Electronic Supplementary Material (ESI) for Lab on a Chip.
This journal is © The Royal Society of Chemistry 2020

Organotypic primary blood vessel models of clear cell renal cell carcinoma for single-patient clinical trials

Authors

M. Virumbrales-Muñoz[1,2], J. Chen[3], J. Ayuso[1,2,4], M. H. Lee[5], E. J. Abel[5], D. J. Beebe[1,2,*]

Affiliations

1. Department of Pathology and Laboratory Medicine. University of Wisconsin, Madison, 1111 Highland Avenue, Madison, Wisconsin 53705, United States
2. University of Wisconsin Carbone Cancer Center, Wisconsin Institutes for Medical Research, 1111 Highland Ave., Madison, WI 53705
3. Department of Biomedical Engineering, Wisconsin Institutes for Medical Research, University of Wisconsin-Madison, 1111 Highland Avenue, Madison, Wisconsin 53705, United States.
4. Medical Engineering, Morgridge Institute for Research, 330 N Orchard street, Madison, WI, USA. 53715.
5. Department of Urology University of Wisconsin School of Medicine and Public Health, Madison, 1111 Highland Ave., Madison, 53705 WI, USA.

Corresponding author: David J Beebe. Phone: 608 262 22 60. E-mail: djbeebe@wisc.edu.

Supplementary information

Table S1: Histologic subtype of renal cell carcinoma tumors used for this study. P= patient, T= tumor depth, M= number of distant metastases, N = nodal status, P.A.S.= patient age at surgery.

P	RCC subtype	T	M	N	nuclear grade	P.A.S.
A	clear cell	3b	0	0	3	45
B	clear cell	3a	0	0	3	75
C	clear cell	3a	1	0	4	52

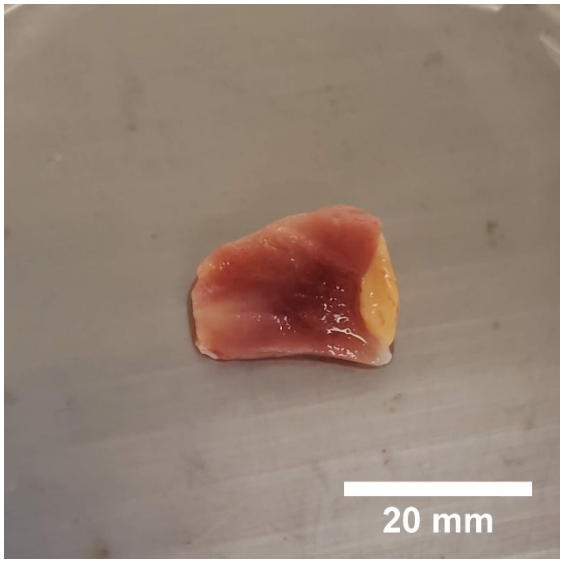

Fig S1: Sample piece of normal tissue used for processing and primary endothelial cell extraction.

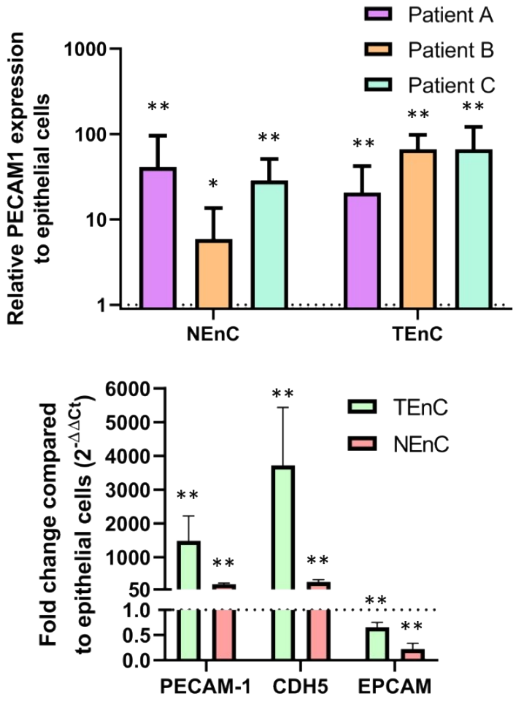

Fig S2: PECAM1 expression fold change of NEnC and TEnC utilized in this paper compared to epithelial cells (top). Expression of PECAM, CDH5 and EPCAM in NEnC and TEnC (pooled samples from 2 patients) compared to negatively selected primary $CD31^-$ cells (bottom).

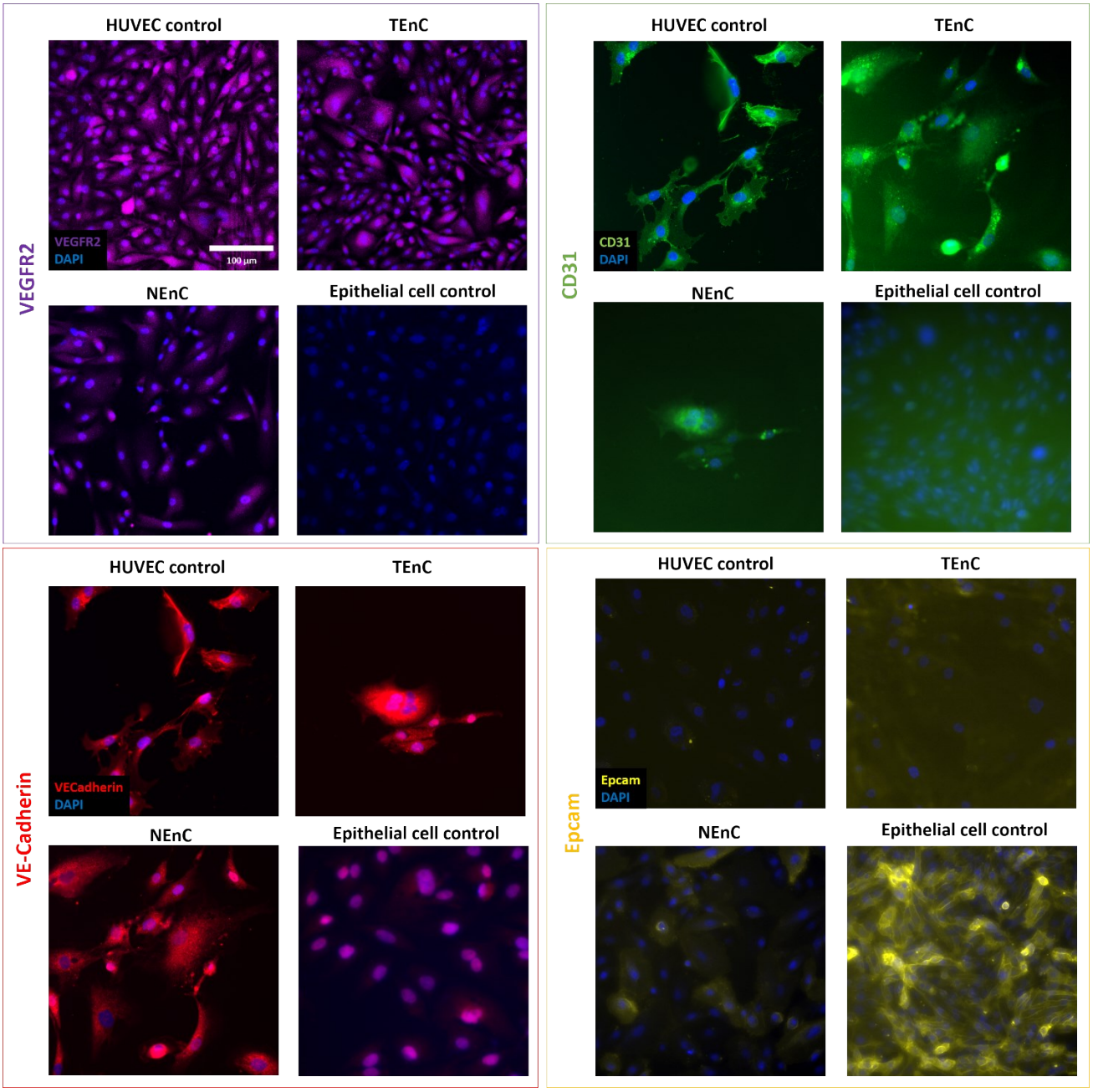

Fig S3: Cell lineage verification via immunofluorescence. Representative images of positively and negatively selected (NEnC cells, TEnC cells and CD31- fraction) cells using CD31 were tested using immunofluorescence for CD31 and Epcam. HUVECs were used as a positive control for the markers. TEnC and NEnC cells were CD31+, VECadherin+, VEGFR2+, Epcam-, as expected. DAPI is used as nuclei stain. Some sets of images were slightly overexposed to illustrate the localization of the staining in the cell. Scalebar (top left image) applies to all images.

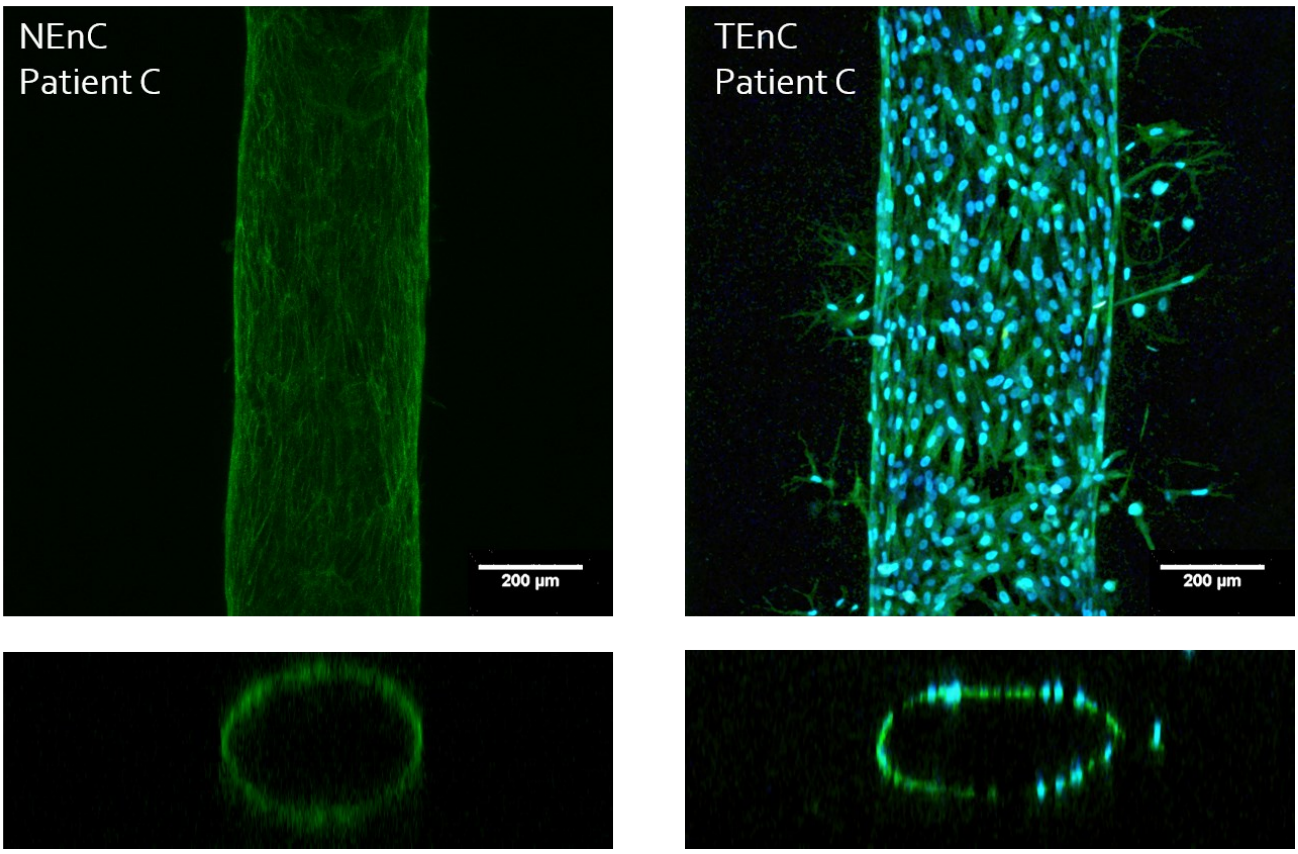

Figure S4: NEnC and TEnC sample lumens from Patient C. Green = Phalloidin staining for F-actin. Cyan = DAPI staining for nuclei. Projected Z-stack images (top) and lumen cross-section (bottom).

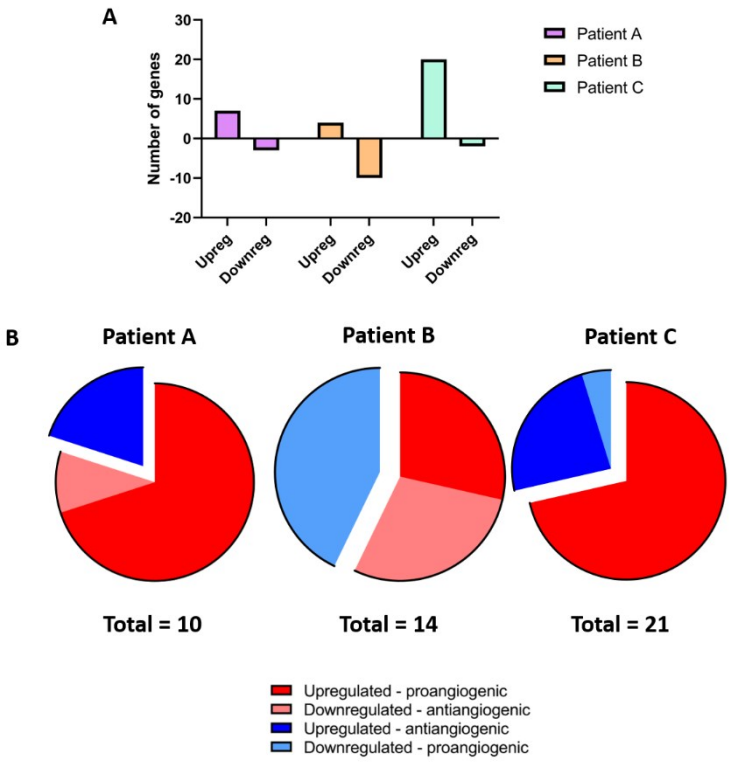

Fig S5: Analysis of the gene expression profiles. A) Graph showing the number of genes significantly upregulated and downregulated for each patient. **B)** Pie charts breaking down the contribution of each gene to angiogenesis. Light and dark red sections indicate significantly dysregulated genes that contribute to angiogenesis, either by being upregulated (proangiogenic) or downregulated (anti-angiogenic). Light and dark blue sections indicate significantly dysregulated genes that decrease the angiogenic response (i.e., the opposite regulation than depicted in red shades).

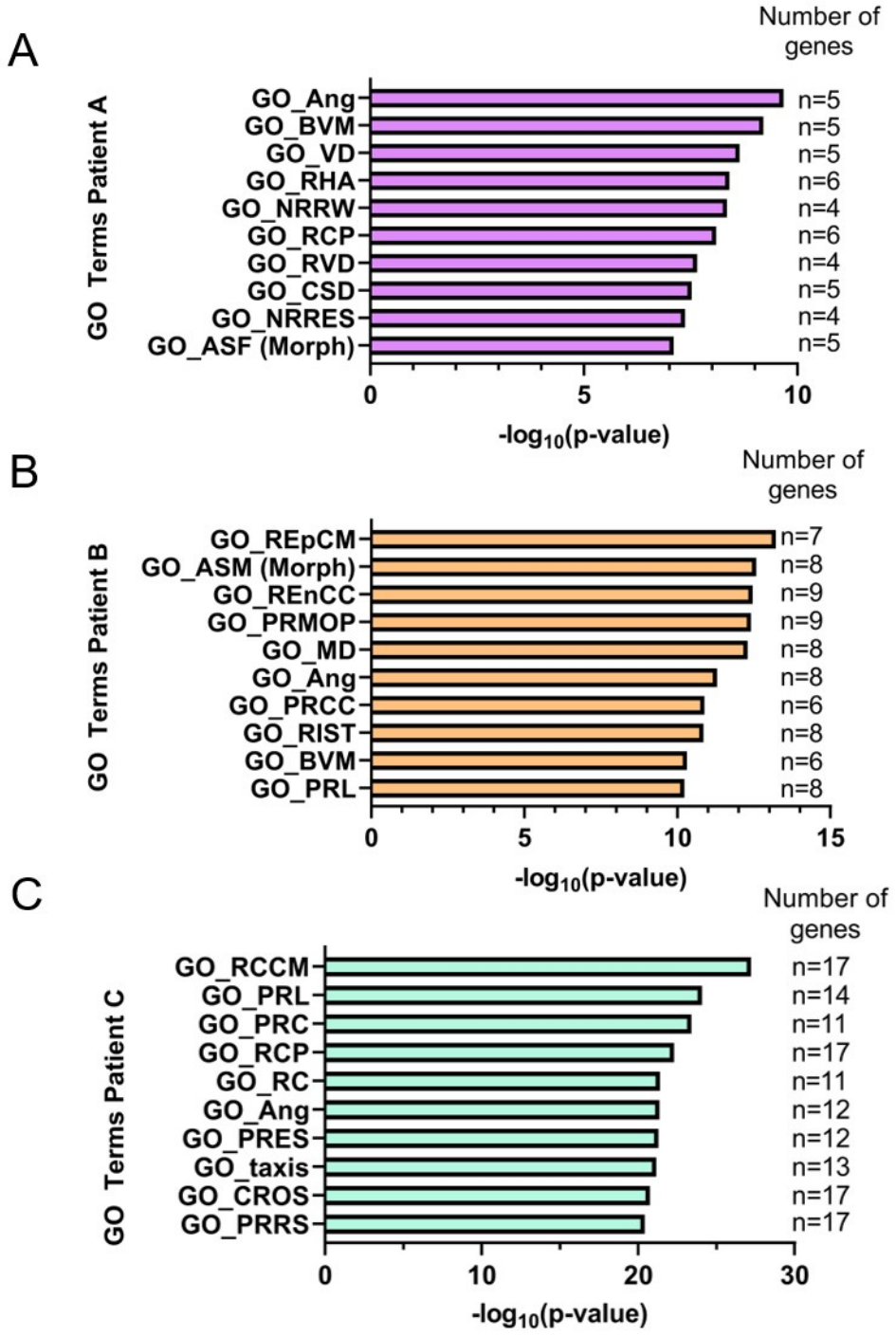

Fig S6: GSEA analysis of the gene expression profiles. A-C) Resulting gene sets were investigated to compute expression profiles for patients A, B, and C. Abbreviations: **Ang** = Angiogenesis; **BVM** = Blood vessel morphogenesis; **VD** = Vasculature development; **RHA** = regulation of hydrolase activity; **NRRW** = negative regulation of response to wounding; **RCP** = regulation of cell proliferation; **RVD** = regulation of vasculature development; **CSD** = circulatory system development; **NRRES** = negative regulation of response to external stimulus; **ASF (Morph)** = anatomic structure formation (morphogenesis); **REpCM** = regulation of epithelial cell migration; **REnCC** = regulation of

endothelial cell chemotaxis; **PRMOP** = positive regulation of multicellular organism process; **MD** = macrophage differentiation**; PRCC** = positive regulation of cell communication; **RIST** = regulation of intracellular signal transduction; **BVM** = blood vessel morphogenesis; **PRL** = positive regulation of locomotion; **RCCM** = regulation of cellular component movement; **PRC** = positive regulation of chemotaxis; **RC** = regulation of chemotaxis; **PRES** = positive regulation of external stimulus; **CROS** = cellular response to organic substance; **PRRS** = positive regulation of response to stimulus.

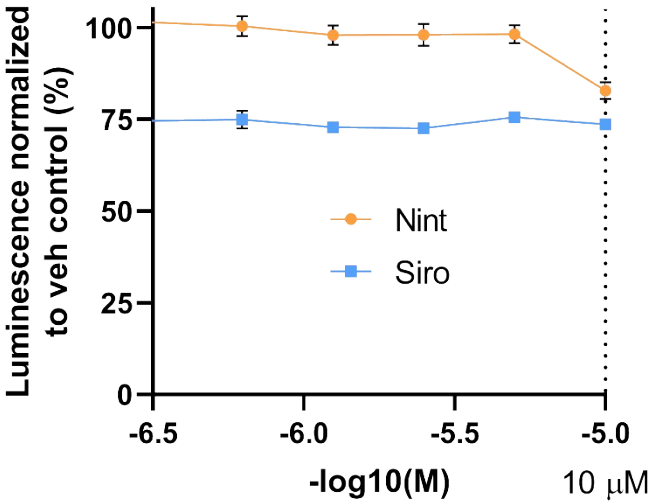

Figure S7: Dose-response of nintedanib (nint) and sirolimus (siro) in primary cells. CellTiterglo values were normalized to non-treated controls for different drug concentrations.

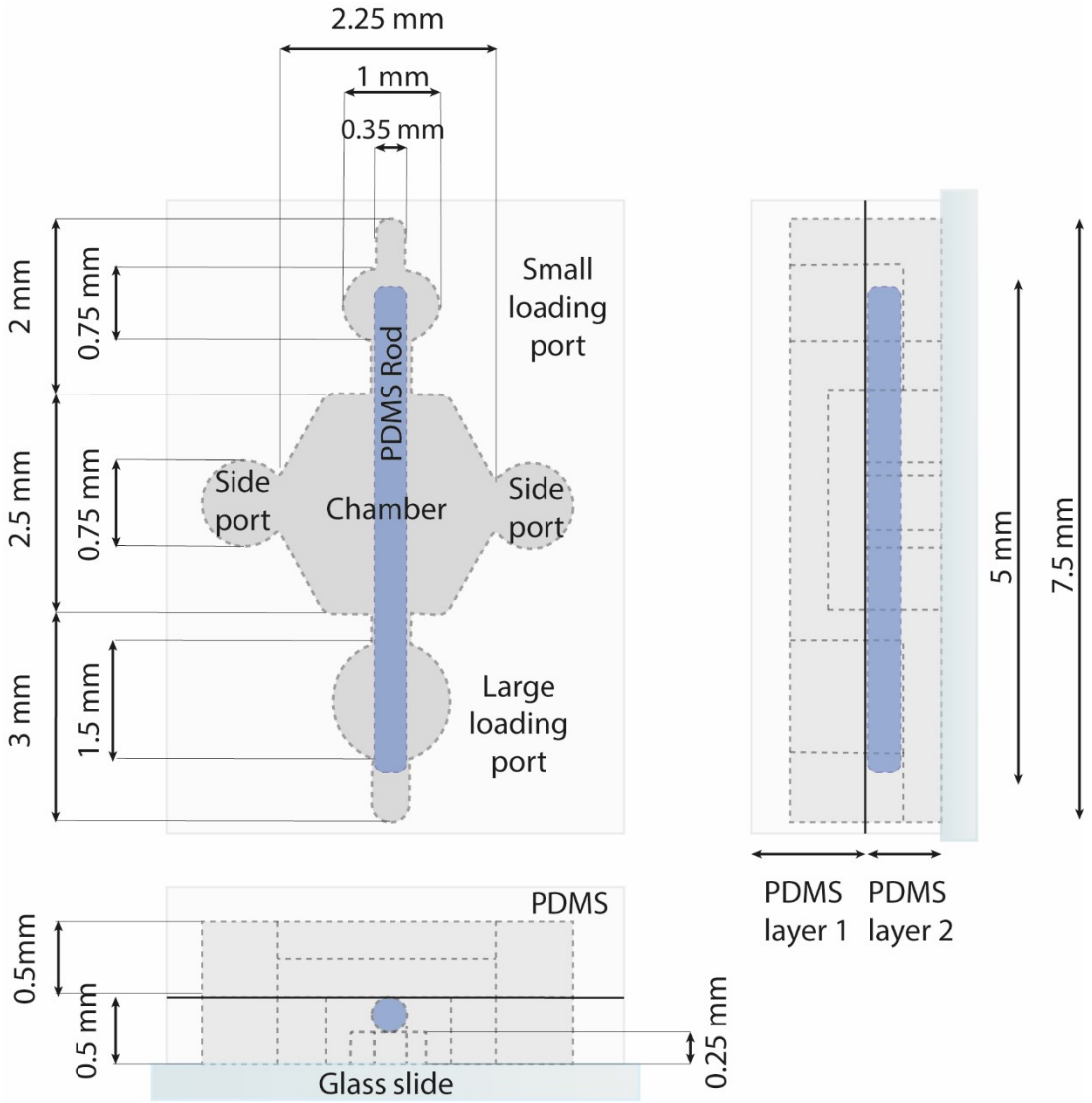

Figure S8: Detailed view of the LumeNEXT device composition and dimensions.

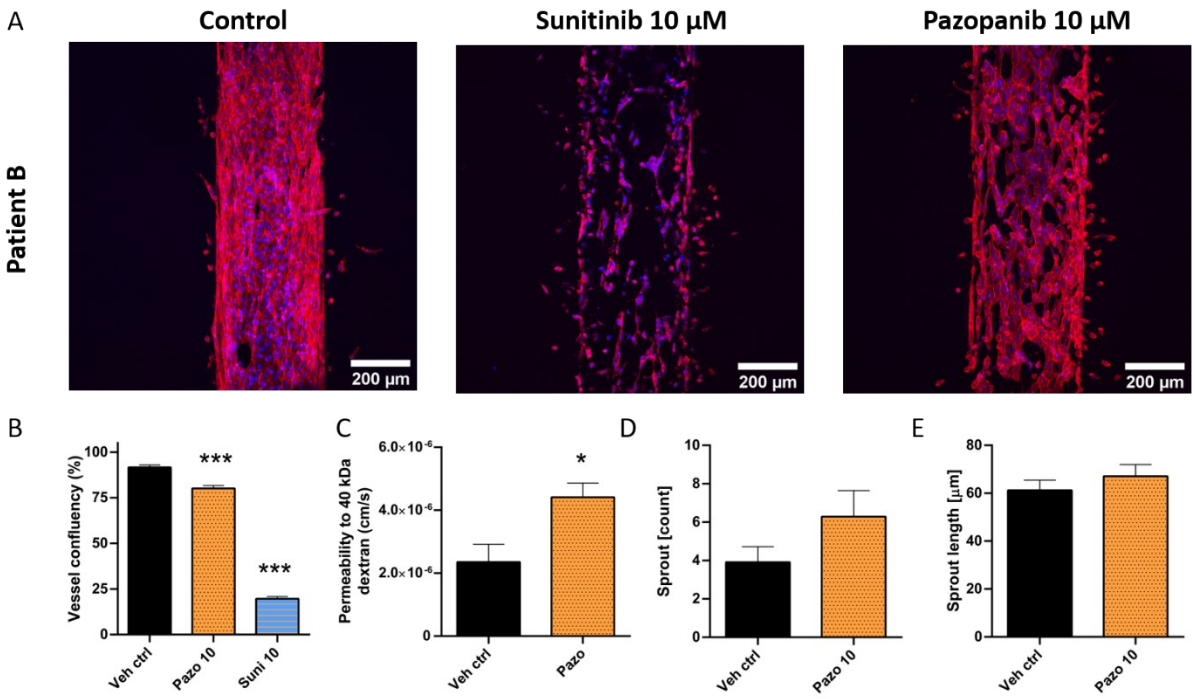

Figure S9: Test standard of care anti-angiogenic drugs on Patient B TEnC vessel models. A) Representative images of control and drug-treated TEnC vessel models. 10 µM of Sunitinib and Pazopanib (Pazo) were used and compared to vehicle control conditions. Vessels are stained with TRITC-Phalloidin (Red) and DAPI (Blue) B-E) Functional characterization of drug response to Sirolimus and Nintedanib using the following readouts: B) sprout length, C) sprout count, D) Permeability values to 40 kDa fluorescently conjugated dextran. N = at least 6 vessels from at least 2 independent experiments. Scalebar = 200 µm. Bars represent average ± S.E.M. One-to-one comparisons were performed via Students' t test, whereas multiple comparisons were performed using one-way ANOVA with Tukey post-hoc test. *$p<0.05$; ***$p < 0.001$.