Supplementary Information

Cell culture media.

Table S1. Cell culture media used in the study

Expansion medium for myogenic progenitors derived from hESCs, hiPSCs, and mESCs	Iscove's Modified Dulbecco's Medium (IMDM) containing 2 mM glutamax (Invitrogen) and supplemented with 15% fetal bovine serum (Atlanta Biological), 10% horse serum (Gibco), 1% chicken embryo extract (US Biological), 50 µg/ml ascorbic acid (Sigma-Aldrich), 4.5 mM monothioglycerol (MP Biomedicals), 50 U/ml Penicillin/Streptomycin (Invitrogen), 5 ng/ml bFGF (R&D Systems), and 0.75 µg/ml doxycycline (Sigma-Aldrich).
Expansion medium for primary human skeletal myoblasts	Hyclone Ham's/F-10 medium supplemented with 20% fetal bovine serum (Atlanta Biological), 2 ng/ml bFGF (R&D Systems), 40 ng/ml Dexamethasone (Cayman Chem), 1.15 µM β-Mercaptoethanol (Life technologies) and 50 U/ml Penicillin/Streptomycin (Invitrogen)
Myogenic differentiation medium for primary human skeletal myoblasts and myogenic progenitors	Knockout DMEM (Invitrogen) supplemented with 20% knockout serum replacement (Invitrogen), 1% MEM non-essential amino acids solution (Invitrogen), and 50 U/ml penicillin/streptomycin (Invitrogen).

derived from hESCs, hiPSCs, and mESCs	
Expansion medium for C2C12 cells	High glucose DMEM (Invitrogen) supplemented with 2 mM glutamax (Invitrogen), 20% fetal bovine serum (Atlanta Biological), and 50 U/ml penicillin/streptomycin (Invitrogen).
Myogenic differentiation medium for C2C12 cells	High glucose DMEM (Invitrogen) supplemented with 2% horse serum (Gibco) and 50 U/ml penicillin/streptomycin (Invitrogen)
Growth medium for 3T3 fibroblasts	High glucose DMEM (Invitrogen) supplemented with 10% fetal bovine serum (Atlanta Biological) and 50 U/ml penicillin/streptomycin (Invitrogen)
Growth medium for HUVECs	EGM-2 BulletKit (Lonza)

Characterization of DMD hiPSC-derived myogenic progenitors carrying the Δex31 mutation.

The human iPS cell line DMD Δex31 was generated at the Pluripotent Stem Cell Facility, (Cincinnati Children's Hospital Medical Center) using non-integrating episomal plasmids. After isolation and expansion, single clones were characterized using different approaches to ensure their pluripotent nature.

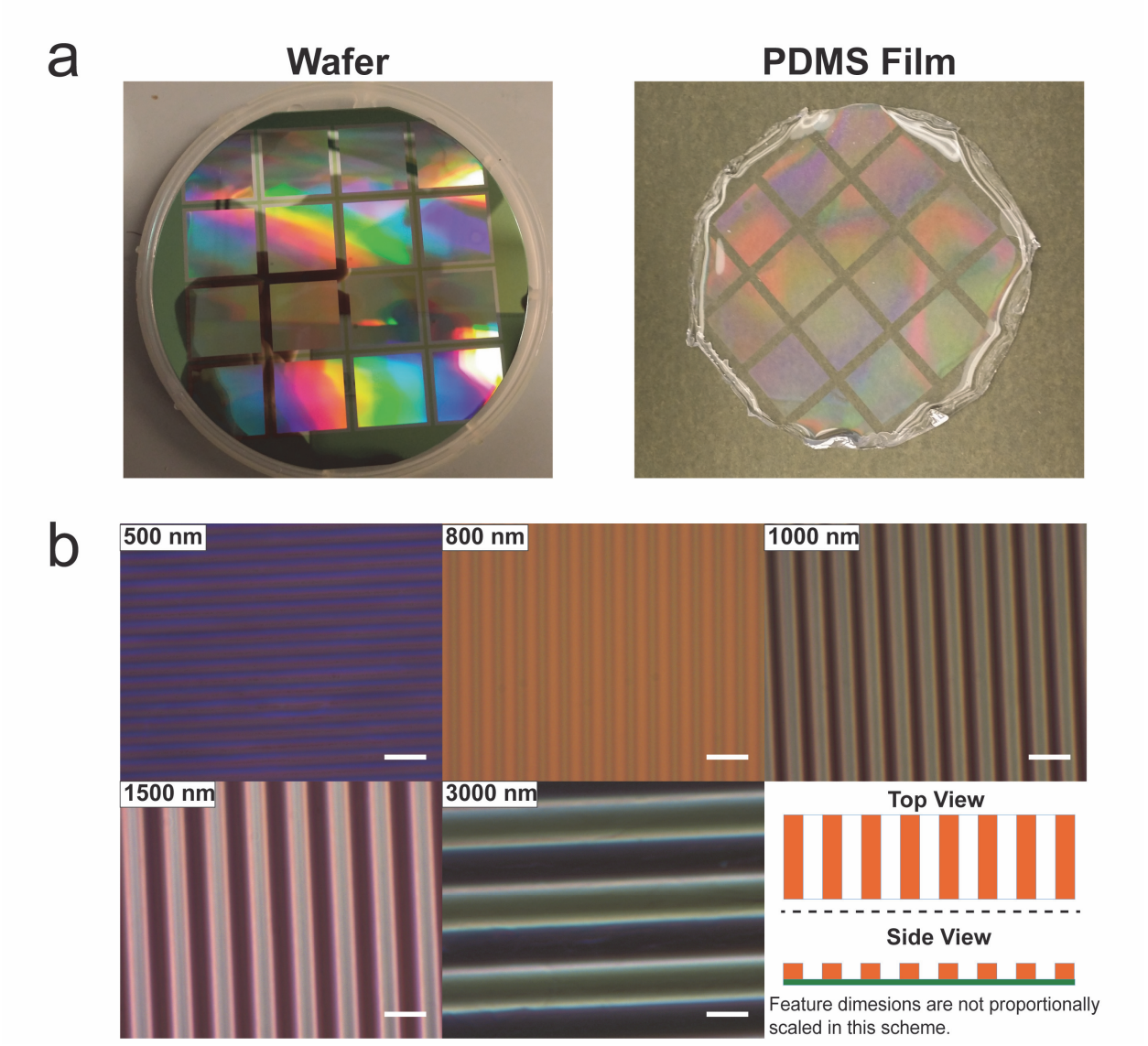
Immunostaining. Human iPS cells growing in 24well plates were fixed with 4% paraformaldehyde/PBS solution for 20 minutes at +4°C, permeabilized with 0.3%Triton X-100/PBS for 15 min at RT, blocked with 3%BSA/PBS for 1 hour at RT and incubated with the primary antibody diluted in 3%BSA/PBS over-night at +4°C. Following 3 washes with PBS, cells were blocked with 3%BSA/PBS for 1 hour at RT and incubated with the secondary antibody + DAPI (for nuclei staining) diluted in 3%BSA/PBS for 1 hour at RT. Following 3 washes with PBS, cells were imaged using an inverted fluorescent microscope (Zeiss). The following antibodies were used in this study:

OCT3-4 - Santa Cruz Biotech. sc-5279
NANOG - Santa Cruz Biotech. sc-374103
SOX2 - Santa Cruz Biotech. sc-17320
SSEA4 - Santa Cruz Biotech. sc-21704.
Alexa-555 goat anti-mouse and Alexa-555 goat anti-rabbit - ThermoFisher.

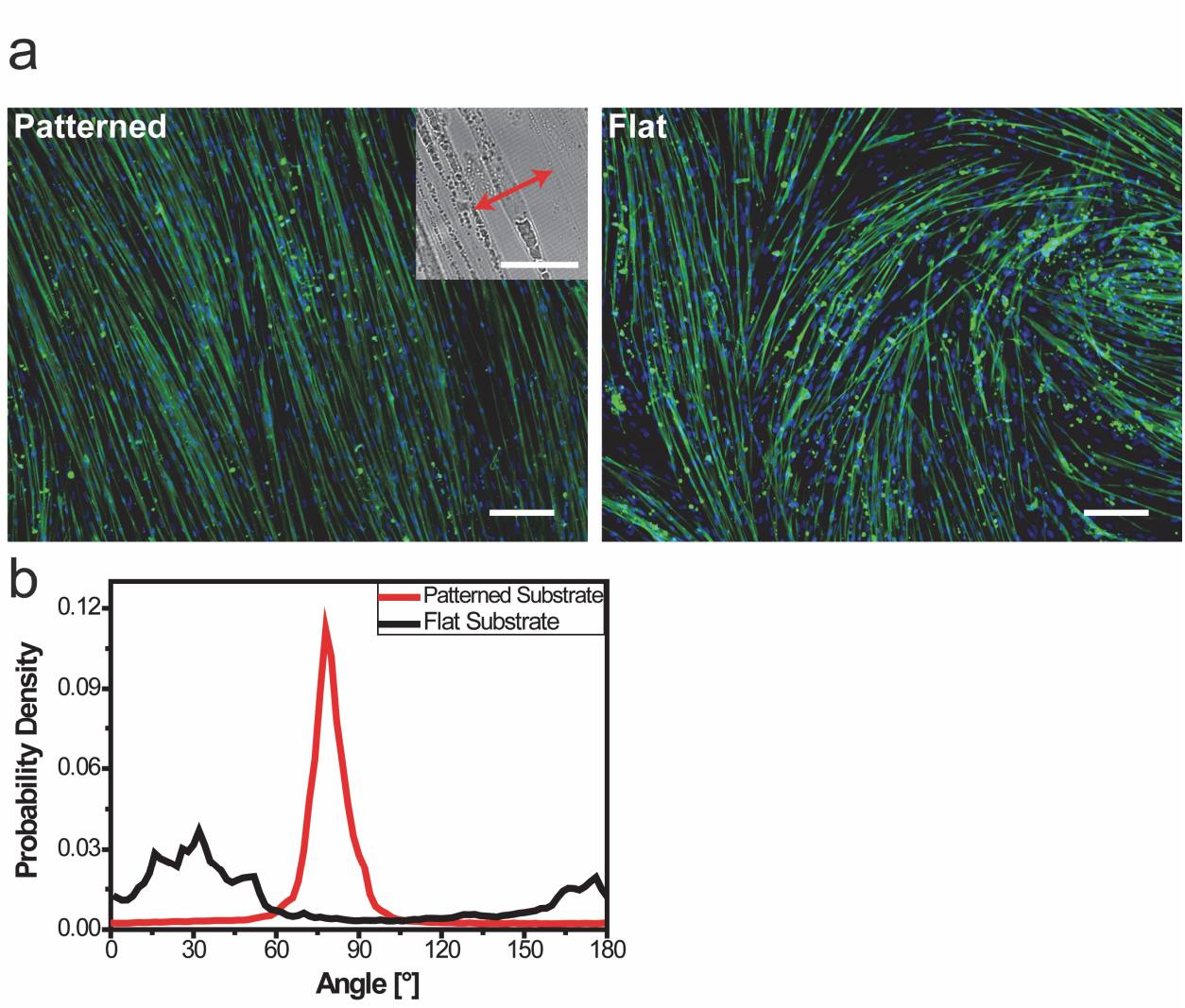
Alkaline phosphatase activity. Human iPS cells growing in 24well plates were assessed for Alkaline Phosphatase activity using the AP kit (Thermo Scientific – TA-060-AL) following the manufacturer's instructions. Before imaging, nuclei were stained with DAPI. Pictures were acquired using an inverted fluorescent microscope (Zeiss).

Karyotype. Cells were subjected to cytogenetic analysis to exclude major abnormalities arising from the clonal selection. The cytogenetic analysis was performed in the Cytogenomics Shared Resource at the University of Minnesota.

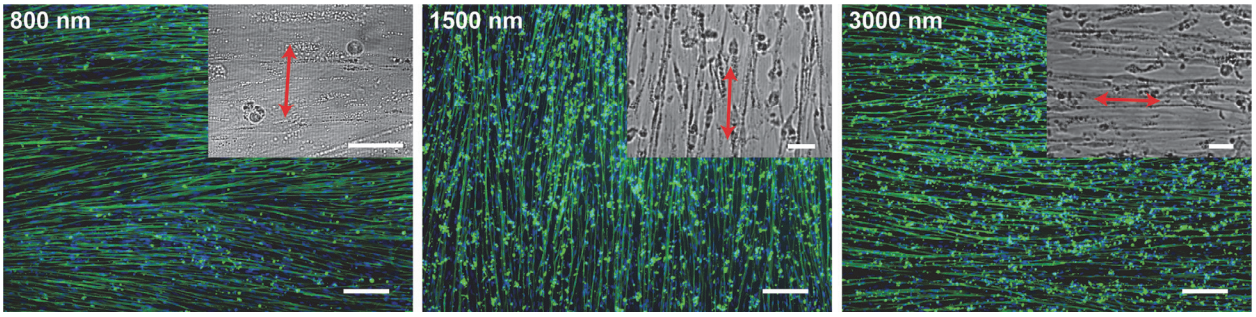
Teratoma assay. DMD Δex31 human iPS cell were harvested using Accumax and, after rinsing the cell pellet with PBS, 10^6 cells were resuspended in 40µl of a 1:1 solution of Matrigel and DMEM-F12. Cells were then injected in the Quadriceps of 6-8 weeks old NOD-scid IL2Rg^{null} mouse (NSG - Jackson lab). After approximately 1-2 months, teratoma was harvested, fixed and embedded for histological analysis. Cryosections were subjected to Hematoxylin-Eosin staining and imaged on an up-right microscope (Zeiss).



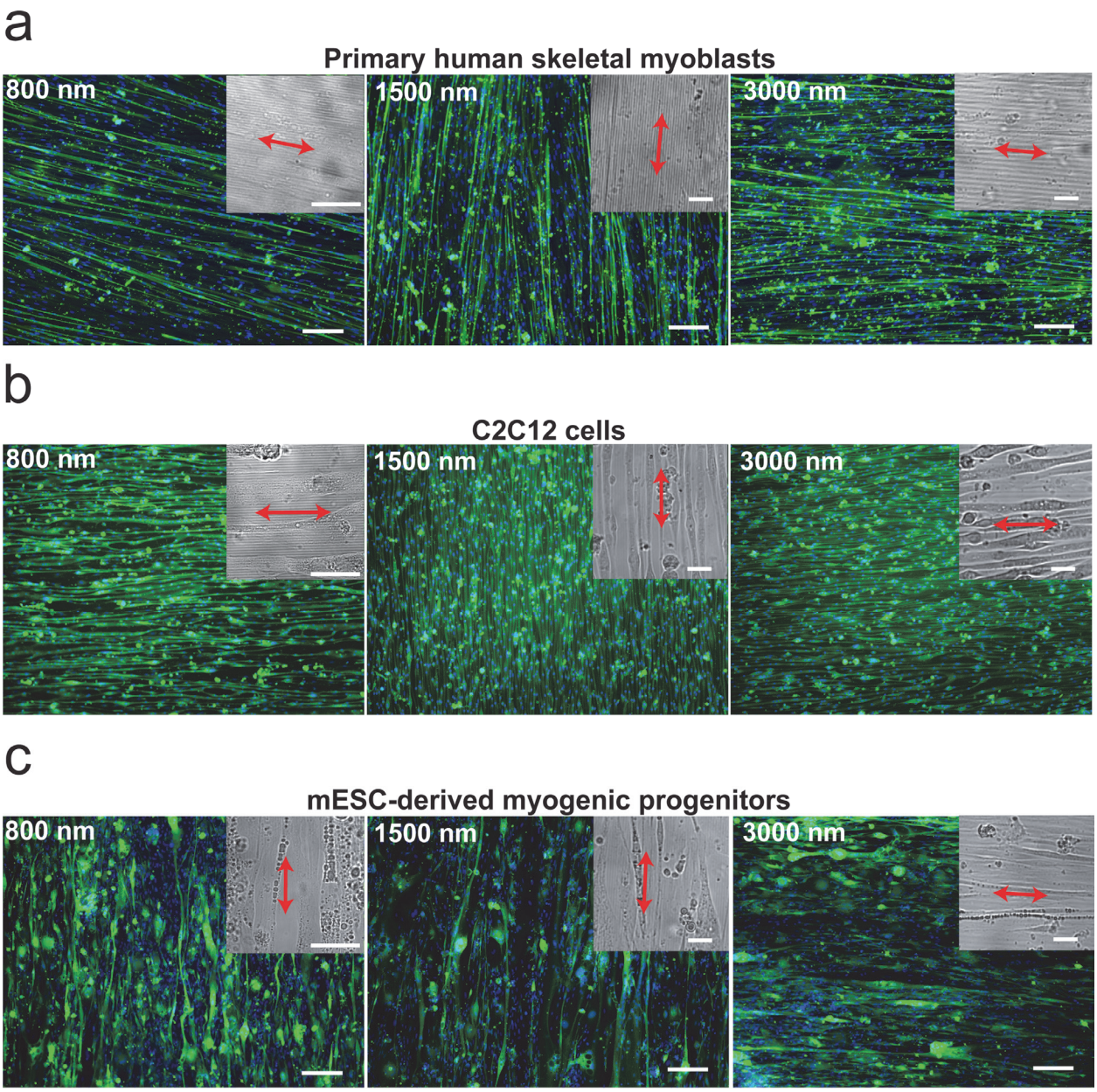
Supplementary Figure 1. Fabrication of topographically patterned culture substrates. (a) Each pattern was fabricated on a silicon wafer and transferred to PDMS films through soft lithography. Topographically patterned regions show iridescent colors. (b) Wafer templates patterned with parallel grooves of various feature sizes (groove and ridge widths) were confirmed by optical microscopy (Scale bar = 3 μm).



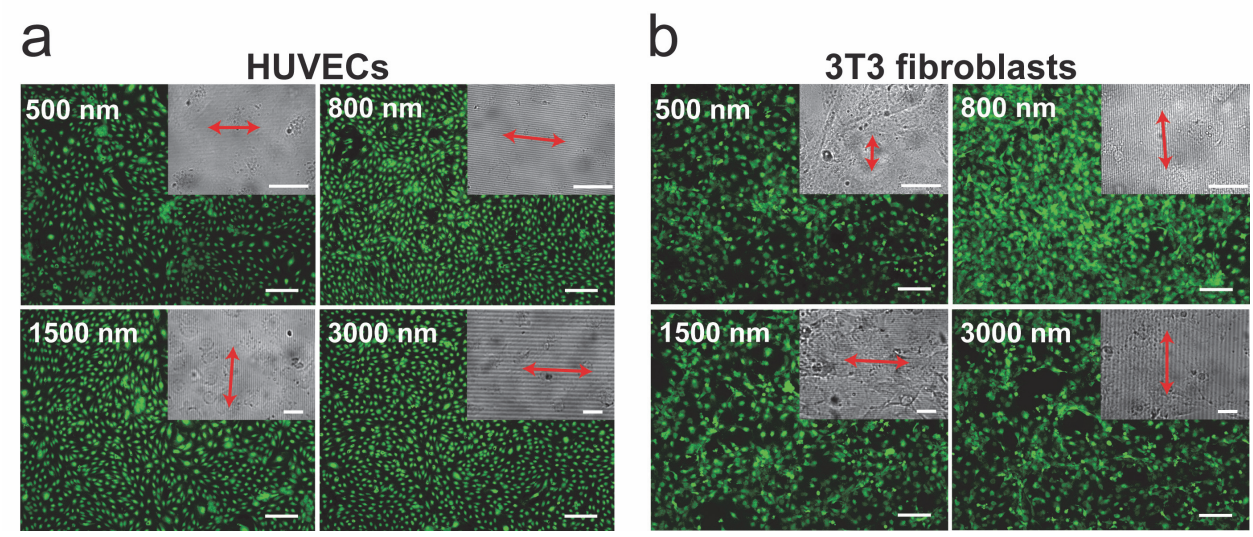
Supplementary Figure 2. Alignment and orientation of myotubes differentiated from hESC-derived myogenic progenitors on Matrigel-functionalized, 800-nm grooved substrates after 4 weeks of culture. (a) Myotubes cultured on nanogrooved substrates aligned in a consistent direction throughout each entire substrate, while those cultured on flat control substrates oriented randomly. Cells were immunofluorescently stained for sarcomeric α-actinin (green) and nuclei were counter-stained with Hoechst 33342. Scale bar, 200 μm. Inset: the nanogroove direction (arrowed red line) was determined via bright-field imaging, revealing nearly perpendicular orientation of the aligned myotubes relative to nanogrooves. Inset scale bar, 30 μm. (b) The p.d.f of myotube orientations has a single, narrow, and high peak near 90° for cells cultured on the nanogrooved substrates, but not for cells cultured on flat control substrates.



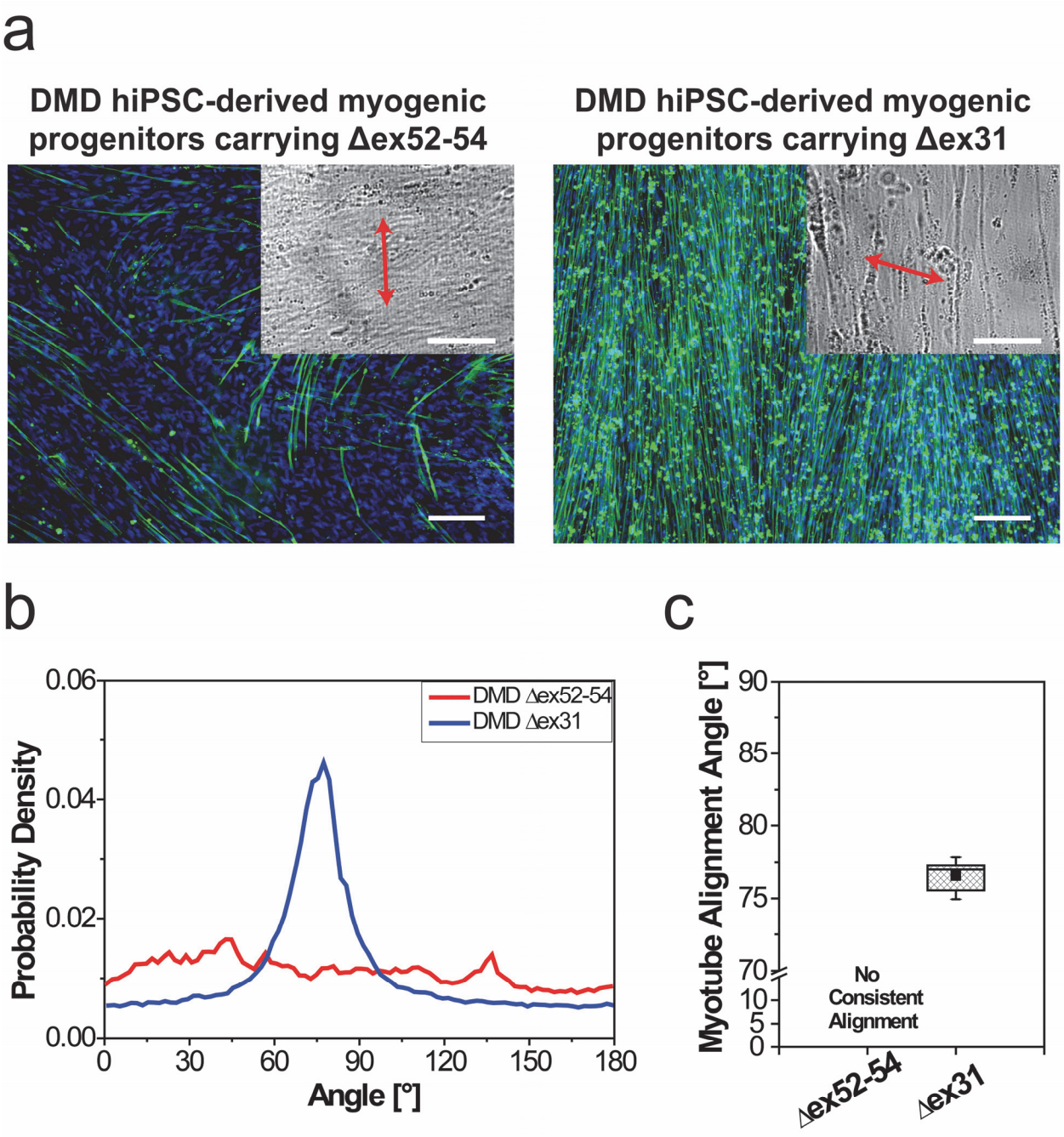
Supplementary Figure 3. Myotubes differentiated from non-diseased hiPSC-derived myogenic progenitors aligned nearly perpendicular to 800-nm wide grooves but parallel to 1500-nm or 3000-nm wide grooves on Matrigel-functionalized substrates. Images are shown as in Supplementary Fig. 2a. Scale bar, 200 μm; inset scale bar, 30 μm.



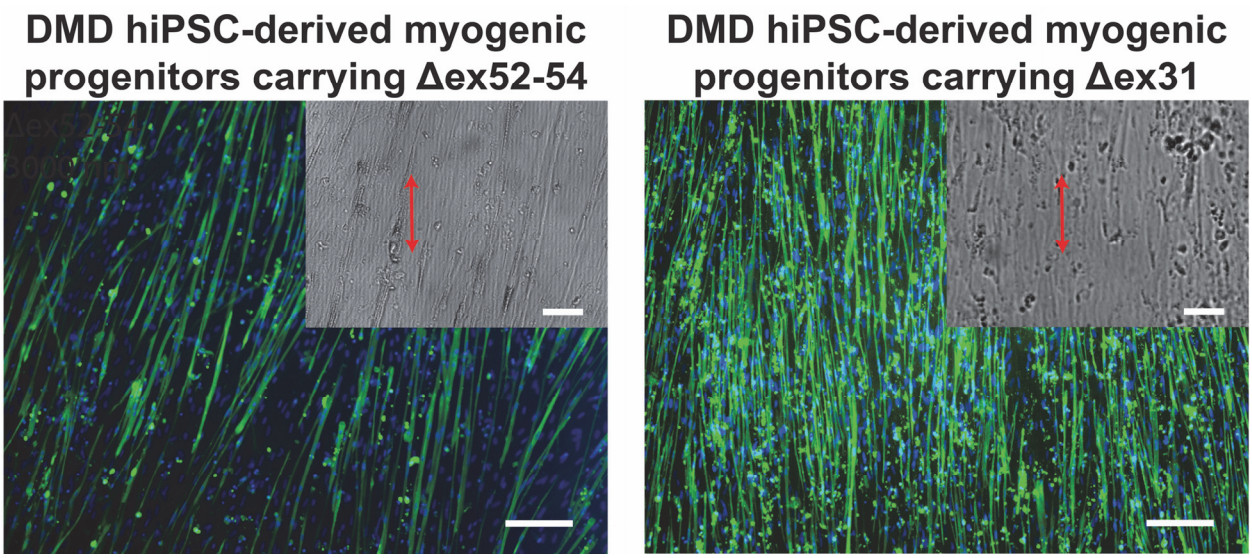
Supplementary Figure 4. Myotubes differentiated from primary human myoblasts (a), C2C12 cells (b), and mESC-derived myogenic progenitors (c) aligned nearly parallel to Matrigel-modified substrates patterned with 800-nm, 1500-nm, and 3000-nm wide grooves. Images are shown as in Supplementary Fig. 2a. Scale bar, 200 μm; inset scale bar, 30 μm.



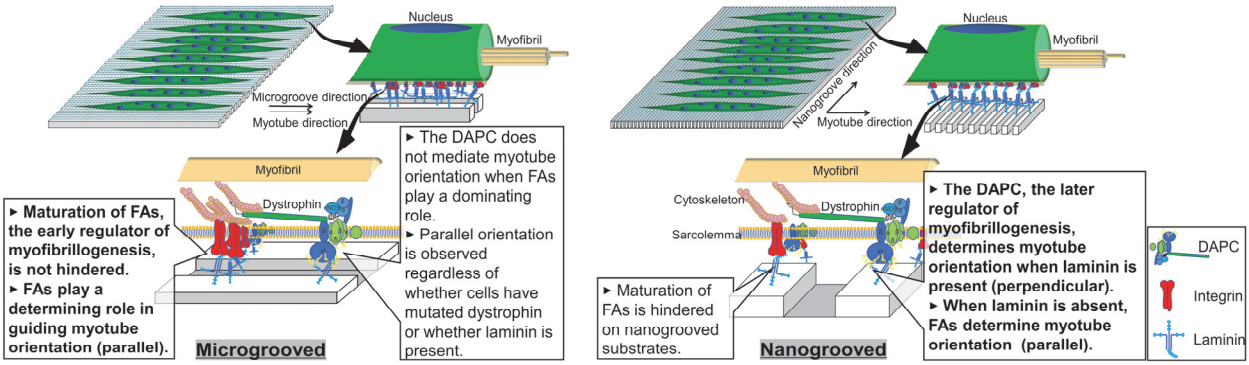
Supplementary Figure 5. HUVECs (a) and 3T3 fibroblasts (b) cultured on Matrigel-functionalized, grooved substrates did not elongate in the direction perpendicular to the grooves regardless of the feature size. Insets: bright field images used to determine groove directions (arrowed red lines). Cells were cultured for 2 weeks, followed by staining with calcein-AM (green). Scale bar, 200 μm; inset scale bar, 30 μm.



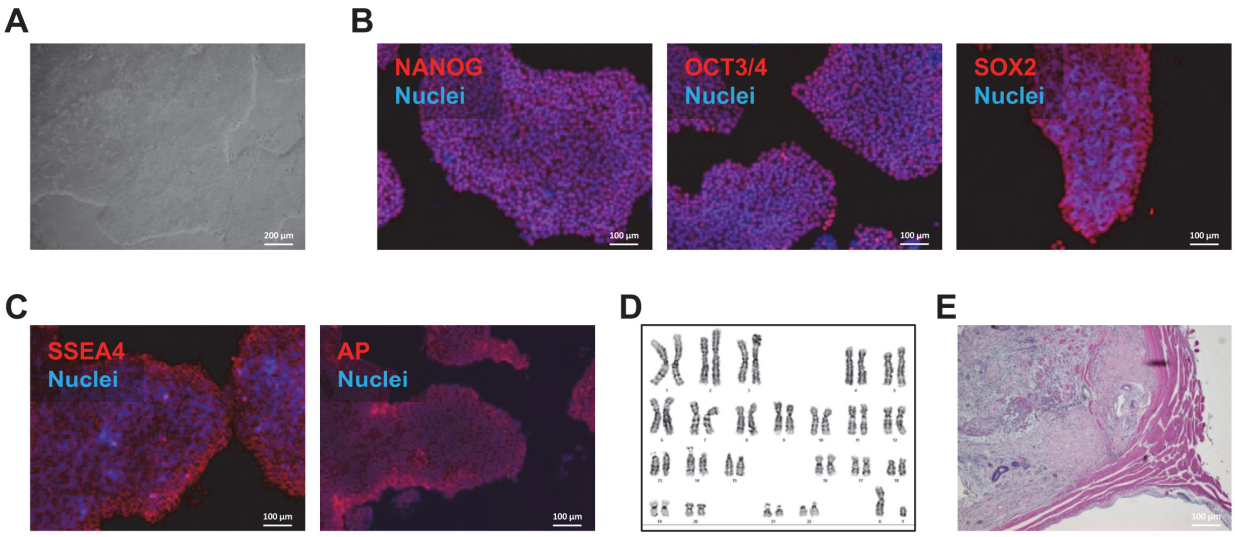
Supplementary Figure 6. Myogenic progenitors derived from a different hiPSC clone from each of the two DMD patients were used to repeat the experiments on 800-nm grooved, Matrigel-functionalized substrates by an investigator blinded to the cell groups. (a) The myotubes from DMD hiPSCs carrying the Δex52-54 mutation oriented randomly and those from DMD hiPSCs carrying the Δex31 mutation aligned in a consistent direction deviated from 90°. (b) The curves of the p.d.f. of myotube orientations for the two cell types are distinct. (c) The p.d.f. curve having a single peak was fitted to a Gaussian function and the revealed mean myotube orientation angle is shown as a box-and-whisker plot (n=9). The box-and-whisker plot is shown as in Figure 2c. Cells were cultured for 2 weeks after differentiation induction.



Supplementary Figure 7. Myotubes differentiated from DMD hiPSC-derived myogenic progenitors cultured on Matrigel-functionalized, 3000-nm grooved substrates aligned nearly parallel to grooves. Images are shown as in Supplementary Fig. 2a. Scale bar, 200 μm; inset scale bar, 30 μm.



Supplementary Figure 8. A hypothetical model of the roles of the DAPC and FAs in mediating topography-responsive myotube orientation. The model explains the influences of the groove feature size, the substrate-bound cell adhesion molecules, and dystrophin mutations on myotube orientation as observed in the study.



Supplementary Figure 9. Characterization of the DMD Δex31 human iPS cell line. A) Phase-contrast morphology of DMD Δex31 iPS cells. B-C) Immunofluorescence for pluripotency markers NANOG, OCT3/4, SOX2 (B), SSEA4 and Alkaline phosphatase activity (C). Scale bar: 100µm. D) Karyotype. E) Teratoma formation.