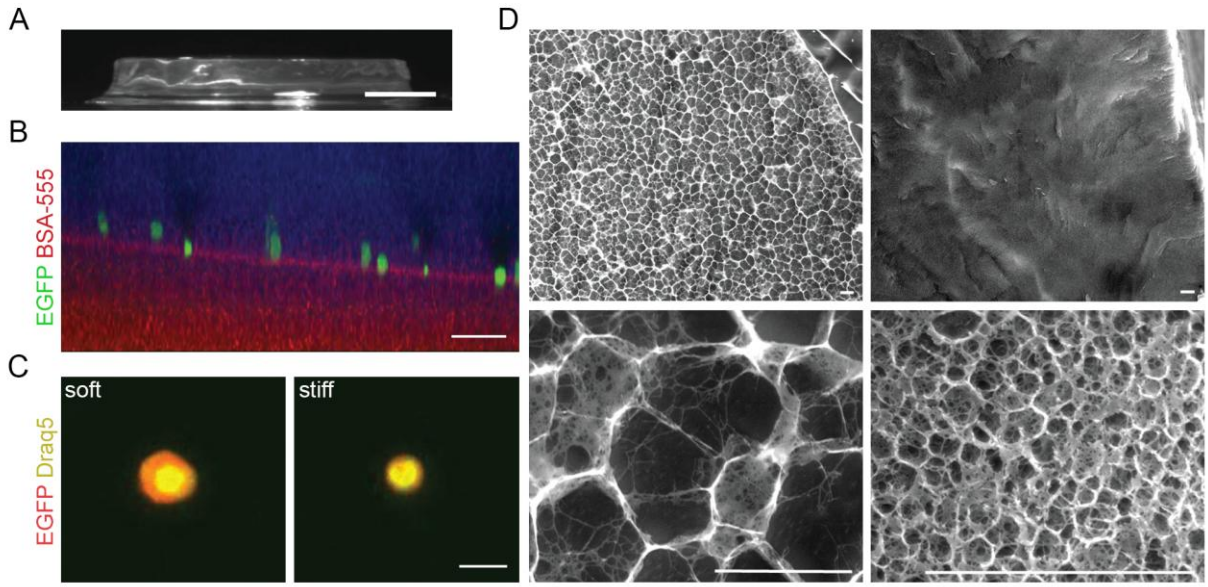
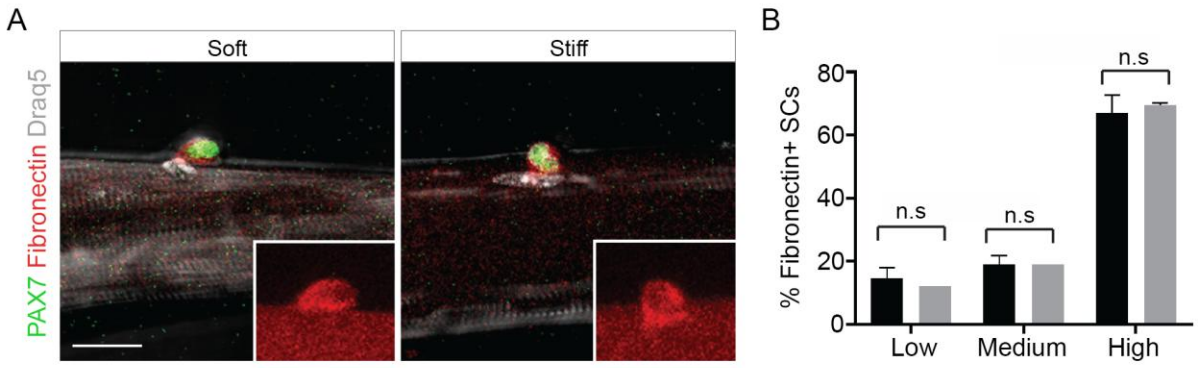
Supplemental Materials

Molecular Biology of the Cell

Moyle et al.



Supplemental Figure S1. Characterization of the 3D artificial niche culture platform. (A) Representative photograph showing side profile of an agarose gel. Scale bar, 2.5 mm. **(B)** Confocal image of green fluorescent protein $(GFP)^+$ myoblasts in 3D culture between the interface of two agarose gels pre-mixed with bovine serum albumin-conjugate with AlexaFluor 546 (bottom) and AlexaFluor 647 (top). Scale bar, 50 µm. **(C)** Confocal images of $EGFP^+$ myoblasts (red) fixed and counterstained with the nuclear stain Draq5 (yellow) 24 hours post-embedding in soft (left panel) or stiff (right panel) agarose gels. Scale bar, 20 µm. **(D)** Cryo-field emission scanning electron microscope images of 0.5% (left panels) compared to 3% weight per volume agarose hydrogel (right panels) at low (top) and high (bottom) magnifications. Scale bars, 5 µm.



Supplemental Figure S2. Three-dimensional niche stiffness does not alter satellite cell fibronectin production. (A, B) Single fibers embedded within soft or stiff 3D agarose hydrogels for 24 hours and fixed 36 hours post-isolation. **(A)** Representative confocal images immunostained for Pax7 (green) and Fibronectin (red). Scale bar, 50 µm. **(B)** Bar graph of the relative expression of fibronectin expressed by SCs embedded within soft (black) and stiff (grey) 3D niches. N = 2 animals/ condition, with at least 5 fibers analyzed per animal.