Suppl Figure 7



SFigure 7. Bevacizumab is transcytosed across normal brain ECs and TECs. Normal primary brain ECs or ECs isolated from GBM (TECs) were plated onto collagen in regular media. **A&B**, At three days post-plating the confluent monolayer was treated with bevacizumab, and at the indicated times media was collected from the lower chamber and subjected to an ELISA assay for human IgG to quantitate transcytosis (A). Alternatively, at three days post-plating the brain ECs or TECs was treated with 70-kDa-FITC-Dextran and the permeability of the monolayer determined by measurement of the fluorescence in the bottom chamber over time and expressed as the volume crossing

the monolayer (B). In A&B, open diamonds denote absence of ECs on the filter; closed triangles denotes the average of two isolates of normal brain ECs; and closed circles denotes TECs. **C&D**, The day following plating, bevacizumab was added to the media and at 30 min the cells were washed, fixed, reacted with Alexa-488-anti-human IgG, followed by DAPI nuclear stain and microscopy. Scale bars denote 20-µm.