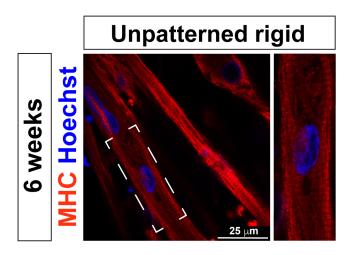
SUPPLEMENTARY INFORMATION

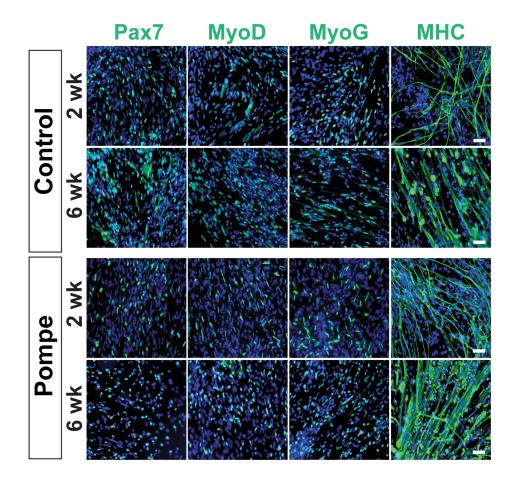
Micropatterned substrates with physiological stiffness promote cell maturation and Pompe disease phenotype in human induced pluripotent stem cell-derived skeletal myocytes

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Supplementary Figure 1. Striated patterns in the myotubes cultured on unpatterned rigid substrates at 6 weeks.



Supplementary Figure 2. Skeletal muscle differentiation using Pompe iPSC-derived myogenic progenitors plated on unpatterned rigid substrates. Representative images of immunocytochemistry for myogenic markers Pax7, MyoD, myogenin (MyoG) and myosin heavy chain (MHC) in the differentiated myocytes prepared from control and Pompe iPSCs. Scale bar = $50 \mu m$.

Supplemental Video information

Supplementary Video. 1A, B, C, D, E, F. Spontaneous contraction of iPSC-derived myotubes on micropatterned soft substrate. Sequential videos of spontaneous contraction of control iPSC-derived myotubes at 4 weeks of differentiation (A and B) and at 6 weeks of differentiation (C-E). Weak contractions were first observed at ~ 4 weeks of differentiation on both conditions. Myotubes were aligned and contracted in any direction on unpatterned rigid substrate (A and C); whereas bundle-like structure myotubes were formed and contracted in one direction along with the lanes of soft substrates (B, D, and E). Myotubes formed from the Pompe disease iPSC line also featured contractions aligned with the direction of the micropattern, although the frequency and strength of the contractions were weaker than the control line (F).

Supplementary Video. 2A, B, C, D. Myogenic progenitor migration and myotube fusion on micropatterned soft substrate. Time-lapse videos were taken for 2-day intervals starting from the first day of plating (A and B) as well as day 10 post-plating (C and D). The micropatterns allow for clear tracking of myogenic progenitor migration and early fusion events. Myotube elongation can be clearly noted in the cells on day 10 post-plating.

Supplemental Videos can be viewed and downloaded at the following box folder:

https://uwmadison.box.com/s/gvkwqiyz1m9x01zn7fwwbs8czaqlzr8e