

Supplementary Materials: Single-Cell Receptor Quantification of an in Vitro Coculture Angiogenesis Model Reveals VEGFR, NRP1, Tie2, and PDGFR Regulation and Endothelial Heterogeneity

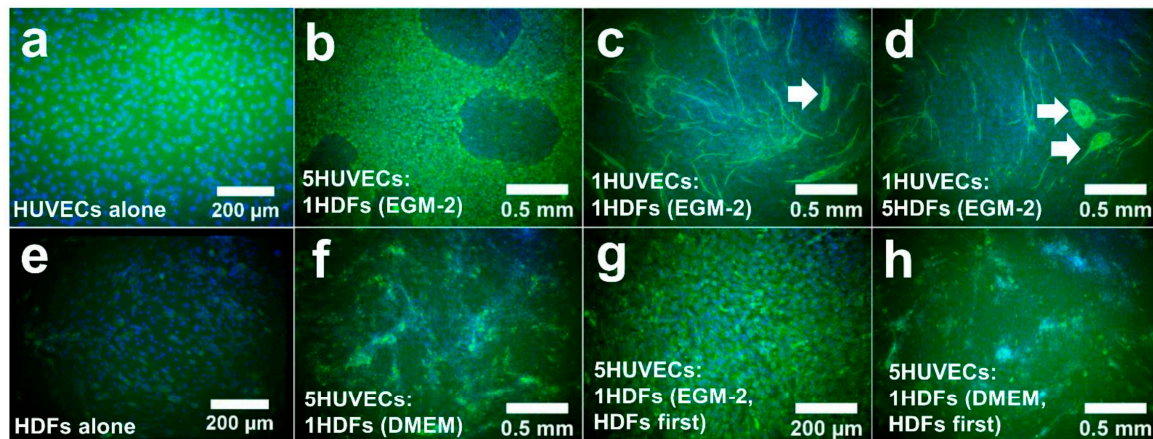


Figure S1. Human umbilical vein endothelial cell (HUVEC)–human dermal fibroblast (HDF) coculture tubule formation dependent on seeding conditions. Fluorescence images of monocultures and cocultures in which the HUVECs stained green with phycoerythrin (PE)-conjugated CD31 antibody and cell nucleus were stained with DAPI (blue) were taken on day 17. (a) HUVEC monocultures. 10 \times . (b) HUVECs and HDFs were seeded at a ratio of 5:1 and cultured in endothelial growth media (EGM)-2. 4 \times . (c) HUVECs and HDFs were seeded at a ratio of 1:1 and cultured in EGM-2. 4 \times . On the right side is a plaque or cluster of HUVECs (arrow) from which tubular sprouts emerge. 4 \times . (d) Similar to (b) and (c), but with a seeding ratio of 1:5 for HUVEC:HDF. Larger plaques of HUVECs (arrows) and fewer tubular structures than (c) were observed. 4 \times . (e) HDF monocultures. 10 \times . (f) HUVECs and HDFs were seeded at a ratio of 5:1 and cultured in fibroblast media, Dulbecco's modified eagle media (DMEM) supplemented with 5% fetal bovine serum (FBS) and 1% antibiotics. 4 \times . (g) HDFs were seeded three days before seeding HUVECs at a 5:1 ratio and cocultures were maintained in EGM-2. 10 \times . (h) Similar to (g), but cocultured were maintained in DMEM media. 4 \times .

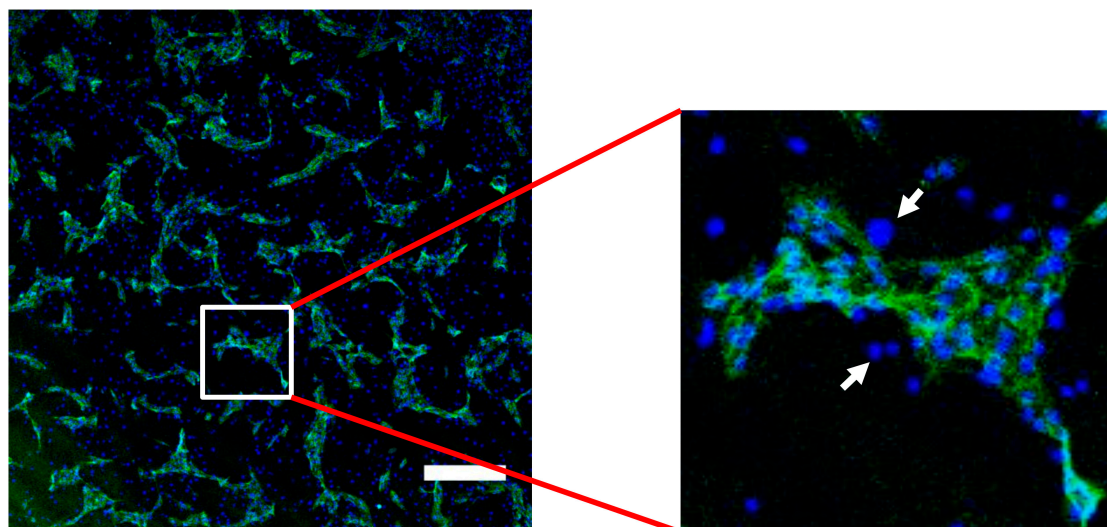


Figure S2. Representative fluorescence image of the 24-hour-old HUVEC–HDF cocultures. Green: CD31; Blue: DAPI. Arrows in enlarged image (right panel) showed nucleus of HDFs (CD31⁻) congregating around a cluster of HUVECs (CD31⁺). Scale bar: 0.5 mm.

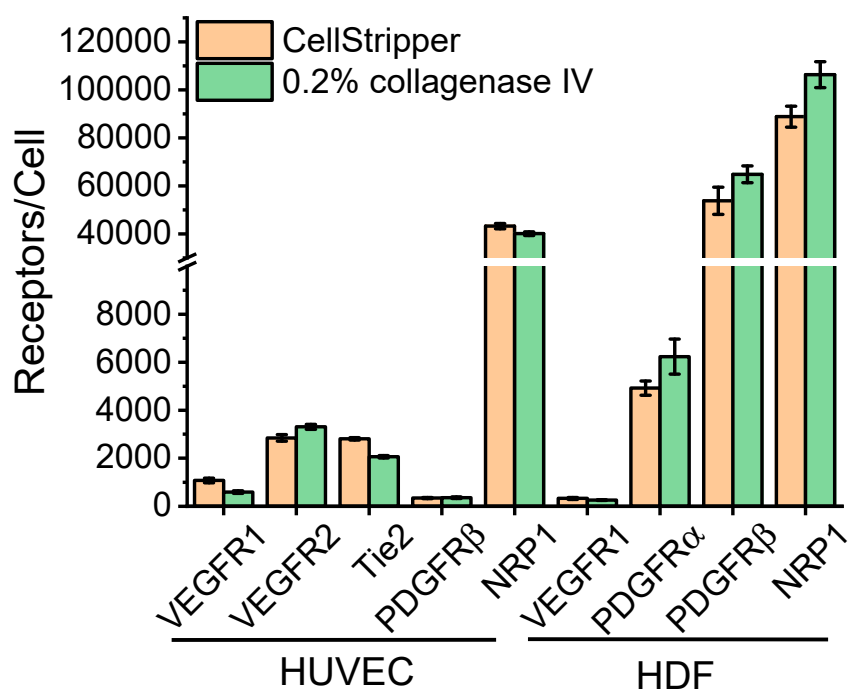


Figure S3. Comparison of receptor concentrations on monocultured cells dissociated using CellStripper™ and cells dissociated using 0.2% collagenase type IV with intermittent vortexing (see Methods and Materials). Mean \pm SEM of replicates were compared using ANOVA Tukey test and no significant changes were observed ($p > 0.05$). NRP: Neuropilin; PDGFR: Platelet-derived growth factor; VEGFR: Vascular endothelial growth factor receptor.