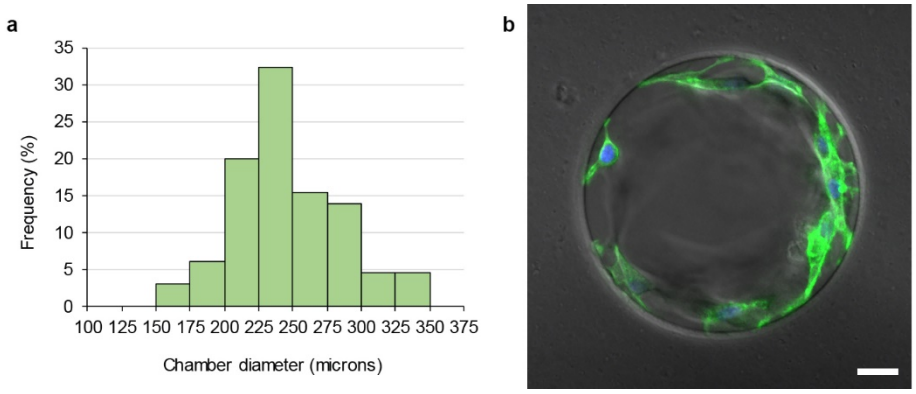
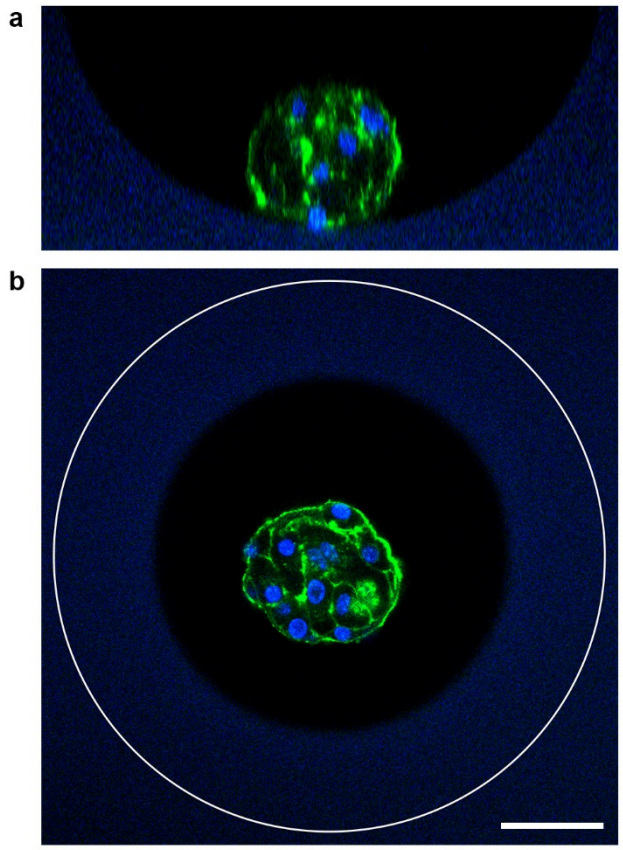
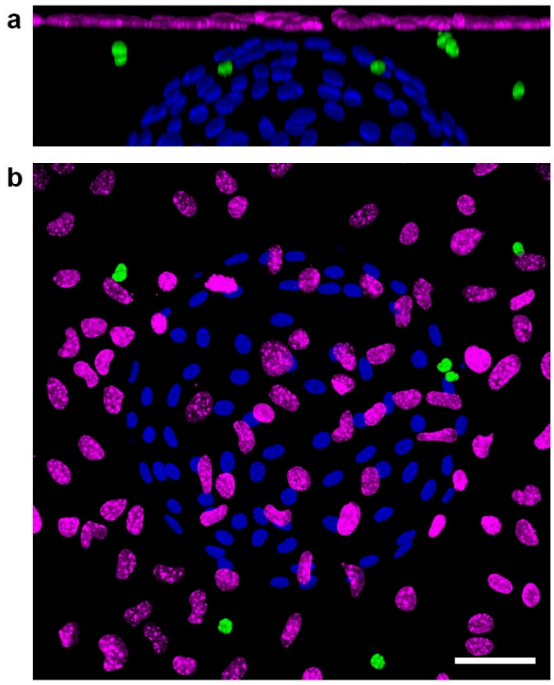
SUPPLEMENTARY INFORMATION



Supplementary Fig. 1 | Poor monolayer formation in small diameter chambers. (a) Histogram showing the distribution of chamber diameters from 65 successfully formed monolayers. The frequency of sub-200 μm monolayers is low compared to the frequency of sub-200 μm beads in the initial mixture (Figure 2b). **(b)** mT/nG **c**ells in an ~135 μm diameter chamber attached at multiple points rather than growing conformally along the chamber's inner surface. Scale bar: 20 μm.



Supplementary Fig. 2 | Solid spheroid formation in non-adhesive microchambers. (**a)** Side view and (**b)** top view of an LEC aggregate at the bottom of a chamber stained with DAPI (blue) and phalloidin (green). Blue background signal shows the surrounding gel. White circle indicates the size of the chamber. Cells aggregated into solid spheroids in the absence of adhesive cues provided by Matrigel. Scale bar: 50 μm.



Supplementary Fig. 3 | Co-culture-like conditions. 3D-rendered **(a)** side view and **(b)** top view of LEC nuclei labelled with DAPI in three compartments: a monolayer on the surface of the hydrogel (magenta), individual cells dispersed within the hydrogel (green), and cells lining a microchamber in the hydrogel (blue). Scale bar: 50 μm.