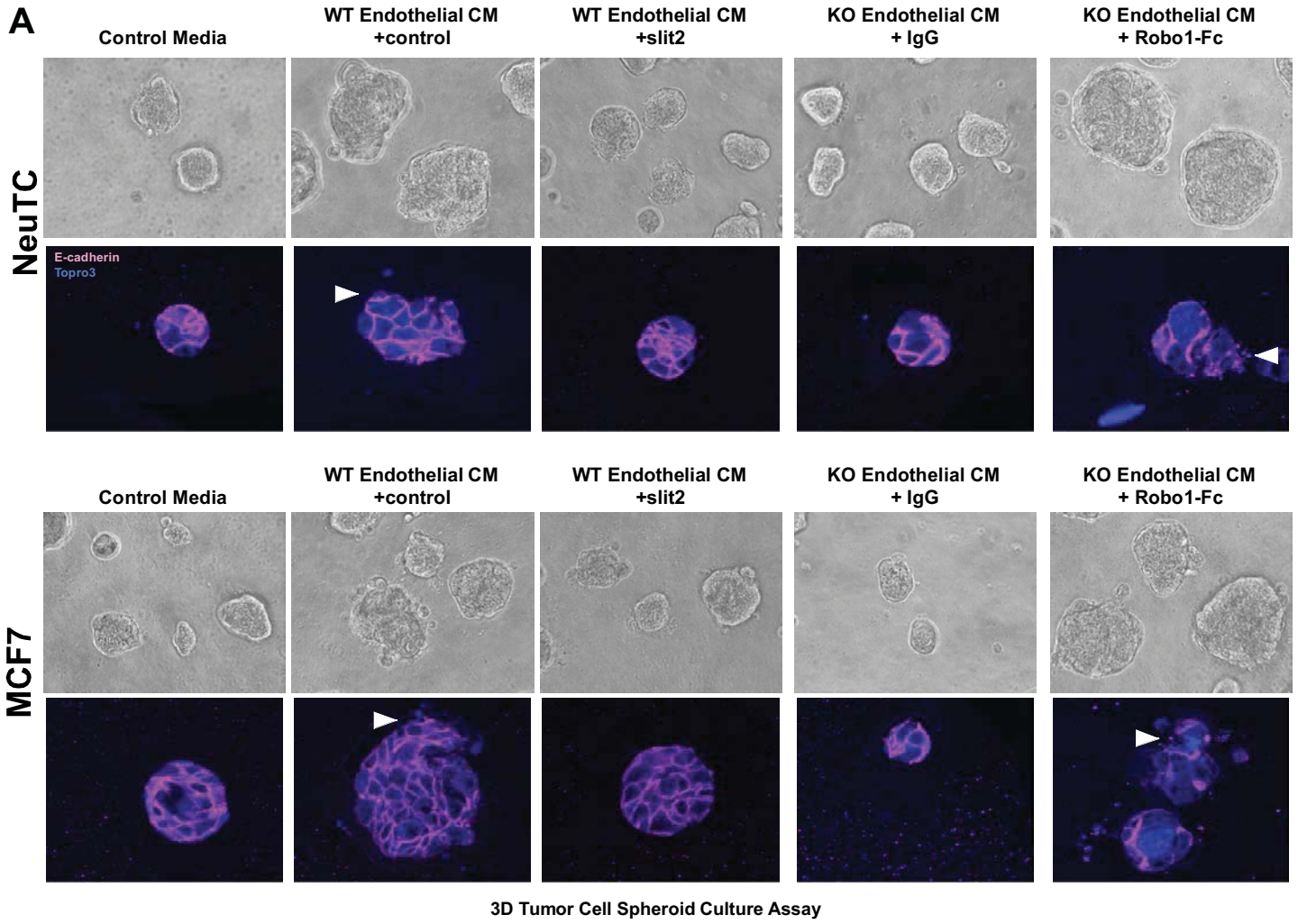
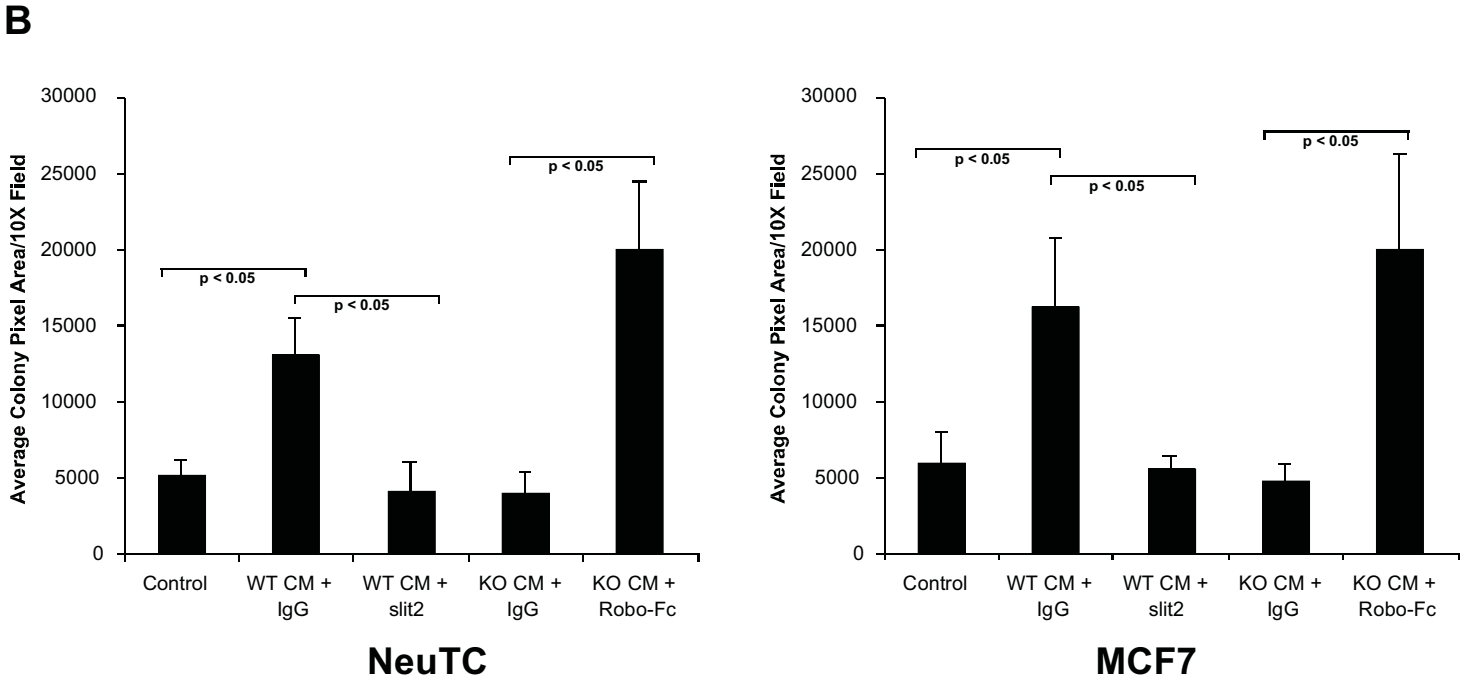
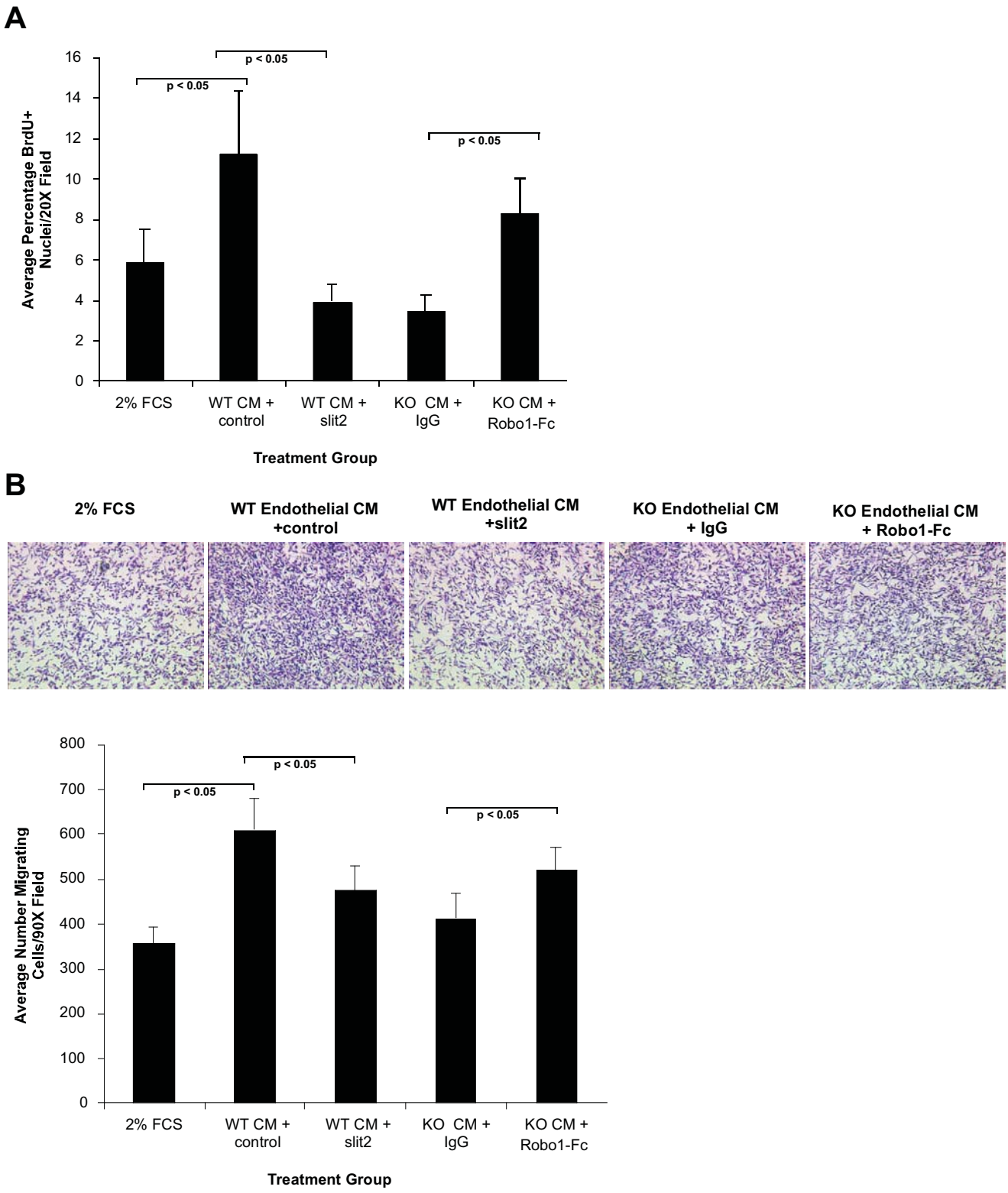
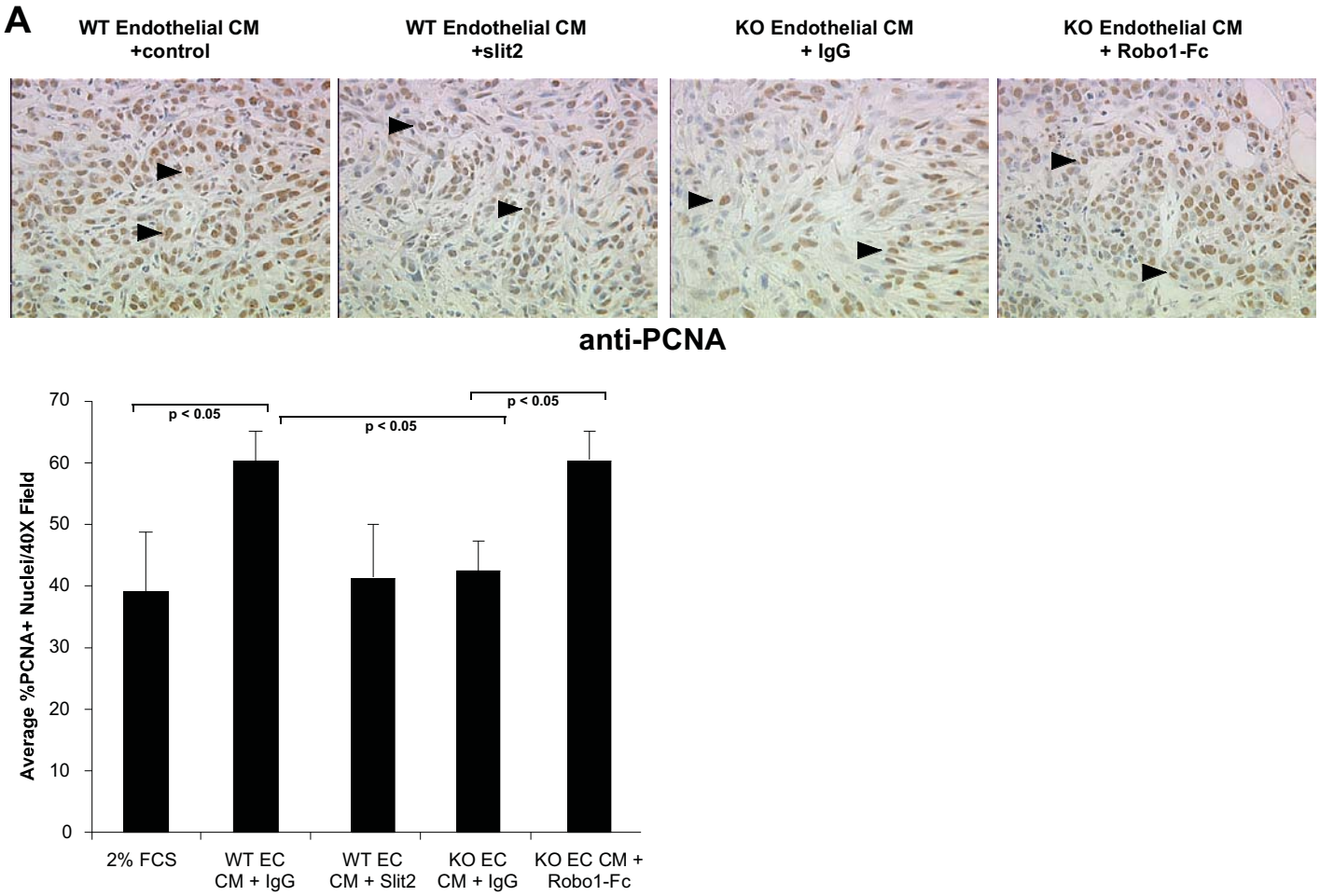


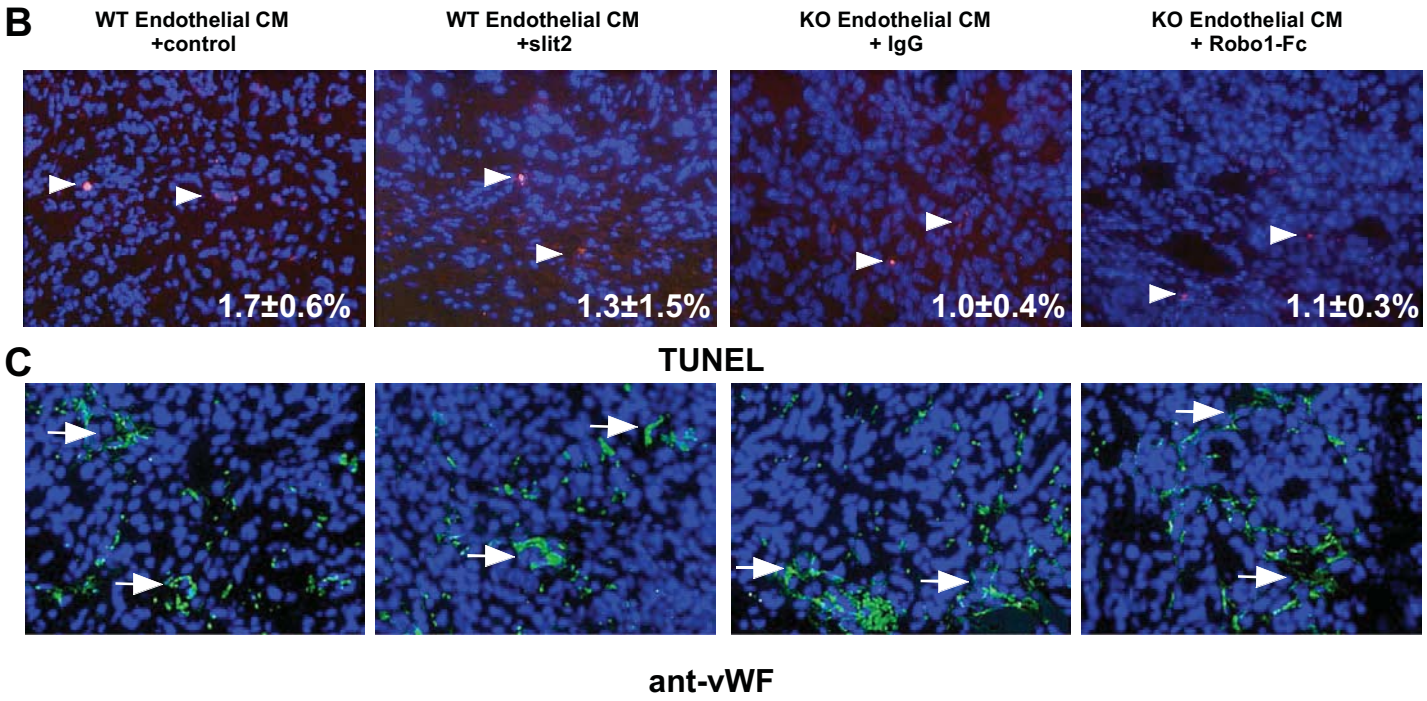


Brantley-Sieders et al., Supplemental Figure S1



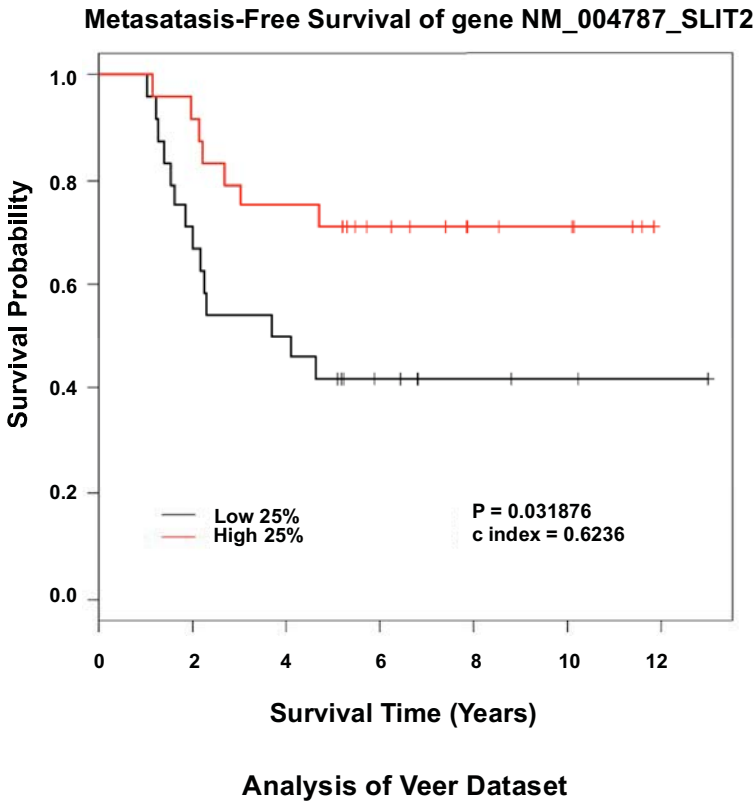
Brantley-Sieders et al., Supplemental Figure S2





Brantley-Sieders et al., Supplemental Figure S3

A



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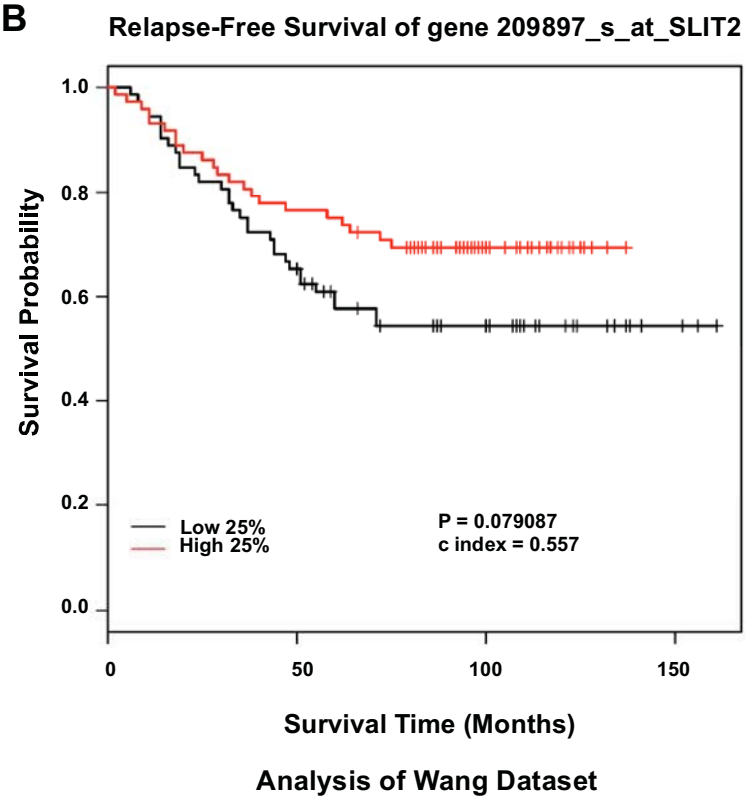

Brantley-Sieders et al., Supplemental Figure S4

Supplemental Figure Legends

Supplemental Figure S1: Modulating Slit2 activity in endothelium affects angiocrine-mediated tumor spheroid colony size and morphology in the context endothelial EphA2 receptor function. (A) Upper panels show photomicrographs of NeuTC tumor cell (line derived from MMTV-Neu transgenic mouse mammary tumors) spheroids, and human MCF7 breast tumor cell spheroids cultured in control medium (2% FCS), WT EC CM ± recombinant Slit2/control IgG, or KO EC CM ± soluble Robo1-Fc/control IgG for 5 days. Lower panels show confocal images of spheroids stained with E-cadherin (pink) and Topro3 (blue) nuclear counterstain. Arrowheads indicate invasive protrusions. (B) Colony size was quantified based on pixel area of 4 independent colonies/photomicrograph in replicate cultures from 3 to 5 independent experiments.

Supplemental Figure S2: Modulating Slit2 activity in endothelium affects angiocrine-mediated tumor cell growth motility in the context of endothelial EphA2 receptor function. (A) MDA-MB-231 human breast tumor cells were cultured in control base medium (2% FCS), WT EC CM ± recombinant Slit2/control IgG, or KO EC CM ± soluble Robo1-Fc/control IgG, and proliferation scored based on BrdU incorporation. (B) MDA-MB-231 cell migration in response to control base medium (2% FCS), WT EC CM ± recombinant Slit2/ control IgG, or KO EC CM ± soluble Robo1-Fc/control IgG was quantified by transwell assay. Upper panels show photomicrographs of crystal violet-stained tumor cells (purple) that migrated to the lower surface of transwell filters. Data are a representation of 3 independent experiments using conditioned medium from two independent WT versus KO EC isolates for all cell culture experiments.

Supplemental Figure S3: Modulating Slit2 activity in endothelial conditioned medium affects angiocrine-mediated tumor cell proliferation *in vivo* in the context of endothelial EphA2 receptor function. 4T1 tumor cells were ad-mixed with 5X concentrated CM from WT or KO EC ± recombinant Slit2/Robo1-Fc/control IgG versus

control base medium, resuspended in growth factor-reduced Matrigel, and injected subcutaneously into the dorsal flank of recipient mice. The resulting tumors were harvested 7 days post-injection for analysis. (A) Upper panels show photomicrographs of proliferating cell nuclear antigen (PCNA)-stained tumor sections from 4T1 cells co-injected with WT EC CM ± recombinant Slit2/control IgG or KO EC CM ± soluble Robo1-Fc/control IgG into nude female mice. Arrowheads indicate PCNA+ (brown) nuclei. Lower panel shows quantification of proliferation based on percentage PCNA+ nuclei. (B) Photomicrographs of TUNEL-stained tumor sections, including quantification of apoptosis based on percentage TUNEL+ nuclei. Arrowheads indicate TUNEL+ (red) nuclei. (C) Photomicrographs of von Willebrand (vWF) stained tumor sections. Arrows indicate vWF+ (green) tumor blood vessels.

Supplemental Figure S4. Slit2 expression patterns in human tissue support the tumor suppressive function of Slit2 in human breast cancer. Kaplan-Meier kinetic analyses of the (A) Veer and (B) Wang breast tumor datasets, with microarray profiles of 117 and 286 human breast tumor samples, respectively, and associated clinical data. The impact of elevated *slit2* expression on metastasis or relaspe-free survival was analyzed by Log-Rank tests.