SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure S1. BMP2 and BMP7 are upregulated in patient gliomas. (A-B) Publicly available REMBRANDT expression data demonstrating that BMP2 and BMP7 are upregulated in patient gliomas (Oligo=oligodendroglioma). *, p<0.05; **, p<0.01; ***, p<0.001.

Supplemental Figure S2. Validation of CD133 as a marker for glioma CSC enrichment. (A) CD133+ fractions following sorting by CD133 magnetic beads. (B) Western Blot demonstrating differential expression of the stem marker Olig2 following sorting for CD133. RT-PCR for (C) Sox2, (D) Olig2 and (E) Nanog demonstrating differential expression in CD133+ and CD133- populations. (F-H) In vitro limiting dilution assays demonstrating increased sphere formation potential in CD133+ populations. (I-K) Quantification of F-H. (L,M) In vivo limiting dilution assay demonstrating decreased tumor latency and increased tumor formation in immunocompromised mice for CD133+ populations. *, p<0.05; **, p<0.01; ***, p<0.001.

Supplemental Figure S3. BMPs and BMP receptors are not consistently differentially expressed in CSC and non-stem glioma cell populations. (A) Western Blot of BMP2 and BMPR1b in matched CSC/non-stem glioma cell populations. RT-PCR expression data of (B) BMP4, (C) BMP7, (D) BMPR1a and (E) Chrd in matched CSC/non-stem glioma cell populations. *, p<0.05; **, p<0.01; ***, p<0.001.
Supplemental Figure S4. Gremlin1 colocalizes with CSC populations in cultured neurospheres. Immunofluorescent staining for Gremlin1 in cultured xenograft spheres with Sox2 and Olig2. Scale bar: 10µm.

Supplemental Figure S5. Gremlin1 does not colocalize with differentiation markers in glioblastoma. Immunofluorescent staining for Gremlin1 in three patient-derived xenografts and a primary human specimen in conjunction with (A) endothelial marker CD31, (B) astrocyte marker GFAP, neuronal markers (C) Map2 and (D) Tuj1, and (E) mature oligodendrocyte marker PLP. White arrows indicate colocalization; orange arrows indicate disparate staining. Scale bar: 10µm.

Supplemental Figure S6. Exogenous Gremlin1 blocks BMP2-mediated differentiation in CSCs. (A) RT-PCR for Olig2 and GFAP expression following three days of BMP2 and/or Gremlin1 treatments. (B) Immunofluorescent staining for stem marker Sox2 and astrocyte marker GFAP following six days of BMP2 and/or Gremlin1 treatments. *, p<0.05; **, p<0.01; ***, p<0.001. Scale bar: 10 µm.

Supplemental Figure S7. Overexpression of Gremlin1 does not change neuronal or oligodendrocyte marker expression. RT-PCR for (A) Map2, (B), Tuj1, and (C) GalC mRNA expression following Gremlin1 overexpression in four CSC populations.
Supplemental Figure S8. Gremlin1 knockdown in CSCs increases GFAP expression and decreases CD133 surface marker expression. (A) RT-PCR for GFAP expression following Gremlin1 knockdown. (B) Quantification and (C,D) FACS plots demonstrating a decrease in the CD133+ population following Gremlin1 knockdown in (C) 3691 and (D) IN528 CSCs.

Supplemental Figure S9. Gremlin1 knockdown decreases self-renewal in 3691 CSCs. (A) In vitro limiting dilution assay following ten days of Gremlin1 knockdown in 3691 CSCs. (B) Quantification of data in A.

Supplemental Figure S10. Gremlin1 knockdown does not cause apoptosis. Relative Caspase activity in 3691 and IN528 CSCs infected with Gremlin1 shRNAs.

Supplemental Figure S11. Gremlin1 knockdown-derived tumors re-express Gremlin1 in a subset of cells. Staining for Gremlin1 in tumors derived from NT shRNA or Gremlin1 shRNA-infected 3691 CSCs. Scale bar: 10µm.

Supplemental Figure S12. Gremlin1 mRNA expression is correlated with patient survival. Kaplan-Meier curve of patient survival based on publicly available microarray and survival data from The Cancer Genome Atlas.
Supplemental Figure S13. Gremlin1 knockdown activates BMP signaling. Ingenuity Map of BMP target genes with Gremlin1 shRNA gene expression data overlay. Predicted relationships: Orange line – activation; Blue line – inhibition; Yellow line – findings inconsistent with state of downstream molecule; Gray line – effect not predicted. Predicted expression changes: Red and Orange – activation; Blue – inhibition. All gene expression changes on this map are consistent with BMP activation except for BMP4 and MEF2C.

Supplemental Figure S14. Xenografts used in this study have differing p53 statuses. (A) Sequencing data of exons 4-9 of the TP53 gene indicating that IN528 is p53 mutant, while 3691 and 3565 are likely p53 wild-type. (B) Staining for p53 in tumor specimens confirming that IN528 is p53 mutant (positive staining), while 3691 and 3565 are p53 wild-type.