Supporting Information

Transient Support of Fibroblasts is Sufficient to Drive Functional Vascularization in Engineered Tissues

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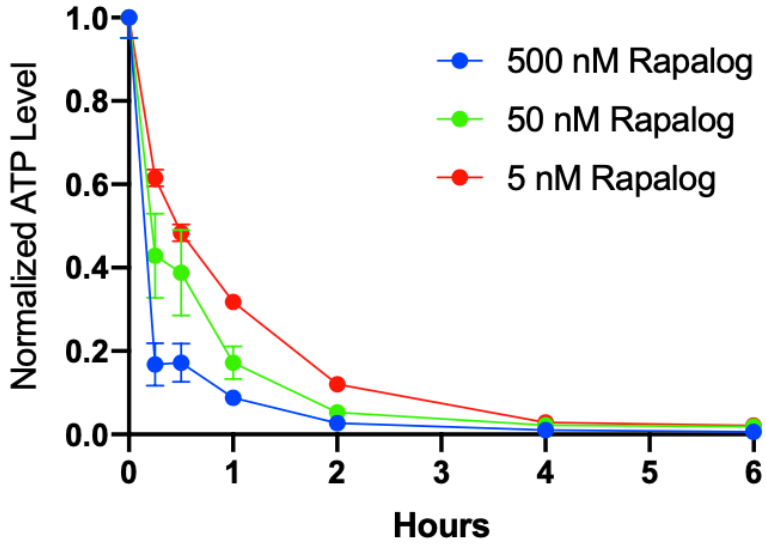

Figure S1. iCasp9-HDFs initiate apoptosis upon the addition of CID. Different concentrations of CID (5, 50, and 500 nM) were added to a 2D culture of iCasp9-HDFs, and the ATP level was measured at different timepoints for 6 hours.

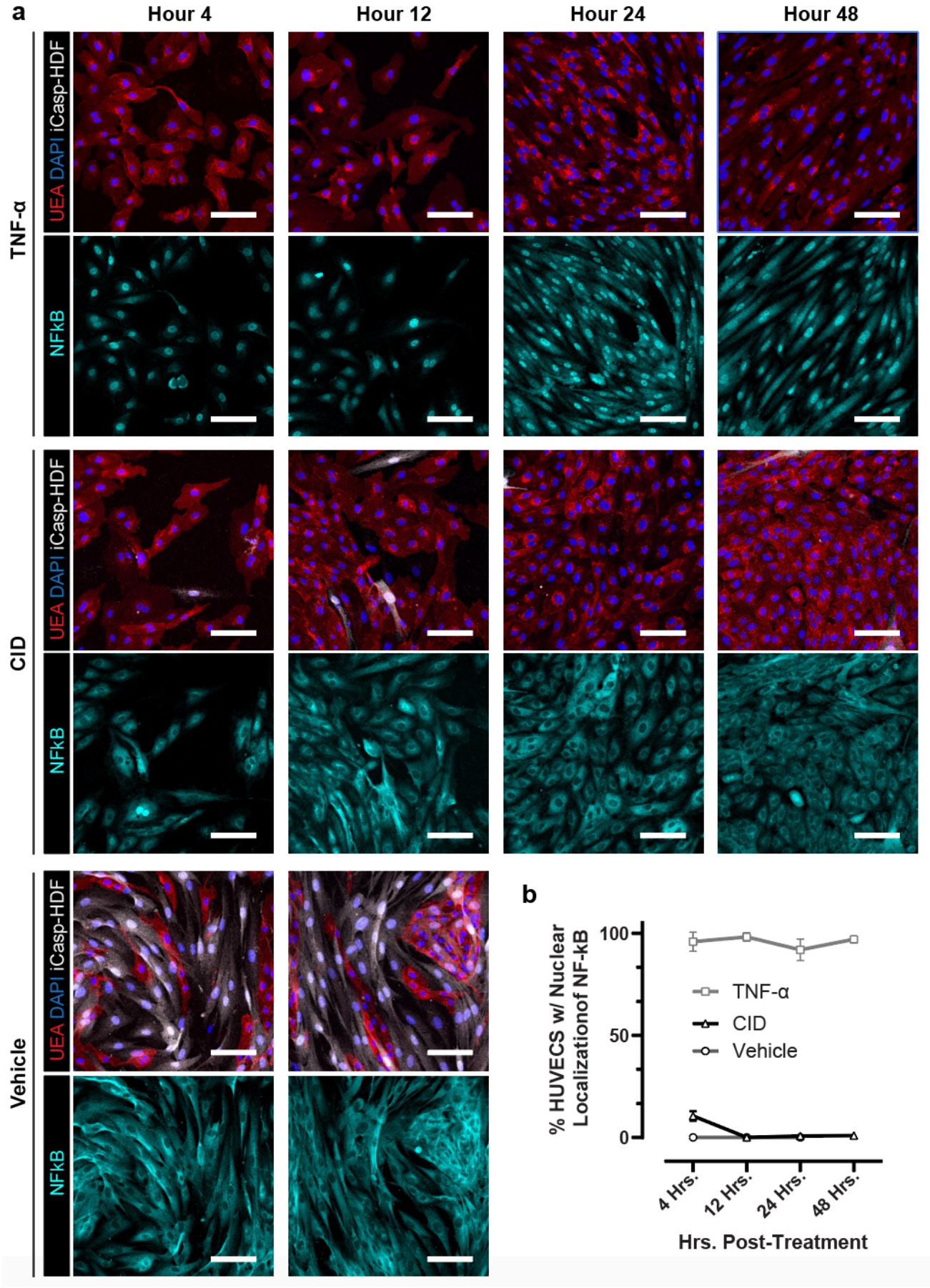
a
Hour 4
Hour 12
Hour 24
Hour 48
TNF-α
CID
Vehicle
UEA DAPI iCasp-HDF
NFkB
b
% HUVECS w/ Nuclear Localizationof NF-kB
100
50
0
TNF-α
CID
Vehicle
4 Hrs.
12 Hrs.
24 Hrs.
48 Hrs.
Hrs. Post-Treatment

Figure S2. Apoptotic bodies of iCasp9-HDFs do not persistently induce NFkB activation in HUVECs. a) Representative images of HUVECs treated with TNF-α (10 ng/mL), HUVEC (UEA, red) and iCasp9-HDF (GFP, white) coculture treated with CID, and coculture treated with vehicle at different time points post-treatment. Samples were stained with NFkB (cyan) to visualize localization. b) quantification of UEA+ HUVECs with nuclear localization of NFkB. Scale bars = 100 μm.

Movie S1. Time-lapse movie of self-assembled HUVEC vessels (LifeAct-Ruby, red) cocultured with iCasp-HDFs (GFP, white). After the 7-day co-culture, the tissues were treated with either vehicle control (left) or CID (right) at the beginning of the image capture.

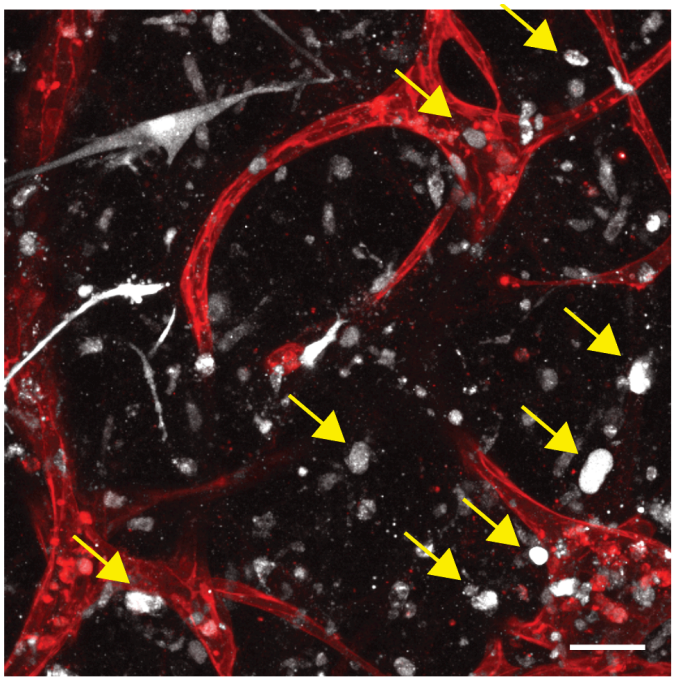

Figure S3. Maximum projection image of device after CID treatment. Apoptotic bodies of iCasp9-HDFs and a small population of non-apoptosed iCasp9-HDFs (GFP, white) remain in coculture matrix with HUVECs (UEA stain, red). Arrows show some of the apoptotic bodies. GFP signal was intentionally brightened to highlight the dimmer, apoptotic bodies. Scale bar = 50 µm.

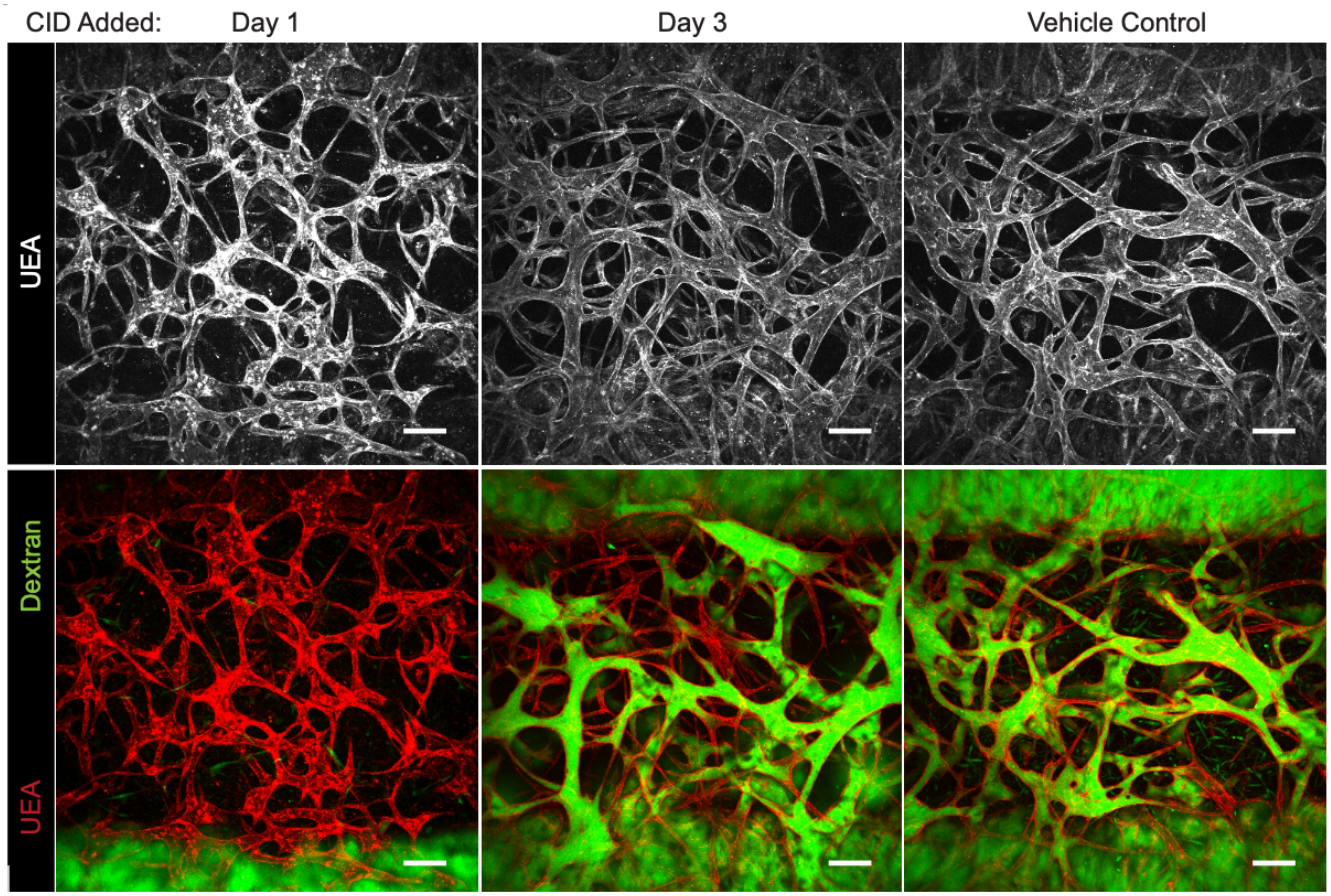

Figure S4. Transient support of human lung fibroblasts (HLFs) is sufficient to drive functional vascular morphogenesis. CID was added to the HUVEC/iCasp9-HLF co-culture devices on day 1 or day 3, and all devices were fixed at day 7. Top row shows UEA-stained HUVECs (white) at day 7. Bottom row shows merged images of UEA-stained HUVECs (red) and FITC-conjugated dextran (green, along with low GFP signal from iCasp9-HLFs). Scale Bars = 150 μm.

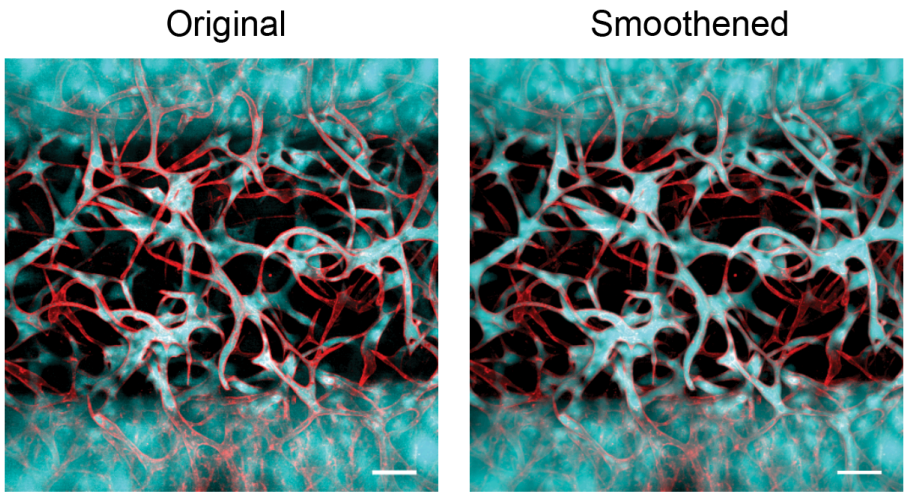

Figure S5. Dextran-perfused (cyan) devices at day 7 without smoothening (left) and after smoothening with a gaussian-filter (right). Scale bars = 150 µm.