Supplementary information

Pattern type	Area (cm^2)
Hybrid 30^0	0.00195
Hybrid 60^0	0.002516
Hybrid 90^0	0.003463
Line 300 µm	0.006
Line 150 µm	0.003
Line 80 µm	0.0016
Line 40 µm	0.0008
Line 20 µm	0.0004
Torus ID 200 µm	0.000703
Torus ID 100 µm	0.00045239
Torus ID 40 µm	0.00030159

Table S1: Area of the different patterns used in the study.

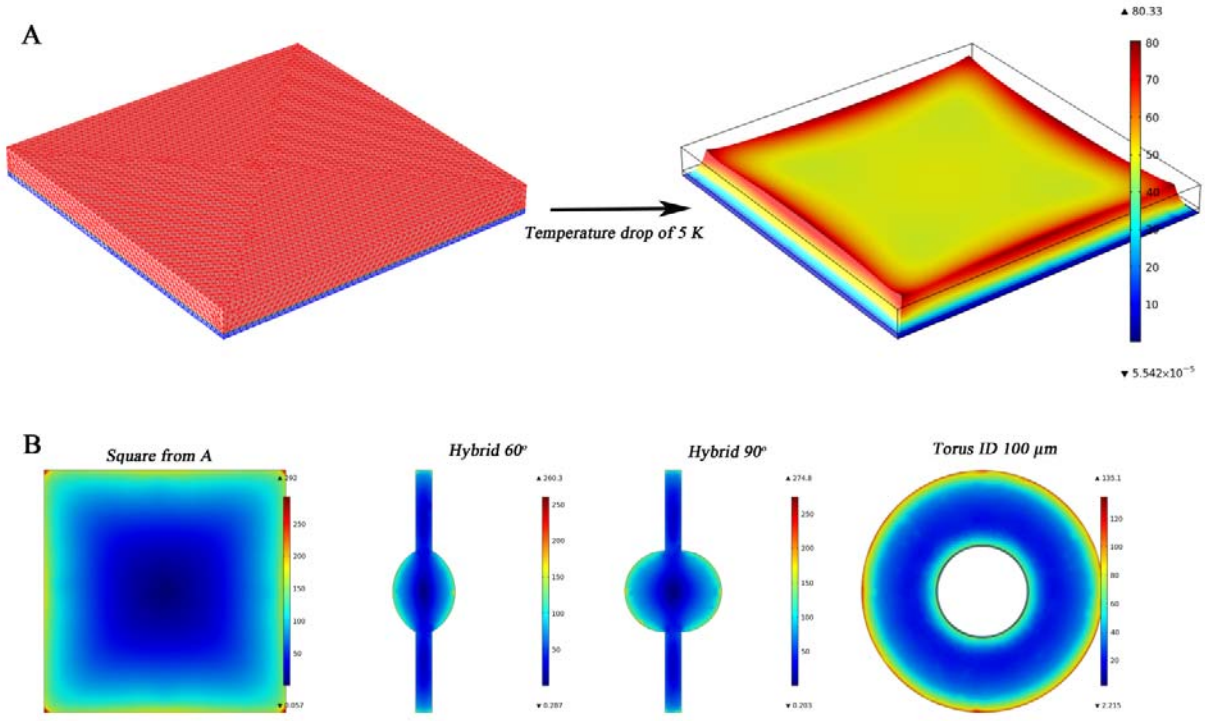


Figure S1: Mechanical stress distribution of the cell microislands. (A) The two layer model showing the passive (bottom, blue) and the contractile (top, red) layers. The surface deformation (shrinkage) and the displacement field of the square after a 5 K temperature drop can be clearly seen. Each side of the square was 1000 µm (B) von Mises stress after the temperature drop for the different structures. The maximum stress is concentrated at the corners and the periphery of the different structures.

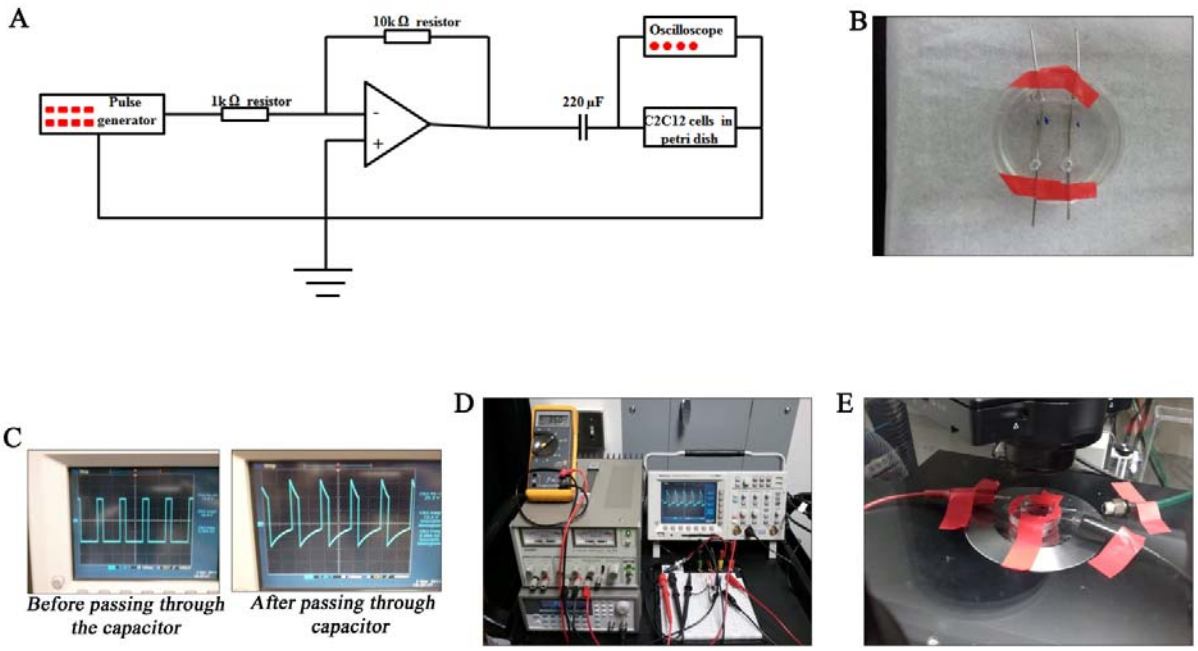


Figure S2: Setup for stimulating the contraction of C2C12 myotubes. (A) Circuit diagram of the electrical pulse stimulation (EPS) setup. (B) Lid with platinum (Pt) electrodes embedded on it. For all EPS studies, the lid would be replaced on to the petri dish for which the stimulation was to be performed after sterilizing the electrodes with 70% ethanol. (C) The generation of biopolar pulses from a monopolar pulse. This phenomenon helped to minimize the electrolysis of the medium. (D) The entire setup for EPS (E) EPS in action on a petri dish.

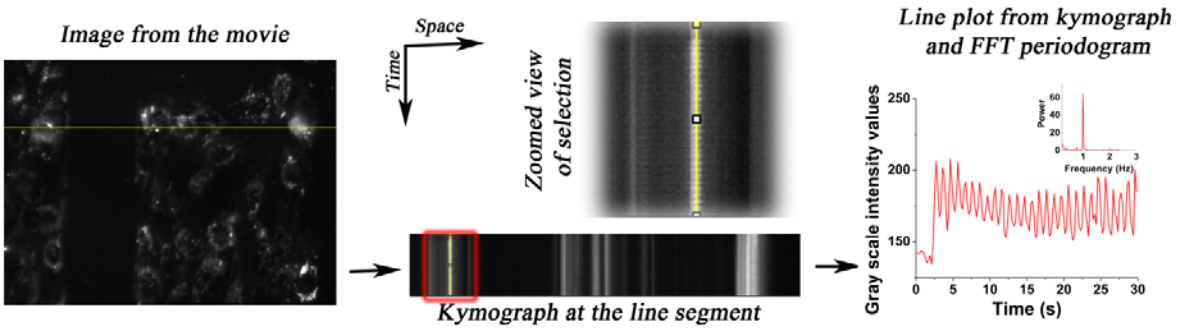


Figure S3: Generation of the contraction frequency of myotubes. The movie file was exported into ImageJ and a line segment was drawn across the entire image. Using the *multiple kymograph* plug-in of ImageJ a kymograph was generated. A line segment was drawn over the kymograph giving gray scale intensity values. A fast Fourier transform (FFT) of the resultant intensity values gave the frequency of contraction (power spectrum) of myotubes.

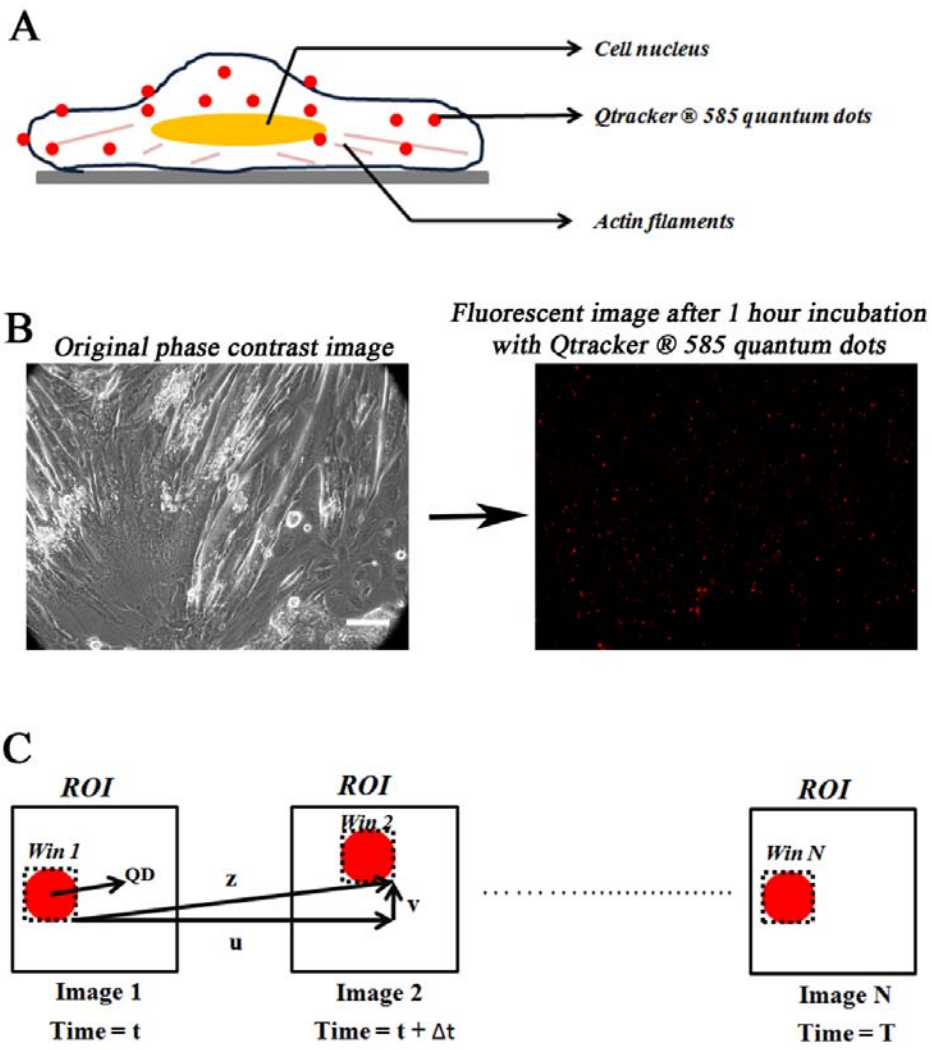
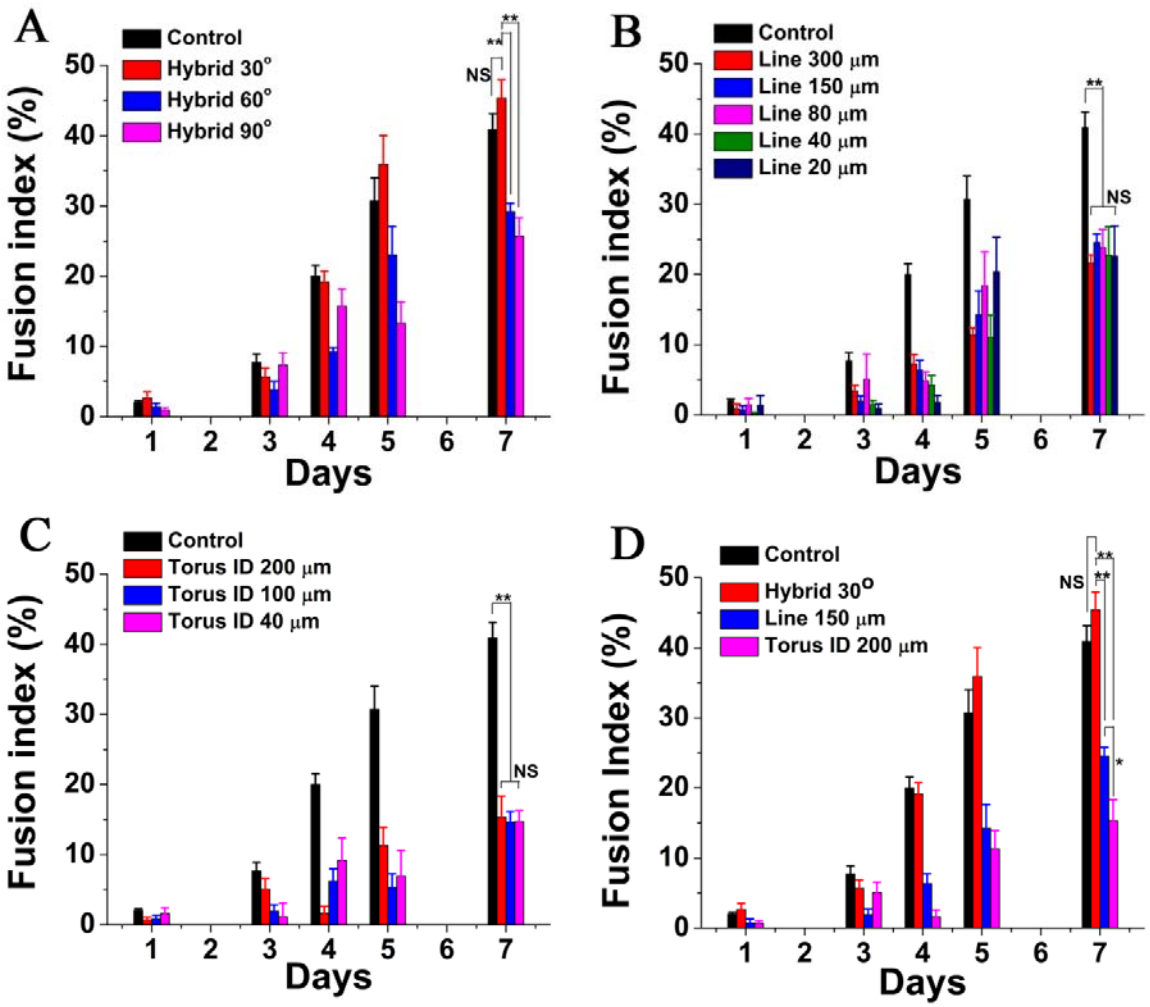


Figure S4: Technique used for the characterization of displacement of the myotubes after stimulating them with EPS. (A) Schematic of the cell loaded with with Qtracker ® 585 quantum dots (QDs) after 1 hour. (B) Phase contrast image of the control sample after 7 days in differentiation media. The cells were incubated with Qtracker ® 585 QDs for 1 hour and imaged under the tetramethyl rhodamine isothiocyanate (TRITC) filter showing the endocytosis of QDs by the C2C12 cells. (B) Digital image correlation (DIC) method used for quantifying the displacement of QDs and hence the myotube in two successive images taken a initial time (t) and time (t+Δt). Δt is the frame rate of the camera at was around 0.21 s or 0.05 s. The x-component of displacement is represented by the vector *u* while the y-component is shown by *v*. The net displacement of the QD is given by the vector *z* which also shows the displacement of the myotubes (Scale bar = 100 μm).



<u>Figure S5</u>: Quantification of the fusion index for the C2C12 cells on the different micropatterned cell islands combining experiment one and experiment two. Fusion index was calculated as the ratio of nuclei number in myocytes with two or more nuclei versus the total number of nuclei. (A) Hybrid patterns with different arc degrees (30°, 60°, and 90°), (B) Linear cell islands (line patterns) with different width (300 μm, 150 μm, 80 μm, 40 μm, and 20 μm), (C) Toroid cell islands (torus patterns) with different inner diameter (40 μm, 100 μm, and 200 μm). (D) The highest from each of the different cell islands are plotted together to show the geometry that maximized the fusion index of C2C12 cells - hybrid 30°. Significance ** $p < 0.01$, *$p < 0.05$, and *NS = not significant*. Data is represented as mean ± S.E.M (n = 10 patterns).

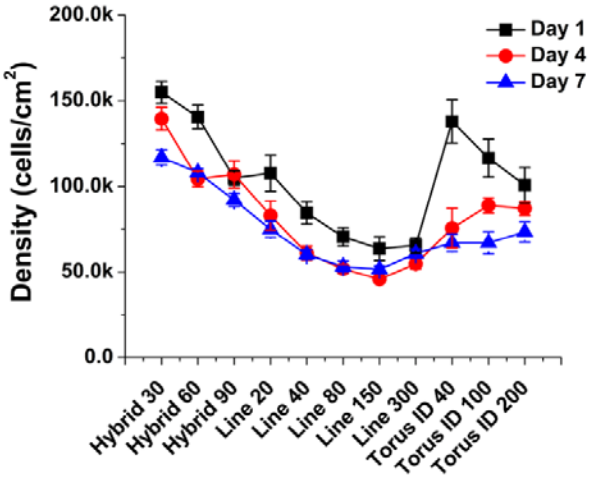


Figure S6: Density of the C2C12 cells as a function of time on the different micropatterns. Cell density was calculated by Cell density was calculated by dividing the number of cells in each pattern (counting the nuclei) by the area of the pattern (Table S1 shows the area of the different patterns).