

Cell Reports Methods, Volume 2

Supplemental information

Functional microvascularization

of human myocardium *in vitro*

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Supplementary Figures

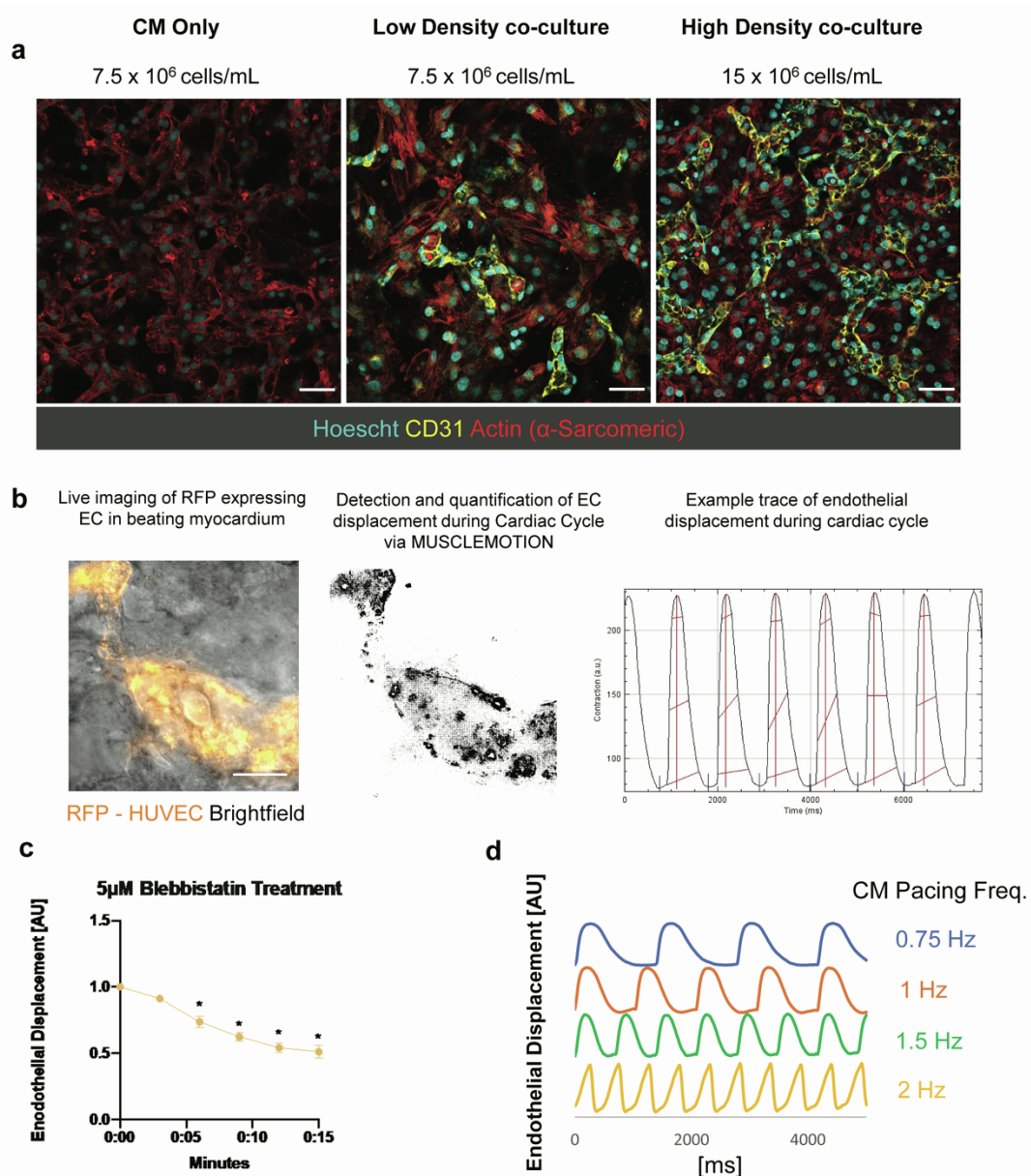


Fig. S1 Fibrin hydrogel culture scaffold enables mechanical coupling between EC and CM **a**, Optimisation of cell density for confluence in 3D fibrin hydrogel. hiPSC-CM (red) and hCMVEC (yellow) were visualised with immunofluorescent confocal microscopy in low and high density co-cultures to establish requisite conditions for confluence in 3D fibrin hydrogel. Scale bar = 50 μm. **b**, Experimental outline of live imaging of RFP-HUVEC to detect and quantify physical displacement of EC by CM contractility. Scale bar = 50 μm. **c**, Reduction of RFP-HUVEC displacement via inhibition of CM contractility via Blebbistatin treatment. Each

datapoint represents average value from 6 dishes, N=3. **d**, EC displacement rate depends on CM electrical simulation frequency. Data is shown as mean \pm SEM. $^* = p < 0.05$. Related to figure 1 and 2 of main text.

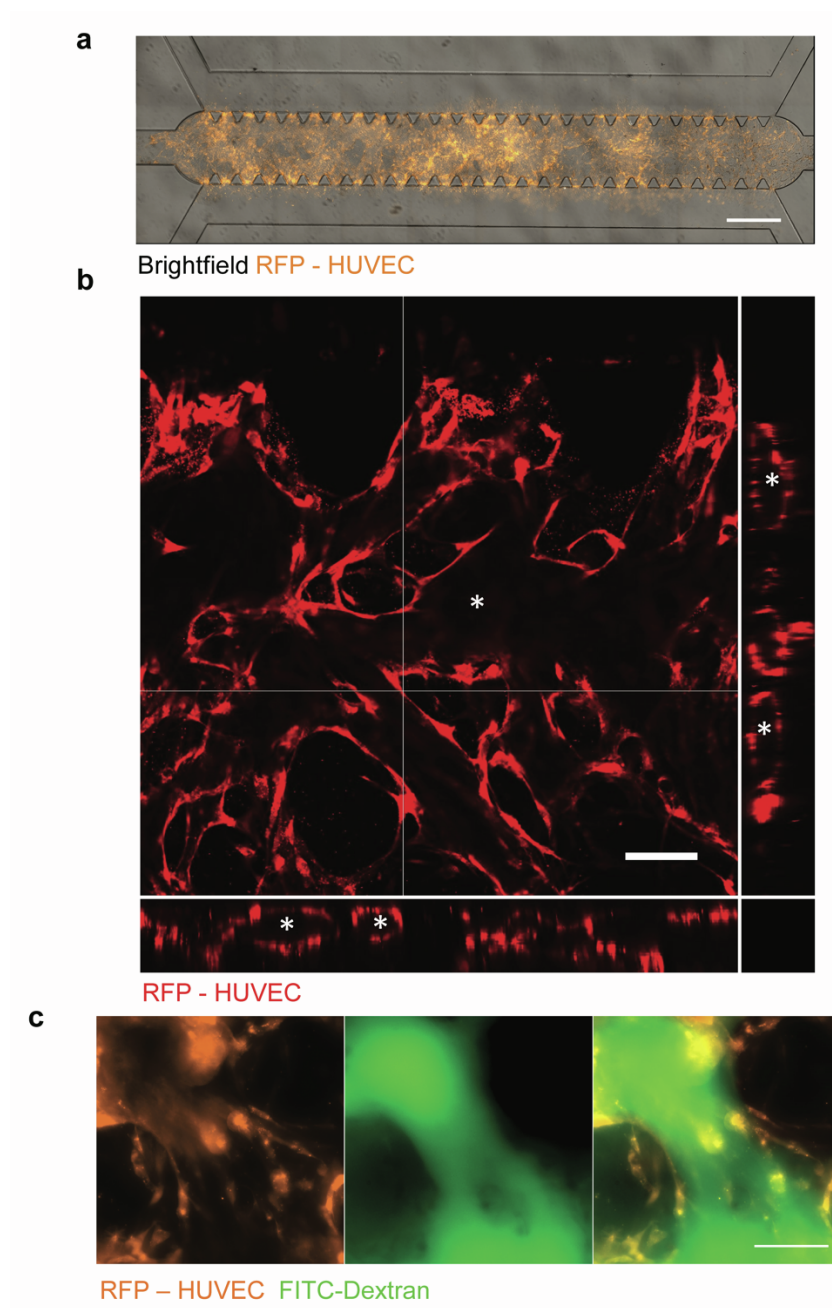


Fig. S2 Organisation of microvascular network in EC-FB microfluidic co-culture

a, Widefield tile showing distribution of RFP-HUVEC in microfluidic chip after 1 week coculture with hLVFB. Scale bar = 1mm **b**, Confocal z-stack showing endothelial network with

continuous open lumen. White asterisks indicate open luminal spaces. Scale bar = 50 μm . **c**,
Perfusion of 40kDa FITC-Dextran through microvasculature in EC-FB co-culture. Scale bar =
50 μm . Related to figures 3 and 4 of main text.