Supplementary Information

Lymphatic endothelial cells support tumor growth in breast cancer

Esak Lee[a], Niranjan B. Pandey[a], and Aleksander S. Popel[a,b]

[a]Department of Biomedical Engineering, Johns Hopkins University School of Medicine, Baltimore, MD 21205, United States.

[b]Department of Oncology and the Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins University School of Medicine, Baltimore, MD 21231, United States.

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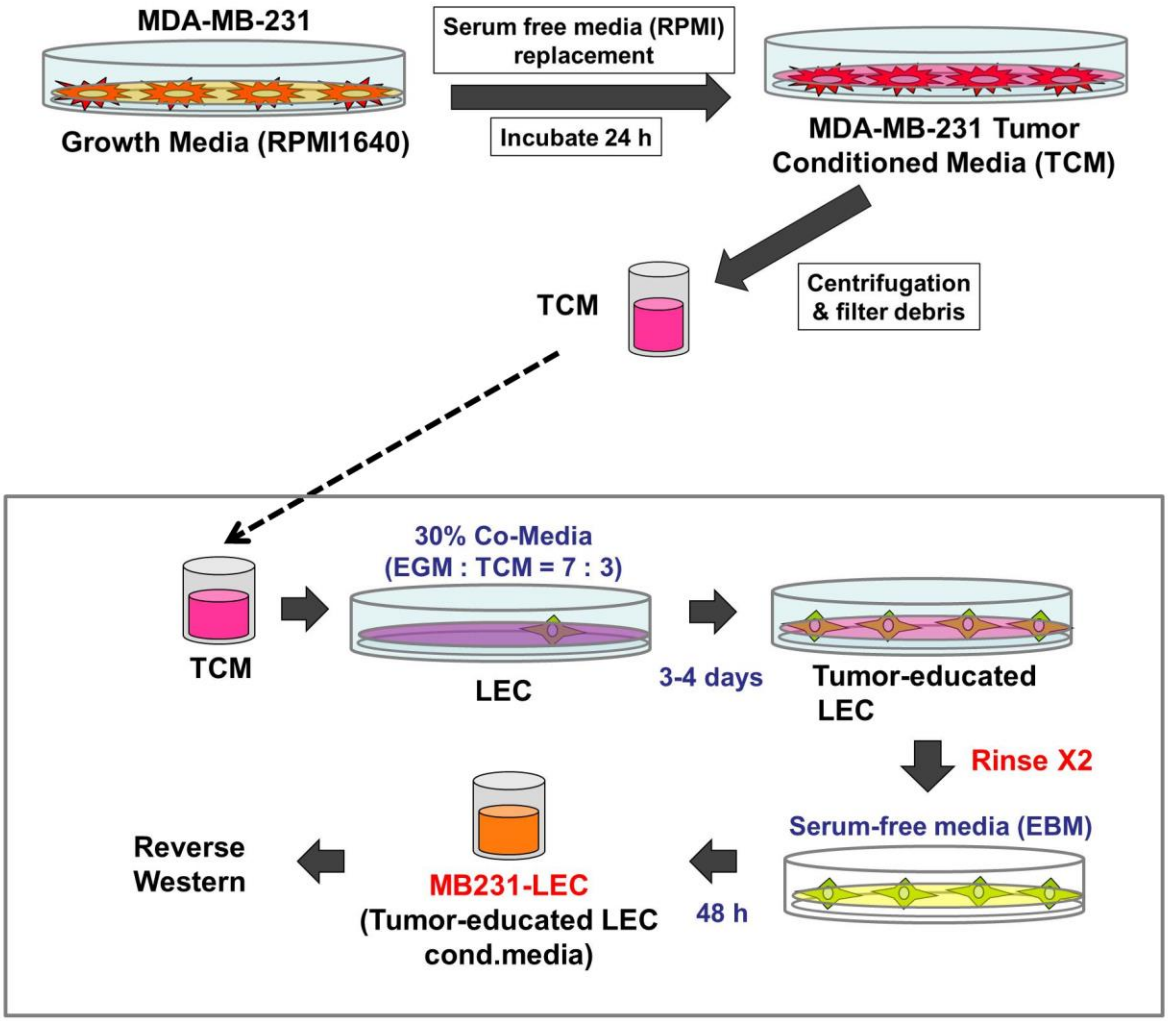
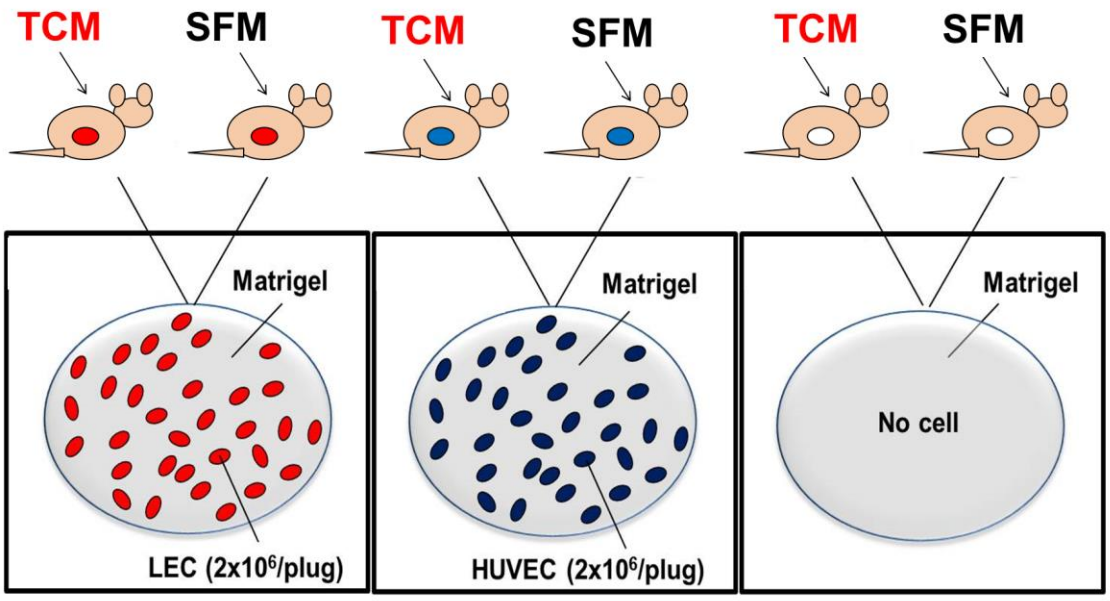
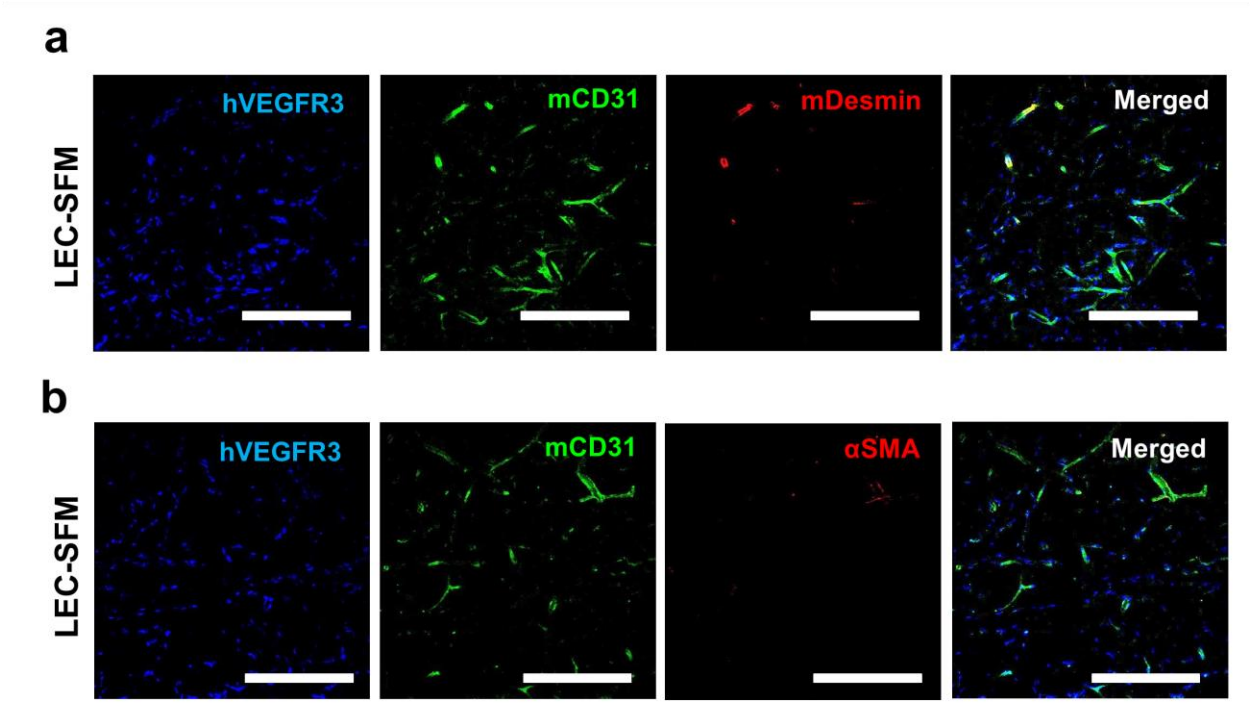
Supplementary Figures



Supplementary Figure S1: Tumor-EC education model. For tumor-EC education model, tumor-conditioned media (TCM) was prepared from MDA-MB-231 cell culture. Normal EC (LEC, MEC, and HUVEC) are exposed to 30% TCM co-culture media (TCM:EGM = 3:7). During 3 or 4 days, EC are propagated in the co-culture environment at which time the EC is being educated by the TCM. Tumor-educated EC was rinsed and serum-free media (EBM) was added and the factors secreted from the tumor-educated EC allowed to accumulate. The media collected at this time is referred to as tumor-educated EC conditioned media, which is analyzed with reverse western assays.



Supplementary Figure S2: LEC-included matrigel plug assays. High-concentrate matrigel containing LEC or HUVEC (2×10^6/gel) and heparin (10 units/gel) was injected subcutaneously. No cell group was also included as a control. TCM or SFM (50 μl/injection) was subcutaneously dosed daily for 10 days, the mice were euthanized, and the gel plugs were excised and analyzed.



Supplementary Figure S3: Anti-desmin and anti-αSMA antibodies have no immunoreactivity with LEC and BEC. We employed anti-desmin and anti-αSMA antibodies to detect pericytes infiltrated into the LEC-TCM matrigel plugs, compared to the LEC-SFM group (Fig. 4). To demonstrate that αSMA & Desmin immunoreactivity is not observed in the LEC control, we stained LEC-SFM matrigel plugs with the anti-desmin and anti-αSMA antibodies. The LEC-SFM matrigel plugs include human LEC (hVEGFR3) and some mouse BV (mCD31). **(a)** Two EC components are not detected with anti-desmin antibodies. **(b)** Two EC components are not detected with anti-αSMA antibodies. These data show that anti-desmin and anti-αSMA antibodies are specific for pericytes in our models. Scale bars represent 200 μm.

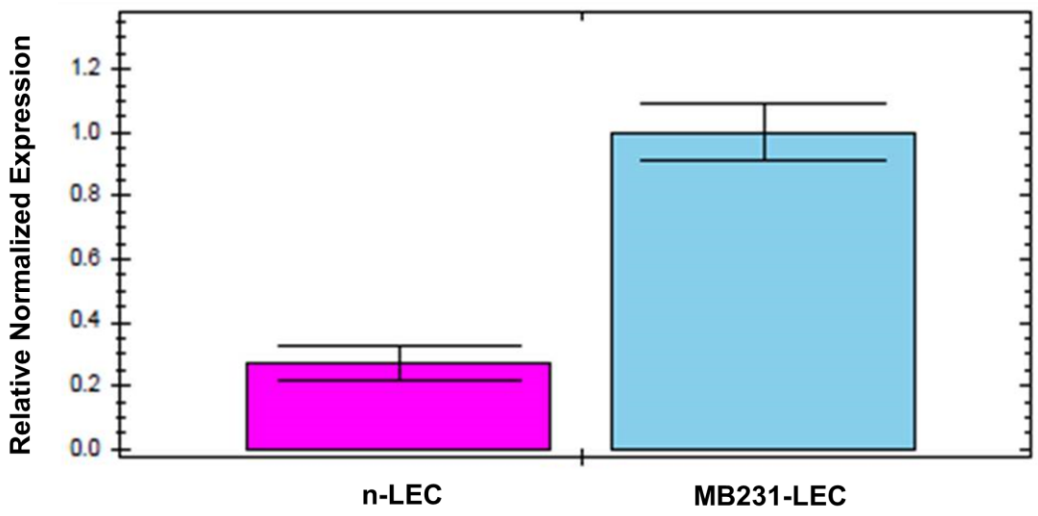
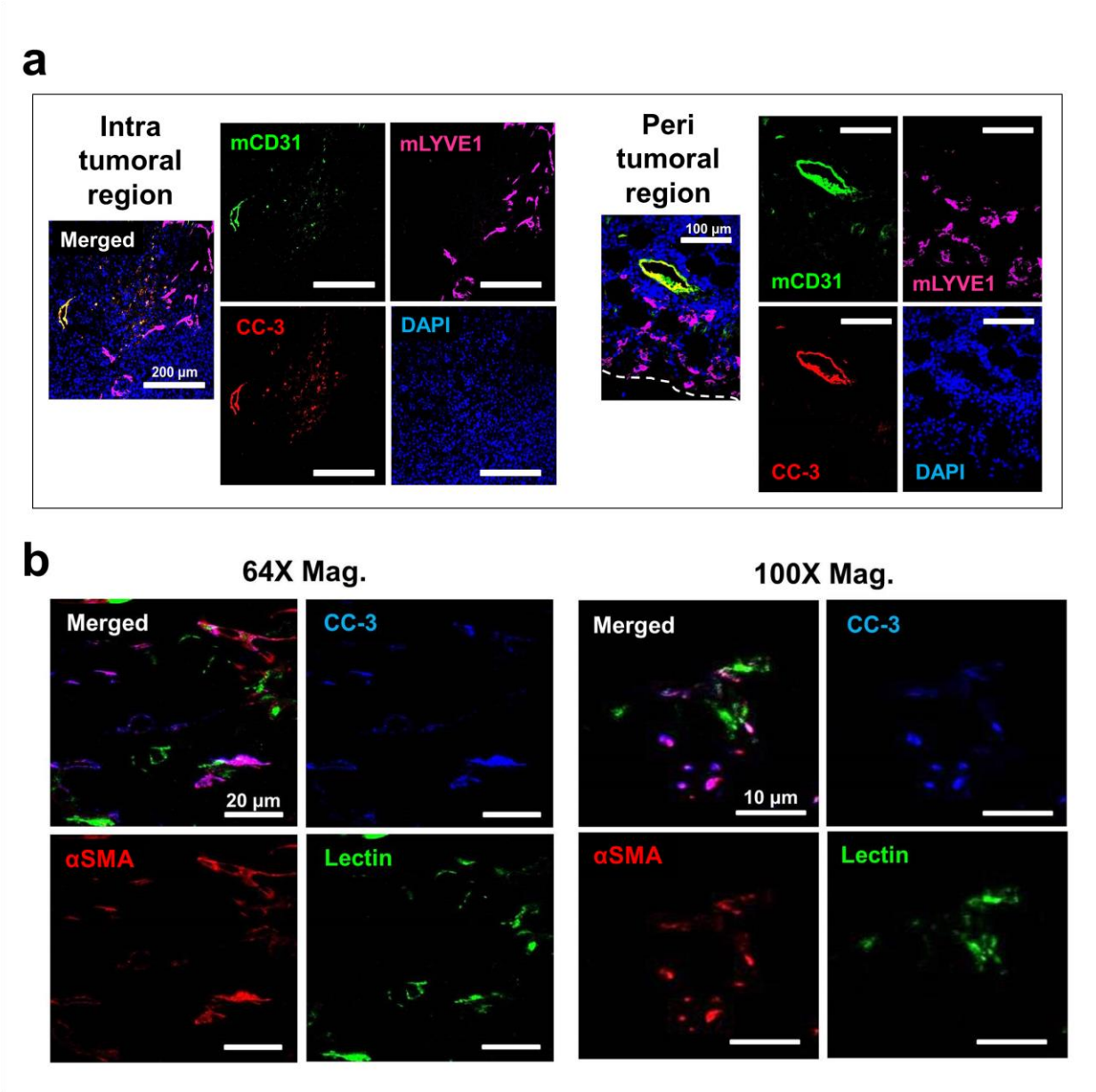


Figure S4: Real Time PCR for *hPDGFB* gene expression in LEC. Angiogenesis reverse array data (Fig. 1) showed that EGF increased around 600%, PDGF-BB increased around 280% in MB231-LEC, compared to normal LEC. As the change in PDGF-BB was relatively smaller than EGF, we additionally performed quantitative RT-PCR for gene expression of *hPDGFB* to account for the protein expression data. Duplicate experiments showed that about 3.4 times more enhanced *hPDGFB* gene expression in MB231-LEC.



Supplementary Figure S5: Pericytes around blood vessels experience apoptosis in MB231 tumors. (a) MB231 tumors were analyzed. Intra- and peri-tumoral regions were stained with anti-cleaved caspase 3 (CC-3), mCD31, and mLYVE-1 antibodies to detect apoptotic cells, mouse blood and lymphatic vessels (mBV and mLV). In both regions, CC-3 positive regions were found only around mBV not around mLV. (b) MB231 tumors were further analyzed by staining with anti-cleaved caspase 3 (CC-3),

anti-αSMA, and anti-lectin antibodies. More high resolution imaging (64x and 100x mag.) reveals that the CC-3 proteins are colocalized with αSMA-positive pericytes around the periphery of mBV. The length of scale bars is presented in the figure images.