SUPPLEMENTAL MATERIALS AND METHODS

Immunohistochemistry and immunofluorescence. The immunohistochemistry of the glioma TMA was performed using a rabbit polyclonal moesin antibody (Q480/3150; Cell Signaling Technology, Danvers, MA) according to the described protocol (21). The intensity of cytoplasmic and membrane moesin staining was assessed by using the ImageJ program (National Institutes of Health, Bethesda, MD) in ten randomly selected fields from each specimen. Since each TMA included two different tumor sites from each case, immunohistochemistry evaluation was performed after examining both specimens. Similarly, the confocal immunofluorescence analysis of the glioma TMA with P-ERM antibody was performed as described (20). The immunohistochemistry of mouse brain tumor sections was performed with Ki-67 antibody (550609; BD Biosciences, LaJolla, CA). The deconvolution immunofluorescence analysis of formaldehyde-fixed cells was performed as described (21) with the primary antibodies: mouse monoclonal moesin (M36830) and β-catenin (610153) (BD Biosciences), rabbit polyclonal P-ERM (3141) and moesin (3150), and mouse monoclonal CD44 (3570) (Cell Signaling Technology). Rhodamine phalloidin (R415; Invitrogen, Carlsbad, CA) and DAPI were used for actin and nucleus counterstaining, respectively.

Protein analysis and antibodies. The antibodies were obtained as follows: actin, nestin (Mab5326), CD133 (Mab4399) (Chemicon/Millipore, Billerica, MA), Olig2 (R&D Systems, Minneapolis, MN), Erk1 (C-16), Erk2 (C-14), Myc (9E10 and A14), Moesin (C-15), Radixin (C-15), NF2 (A-19), PARP-1/2 (H-250) (Santa Cruz Biotechnology, Santa Cruz, CA), phospho-ERM (3141), CD44 (3150) phospho-Akt (Ser473) (9271), Akt (9272), c-JUN (9165), phospho-p38 MAPK (Thr180/Tyr182) (9215), p38 MAPK (9212), EGFR (2232), Sox2 (4900), phospho-β-catenin Ser552 (Cell Signaling Technology), Myc, N-cadherin (Invitrogen), β-catenin (610153), ezrin (30252), NHERF1 (BD Biosciences).

Plasmids and small hairpin (sh)RNAs. Myc-tagged moesin (Myc-Moesin) and ezrin (Myc-Ezrin) (21) were further cloned in pCX (blasticidin resistance) retroviral vector (22). Moesin shRNAs #3 (GCTCGTATGCTGTCCAGTCTAA), #4 (GGAGGATGCTGTCCTGGAATA), #7 (GGATGAGCAGGATGAGAAT) and radixin shRNA #3 (GAACTGGCATGAAGAACATA), #8 (AGCAGGCCGTGATAAGTAC) were designed and cloned in the pSIREN-RetroQ retroviral vector (shV) (Clontech, Mountain View, CA). Ezrin shRNAs cloned in the same retroviral vector were previously described (21).

RNA extraction and quantitative real time PCR analysis. 3x10^6 control or Myc-Moesin overexpressing LN229 cells were grown in complete culture medium until 60-70% confluence. Total cellular RNA was isolated using the RNeasy Mini Kit (QIAGEN, Hilden, Germany) according to the manufacturer’s instructions. To remove any residual genomic DNA, RNA samples were treated with DNase (QIAGEN, Hilden, Germany). 1μg total RNA was reverse transcribed into first strand cDNA, using an RT2 First Strand Kit (SA Biosciences). The resulting cDNA was subjected to SYBR-Green based quantitative PCR analysis (SA Biosciences, Frederick, MD) following manufacturer’s protocol. RT2 Profiler PCR 96-well arrays were run on a Chrom4 system for real time PCR detection (Biorad Laboratories, Hercules, CA). Each array contains duplicate wells that were loaded with extracts from control and moesin-overexpressing cells. Duplicate arrays were run to ensure reproducibility. Data analysis was
performed using the manufacturer’s integrated web-based software package for the PCR Array System using cycle threshold (Ct)-based fold-change calculations.

**GSC neurosphere formation assays.** For GSC neurosphere assays, neurospheres were dissociated with accutase (Sigma-Aldrich Corp.) and $10^8$ single cells were seeded in triplicates in 60-mm low-binding dishes containing 6 ml neurosphere growth medium (DMEM/F12 supplemented with B27 (Invitrogen), 20 ng/ml epidermal growth factor and 20 ng/ml basic fibroblast growth factor (R&D Systems, Minneapolis, MN)). Neurospheres were allowed to form for either 3 days for proliferation assays or 5 days for neurosphere renewal assays (24). For proliferation, neurospheres were collected by centrifugation and dissociated with accutase. Single cells were resuspended in 1 ml neurosphere growth medium, distributed at 100 μl per well in quintuplicate in 96-well plates, and subjected to MTT proliferation assay. The absorbance was measured in a Mithras LB-94 plate reader (Berthold Technologies, Bad Wildbad, Germany) at 570-nm wavelength.

**Statistical analysis.** The Western blot quantification and analysis, mouse survival analysis and statistical analysis using t-test were described in detail elsewhere (19). The correlation analysis of the expression levels over the 17-glioma data sets was performed by using the Spearman nonparametric correlation test. The specimen numerical differences between glioma grades for P-ERM labeling of the TMA were analyzed by Chi-square test. Statistical significance was considered for P<0.05. Data are representative of at least two or three independent experiments with essentially similar results.