

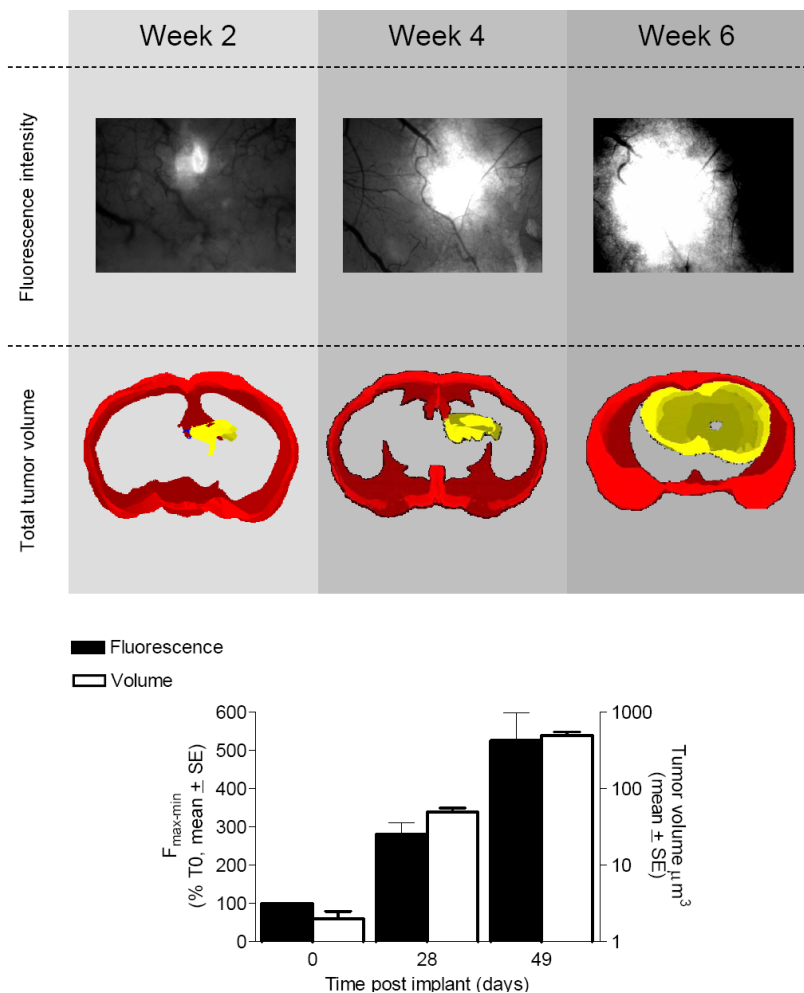
## Supplemental Data

### A Perivascular Niche for Brain Tumor Stem Cells

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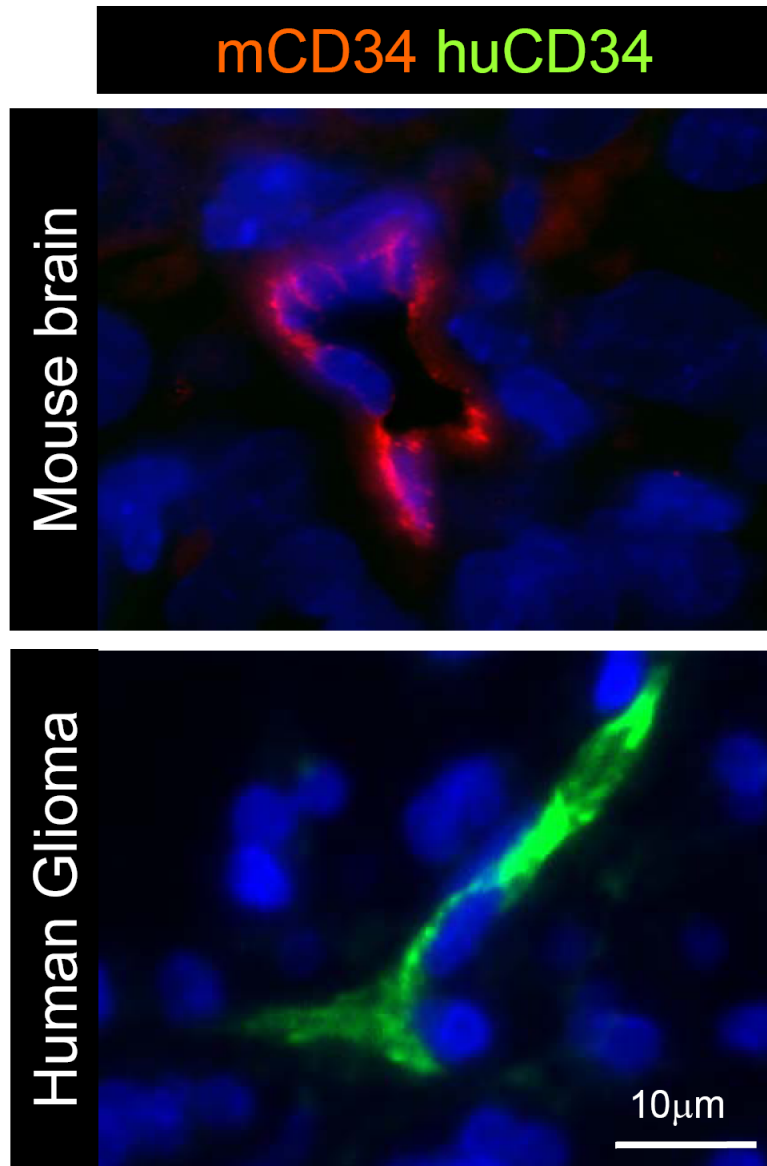
#### Figure S1. Autofluorescent Orthotopic Brain Tumor Xenograft Model

Upper panels: intravital fluorescence photomicrographs of three separate Daoy xenografts that were imaged 2, 4 and 6 weeks after orthotopic transplantation of  $1 \times 10^6$  GFP-labeled Daoy cells under cranial windows into the cerebral cortex of nude mice. Middle panels: tumors in the upper panels were resected immediately following imaging at the indicated time points and subject to concurrent 3D histologic reconstruction analysis. Bottom graph: summary of the concurrent analysis of three serial tumor fluorescence and 3D histologic reconstruction studies confirms that tumor fluorescence reliably reports tumor burden.



**Figure S2. Confirmation of the Species Specificity of Anti-CD34 Antibodies**

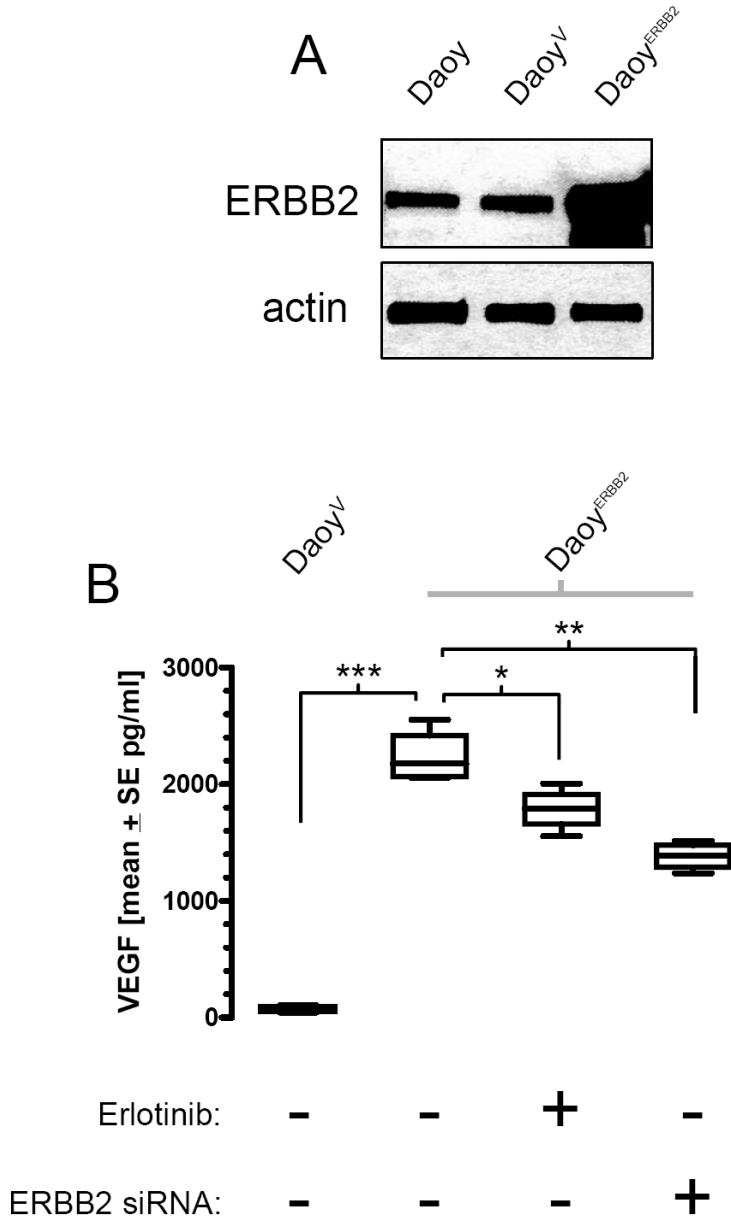
Co-immunofluorescence analysis of normal mouse brain (top) and a human glioma (bottom) that were co-stained with mouse (red) and human (green) specific anti-CD34 antibodies.



**Figure S3. ERBB2 Elicits a Proangiogenic Signal in Daoy Cells**

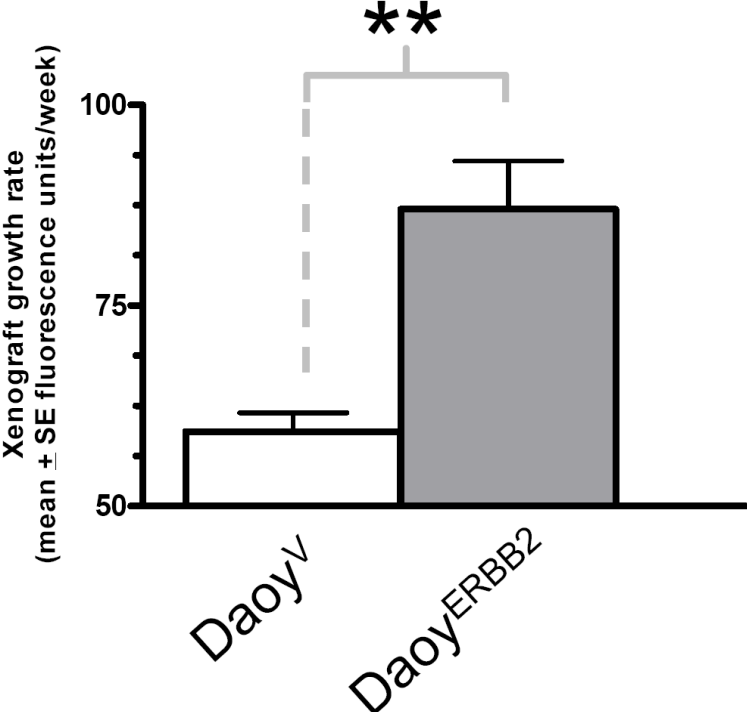
(A) Western blot analysis of ERBB2 expression in Daoy parental, Daoy<sup>V</sup>, and Daoy<sup>ERBB2</sup> cells.

(B) ERBB2 signaling increases VEGF secretion (measured by ELISA) by Daoy<sup>ERBB2</sup> cells relative to Daoy<sup>V</sup> cells. VEGF secretion by Daoy<sup>ERBB2</sup> cells is inhibited by Erlotinib or an anti-ERBB2 siRNA (\*=P<0.05; \*\*=P<0.005; \*\*\*=P<0.0005, Exact Wilcoxon test).



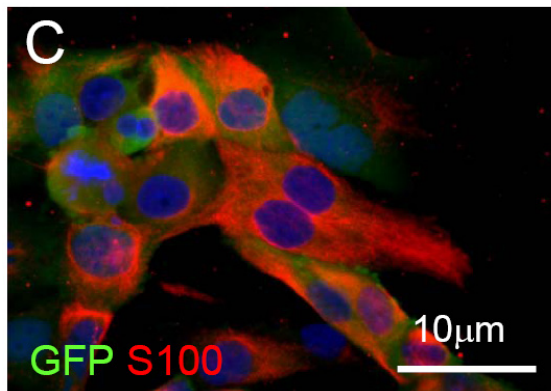
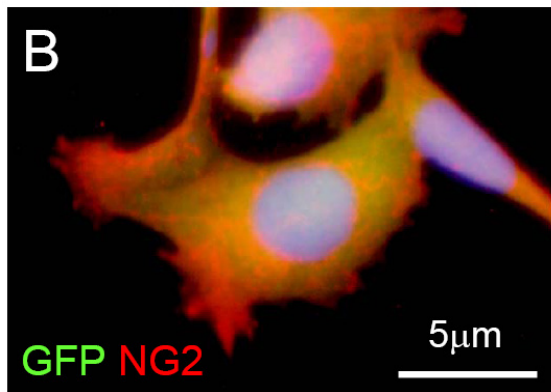
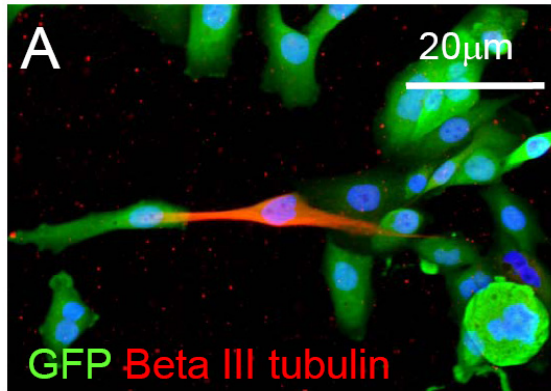
**Figure S4. Growth Rate of Daoy Xenografts for the First Four Weeks following Orthotopic Transplantation**

Growth rate is measured as increase per week of tumor fluorescence units measured by intravital fluorescence microscopy (\*\*=P<0.005).



**Figure S5. Daoy Xenograft-Derived Tumor Sphere Forming Cells Display Evidence of Multipotency**

Immunofluorescence analysis of Daoy<sup>V</sup> tumor sphere cells that were transferred to culture conditions that force differentiation. Cells displayed aberrant differentiation along neuronal (A), oligodendroglial (B) and astrocytic (C) lines.



**Figure S6. Upregulation of ERBB2 in Daoy Cells and Treatment of These Cells with Erlotinib and Bevacizumab Does Not Impact the Proliferation, Survival, or Self-Renewal of These Cells in Culture**

Graphs report cell growth curves (A), cell cycle distribution (FACS of propidium iodide content of nuclei) (B), apoptosis (FACS analysis of annexin V staining) (C), or self-renewal (serial tumor sphere forming assay) (D) of Daoy<sup>ERBB2</sup> and Daoy<sup>V</sup> cells. Exposure of these cells to Erlotinib or Bevacizumab in culture does not impact the self-renewal of Daoy<sup>V</sup> or Daoy<sup>ERBB2</sup> (E).

