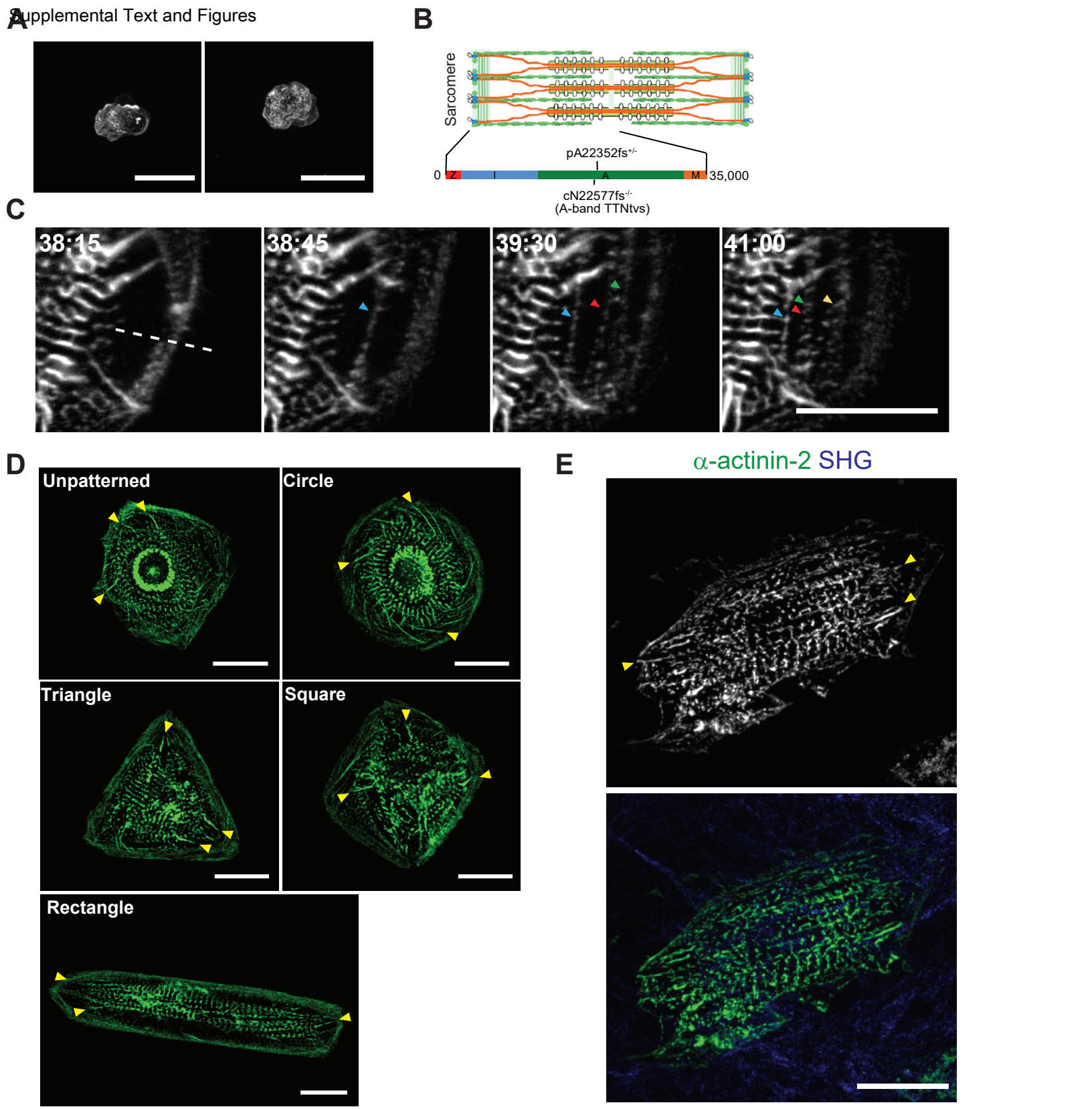

A
B
Sarcomere
pA22352fs+/-
0
Z
I
A
M
35,000
cN22577fs-/-
(A-band TTNtvs)
C
38:15
38:45
39:30
41:00
D
Unpatterned
Circle
Triangle
Square
Rectangle
E
α-actinin-2 SHG

Figure S1

Figure S1. Related to Figure 1. (A) Representative images of cardiomyocytes detached from the substrate using trypsin, fixed and stained for actinin, indicating sarcomere disassembly. Scale bars 20 µm. (B) Schematic representation of the cardiac sarcomere with actinin (light green), TTN (orange), thick filaments (white heads), and thin filaments (green). TTN protein segments (z-disc, red; I-band, blue; A-band, green; M-band, gold) with location of human (“p” patient-derived; “c” CRISRP/CAS9-generated) mutations. (C) Time lapse images of WT cells expressing GFP actinin, colored arrows indicate the appearance of transverse actinin fibers. Scale bar 20 µm. (D) Representative images of GFP actinin WT cells plated on unpatterned or fibronectin patterned islands of various geometries displaying centripetal fibers as indicated by yellow arrows. Scale bars 20 µm. (E) (Top) representative image of a cardiomyocyte in a 3D cardiac microtissues (collagen: SHG, second harmonic generation) in engineered heart microtissues, arrows indicate the appearance of centripetal actinin fibers (green). Scale bars 20µm.

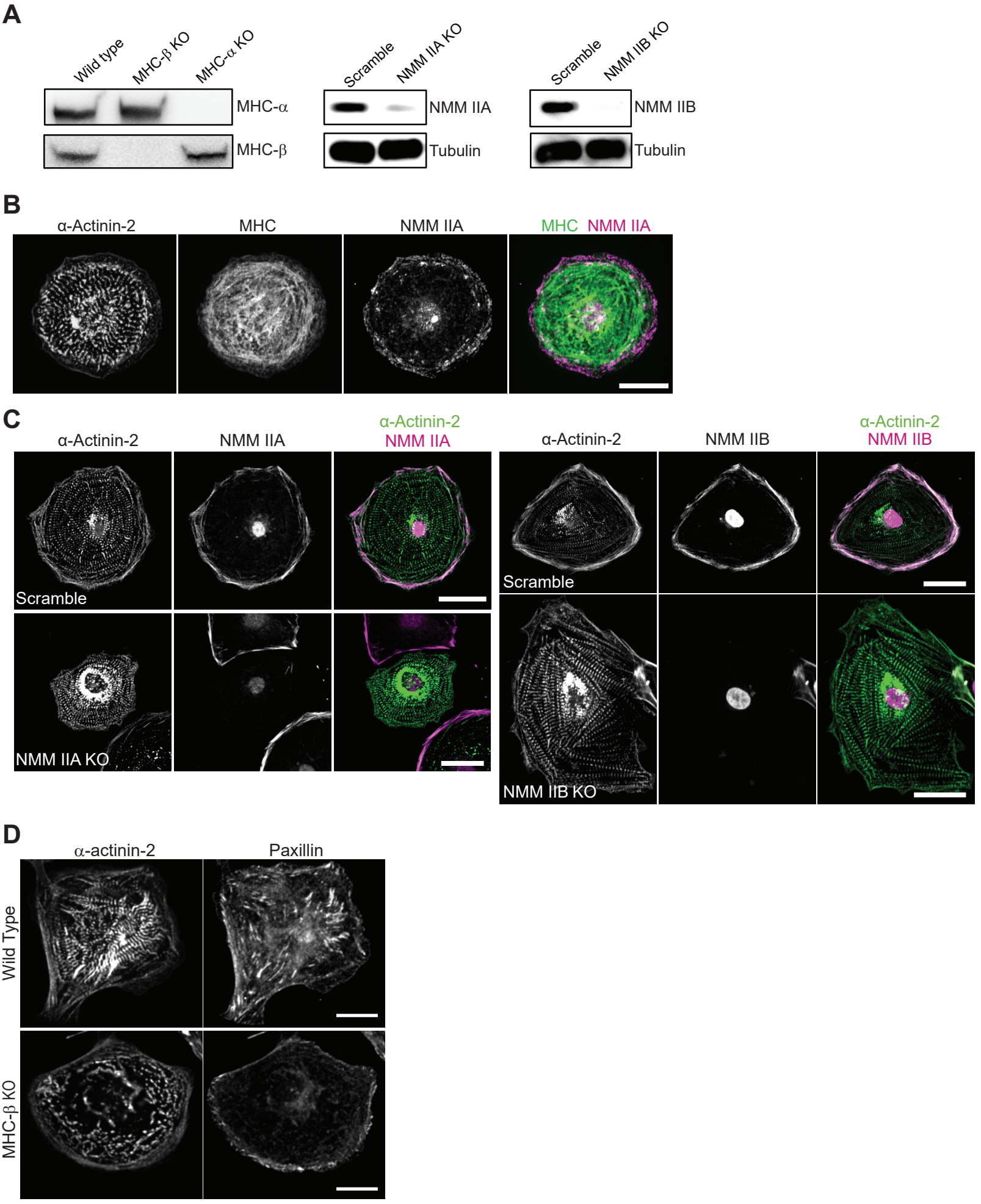

A
Wild type
MHC-β KO
MHC-α KO
MHC-α
MHC-β
Scramble
NMM IIA KO
NMM IIA
Tubulin
Scramble
NMM IIB KO
NMM IIB
Tubulin
B
α-Actinin-2
MHC
NMM IIA
MHC NMM IIA
C
α-Actinin-2
NMM IIA
α-Actinin-2
NMM IIA
Scramble
NMM IIA KO
α-Actinin-2
NMM IIB
α-Actinin-2
NMM IIB
Scramble
NMM IIB KO
D
α-actinin-2
Paxillin
Wild Type
MHC-β KO

Figure S2

Figure S2. Related to Figure 3. (A) Western blots (left to right) of WT, MHC-α, MHC-β KO, NMM IIA and NMM IIB knock out cells normalized for protein loading, and probed with antibodies to MHC-α, MHC-β, NMM IIA and NMM IIB. (B) Image of cardiomyocyte patterned on a circular fibronectin island and stained for (left to right) actinin, myosin heavy chain (pan MHC), NMM IIA, showing the differential localization of the myosins. Scale Bar 20 μm. (C) (left) Representative image of scramble (top) and NMM IIA KO (bottom) cells fixed and stained for actinin and NMM IIA. Scale bars 20 μm. (right) Representative image of scramble (top) and NMM IIB KO (bottom) cells fixed and stained for actinin and NMM IIB using an Alexa Fluor568 direct conjugation. Scale bars 20 μm. (D) Top and bottom panel (left to right) representative images of cells stained for actinin and paxillin in wild type and MHC-β KO cells. Scale bars 20 μm

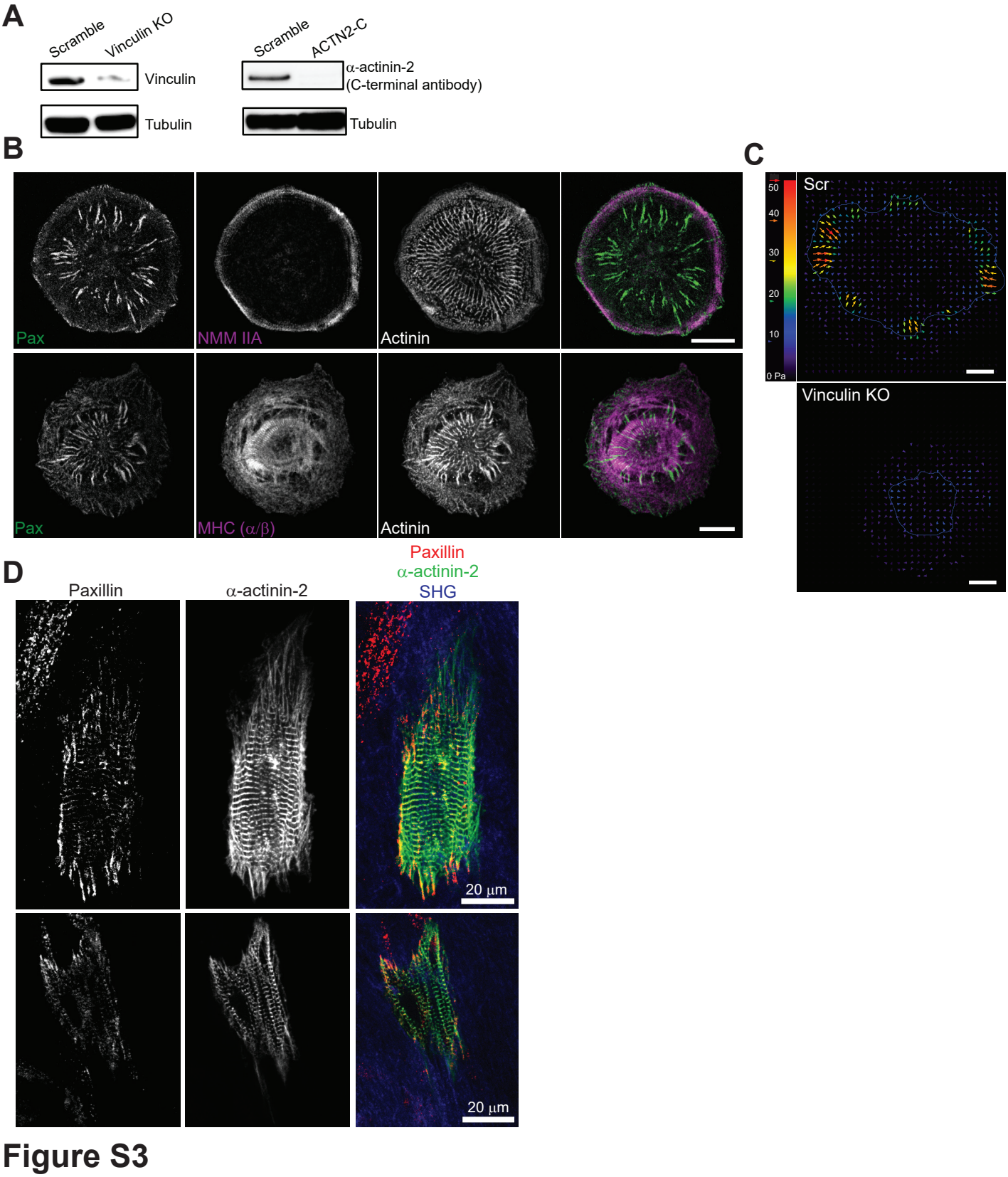
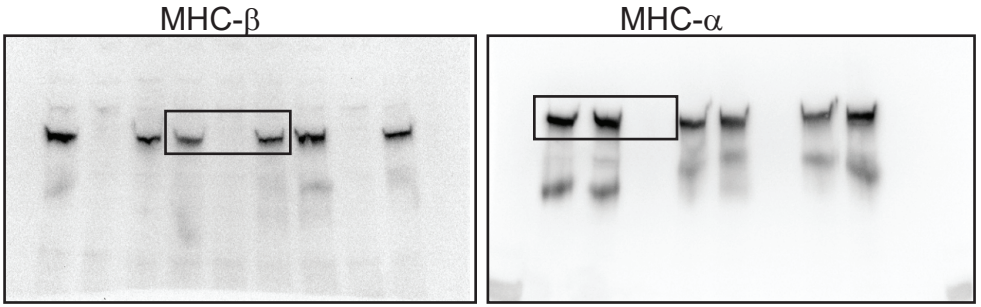

A
Scramble
Vinculin KO
Vinculin
Tubulin
Scramble
ACTN2-C
α-actinin-2
(C-terminal antibody)
Tubulin
B
Pax
NMM IIA
Actinin
Pax
MHC (α/β)
Actinin
C
Scr
50
40
30
20
10
0 Pa
Vinculin KO
D
Paxillin
α-actinin-2
Paxillin
α-actinin-2
SHG
20 μm
20 μm

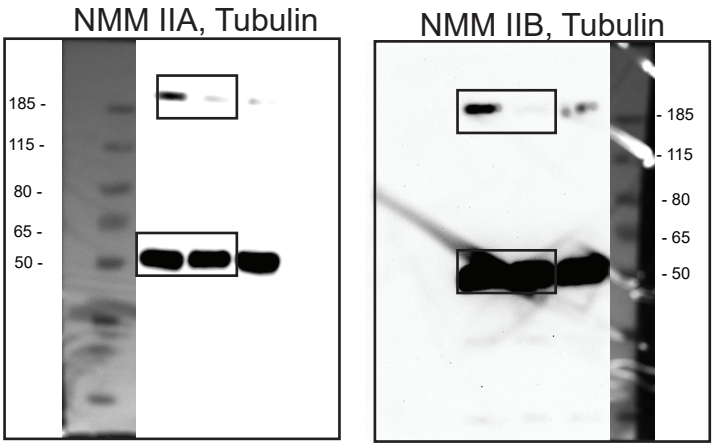
Figure S3

Figure S3. Related to Figure 4. (A) Western blots (left to right) of Scramble, vinculin KO and ACTN2-C KO cells normalized for protein loading, and probed with antibodies to vinculin and actinin-2 (c terminal). (B) Cardiomyocytes patterned on circular fibronectin islands fixed and stained for paxillin (green), NMM IIA (purple, top panel), muscle myosin (pan-MHC, purple, bottom panel) and actinin (white) show the existence of muscle myosin, but not non-muscle myosin, in centripetal fibers and protocostameres. Scale bar 20 µm. (C) Representative stress maps of wild type and vinculin KO cells. Scale bars 20 µm. (D) Top and bottom panel (left to right) representative images of cardiomyocytes within 3D engineered cardiac microtissues stained for actinin (green) and paxillin (red). Collagen visualized by second harmonic generation (blue, SHG). Scale Bar 20 µm.

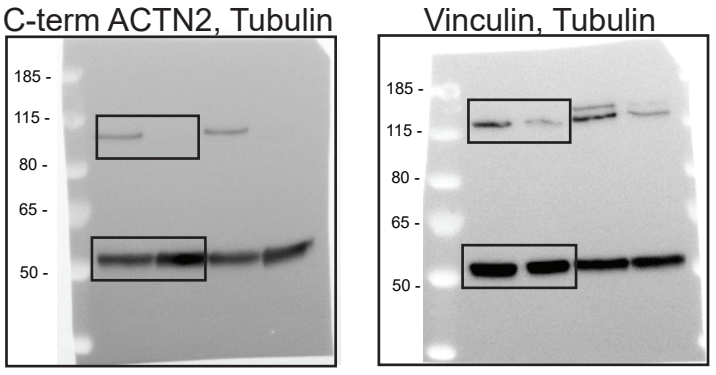
Suppl. Fig. 2A



Suppl. Fig. 2A



Suppl. Fig. 3A



Supplementary Figure 4

Figure S4. Related to Figures 3 and 4. Uncropped Western blot images.

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