**SUPPLEMENTARY MATERIAL**

# Early tension regulation coupled to surface myomerger is necessary for the primary fusion of C2C12 myoblasts

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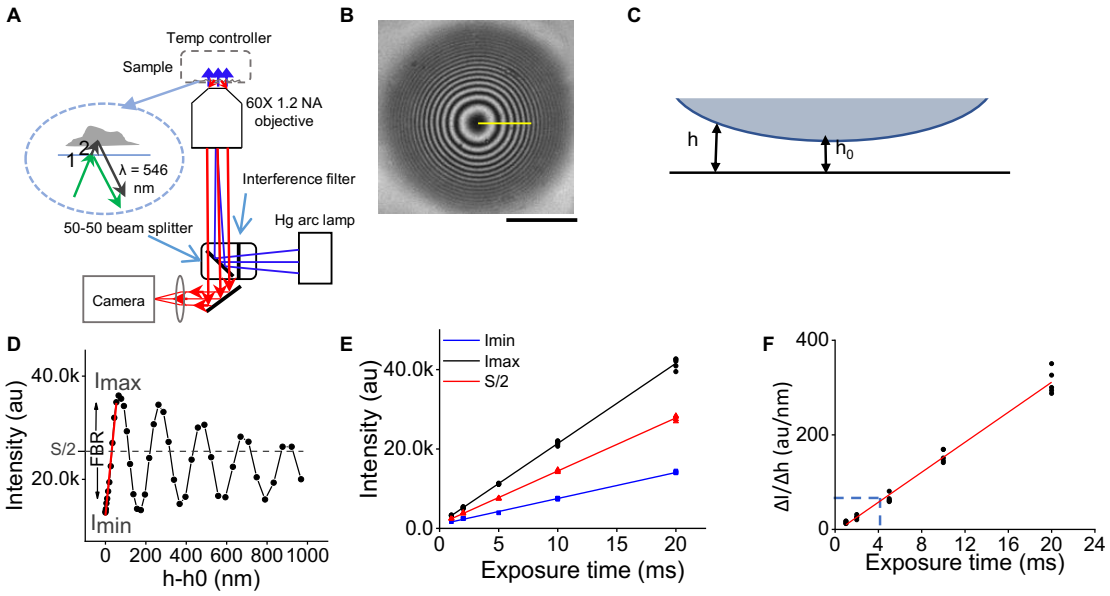


***Figure S1: Schematic and calibration Interference Reflection Microscopy (IRM) techniques*** *(A) Schematic of IRM (B) IRM images of a 60 µm polystyrene bead attached on the glass surface showing interference pattern (C) Cross-sectional representation of bead attached on the glass surface displaying the profile of its distance from coverslip (height with $h_0$ being the reference and h- $h_0$ used as relative height. (D) Intensity vs relative height profile derived from the bead in (B) along the yellow line. Intensity minima ($I_{min}$), maxima ($I_{max}$) and S/2 (background intensity) were marked out. (E) Dependence of $I_{min}$ , $I_{max}$ and S/2 on exposure times (F) Line profile of slope (intensity to height conversion) vs exposure times. These calibrations are used to estimate the conversion to be used in images of cells. Cell's $I_{max}$, $I_{min}$, S/2 are compared against E and F to find the conversion factor to be used. Exposure time is altered to change the signal mimicking altered reflectivity* (Biswas, Alex and Sinha, 2017).

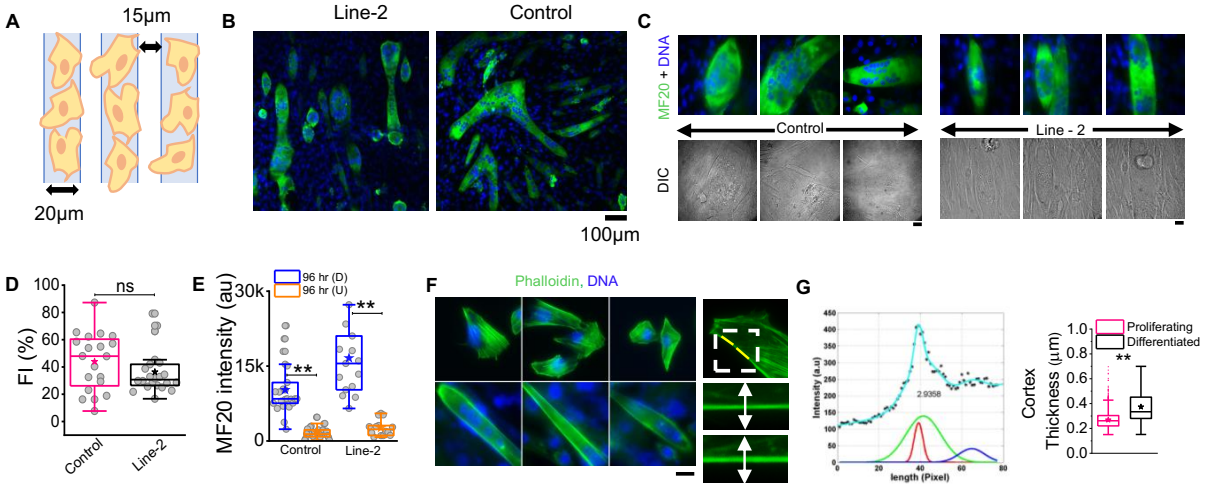


***Figure S2: Fusion index and cortex thickness analysis*** *(A) Schematic of micropatterning on Line-2 micropatterns (B) MF20 labelling on samples differentiating on micropatterned coverslips (Line-2) and non-patterned coverslip (Control) (C) MF20 labelling of myotubes on 5th day of differentiation (96 hr D) and corresponding DIC images of the same (bottom panel) (D) Fusion index calculation; n = 40 independent frames used to measure 50 myotubes (E) Comparison of MF20 change in micropatterned and non-micropatterned substrates (F) Measurement of cortex thickness* (Kumar, Saha and Sinha, 2019) *involved confocal imaging of mononucleated cells (cells in GM) and myotubes labelled with Phalloidin to visualize F-actin. Typical region of interest (ROI) shown in yellow straightened line (80 x 77 pixels) and zoomed image of cortex straightened along such lines (G) a typical multi term gaussian fitting graph; 38 ROIs from 16 proliferating cells and 34 ROIs from 13 myotubes used to compare cortex thickness. Scale bar = 20 µm.*

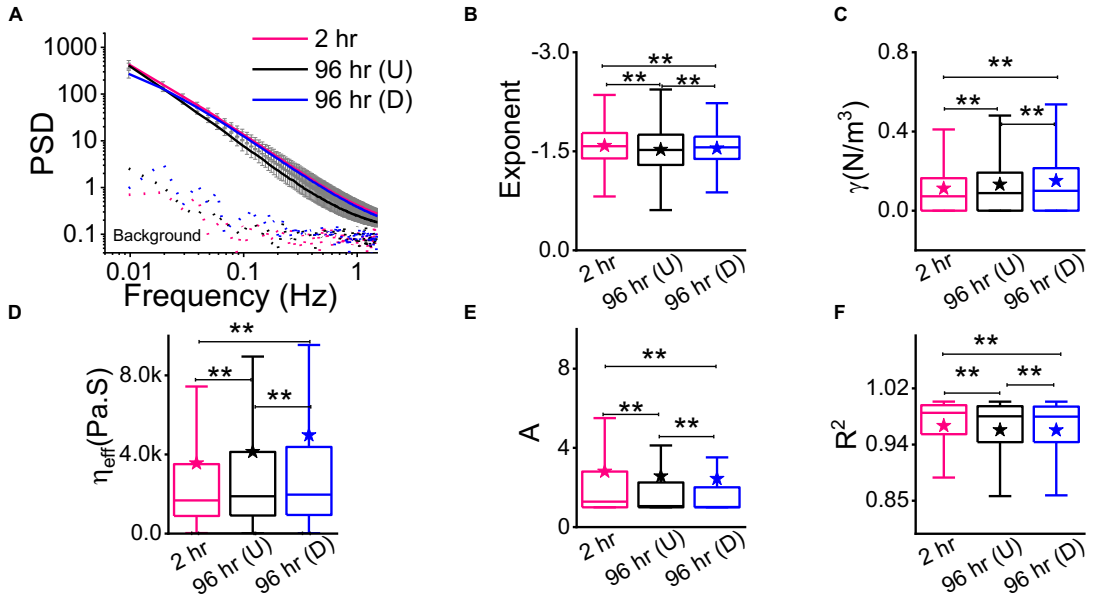


***Figure S3: Power spectral density (PSD) and mechanical parameters extracted*** *(A) Comparison of PSDs from 2 hr, 96 hr (U) and 96 hr (D) cells (B) Exponent calculated from a linear fit to log (PSD) versus log(f)plot for frequencies ranging from 0.04 to 0.4 Hz. (C) Confinement (D) Effective cytoplasmic viscosity (E) Active temperature and (F) $R^2$ value from the fitted data. Cells and FBR numbers correspond to those in Figure 2. Mann-Whitney U statistical significance test is performed, ** denotes p value < 0.001.*

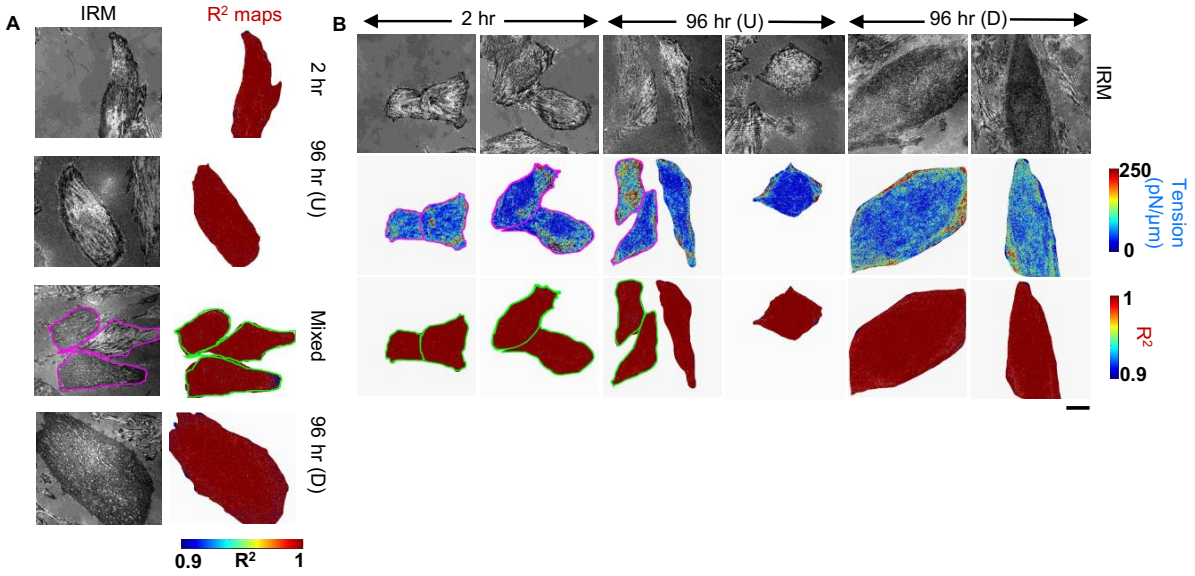


***Figure S4: Tension and $R^2$ maps of three different population of cells*** *(A) Respective $R^2$ maps of tension mapped cells shown in Figure 3 (B) IRM images, and its corresponding tension and $R^2$ maps of 2 hr, 96 hr (U) and 96 hr (D) cells. Scale bar = 20 µm.*

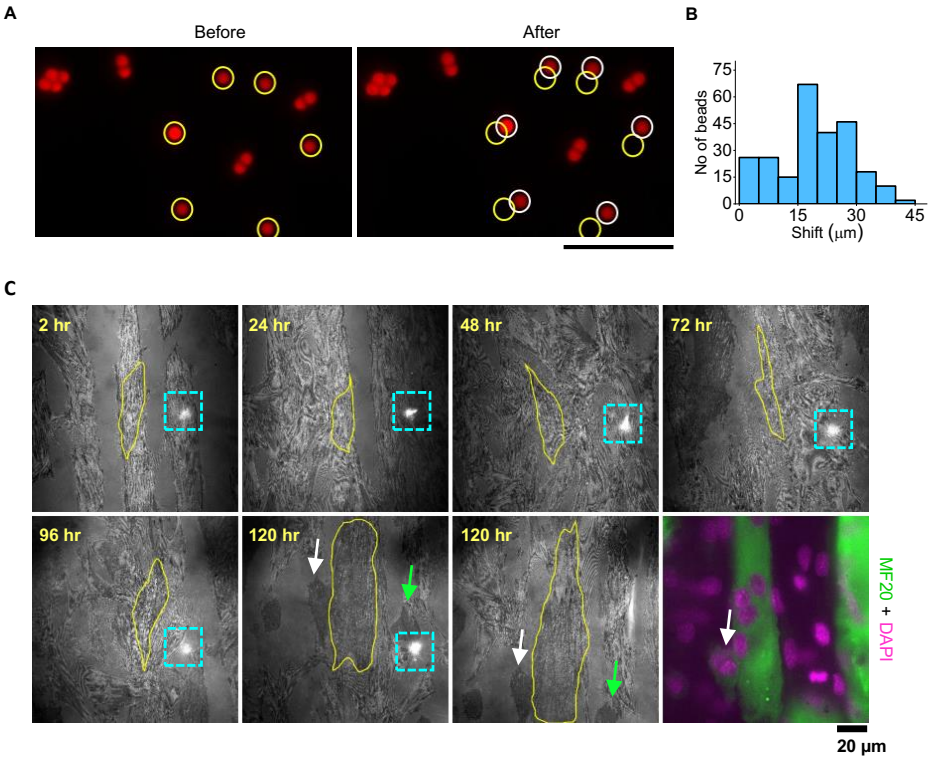


***Figure S5: Tracking beads using transformation matrix*** *(A) Epifluorescence image of 2 µm beads from a particular image field with two different attempts together as before and after to assess displacement from centroids (B) Distribution of average shift measured for beads between consecutive attempts of any particular experiment; n = 4 independent experiments used to obtain 41 beads with 16 different attempts (C) Same cell tracking successfully using transformation matrix, cyan boxes in each day's image denoted the same artifacts to understand the same place, green arrows in last two frames showing upper and lower portion of same myotube which finally labelled by MF20. Scale Bar = 20 µm.*

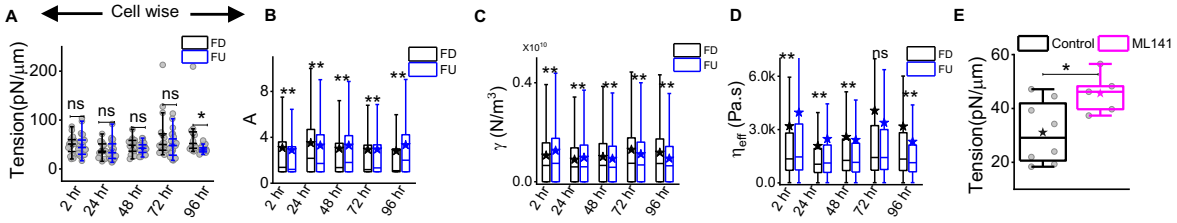


***Figure S6: Cell-wise comparison and other mechanical parameters comparison from single-cell tracking*** *(A) Membrane tension comparison between FD and FU cells across time points (B) Active temperature comparison (C) Confinement comparison (D) Effective cytoplasmic viscosity comparison; Numbers of cells and FBRs are similar as in Figure 5. (E) Cell-wise tension comparison between control and ML141 treated C2c12 cells in growth media. Mann-Whitney U statistical significance test is performed, ** denotes p value < 0.001.*

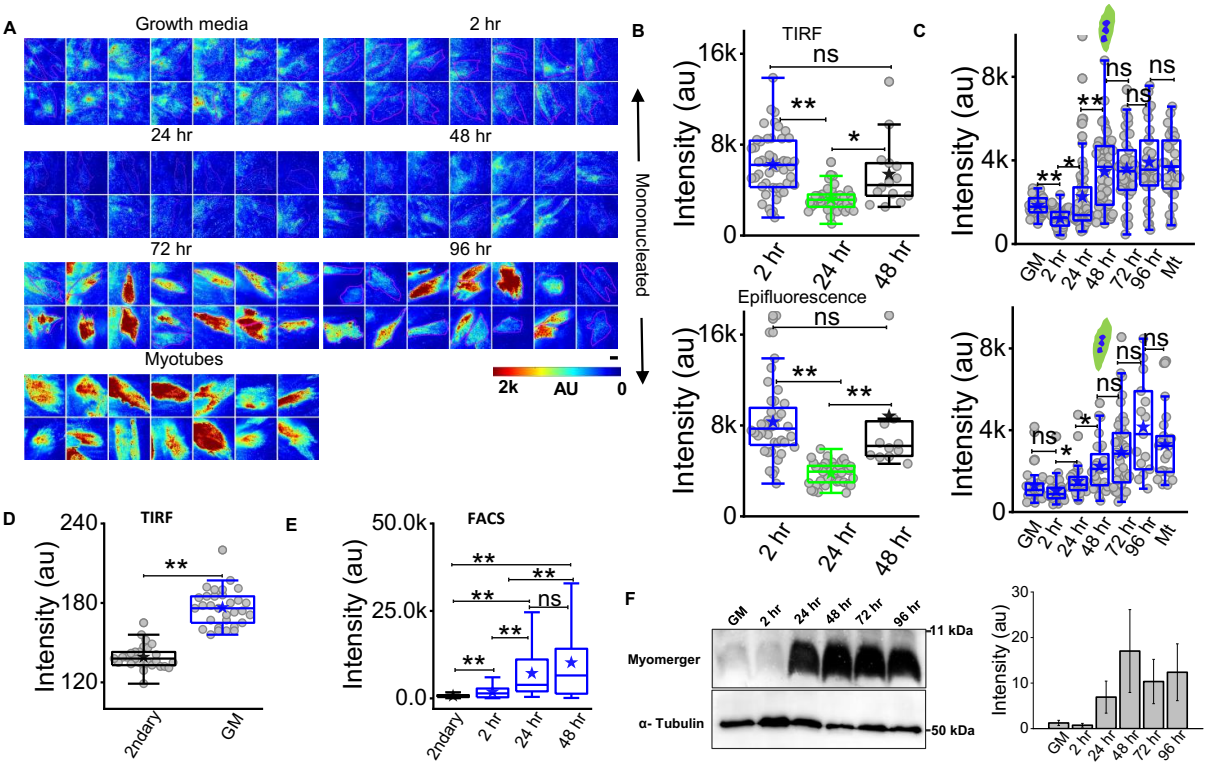


***Figure S7: Heterogenous expression of myomerger at different time points*** *(A) Cell surface expression of myomerger shown as heatmaps from immunostaining data from a representative single-set (B) Comparison of myomerger intensity of a particular set measured using TIRF (top) and epifluorescence (bottom) displaying similar trends n = 1 try used to get 48 cells (2 hr), 43 cells (24 hr) and 13 cells (48 hr) (C) Surface myomerger intensity comparison of different time points for two sets which shown myotubes at 48$^{th}$ hr (D) Secondary signal comparison in TIRF (E) FACS data showing myomerger expression at different time points including secondary-only control (F) Western blot showing whole cell expression of myomerger at different time points and its intensity comparison. Cells from different time points were scraped in cold PBS and then centrifuged at 3500 rpm for 5 min, cell pellet were then lysed using lysis buffer (mixture of RIPA and protease inhibitor cocktail) for 45 min in ice cold condition. Lysed cells were sonicated for 5 seconds with 30 seconds interval for 6 cycles. The whole cell lysate (WCL) then centrifuge in 4° C at 3500 rpm for 10 min. The whole cell lysate was then collected as supernatant. The WCL was then used first for estimating the total protein concentration with Bradford assay. Same protein amounts were loaded in SDS PAGE well. NuPage loading dye along with β-marcaptoethanol was used as a loading buffer. Before loading, samples were incubated with loading buffer at 37° C for 30 min. 0.2 μm PVDF membrane was used for transferring of protein from gel*

*to membrane. After transfer membrane kept in blocking solution using 5% skimmed milk in TBST (Tris buffer saline with 0.1 % tween 20) for 3 hr. Primary antibody for myomerger (Anti-sheep ESGP antibody 1:500 dilution) and Alpha-Tubulin (Anti-rabbit Alpha tubulin 1:20000 dilution) were added after blocking and kept at 4°C shaker for overnight. Secondary antibody for respective proteins (Rabbit Anti-sheep IgG- AP and Goat Anti-Rabbit HRP) were then added after several washing steps with TBST buffer. Secondary treatment was done for 2 hr. Finally blots were visualized using chemidoc system. Scale bar = 10 µm, Mann-Whitney U statistical significance test is performed, ** denotes P value < 0.001, * denotes P value < 0.05 and ns denotes not significant.*

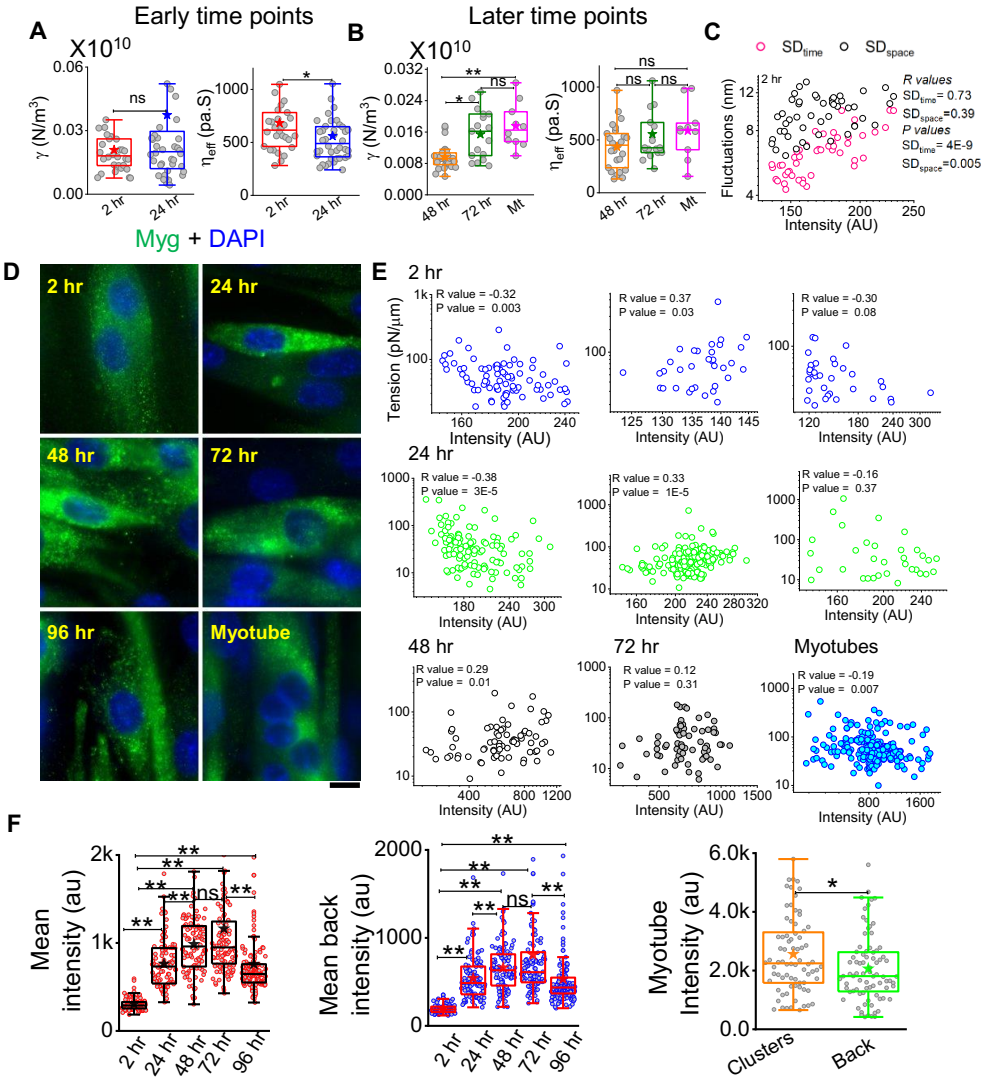


***Figure S8: Other mechanical parameters, local correlation and myomerger cluster-to-background ratio.*** *(A, B) Confinement and effective viscosity - fitting parameters of fbr-wise fluctuations data used to derive tension in Figure 7. Mann-Whitney U statistical significance test is performed, ** denotes p value < 0.001, * denotes p value < 0.05 and ns denotes not significant.(C) Correlation of fluctuations with myomerger intensity at the same region – for typical regions of different samples. (D) Images of myomerger IF at different time points (E) Correlation of tension with intensity at other typical regions (F) left: Mean intensity of clusters detected in IF images of myomerger at different time points; centre: mean intensity of the diffused background around clusters; right: mean intensity of clusters and diffused background for myotubes. Satistics of data is same as for Figure 8G.*

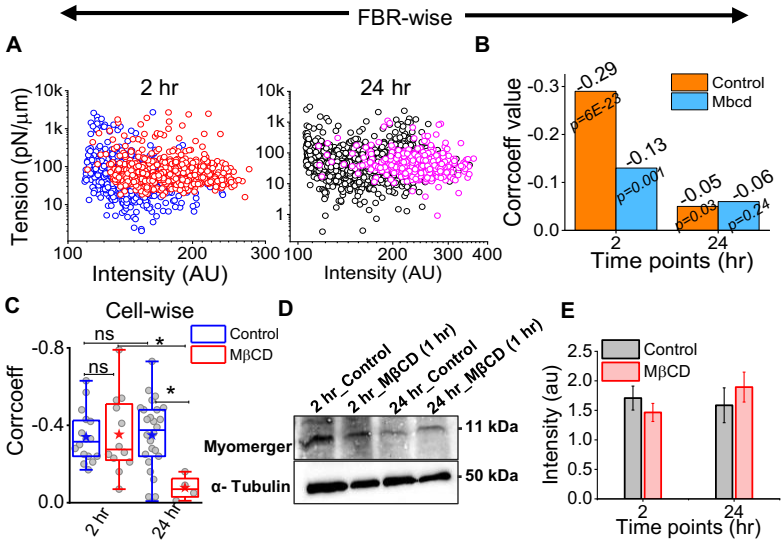


***Figure S9: Effect of cholesterol depletion on correlation coefficient.*** *(A) Typical scatter plot for intensity and tension from FBRs for single cells at 2 hr (left) or 24 hr (right) in control (blue/black) or MβCD-treated condition (pink/red). Note that these are not same cells before and after cholesterol depletion (B) Correlation coefficients and p-values for scatter plots shown in (A). (C) Box-plot comparison of correlation coefficients of those scatters that show negative correlation (even if ns). The reduced coefficient is more prominent for 24 hr (D) Western blot showing the expression pattern of myomerger upon MβCD treatment (E) Intensity of myomerger from WB.*

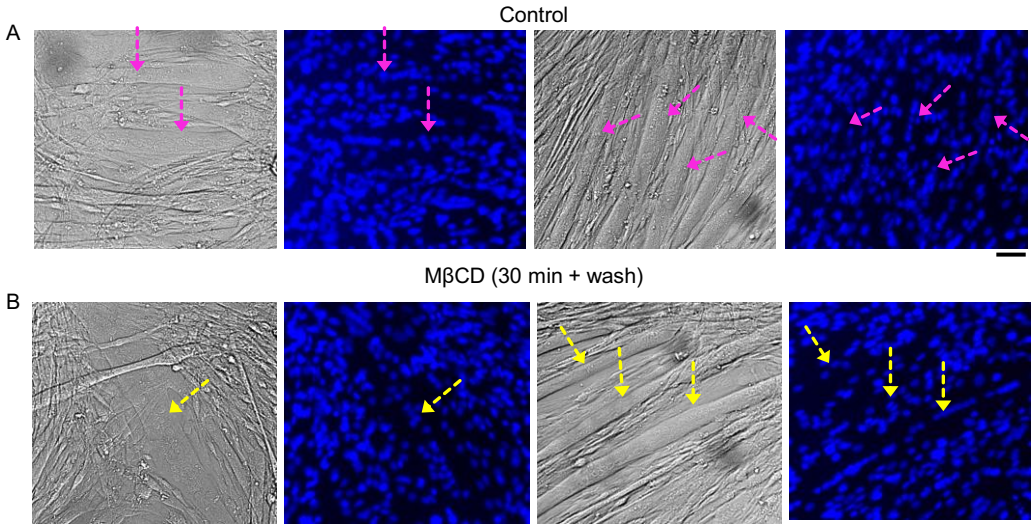


***Figure S10: Effect of pulsed cholesterol depletion on fusion.*** *(A) DIC (grey and DAPI staining (blue) for control cells after 96 hr of DM treatment (B) DIC (grey and DAPI staining (blue) for cells treated with MβCD at 24 hr but only for 30 min after which media is replaced by DM again. Images are after 96 hr of DM treatment. Scale bar = 50 μm.*

***Table S1: List of parameters measured and reported in plots in figures.***

*P values are calculated using Mann-Whitney unless mentioned tohave been also calculated using Linear Mixed-effect Model (LMM)*

| Figure 1D (left) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Parameters** | **Condition** | **$N_{cells}$** | **$n_{rois}$** | **Mean** | **SD** | **SEM** | **Median** | **P value** |
| | | | | | | | | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Myoblasts | 32 | 1526 | 138 | 35 | 6.13 | 131 | |
| MF20 intensity | 96 hr (U) | 25 | 626 | 1834 | 1406 | 281.26 | 1659 | 4.00E-10 |
| | 96 hr (D) | 24 | 1647 | 10128 | 4831 | 1007.35 | 8545 | 1.00E-08 |
| | | | | | | | | |
| **Figure 2E (left)** | | | | | | | | |
| **Parameters** | **Condition** | **$N_{cell}$** | **$N_{FBR}$** | **Mean** | **SD** | **SEM** | **Median** | **P value (wrt 2hr)** |
| | 2hr | 52 | 947 | 4.3 | 0.9 | 0.03 | 4.3 | |
| SD time (nm) | 96 hr (U) | 40 | 712 | 3.7 | 0.7 | 0.03 | 3.7 | 0* |
| (12x12 | | | | | | | | |
| pixels) | 96 hr (D) | 32 | 948 | 3.4 | 0.9 | 0.03 | 3.4 | 0** |
| | | | | | | | | |
| **Figure 2E (right)** | | | | | | | | |
| **Parameters** | **Condition** | **$N_{cell}$** | **$N_{FBR}$** | **Mean** | **SD** | **SEM** | **Median** | **P value (wrt 2hr)** |
| SD space (nm) | 2hr | 52 | 947 | 7.8 | 2 | 0.07 | 7.6 | |
| (12x12 pixels) | 96 hr (U) | 40 | 712 | 6.6 | 1.5 | 0.05 | 6.4 | 0 |
| | 96 hr (D) | 32 | 948 | 5 | 1.3 | 0.04 | 5 | 0 |
| **Figure 2G (left)** | | | | | | | | |
| **Parameters** | **Condition** | **$N_{cell}$** | **$N_{FBR}$** | **Mean** | **SD** | **SEM** | **Median** | **P value (wrt 2hr) (LMM)** |
| Tension (pN/µm) | 2hr | 52 | 14269 | 259 | 746 | 6.3 | 61.9 | |
| (4x4 pixels) | 96 hr (U) | 40 | 20090 | 327 | 834 | 5.9 | 82 | 0.6 |
| | 96 hr (D) | 32 | 35041 | 398 | 1043 | 5.6 | 90.2 | 0.01 |
| | | | | | | | | |
| **Figure 2G (right)** | | | | | | | | |
| **Parameters** | **Condition** | **$N_{cell}$** | **$N_{FBR}$** | **Mean** | **SD** | **SEM** | **Median** | **P value (wrt 2hr)** |
| Tension (pN/µm) | 2hr | 52 | 14269 | 259 | 746 | 6.3 | 61.9 | |
| (4x4 pixels) | 96 hr (U) | 40 | 20090 | 327 | 834 | 5.9 | 82 | <0.001 |
| | 96 hr (D) | 32 | 35041 | 398 | 1043 | 5.6 | 90.2 | <0.001 |
| | | | | | | | | |
| **Figure 3B** | | | | | | | | |
| **Parameters** | 2hr | 52 | 52 | 1.2 | 0.16 | 0.024 | 1.19 | |
| SD ($SD_{time}$) | 96 hr (U) | 40 | 40 | 1 | 0.17 | 0.028 | 0.99 | 1.8E-5 |
| (nm) | 96 hr (D) | 32 | 32 | 0.77 | 0.15 | 0.025 | 0.76 | 2.7E-13 |
| | 96 hr (D) | 32 | 32 | 0.77 | 0.15 | 0.025 | 0.76 | 2.7E-13 |
| **Figure 3C** | | | | | | | | |
| **Parameters** | **Condition** | **N** | **n** | **Mean** | **SD** | **SEM** | **Median** | **P value (wrt 2hr)** |
| SD ($SD_{space}$) | 2hr | 52 | 52 | 0.28 | 0.06 | 0.008 | 0.28 | |
| (nm) | 96 hr (U) | 40 | 40 | 0.3 | 0.07 | 0.012 | 0.28 | 0.376 |
| | 96 hr (D) | 32 | 32 | 0.24 | 0.05 | 0.008 | 0.26 | 0.007 |
| SD (Tension) | **Condition** | **N** | **n** | **Mean** | **SD** | **SEM** | **Median** | **P value (wrt 2hr)** |
| (pN/µm) | 2hr | 52 | 52 | 681 | 414 | 59.81 | 605 | |
| | 96 hr (U) | 40 | 40 | 787 | 248 | 40.75 | 740 | 0.005 |
| | 96 hr (D) | 32 | 32 | 877 | 375 | 65.2 | 817 | 0.002 |
| **Figure 4C** | | | | | | | | |
| **Parameters** | **Condition** | **$N_{cell}$** | **$n_{FBR}$** | **Mean** | **SD** | **SEM** | **Median** | **P value (wrt 2hr)** |
| | **(Finally differentiated)** | | | | | | | |
| Tension (pN/µm) | 2hr | 1 | 2563 | 171 | 408 | 8.07 | 47.6 | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 24hr | 1 | 2560 | 157 | 491 | 9.72 | 36.23 | 0 |
| | 48hr | 1 | 2037 | 197 | 419 | 9.28 | 50.18 | 0.183 |
| | 72hr | 1 | 3053 | 338 | 684 | 12.39 | 74.35 | 1.00E-41 |
| | 96hr | 1 | 2209 | 264 | 656 | 13.95 | 73.2 | 3.00E-31 |
| $SD_{time}$ (nm) | 2hr | 1 | 3385 | 6.01 | 1.36 | 0.023 | 5.91 | |
| | 24hr | 1 | 6239 | 7.07 | 2.03 | 0.025 | 6.89 | 2.00E-142 |
| | 48hr | 1 | 6798 | 6.34 | 1.2 | 0.014 | 6.26 | 3.00E-32 |
| | 72hr | 1 | 6711 | 4.78 | 0.71 | 0.008 | 4.78 | 0 |
| | | 1 | 5089 | 4.44 | 0.71 | 0.009 | 4.38 | 0 |
| **Figure 4D** | | | | | | | | |
| **Parameters** | **Condition** | **$N_{cell}$** | **$n_{FBR}$** | **Mean** | **SD** | **SEM** | **Median** | **P value (wrt 2hr)** |
| | **(Finally undifferentiated)** | | | | | | | |
| Tension (pN/μm) | 2hr | 1 | 1342 | 193 | 453 | 12.38 | 46.69 | |
| | 24hr | 1 | 560 | 314 | 774 | 32.74 | 74.29 | 6.00E-12 |
| | 48hr | 1 | 2346 | 173 | 489 | 10.09 | 45.3 | 0.493 |
| | 72hr | 1 | 2750 | 193 | 495 | 9.44 | 54.99 | 0.0012 |
| | 96hr | 1 | 2997 | 152 | 446 | 8.15 | 43.13 | 0.009 |
| **Parameters** | **Condition** | **$N_{cell}$** | **$n_{FBR}$** | **Mean** | **SD** | **SEM** | **Median** | **P value (wrt 2hr)** |
| | **(Finally undifferentiated)** | | | | | | | |
| $SD_{time}$ (nm) | 2hr | 1 | 3020 | 6.63 | 1.53 | 0.027 | 6.58 | |
| | 24hr | 1 | 2638 | 5.25 | 1.4 | 0.028 | 5.08 | 0 |
| | 48hr | 1 | 4615 | 5.34 | 1.47 | 0.021 | 5.11 | 0 |
| | 72hr | 1 | 6172 | 5.52 | 1.43 | 0.018 | 5.53 | 0 |
| | 96hr | 1 | 7118 | 6.36 | 1.71 | 0.02 | 6.17 | 0 |
| **Figure 5A** | | | | | | | | |
| **Parameters** | **Condition** | **N** | **n** | **Mean** | **SD** | **SEM** | **Median** | **P value (wrt 2hr)** |
| | **(FD+FU)** | | | | | | | |
| Tension | 2hr | 24 | 24 | 62.12 | 16.12 | 3.29 | 57.31 | |
| (pN/μm) | 24hr | 24 | 24 | 49.32 | 14.22 | 2.9 | 48.8 | 0.006 |
| | 48hr | 24 | 24 | 50.03 | 10.37 | 2.12 | 48.92 | 0.004 |
| | 72hr | 24 | 24 | 68.31 | 39.64 | 8.09 | 53.75 | ns |
| | 96hr | 13 | 13 | 67.38 | 45.36 | 12.58 | 49.86 | ns |
| **Figure 5B** | | | | | | | | |
| **Parameters** | **Condition** | **N** | **n** | **Mean** | **SD** | **SEM** | **Median** | **P value (wrt** |
| | **(Finally differentiated)** | | | | | | | |
| Relative | 2hr | 16 | 16 | 1.22 | 0.26 | 0.07 | 1.29 | |
| $SD_{time}$ (nm) | 24hr | 16 | 16 | 1 | 0.18 | 0.04 | 1.05 | 0.001 |
| | 48hr | 16 | 16 | 0.92 | 0.24 | 0.06 | 0.82 | ns |
| | 72hr | 16 | 16 | 0.84 | 0.19 | 0.06 | 0.78 | ns |
| | 96hr | 12 | 12 | 1.22 | 0.27 | 0.07 | 1.28 | 0.01 |
| | **(Finally undifferentiated)** | | | | | | | |
| Relative | 2hr | 24 | 24 | 1.18 | 0.3 | 0.06 | 1.14 | |
| $SD_{time}$ (nm) | 24hr | 24 | 24 | 1.02 | 0.2 | 0.04 | 0.97 | ns |
| | 48hr | 24 | 24 | 0.99 | 0.22 | 0.04 | 0.98 | ns |
| | 72hr | 24 | 24 | 1.02 | 0.22 | 0.05 | 0.98 | ns |
| | 96hr | 17 | 17 | 1.18 | 0.3 | 0.06 | 1.14 | ns |
| **Figure 5C** | | | | | | | | |
| **Parameters** | **Condition** | **N** | **n** | **Mean** | **SD** | **SEM** | **Median** | **P value (wrt 2hr)** |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **(Finally differentiated)** | | | | | | | |
| Relative | 2hr | 12 | 12 | 1 | 0 | 0 | 1 | |
| Tension (pN/µm) | 24hr | 12 | 12 | 0.8 | 0.24 | 0.07 | 0.75 | 0.003 |
| | 48hr | 12 | 12 | 0.9 | 0.19 | 0.05 | 0.89 | ns |
| | 72hr | 12 | 12 | 1.3 | 0.66 | 0.19 | 1.11 | ns |
| | 96hr | 8 | 8 | 1.6 | 1.05 | 0.37 | 1.26 | ns |
| | **(Finally undifferentiated)** | | | | | | | |
| Relative | 2hr | 12 | 12 | 1 | 0 | 0 | 1 | |
| Tension (pN/µm) | 24hr | 12 | 12 | 0.9 | 0.33 | 0.09 | 0.96 | ns |
| | 48hr | 12 | 12 | 0.8 | 0.19 | 0.06 | 0.76 | ns |
| | 72hr | 12 | 12 | 0.9 | 0.38 | 0.11 | 0.95 | ns |
| | 96hr | 5 | 5 | 0.7 | 0.24 | 0.11 | 0.68 | 0.02 |

## Figure 5G

| **Parameters** | **Condition** | **$N_{Cell}$** | **n** | **Mean** | **SD** | **SEM** | **Median** | **P value (wrt control)** |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | | |
| Fusion Index (%) | Control | 16 | 16 | 39.54 | 11.98 | 2.99 | 39.35 | |
| | ML141 | 16 | 16 | 15.09 | 11.65 | 2.91 | 10.71 | 4.00E-05 |
| | MβCD | 16 | 16 | 0 | 0 | 0 | 0 | 3.00E-07 |
| Tension | Control | 12 | 9 | 34.27 | 15.74 | 5.25 | 28.78 | |
| (pN/µm) | MβCD | 12 | 15 | 53.99 | 12.58 | 3.25 | 55.88 | 0.007 |

## Figure 5H (left)

| **Parameters** | **Condition** | **$N_{Cell}$** | **$n_{FBR}$** | **Mean** | **SD** | **SEM** | **Median** | **P value (wrt 2 hr)** |
|---|---|---|---|---|---|---|---|---|
| | **(Finally differentiated)** | | | | | | | |
| $SD_{time}$ | 2 hr | 12 | 82893 | 5.57 | 1.81 | 0.00629 | 5.26 | |
| (nm) | 24 hr | 12 | 57773 | 6.78 | 1.91 | 0.008 | 6.61 | 0 |
| | 48 hr | 12 | 85871 | 5.38 | 1.42 | 0.005 | 5.24 | 0 |
| | 72 hr | 12 | 66018 | 5.63 | 1.7 | 0.007 | 5.51 | 0 |
| | 96 hr | 8 | 91448 | 5.07 | 1.42 | 0.005 | 4.93 | 0 |
| | **(Finally undifferentiated)** | | | | | | | |
| $SD_{time}$ | 2 hr | | 87626 | 5.73 | 1.74 | 0.006 | 5.59 | |
| (nm) | 24 hr | | 71161 | 6.46 | 1.89 | 0.007 | 6.27 | 0 |
| | 48 hr | | 109153 | 6.36 | 1.72 | 0.005 | 6.21 | 0 |
| | 72 hr | | 91307 | 5.79 | 1.68 | 0.005 | 5.66 | 0 |
| | 96 hr | | 28950 | 6.35 | 1.71 | 0.01 | 6.21 | 0 |

## Figure 5H (right)

| **Parameters** | **Condition** | **$N_{Cell}$** | **n** | **Mean** | **SD** | **SEM** | **Median** | **P value (wrt 2 hr)** |
|---|---|---|---|---|---|---|---|---|
| | **(Finally differentiated)** | | | | | | | |
| Tension | 2 hr | | 33007 | 229.29 | 664.91 | 3.18 | 53.33 | |
| (pN/µm) | 24 hr | | 24452 | 168.19 | 463.52 | 2.96 | 41.4 | 0 |
| | 48 hr | | 22179 | 227.41 | 668.59 | 4.49 | 51.42 | 0.005 |
| | 72 hr | | 35653 | 321.78 | 951.79 | 5.04 | 65.32 | 4.00E-111 |
| | 96 hr | | 40465 | 279.45 | 754.11 | 3.75 | 64.06 | 5.00E-107 |
| | **(Finally undifferentiated)** | | | | | | | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Tension | 2 hr | | 34726 | 256.62 | 726.9 | 3.9 | 63.03 | |
| (pN/µm) | 24 hr | | 25030 | 208.86 | 568.45 | 3.593 | 49.33 | 0 |
| | 48 hr | | 39562 | 186.82 | 497.79 | 2.502 | 47.003 | 0 |
| | 72 hr | | 39910 | 231.81 | 696.72 | 3.487 | 55.39 | 0 |
| | 96 hr | | 11450 | 160.36 | 448.96 | 4.196 | 42.64 | 0 |
| **Figure 6C** | | | | | | | | |
| **Parameters** | **Condition** | **n** | **Mean** | **SD** | **SEM** | **Median** | **P value (wrt 2 hr)** | |
| | | | | | | | | |
| Myomerger | GM | 52 | 546 | 173 | 24.05 | 553 | 1.00E-06 | |
| TIRF | 2 hr | 50 | 400 | 94.15 | 13.31 | 395 | | |
| | 24 hr | 52 | 312 | 96.15 | 13.33 | 313 | 4.00E-05 | |
| | 48 hr | 51 | 444 | 132 | 18.55 | 431 | 0.10969 | |
| | 72 hr | 53 | 906 | 527 | 72.47 | 788 | 5.00E-09 | |
| | 96 hr | 51 | 766 | 512 | 71.75 | 668 | 0.003 | |
| | Myotubes | 20 | 1149 | 607 | 135.94 | 1169 | 2.00E-06 | |
| **Figure 7B and 7C** | | | | | | | | |
| **Parameters** | **Condition** | **n** | **Mean** | **SD** | **SEM** | **Median** | **P value** | |
| Myomerger Intensity (au) | 2 hr | 32 | 203 | 35.44 | 6.26 | 202 | | |
| | 24 hr | 31 | 375 | 183 | 33.01 | 357 | 5.00E-04 | |
| Tension (pN/µm) | 2 hr | 32 | 44.19 | 24.26 | 4.29 | 38.89 | | |
| | 24 hr | 31 | 30.79 | 13.98 | 2.51 | 27.53 | 0.0025 | |
| Myomerger intensity (au) | 48 hr | 38 | 497 | 241 | 39.24 | 443 | | |
| | 72 hr | 25 | 1076 | 947 | 189 | 755 | 6E-4 (wrt 48 hr) | |
| | | | | | | | | |
| | Myotubes | 10 | 818 | 859 | 271 | 504 | 0.15885 (wrt 48 hr) | |
| Tension (pN/µm) | 48 hr | 38 | 44.7 | 61.89 | 10.04 | 31.76 | | |
| | 72 hr | 25 | 67.86 | 63.12 | 12.62 | 49.09 | 0.0014 (wrt 48 hr) | |
| | Myotubes | 10 | 45.27 | 14.44 | 4.57 | 44.67 | 0.038 (wrt 48 hr) | |
| **Figure 8B (left)** | | | | | | | | |
| **Parameters** | **Condition** | **$N_{cells}$** | **Mean** | **SD** | **SEM** | **Median** | **P value** | |
| | | | | | | | **(wrt control)** | |
| Intensity (au) | Control_2 hr | 11 | 135 | 8.05 | 2.43 | 133.79 | | |
| | MβCD_2 hr | 9 | 173 | 32.57 | 10.85 | 173.34 | 0.001 | |
| | Control_24 hr | 10 | 212 | 24.26 | 7.67 | 215.38 | | |
| | MβCD_24 hr | 8 | 235 | 33.43 | 11.82 | 241.92 | ns | |
| **Figure 8B (right)** | | | | | | | | |
| **Parameters** | **Condition** | **$N_{cells}$** | **Mean** | **SD** | **SEM** | **Median** | **P value** | |
| | | | | | | | **(wrt control)** | |
| Tension (pN/µm) | Control_2 hr | 11 | 49.76 | 19.34 | 5.83 | 45.89 | | |
| | MβCD_2 hr | 9 | 75.98 | 30.49 | 10.16 | 70.03 | 0.02 | |
| | Control_24 hr | 10 | 45.16 | 32.35 | 10.23 | 33.35 | | |
| | MβCD_24 hr | 8 | 46.69 | 18.39 | 6.5 | 42.03 | ns | |

| Figure 8G | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Parameters** | **Condition** | **$N_{clusters}$** | **$n_{rois}$** | **Mean** | **SD** | **SEM** | **Median** | **P value** |
| | | | | | | | | **(wrt 2 hr)** |
| Cluster ratio | 2 hr | 90 | 10 | 1.56 | 0.17 | 0.02 | 1.53 | |
| | 24 hr | 100 | 10 | 1.51 | 0.15 | 0.01 | 1.5 | ns |
| | 48 hr | 100 | 10 | 1.54 | 0.19 | 0.02 | 1.51 | ns |
| | 72 hr | 108 | 10 | 1.5 | 0.16 | 0.01 | 1.5 | 0.001 |
| | 96 hr | 150 | 10 | 1.48 | 0.16 | 0.01 | 1.48 | 2.00E-16 |
| **Figure 8H** | | | | | | | | |
| **Parameters** | **Condition** | **$N_{clusters}$** | **$n_{rois}$** | **Mean** | **SD** | **SEM** | **Median** | **P value** |
| | | | | | | | | **(wrt 2 hr)** |
| Cluster numbers | 2 hr | 90 | 10 | 1.04 | 0.21 | 0.022 | 1.04 | |
| | 24 hr | 100 | 10 | 0.99 | 0.32 | 0.031 | 1.02 | ns |
| | 48 hr | 100 | 10 | 0.91 | 0.24 | 0.024 | 0.93 | 6.00E-05 |
| | 72 hr | 110 | 10 | 0.72 | 0.26 | 0.025 | 0.76 | 0.001 |
| | 96 hr | 150 | 10 | 0.73 | 0.24 | 0.02 | 0.75 | |
| | Myotubes | 80 | 10 | 0.33 | 0.29 | 0.032 | 0.27 | 2.00E-16 |
| **Figure 8J (right)** | | | | | | | | |
| **Parameters** | **Condition** | **$N_{clusters}$** | **$n_{rois}$** | **Mean** | **SD** | **SEM** | **Median** | **P value** |
| | | | | | | | | **(wrt control)** |
| Cluster numbers | Control_2 hr | 30 | 10 | 0.43 | 0.15 | 0.03 | 0.43 | |
| | MβCD_2 hr | 30 | 10 | 0.34 | 0.22 | 0.04 | 0.31 | ns |
| | Control_24 hr | 39 | 10 | 1.31 | 0.07 | 0.01 | 1.29 | |
| | MβCD_24 hr | 49 | 10 | 1.24 | 0.08 | 0.01 | 1.23 | 5.00E-07 |
| **Figure 8J (left)** | | | | | | | | |
| **Parameters** | **Condition** | **$N_{clusters}$** | **$n_{rois}$** | **Mean** | **SD** | **SEM** | **Median** | **P value** |
| | | | | | | | | **(wrt control)** |
| Cluster ratio | Control_2 hr | 29 | 10 | 1.36 | 0.09 | 0.01 | 1.35 | |
| | MβCD_2 hr | 28 | 10 | 1.24 | 0.07 | 0.01 | 1.23 | 2.00E-06 |
| | Control_24 hr | 39 | 10 | 1.31 | 0.07 | 0.01 | 1.29 | |
| | MβCD_24 hr | 49 | 10 | 1.24 | 0.08 | 0.01 | 1.23 | 1.00E-08 |
| **Figure S2B** | | | | | | | | |
| **Parameters** | **Condition** | **n** | **Mean** | **SD** | **SEM** | **Median** | **P value (wrt 2 hr)** | |
| | | | | | | | | |
| Fusion Index | Myotubes | 50 | 30.75 | 13.27 | 1.88 | 28.23 | | |
| **Figure S3** | | | | | | | | |
| **Parameters** | **Condition** | **n** | **Mean** | **SD** | **SEM** | **Median** | **P value (wrt 2 hr)** | |
| | | | | | | | | |
| **Figure S3B** | | | | | | | | |
| Exponent | 2 hr | 42815 | -1.58 | 0.33 | 0.0016 | -3.55 | | |
| | 96 hr (U) | 40148 | -1.52 | 0.34 | 0.0017 | -2.86 | 3.00E-162 | |
| | 96 hr (D) | 85129 | -1.55 | 0.25 | 0.0008 | -2.86 | 2.00E-77 | |
| **Figure S3C** | | | | | | | | |
| γ ($N/m^3$) | 2 hr | 14269 | 0.112 | 0.149 | 0.0012 | 0.073 | | |
| | 96 hr (U) | 20090 | 0.133 | 0.175 | 0.0012 | 0.089 | 6.00E-18 | |
| | 96 hr (D) | 35041 | 0.151 | 0.199 | 0.001 | 0.1 | 5.00E-60 | |
| **Figure S3D** | | | | | | | | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| $\eta_{eff}$ (Pa.s) | 2 hr | 14269 | 3560 | 7261 | 60.79 | 1676 | | |
| | 96 hr (U) | 20090 | 4138 | 8249 | 58.2 | 1886 | 7.00E-14 | |
| | 96 hr (D) | 35041 | 4981 | 12998 | 69.44 | 1969 | 3.00E-40 | |
| **Figure S3E** | | | | | | | | |
| A | 2 hr | 14269 | 2.81 | 3.01 | 0.025 | 1.28 | | |
| | 96 hr (U) | 20090 | 2.57 | 2.94 | 0.02 | 1.06 | 0 | |
| | 96 hr (D) | 35041 | 2.45 | 2.89 | 0.015 | 1.01 | 0 | |
| **Figure S3F** | | | | | | | | |
| $R^2$ | 2 hr | 14269 | 0.96 | 0.046 | 4.00E-04 | 0.98 | | |
| | 96 hr (U) | 20090 | 0.96 | 0.05 | 4.00E-04 | 0.98 | 0 | |
| | 96 hr (D) | 35041 | 0.96 | 0.05 | 3.00E-04 | 0.98 | 0 | |
| **Figure S4B** | | | | | | | | |
| **Parameters** | **Condition** | **n** | **Mean** | **SD** | **SEM** | **Median** | **P value (wrt 2 hr)** | |
| | **(Finally differentiated)** | | | | | | | |
| A | 2 hr | 33007 | 3.08 | 3.14 | 0.017 | 1.38 | | |
| | 24 hr | 24452 | 3.5 | 3.16 | 0.02 | 2.17 | 3.00E-99 | |
| | 48 hr | 22179 | 3.01 | 3.08 | 0.021 | 1.36 | 0.07 | |
| | 72 hr | 35653 | 2.93 | 3.07 | 0.016 | 1.19 | 9.00E-12 | |
| | 96 hr | 40465 | 2.8 | 3.03 | 0.015 | 1.09 | 0 | |
| | **(Finally undifferentiated)** | | | | | | | |
| A | 2 hr | 34726 | 2.88 | 3.02 | 0.016 | 1.2 | | |
| | 24 hr | 25030 | 3.29 | 3.16 | 0.02 | 1.72 | 3.00E-64 | |
| | 48 hr | 39562 | 3.29 | 3.14 | 0.016 | 1.81 | 1.00E-95 | |
| | 72 hr | 38873 | 2.95 | 3.03 | 0.015 | 1.34 | 2.00E-04 | |
| | 96 hr | 11450 | 3.33 | 3.07 | 0.029 | 2.01 | 9.00E-75 | |
| **Figure S4C** | | | | | | | | |
| **Parameters** | **Condition** | **n** | **Mean** | **SD** | **SEM** | **Median** | **P value (wrt 2 hr)** | |
| | **(Finally differentiated)** | | | | | | | |
| $\gamma$ ($N/m^3$) | 2 hr | 33007 | 3.08 | 3.14 | 0.017 | 1.38 | | |
| | 24 hr | 24452 | 3.5 | 3.16 | 0.02 | 2.17 | 3.00E-99 | |
| | 48 hr | 22179 | 3.01 | 3.08 | 0.021 | 1.36 | 0.07 | |
| | 72 hr | 35653 | 2.93 | 3.07 | 0.016 | 1.19 | 9.00E-12 | |
| | 96 hr | 40465 | 2.8 | 3.03 | 0.015 | 1.09 | 0 | |
| **Parameters** | **Condition** | **n** | **Mean** | **SD** | **SEM** | **Median** | **P value (wrt 2 hr)** | |
| | **(Finally undifferentiated)** | | | | | | | |
| $\gamma$ ($N/m^3$) | 2 hr | 34726 | 2.88 | 3.02 | 0.016 | 1.2 | | |
| ($X10^{10}$) | 24 hr | 25030 | 3.29 | 3.16 | 0.02 | 1.72 | 3.00E-64 | |
| | 48 hr | 39562 | 3.29 | 3.14 | 0.016 | 1.81 | 1.00E-95 | |
| | 72 hr | 38873 | 2.95 | 3.03 | 0.015 | 1.34 | 2.00E-04 | |
| | 96 hr | 11450 | 3.33 | 3.07 | 0.029 | 2.01 | 9.00E-75 | |
| **Figure S4D** | | | | | | | | |
| **Parameters** | **Condition** | **n** | **Mean** | **SD** | **SEM** | **Median** | **P value (wrt 2 hr)** | |
| | **(Finally differentiated)** | | | | | | | |
| $\eta_{eff}$ (Pa.s) | 2 hr | 33007 | 3194 | 8422 | 46.36053 | 1351.2 | | |
| | 24 hr | 24452 | 2081 | 5105 | 32.64666 | 1051.05 | 0 | |
| | 48 hr | 22179 | 2587 | 5088 | 34.17101 | 1265.9 | 4.00E-13 | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 72 hr | 35653 | 4066 | 12227 | 64.75784 | 1427.9 | 9.00E-12 | |
| | 96 hr | 40465 | 3177 | 7797 | 38.76384 | 1340.6 | ns | |
| | **(Finally undifferentiated)** | | | | | | | |
| $\eta_{eff}$ (Pa.s) | 2 hr | 34726 | 3958 | 10257 | 55.04 | 1461 | | |
| | 24 hr | 25030 | 2475 | 7053 | 44.58 | 1119 | 0 | |
| | 48 hr | 39562 | 2420 | 5528 | 27.79 | 1156 | 0 | |
| | 72 hr | 38873 | 3387 | 7821 | 39.67 | 1427 | 0.01 | |
| | 96 hr | 11450 | 2318 | 4760 | 44.49 | 1137 | 0 | |

## Supplementary References


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