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Supplemental Information

Local Arrangement of Fibronectin by Myofibroblasts Governs Peripheral Nuclear Positioning in Muscle Cells

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Figure S1

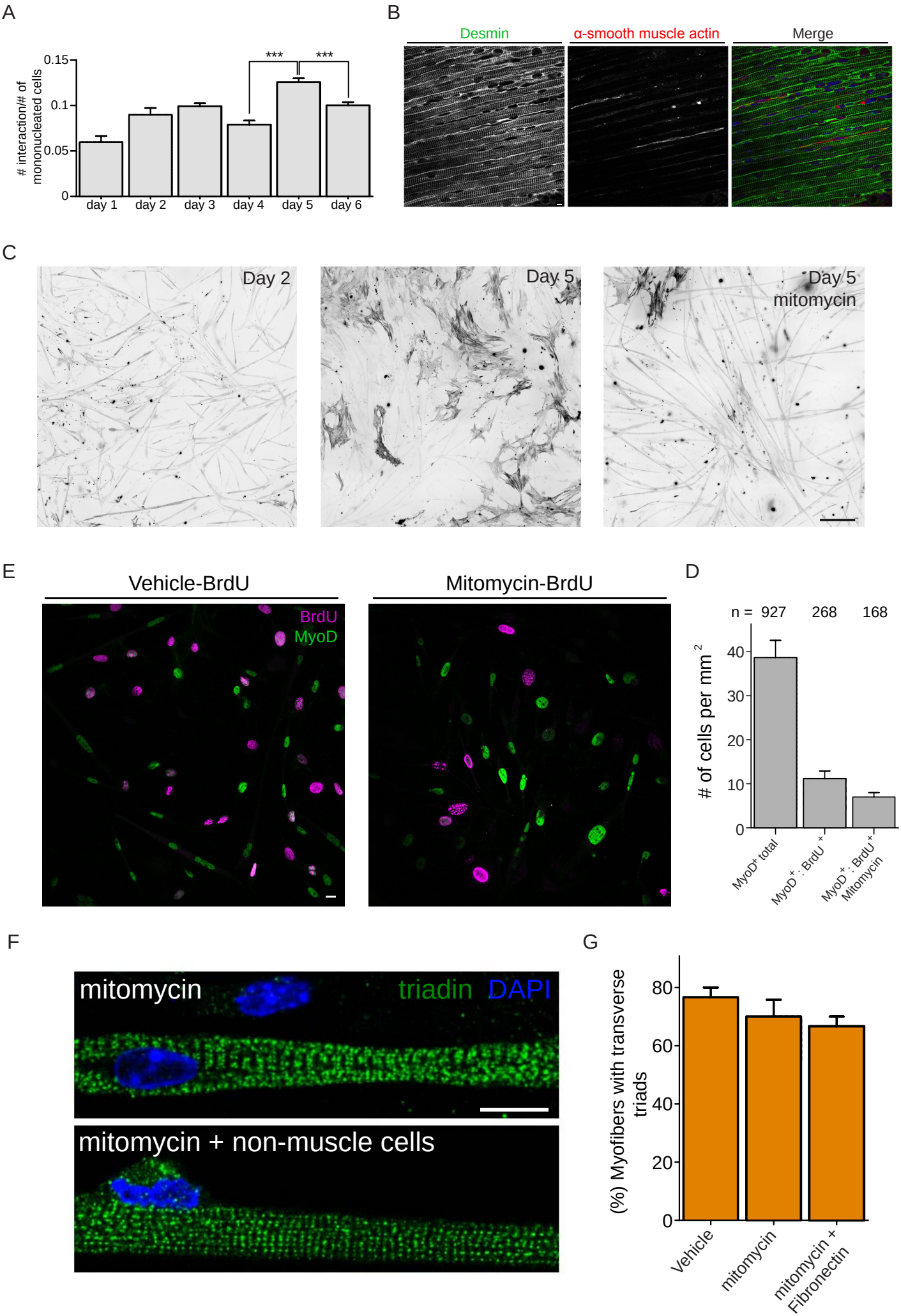
A
interaction/# of mononucleated cells
0.15
0.1
0.05
0
***
***
day 1
day 2
day 3
day 4
day 5
day 6
B
Desmin
α-smooth muscle actin
Merge
C
Day 2
Day 5
Day 5 mitomycin
E
Vehicle-BrdU
Mitomycin-BrdU
BrdU
MyoD
D
n = 927 268 168
# of cells per mm 2
40
30
20
10
0
MyoD+ total
MyoD+ : BrdU+
MyoD+ : BrdU+ Mitomycin
F
mitomycin
triadin DAPI
mitomycin + non-muscle cells
G
(%) Myofibers with transverse triads
80
60
40
20
0
Vehicle
mitomycin
mitomycin + Fibronectin

Figure S1, related to Figure 1.

A. Quantification of number of interaction between myotubes and non-muscle cells per hour normalized on number of mononucleated cells during muscle development. Data from 3 independent experiments with n = 19 myotubes quantified. Error bars correspond to s.e.m.

B. Representative immunofluorescence image of newborn mice muscle using a clearing protocol and stained for desmin (green), α-smooth muscle actin (myofibroblasts, red) and DAPI (nucleus, blue). Yellow arrowheads indicate mononucleated myoblasts in between myofibers. Scale bar, 10μm.

C. Representative inverted immunofluorescence image of cultures at day 2, 5 or cultures at day 5 treated with mitomycin, and stained for α-smooth muscle actin (myofibroblasts). Scale bar, 200μm.

D. Representative immunofluorescence image of 1-day cultures treated with vehicle or mitomycin and stained for BrdU (proliferation, magenta) and MyoD (muscle cells, green). Scale bar, 10μm.

E. Quantification of mononucleated cells per mm^2 positive for MyoD alone and for BrdU in vehicle and mitomycin treated cultures. Data collected from 3 independent experiments with at least 12 areas of 322 mm^2 for each condition.

F. Representative immunofluorescence image of 10-day myofibers treated with mitomycin or mitomycin supplemented with non-muscle cells and stained for transverse triads (triadin, green) and DAPI (nucleus, blue). Scale bar, 10μm.

G. Quantification of transverse triad organization in 10-day myofibers from cultures treated with mitomycin or mitomycin supplemented with non-muscle cells. Data from 3 independent experiments were combined and error bars represent s.e.m from 30 myofibers for each cohort. Unpaired t-test was used to determine statistical significances.

Figure S2

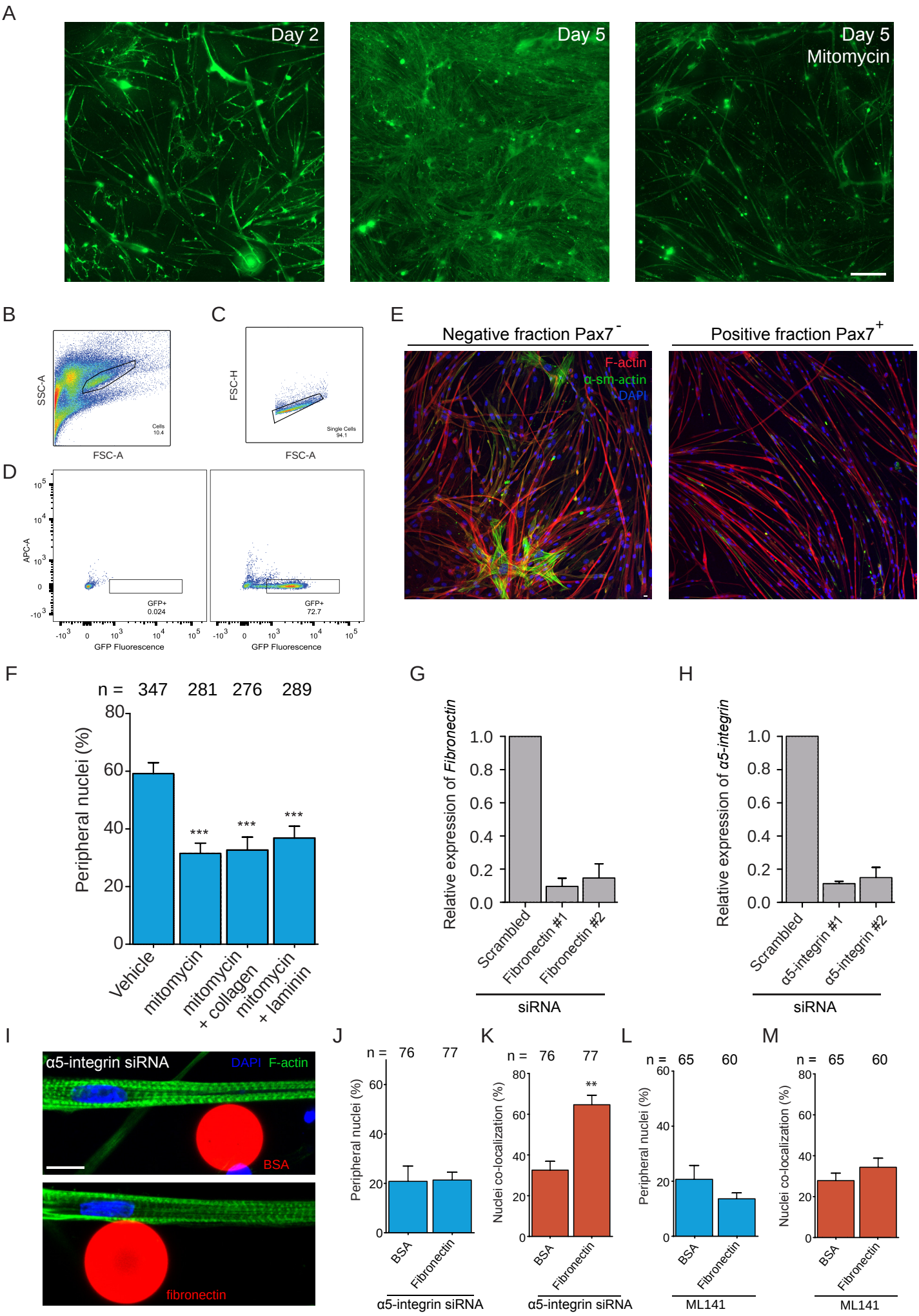
A
Day 2
Day 5
Day 5
Mitomycin
B
SSC-A
FSC-A
Cells
10.4
C
FSC-H
FSC-A
Single Cells
94.1
D
APC-A
GFP+
0.024
GFP+
72.7
GFP Fluorescence
GFP Fluorescence
E
Negative fraction Pax7-
Positive fraction Pax7+
F-actin
α-sm-actin
DAPI
F
n = 347 281 276 289
Peripheral nuclei (%)
Vehicle
mitomycin
mitomycin + collagen
mitomycin + laminin
G
Relative expression of Fibronectin
Scrambled
Fibronectin #1
Fibronectin #2
siRNA
H
Relative expression of α5-integrin
Scrambled
α5-integrin #1
α5-integrin #2
siRNA
I
α5-integrin siRNA
DAPI F-actin
BSA
fibronectin
J
n = 76 77
Peripheral nuclei (%)
BSA
Fibronectin
α5-integrin siRNA
K
n = 76 77
Nuclei co-localization (%)
BSA
Fibronectin
α5-integrin siRNA
L
n = 65 60
Peripheral nuclei (%)
BSA
Fibronectin
ML141
M
n = 65 60
Nuclei co-localization (%)
BSA
Fibronectin
ML141

Figure S2, related to Figure 2 and 3.

A. Representative immunofluorescence image of 10-day myofibers generated from *Pax7-nGFP* mice and sorted as a negative (left) and positive (right) fraction. Fibronectin was added to the positive fraction. Cultures were stained for F-actin (phalloidin, red), α-smooth muscle actin (myofibroblasts, green) and DAPI (nucleus, blue). Scale bar, 10μm.

B. SSC-A/FCS-A gate was used to sort purified cells according to cell size. SSC-A, side scatter area; FCS-A, forward scatter area.

C. FCS-H/FCS-A gate was used to exclude possible doublets. FCS-H, forward scatter height; FCS-A, forward scatter area.

D. Representative FACS profiles of unmarked cells (Left) from *wt* mice and GFP positive cells (right) from *Pax7-nGFP* mice. GFP selection gate represented for both populations. Values on plots represent percentage of sorted GFP positive cells from singlet population.

E. Representative immunofluorescence image of cultures at day 2, 5 or cultures at day 5 treated with mitomycin and stained for fibronectin (green). Scale bar, 200μm.

F. Quantification of peripheral nuclei in 10-day myofibers treated with vehicle, mitomycin or mitomycin supplemented with collagen or laminin. Data from 3 independent experiments were combined and error bars represent s.e.m from indicated *n* nuclei for each cohort. Unpaired t-test was used to determine statistical significances, where * $P < 0.05$, *** $P < 0.001$.

G. Relative expression levels of α5-integrin mRNA in day 2 myotubes knocked down for scrambled or α5-integrin. Data from 3 independent experiments were combined for each condition. Error bars represent the s.e.m.

H. Relative expression levels of Fibronectin mRNA in day 2 myotubes knocked down for scrambled or fibronectin. Data from 3 independent experiments were combined for each condition. Error bars represent the s.e.m.

I. Representative immunofluorescence z-projection images of 10-day myofibers knocked down for α5-integrin and cultured with 20 μm beads coated with either BSA or fibronectin and stained for F-actin (myofibrils, green) and DAPI (nucleus, blue). Scale bar, 10μm.

J. Quantification of number of peripheral nuclei from 10-day myofibers knocked down for α5-integrin and in contact with BSA or fibronectin coated beads. Data from 3 independent experiments were combined and error bars represent s.e.m from indicated *n* nuclei for each cohort. Unpaired t-test was used to determine statistical significances.

K. Quantification of number of nuclei from 10-day myofibers knocked down for α5-integrin in proximity to BSA or fibronectin coated beads. Data from 3 independent experiments were combined and error bars represent s.e.m from indicated *n* nuclei for each cohort. Unpaired t-test was used to determine statistical significances, where ** $P < 0.01$.

L. Quantification of number of peripheral nuclei from 10-day myofibers treated with ML141 and in contact with BSA or fibronectin coated beads. Data from 3 independent experiments were combined and error bars represent s.e.m from indicated *n* nuclei for each cohort. Unpaired t-test was used to determine statistical significances.

M. Quantification of number of nuclei from 10-day myofibers treated with ML141 in proximity to BSA or fibronectin coated beads. Data from 3 independent experiments were combined and error bars represent s.e.m from indicated *n* nuclei for each cohort. Unpaired t-test was used to determine statistical significances.

Figure S3

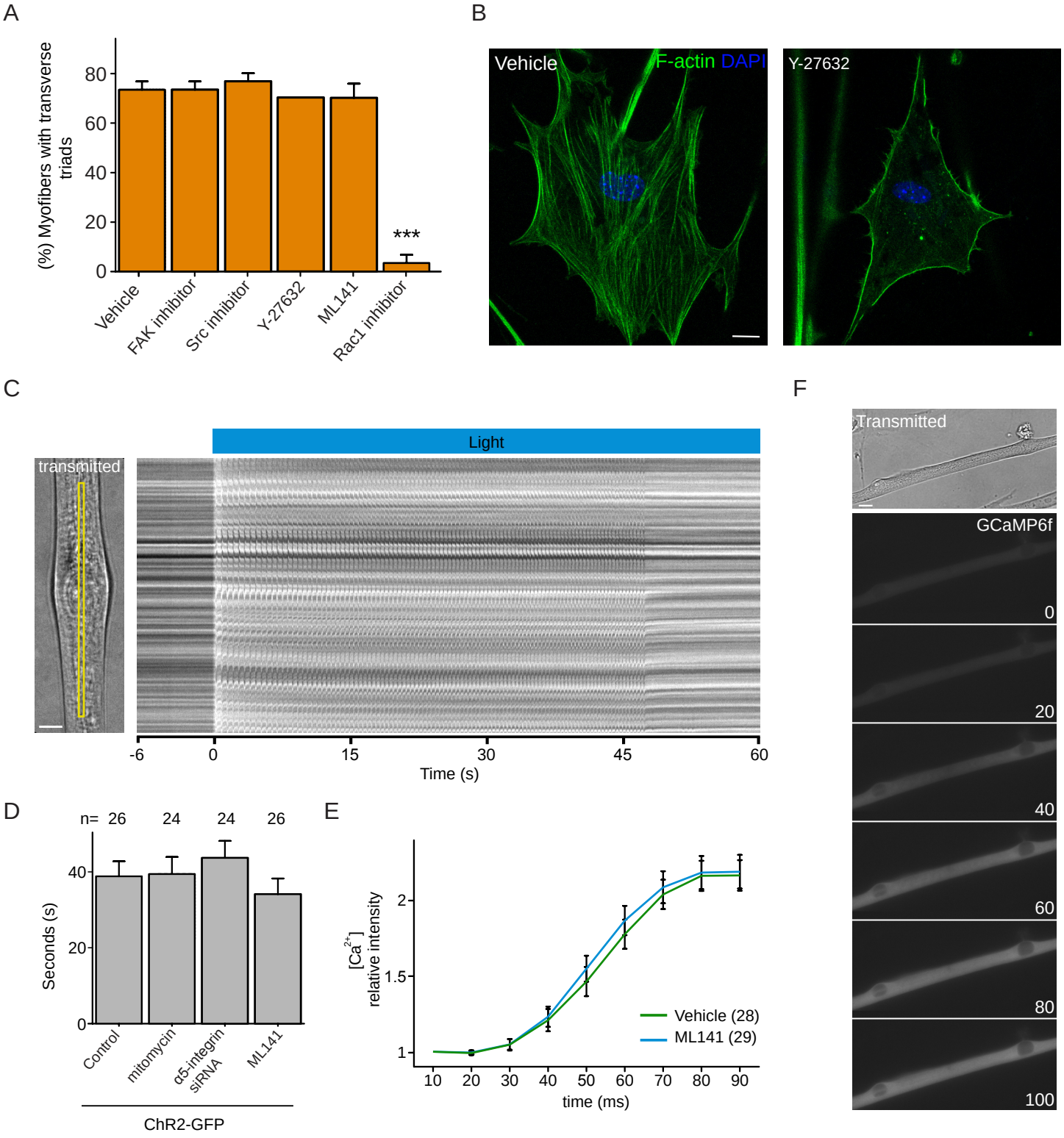
A
(%) Myofibers with transverse triads
80
60
40
20
0
***
Vehicle
FAK inhibitor
Src inhibitor
Y-27632
ML141
Rac1 inhibitor
B
Vehicle
F-actin DAPI
Y-27632
C
transmitted
Light
-6
0
15
30
45
60
Time (s)
D
n= 26
24
24
26
Seconds (s)
40
20
0
Control
mitomycin
α5-integrin siRNA
ML141
ChR2-GFP
E
[Ca2+] relative intensity
2
1.5
1
10
20
30
40
50
60
70
80
90
time (ms)
Vehicle (28)
ML141 (29)
F
Transmitted
GCaMP6f
0
20
40
60
80
100

Figure S3, related to Figure 4.

A. Quantification of transverse triad organization in 10-day myofibers from cultures treated with vehicle, FAK inhibitor, Src inhibitor, Y-27632, ML141 or Rac1 inhibitor. Data from 3 independent experiments were combined and error bars represent s.e.m from 30 myofibers for each cohort. Unpaired t-test was used to determine statistical significances, where *** $P < 0.001$.

B. Representative immunofluorescence image of non-muscle cell treated with either vehicle or Y-27632 and stained for F-actin (stress fibers, green) and DAPI (nucleus, blue). Scale bar, 10μm.

C. Representative kymograph from the yellow rectangle of a myofiber overexpressing ChR2-GFP and induced to repeatedly contract. Scale bar, 10μm.

D. Quantification of duration of repeated contraction in myofibers treated with vehicle, mitomycin, ML141 or knocked down for α5-integrin and overexpressing ChR2-GFP and opotogenetically induced to contract. Data from 3 independent experiments were combined and error bars represent s.e.m from indicated *n* myofibers for each cohort. Unpaired t-test was used to determine statistical significances.

E. Quantification of relative intensity in myofibers transfected with GCaMP6f and optogenetically induced to contract. Data from 3 independent experiments were combined and error bars represent s.e.m from indicated *n* myofibers for each cohort. Unpaired t-test was used to determine statistical significances.

F. Time lapse images of 4.5-day myofiber transfected with GCaMP6f (grey) and ChR2-GFP and exposed to blue light. Time in milliseconds. Scale bar, 10μm.

Figure S4

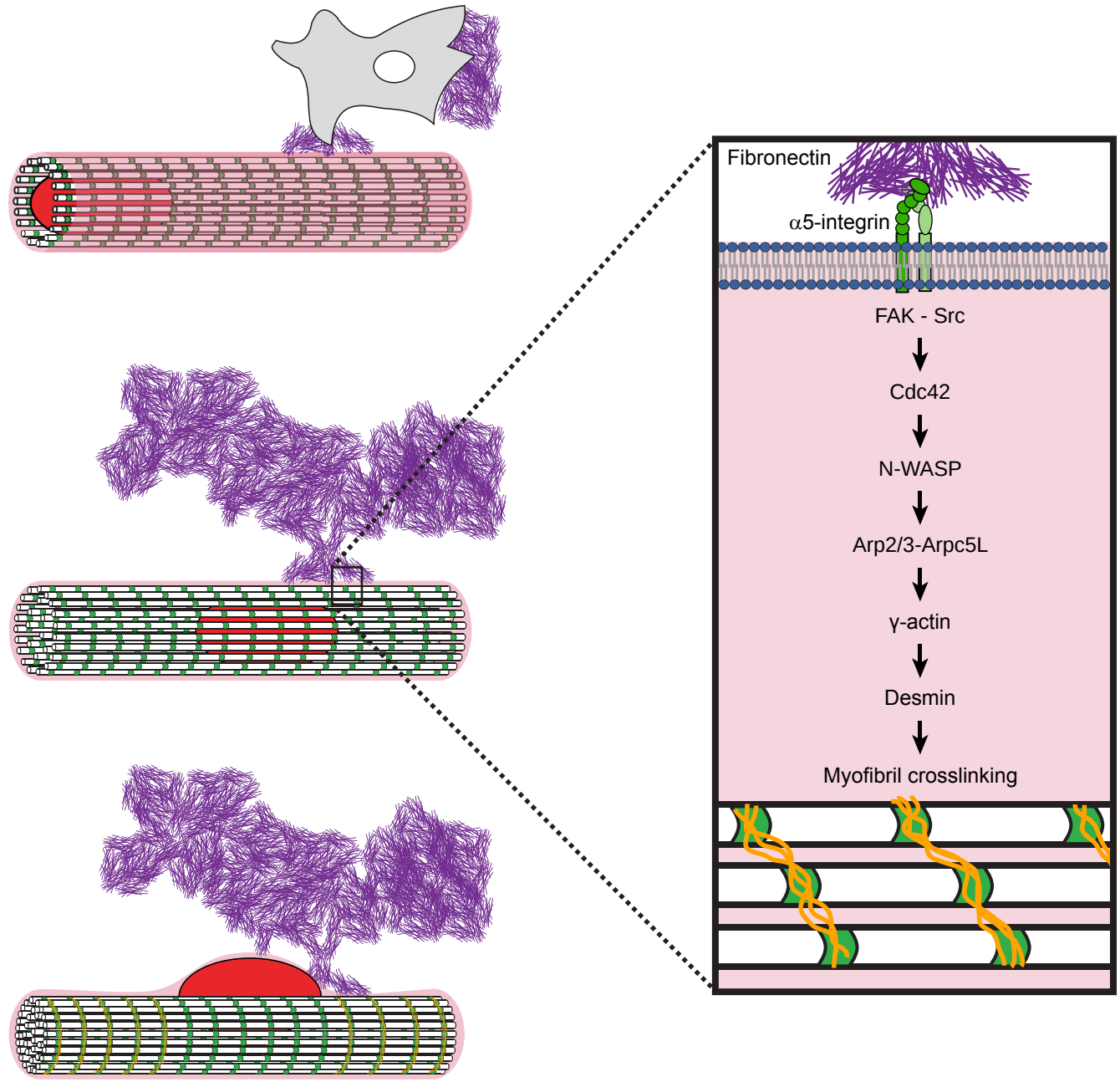
Fibronectin
α5-integrin
FAK - Src
Cdc42
N-WASP
Arp2/3-Arpc5L
γ-actin
Desmin
Myofibril crosslinking

Figure S4, related to Figure 1, 2, 3 and 4.

Model depicting local fibronectin-mediated attraction of nuclei to the periphery of myofibers. The left panel illustrates how myofibroblasts interact with developing myotubes and deposit fibronectin at their surface triggering nuclei to migrate to the fibronectin site and move to the periphery. This mechanism is mediated by a fibronectin-Cdc42 pathway shown in the right panel.