Supporting Information

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SI Materials and Methods

Tissue Culture. Primary human and mouse astrocyte tissue cultures were purchased and maintained according to the manufacturer's protocol (Sciencell Research Laboratories). An in vitro scratch assay was used for the injury of astrocytes, as previously described (1–3). Monolayer cells were scraped with a sterile pipette tip. Cells were then incubated in fresh growth medium. Cellular morphology and healing of the wound were subsequently measured and recorded.

Primary glioma cell cultures were maintained under conditions described previously (4, 5). Cultures were dissociated into a single-cell suspension using TrypLE Express (Invitrogen) for transfection and assayed for the formation of clones. Cells were resuspended at 50,000 cells/mL in DMEM/F12 supplemented with 50 ng/mL EGF, 20 ng/mL bFGF, and B27 (Invitrogen).

Glioma Tumor Samples and Tissue Dissection. Tissue samples were collected at the Cleveland Clinic Foundation. All tissue samples and clinical information were obtained as part of an Institutional Review Board-approved study. Frozen tissue samples included grade II astrocytomas ($n = 7$), grade III astrocytomas ($n = 7$), grade IV astrocytomas ($n = 2$), and normal brain ($n = 1$). Tissue dissection was performed as previously described (6).

Immunofluorescence Analysis. Cells and tissue slices were fixed in Histochoice (Mandel) and labeled with primary antibodies overnight. Cell nuclei were counterstained with Hoechst 33342 (Invitrogen). Specimens were visualized using a Zeiss LSM 510 confocal microscope.

Western Blot Analysis. Western blot analysis was performed as previously described (7). Dissected tissue and cells were lysed in RIPA lysis buffer (Thermo Scientific). To investigate subcellular distribution of proteins, nuclear, cytoplasmic and membrane fractions were enriched using Qproteome Cell Compartment Kit (Qiagen). Proteins were quantified and equal amounts were resolved on NuPAGE gels (Invitrogen). Samples were then transferred to PVDF membranes, blocked with 5% dried skim milk and blotted with primary antibodies. Protein expression was detected by chemiluminescence. Antibodies used in this study include GFAP (1:2,000; Sigma), Nestin (1:2,000; Abcam), sex-determining region Y-box 2 (SOX2, 1:1,000; Cell Signaling), EGFR (1:1,000; Cell Signaling), ciliary neurotrophic factor receptor (CNTFR, 1:1,000; Abcam), NcoR (1:1,000; Thermo Scientific), Phospho-β-Catenin (Ser33/37/Thr41, 1:1,000; Cell Signaling), Phospho-β-Catenin (Ser675, 1:1,000; Cell Signaling), β-Catenin (1:2,000; Cell Signaling), Vimentin (1:1,000; Dako), *N*-Cadherin (1:1,000; Abcam), VE-Cadherin (1:1,000; Abcam), and β-Actin (1:2,000; Sigma).

Immunoprecipitation. Immunoprecipitation was performed as previously described with minor modifications (8). Two-hundred micrograms of whole-cell lysates were precipitated using Dyna-Beads Protein G (Invitrogen) with antibodies against *N*-Cadherin, VE-Cadherin, or β-Catenin. Precipitated products were analyzed by Western blot analysis.

RNA Interference. RNA interference against endogenous β-Catenin was carried out with Stealth siRNA (VHS50819, VHS50822, Invitrogen). Stealth siRNA oligo (100 pmol) was transfected into primary glial cell cultures in 35-mm tissue culture dishes or 50,000 primary glial cells using 5 μL RNAiMAX reagent (Invitrogen). Interference rate was measured through Western blot on transfected lysate. Cell proliferation, tumor sphere formation, tumor sphere size measurement, and protein patterns of expression were subsequently investigated.

Real-Time PCR. RNA was extracted from cells using a PureLink RNA Mini kit (Ambion). Genomic DNA was removed through DNase I digestion (Qiagen). Total RNA was reverse-transcribed into cDNA using a Supercript III cDNA synthesis kit (Invitrogen). The cDNA products were used for quantitative real-time PCR with the ready-to-use TaqMan gene expression assay (4414133, 4414077; Applied Biosystems). Real-time PCR was performed in 96-well plates with an ABI7500 Real-time PCR system. Gene-expression changes were calculated using a ΔΔCt method with statistical analysis (9).

1. Ishiuchi S, et al. (1998) In vitro neuronal and glial production and differentiation of human central neurocytoma cells. *J Neurosci Res* 51:526–535.
2. Liang C-C, Park AY, Guan J-L (2007) In vitro scratch assay: A convenient and inexpensive method for analysis of cell migration in vitro. *Nat Protoc* 2:329–333.
3. Wu BY, Yu AC (2000) Quercetin inhibits c-fos, heat shock protein, and glial fibrillary acidic protein expression in injured astrocytes. *J Neurosci Res* 62:730–736.
4. Bao S, et al. (2006) Stem cell-like glioma cells promote tumor angiogenesis through vascular endothelial growth factor. *Cancer Res* 66:7843–7848.
5. Park DM, et al. (2007) N-CoR pathway targeting induces glioblastoma derived cancer stem cell differentiation. *Cell Cycle* 6:467–470.
6. Furuta M, et al. (2004) Protein patterns and proteins that identify subtypes of glioblastoma multiforme. *Oncogene* 23:6806–6814.
7. Yang C, et al. (2011) Missense mutations in the NF2 gene result in the quantitative loss of merlin protein and minimally affect protein intrinsic function. *Proc Natl Acad Sci USA* 108:4980–4985.
8. Lu J, et al. (2010) Decreased glucocerebrosidase activity in Gaucher disease parallels quantitative enzyme loss due to abnormal interaction with TCP1 and c-Cbl. *Proc Natl Acad Sci USA* 107:21665–21670.
9. Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* 25:402–408.

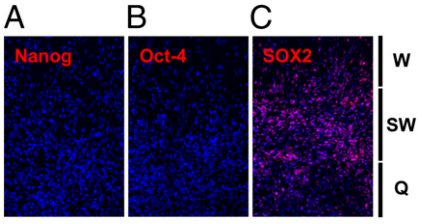


Fig. S1. Expression of pluripotency biomarkers resulting from injury to astrocytes. (*A*) Immunofluorescence staining for expression of Nanog (red) in migratory, proliferative and quiescent astrocytes following injury demonstrating minimal expression of Nanog. (*B*) Immunofluorescence staining for the expression of Oct4 (red) in migratory, proliferative and quiescent astrocytes following injury. (*C*) Immunofluorescence staining for expression of SOX2 (red) in migratory, proliferative and quiescent astrocytes following injury demonstrating up-regulation of expression following injury. Q, quiescent region; SW, submarginal wound region; W, wound region. (Magnification: 200×.)

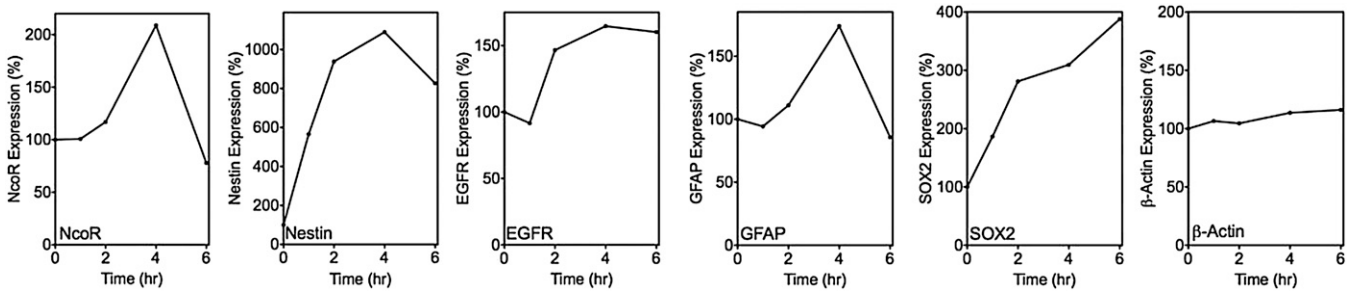


Fig. S2. Temporal quantification of biomarker expression in astrocytes following injury. NcoR, Nestin, EGFR, GFAP and SOX2 protein expression was quantified based on Western blot analysis. β-Actin was used as an internal control.

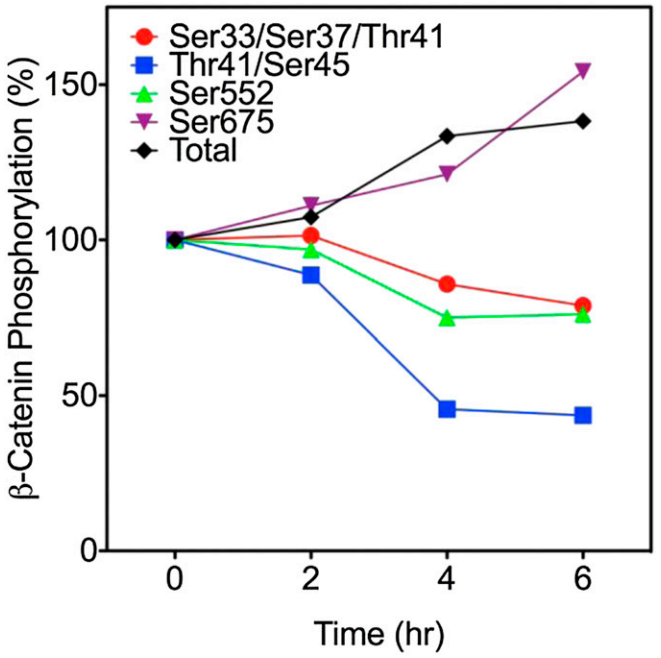


Fig. S3. Quantification of β-catenin phosphorylation after scratch injury. Quantification of Western blot analysis demonstrating an increased expression of total β-catenin. β-catenin with phosphorylation at the Ser-675 site was upregulated following injury.

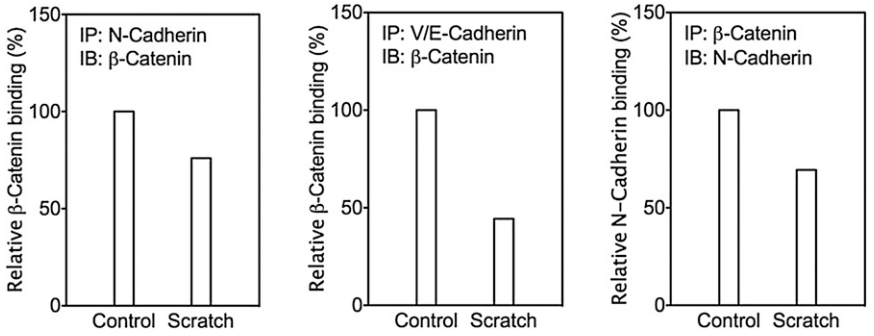


Fig. S4. Quantification of the association between cadherins and β-catenin in astrocytes after injury. Co-immunoprecipitation (IP) and immunoblot (IB) analyses were quantified, which demonstrated decreased affinity for various cadherins with β-catenin following injury.

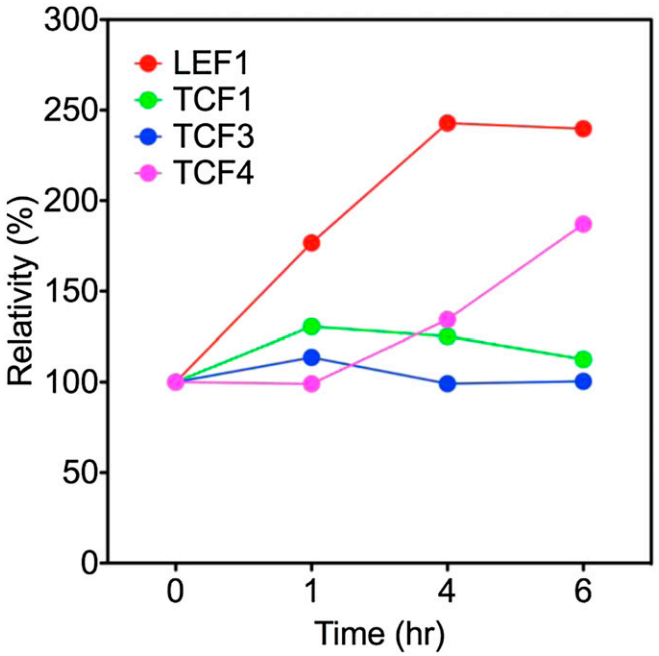


Fig. S5. Quantification of LEF/TCF transcription factors in astrocytes following injury. Western blot analyses were quantified, which demonstrated an increased expression of LEF1 (red) and TCF4 (pink) in astrocytes following injury. TCF3 (blue) and TCF1 (green) levels were unchanged.

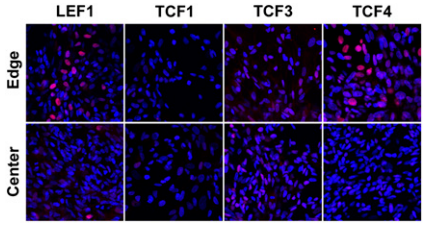


Fig. S6. Measurement of T-cell factors (TCFs) and lymphoic enhancer factor (LEF) family transcription factors following injury of astrocytes. Immunofluorescence staining for downstream β-catenin targets LEF1, TCF1, TCF3, and TCF4 (red) at the margin of the wound (*Upper*) and submarginal wound region (*Lower*) of astrocytes following injury demonstrating increased expression of LEF1 and TCF4. (Magnification: 200×.)

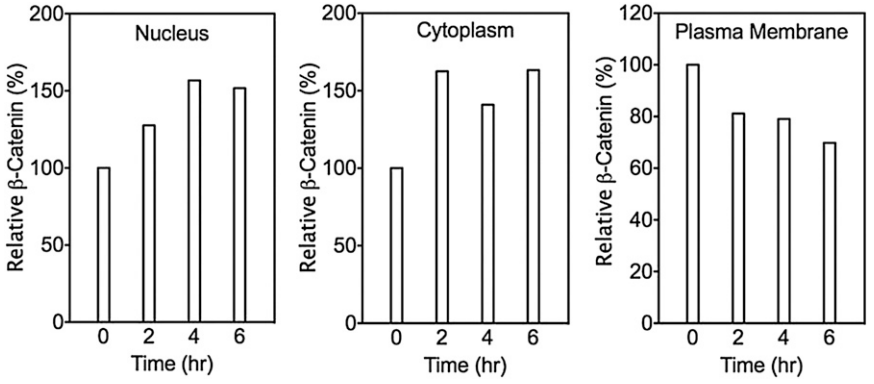


Fig. S7. Quantification of β-catenin in different cellular compartments after injury. Quantification of Western blot analyses demonstrating an increase in nuclear and cytoplasmic β-catenin expression following injury, with a decrease in membrane-associated β-catenin in the same assay.

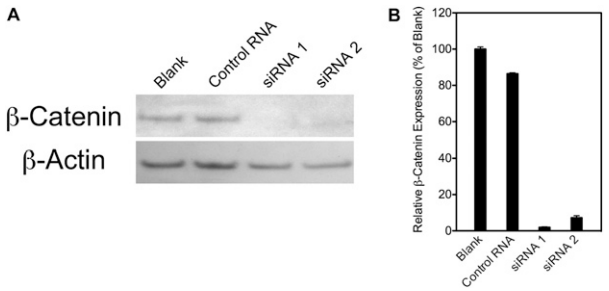


Fig. S8. Measuring knockdown of β-catenin following siRNA transfection. (*A*) Western blot analysis demonstrating decreased expression of β-catenin following knockdown with two different siRNA constructs. (*B*) Quantification of expression of β-catenin following knockdown with siRNA constructs.

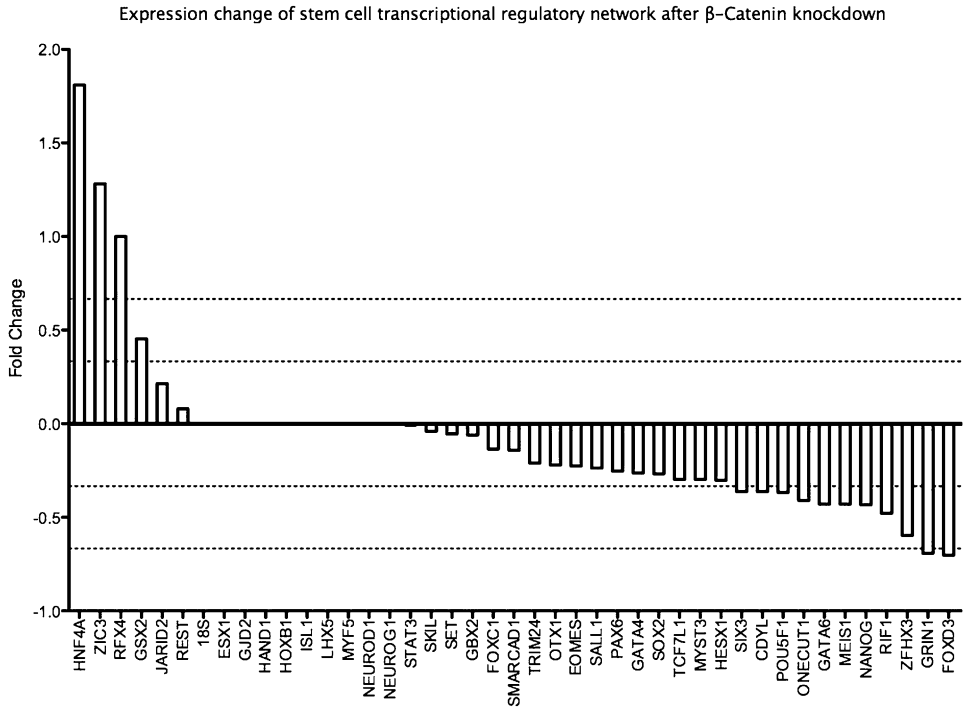


Fig. S9. Changes in gene expression of primary glioma cells following inhibition of β-catenin. Changes in expression in various markers known to be associated with stem cells and primary glioma cells following siRNA knockdown of β-catenin.

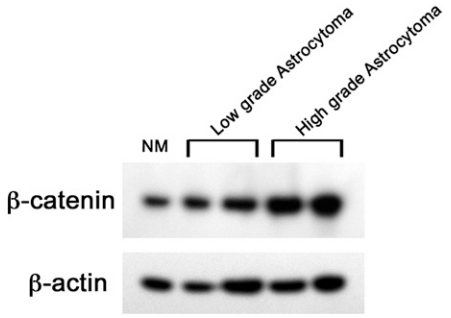


Fig. S10. Expression of β-catenin in astrocytomas of varying grade. Western blot analysis demonstrating positive correlation of expression of β-catenin in astrocytomas with the increase of histological grade.

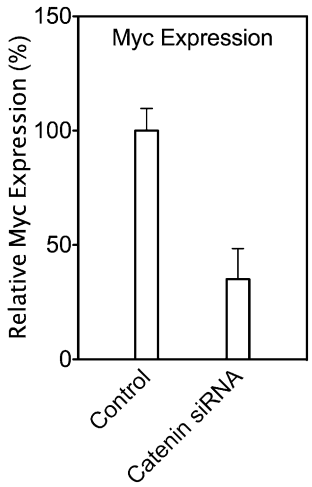


Fig. S11. c-Myc expression following β-catenin inhibition. Quantification of c-Myc expression demonstrating decreased c-Myc expression after inhibition of β-catenin.

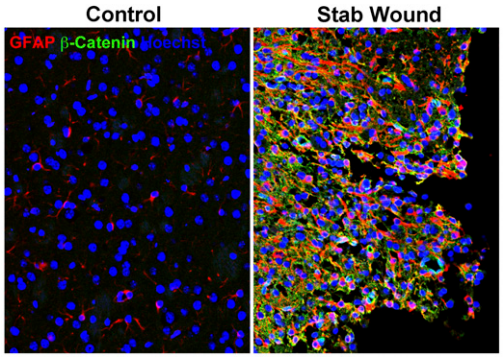
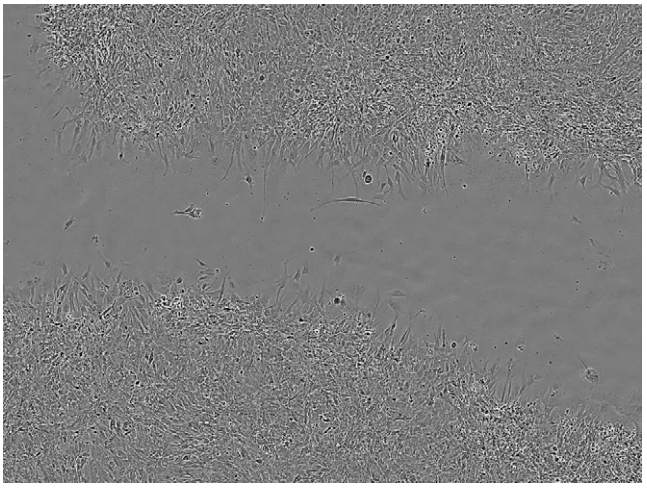
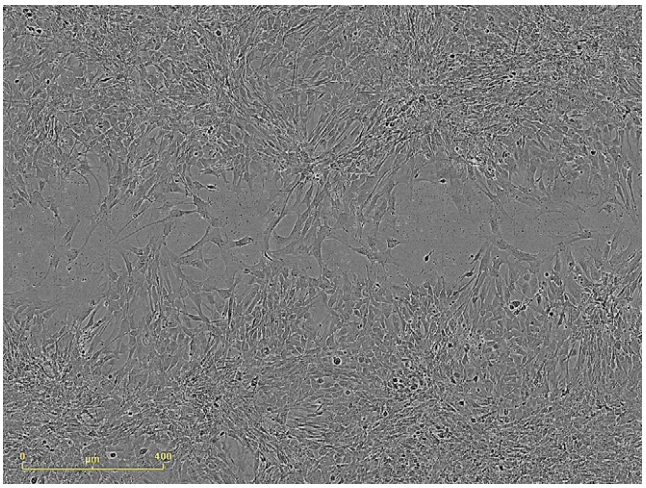


Fig. S12. Expression of GFAP and β-catenin under in vivo stab-wound injury in mouse brain. Immunofluorescence staining for expression of GFAP (red) and β-catenin (green) in cerebral cortex treated with stab wound injury. Contralateral uninjured cortex was stained as control. Cell nuclei were labeled with Hoechst 33342. (Magnification: 200×.)



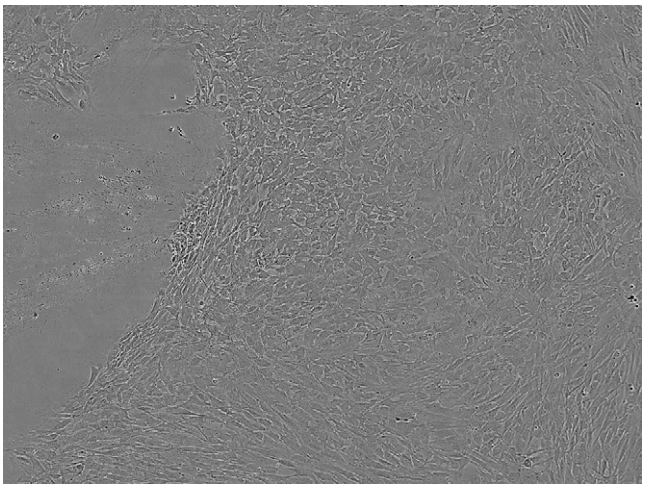
Movie S1. Real-time imaging of human and mouse astrocytes following injury in vitro. Primary culture and serial imaging of mouse astrocytes following injury demonstrating astrocyte migration into the wound space. (Magnification: 100×.)

Movie S1



Movie S2. Real-time imaging of human and mouse astrocytes following injury in vitro. Primary culture and serial imaging of human fetal astrocytes following injury demonstrating infiltration of reactive astrocytes into the space created by injury. (Magnification: 100×.)

Movie S2



Movie S3. Real-time imaging of human and mouse astrocytes following injury in vitro. Human adult astrocyte culture and serial imaging following injury. (Magnification: 100×.)

Movie S3