The anti-vascular endothelial growth factor receptor-1 monoclonal antibody D16F7 inhibits glioma growth and angiogenesis *in vivo*

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SUPPLEMENTAL FIGURE LEGENDS

Figure S1

D16F7 mAb inhibits VEGFR-1 phosphorylation in response to PlGF and VEGF-A in C6-MF3 cells. A) Immunoblot of total or phosphorylated VEGFR-1 at tyrosine 1213 (pVEGFR-1) in untreated or D16F7 mAb pre-treated C6-MF3 (5 μg/ml) cells in response to PlGF or VEGF-A. Protein extracts were prepared from glioma cells pretreated with 5 μg/ml D16F7 for 30 min and then exposed for 10 min to PlGF or VEGF-A (100 ng/ml) at 37°C. **B**) Histogram represents the densitometric quantification of immunoblot band intensities, expressed as pVEGFR-1/VEGFR-1 protein ratio relative to untreated cells, after normalization for β-actin expression in the samples. Normalized pVEGFR-1/VEGFR-1 protein ratio in untreated control was considered equal to 1. NS, non-stimulated cells; NT, untreated cells.

Figure S2

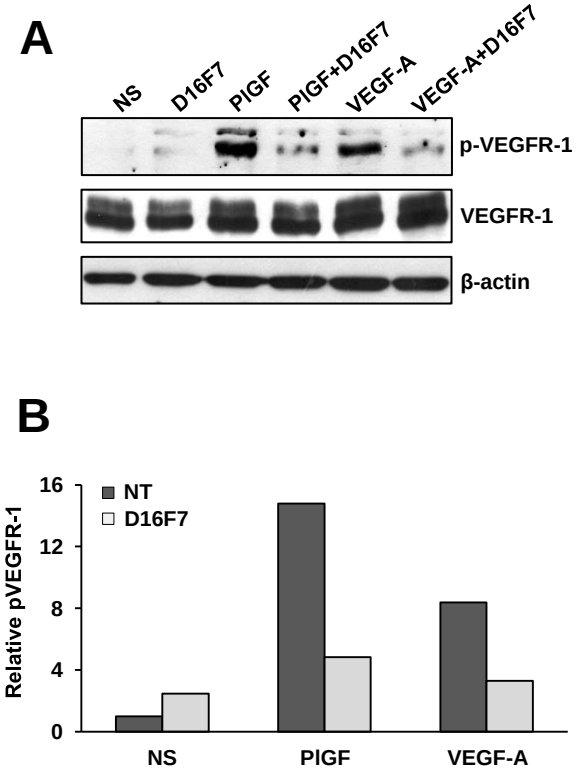
D16F7 mAb specifically recognizes both human and murine VEGFR-1 protein in a concentration-dependent manner. A) The specificity of D16F7 mAb (4 μg/ml) binding to VEGFR-1 was evaluated by a modified ELISA using plates coated with human or mouse soluble VEGFR-1 containing the extracellular domain of the receptor or VEGFR-2/Fc

chimera (negative control). D16F7 binding was compared to the signal obtained with a mouse IgG1 control mAb. The mAb binding was assessed using alkaline phosphatase-conjugated anti-mouse antibody and results were expressed as arithmetic mean ± SD (n=4) of absorbance at 405 nm after subtraction of background absorbance measured in BSA coated wells. **B**) The binding of D16F7 to the human and mouse forms of VEGFR-1 was performed as described in panel A, but using graded concentrations of the mAb.

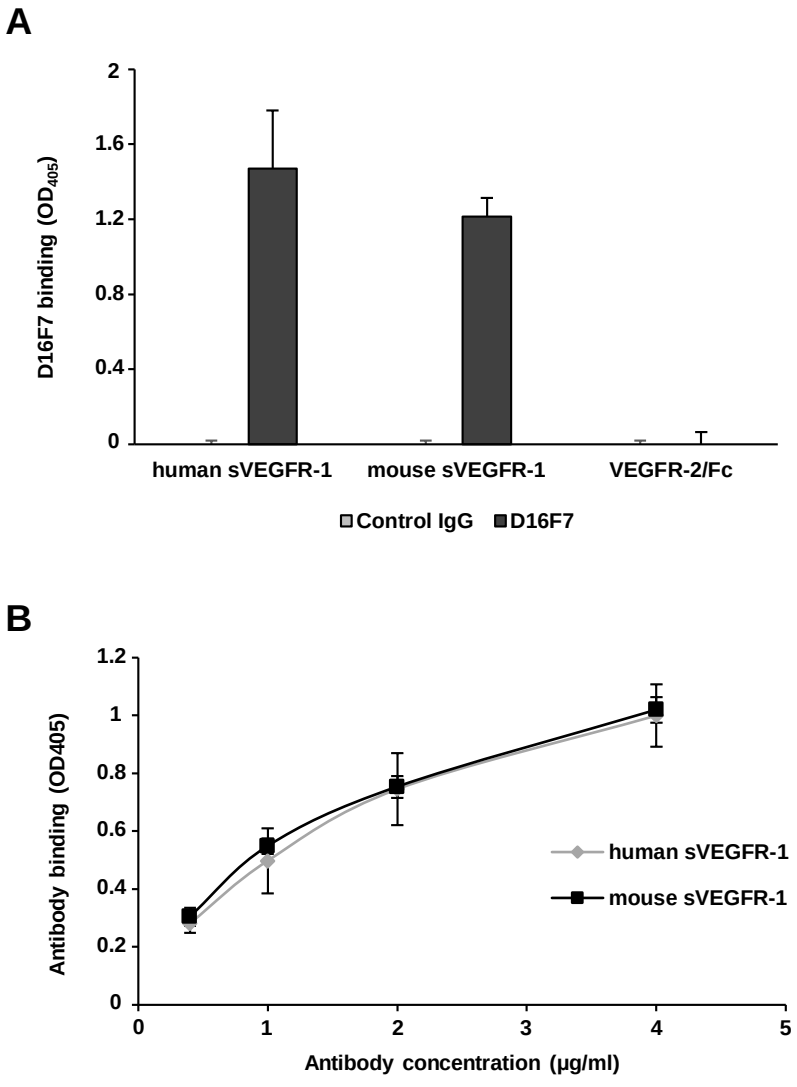
No statistically significant differences were detected between D16F7 binding to murine and human VEGFR-1 (Student's *t* test analysis: p=0.6).

Figure S3

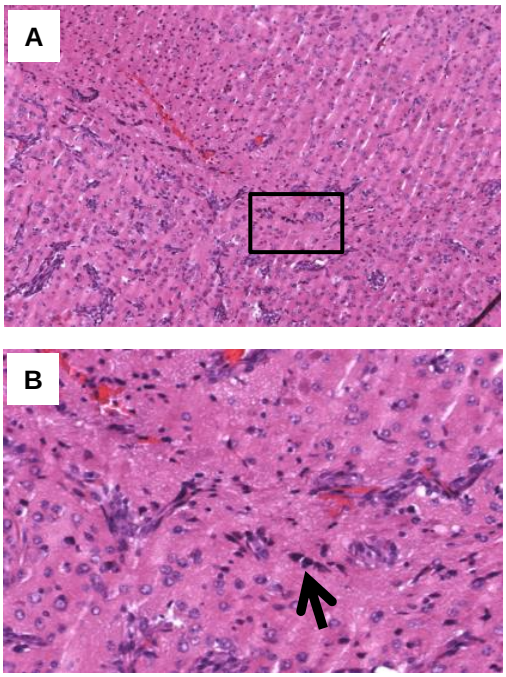
Histological analysis of brain sections from long-term surviving mice treated with D16F7. Hematoxylin-eosin staining of brain sections obtained from long-term surviving mice treated with 20 mg/kg D16F7 mAb showing a residual tumor area (square) (**A**; 4x magnification). High magnification displays residual tumor cells (arrow) (**B**; 20x magnification).



Supplemental Figure 1



Supplemental Figure 2



Supplemental Figure 3