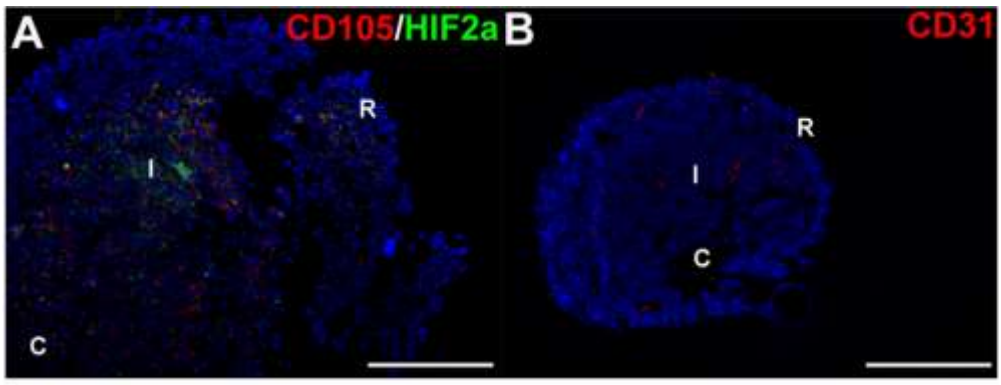
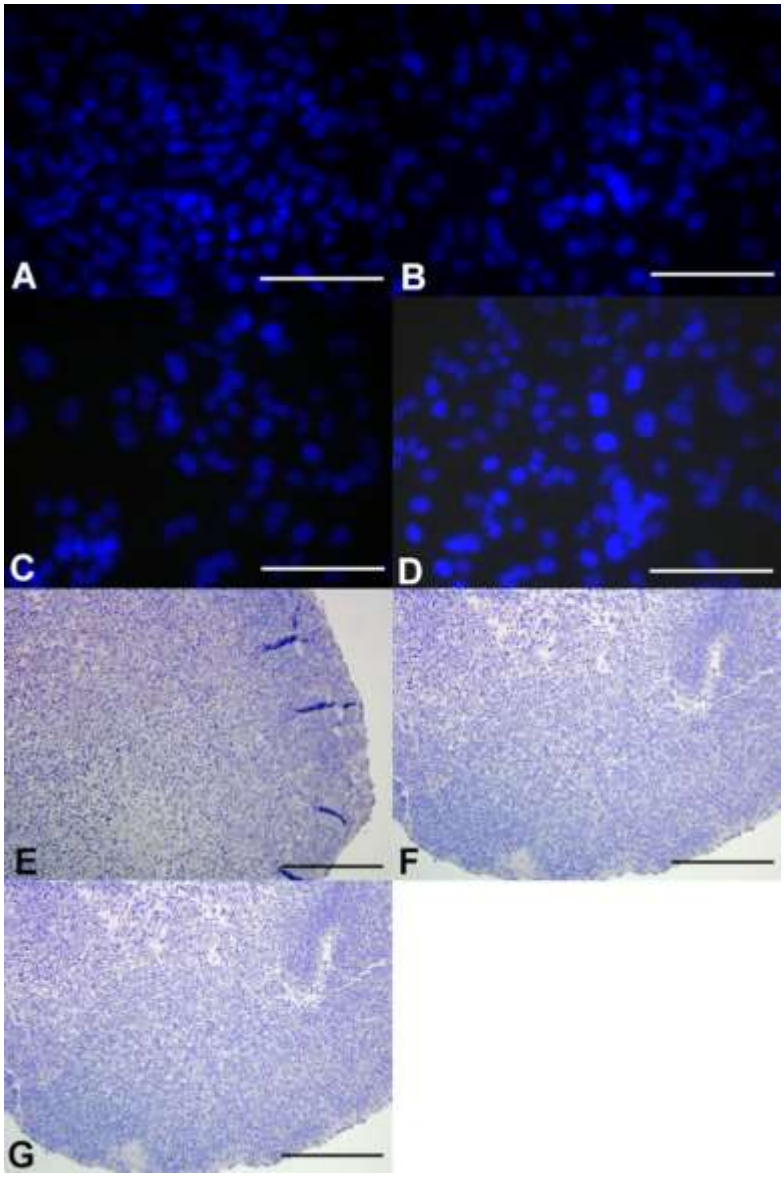
Endothelial-like malignant glioma cells in dynamic three dimensional culture identifies a role for VEGF and FGFR in a tumor-derived angiogenic response

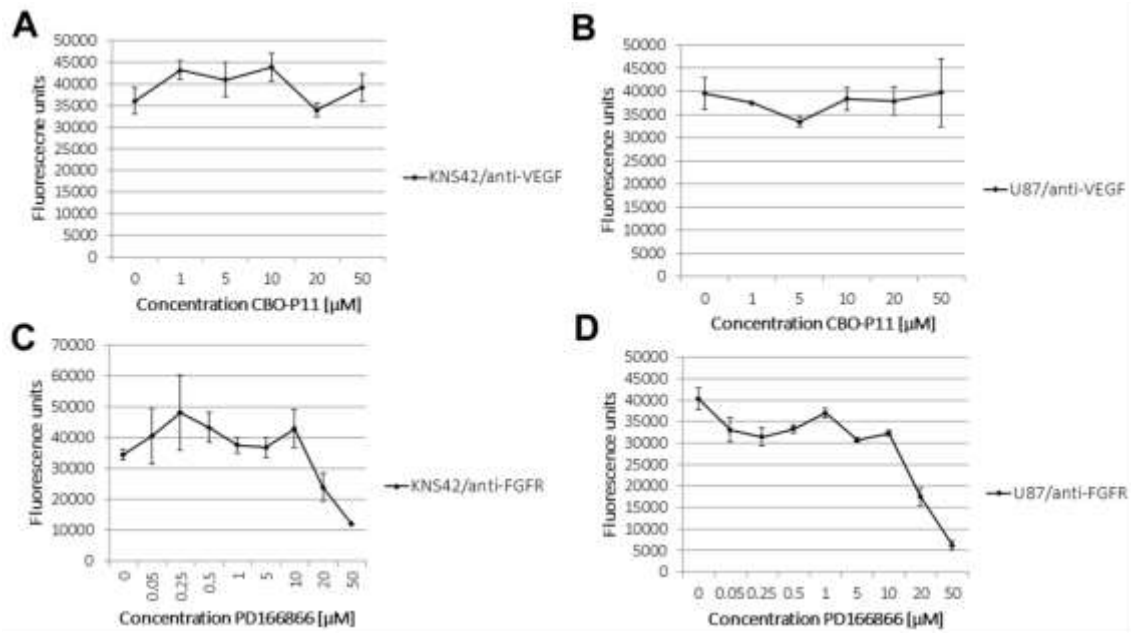
Supplementary Material



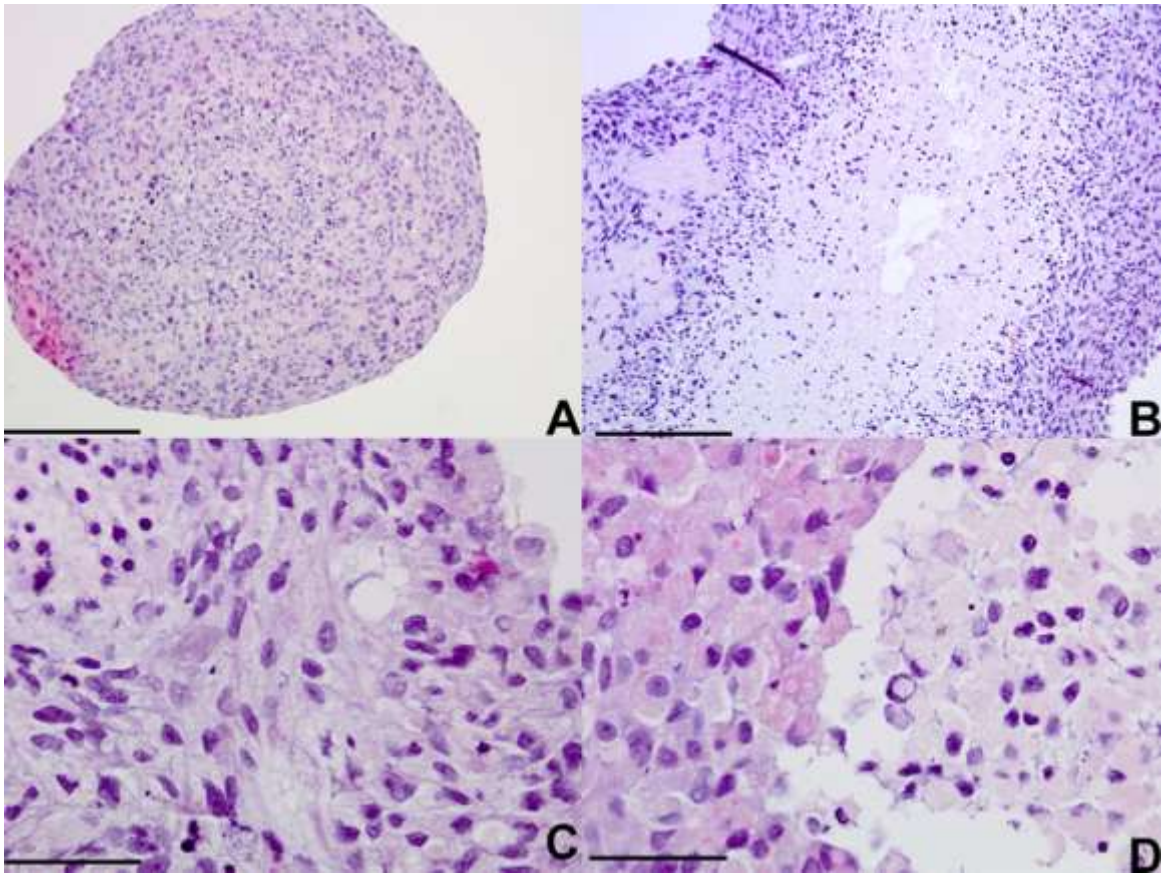
Supplementary Figure 1: Whole-field RCCS U87 aggregate views of CD105 and CD31 positive cells. (A) Location of CD105 positive cells in relation to aggregate location and to HIF-2α positive cells. (B) Location of CD31 positive cells in relation to aggregate location. Aggregate core, rim and interface regions are denoted as *C*, *R* and *I* respectively. *Scale bar = 200μm.*



Supplementary Figure 2: Negative staining for CD105 and CD31 in monolayers and stem cell aggregates (CD31). (A) Merged image with no positive (red) staining for CD105 on immunofluorescence (IF) of a KNS42 2D monolayer. (B) Merged image with no positive CD105 staining (red) on IF of a U87 2D monolayer. (C) Merged image with no positive CD31 staining (red) on IF of a KNS42 2D monolayer. (D) Merged image with no positive CD31 staining (red) on IF of a U87 2D monolayer. In *A-D* monolayer cells were cultured for 72 hours prior to staining with either endothelial marker. (E-G) Immunohistochemistry against CD31 on aggregates of ESC (E), NSC (F) and NSC with OCT4 overexpression (G) with no discernible positive staining. *Scale bar A-D = 25µm E-G = 200µm*.



Supplementary Figure 3: KNS42 and U87 RCCS aggregate viability upon exposure to either VEGF inhibitor (CBO-P11) or FGFR inhibitor (PD166866). (A-B) Exposure of either glioma aggregate to up to 50 µM VEGF inhibitor resulted in no impairment of cell viability. (C-D) Exposure of either glioma aggregate to FGFR inhibitor, resulted in marked loss of cell viability at concentrations above 10 µM. Based on this data, 15 µM and 10 µM were chosen as VEGF inhibitor and FGFR inhibitor concentrations respectively, by which to monitor anti-angiogenic response without dramatically impairing cell viability.



Supplementary Figure 4: Hematoxylin and eosin stained sections of anti-angiogenic drug treated aggregates. (A) KNS42 aggregate treated with 15μM PD166866 (FGFR inhibition) (B) KNS42 aggregate treated with 10μM CBO-P11 (VEGF inhibition) (C) U87 aggregate, FGFR inhibition (D) U87 aggregate, VEGF inhibition. *Scale bar A&B = 200μm C&D = 25μm.*

Supplementary Table 1: Genes significantly differentially expressed between 3D and 2D KNS42 GBM cultures.

Supplementary Table 2: Genes significantly differentially expressed between 3D and 2D U87 GBM cultures.

Supplementary Table 3: Full list of genes differentially expressed between a GBM primary tumor explant T7 / 11 cultured in the RCCS for 3 weeks and a monolayer culture derived from the T7 / 11 explant and cultured for six passages.

Supplementary Table 4: Genes significantly differentially expressed between HBMEC / KNS42 RCCS co-culture and KNS42 RCCS culture.

Supplementary Table 5: Genes significantly downregulated in KNS42 aggregates in response to VEGF inhibition with the VEGF inhibitor CBO-P11 relative to untreated aggregates.

Supplementary Table 6: Genes significantly downregulated in KNS42 aggregates in response to FGF inhibition with the FGF receptor tyrosine kinase inhibitor PD166866 relative to untreated aggregates.

Supplementary Table 7: Genes significantly differentially expressed between U87 aggregates treated with the VEGF inhibitor CBO-P11 and untreated aggregates.

Supplementary Table 8: Genes significantly differentially expressed between U87 aggregates treated with the FGFR inhibitor PD166866 and untreated aggregates.

For Tables S1 to S8, please see the attached Excel file.