Supplemental Information

1. Supplemental Data

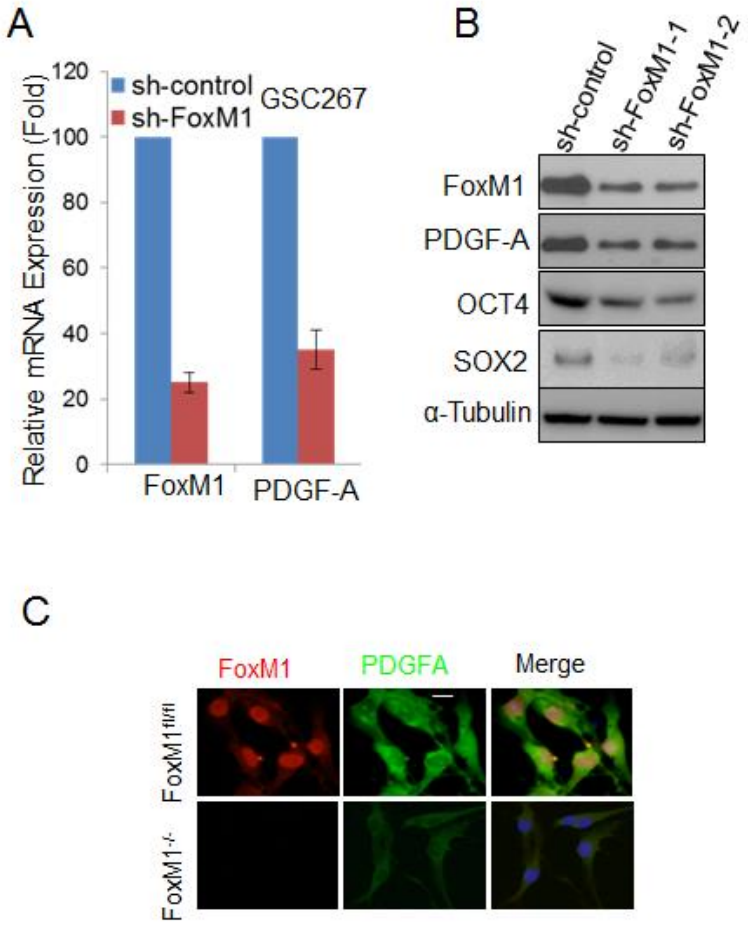


Figure S1. (A) FoxM1 and PDGF-A mRNA levels were detected using real-time PCR in stable FoxM1-knockdown or sh-control GSC267 cells. (B) FoxM1, PDGF-A, OCT4 and SOX2 protein levels were detected by Western blotting in stable FoxM1-knockdown or sh-control GSC267 cells. (C) PDGF-A and FoxM1 proteins in immortalized $FoxM1^{fl/fl}$ and $FoxM1^{-/-}$ NSCs were determined by immunefluorescence staining. Bar is 20μm.

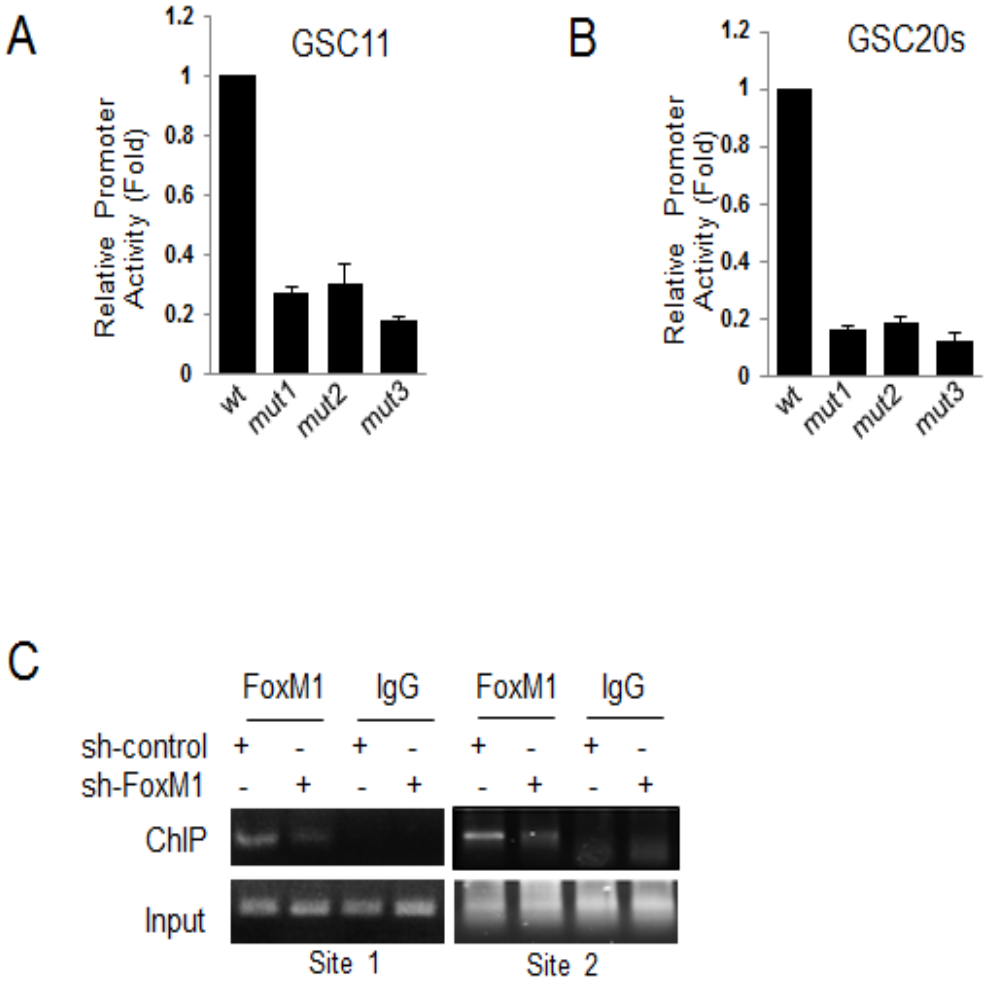
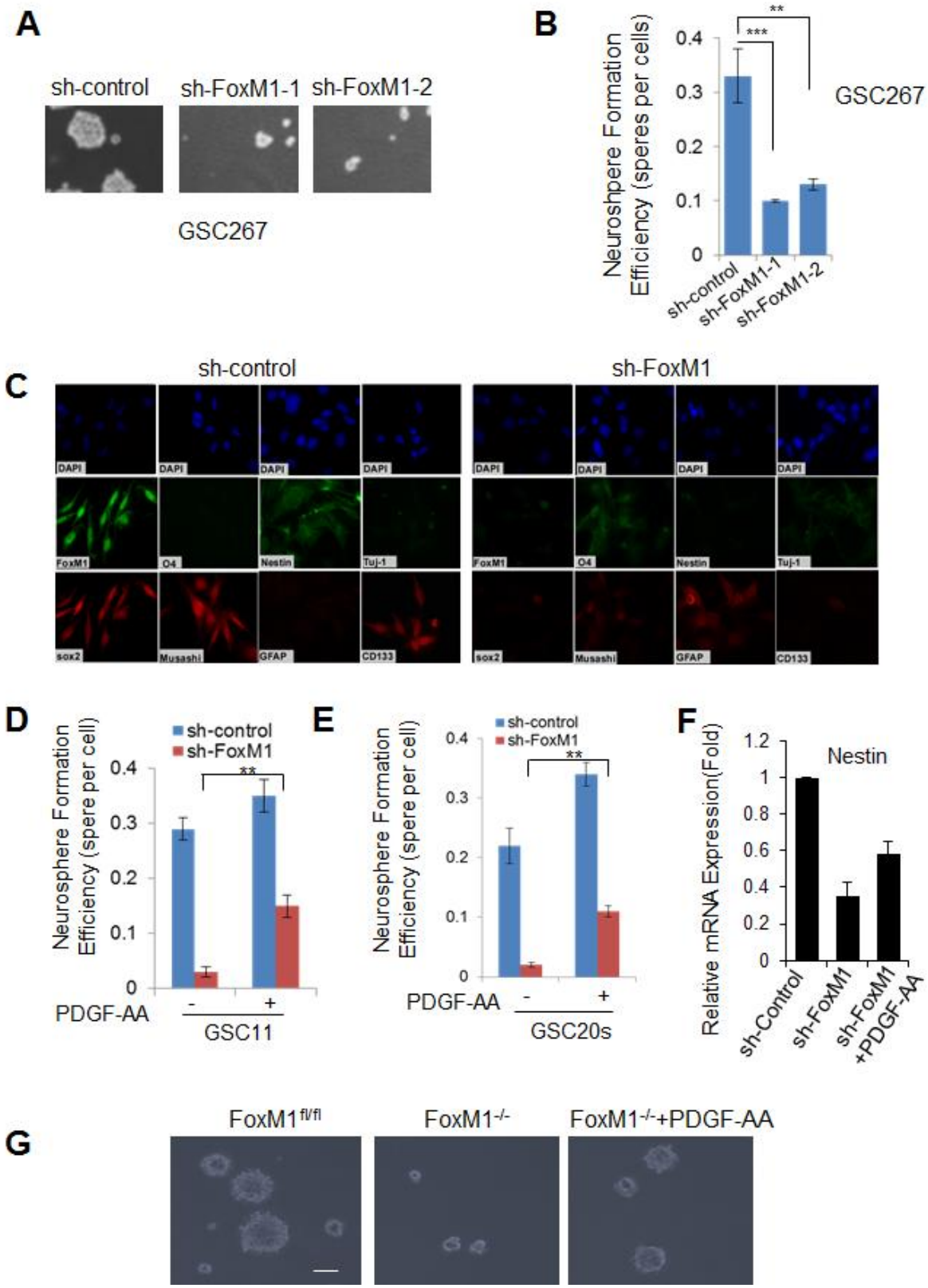


Figure S2. (A & B) The relative promoter activities driven by different *PDGF-A* promoter mutants were determined by dual luciferase reporter assay in GSC11 (A) or GSC20s (B). Values are mean ± SD for triplicate samples. (C) ChIP assays were performed in GSC11 cells expressing sh-control or sh-FoxM1.


A
sh-control
sh-FoxM1-1
sh-FoxM1-2
GSC267
B
Neuroshpere Formation Efficiency (speres per cells)
**
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GSC267
sh-control
sh-FoxM1-1
sh-FoxM1-2
C
sh-control
sh-FoxM1
DAPI
FoxM1
O4
Nestin
Tuj-1
sox2
Musashi
GFAP
CD133
D
Neurosphere Formation Efficiency (spere per cell)
sh-control
sh-FoxM1
PDGF-AA
GSC11
E
Neurosphere Formation Efficiency (spere per cell)
sh-control
sh-FoxM1
PDGF-AA
GSC20s
F
Relative mRNA Expression(Fold)
Nestin
sh-Control
sh-FoxM1
sh-FoxM1 +PDGF-AA
G
FoxM1fl/fl
FoxM1-/-
FoxM1-/-+PDGF-AA

Figure S3. (A) Photographs of neurosphere of GSC267 cells expressing control or FoxM1 shRNAs. (B) Neurosphere formation efficiency of the cells in (A). Values are mean ± SD for triplicate samples. $P<0.001$ (C) FoxM1 knocking-down in GSC11 cells suppressed the expression of stem cell markers Nestin and CD133, but increased the expression of differentiation makers O4, GFAP and Tuj-1 as determined by immunefluorescence staining. (D & E) The neurosphere formation efficiency (spheres/cells plated) were analyzed in GSC11- or GSC20s-sh-control and sh-FoxM1 treated with or without PDGF-AA (20ng/ml). $P<0.001$ (F) Nestin mRNA relative expression was determined by real-time RT-PCR in GSC11-sh-control and sh-FoxM1 treated with or without PDGF-AA (50ng/ml). (G) Photographs of neurosphere formation of $FoxM1^{fl/fl}$ and $FoxM1^{-/-}$-NSCs treated with or without PDGF-AA (50ng/ml). Bar is 10μm.

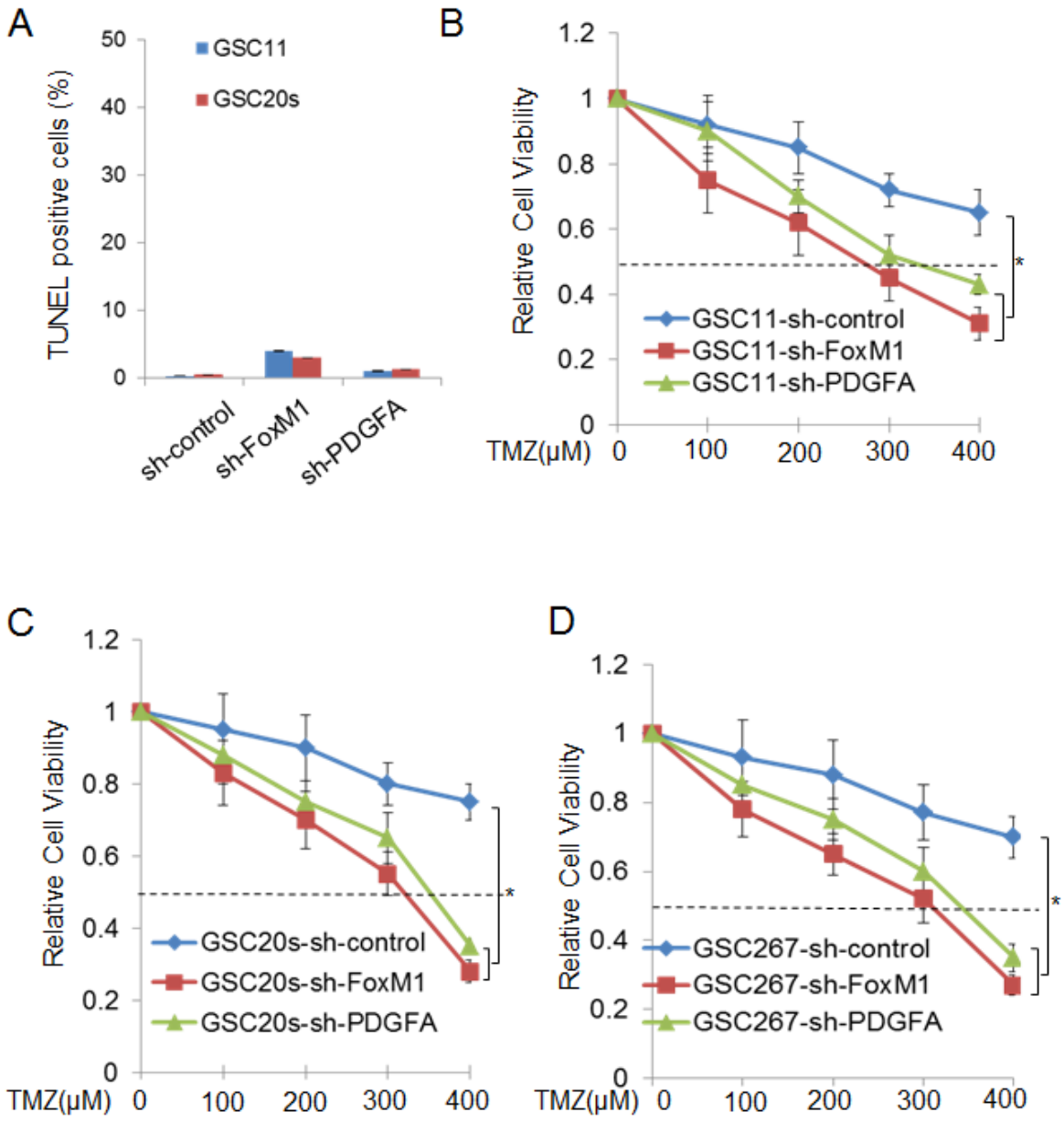

Figure S4. (A) The percentages of TUNEL positive cell in GSC11- and GSC20s-sh-control, sh-FoxM1 or sh-PDGFA were determined by TUNEL apoptosis assay. (B-D) GSC11-, GSC20s- and GSC267-sh-control, sh-FoxM1 or sh-PDGFA cells were treated with TMZ (0, 100, 200, 300, and 400μM), respectively. Relative cell viability was determined by MTT assay in GSC11 (B), GSC20s (C) and GSC267 (D). Values are mean ± SD for triplicate samples. **Dotted line** indicates 50% cell viability. P<0.01.

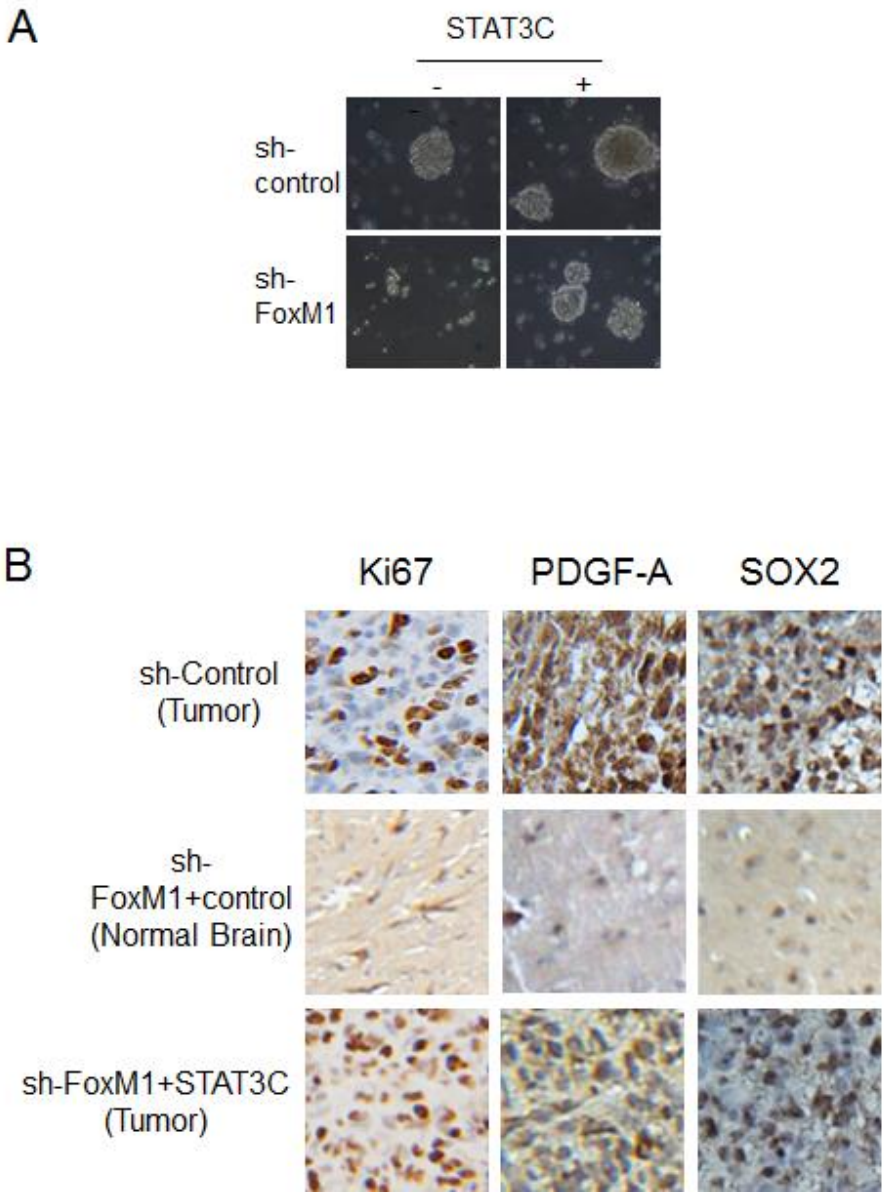

Figure S5. (A) Photographs of neurosphere formation of GSC11-sh-control and GSC11-sh-FoxM1 cells rescued by STAT3C. (B) Sections of normal brain from GSC11-sh-FoxM1+control group or tumors produced by GSC11-sh-Control and GSC11-sh-FoxM1+STAT3C were subjected to immunohistochemistry staining for Ki67, PDGF-A and SOX2. Representative pictures are shown

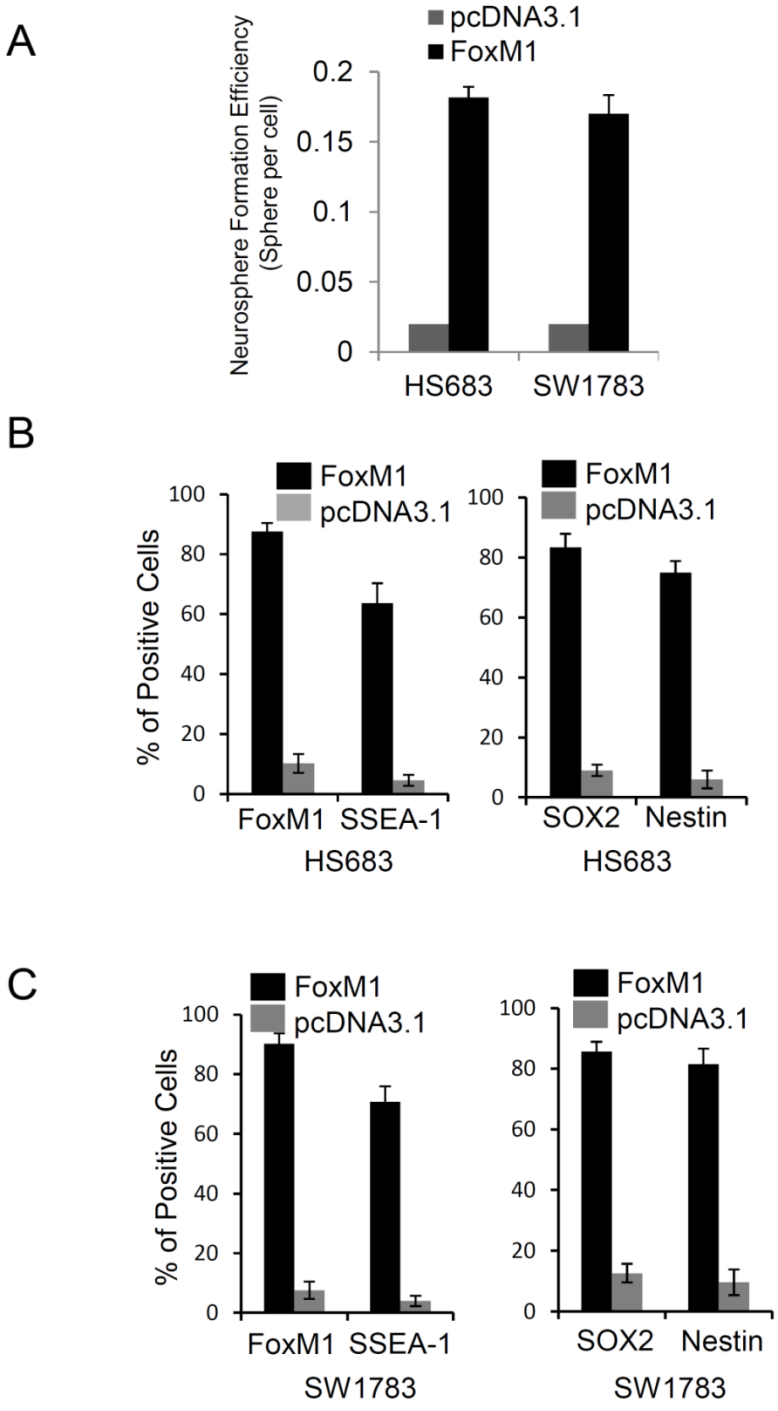

Figure S6. (A) The neurosphere formation efficiency (spheres/cells plated) was analyzed in SW1783 and HS683 cells expressing FoxM1 or pcDNA3.1 cells cultured in neurosphere medium. (B, C) Percentage of cells expressing indicated markers was analyzed in cells indicated above.

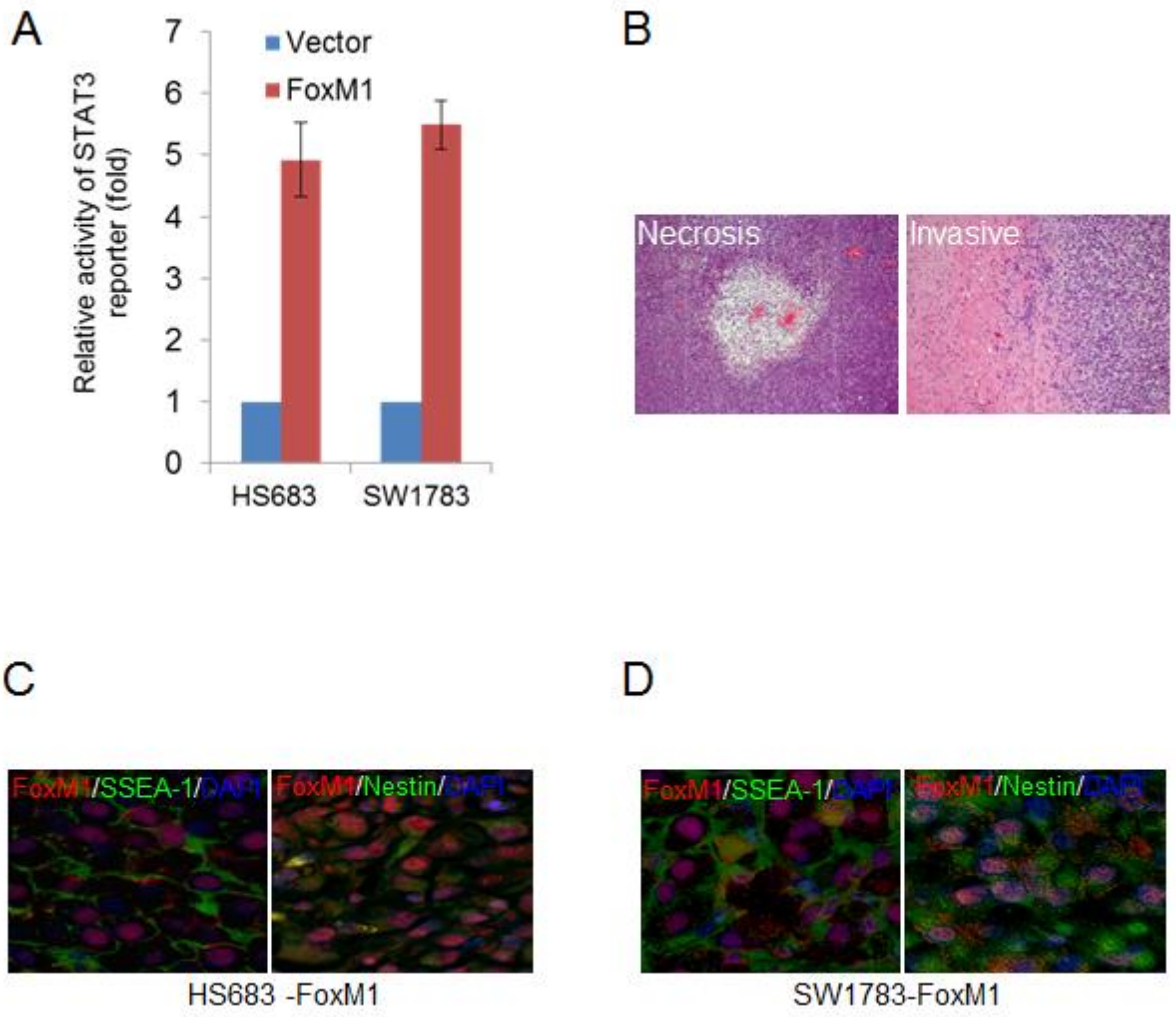

Figure S7. (A) Relative activity of STAT3 reporter was determined by dual luciferase reporter assay in SW1783 and Hs683 cells expressing FoxM1 or pcDNA3.1 cultured in neurosphere medium. (B) Sections of tumors produced by Hs683-FoxM1 cells or brain tissues from mice injected with Hs683-vector cells were HE-stained. (C&D) Sections of tumors produced by Hs683-FoxM1 (B) and SW1783-FoxM1 cells (C) were immunofluorescence-stained for FoxM1, SSEA-1, and Nestin. Bar is 20 μm.

2. Supplemental Materials and Methods

Immunofluorescent staining and confocal microscopy

GSCs were seeded on chamber slides pre-coated with poly-L-ornithine and fibronectin. Cells or tissue sections cut from frozen GBM specimens were fixed with 4% paraformaldehyde, permeabilized with 0.1% Triton X-100 in PBS for 2 min (PBS-T), quenched using 50 mM NH_4Cl in PBS-T, and blocked with 1% BSA in PBS-T. Immunostaining was performed using the appropriate primary and secondary antibodies. Nuclei were stained with DAPI (Gold antifade with DAPI, Invitrogen), and images were acquired using an Olympus FluoView FV1000 confocal microscope.

Lentivirus production and establishment of stable cell lines

Lentivirus was produced with PRNAT-U6.2-shFoxM1 or PRNAT-U6.2-shControl by using the Invitrogen lentivirus packing mix system as described (Zhang et al. 2011). GSC11, GSC20s and GSC267cells were infected with the lentivirus with 6 μg/ml polybrene for 24 hr. After 72 hr of transduction, cells were selected with 500 μg/ml G418 (Invitrogen) for 14 days to establish stable cell lines.

Preparation of NSCs from FoxM1$^{fl/fl}$ mice, immortalized and deletion of FoxM1 from the cells

Primary NSCs were isolated from the subventricular zones of FoxM1$^{fl/fl}$ mice and maintained in stem cell medium as previously described (39). The cells were then immortalized by transfection of SV-40 large and small T-antigen vector. Specifically, the cells were transfected with the plasmid pSV3neo containing the SV40 large and small T-antigen early regions and the neomycin gene which confers resistance to G418. Control cells were transfected with pSV3neo which does

not contain T-antigen. Resistant colonies were selected by culturing the cells with G418 for 14 days.

To delete the floxed alleles of FoxM1, the adenoviral vector expressing Cre recombinase (Ad-CMV-Cre; Vector Biolabs) was transduced to the NSCs. An adenoviral vector expressing GFP (Ad-CMV-GFP; Vector Biolabs) was used as a control.

Real-time reverse transcription-PCR

TRIzol (Invitrogen) was used for RNA extraction and residual DNA was removed by DNaseI treatment (Invitrogen). cDNA was generated with a Reverse Transcription kit (Invitrogen) using oligo dT primers. Relative transcript expression levels were measured by quantitative real-time PCR using a SYBR Green-based method on a MiniOpticon real-time PCR system (BioRad) using the following primers: FoxM1: sense 5'- AA CAATAGCCTATCCAACA -3', antisense 5'- CTCCATCTCTTGCTTGAT -3'; GFAP: sense 5'- AGGAACATCGTGGTGAAGA -3', antisense 5'- ATCCTTGTGCTCCTGCTT -3'; PDGF-A: sense 5'- AAGTCCAGGTGAGGTTAG -3', antisense 5'- TCCTCTTCCCGATAA T CC -3'; STAT3: sense 5'- CACCTTCCTGCTAAGATTCA-3', antisense 5'- TTACCG CTGATG TCCTTC-3'; Nestin: sense 5'- GAAGAGGACCAGAGTATT -3', antisense 5'- TTCAAGAG TTC TCA ATGTC -3'; Tuj-1: sense 5'-GAGCGGATCAGCGTCTAC-3', antisense 5'-TTCC AGGTCCACCAGAATG-3'; GAPDH: sense 5'- CGCTCTCTGCTCCTCCTGTT -3', antisense 5'- CCATGGTGTCTGAGCGATGT -3'.

ChIP assays

For ChIP assay, 2×10^6 cells were prepared with a ChIP assay kit (Cell Signaling Technology) according to the manufacturer's instructions. The resulting precipitated DNA samples were analyzed by PCR to amplify the putative FoxM1 binding region 1 (−544 to −535bp) or binding region 2 (−211 to −201 bp) of the PDGF-A promoter using the primers 5'-CGACTGGAGCTCGCTCCC-3' and 5'-GGGACCGGCTCTCTGGCC-3' or the primers 5'-GCCGCAGGATTGCAGCTG-3' and 5'-CGAACCCCGAGCGCTTCC-3', respectively. The resulting precipitated DNA samples were analyzed by PCR to amplify the putative TCF4 binding region (−571 to −565 bp) of the STAT3 promoter using the primers 5'-GGTTAAATCCACTACCCTCTC-3' and 5'-GGGAATCAGCTAGTTAGATAGTC-3'.

***In vitro* cell proliferation assay and apoptosis assay**

For the cell proliferation assay, $1x10^5$ cells were plated in 6-well plates. Three days later, viable cells were counted with trypan blue and a hemacytometer. Data represent the means ± SD of three independent experiments. For TUNEL assay, cells were gently harvested. Cells were washed, and fixed in 4% paraformaldehyde. Cells were kept in permeabilization solution (0.1% Triton X-100, 0.1% sodium citrate) for 2 minutes on ice and then were underwent TdT enzyme reaction at 37°C for 1 hour. The cells were then washed with PBS twice and the samples were subjected to flow cytometry for apoptosis analysis. Quantitative results were analyzed by Student's t-test (two-tailed); $P<0.05$ was considered statistically significant.

TMZ treatment and cell survival assay

TMZ was obtained from Sigma-Aldrich (T2577) and dissolved in dimethyl sulfoxide. Cells were pre-incubated with various concentrations of TMZ for 4 days before cells were

collected. Each experiment was performed in triplicate. Cell viability was determined by using a standard tetrazolium bromide (MTT) assay.