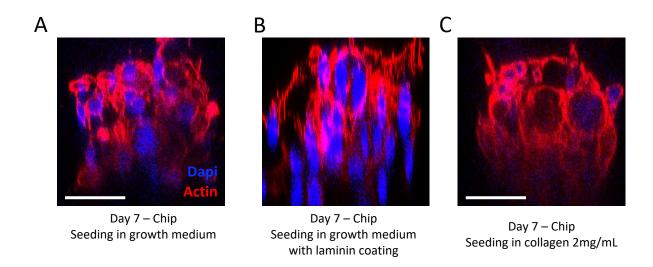
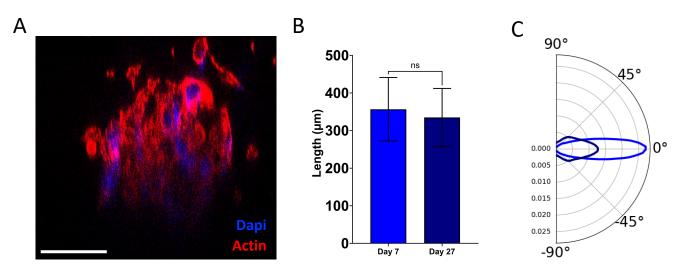
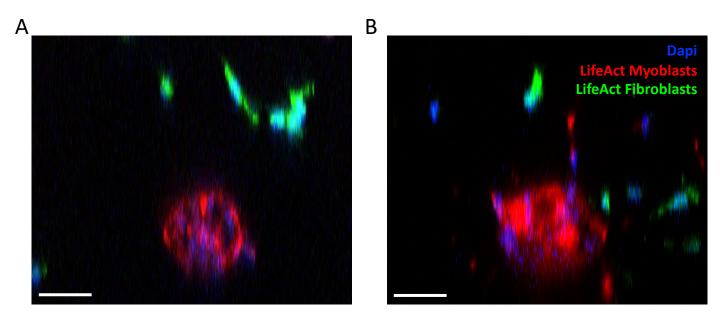
Electronic Supplementary Material (ESI) for Lab on a Chip. This journal is © The Royal Society of Chemistry 2024



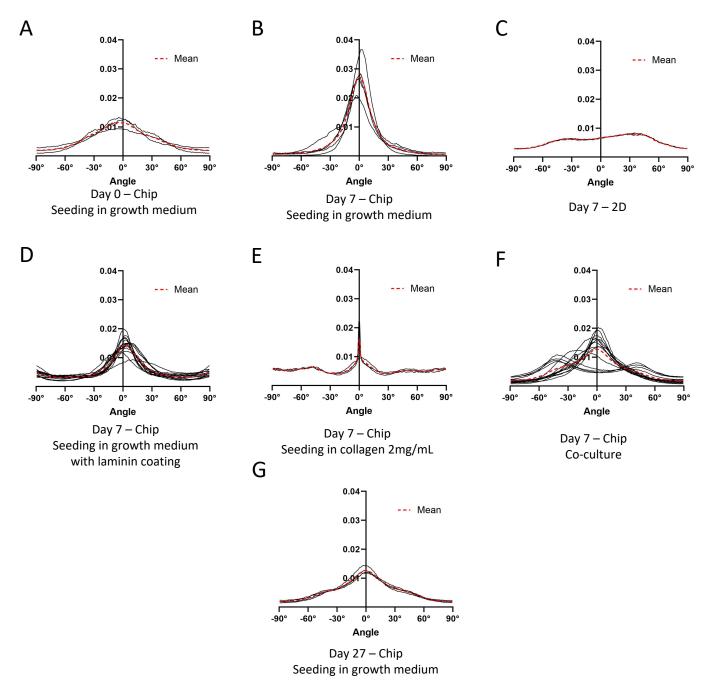
**Supplementary Figure 1.** Cross-sections of tissues cultured for 3 days in proliferation and 7 days in differentiation medium after being seeded (A) in growth medium, (B) in growth medium with laminin coating and (C) in collagen 2mg/mL (Scale bar=  $50\mu$ m, nuclei in blue and actin in red)



**Supplementary Figure 2.** (A) Cross-section of a tissue cultured for 3 days in proliferation and 27 days in differentiation (Scale bar=  $50\mu m$ , nuclei in blue and actin in red), (B) average length of the myotubes and (C) actin orientation distribution at days 7 and 27



**Supplementary Figure 3.** Cross-sections of tissues co-cultured with fibroblasts for (A) 3 days in proliferation, and (B) 3 days in proliferation and 27 days in differentiation (Scale bar=  $50\mu m$ , nuclei in blue, LifeAct myoblasts in red and Lifeact fibroblasts in green)



**Supplementary Figure 4.** Raw data plots of the actin orientation distribution and the corresponding mean curve for (A) 3D tissues at day 0 and (B) day 7 of differentation seeded in growth medium, (C) 2D tissues at day 7, (D) 3D tissues at day 7 seeded in growth medium with laminin coating, (E) 3D tissues at day 7 seeded in collagen 2mg/mL, (F) 3D tissues at day 7 co-cultured with fibroblasts and (G) 3D tissues at 27 day seeded in growth medium