Supplementary Figure Legends

Supplementary Figure 1. **Bevacizumab Treatment Promotes Invasiveness Of GBM.** (**a**) Immunofluorescent staining for U87MG cells (human vimentin, red) in xenograft sections from mice after 1 month of control (left) or bevacizumab (right) treatment. Increased parenchymal invasion is observed in the experimental group (right). (**b**) Immunofluorescence for tumor cells (human mitochondria or human vimentin, green) and brain vasculature (glut-1 or collagen type IV, red) shows extensive invasion by vessel cooption in the SF8106 bevacizumab-resistant orthotopic xenograft model. Scale bars,120 μm.

Supplementary Figure 2. Increased Expression Of Beta1 In The U87-BEV Cell Line. In support of **figure 1**. Mice with subcutaneous U87MG glioma xenografts were treated with bevacizumab or human IgG (10 mg/kg IP, biweekly) for 4 weeks. Resistant and control tumors were isolated and cell lines created from them: U87-BEV and U87-IGG, respectively. (**a**) Western blot demonstrated increased levels of β1 integrin and its potential heterodimer partners, αV and α5 integrin, in 4 different U87-BEV xenografts compared to 4 different U87-IGG xenografts but no change in expression of α4 or β3 integrins. Numbers represent band intensities normalized to GAPDH. (**b**) FACS for β1 integrin (CD29, clone K20) demonstrates increased cell surface expression of β1 integrin in the U87-BEV line compared to control U87-IGG. (c) Sequential images acquired at 30 second intervals during FRAP of SF7996 bevacizumab-responsive cells (left) and SF8106 bevacizumab-resistant (right) cells expressing a beta1-GFP fusion

protein. Quantification of fluorescence revealed more rapid recovery to a higher plateau in SF8106 than SF7996, as shown in Figure 1e.

Supplementary Figure 3. Beta1 Integrin Expression In GBM Associated With Cellular Insults And Mesenchymal Phenotype. (**a**) Immunofluorescent staining for β1 integrin in clinical specimens after development of bevacizumab resistance correlates with CA9-positive (surrogate marker for hypoxia) regions in adjacent sections (hatched area). Scale bar, 100 μm. (**b**) Immunofluorescence demonstrates striking immunoreactivity for beta1 integrin in the tumor core of bevacizumab-treated mice. Scale bar, 120 μm. (**c**) Experimental hypoxia for 24 or 48 h results in significant, but transient, upregulation of β1 integrin by FACS in the U87-MG glioma line (*$p < 0.01$, ANOVA with post-hoc Dunnett test). Scale bar, 100 μm. (**d**) Experimental hypoxia results in increased cell surface expression of β1 integrin in human adenocarcinoma lines and primary glioma cells by FACS (anti-CD29, clone TS-2/16; * $p < 0.01$, t-test).

Supplementary Figure 4. Establishment And Characterization Of Stable Beta1 Knockdown Clones In The BRG3 Line. The BRG3 line was established from cells derived from an intraoperative tumor specimen (SF8106). (**a**) BRG3 cells grown as a subcutaneous xenograft show no response to standard bevacizumab treatment (10 mg/kg IP, biweekly) confirming stable resistance to antiangiogenesis *in vivo*. (**b**) FACS for cell surface β1 integrin in mass cultures of BRG3 cells transduced with shRNA targeting β1 (shB1) confirms approximately 70% knockdown compared to mass cultures of BRG3 cells transduced with control shRNA (shCTRL). As an additional control, mass

cultures of BRG3 cells transduced with beta3 integrin-targeted shRNA (shB3) were analyzed for β1 integrin expression, which was not reduced in these cells (anti-CD29, clone TS-2/16; *$p < 0.01$, ANOVA with post-hoc Dunnett test). (**c**) β1 integrin knockdown clones (shB1-c1, shB1-c4, and shB1-c6) were isolated by limiting dilution and shown to express less than 10% cell surface β1 compared to shCTRL cells (* $p < 0.01$, t-test; ANOVA with post-hoc Dunnett test). (d) Western blot confirming knockdown of β1 integrin in knockdown clones (shB1-c1, shB1-c4, and shB1-c6). Numbers represent band intensities normalized to GAPDH.

Supplementary Figure 5. Beta1 integrin knockdown in resistant GBM cells inhibits mesenchymal phenotype. (**a**) Brightfield microscopy demonstrates a morphological mesenchymal to epithelial shift in the BRG3 knockdown clones compared to control. Representative cells are outlined in black dashes. Scale bar, 100 μm. (**b**) Cell area is increased in β1 integrin knockdowns consistent with epithelial shift (*$p < 0.01$, ANOVA with post-hoc Dunnett test). (**c**) β1 integrin knockdowns demonstrate decreased dendricity factor consistent with epithelial polygonal morphology (*$p < 0.01$, ANOVA with post-hoc Dunnett test). (**d**) β1 integrin knockdown does not promote cell death of BRG3 cells in 2-
dimensional culture.

Supplementary Figure 6. **Inhibitory Anti-Beta1 Mab OS2966 Attenuates Adhesion And Proliferation Of BRG Cells.** In support of **Figure 2**. (**a**) Adhesion assay of BRG3 cells on fibronectin (FN) or laminin (LN) demonstrates potent inhibition in the presence

of 10 μg/ml OS2966 compared to IgG control mAb (*$p < 0.01$, t-test). (**b**) Dose-dependent inhibition of proliferation of BRG2 cells in the presence of OS2966 as shown by FACS for Ki67 (*$p < 0.01$, ANOVA with posthoc Dunnett test).

Supplementary Figure 7. **A Role For Beta1 In Spheroidal Growth Of U87MG Glioma Cell Line.** In support of **Figure 3**. (**a**) Freshly isolated U87-BEV cells (right) tend towards rapid growth as spheres when cultured in complete media with serum. Scale bar, 120 μm. (**b**) FACS for cell surface β1 integrin in U87MG cells confirms approximately 70% knockdown compared to shCTRL cells; as an additional control, shB3 cells were analyzed (anti-CD29, clone TS-2/16; *$p < 0.01$, ANOVA with posthoc Dunnett test). Spheroidal growth is impaired in the knockdown cells (see **Fig. 5c**) (**c**) Wild-type U87MG cells grown in acidified media (pH 6.6) demonstrate impaired ability to form spheroids in the presence of OS2966. Scale bar, 120μm.

Supplementary Figure 8. **Beta1 Inhibition In Xenograft Models.** Related to **Figure 5**. Successful intracranial delivery of OS2966 to mouse brain. OS2966 (red) was detected throughout the rostro-caudal axis of the ipsilateral parenchyma of nude mice after 28 days of intratumoral infusion via Model 104 Alzet pump. Scale bar, 120 μm. “X” denotes cannula site. Axial histology slide of the mouse brain at the level of the dorsal hippocampus courtesy of MBL.org.