*β*1,4-Galactosyltransferase V activates Notch1 signaling in glioma stem-like cells and promotes their transdifferentiation into endothelial cells

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Running title: *β*1, 4GalT V repression decreases glioma angiogenesis

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*β*1,4GalT V; glioblastoma; glioma stem-like cell; endothelial cell; transdifferentiation; Notch1

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Supplement Fig.1

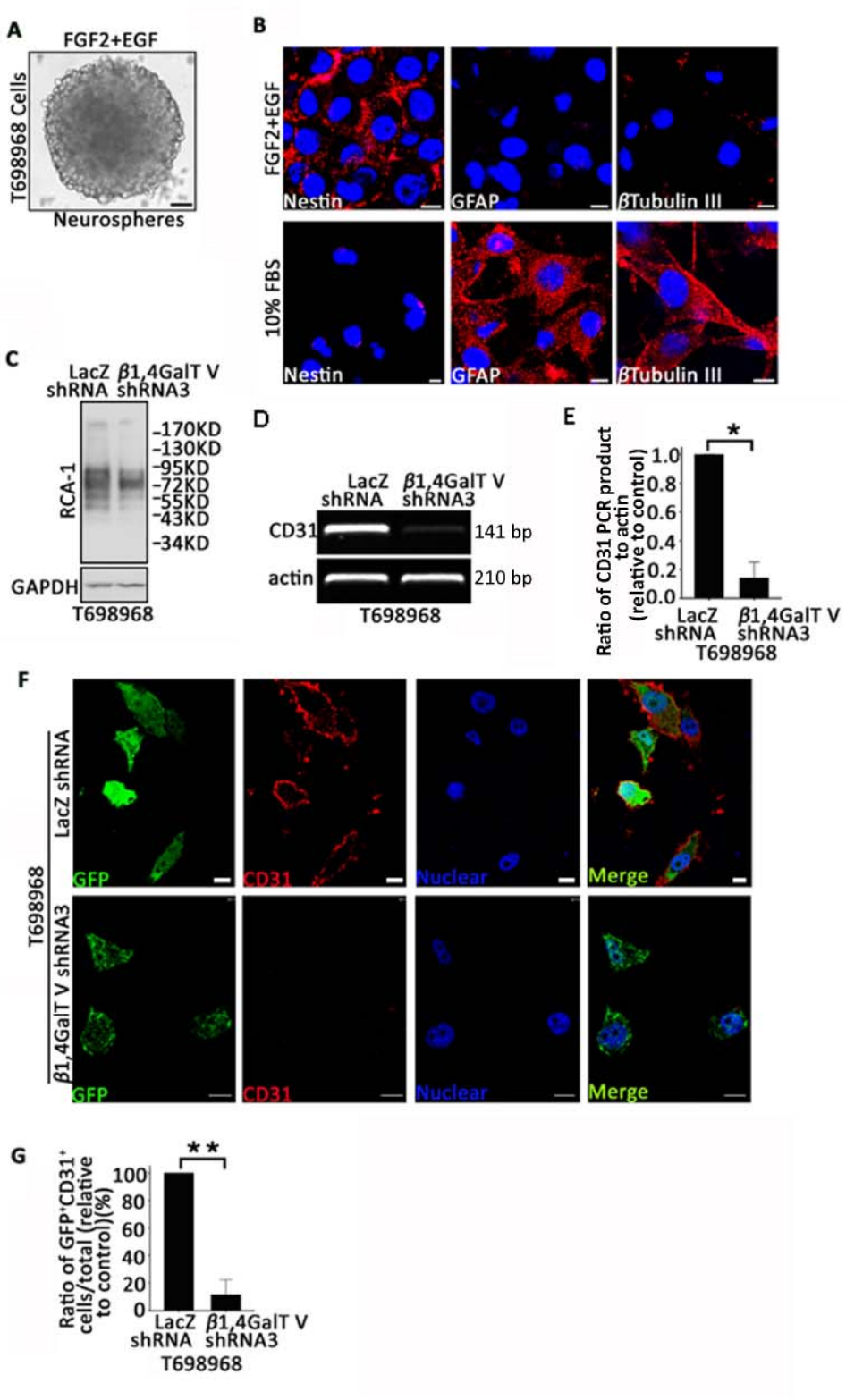

Figure S1. β1, 4GalT V depletion inhibits the transdifferentiation of glioma stem-like

cells *in vitro*.

(A) Neurospheres formed by T698968 cells are shown. Scale bar, 20 μm. (B) Immunostaining assay showed expression of Nestin, GFAP and β-tubulin Ⅲ of T698968 cells cultured in serum-free medium or in medium containing 10% FBS. Scale bar, 10 μm. (C) Proteins were separated by SDS-PAGE. The binding to RCA-1 lectin was analyzed by lectin blot in T698969 cells. GAPDH expression served as a loading control. (D) RT-PCR analysis showed that reduction of β1,4GalT V expression suppressed the CD31 mRNA expression in T698968 cells. Actin expression served as loading control. (E) Relative densities of CD31 PCR product level in (D) were quantified using densitometry. Values are normalized to that of T698968 cells expressing LacZ shRNA. Results are expressed as Mean ± SD (n = 3; $*p < 0.05$). (F) Immunofluorescence staining of human endothelial cells marker CD31 and GFP in T698968 cells expressing control- or β1,4GalT VshRNA cultured in human endothelial SFM. Scale bar, 10 μm. (G) The number of $GFP^{+}CD31^{+}$ cells in (F) was quantified using cell counting. Values are normalized to that of T698968 cells expressing LacZ shRNA. Results are expressed as Mean ± SEM (n = 3; **p < 0.01).

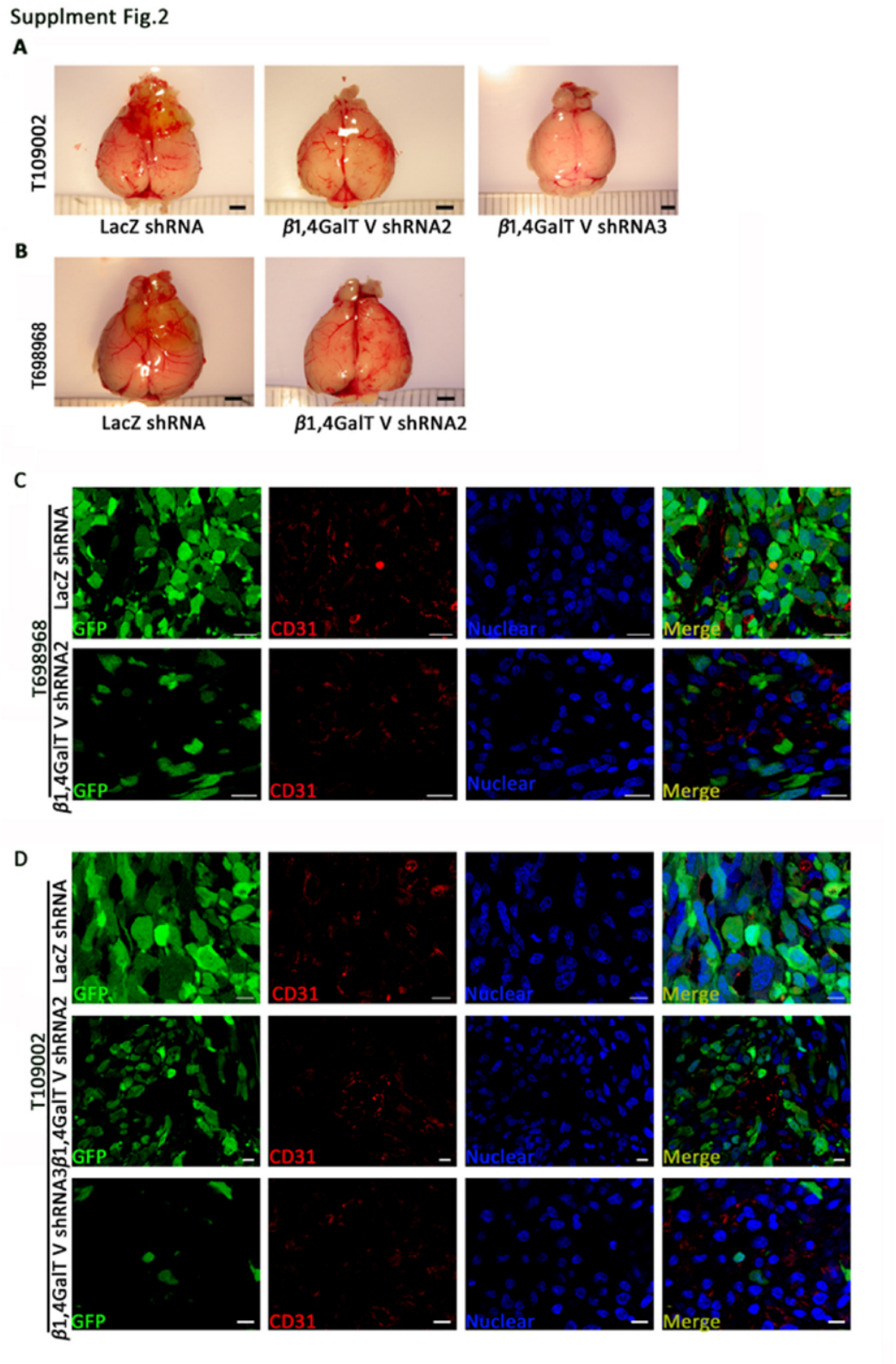

Figure S2. Reduction of *β*1, 4GalT V expression inhibits gliomagenesis and transdifferentiation of glioma stem-like cells into endothelium *in vivo*.

(A-B) Nude mice were injected with T109002 (A) or T698968 (B) cells expressing control or *β*1,4GalT V shRNA. Four weeks later, photos were taken for the xenograft. Scale bar, 2 mm.

(C-D) Confocal immunofluorescence analysis showed co-localization of human endothelilal cell marker CD31 and tumor cell marker GFP in tumor xenograft formed by T698968 (C) and T109002 (D) cells expressing control or β1,4GalT V shRNA. Scale bar, 10 μM.