Supplementary Information

Comparing GFP and TERT-HUVECs

Primary human umbilical endothelial cells (HUVECs) are often used to vascularize *in vitro* tissues. However, they suffer from the same drawbacks of any other cell line: limited passage numbers and constant need for sourcing. To combat these issues, HUVECs can been transduced with the telomerase reverse transcriptase gene (TERT) which permits the maintenance of their differentiated phenotypes and extends their lifespan[1–3]. Although it has been shown that TERT-HUVECs exhibit similar growth rates and barrier properties their primary counterparts[1,2], we wished to confirm this in our model system. We first seeded either GFP-HUVECs or TERT-HUVECs into the vascular channel of our hydrogel, allowed them to proliferate and form a confluent luminal vessel, then seeded PSCs only the surface of the gel on day 7. Cultures were switched to compartmentalized media consisting of ST-PSCM above the ST monolayer and ECGM2 in the vascular channel. Permeability to 4 kDa and 65 kDa dextran was evaluated on day 6, before PSCs were added and compartmentalized culture was commenced, as a baseline. Endothelial cell only conditions were included to isolate the effects of ST coculture from the effects of compartmentalized media culture (**Figure S2**).

Firstly, the TERT-HUVEC condition exhibited higher 4 kDa and 65 kDa dextran permeation rates (682.3 $\pm$ 112.6 pmol/hr and 14.82 $\pm$ 6.51 pmol/hr, respectively) than the GFP-HUVECs condition (489.2 $\pm$ 34.7 pmol/hr and 8.26 $\pm$ 1.04 pmol/hr, respectively) on day 6. However, the vessels constructed of both cell types degraded over time and did not maintain their barrier integrity to 4 kDa (1089 $\pm$ 169 pmol/hr for TERT-HUVECs pmol/hr and 756.1 $\pm$ 93.2 pmol/hr for GFP-HUVECs) or 65 kDa dextrans (30.74 $\pm$ 9.29 pmol/hr for TERT-HUVECs pmol/hr and 27.04 $\pm$ 5.61 pmol/hr for GFP-HUVECs). Interestingly, when cultured with STs, this barrier degradation was no longer apparent in the 65 kDa permeability assay (8.96 $\pm$ 2.87 pmol/hr for GFP-HUVECs + STs and .22 $\pm$ 2.71 pmol/hr for TERT-HUVECs + STs), and most notably, the barrier seemed to improve from day 6 to day 15 when testing for 4 kDa dextran vascular diffusion (367.2 $\pm$ 67.4 pmol/hr for GFP-HUVECs + STs and 319.4 $\pm$ 61.8 pmol/hr for TERT-HUVECs + STs). These findings indicate that trophoblast coculture enhances the barrier integrity of both endothelial cell types examined in this study. This agrees with previous findings by Lee *et al.* that showed that PSC syncytialization triggered an increase in endogenous progesterone immunomodulatory binding factor 1 (PIBF1) secretion, which was later shown to enhance endothelial migration and tube formation of endothelial cells in a dose-dependent manner[4]. The improvements in vascular endothelial integrity that we observed can be likened to those caused by endothelial cell coculture with other support cells such as fibroblasts and pericytes, highlighting the importance of paracrine and endocrine signaling within the placenta[5]. Finally, GFP-HUVECs exhibited superior baseline permeability, thus serving as the preferred choice for all subsequent experiments detailed in this work. Overall, these findings demonstrated the beneficial impact of STs on HUVEC culture, prompting an intriguing inquiry into how trophoblasts at various differentiation stages influence blood vessel behavior.

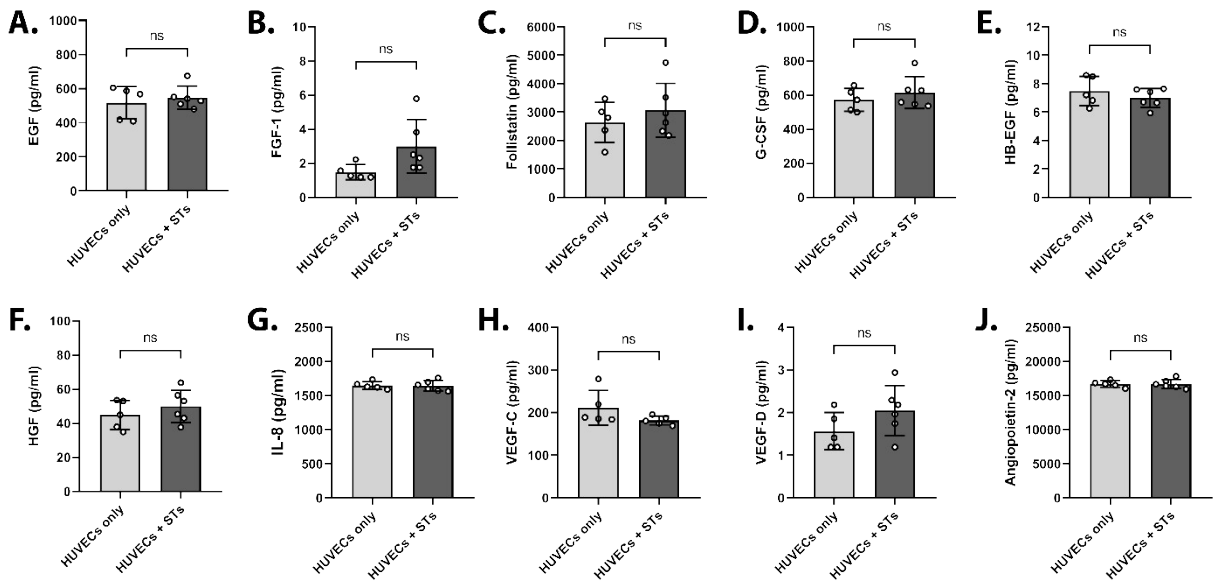

Figure S1. The effects of ST coculture with HUVECs on the secretion of angiogenic cytokines. A-J. *Angiogenic cytokine secretion of HUVECs only and HUVECs + ST barrier models 24 hrs after media change. (N=5-6, t-tailed t-test, *p<0.05, **p<0.01, ***p<0.001).*

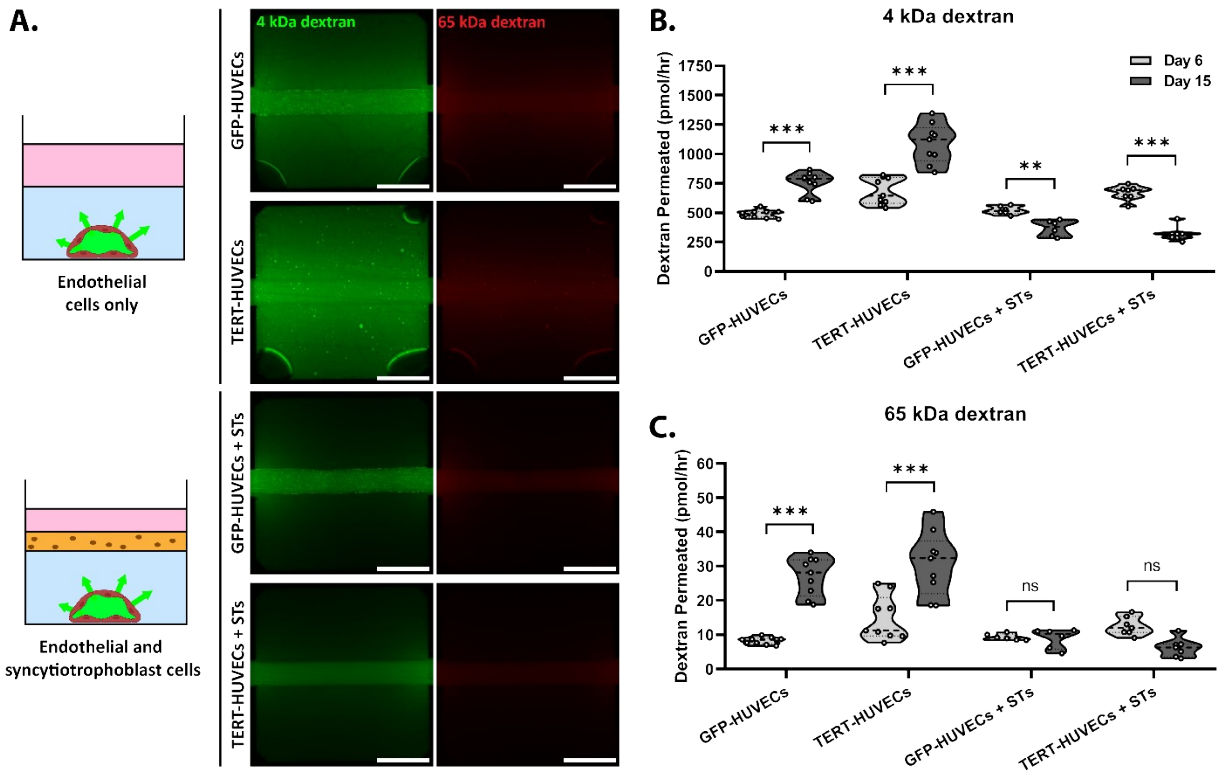

Figure S2. Comparing TERT-HUVECs with GFP-HUVECs in compartmentalized media and ST coculture. A*. Images of 4 kDa and 65 kDa dextran diffusion through vasculature after 1 hour of perfusion on the rocker on day 15 of culture.* ***B.-C.*** *Diffusion of 4 kDa and 65 kDa dextrans through the vascular channel on day 6 and day 15 (N=7-9, t-test, **p<0.01, ***p<0.001).*

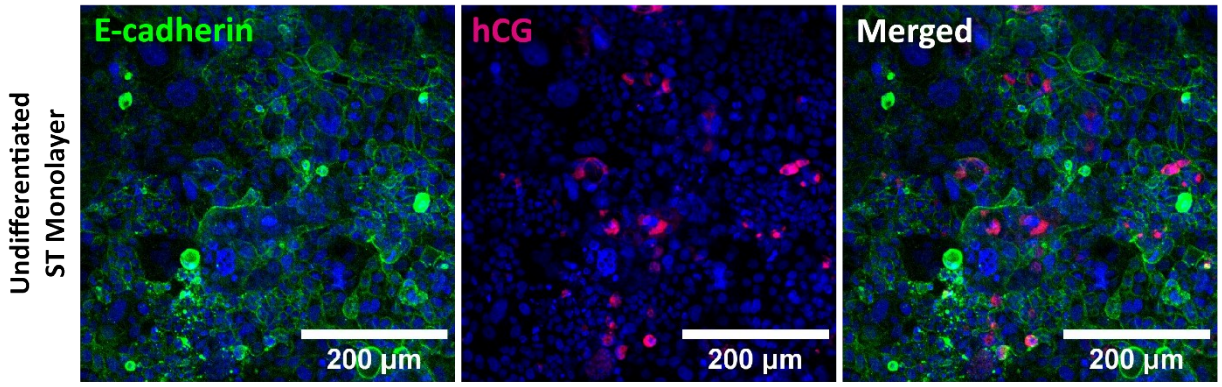

Figure S3. E-cadherin (green) and hCG (red) immunostaining of undifferentiated ST monolayer in HUEVC+ST coculture condition within the AngioPlate barrier model.

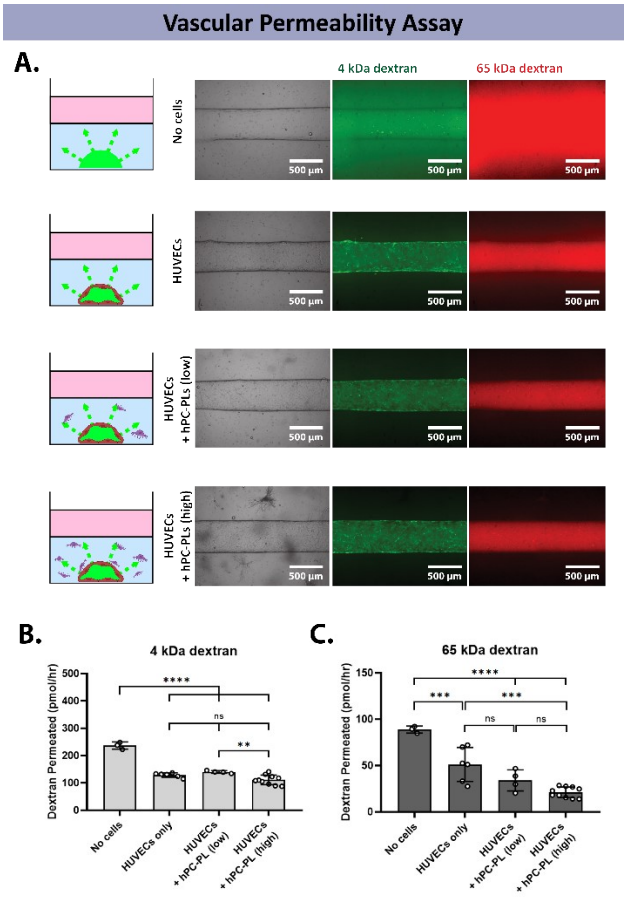

Figure S4. Effects of pericytes on vascular permeability of placental barrier model without STs on day 7 of culture. **A.** Brightfield images of vascular channel for the following conditions: no cell control, HUVECs only and HUVECs + hPC-PLs at high and low densities. Fluorescent images of 4kDa (green) and 65 kDa (red) dextran permeation through endothelial channel after 1 hour of perfusion. **B.** Vascular barrier permeability to 4 kDa dextran. (N=3-10, one-way ANOVA, *p<0.05, **p<0.01, ***p<0.001) **C.** Vascular barrier permeability to 65 kDa dextran. (N=3-7, one-way ANOVA, *p<0.05, **p<0.01, ***p<0.001).

References

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