SUPPLEMENTAL MATERIALS AND METHODS

Recombinant BMP and Gremlin1 Treatments

CSCs or non-stem glioma cells were treated with 25 ng/mL BMP2 in the presence or absence of 1 µg/mL Gremlin1 (R&D Systems). Cells were plated in Neurobasal media without EGF and FGF prior to performing treatments.

Real-Time PCR

Total RNA was isolated using the RNeasy isolation kit (Qiagen) or Trizol (Invitrogen) and reverse transcribed into cDNA using the qScript cDNA SuperMix (Quanta Biosciences). mRNA was labeled with SYBR Green MasterMix (Applied Biosystems). Sequences of primers used in this study are given in Supplemental Table 2. Levels of mRNA were measured using an ABI-7900 RT-PCR system (Applied Biosystems). Expression values were normalized to β-Actin or GAPDH. For Figures 1D and 1E, these expression values were reported. For all other figures, expression values were further normalized to a control population.

Immunoblots and ELISAs

20-40 µg of cell lysate was resolved on commercially available gradient SDS-PAGE gels (BioRad) or homemade 12% SDS-PAGE gels, transferred to
polyvinylidenedifluoride membranes (Millipore) and detected using an enhanced chemiluminescence system (Pierce Biotechnology) or fluorescence-based system (IRDye®, Li-Cor). Primary antibodies that were used included Rabbit anti-P-Smad 1/5/8 (Cell Signaling), Rabbit anti-Smad1 (Cell Signaling), Goat anti-Gremlin1 (R&D), Mouse anti-p21 (Cell Signaling), Rabbit anti-p27 (Santa Cruz), Rabbit anti-p57 (Santa Cruz), Mouse anti-c-myc (Santa Cruz), Rabbit anti-BMPR1b (Abgent), Rabbit anti-BMP2 (Abcam), Mouse anti-Tubulin (Millipore) and Mouse anti-Actin (Sigma). A Gremlin1 ELISA Kit (USCNK) was used to perform ELISAs on 1 mL of media harvested from 50,000 cells for 24 hours, following the manufacturer’s instructions.

**Cell Viability Assay and Caspase Activity Assay**

CSCs infected with shRNA-expressing lentivirus or treated with recombinant proteins were plated in 96 wells at 500 cells per well. Cell titers were determined after a number of days using the CellTiter-Glo Luminescent Cell Viability Assay Kit (Promega). Caspase activity was determined 2 days and 3 days following transduction using the Caspase-Glo 3/7 Assay Kit (Promega). Caspase activity was normalized to Cell Titer activity to account for differences in cell number.

**Cell Cycle Analysis**

CSCs infected with shRNA-expressing lentivirus were fixed two or three days after transduction in 70% ethanol. Fixed cells were labeled in propidium iodide-staining
buffer (0.25% Triton in PBS, 25 µg/mL RNAseA, and 10 µg/mL propidium iodide) for one hour. FACS analyses were performed on the FACS Scan Analyzer (BD, San Jose, CA) and ModFit (Verity Software House) analysis was performed to determine cell cycle fractions.

**P53 sequencing**

Genomic DNA was isolated from 2,000,000 CSCs on the Qiagen BioRobot EZ1 workstation using the Qiagen EZ1 DNA Tissue kit (Qiagen) following the manufacturer’s protocol. DNA was amplified by standard PCR using primers against exon 4-9 of TP53 as listed in Supplemental Table 3, for 30 cycles with an annealing temperature of 55°C. Sequencing was carried out on an ABI 3730xl DNA analyzer (Applied Biosystems). Mutations in exons 4-9 were evaluated using Finch TV software (Geospiza, Seattle, WA) and aligned with the complete sequence of the human p53 gene using Clustal W alignment software (Larkin et al. 2007).

**Statistical Analyses**

Grouped data are presented as mean +/- standard error. The difference between groups was determined by ANOVA or Student’s t-test using GraphPad Prism software. For survival analysis, survival was represented with Kaplan-Meier survival curves and p-values were calculated using a log-rank test.
Supplemental Reference