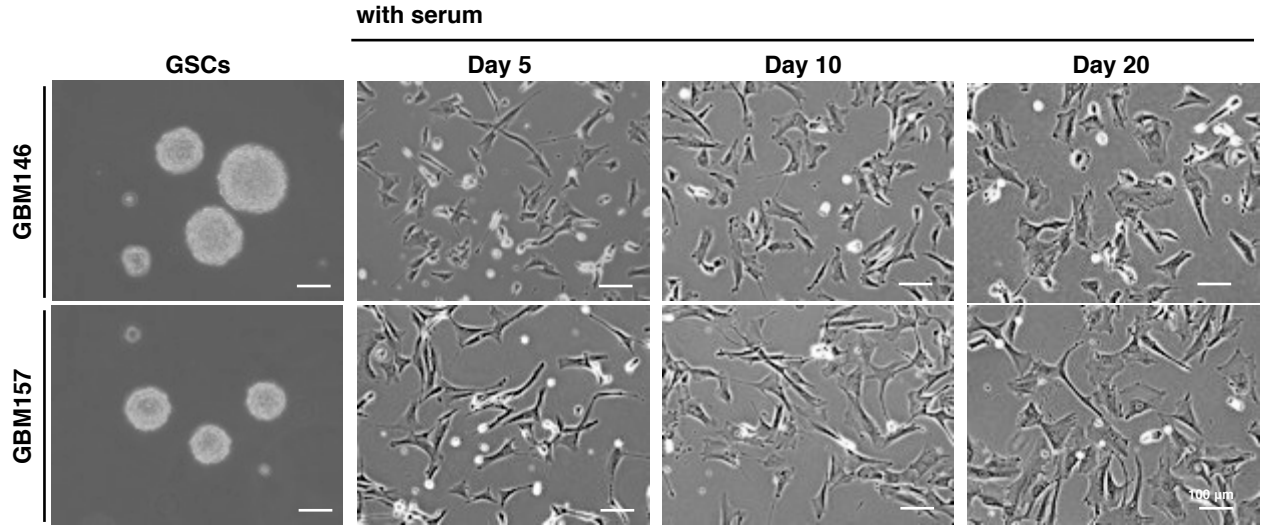
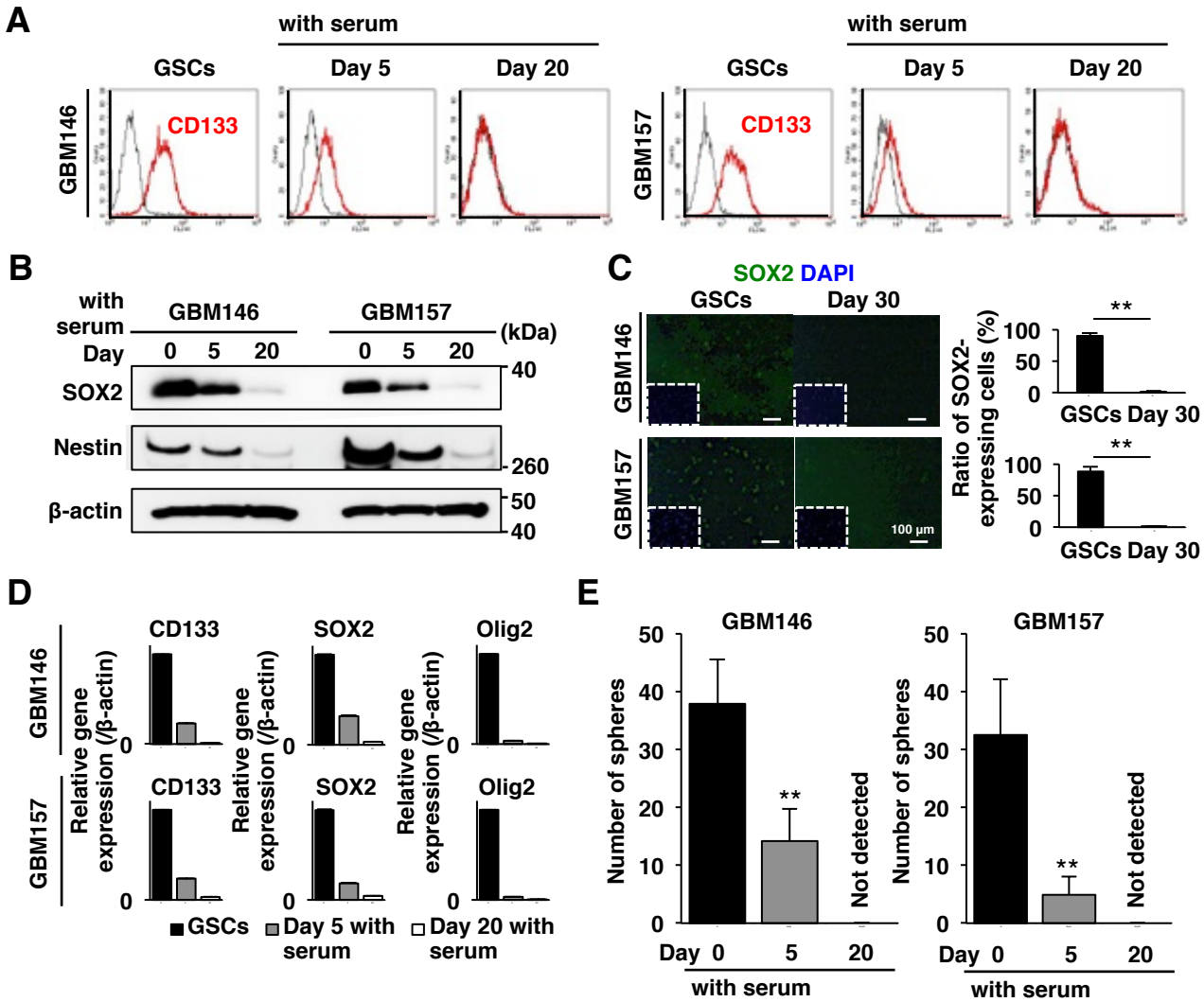
Supplementary Figures

Senescence from glioma stem cell differentiation promotes tumor growth

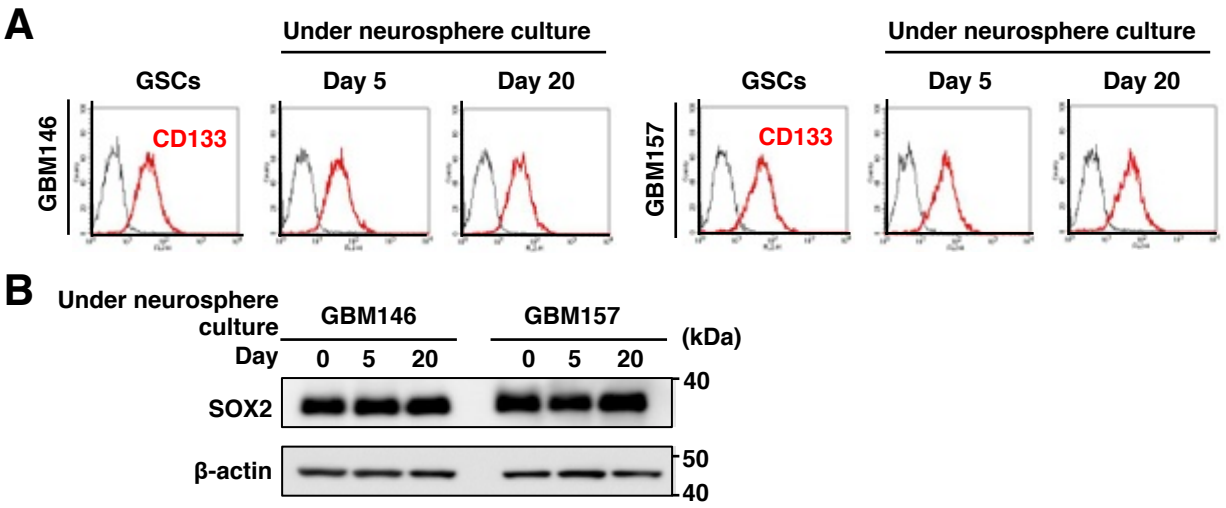
Rie Ouchi, Sachiko Okabe, Toshiro Migita, Ichiro Nakano & Hiroyuki Seimiya



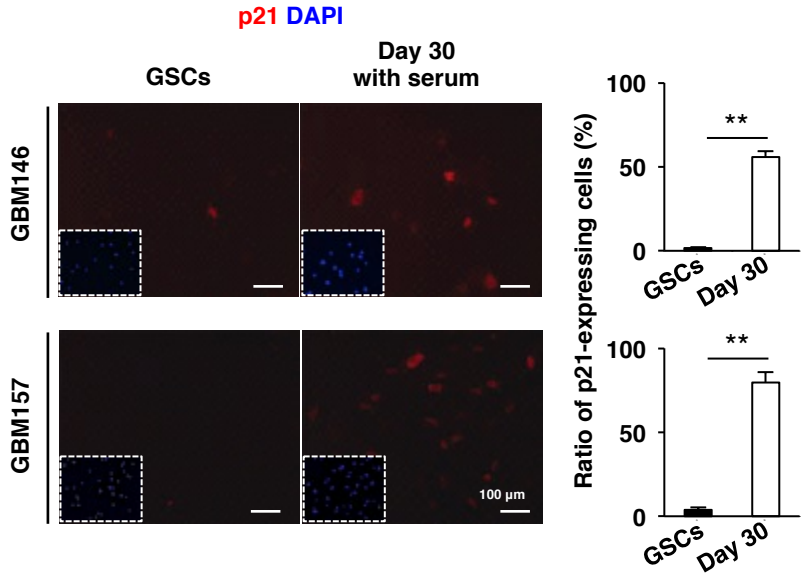
Supplementary Figure S1. GSCs are adherent and sharp in serum-containing medium. Morphological change of glioma stem cells (GSCs) at the indicated time after serum exposure. Cells were cultured in sphere medium (GSCs) or in serum-containing medium.



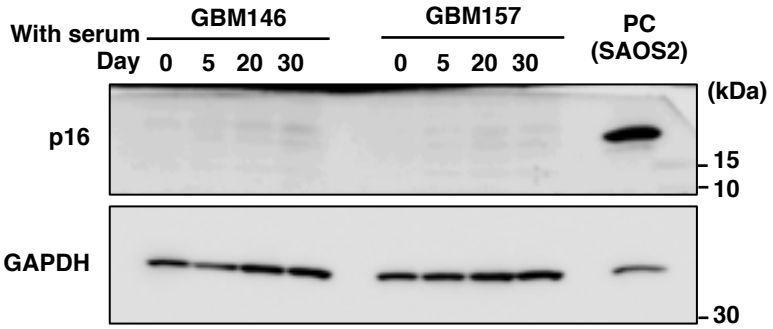
Supplementary Figure S2. Differentiation of GSCs to NSGCs upon serum stimulation. (A) Flow cytometry of CD133-positive cells. *Grey*: normal immunoglobulin as negative control. (B) Western blot analysis of SOX2 and Nestin. β-actin was used as an internal control. (C) Immunofluorescence staining with anti-SOX2 antibody (green). DNA was counterstained with DAPI (blue). Right graphs indicate quantitation of the SOX2-expressing cells ($n > 90$). Error bars, standard deviations. Statistical evaluations were performed using the Welch *t* test. **, $P < 0.01$. (D) Quantitation of *CD133*, *SOX2* and *Olig2* transcripts by RT-qPCR. The expression value for each gene was normalized with that of *β-actin*. (E) Sphere-forming assay. Cells were cultured in serum-containing medium for 5 or 20 days, and subsequently transferred into serum-free, sphere culture medium. The number of spheres was counted at day 7 of culture. Error bar, standard deviation. Statistical evaluations were performed using the Welch *t* test. **, $P < 0.001$.



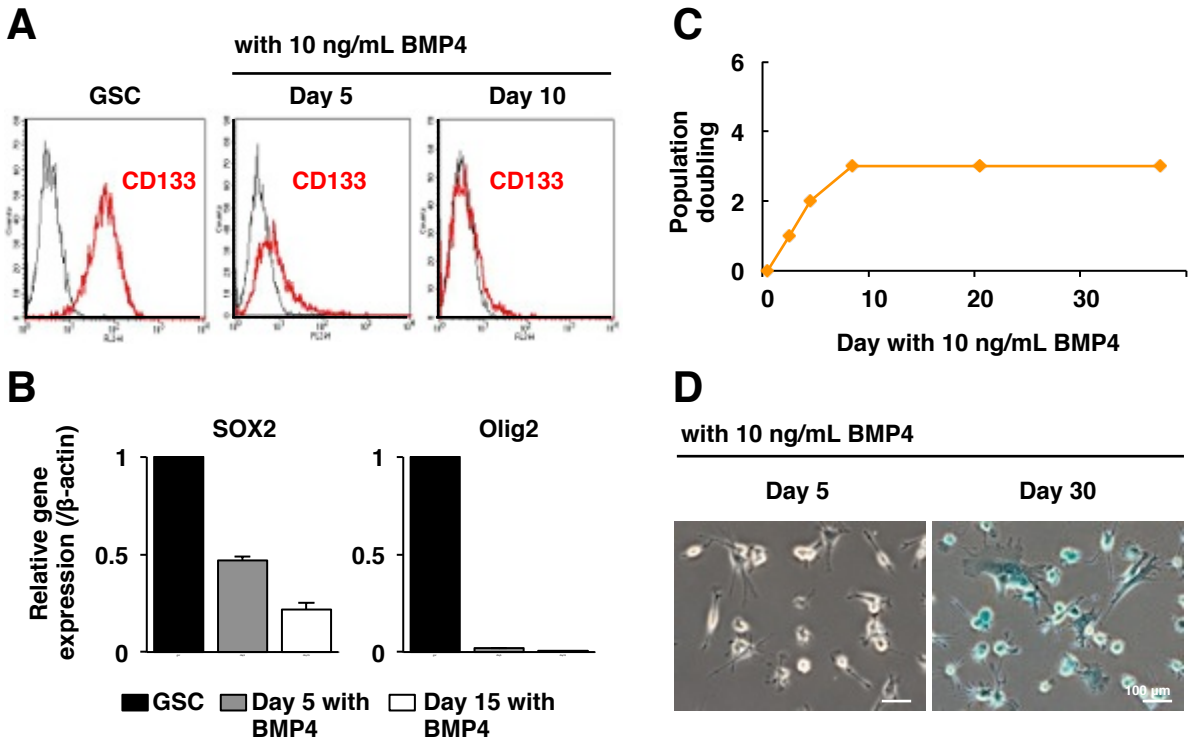
Supplementary Figure S3. Stable expression of GSC markers under neurosphere culture. GBM146 and GBM157 GSCs were cultured for 0, 5, or 20 days in neurosphere culture. (A) Flow cytometry with anti-CD133 antibody (red). Grey: normal immunoglobulin as negative control. (B) Western blot analysis of SOX2. β-actin was used as an internal control.



Supplementary Figure S4. p21 upregulation in serum-treated glioma stem cells. Glioma stem cells (GSCs) and non-stem glioma cells (NSGCs) cultured in serum-containing medium for 0 and 30 days, respectively, were subjected to immunofluorescence staining with anti-p21 antibody (red). DNA was counterstained with DAPI (grey). Graphs on right indicate quantitation of the p21-expressing cells ($n > 90$). Error bars, standard deviations. Statistical evaluations were performed using the Welch *t* test. **, $P < 0.01$.



Supplementary Figure S5. Lack of p16 induction during senescence of NSGCs. Cells were cultured in serum-containing medium for the indicated time periods. Cell lysates were prepared and subjected to western blot analysis with anti-p16 and anti-GAPDH antibodies. PC: SAOS2 cells as positive control for p16 expression.



Supplementary Figure S6. BMP4 induces differentiation and subsequent senescence of GSCs. GBM157 GSCs were cultured in BMP4-containing (10 ng/mL), serum-free medium for the indicated time periods. Then, the cells were subjected to the following experiments: (A) Flow cytometry with anti-CD133 antibody (red). Grey: normal immunoglobulin as negative control. (B) RNA expression of *SOX2* and *Olig2* measured by RT-qPCR. The expression value for each gene was normalised with that of *β-actin*. (C) A growth curve of NSGCs in BMP4-containing culture. (D) Senescence-associated β-galactosidase (SA-β-gal) staining. Blue color indicates senescence.