Supporting Information

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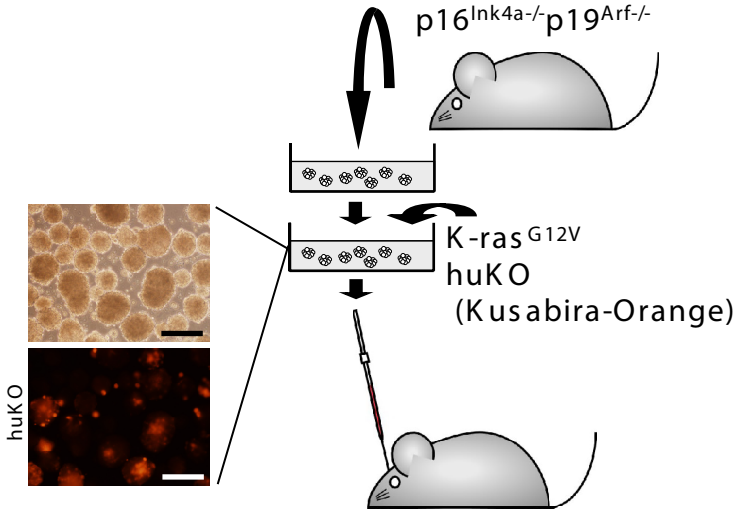

Fig. S1. Generation of the brain tumor model. Neurosphere cells derived from $p16^{Ink4a-/-}/p19^{Arf-/-}$ neonates were infected with retrovirus carrying the mutant K-ras ($K\text{-}ras^{G12V}$) and huKO genes. These cells were then inoculated into the brains of WT recipient mice. Neurosphere cells (*Upper*, bright field; *Lower*, huKO fluorescence) are shown. (Scale bars: 200 μm.)

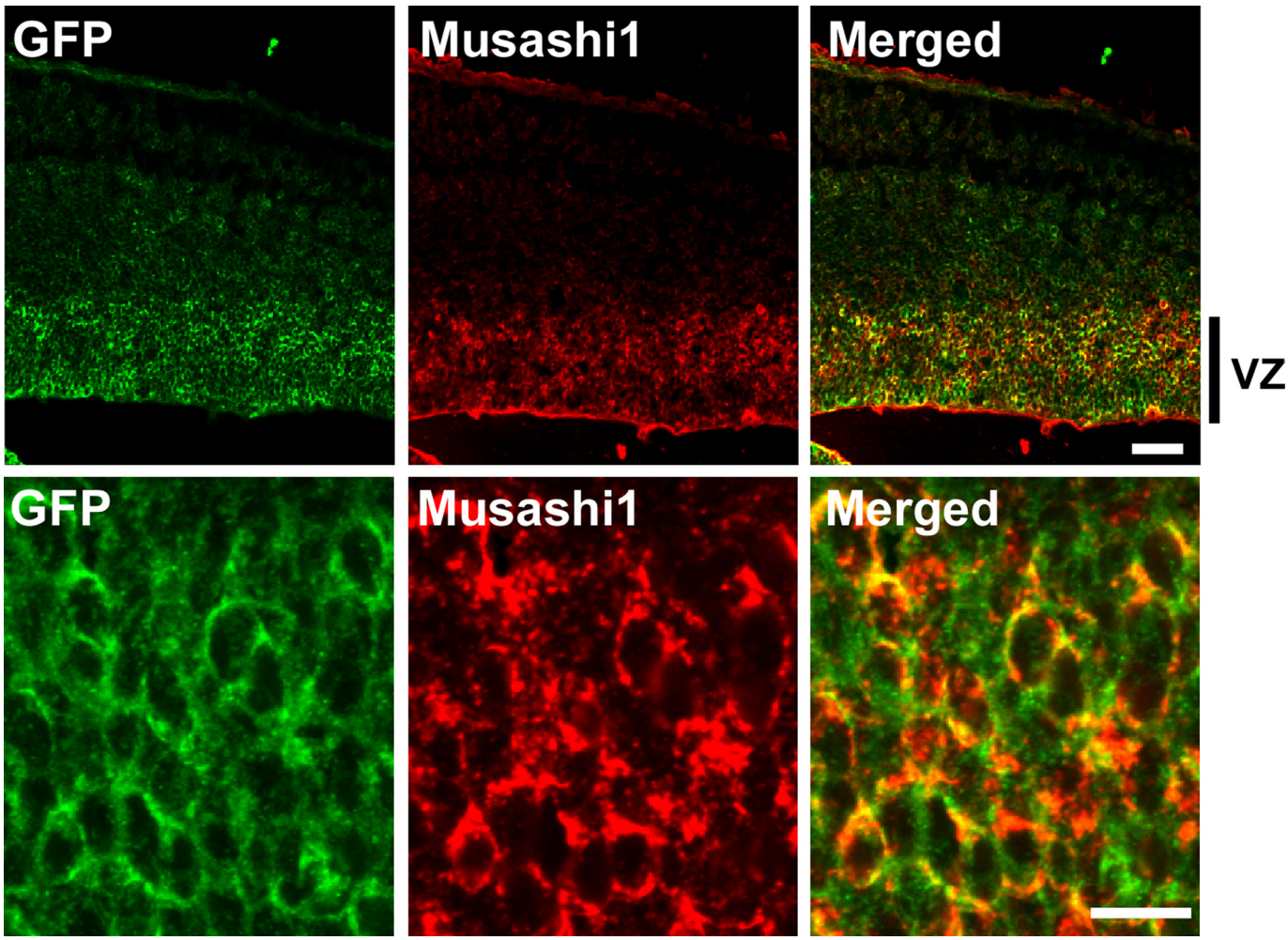

Fig. S2. NSC/NPCs in E14.5 NS-GFP-Tg mice. Coronal sections of the forebrains of E14.5 NS-GFP-Tg mice were subjected to immunofluorescence analysis with anti-GFP (green) and anti-musashi-1 (14H-1, red) [Kaneko, Y., et.al. (2000) *Dev Neurosci* 22:139–153]. VZ, ventricular zone. (*Bottom*) Higher magnification views of the images in *Upper*. (Scale bars: *Upper*, 100 μm; *Lower*, 10 μm). For immunostaining with the biotinylated anti-musashi-1 antibody, the tyramide signal amplification (TSA) system was used according to the manufacturer's protocol (Perkin Elmer).

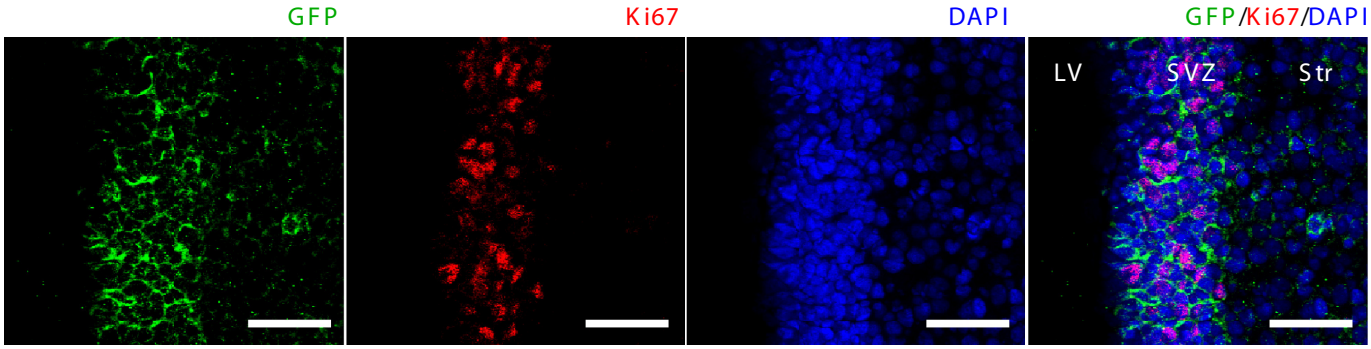

Fig. S3. NSC/NPCs in postnatal NS-GFP-Tg mice. Coronal sections of forebrains of P3 NS-GFP-Tg mice were subjected to immunofluorescence analysis with anti-GFP (green), anti-Ki67 (red) and DAPI (nuclear staining, blue). SVZ, subventricular zone; Str, striatum; LV. Lateral ventricle. (Scale bars: 50 μm.)

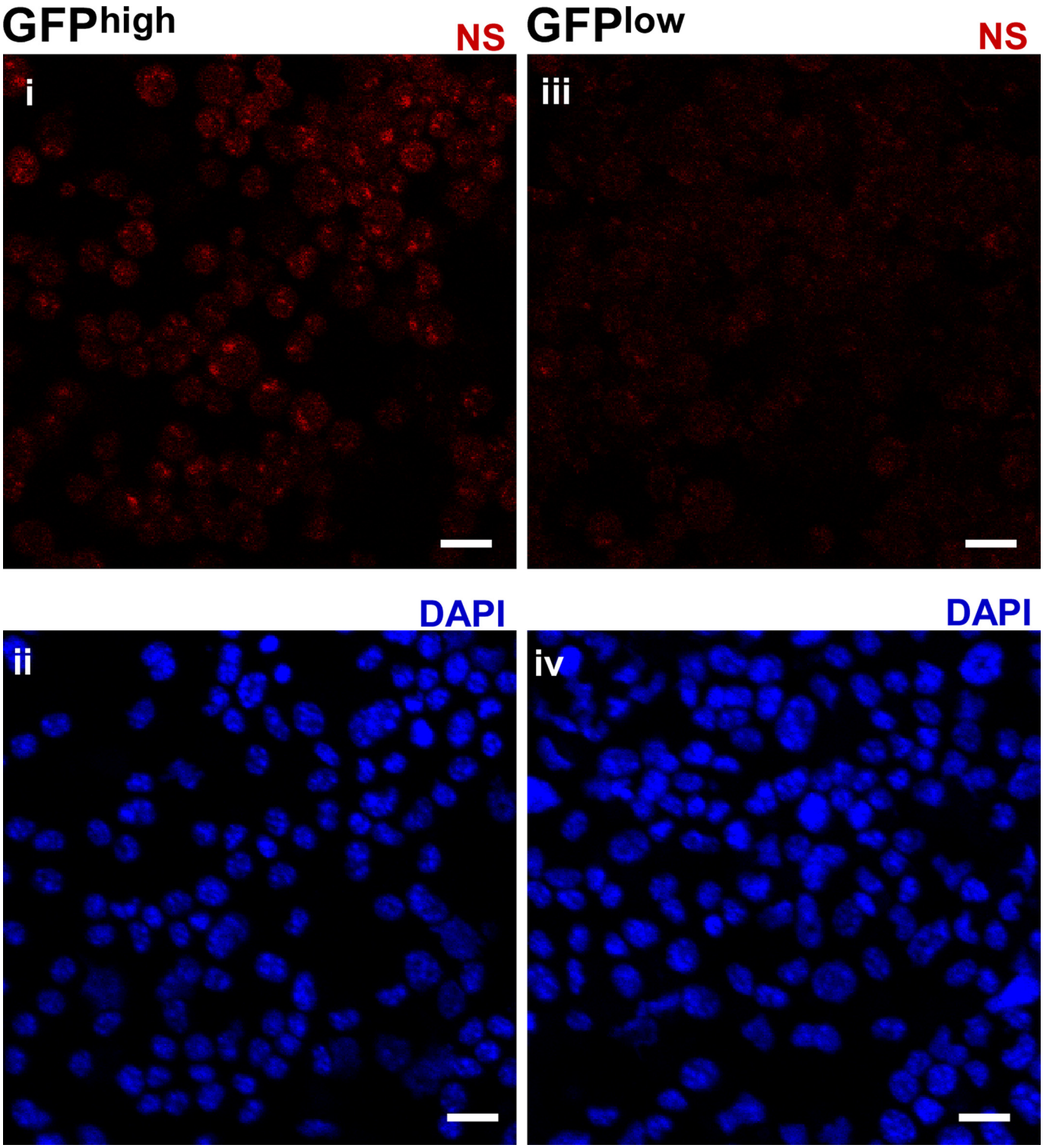

Fig. S4. Endogenous NS expression by GFP^{high} or GFP^{low} tumor cells. Cytospin smears of sorted GFP^{high} or GFP^{low} cells were fixed and stained with (*i* and *iii*) anti-NS antibody (red) or (*ii* and *iv*) DAPI (blue, nuclear staining). (Scale bars: 20 μm.)

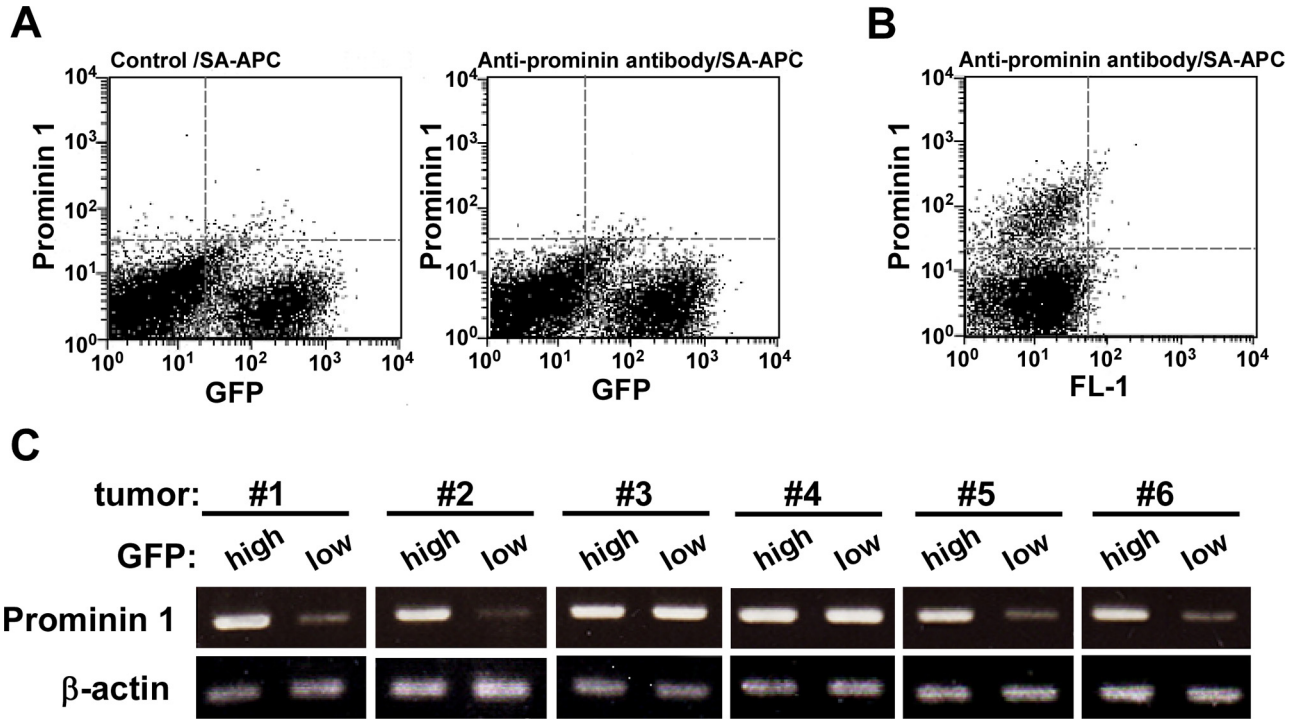

Fig. S5. Prominin 1 expression in GFP^{high} or GFP^{low} tumor cells. (*A*) Flow cytometry analysis of brain tumor cells with anti-prominin 1 antibody. Dissociated brain tumor cells were stained with biotin-conjugated anti-prominin 1 antibody (13A4, eBioscience), followed by staining with streptavidin (SA)- allophycocyanin (APC) (*Right*). Simultaneously, tumor cells were incubated with only SA-APC as control (*Left*). (*B*) SVZ cells from P3 C57BL/6 mice were stained with anti-prominin 1 antibody as shown in *A*. Expression of prominin protein was not detected in tumor cells, despite the fact that the antibody recognized prominin protein in normal brain tissue. Note: We assume that the epitope for recognition by antibody in brain tumors may differ or be masked compared with normal tissue. Alternatively, prominin 1 protein expression may be extremely low in tumors. (*C*) Expression of prominin 1 mRNA in brain tumor cells. Total RNA was purified from GFP^{high} and GFP^{low} cells isolated from three independent original tumors and prominin 1 mRNA levels were evaluated by RT-PCR.β-actin, control. Prominin 1 mRNA levels in GFP^{high} tumor cells were higher than that seen in GFP^{low} cells in tumors 1, 2, 5, and 6, but a similar correlation was not observed in tumors 3 and 4.

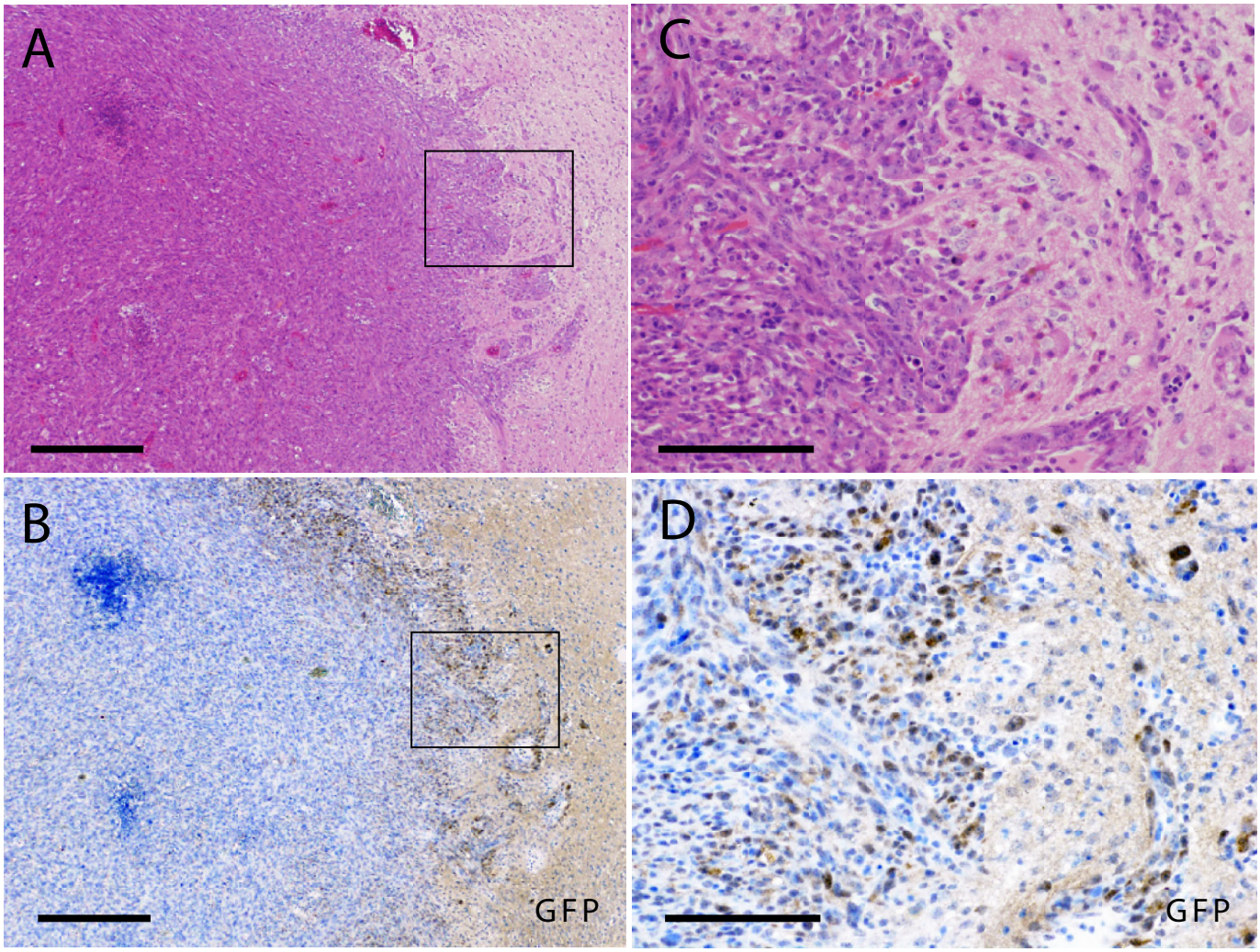

Fig. S6. Localization of T-ICs in the original brain tumor. Serial sections of one of the original tumors were subjected to: (*A* and *C*) H&E staining and (*B* and *D*) anti-GFP staining. Magnified views of the areas indicated by the squares in *A* and *B* are shown in *C* and *D*, respectively. (Scale bars: *A* and *B*, 1 mm; *C* and *D*, 200 μm).

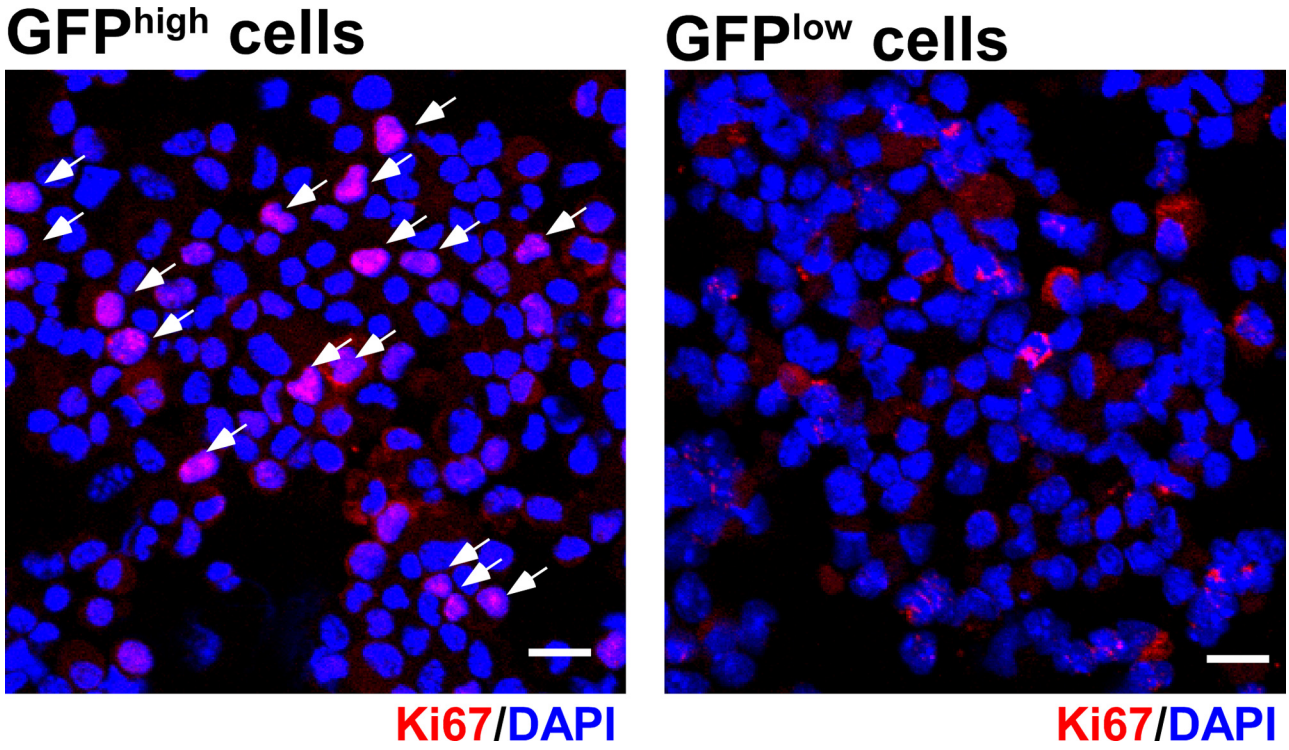

Fig. S7. Cell cycle status of GFP^{high} and GFP^{low} tumor cells. Cytospin smears of sorted GFP^{high} and GFP^{low} cells were fixed and immunostained to detect Ki67 (red). Blue, nuclear marker DAPI. Representative data of 3 independent experiments are shown. (Scale bars: 20 μm.) Arrows, Ki67^{+} cells.

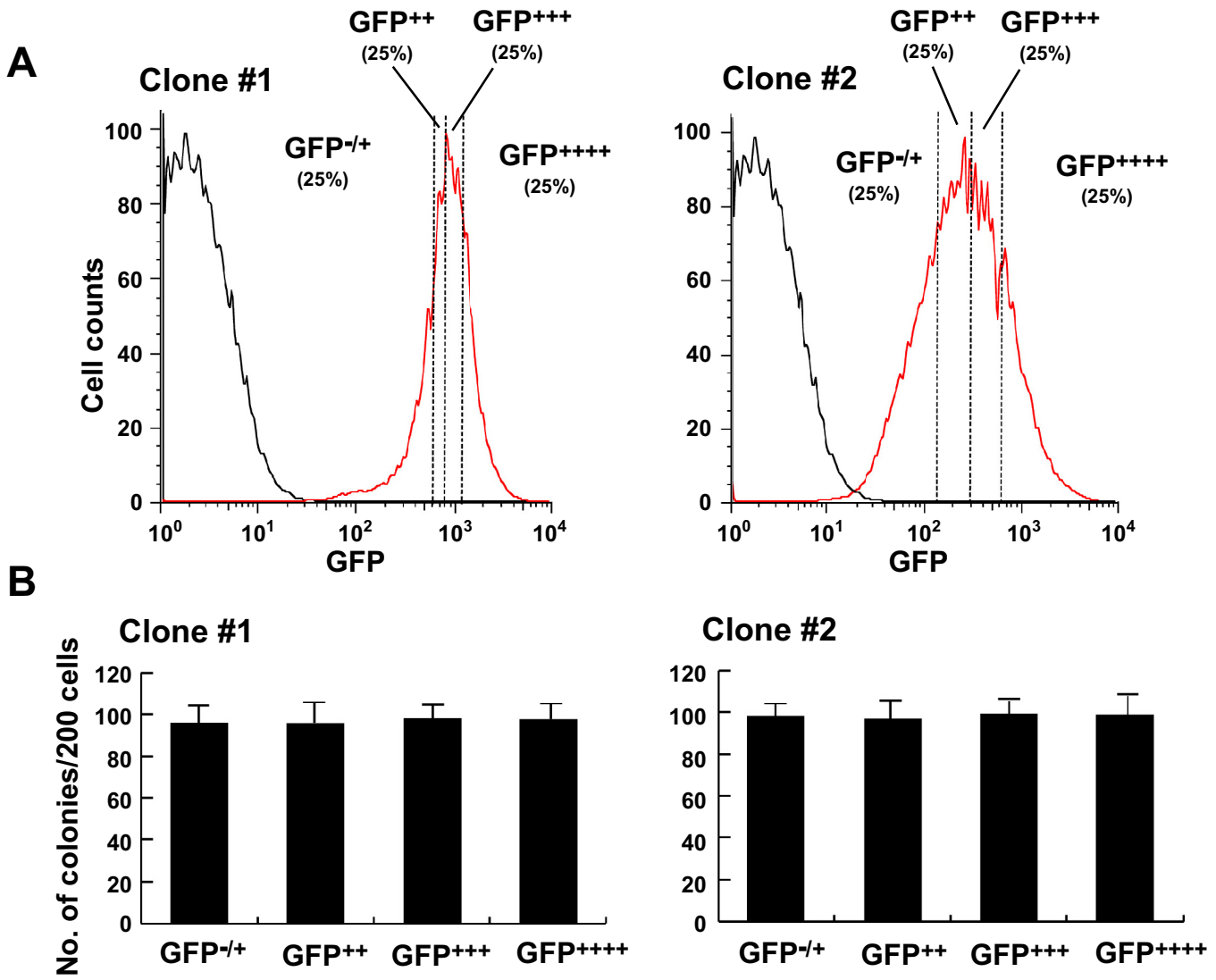

Fig. S8. GFP expression in NS-GFP C6. The NS-GFP construct was introduced into the C6 glioma cell line and single clone-derived lines were established by limiting dilution. (*A*) Fractionation of GFP-expressing cells from NS-GFP C6 cells. Cells were fractionated by flow cytometry into 4 subpopulations, $GFP^{-/+}$, GFP^{++}, GFP^{+++} and GFP^{++++}, based on GFP fluorescence intensity as indicated. Black peak, control C6; red peak, NS-GFP C6. Two representative clones are shown. (*B*) Colony forming capacity of fractionated NS-GFP C6 cells. Fractionated cell subpopulations from (*A*) were cultured. Data shown are the mean number ± SD. of colonies generated per 200 cells ($n = 3$).