

## **Additional details on methods**

### **Image selection:**

For all 3D conditions (ULA, agarose, rigid scaffold), quantitative morphometrics (equivalent diameter, area, perimeter, circularity, solidity) were extracted only from fully contained objects that met pre-specified quality criteria; edge-touching or truncated objects were excluded from size analyses.

Acquisition and calibration. Bright-field images were acquired under harmonised settings (identical objective/magnification, illumination/exposure/gain, pixel size, and display range). Spatial calibration was applied uniformly from the microscope scale bar, enabling size computation in micrometres.

### **inclusion/exclusion (applies to all methods).**

Include only objects with a closed, continuous boundary fully contained within the frame (no edge contact), adequate focus for boundary detection ( $\geq 3$  adjacent z-planes for scaffold), and meeting our a priori morphometric thresholds (circularity  $\geq 0.85$ ; solidity  $\geq 0.9$ ; minimum equivalent diameter  $\geq 50 \mu\text{m}$  for size analyses).

Exclude edge-touching/truncated objects, overlapping/merging aggregates, tethered fragments, or out-of-focus candidates.

**Scaffold-specific handling:** On the rigid scaffold, imaging was performed in situ to avoid mechanical disturbance. Because pores/struts can create bright contours, a candidate object was accepted only if a cellular boundary could be traced independently of the strut edge and remained stable across z-planes. Representative figure panels show cropped views for readability; all measurements were taken from the corresponding full-field frames acquired at the same session.

**Longitudinal panels and mobile aggregates.** For the longitudinal examples (day 4, 5 and 6), the same well/field was followed at each time point. Apparent xy shifts of circled objects

reflect free movement in U-bottom wells and gentle medium exchange, not a change in the object images. Only objects that remained fully contained at a given time point were measured at that time; if an object became edge-touching later, it was excluded for that time point (and the reduced number of replicates is reflected in the legend/Methods) and was not included in the presented images; however, it will be reported

**Fluorescence images:** Fluorescent panels were used qualitatively (e.g., Hoechst nuclear distribution) and were not used for size quantitation. All quantitative size metrics derive from the harmonised bright-field workflow described above.