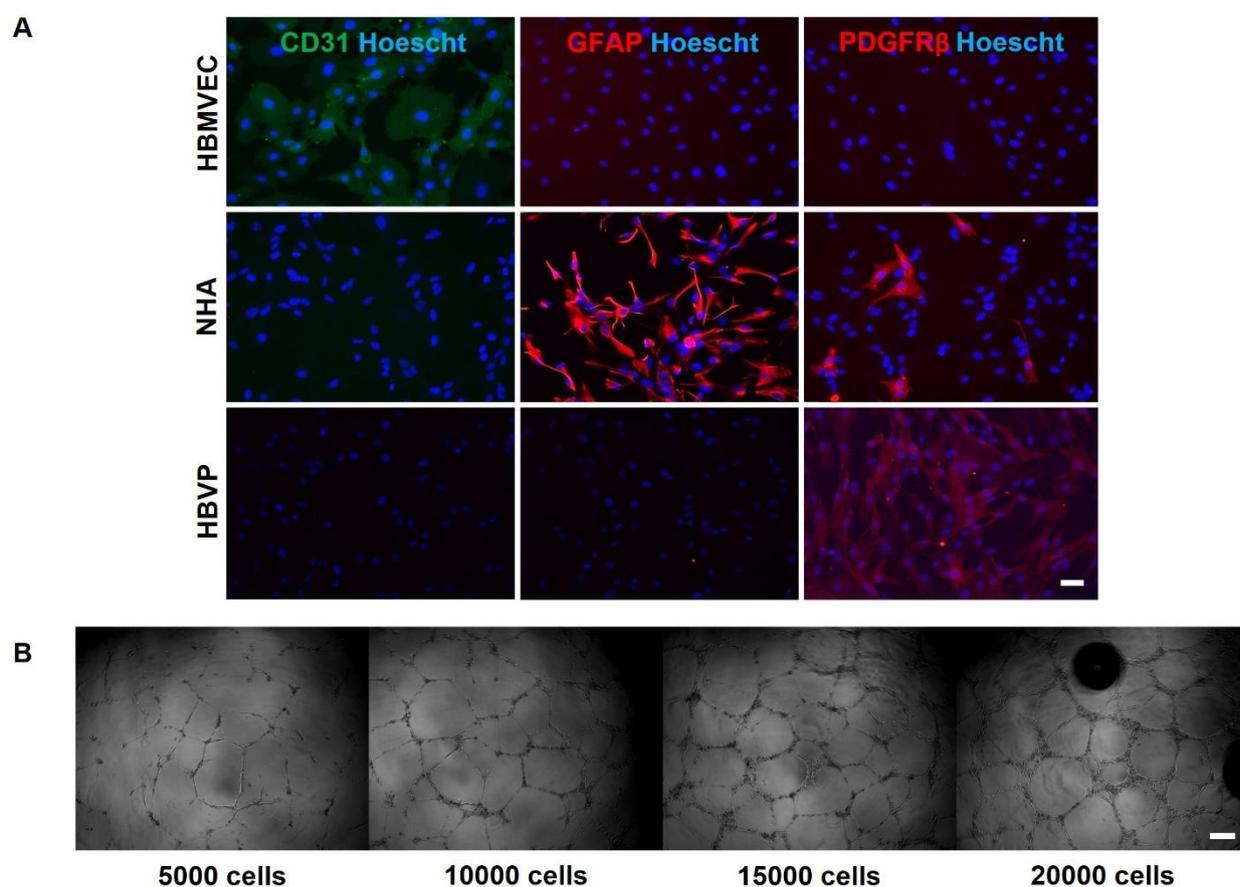


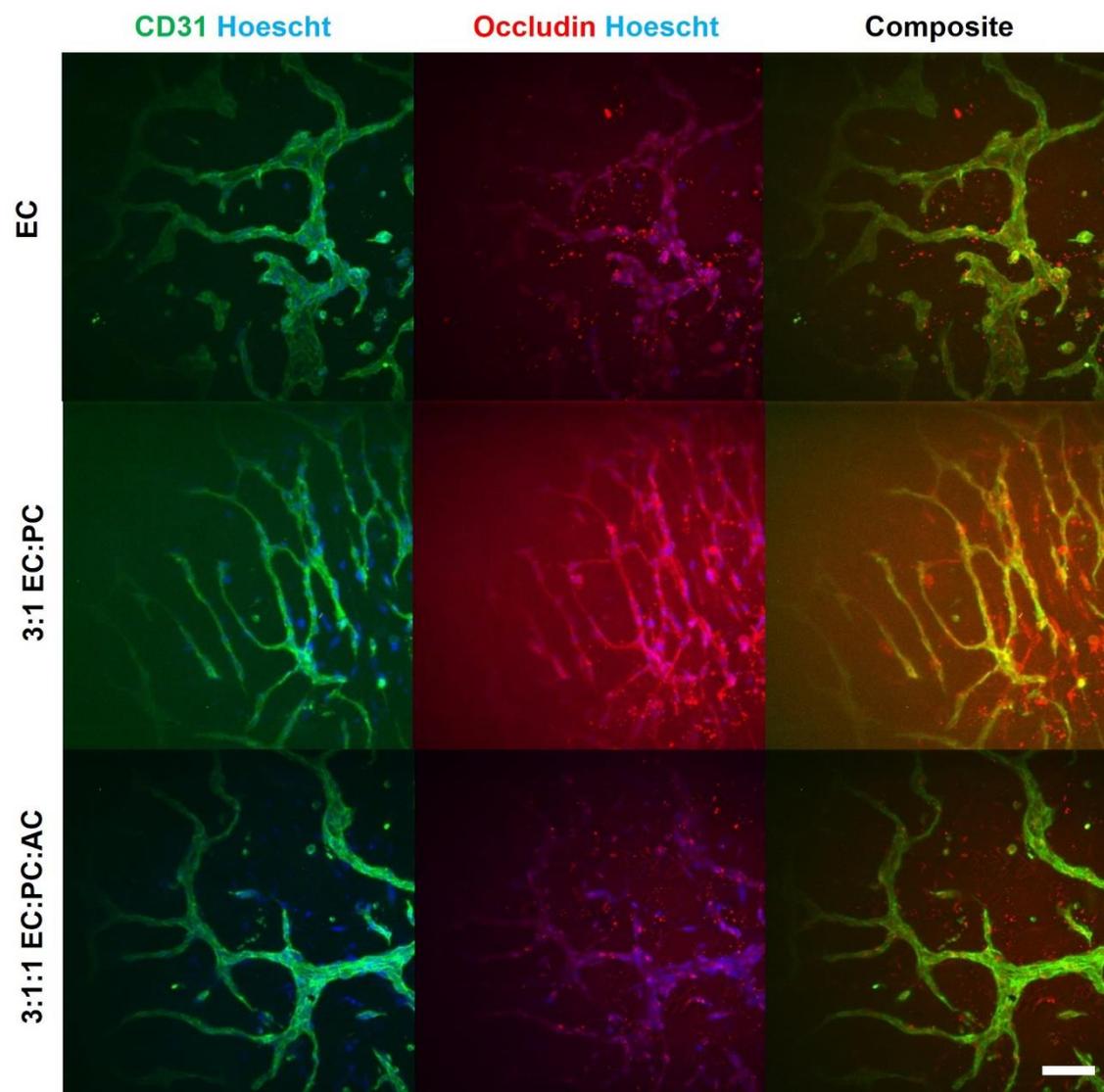
Supporting Information

**Perivascular Stromal Cells Instruct Glioblastoma Invasion, Proliferation, and  
Therapeutic Response within an Engineered Brain Perivascular Niche Model**

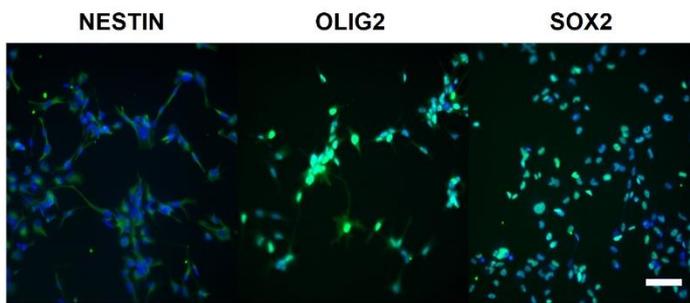
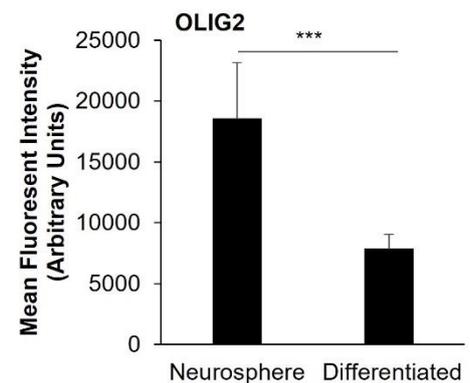
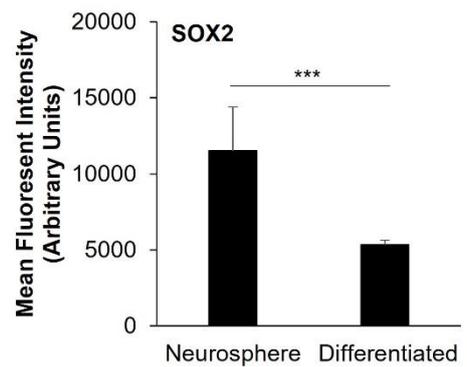
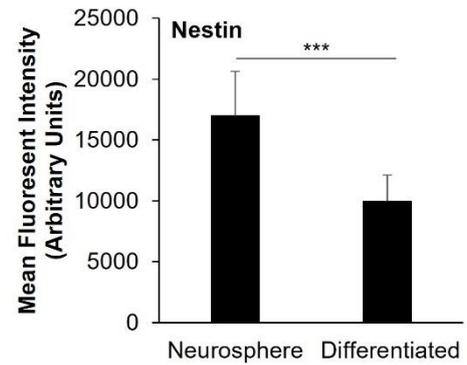
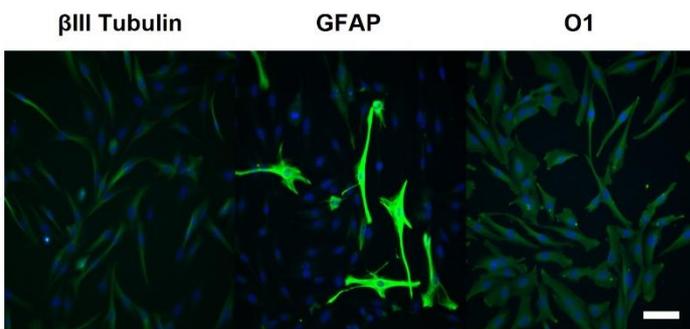
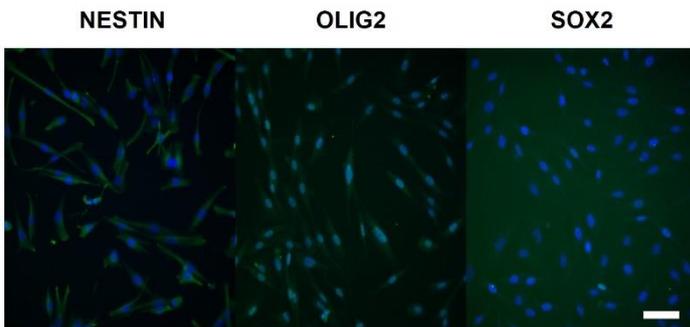
*Mai T. Ngo, Jann N. Sarkaria, Brendan A.C. Harley\**



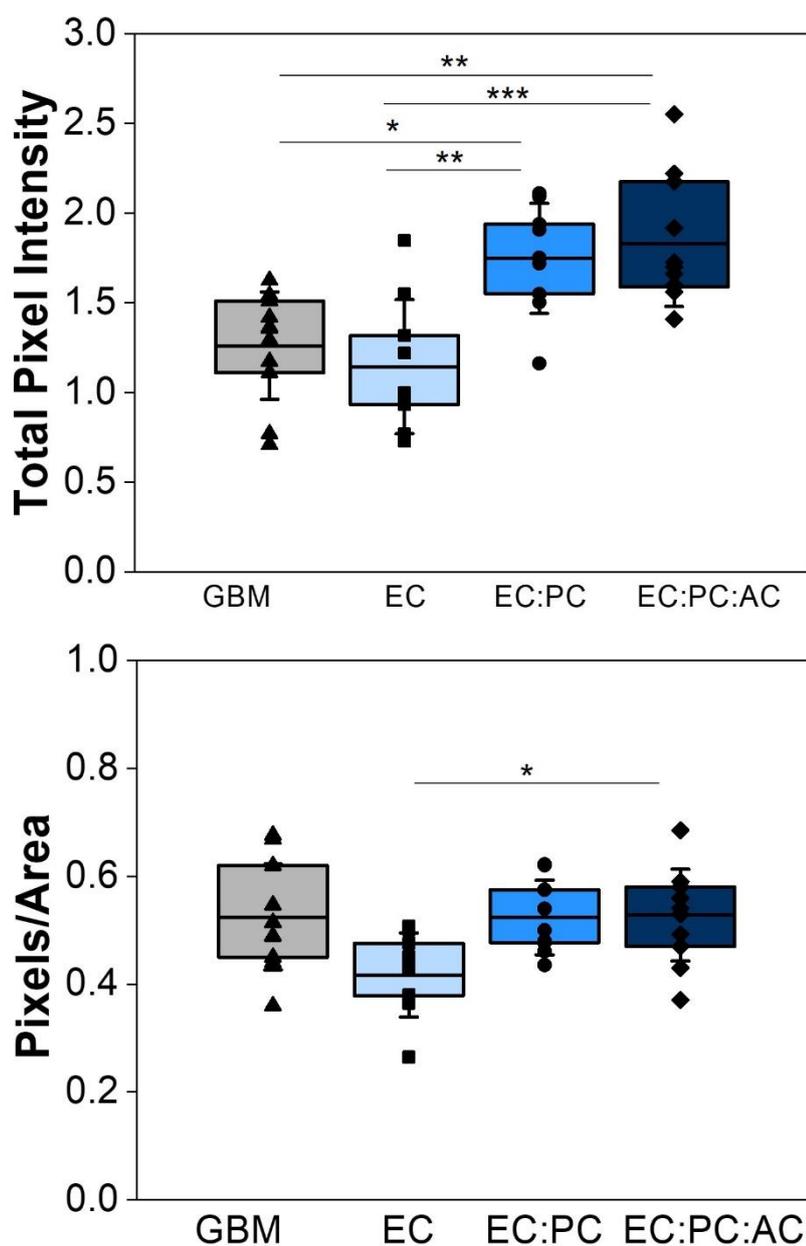
**Figure S1.** (A) Immunofluorescent markers for human brain microvascular endothelial cells (HBMVEC), normal human astrocytes (NHA), and human brain vascular pericytes (HBVP). Cells were fixed 48 hours after seeding. Scale bar = 50  $\mu$ m. (B) Matrigel tube formation assay for brain microvascular endothelial cells. Images were taken 16 hours after cell seeding. Scale bar = 100  $\mu$ m



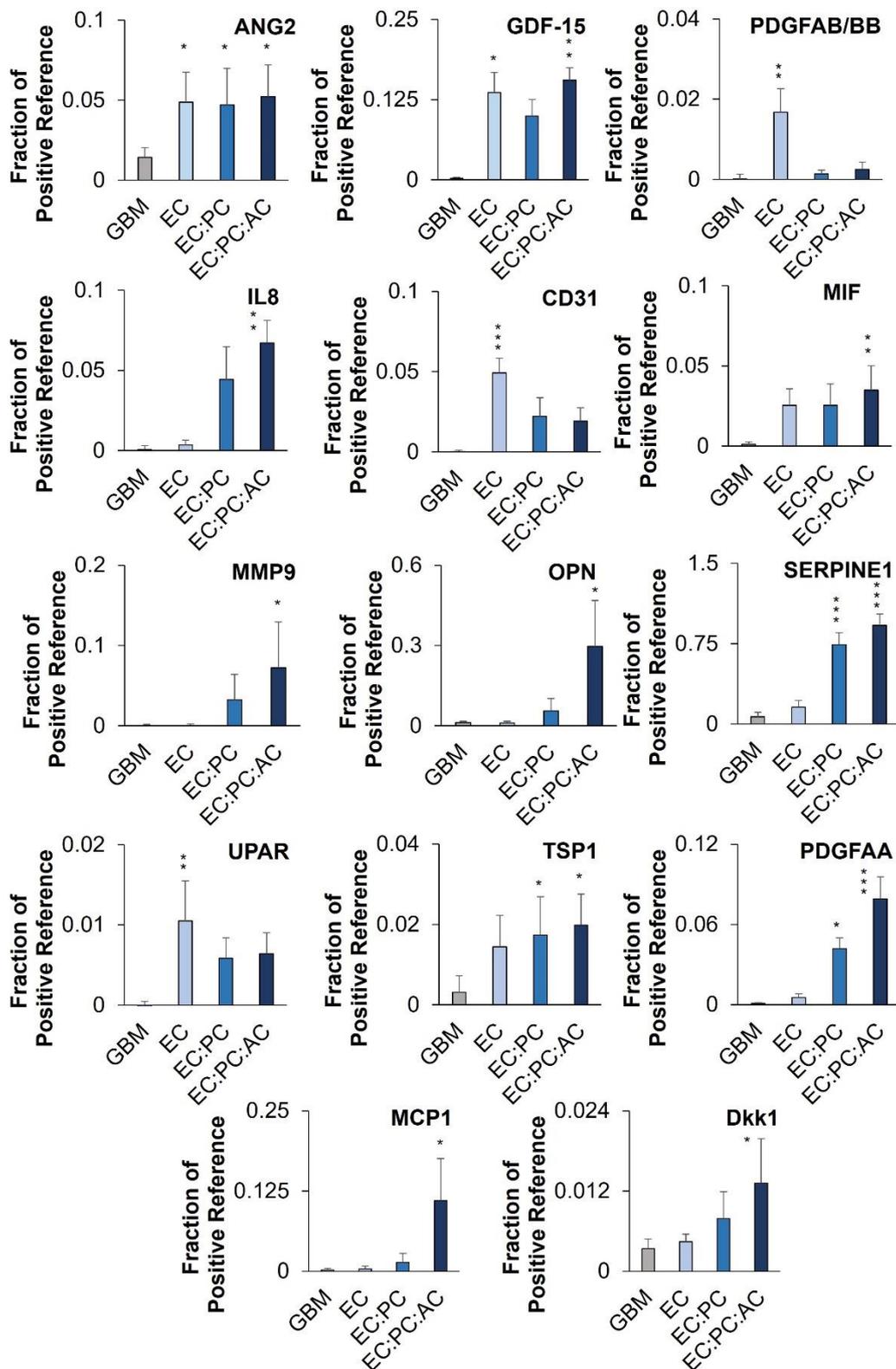
**Figure S2.** Immunofluorescent staining for occludins in microvascular cultures. Scale bar = 100  $\mu\text{m}$ .

**A Neurosphere Culture****B Differentiated Culture**

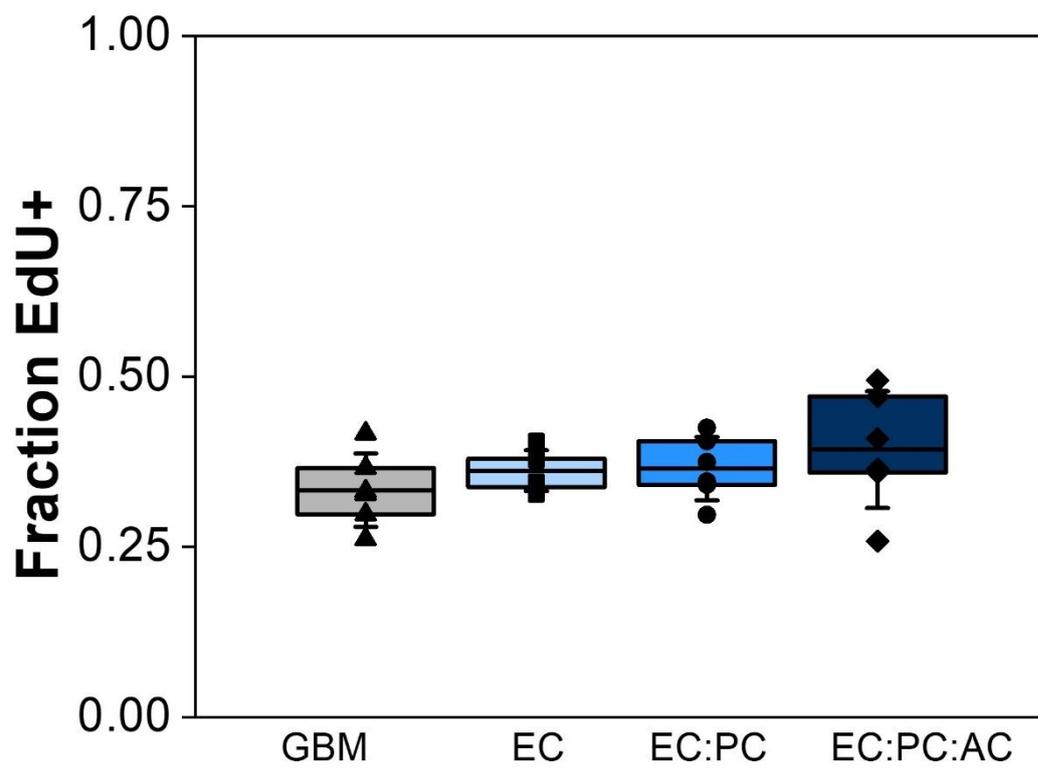
**Figure S3.** (A) Immunofluorescent staining for stem markers in GBM6 cells that have been grown in neurosphere culture and plated on laminin. Scale bar = 100  $\mu$ m. (B) Immunofluorescent staining for stem and differentiated markers for GBM6 cells grown in media containing FBS for two weeks. Scale bar = 100  $\mu$ m. (C) Quantification of stem marker expression (assessed via mean fluorescent intensity) between neurosphere and differentiated cultures. Data analyzed using Mann-Whitney tests; \*\*\* $p$ <0.001.



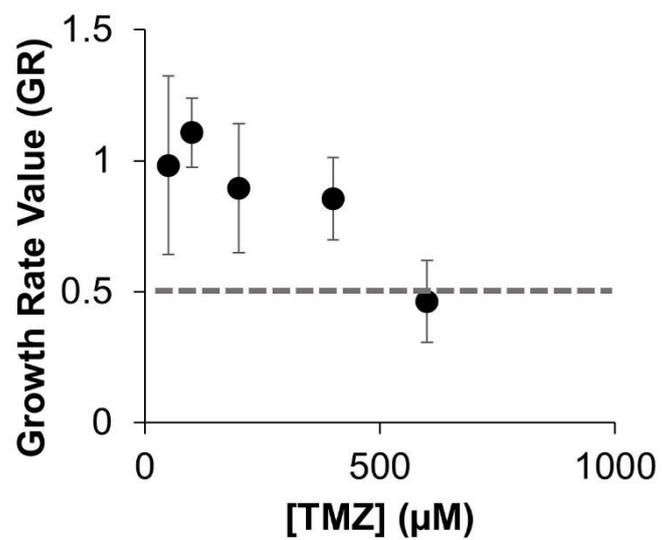
**Figure S4.** Quantification of total pixel intensity and total pixel intensity normalized to outgrowth area in spheroid invasion cultures. Data analyzed using one-way ANOVA with Scheffe's post-hoc; \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ ,  $N = 9 - 11$  hydrogels.



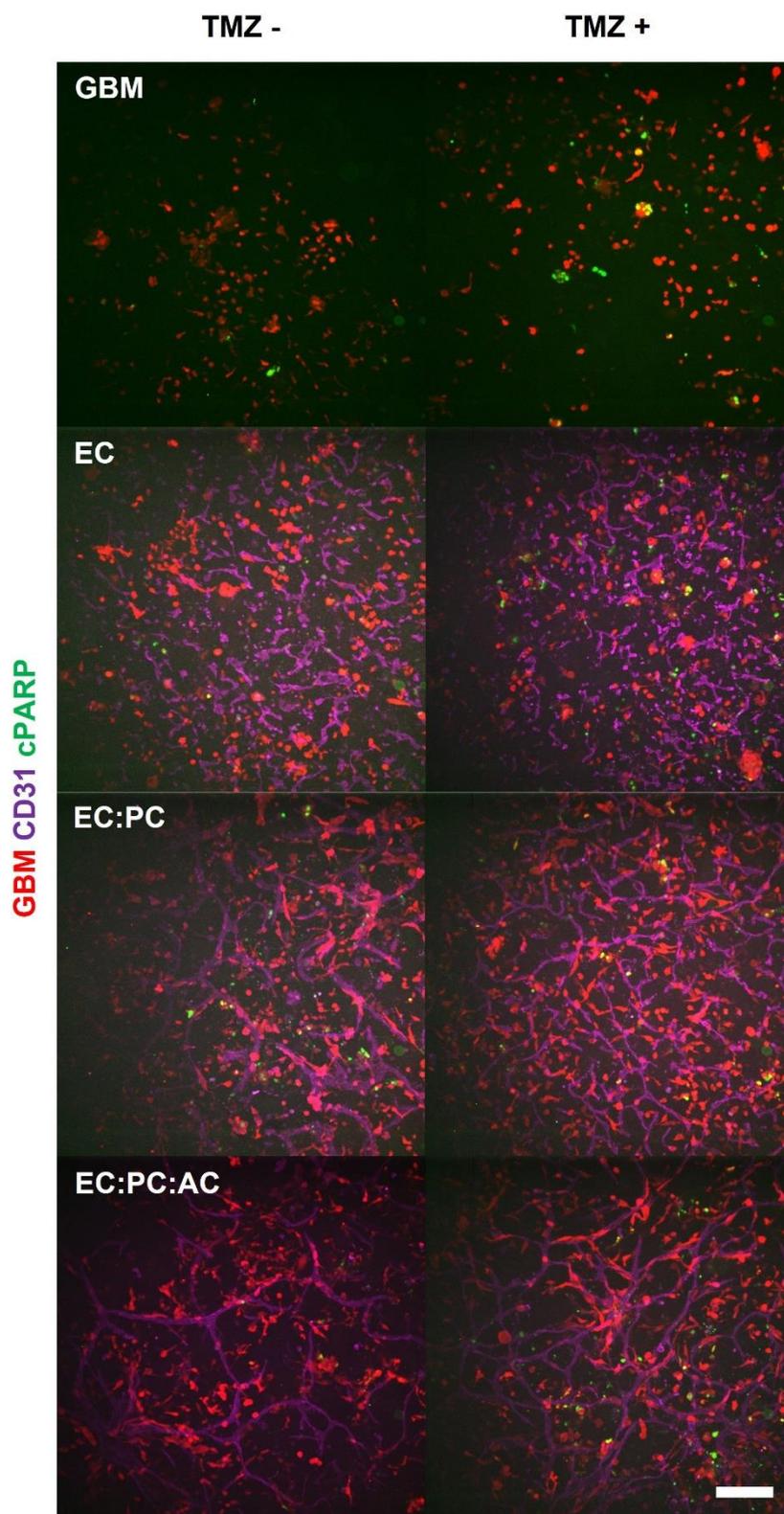
**Figure S5.** Quantitative comparison of proteins secreted by spheroid invasion cultures. Data analyzed using one-way ANOVA with Tukey's post-hoc or Kruskal-Wallis test with Dunn's post-hoc; \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$  compared to GBM6-only cultures;  $N = 5$  conditioned media samples.



**Figure S6.** Quantification of the fraction of RFP-expressing GBM6 tumor cells that incorporate EdU in a 24-hour pulse. N = 6 hydrogels.

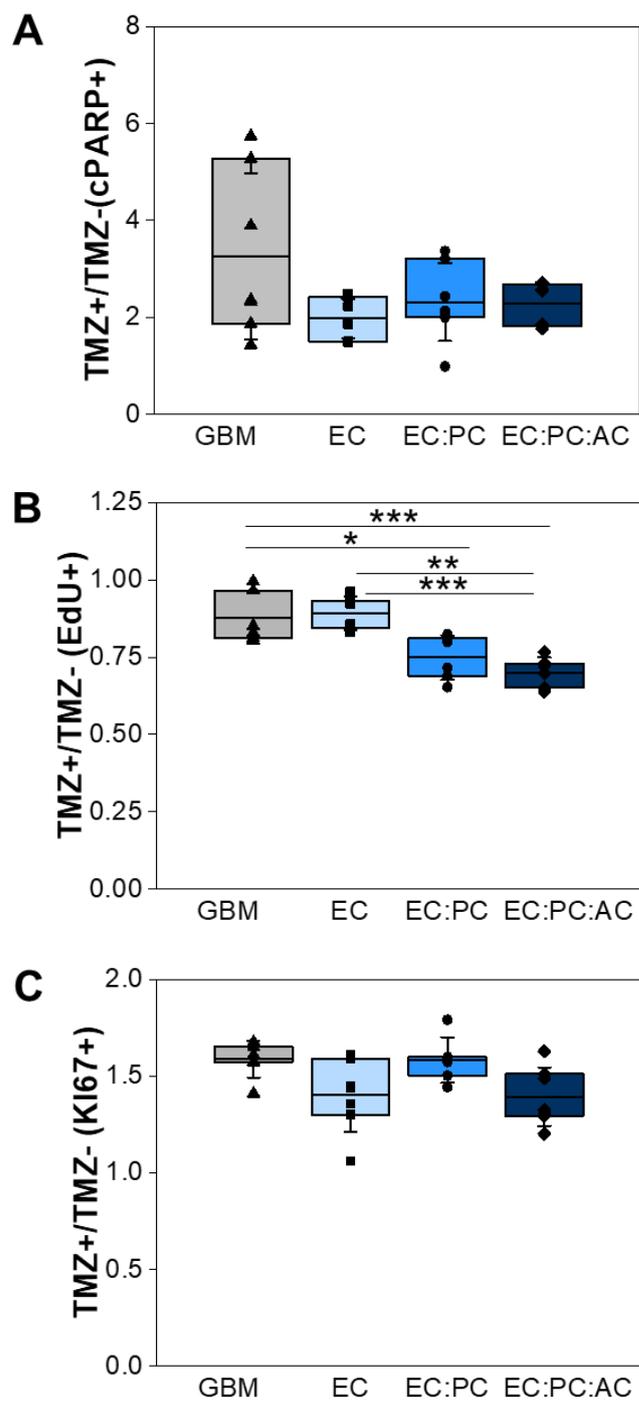


**Figure S7.** Dose-response curve for GBM12 cells treated with temozolomide for 48 hours. N = 4 - 6 hydrogels.

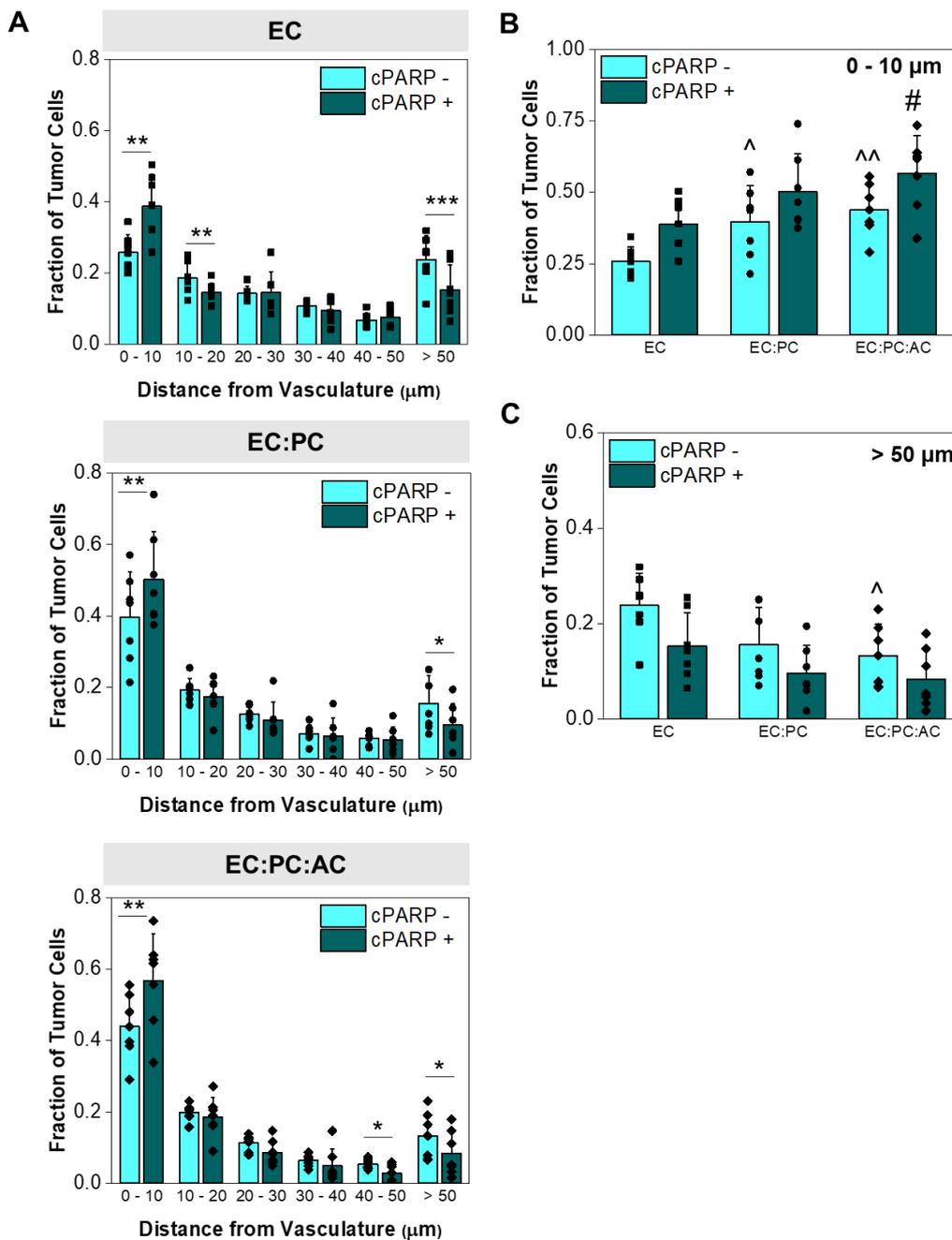


**Figure S8.** Representative images of tumor-vascular co-cultures with and without 48 hours of temozolomide treatment. cPARP staining was performed at the end of the treatment period.

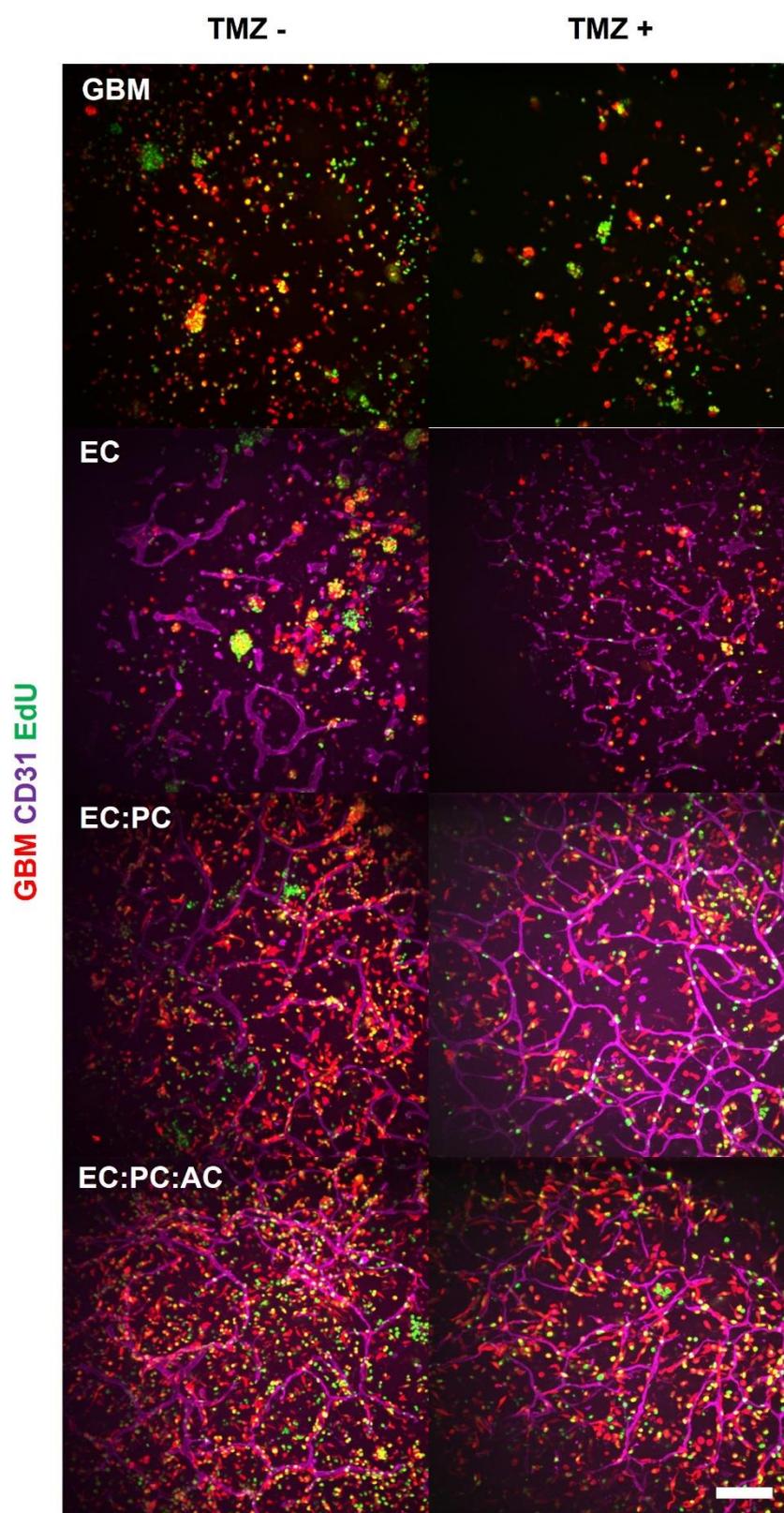
Scale bar = 200  $\mu\text{m}$ .



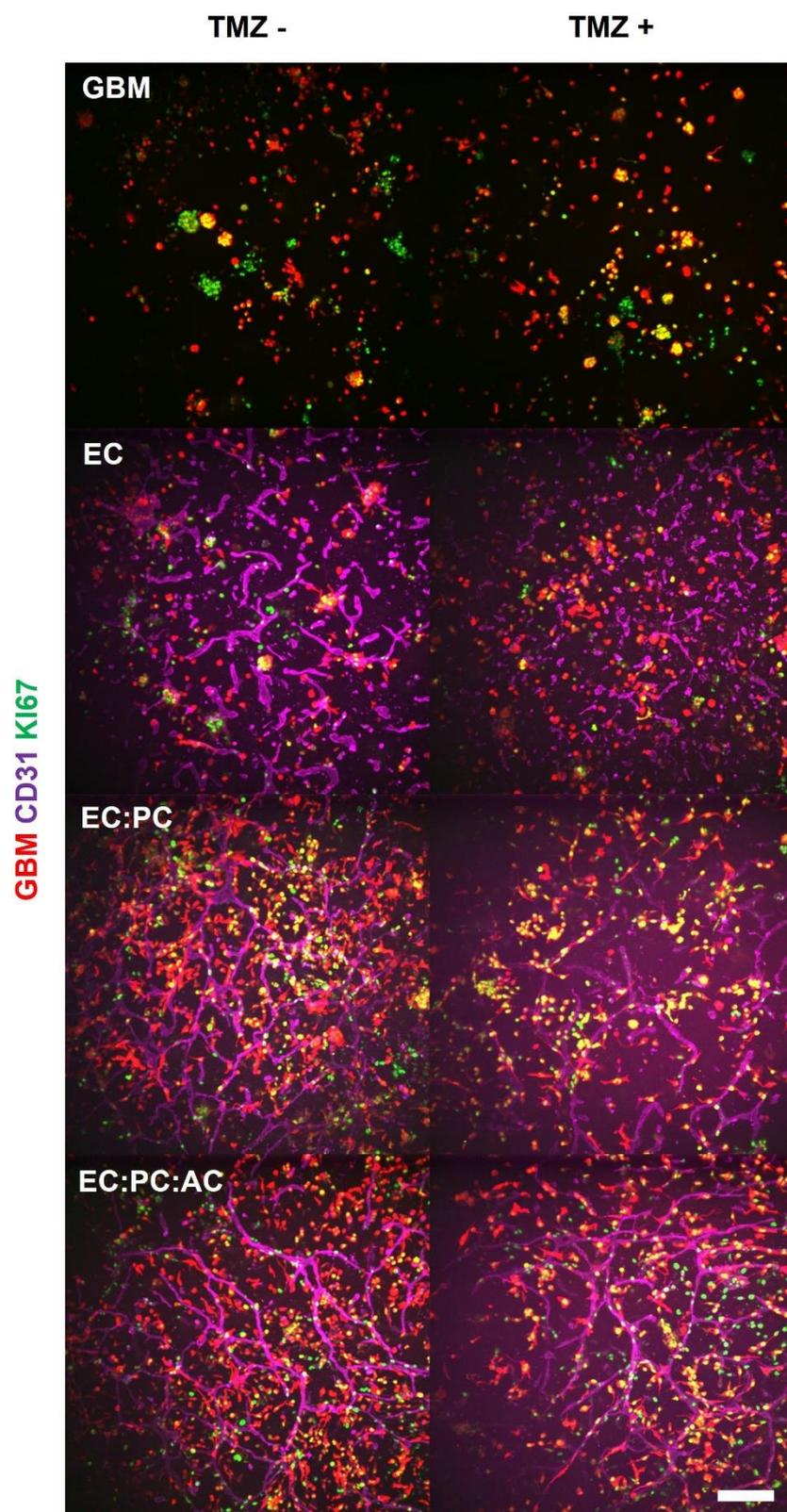
**Figure S9.** Calculated ratios for **A)** cPARP, **B)** EdU, and **C)** KI67 expression between TMZ-treated (TMZ+) and DMSO control (TMZ-) groups. \*  $p < 0.05$ , \*\*  $p < 0.01$ , and \*\*\*  $p < 0.001$  between groups.



**Figure S10.** A) Comparing the proximity of cPARP- vs. cPARP+ GBM12 cells to the microvascular network after temozolomide treatment. Data points represent the fraction of tumor cells that are a specified distance from the microvascular network in a single hydrogel. Data analyzed using paired-sample t-test or Wilcoxon signed-rank test; \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$  B) Comparing the fraction of cPARP- and cPARP+ GBM12 cells that are within 10  $\mu\text{m}$  of the microvascular network. Data analyzed using one-way ANOVA with Tukey's post-hoc;  $\wedge p < 0.05$ ,  $\wedge\wedge p < 0.01$  compared to EC, cPARP-; # $p > 0.05$  compared to EC, cPARP+ C) Comparing the fraction of cPARP- and cPARP+ GBM12 cells that are greater than 50  $\mu\text{m}$  from the microvascular network. Data analyzed using one-way ANOVA with Tukey's post-hoc; # $p < 0.05$  compared to EC, cPARP-; N = 7 hydrogels



**Figure S11.** Representative images of tumor-vascular co-cultures with and without 48 hours of temozolomide treatment. EdU staining occurred after a 24-hour pulse of EdU after temozolomide treatment. Scale bar = 200  $\mu\text{m}$ .



**Figure S12.** Representative images of tumor-vascular co-cultures with and without 48 hours of temozolomide treatment. KI67 staining was performed at the end of the treatment period. Scale bar = 200  $\mu\text{m}$ .