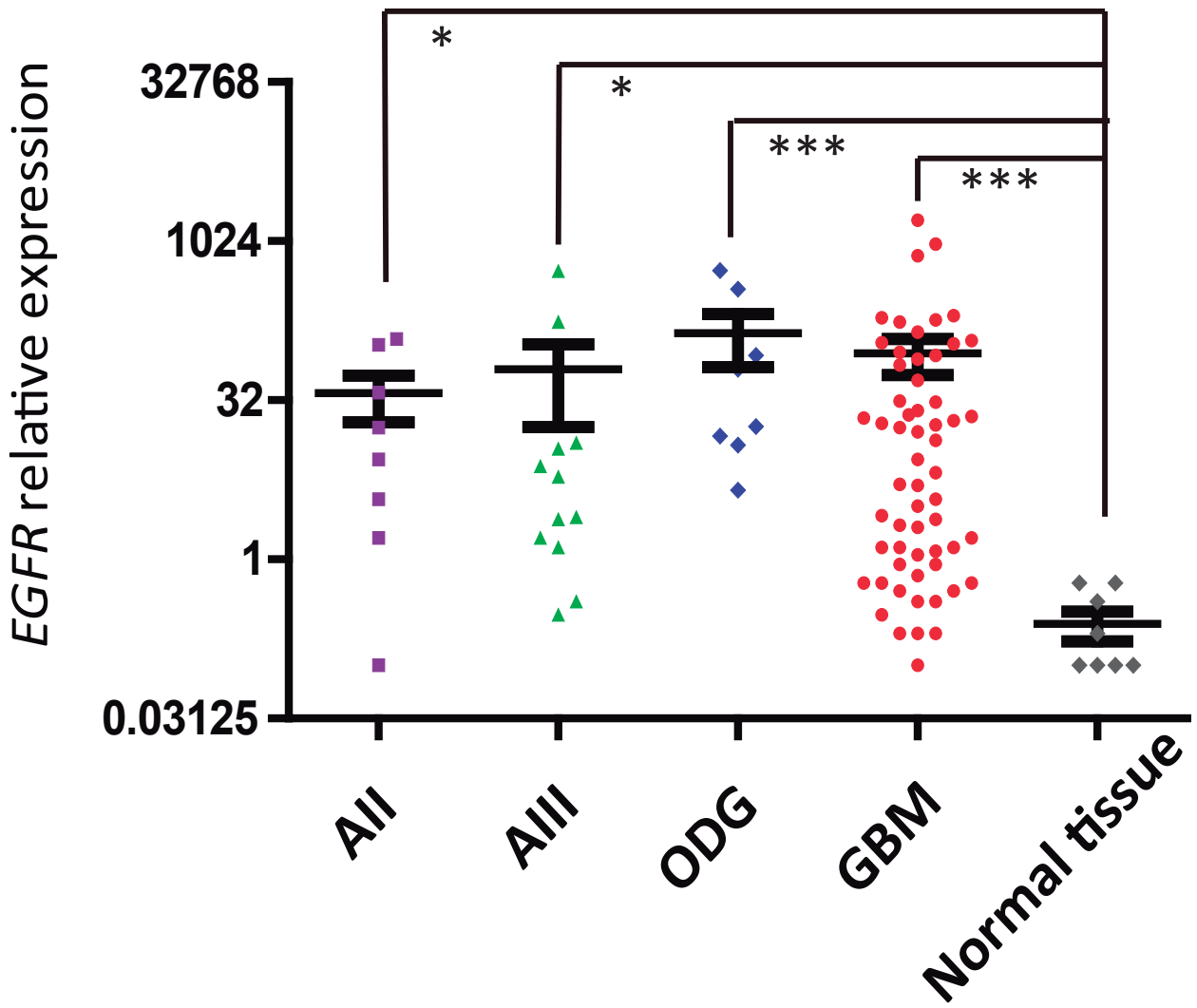


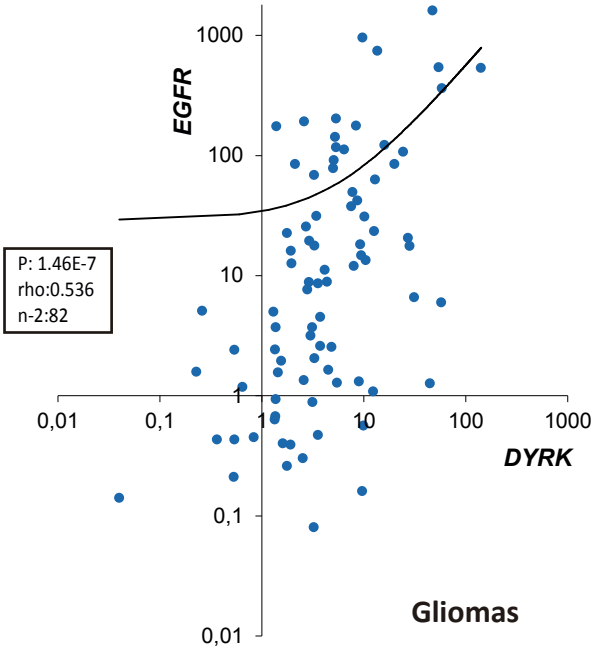
Supplementary Figure 1. shDYRK1A does not affect EGFR gene expression or proliferation in U87 cells. (A) **U87 cells were** infected with lentivirus expressing shControl or shDYRK1A. DYRK1A and EGFR expression were assesed by RT-PCR. (B) Infected cells were incubated in the presence of BrdU, fixed and stained. The graph shows the quantification of BrdU positive cells.



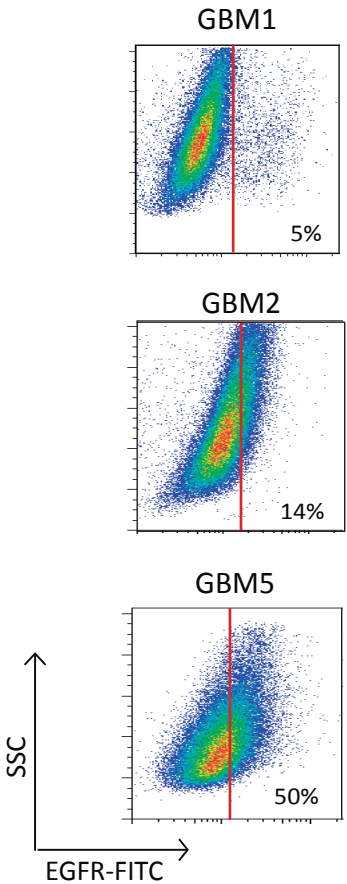
Supplementary Figure 2. U87 sh-DYRK1A cells have less tumorigenic capacity. Images showing the tumors formed 30 days after the implantation of 1,000 U87-shControl cells expressing GFP, together with 9,000 U87-shDYRK1A cells. 60% of cells express GFP in the tumors formed. Scale bar, 50μm



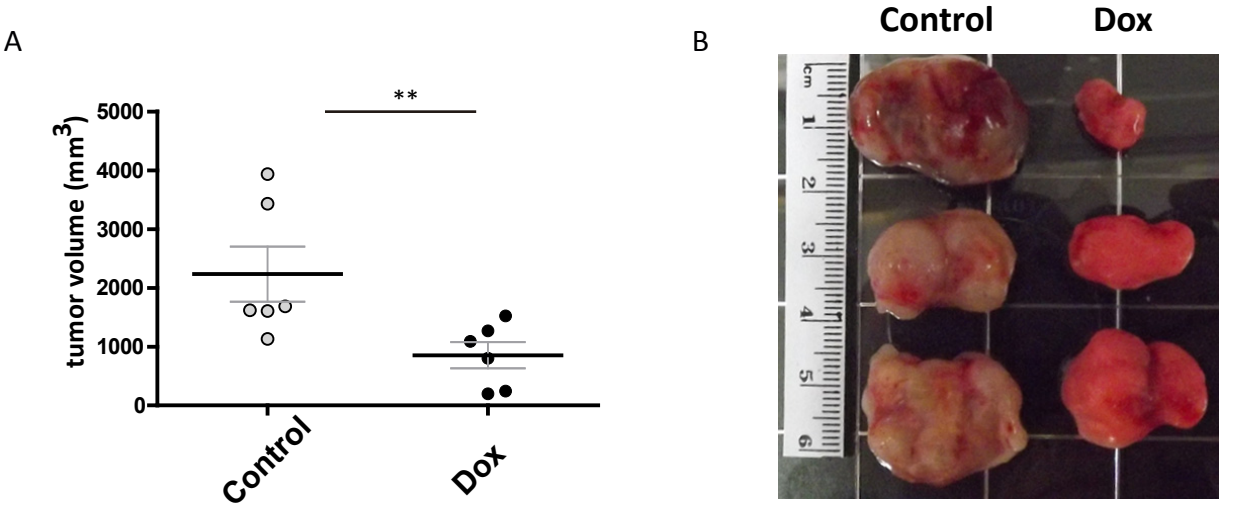
Supplementary Figure 3. Analysis of *EGFR* expression in gliomas. *EGFR* transcript levels were determined by RT-PCR in glioma and normal tissue (obtained from surgery on epileptic patients). *HPRT* levels were used as an internal normalization control. A, astrocitoma; ODG, oligodendroglioma; GBM, glioblastoma.



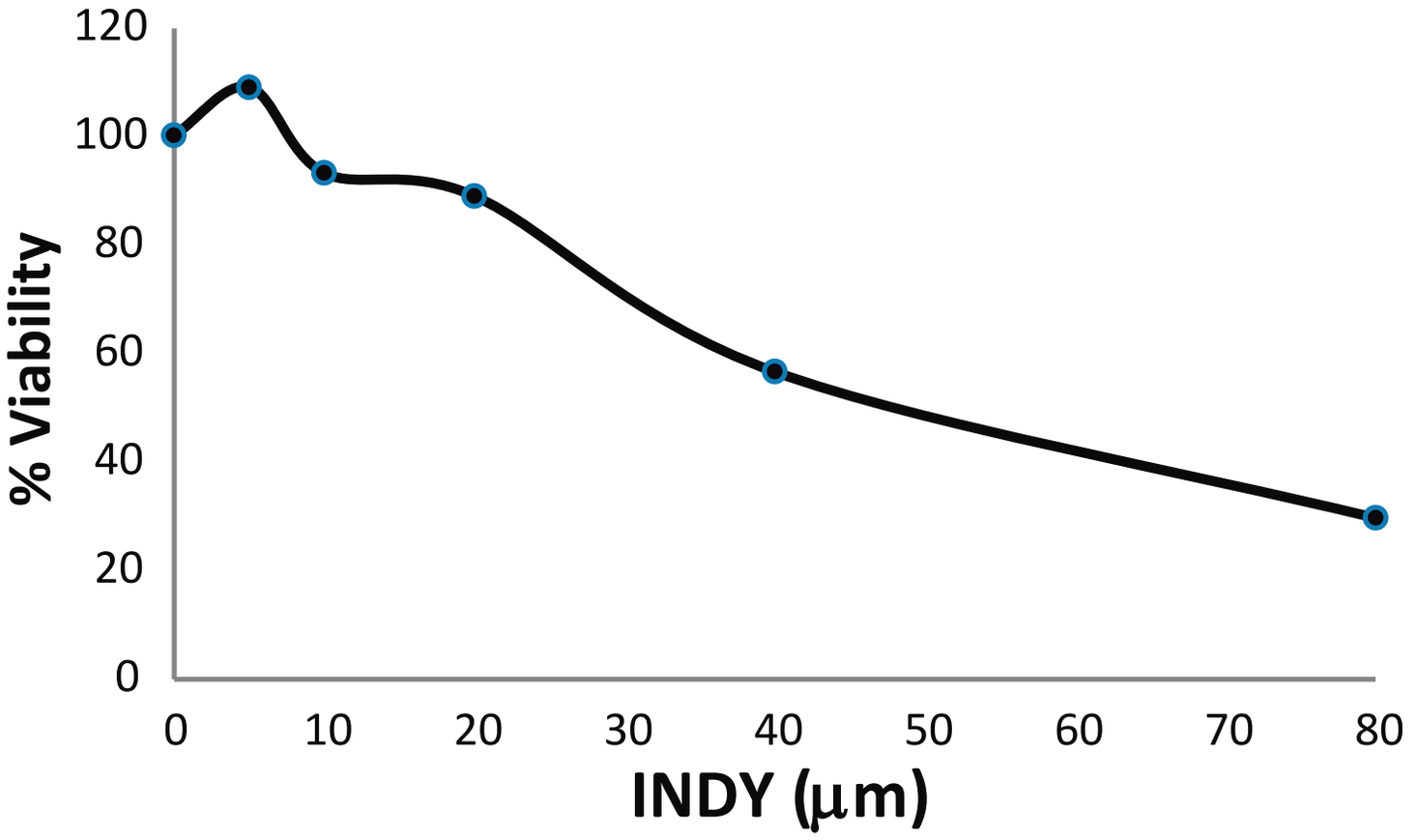
Supplementary Figure 4. *DYRK1A* **expression correlates with that of** *EGFR* **in glioma samples.** A, correlation between *DYRK1A* and *EGFR* transcript levels in a pool of different grade gliomas. Spearman´s rank correlation parameters are presented in the boxes. GBM, glioblastoma.



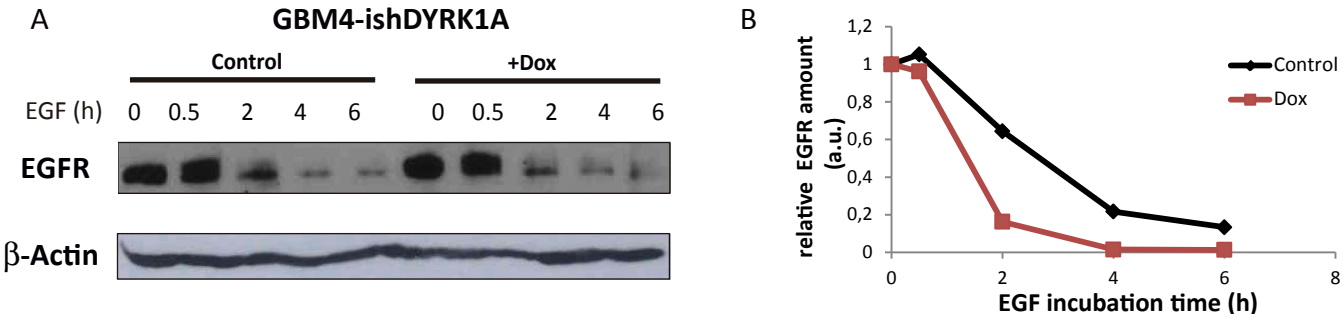
Supplementary Figure 5. Flow-cytometry characterization of GBM-TICs. Representative FACS analysis after EGFR staining of three different primary lines. The percentage of positive cells is presented.



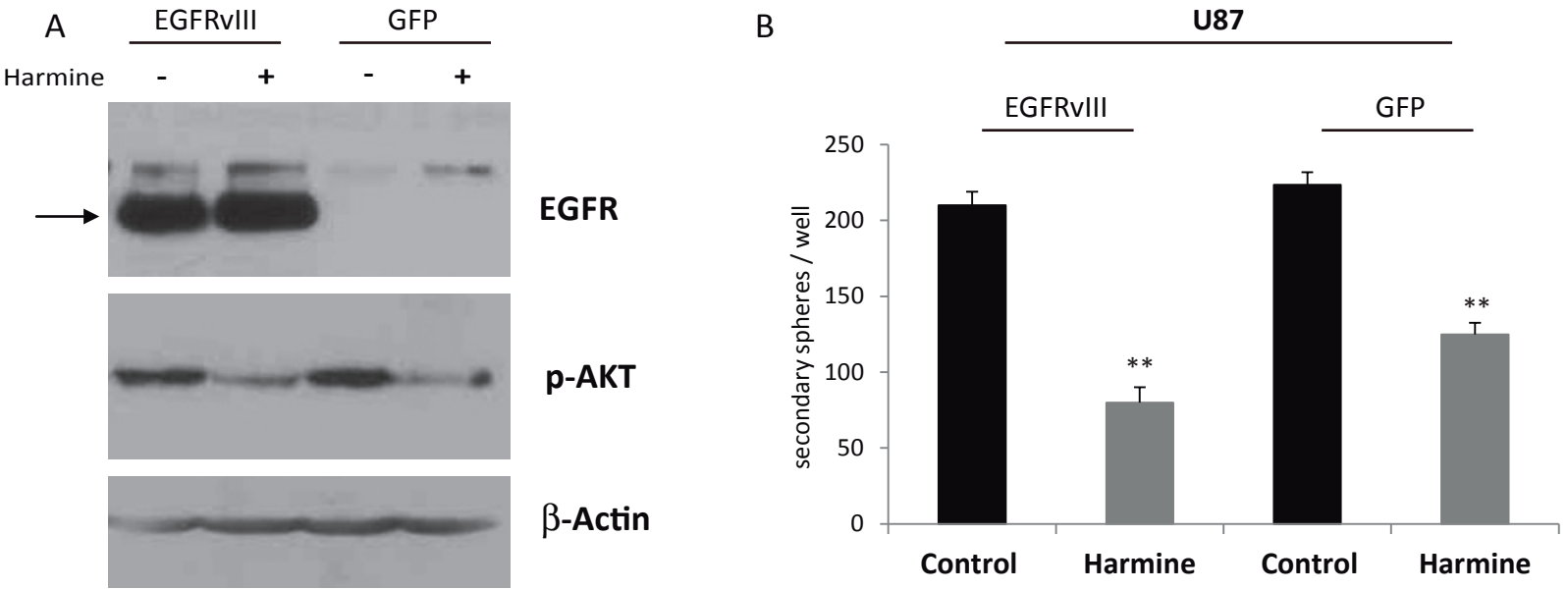
Supplementary Figure 6. Conditional DYRK1A interference affects the tumorigenic capacity of GBM-TICs. (A) 3.5x106 GBM5-ishDYRK1A were inoculated into the flank of nude mice. Two weeks later Dox was added to one set of mice in the drinking water. The graph represents the tumov volume measured at the final end point. B, representative images of tumors meassured in A. The tumors from Dox-treated animals show a clear red color from red fluorescent protein (RFP) expression.



Supplementary Figure 7. INDY impairs the self-renewal capacity of SVZ-NSCs. SVZ-neurospheres were incubated for two days in the presence of different concentrations of INDY. Viability of the cells was meassured by colorimetric WST-1 assay. The % inhibition with respect to cells exposed to DMSO alone is represented in the graph.



Supplementary Figure 8. DYRK1A interference stimulates EGFR degradation in GBM cells. (A) WB analysis of GBM4 cells infected with inducible shDYRK1A (GBM4-ishDYRK1A), deprived of growth factors for 12h in the presence or in the absence of Dox, and stimulated with EGF (100ng/ml) for the indicated times. (B) quantification of the levels of EGFR relative to β-Actin is shown on the graph.



Supplementary Figure 9. EGFRvIII does not rescue DYRK1A inhibition in U87 cells. (A) WB analysis of U87 cells infected with a lentivirus expressing EGFRvIII or GFP. The presence of the vIII isoform is indicated in the WB (arrow). Cells were treated with Norharmane (Control) or Harmine for 3 days and phospho-AKT (p-AKT) levels were determined to quantify EGFR pathway activation, using β-Actin as a control. (B) Quantification of the formation of secondary spheres of U87 cells expressing EGFRvIII or GFP after treatment with Norharmane or Harmine for three days.

SUPPLEMENTARY EXPERIMENTAL PROCEDURES

cDNA preparation and qRT-PCR

Representative sections from tissue used for RNA extraction were stained with hematoxilina/eosine, reviewed uniformly and classified as oligodendrogliomas, astrocytomas or glioblastomas. Total RNA was extracted from the tissue using the Trizol reagent (Life Technologies) and the RNeasy Kit (Qiagen), and it was digested with RNase free DNase I (Qiagen) according to the manufacturer's instructions. A small aliquot of RNA was separated for quantification and for quality control (1% agarose gel). Total RNA (1µg) was reverse transcribed in a total volume of 20 µl containing: 4 µl of 5x first strand buffer, 1 µl of RNase inhibitor (Invitrogen), 2 µl of 0.1 M DTT, 2 µl of 10 mM dNTPs and 1 µl of SuperScript II Reverse Transcriptase (Invitrogen). The samples were incubated at 42ºC for 1 h and as a negative control RT was performed without the RNA template. The product of this reverse transcription was diluted ten-fold with nuclease-free water and stored at -20ºC.

Quantitative real-time PCR was performed using the Light Cycler 1.5 (Roche) with the SYBR Premix Ex Taq (Takara) and using HPRT as an internal control of expression. The primers used for each reaction are indicated in Supplementary Table 1. Reactions were performed in LightCycler® Capillaries in a final volume of 10 µl containing: SYBR Premix Ex Taq II (5µl) (Takara), 10 µM forward and reverse primers (0.2 µl), 2 µl of cDNA template (ten-fold diluted) and nuclease-free water (2.6µl). Cycling conditions included an initial denaturation step of 10 min at 95ºC, followed by 45 cycles of 10 sec at 95ºC, 10 sec at the primer hybridization temperature and 10 sec at 72ºC. The cDNA from normal tissue, adjacent to one of the tumors, was used as to calibrate all RT-PCRs. Gene expression was quantified by the delta-delta Ct method.

Gene dosage analysis. Genomic DNA (gDNA) was isolated from frozen GBM tissue samples with a Qiagen kit following manufacturer instructions. RT-PCR was performed on gDNA (10 ng) to detect *EGFR* gene amplification. Glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) was used as an internal gene dosage control.

Immunohistochemical analysis

Immunohistochemical staining of DYRK1A and EGFR was performed on 5 µm thick sections from paraffin-embedded tumors. DYRK1A and EGFR staining was undertaken by antigen retrieval (pressure cooking) in 10 mM sodium citrate [pH 6.0], followed by the cooling of tissue sections to room temperature prior to adding the primary antibodies. A Rabbit anti-human N-terminal DYRK1A polyclonal antiserum (Abgent) was diluted 1:50 in Blocking Solution (Zymed; Invitrogen) and a rabbit anti-human EGFR polyclonal antiserum (Cell Signaling Technology) was diluted 1:300 in the same Blocking Solution. After incubation overnight at 4ºC, immunodetection was performed with the Avidin/Biotin Blocking Kit from Zymed Laboratories

(Zymed, Invitrogen) and peroxidase activity was visualized using 3,3-diaminobenzidene (DAB) chromogen as the substrate. Sections were counterstained with hematoxylin

Cell proliferation and viability assays.

Cell proliferation was determined by assessing 5-Bromo-2′-deoxyuridine (BrdU; 2 μg/mL) (Sigma-Aldrich) incorporation and after treating cells with harmine or control for 3 (GBM-TICs) or 2 days (SVZ-NSCs). Neurospheres were attached to Matrigel (BD Biosciences)-coated coverslips, fixed with 4% PFA and stained with anti-BrdU antibodies. For cell viability assays, 10,000 SVZ cells were seeded in a 96-well microplate and three days later, harmine or INDY was added at different concentrations and cell viability assessed 48 h after using the WST-1 reagent in a colorimetric assay (Roche) according to the manufacturer´s instructions.

Self-renewal assays.

Cell cultures were treated for 3 days with harmine, INDY or Dox, or their respective vehicles, and then neurospheres were disaggregated into single cells using Accumax and plated in fresh medium in the absence of the drugs at a clonal density of 2.5 cells/μL in triplicate wells of a 96-well plate. In Figure 5A, a double density was used. The percentage of self-renewing cells was determined 6 days after seeding by counting the number of secondary neurospheres formed.

Flow cytometry.

Neurospheres were disaggregated into individual cells with Accumax and they were then stained with an antibody against the extracellular domain of FITC conjugated to EGFR (Abcam), diluted in PBS-10% BSA (Staining buffer) for 20 min on ice. Cells were washed in PBS, treated with propidium iodide (5 μg/mL, Sigma-Aldrich) in order to distinguish dying cells, and those analyzed by flow cytometry (FACSalibur, Beckton Dickinson) using the FlowJow software.

Protein extracts and immunoblot analysis

For immunoblotting, cells were collected and rinsed in cold phosphate buffered saline (PBS). Protein extracts were prepared by re-suspending the cell pellet in sodium dodecyl sulfate (SDS) buffer (25 mM Tris-HCl [pH 7.4], 1 mM EDTA, 1% SDS, 10 mM sodium pyrophosphate, 20 mM β-glycerol phosphate, 2 mM sodium orthovanadate and a protease inhibitor cocktail [Roche Applied Science]), and incubating the cells for 20 min on ice. The lysed cells were centrifuged at 13,000 *g* for 15 min at 4ºC and the protein concentration was determined using a commercially available colorimetric assay (BCA Protein Assay Kit, Pierce Biotechnology) following the manufacturer's indications. Approximately 20 to 50 μg of protein were resolved by 7.5% or 10% SDS-PAGE and they were then transferred to a nitrocellulose membrane (Hybond-ECL, Amersham Biosciences). The membranes were blocked for 1 h at room temperature in 5% (w/v) skimmed milk or 5% (w/v) bovine serum albumin (BSA) in TBS-T (10 mM Tris-HCl [pH 7.5], 100 mM NaCl, and 0.1% Tween-20), and then incubated overnight at 4ºC with the corresponding primary antibody diluted in 5% skimmed milk or 5% BSA in TBS-T. The primary antibodies and

the dilutions at which they were used are shown in Supplementary Table 2. After washing 3 times with TBS-T, the membranes were incubated for 1 h at room temperature with their corresponding secondary antibody (Supplementary Table 2) diluted in 5% skimmed milk in TBS-T. Antibody binding was detected by enhanced chemiluminiscence with ECL (Pierce).

Preparation of mouse tissue sections

Animals were injected intraperitoneally with BrdU (50mg/Kg) in saline solution 2 h before being sacrificed.

Intracranial xenografts. Animals were deeply anesthetized with isoflurane (Schering-Plough) and transcardially perfused with 0.9% (w/v) sodium chloride and 4% paraformaldehyde (PFA, Merck) in 0.1 M PB. After removing the brain, post-fixing it in 4% PFA for 12 h and then rinsing it in 0.1 M PB, coronal vibratome sections (40 µM) or cryostat sections 20 µM were obtained, the latter after cryoprotecting the tissue in a 30% sucrose solution.

Subcutaneous xenografts. Animals were sacrificed by cervical dislocation, and the tumors induced were removed and fixed in 4% PFA for 24 h. The fixed tumors were rinsed in 0.1M PB and cryoprotected in 30% sucrose before obtaining cryostat sections (20 µM).

Immunofluorescent staining

Floating neurospheres were plated onto Matrigel (Bekton-Dickinson: 15mg/ml stock solution diluted 1:100 in DMEM medium [Lonza])-coated glass coverslips (Thermo) and incubated at 37ºC for 15 min. The cells were fixed with 4% PFA and washed 3 times with PB. Neurospheres were blocked for 1 hour in 5% (w/v) BSA and 0.2% Triton X-100 (Sigma-Aldrich) in PBS (PBS-BT) for intracellular antigens, or 5% (w/v) BSA in PB for the membrane antigens, and they were then incubated overnight at 4ºC with the corresponding primary antibody (Supplementary Table 2a) diluted in the same buffer. After washing 3 times with PB, the neurospheres were incubated for 1 hour with their corresponding secondary antibody (Table 2b, Supplementary data). After washing twice with PB, the neurospheres were incubated for 2 min with DAPI (0.1µg/ml) to counterstain the nuclei. To measure BrdU incorporation, neurospheres were incubated for 15 min with 2N HCl at 37ºC, following a 1 min incubation in sodium borate, and they were then placed in the blocking solution as described above.

Vibratome and cryostat sections from xenografted brains were blocked for 1 hour in 2% (w/v) BSA (Sigma) and 0.2% Triton X-100 in PBS and then incubated for 48 hours at 4ºC with the corresponding primary antibody (Table 2, Supplementary data) diluted in PBS-BT. After washing 3 times with PB, sections were incubated for 1 hour with their corresponding secondary antibody (Supplementary Table 2) diluted in PBS-BT. After washing twice with PB, sections were incubated for 5 minutes with 1mg/ml DAPI for nuclear counterstaining. To detect BrdU, the vibratome sections were incubated with pre-heated 2N HCl for 15 min at 90ºC, followed by a 10 min incubation in 0.1 M sodium borate [pH 8.5], and they were then placed in the blocking solution as described above.

Supplementary Table 1: Primers used for RT-PCR

Gene name	Forward primer 5'-3'	Reverse primer 5'-3'
DYRK1A (cDNA)	GCAATTTCCTGCTCCTCTTG	TTACCCAAGGCTTGTTGTCC
EGFR (cDNA)	CAGCGCTACCTTGTCATTCA	AGCTTTGCAGCCCATTTCTA
EGFR (gDNA)	GTGCAGATCGCAAAAGGTAATCAG	GCAGACCGCATGTGAGGAT
GAPDH (gDNA)	ACCCACTCCTCCACCTTTG	CTCTTGTGCTCTTGCTGGG
HPRT-I (cDNA)	TGACACTGGCAAAACAATGCA	GGTCCTTTTCACCAGCAAGCT

Supplementary Table 2a: Primary antibodies for flow cytometry (FC), immunofluorescence (IF), immunohistochemistry (IHC) and western blotting (WB).

Primary antibody	Host species	Dilution used	Provider
β-actin	mouse	1:1000 (WB)	Sigma
Akt	rabbit	1:1000 (WB)	Cell Signaling
BrdU	mouse	1:300 (IF)	Dako
Caspase-3	rabbit	1:200 (IF)	Cell Signaling
DYRK1A	rabbit	1:200 (IF), 1:500 (WB)	Cell Signaling
DYRK1A	rabbit	1:50 (IHC)	Abgent
EGFR	rabbit	1:500 (WB), 1:200 (IF), 1:300 (IHC)	Cell Signaling
EGFR-FITC	rat	1:10 (FC)	Abcam
GADPH	mouse	1:1500 (WB)	Santa Cruz Biotechnology
GFAP	rabbit	1:300 (IF)	Sigma
MAPK	rabbit	1:1000 (WB)	Cell Signaling
Phospho-Akt	rabbit	1:1000 (WB)	Cell Signaling

Phospho-MAPK	rabbit	1:1000 (WB)	Cell Signaling
Sprouty-2	rabbit	1:1000 (WB)	Millipore
Vimentin (human)	mouse	1:300 (IF)	Santa Cruz Biotechnology

Table 2b: Secondary antibodies for immunofluorescence (IF), immunohistochemistry (IHC) and western blotting (WB).

Secondary antibody	Host species	Dilution used	Provider
Cy3-conjugated anti-goat	donkey	1:1500 (IF)	Jackson Immunoresearch
Cy3-conjugated anti-mouse	goat	1:1500 (IF)	Jackson Immunoresearch
Cy3-conjugated anti-rabbit	donkey	1:1500 (IF)	Jackson Immunoresearch
Dylight 488-conjugated anti-mouse	donkey	1:500 (IF)	Jackson Immunoresearch
Dylight 488-conjugated anti-rabbit	donkey	1:500 (IF)	Jackson Immunoresearch
HRP-conjugated anti-mouse	goat	1:1000 (WB, IHC)	Dako
HRP-conjugated anti-rabbit	donkey	1:5000 (WB)	Amersham