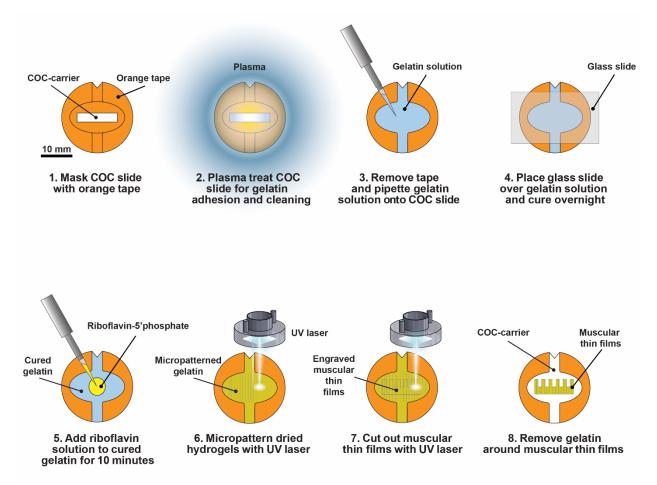
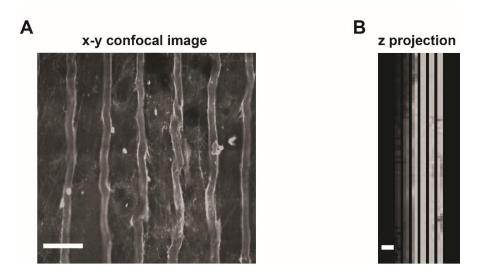
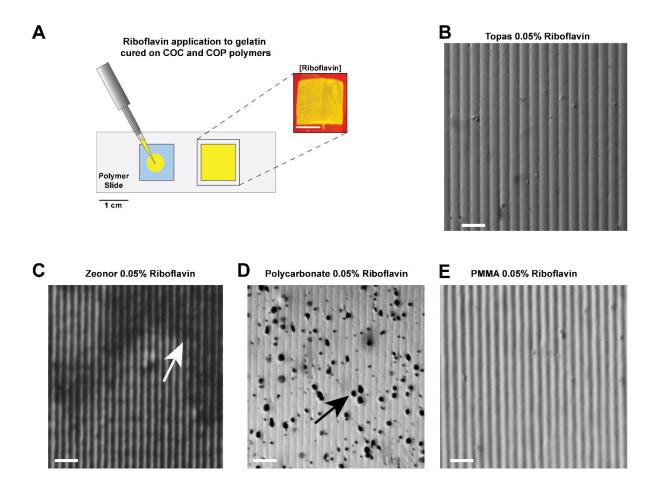
## **Supplemental Information:**



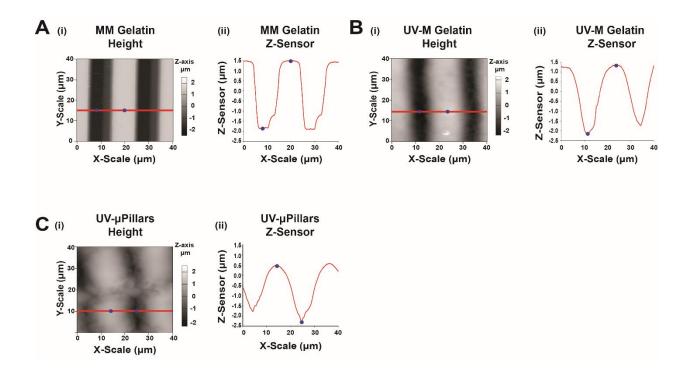
**Supplemental Fig. 1. Fabrication of muscular thin films with UV laser micropatterning.** Schematic of the entire fabrication process for engineering UV-M hydrogels. 1. COC slides were cut and masked with orange tape (3M) using a UV laser engraver. 2. An adhesion window was peeled and plasma treated for cleaning and gelatin attachment. 3. Tape was peeled in the center of the COC slide and a 10% (w/v) gelatin-4% (w/v) microbial transglutaminase solution was added to the surface. 4. A glass slide was placed over the top of the gelatin and gels were cured overnight (12 hours). 5. After surface hydration and removal of the glass slide, 0.05% (w/v) riboflavin-5'phosphate solution was added to the surface of the gelatin. 6. Gelatin hydrogels were dried and UV-micropatterned with a UV laser. 7. MTFs were cut out with the UV laser engraver. 7. Excess gelatin was removed to reveal the thin film cantilevers. Thin film cantilevers were prepared for cell seeding according to the methods section.



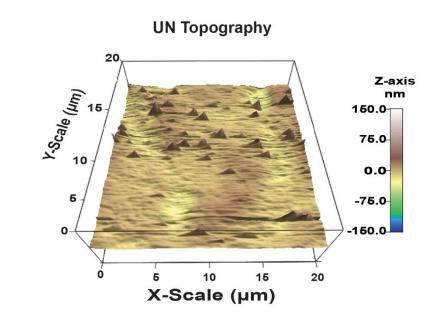
Supplemental Fig. 2. Fibronectin-functionalized gelatin for human iPSC attachment. (A) Confocal image of micromolded gelatin hydrogel surface with 20  $\mu$ m wide lanes separated by 4  $\mu$ m wide ridges covalently functionalized with fibronectin. Grayscale signal: human fibronectin; scale = 25  $\mu$ m. (B) Cross-section of gelatin surface reconstructed from confocal image planes. Grayscale signal: human fibronectin; scale = 2  $\mu$ m, left to right: bottom to surface of the gelatin



Supplemental Fig. 3. The effect of plastic carrier and riboflavin concentration on UV laser micropatterning. (A) Schematic of riboflavin application to cured gelatin to fabricate UV laser micropatterns on polymer carriers. Inset: Image of riboflavin solution added to cured gelatin. Scale bar is 7.5 mm. All polymers were incubated with 0.05% riboflavin (w/v) solution for 10 minutes, washed, dried and then patterned with a UV laser (power=0.16 W, frequency=50 kHz, speed= 80 mm/second. (B) UV-M gelatin on Topas polymer carrier. Scale bar is 50  $\mu$ m. (C) UV-M gelatin on Zeonor polymer carrier. Arrow demarks burned zone. Scale bar is 50  $\mu$ m. (D) UV-M gelatin on Polycarbonate polymer carrier. Arrow demarks burn mark. Scale bar is 50  $\mu$ m. (D) UV-M gelatin on PMMA carrier. Scale bar is 50  $\mu$ m.



Supplemental Fig. 4. Contact mode atomic force microscopy of molded and UV micropatterned hydrogel height. (A-C) Atomic force microscopy topography images of (A) MM, (B) UV-M, and (C) UV- $\mu$ P gelatin with corresponding step-height profiles (i and ii) displayed by the red lines and the height change between locations indicated by blue dots. Refer to Fig 3 for 3D topography and Z-sensor height differences.



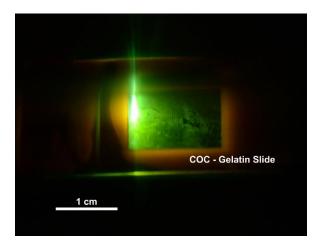
Supplemental Fig 5. Atomic force microscopy of unpatterned gelatin. Atomic force microscopy topography of UN gelatin in liquid contact on a 20  $\mu$ m<sup>2</sup> area and Z-axis range of 300 nm.

UV Laser Micropatterned Gelatin 5 Days	Pacing Rate (Hz)	Diastolic Stress (kPa)	Systolic Stress (kPa)	Twitch Stress (kPa)	N =
	0	$24.7\pm0.6$	$26.5\pm0.7$	$1.8 \pm 0.3$	12 films, 6 chips
	1	$23.4\pm0.8$	$26.5 \pm 1.0$	$3.1 \pm 0.4$	13 films, 6 chips
	2	$24.1\pm0.7$	27.3 ± 1.2	$3.3\pm0.5$	9 films, 5 chips
UV Laser Micropatterned Gelatin 27 Days	1	$12.6 \pm 0.6*$	17.7 ± 0.8 *	$5.1\pm0.6$	3 films, 1 chip
	2	$21.8\pm0.01^{\#}$	$23.2 \pm 0.1^{\#}$	$1.5 \pm 0.2^{\#}$	2 films, 1 chip

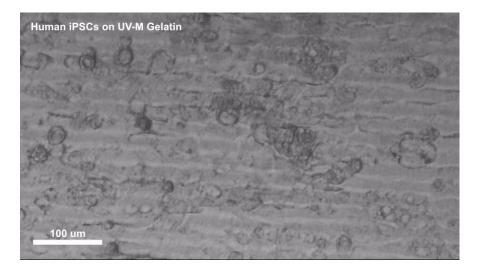
Supplemental Table 1: Muscular thin film contractile stress

\*P<0.05 vs UV laser micropatterned gels at 5 days, same pacing rate  $^{\#}P$  <0.05 vs. 1 Hz pacing at 27 days

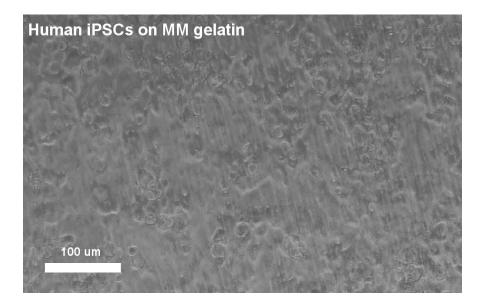
Supplemental Movie 1: UV laser engraving of gelatin hydrogels



Supplemental Movie 2: Human iPSCs cultured on UV-M hydrogels



Supplemental Movie 3: Human iPSCs cultured on MM hydrogels



Supplemental Movie 4: Contractile response of UV-M muscular thin films

