Supporting Information

Mechanical Stimulation of Adhesion Receptors Using Light-Responsive Nanoparticle Actuators Enhances Myogenesis

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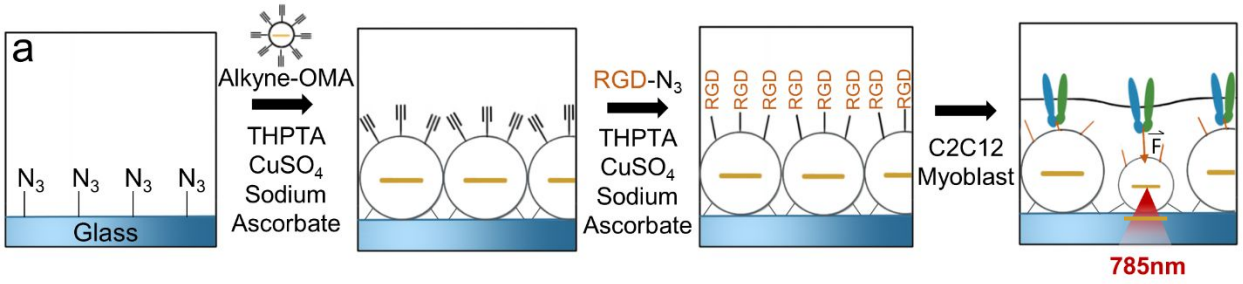
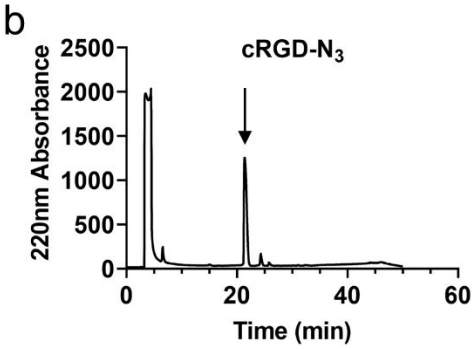
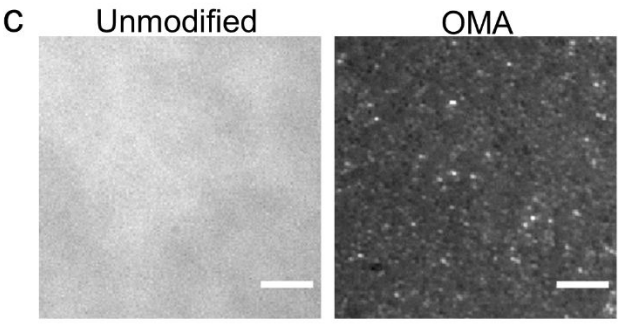
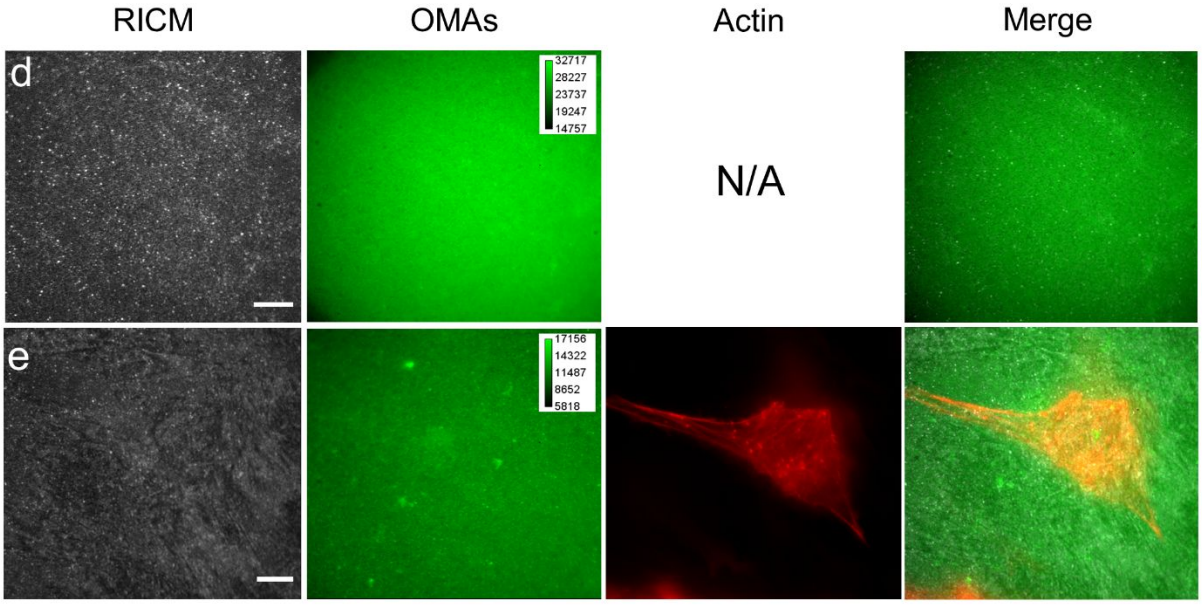
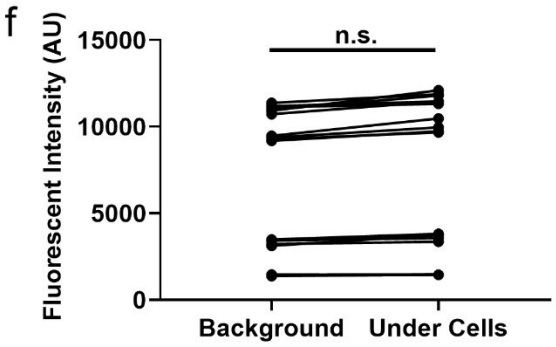

a
N3 N3 N3 N3
Glass
Alkyne-OMA
THPTA
CuSO4
Sodium
Ascorbate
RGD-N3
THPTA
CuSO4
Sodium
Ascorbate
RGD
C2C12
Myoblast
F
785nm


b
cRGD-N3
220nm Absorbance
2500
2000
1500
1000
500
0
0
20
40
60
Time (min)


c
Unmodified
OMA


RICM
OMAs
Actin
Merge
d
32717
28227
23737
19247
14757
N/A
e
17156
14322
11487
8652
5818


f
n.s.
Fluorescent Intensity (AU)
15000
10000
5000
0
Background
Under Cells

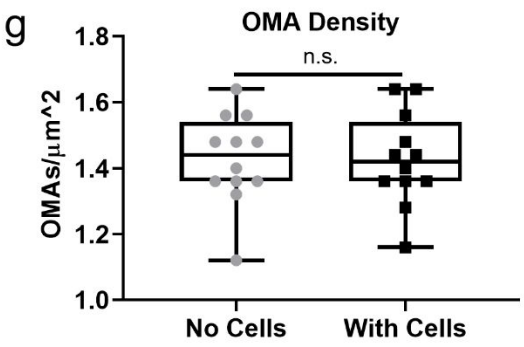

g
OMA Density
n.s.
OMAs/μm^2
1.8
1.6
1.4
1.2
1.0
No Cells
With Cells

Figure S1: Surface preparation and functionality. a) Schematic of the surface preparation protocol for this study. Alkyne-modified OMAs were attached to azide-modified glass cover slips by click chemistry (left and center-left). An additional click reaction attached alkyne-modified cyclic RGDfk molecules to alkyne-OMAs (center-right). Finally, myoblasts were plated and stimulated with NIR light (right). b) HPLC trace from the purification of the azide-functionalized cyclic RGD molecule. The black arrow indicates the expected product absorbance peak at ~21 min. c) Representative RICM images showing a glass surface before (left) and after (right) covalent modification with OMAs. Scale bar = 5 μm. d) Surfaces were modified with FAM-labeled OMAs using the protocol in (a). Representative images are shown, demonstrating an even layer of fluorescence characteristic of a dense particle layer. Scale bar = 10 μm e) Subsequently, m-cherry LifeAct transfected C2C12 cells were cultured on the surfaces. After 16 hours, a continuous layer of OMA particles remained. Scale bar = 10 μm. f) Cell culture surfaces also showed no difference in average fluorescence intensity under the cell and on the glass background. g) Quantification of OMA density of *n=4* microscopic areas on *n=3* independently prepared surfaces showed an average of 1.4 OMAs/μm^2 on surfaces before and after the addition of cells.

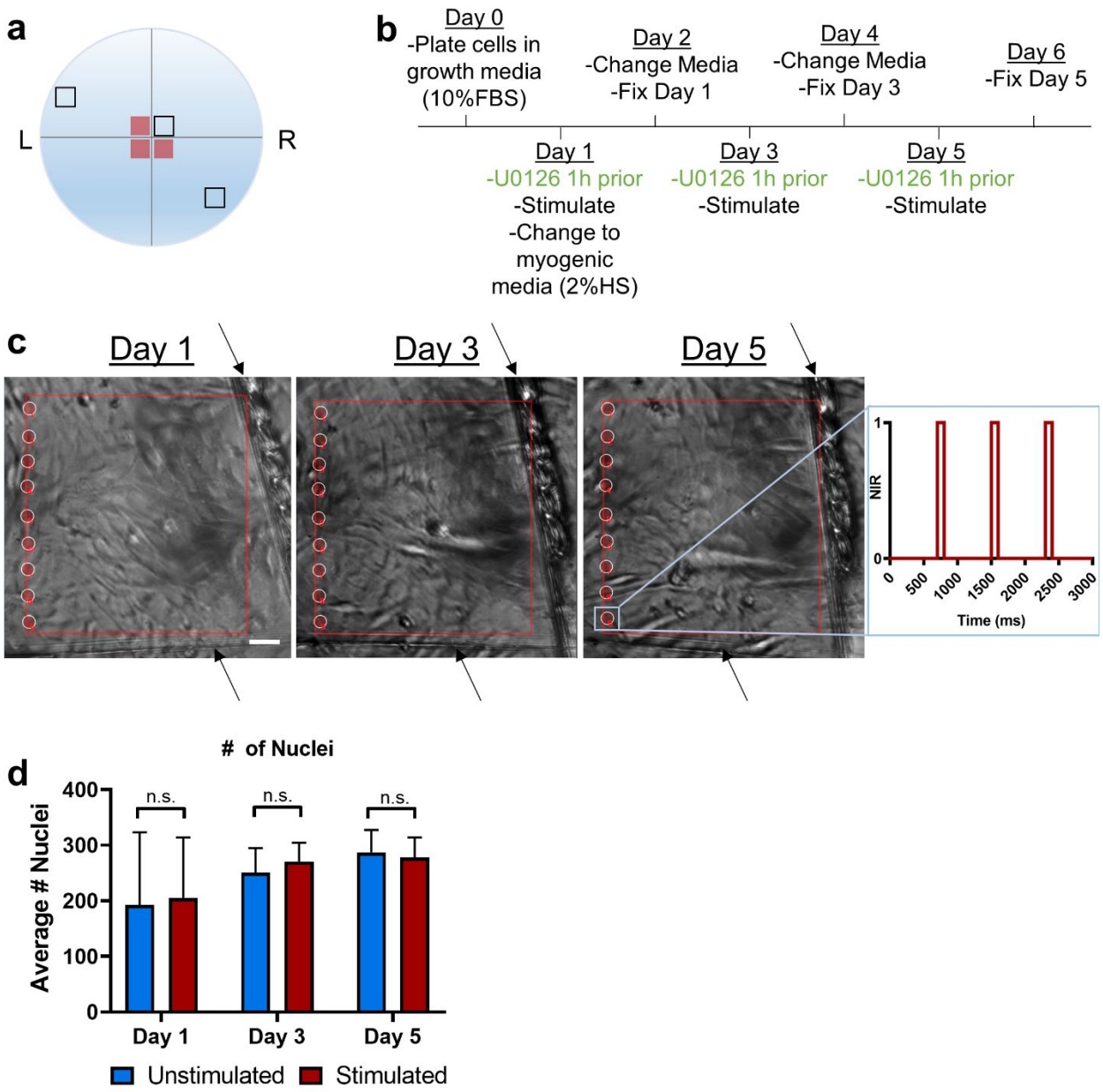


Figure S2: Experimental design for 5-day myogenesis studies. A) Schematic of OMA surface configuration in the myogenesis experiments in this work. Scratch defects were introduced to the underside of glass surfaces modified with OMAs to allow for location of the same microscopic areas during multiple days of stimulation (grey cross). Red squares indicate regions of NIR stimulation, black empty squares indicate example regions of unstimulated measurements. b) A timeline showing the procedure for NIR stimulation and cell maintenance for 5-day myogenesis experiments. Steps listed in green apply to U0126 MEK inhibitor studies. c) Representative brightfield images taken at 20x magnification with the ROE SysCon software overlay shows the location of NIR stimulation points (red circles) on a cell-seeded surface used in these experiments. Scratch defects on the bottom of the glass are visible (black arrows) showing repeated realignment of stimulation areas over the duration of the study. Scale bar = 50 μm. Outset: Representative 100 ms on-time at one of the points in the stimulation pattern,

showing the duty cycle of illumination. d) Bar graph showing average number of nuclei at various timepoints, from at least n=2 stimulated and unstimulated regions of interest from n=3 independently prepared surfaces. A mixed-effects model with multiple comparisons showed no differences between groups at any timepoint, eliminating cell density as a confounding factor in myogenesis.

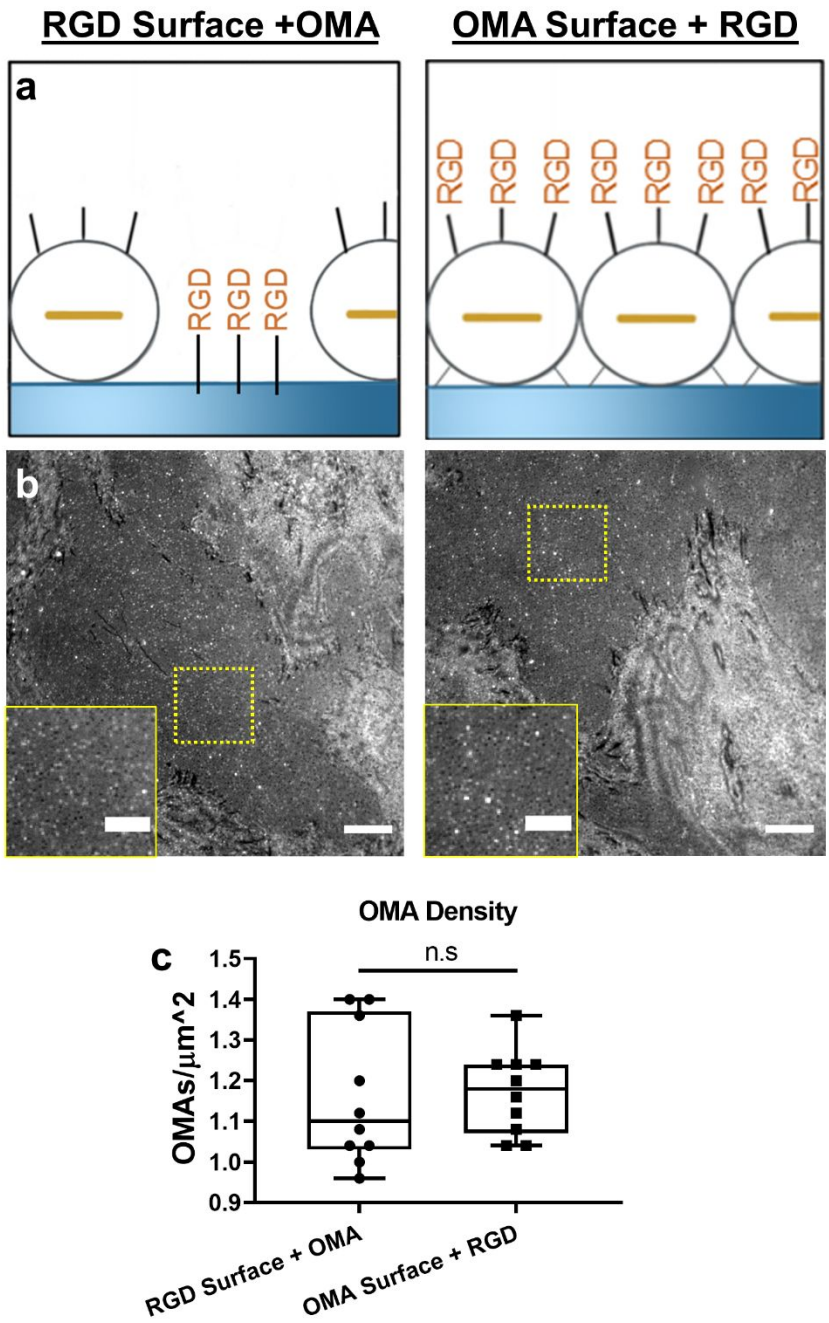


Figure S3: Evidence for integrin-mediated OMA force transmission. a) Schematic of control surfaces prepared with RGD-alkyne (RGD surface + OMA, left) in place of RGD-azide (OMA surface + RGD, right). This was done to cause RGD functionalization of the glass but not OMAs, providing cell adhesion sites with no physical attachment to the OMAs. b) Representative RICM images of cell attachment and OMA density on surfaces prepared with alkyne-RGD (left) or azide-RGD (right). Scale bar = 10 μm; inset scale bar = 5 μm. c) OMA density was not different in *n*=2 independent surface preparations surfaces and *n=5* microscopic areas by a Mann-Whitney nonparametric test (p=0.613).

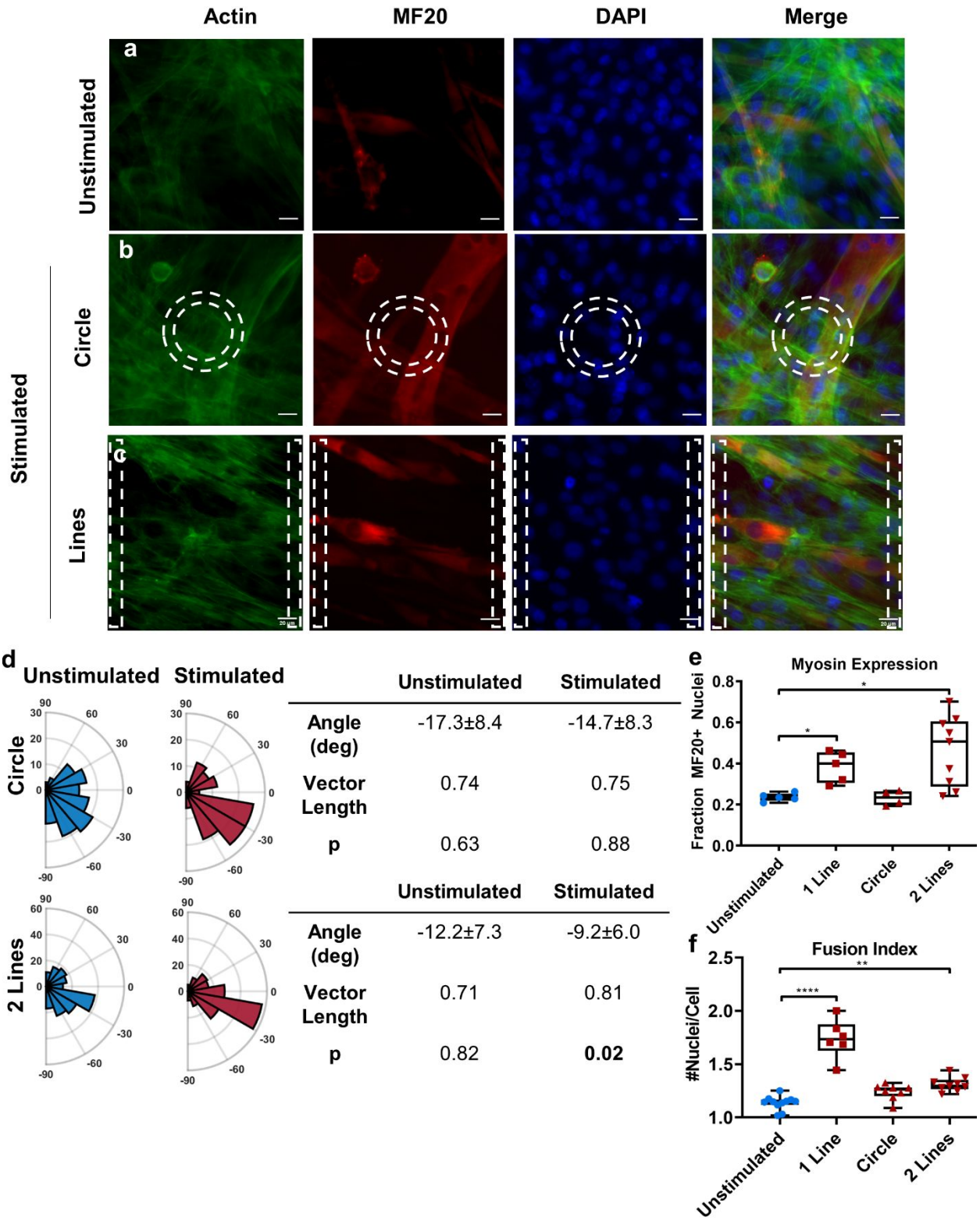
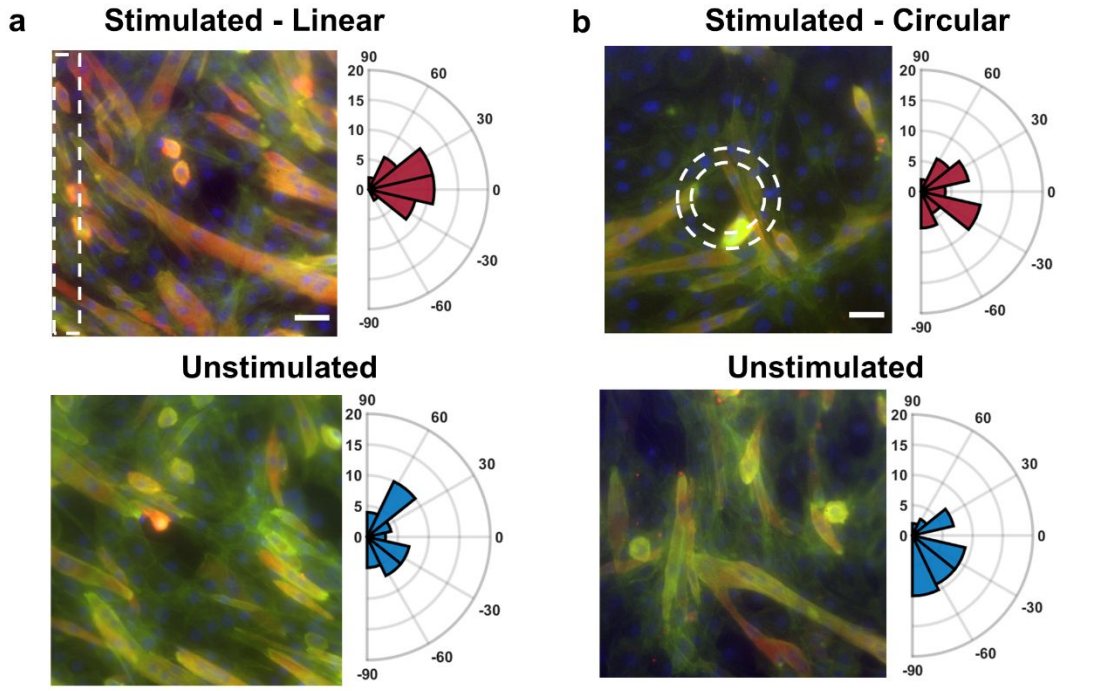


Figure S4: Response of C2C12 myoblasts to different geometries of NIR mechanical stimulation. a-c) Representative images of myocytes stained for actin (phalloidin, green), sarcomeric myosin (anti-MF20, red), and nuclei (DAPI, blue) after 5 days. Cells were exposed to

either no NIR stimulation (a), a circular array of NIR stimulation (b), or two parallel linear arrays of NIR stimulation (c). Each stimulation array (white dotted regions) consisted of 9 NIR illumination spots at 1.1Hz per spot and 15mW laser power. Scale bar = 50 μm. d) Polar histograms show alignment of *n*=120 cells (stimulated and unstimulated) from 3 independent experiments. The 0^{o} angle was set as perpendicular to the long axis of stimulation. Summary values of alignment are tabulated, showing average alignment angle, dispersion vector, and p-value, calculated by Rayleigh's modified v-test for uniformity, with a significance level of 0.05. e) Box plot of myosin expression as quantified by fraction of nuclei contained within MF20 positive cells. *p<0.05 by one-way ANOVA with Tukey's multiple comparisons vs. unstimulated group for: unstimulated *n*=5, 1 line *n*=5, circle *n*=4, 2 lines *n*=9 microscopic areas from 3 independent experiments. f) Box plot of fusion index, quantified by average number of nuclei per cell. ** p<0.01, **** p<0.0001 by Kruskal-Wallis nonparametric ANOVA with Dunn's multiple comparisons vs. unstimulated group for: unstimulated *n*=11, 1 line *n*=6, circle *n*=8, 2 lines *n*=9 microscopic areas from 3 independent experiments. Bars show range of data.



c

Linear	Stimulated	Unstimulated
Every Day n=40	Vector=0.85 Angle= -11.4±11.9 **p=2.3e-5**	Vector=0.65 Angle= -0.3±17.5 p=0.53
Every Other Day n=100	Vector=0.89 Angle= -1.2±7.0 **p=8.3e-9**	Vector=0.67 Angle= -5.7±8.3 p= 0.20

Circular	Stimulated	Unstimulated
Every Day n=40	Vector=0.70 Angle= -5.8±15.9 p=0.42	Vector=0.73 Angle= -28.2±15.2 p=0.98
Every Other Day n=120	Vector=0.75 Angle=-14.7±8.3 p=0.88	Vector=0.74 Angle= -17.3±8.4 p=0.63

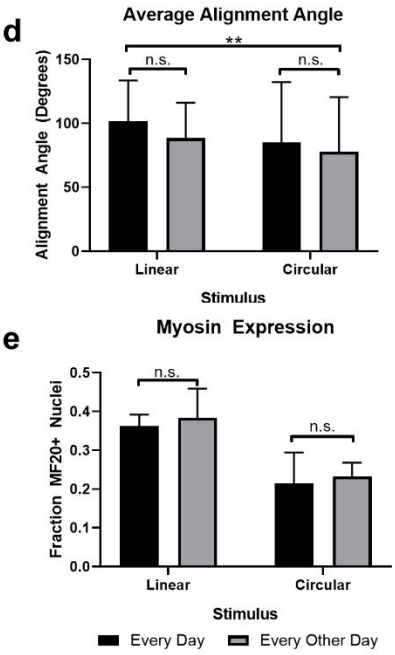


Figure S5: Response of C2C12 myoblasts to daily NIR stimulation. Representative images of cells stained for myosin (red), actin (green), and nuclei (blue) unstimulated and stimulated in a) linear and b) circular geometries, as well as polar histograms showing the distribution of cell orientations in each condition. Scale bar = 50 μm. c) A v-test for uniformity shows in cells stimulated daily and every other day, linearly stimulated cells show a preferred alignment direction, whereas circularly stimulated and unstimulated cells do not. *N=40* for cells on surfaces stimulated daily, *n=100* for cells stimulated linearly every other day, and *n=120* for cells stimulated circularly every other day. d) Average alignment angle was not different between cells stimulated daily or every other day, by Kruskal-Wallis non-parametric ANOVA with Dunn's

multiple comparisons; *n=40* for cells on surfaces stimulated daily, *n=100* for cells stimulated linearly every other day, and *n=120* for cells stimulated circularly every other day. Of note, while the average alignment angles differ only between linear-daily and circular-every other day stimulation patterns, the actual angle of alignment is of less interest than the degree of alignment of cells toward these angles, which varies significantly as evidenced by the v-test outcomes in panel c. e) Fraction of MF20 positive nuclei per surface was also not different between daily and every other day stimulation for *n=2* regions of interest for daily stimulation and *n=5* regions of interest for every other day, by a Kruskal-Wallis non-parametric ANOVA with Dunn's multiple comparisons.

Supplemental Video Captions

Video S1: OMA actuation. RICM imaging shows response of OMAs to NIR stimulation (white circle), as evidenced by contrast change of the collapsing particles. This response is repeated over multiple cycles. Scale bar = 5 μm.

Video S2: OMA actuation after 20 minutes. After 20 minutes of stimulation at 1Hz, OMAs are still responsive in the area of NIR stimulation (white circle) as evidenced by RICM contrast change. Scale bar = 5 μm.