SUPPLEMENTAL MATERIAL

Supplemental Methods

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HiPSC culture

The WTC11 hiPSC line[1] was generously provided by Dr. Bruce Conklin (Gladstone Institute, UCSF) and maintained on plates coated with Matrigel (Corning) in mTeSR1 media (STEMCELL Technologies). When cells reached 70-80% confluency, they were dissociated using 0.48mM EDTA in PBS warmed to 37°C and replated in media containing 10μM Y-27632 ROCK Inhibitor (Tocris).

HiPSC Gene Editing

For *TTN* Exons 2 and 326, gRNA were ligated into vector PX459v2 (Cas9-2A-Puro)[2] and 300,000 WTC hiPSC were transfected with 1μg plasmid using GeneJuice (EMD Millipore) during replating, and selected with 0.5μg/mL puromycin (ThermoFisher) for 2 days beginning the day after transfection. After selection, cells were replated to grow as single-cell colonies. Colonies were screened for non-homologous end joining by amplifying the region around the targeted sgRNA site from 100-300ng genomic DNA using GoTaq Flexi DNA polymerase (Promega) following manufacturer's guidelines and primers listed in Table S2. Following visualization by electrophoresis on a 0.8% agarose gel stained with ethidium bromide, PCR products were sequenced using the forward primer (Eurofins genetics). For Cronos targeting, sgRNA was ligated into PX458 (Cas9-2A-GFP)[2] and 1 million WTC cells were transfected with 5μg of plasmid using Amaxa nucleofector (Human Stem Cell kit 2) in presence of ROCK inhibitor. The next day, cells were FACS sorted for the GFP positive population. Individual colonies were hand-picked several days later and plated into 96 well plates. DNA was extracted using Quick Extract DNA extraction solution (Epicentre#QE09050) and nested PCR was performed. The PCR product was purified using EXO-SAP enzyme (ThermoFisher) and sent for Sanger sequencing analysis (Genewiz). For off-target analysis, primers were designed using Primer-BLAST[3] and used to amplify the area surrounding the predicted off-target cut site using GoTaq Flexi DNA polymerase (Promega) and sequenced using the forward primer (see Table S3 for accession numbers and primers).

Cardiac differentiation

Cardiac differentiation was performed following a previously published protocol[4]. Briefly, cells were plated into a Matrigel-coated plate in mTeSR media containing 10μM Y-27632 ROCK Inhibitor and 1μM Chiron 99021 (Tocris) at a density of $1.3x10^5$ cells/cm^2. The following day (Day 0 of differentiation), the media was changed to RPMI media (Gibco) supplemented with 2% B-27 without insulin (Life Technologies), 1X Matrigel, and 100ng/mL Activin A (R&D Systems). 17 hours later, the media was changed to RPMI supplemented with 2% B-27 without insulin, 5ng/mL BMP4 (R&D Systems), and 1μM Chiron 99021. 2 days later, media was changed to RPMI supplemented with 2% B-27 without insulin and 1μM XAV939 (Tocris). Media was changed to RPMI supplemented with 2% B-27 without insulin two days later. Beginning 7 days after the start of differentiation, cells were maintained in RPMI supplemented with 2% B-27 with insulin (Life Technologies) and 1% penicillin/streptomycin (Invitrogen), and media was changed every 2-3 days.

Cell culture on nanopattern substrates

Nanopatterned coverslips were generated as previously described and coated by incubating in a 5μg/mL human fibronectin (Life Technologies) solution at 4°C overnight[5]. Cells were replated as 23-32 days after the start of differentiation at a density of $1.1x10^4$ cells/cm^2 and fixed and stained 27-31 days (for the day 30 group) or 53-63 days (for the day 60 group) later. For fixation, cells were relaxed in 150mM KCl for 5 minutes at room temperature and then fixed for 10 minutes at room temperature in 4% paraformaldehyde. Staining was performed as described below.

Human tissue immunohistochemistry

The custom Cronos titin antibody was used at a final concentration of 0.6μg/mL. Human fetal cardiac tissue samples were obtained from the Birth Defects Research Laboratory at the University of

Washington, and normal human adult left ventricular samples were obtained from the National Disease Research Interchange. All samples were fixed in 4% paraformaldehyde and paraffin embedded. Samples were rehydrated and underwent antigen retrieval by boiling in citrate buffer (pH=6.0) for 20 minutes and then stained as described below.

Immunostaining

Following three PBS washes, samples were blocked in 1.5% normal goat serum for 1 hour at room temperature. Primary antibodies were diluted in blocking buffer (antibody details in Table S4) and added to samples for overnight incubation at 4°C. The following day, samples were incubated in AlexaFluor-conjugated goat anti-mouse and anti-rabbit antibodies (Life Technologies, diluted 1:100) for 1 hour at room temperature and coverslipped using Vectashield with DAPI (Vector Laboratories).

ChIP data

For hPSC-CM data, ChIP data from ref [6] was mapped to human genome hg38 using BWA-MEM with default parameters. ChIP tracks were generated in IGV using normalized read to genomic content from deepTools Bamcoverage. Enriched regions were called with MACS2 broad peaks with default parameters. For fetal and adult tissue data, data was accessed and visualized on the human genome hg38 using ENCODE[7]. GEO accession numbers for samples analyzed are fetal heart: GSM772735; adult LV1: GSM910580; adult LV2: GSE101357.

Titin protein electrophoresis and Western blots

Pellets of human iPSC-derived cardiomyocytes (29-33 days after the start of differentiation) or small pieces of left ventricular tissue from adult human donor hearts were homogenized in a modified Laemmli buffer containing 8 M urea, 2 M thiourea, 3% (w/v) sodium dodecyl sulfate (SDS), 75 mM DTT, 0.03%

(w/v) bromophenol blue, 10% (v/v) glycerol, and 50 mM Tris-HCl, pH 6.8, heated for 3 minutes at 96°C, and centrifuged at 14,000 rpm[8]. Samples were loaded at a concentration of 20-25 µg (checked by spectroscopic methods) onto agarose-strengthened 1.8% SDS-polyacrylamide gels, proteins separated by electrophoresis (5 mA constant current for 16 hours), and gels stained with Coomassie brilliant blue. For Western blotting following SDS-PAGE, proteins were transferred to Hybond ECL nitrocellulose membranes[8]. Blots were pre-incubated with 0.5% milk powder or 3% bovine serum albumin in Tween Tris-buffered saline (TTBS); 10 mmol/L Tris-HCl; pH 7.6; 75 mmol/L NaCl; 0.1% Tween) for 1 hour at room temperature, followed by incubation with anti-Cronos antibody (dilution, 1:2000) overnight at 4°C. The blot was washed with TTBS and titin signals were visualized using secondary horseradish peroxidase-labeled, goat-anti-rabbit/mouse antibodies (dilution 1:1000; DakoCytomation) and enhanced chemiluminescence staining kit (Amersham Biosciences). Gel images were digitized using the LAS-4000 Image Reader (Fuji Science Imaging Systems).

EHT immunohistochemistry

After videos were taken of EHTs, they were fixed for immunohistochemistry by relaxing in 150mM KCl and fixing in 4% paraformaldehyde for 15 minutes at room temperature, and then embedded in Tissue-Tek O.C.T. compound (VWR) using an ethanol-dry ice bath. Cryoblocks were sectioned at a 5µm thickness and staining was started immediately.

Cronos titin custom antibody generation

A custom rabbit polyclonal antibody was generated by Pierce Custom Antibodies (ThermoFisher). The antigen used for antibody production consisted of the entire N-terminal, Cronos titin-specific sequence (i.e. encoded by the intronic sequence upstream of *TTN* exon 240 as determined by the previously reported 5' RACE data on human cardiac samples[8]) excluding the start methionine: SNAKLFLPSRLA. Plasma obtained from the rabbits 70-72 days following inoculation was affinity purified using the same

antigen and then tested on hiPSC-CMs as a positive control and undifferentiated hiPSCs as a negative control. Staining with the Cronos titin antibody generated a striated pattern in the hiPSC-CMs that correlated with α-actinin co-staining and was absent in both undifferentiated hiPSC negative controls and a secondary-only group.

Fluorescent image acquisition & analysis

Stained samples were imaged on a Nikon 1A confocal or spinning disk confocal. Sarcomere length, nuclear number, and maximum myofibril bundle width was measured manually using FIJI. Circular variance was measured using CytoSpectre[9].

Single cell force mechanics

Single-cell forces were measured using PDMS micropost arrays as previously described using posts with a stiffness of 56.91nN/μm[10]. Wild type and TTN-$Z^{-/-}$-1 hiPSC-CMs were replated 29-33 days after initiation of differentiation onto micropost arrays stamped with laminin (Life Technologies). Videos were collected 6-7 days later while cells were kept at 37°C in Tyrode's buffer containing 1.8mM Ca^{2+} and analyzed to determine force magnitude and kinetics as previously described[11].

Calcium transient measurements

Glass coverslips were coated by incubating in a 5μg/mL human fibronectin (Life Technologies) solution at 4°C overnight. Cells were replated 23-32 days after the start of differentiation at a density of $1.1x10^4$ cells/cm^2 and imaged 21-28 days later. Cells were stained by incubating in 0.2μM Fura-2 AM (Thermo Scientific) at 37°C for 30 min, washed by incubating in Tyrode's buffer for 15 min at 37°C, and then imaged during perfusion with Tyrode's buffer containing 1.8mM Ca^{2+} kept at 37°C while paced at 1Hz.

RT-qPCR

Fetal ventricle, atrium, and kidney samples (90-105 days old) were obtained from the Birth Defects Research Laboratory at the University of Washington and human adult heart failure samples were obtained from the laboratory of Dr. April Stempien-Otero in the Department of Cardiology at the University of Washington. Total RNA was isolated from tissue samples using RNEasy Fibrous Tissue kit (Qiagen), and from cell pellets of day 30 hiPSC-CM using the RNEasy Mini Kit (Qiagen). cDNA was generated using M-MLV reverse transcriptase (Invitrogen) following the manufacturer's protocol and using 250-500ng of RNA per reaction, which was then diluted to a total volume of 200µL. Quantitative PCR was performed using 10µL SYBR Select Master Mix (Thermo Fisher), 1µL primer pairs (5µM each, sequences listed in Table S5), 3µL cDNA, and 6µL ddH_2O on a 7900HT Fast Real-Time PCR System (Applied BioSciences). Thermocycle programs consisted of 40 cycles each with 15 seconds at 95°C, 30 seconds at 60°C and a 30 second extension step at 72°C. Relative expression levels were determined using the $\Delta\Delta C_t$ method with HPRT as the internal housekeeping gene.

Live Cell Imaging & Analysis

On the 6th day after viral transduction, which was one day before imaging, cells were replated onto a Matrigel-coated chambered coverglass at a density of 500 thousand cells/mL. Media was changed to regular RPMI + B27 with insulin + 1% pen/strep 24 hours after replating before imaging. 10-15 cells were tagged for imaging (NIS Elements) based on visible beating or appearance of sarcomere-like structures. Cells were imaged using a Yogokawa spinning-disk confocal (Institute for Stem Cells and Regenerative Medicine Garvey Imaging Core, University of Washington) using a 60x oil lens on a single focal plane every 30 minutes for 12 hours while maintained at 37°C and 5% CO_2. Images were analyzed by hand to measure number of myofibrils in the 1st, 13th, and 25th frames, using the basis of the most sarcomere-like structures observed in the cells. The maximal myofibril bundle width was measured for 3

consecutive Z-disks in 3 regions of the cell and averaged for the value for a single cell in the 1st and 25th frame for each cell. Both measurements were performed in Fiji[12].

Supplemental Tables

Supplemental Table 1. CRISPR sgRNA sequences

sgRNA name	Sequence (5' to 3')	Cell lines in this study created using sgRNA
TTN_Ex2_gRNA1	GCAGCCGTTACAAAGCGTTG	TTN-Z$^{-/-}$-1
TTN_Ex2_gRNA2	GGGTAGTACCGCAACCTTTG	TTN-Z$^{-/-}$-2
TTN_Ex326_gRNA1	GATCCGCCCAAAAACCCTGA	TTN-A$^{-/-}$-1
TTN_Ex326_gRNA2	GTGATAAAGCTGGCCAACGC	TTN-A$^{-/-}$-2
Cronos_gRNA	AATTACTCGTGCTTTTCGAG	Cronos KO-1, Cronos KO-2

Supplemental Table 2. Primer sequences used for genotyping

Name	Sequence (5' to 3')	Reference
TTN_Ex2genotyping_F	CCCTTAGCTGGGACACCCT	Designed on Primer-BLAST[3]
TTN_Ex2genotyping_R	GCAGGGCTTAAACTTGGCGTC	Designed on Primer-BLAST[3]
TTN_Ex326genotyping_F	CCTGAAGGACCTTTGGCTGT	Designed on Primer-BLAST[3]
TTN_Ex326genotyping_R	CCCAGTGATTTCTGAACCACC	Designed on Primer-BLAST[3]

Supplemental Table 3. Genes screened for off-target mutations

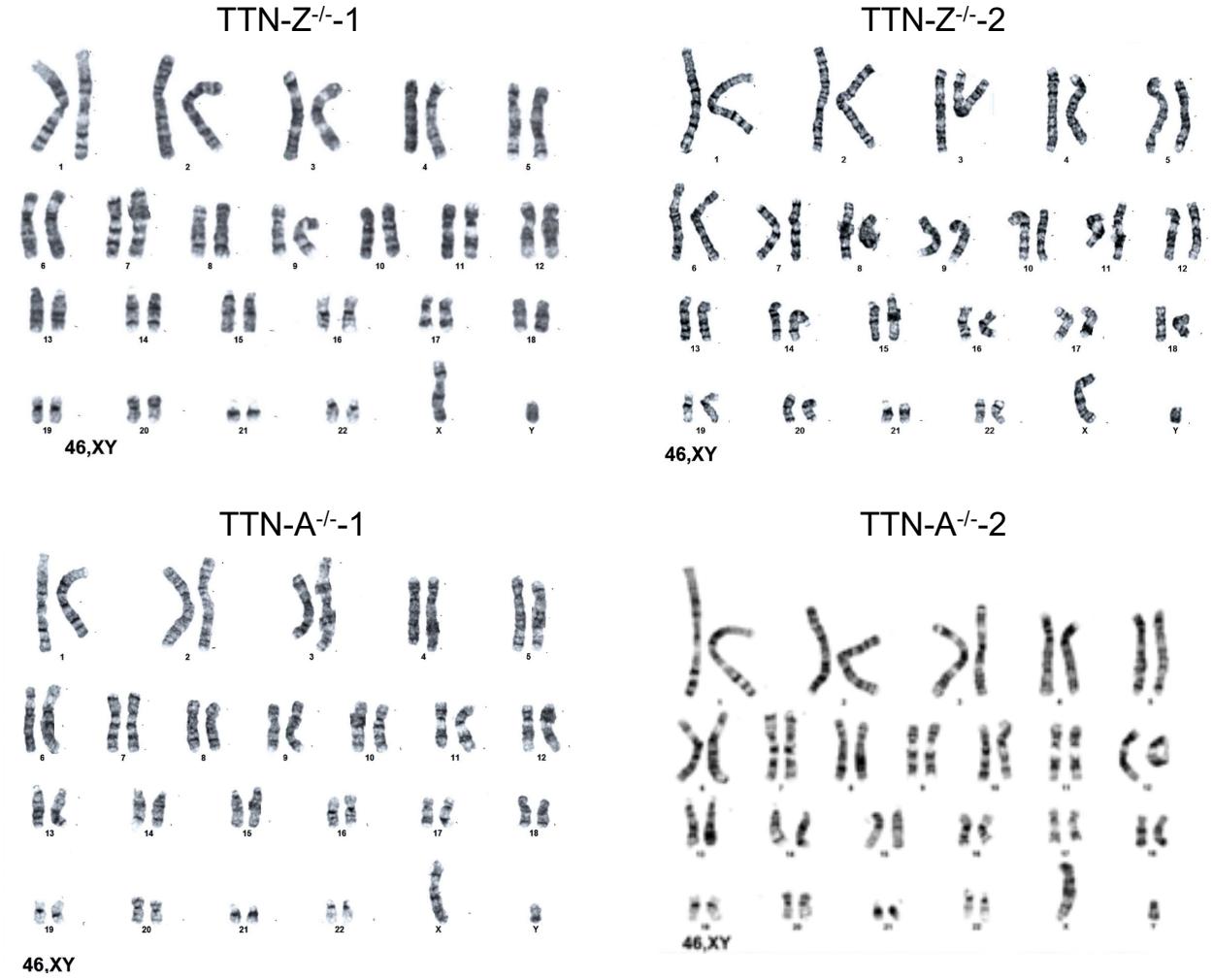
sgRNA	Gene accession number	Forward primer (5' to 3')	Reverse primer (5' to 3')
TTN_Ex2_gRNA1	NM_152335	CCCTCCGCAGTTTGAGATCC	ACACACCTGAAAGGAACCCC
TTN_Ex2_gRNA1	NM_176819	CTCTTGACTGCTACGGGCAT	TCTCCTGGTCTCTGGTCAGG
TTN_Ex2_gRNA1	NM_001024628	GGACACCCGATGGAATGAGG	AGTCTTCCCCGTTGGAGCTA
TTN_Ex2_gRNA1	NM_080872	TGACCACCGTTTCCGTAGTT	TGGTAGACACTGGCTCTCCT
TTN_Ex2_gRNA1	NM_015123	TCAGACCATATGTGCTCCCG	TTCGGGTAAGAACTGGCGTG
TTN_Ex2_gRNA2	NM_002019	AACTCCAGAGGTAGTCAGGCT	AAATATCTCAGCGCGTAGGAC
TTN_Ex2_gRNA2	NM_198827	CAGCAGACACTCTCCAGCTC	ACCTGCATGAGGATGGCAAA
TTN_Ex326_gRNA1	NM_001040113	TGACCAGTCTGTCTGGGAGA	TTCTACCACCAGCTACGGGA
TTN_Ex326_gRNA1	NM_001206966	CTGCAGGTGAGGGGTTTGAT	GGGTCTGGCTTTCTCCACTC
TTN_Ex326_gRNA1	NM_001146336	GTAGGGGCAGTGACAGTGTG	CTGGGCTGGAAACCACTCAT
TTN_Ex326_gRNA1	NM_015668	GGCTATAGTTGGGCTCCACAAA	GTTCCCTGAATGCTCGCAGAA
TTN_Ex326_gRNA1	NM_001278371	ATCACCTGTGACGACGTGTG	CAACCCGTGTACCCAAAGGT
TTN_Ex326_gRNA2	NM_021044	AAACACTTCCATTCAGAGGGTGA	TTTCCCACCTGGGACAACC
TTN_Ex326_gRNA2	NM_022474	TCTTGCTATTTGCTCGGCCA	ATCCCAGGCTGCACATAAGG
TTN_Ex326_gRNA2	NR_102336	AGCCTTGATTCTTTGACTTGCTG	TGTTTGATACCTGGGGCTACTTT
TTN_Ex326_gRNA2	NM_005242	TCTGAGTTTCGAATCGGCGG	ATTCGTAGAGATGGGTGCCG
TTN_Ex326_gRNA2	NM_003887	CAAGATCCTGCCTCTACGGAAT	TGATAGCTGCAACCTCACAAAT
Cronos_gRNA	NM_018424	TCAATTGGTTAAGGGGTTTTATGTT	TTGGCAGAGGCGTCTGATTT
Cronos_gRNA	NM_001448	CTGGTGAACTCCCAGTACCAC	AGTGTGTGGGAGAGAAGAGGT
Cronos_gRNA	NM_080646	ATGTGGACCCACGCAAAGAT	CACAGCCTGCAGGTCTAAGC
Cronos_gRNA	NM_001144884	TGGCATTGAAGAGTAAGCTGC	CAAGGCTGTTGTAAGAAGTCAACA
Cronos_gRNA	NM_018424	GGATTCTGGGGGATTGGCATA	CAGGTTCTGGGCTTGGTTTGT

Supplemental Table 4. Primary antibody details

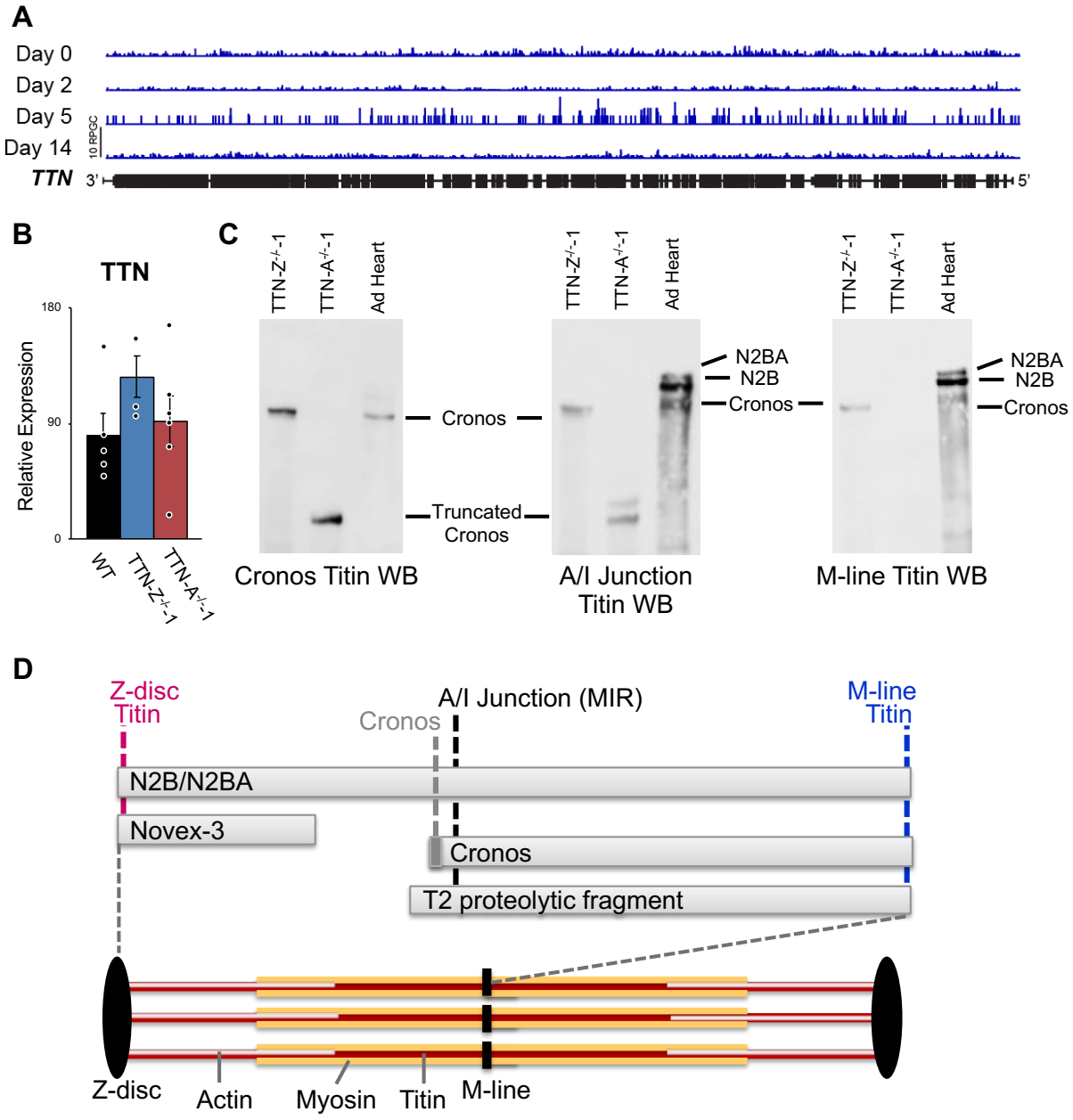
Antibody name	**Species**	**Dilution**	**Company**	**Product #**
Sarcomeric α-actinin	Mouse	1:800	Abcam	ab68167
Titin Z-disk (Z1Z2)	Rabbit	1:300	Myomedix	TTN-Z
Titin I-band (MIR)	Rabbit	1:300	Myomedix	TTN-MIR
Titin M-line (M8M10)	Rabbit	1:300	Myomedix	TTN-M
β-myosin heavy chain	Mouse	1:10	DSHB	BA-D5

Supplemental Table 5. Primer sequences used in RT-qPCR

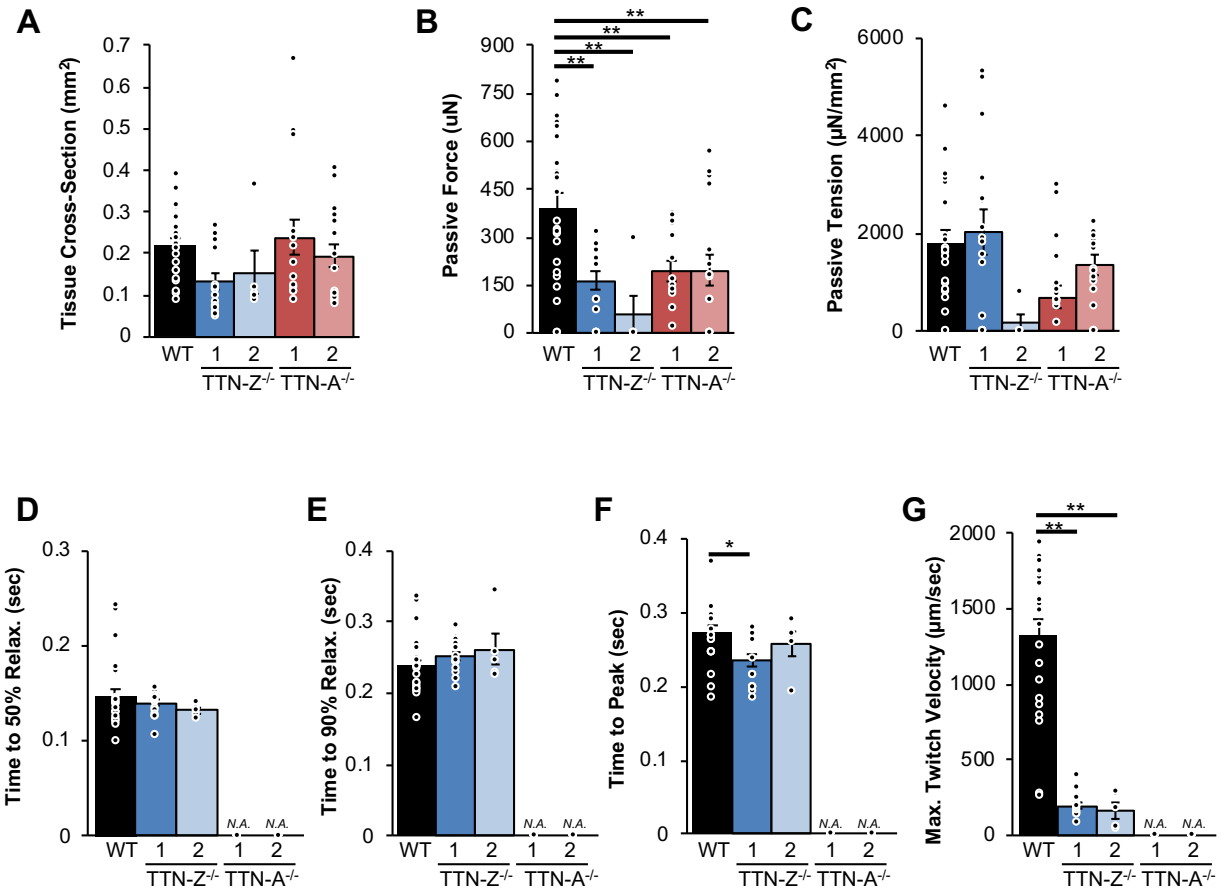
Name	**Sequence (5' to 3')**	**Source**
HPRT_qPCR_F	TGACACTGGCAAAACAATGCA	Harvard Primer Bank[12]
HPRT_qPCR_R	GGTCCTTTTCACCAGCAAGCT	Harvard Primer Bank[12]
FullTTN_qPCR_F	TTTTGCACAACTGTCGCCTG	Designed on Primer-BLAST[3]
CronosandFullTTN_qPCR_R	CTTCGTAGGAGAGCTCGCAG	Designed on Primer-BLAST[3]
CronosTTN_qPCR_F	ACGCAAAGCTGTTTCTTCCC	Designed on Primer-BLAST[3]



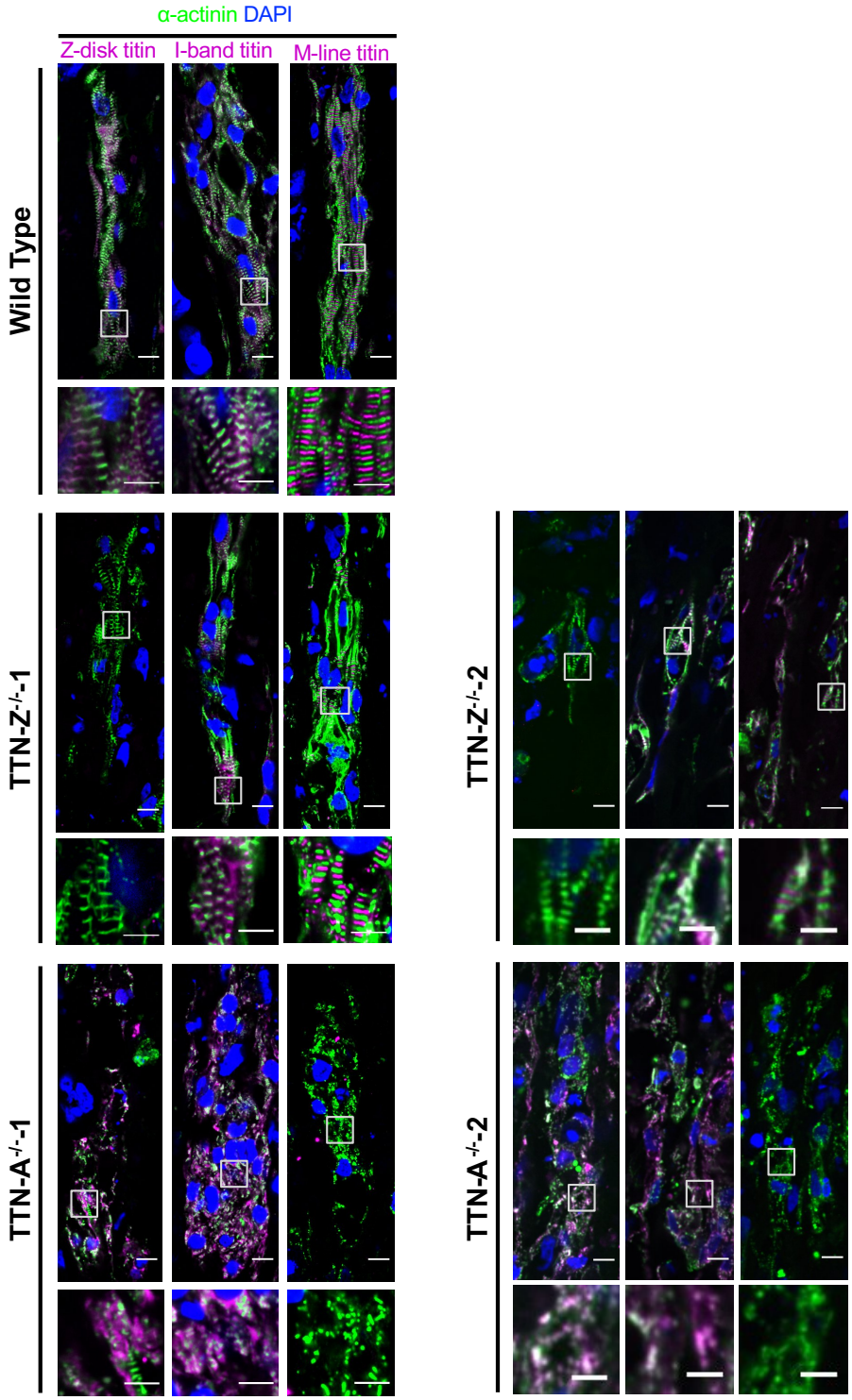
Supplemental Figure 1: Karyotypes of edited hiPSCs. All gene-edited hiPSC lines had normal karyotypes.



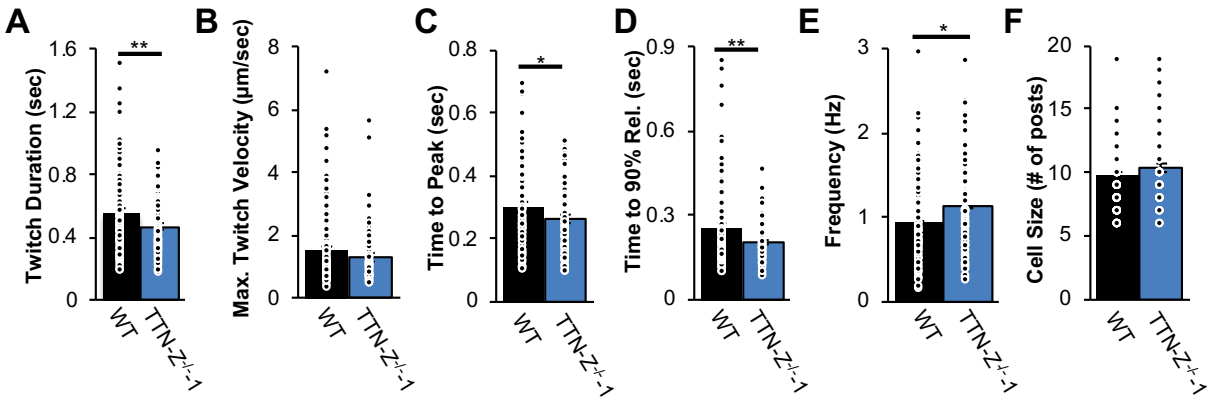
Supplemental Figure 2:H3K27me3 of *TTN* & additional titin western blots. (A) H3K27me3 deposition does not show any prominent peaks in *TTN* during cardiac differentiation of hESCs (data adapted from ref 1). (B) qPCR of day 30 hiPSC-CM indicate that titin transcript levels are not significantly different between WT, TTN-Z$^{-/-}$, and TTN-A$^{-/-}$ cells. (C) Western blots (WB) of hiPSC-CM lysates probing for Cronos titin, the A-band/I-band junction of titin, and M-line titin. (D) Schematic showing the location of antibodies used relative to different titin isoforms expressed in hiPSC-CMs.



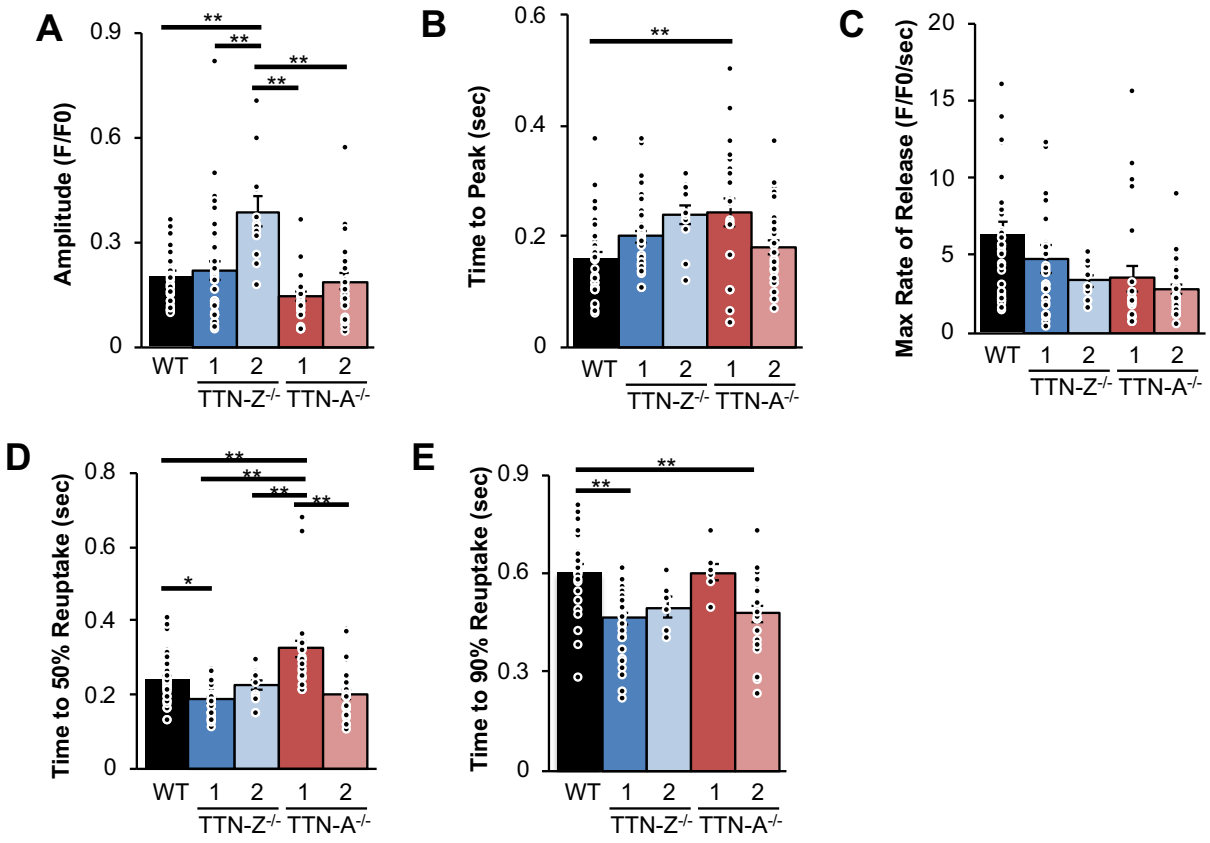
Supplemental Figure 3: EHT measurements of homozygous titin truncation lines. Measurements of EHTs seeded with TTN-$Z^{-/-}$ and TTN-$A^{-/-}$ CMs indicate that (A) tissue cross-sectional area is not significant different compared to WT, (B) passive force is significantly lower in all mutant cell lines but (C) passive tension normalized to cross-sectional area is not significantly different. While relaxation to (D) 50% and (E) 90% were not significantly different between TTN-$Z^{-/-}$ and WT tissues, (F) Time to peak was slightly faster even though (G) maximum twitch velocity was drastically reduced. WT: n=23; TTN-$Z^{-/-}$-1: n=16; TTN-$Z^{-/-}$-2: n=5; TTN-$A^{-/-}$-1: n=16; TTN-$A^{-/-}$-1: n=17. *: adjusted p-value<0.05; **: adjusted p-value<0.01. Nominal P-values were calculated using ANOVAs. Adjusted p-values were calculated using Tukey's post-hoc test and are reported in this figure.



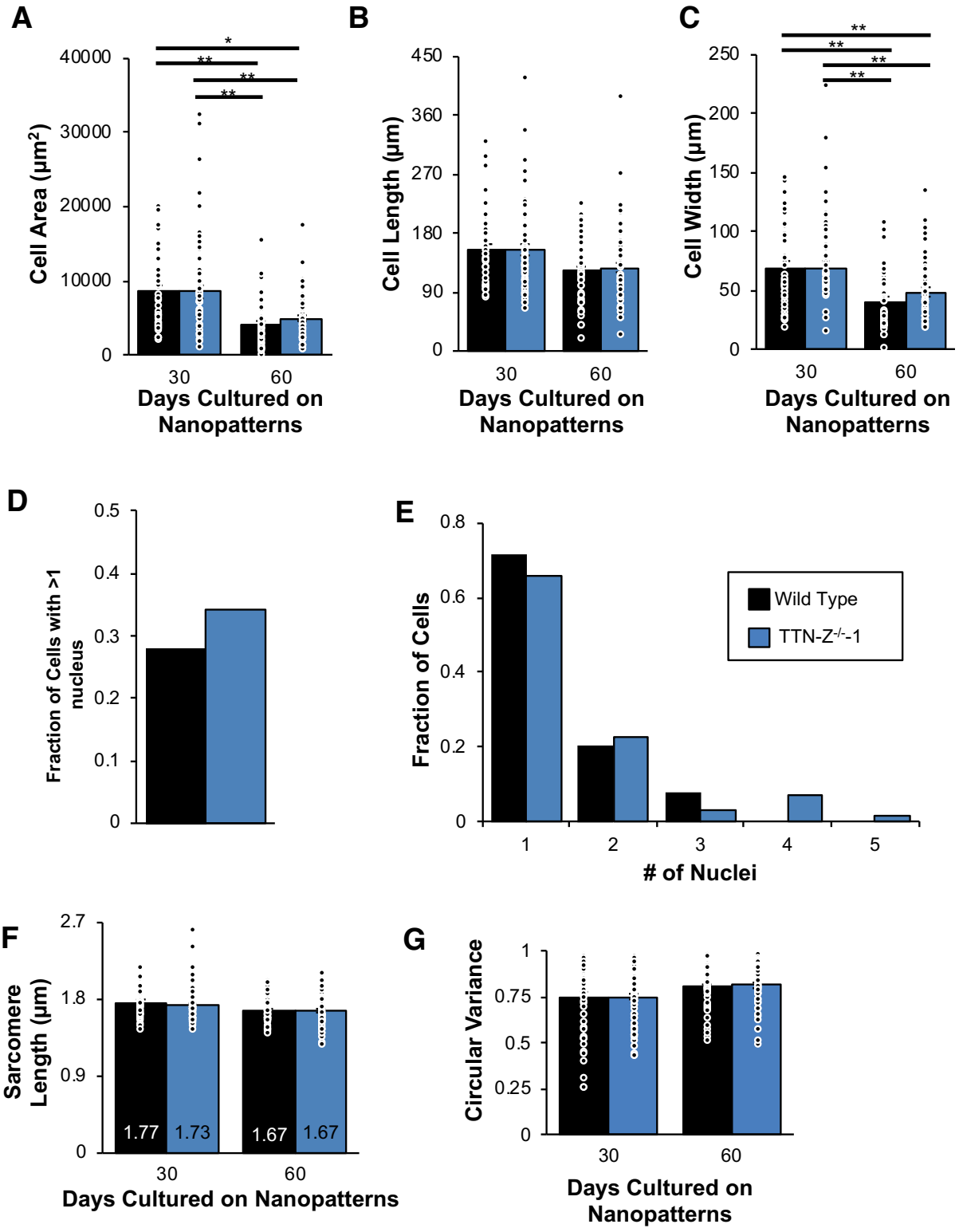
Supplemental Figure 4: Immunohistochemistry of EHTs for titin epitopes. Immunohistochemistry of EHTs seeded with hiPSC-CMs from each cell line using titin antibodies that recognize the Z-disk, distal I-band, and M-line regions of the protein. In each panel, large image: scale bar = 10μm, small image: scale bar = 5μm. White boxes indicate regions magnified in small image.



Supplemental Figure 5: Single-cell force mechanics of WT and TTN-Z$^{-/-}$ CMs. (A) The twitch duration for TTN-Z$^{-/-}$-CMs was significantly shorter than WT. There was no significant difference between WT and TTN-Z$^{-/-}$ cells in (B) maximum twitch velocity. In TTN-Z$^{-/-}$-CMs, (C) time to peak and (D) time to 90% relaxation were significantly shorter and (E) spontaneous beating frequency was significantly higher than wild type. As indicated by (F) number of posts each cell occupied, cell size between groups was not significantly different. WT: n=107; TTN-Z$^{-/-}$-1: n=71. *: adjusted p-value<0.05; **: adjusted p-value<0.01. P-values were calculated using a student's t-test. Adjusted p-values were computed using the Benjamini & Hochberg method.

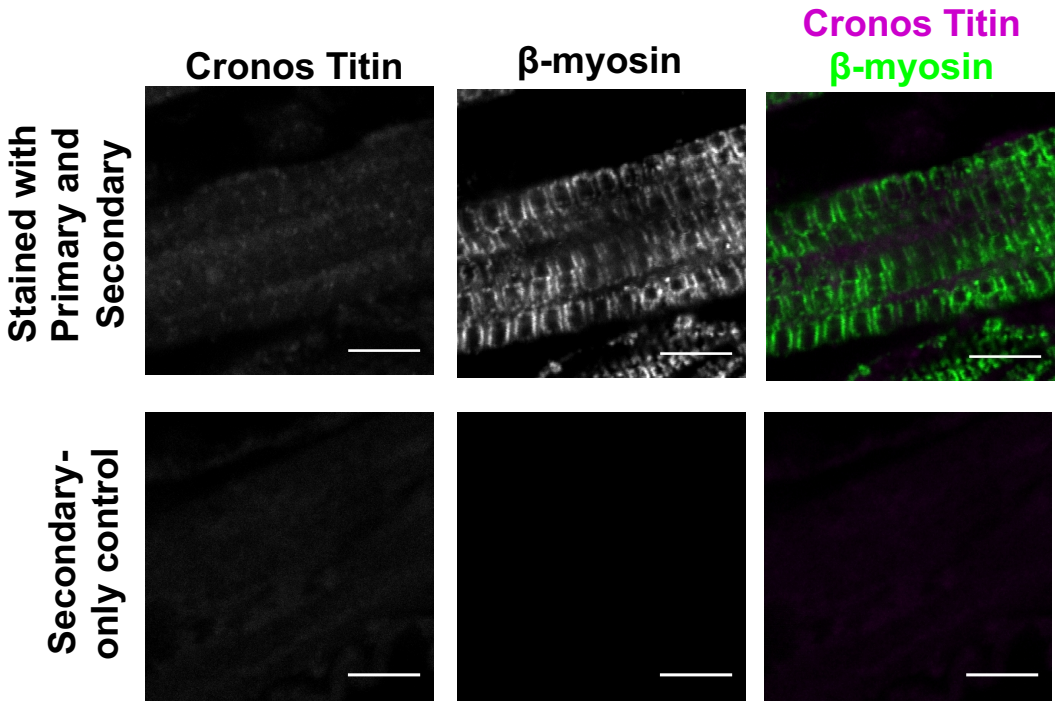


Supplemental Figure 6: Calcium transient measurements of single WT and mutant CMs. Calcium transient measurements of single cells stained with Fura2-AM indicate that (A) amplitude of transient is significantly larger in TTN-$Z^{-/-}$-2 CMs but no different in other groups compared to WT, (B) Time to peak is only significantly different between WT and TTN- $A^{-/-}$-1 CMs, and (C) maximum rate of release is not significantly different between any groups studied. (D-E) Calcium reuptake kinetics are slightly perturbed with an overall trend of faster reuptake by cells carrying titin mutations. WT: n=35; TTN-$Z^{-/-}$-1: n=37; TTN-$Z^{-/-}$-2: n=12; TTN-$A^{-/-}$-1: n=24; TTN-$A^{-/-}$-2: n=28. Error bars indicate standard error. *: adjusted p-value<0.05; **: adjusted p-value<0.01. Nominal P-values were calculated using ANOVAs. Adjusted p-values were calculated using Tukey's post-hoc test and are reported in this figure.

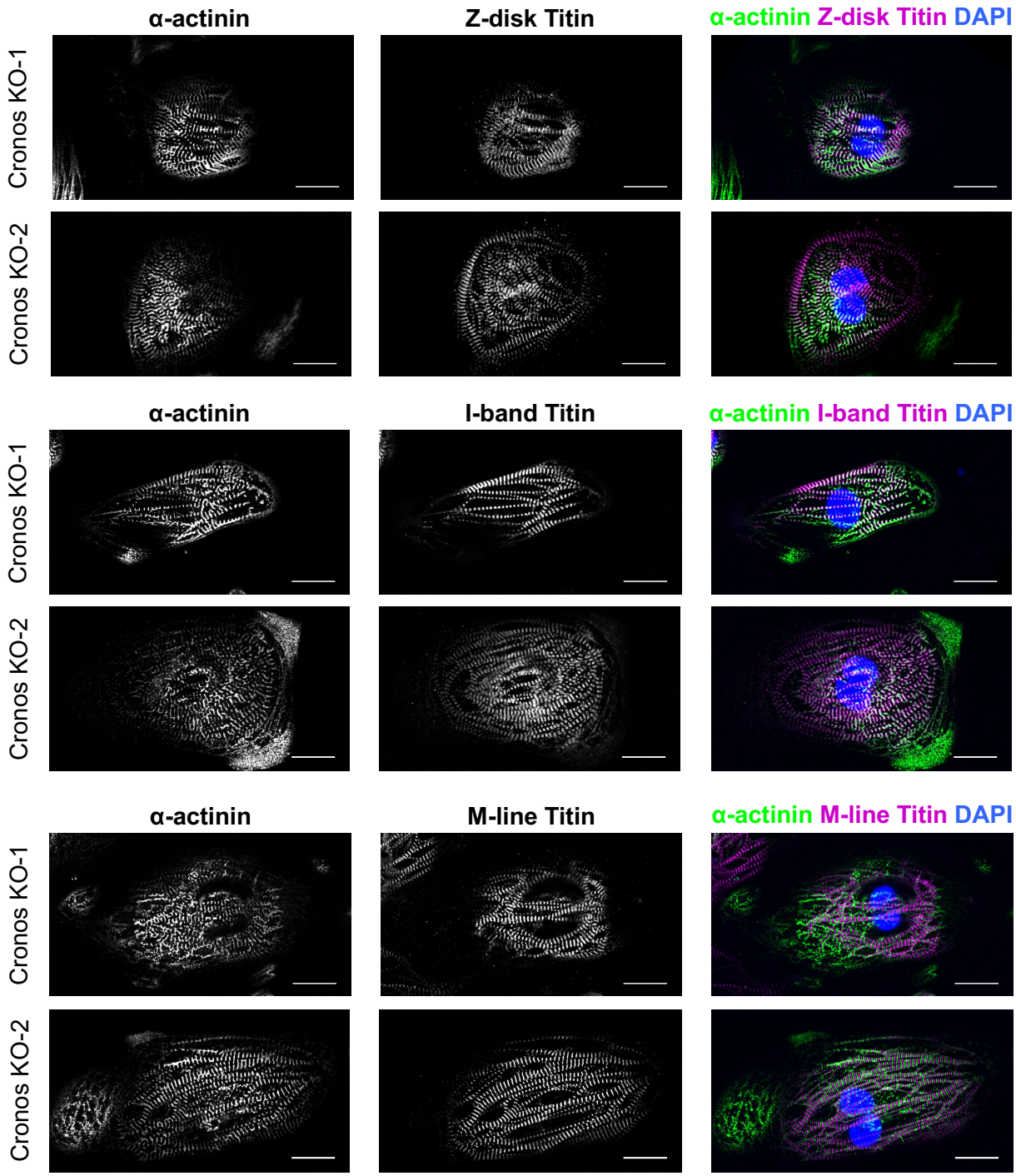


Supplemental Figure 7: Cell size and multinucleation measurements. At each time point, there was no significant difference between WT and TTN-$Z^{-/-}$ cardiomyocytes cultured on nanopatterns for (A) cell area, (B) cell length, or (C) cell width. After 60 days cultured on nanopatterns, (D) there is not a

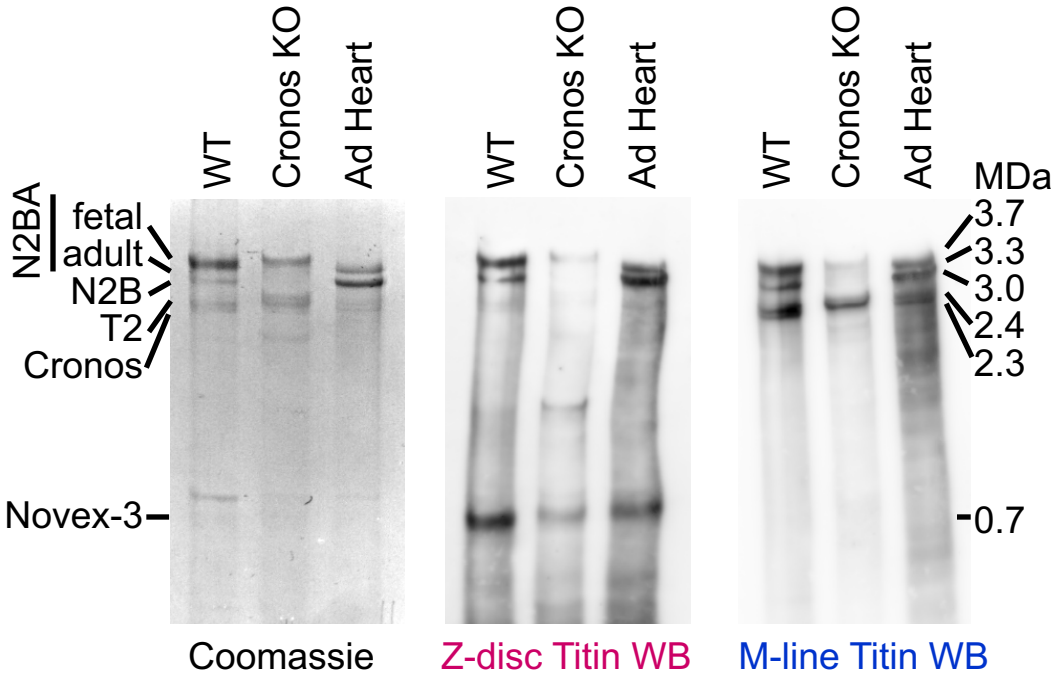
significant difference in fraction of multinucleated cells, but (E) TTN-$Z^{-/-}$-CMs have a higher number of cells with >3 nuclei. (F) Sarcomere length and (G) circular variance were not significantly different at either time point studied. 30 day group: WT: n=41; TTN-$Z^{-/-}$: n=68. 60 day group: WT: n=47; TTN-$Z^{-/-}$: n=56. Error bars indicate standard error. *: adjusted p-value<0.05; **: adjusted p-value<0.01. Nominal P-values were calculated using ANOVAs. Adjusted p-values were calculated using Tukey's post-hoc test and are reported in this figure.



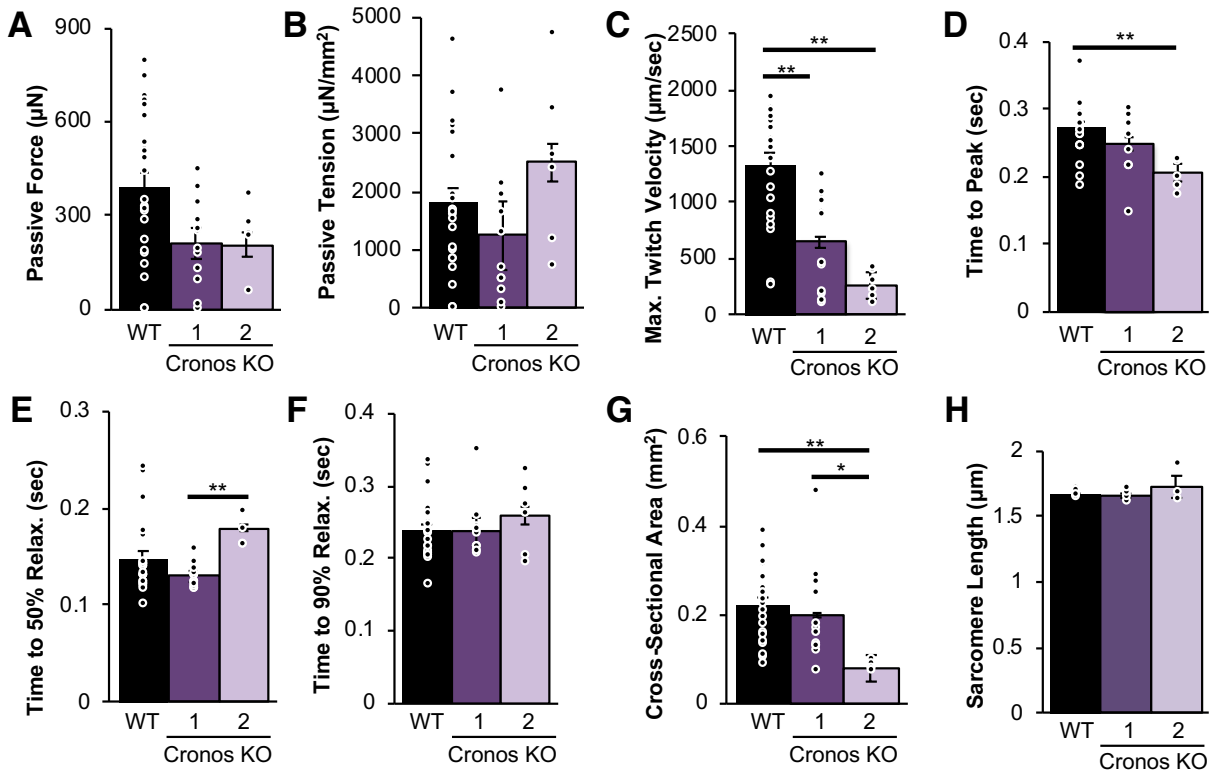
Supplemental Figure 8: Adult human left ventricular tissue immunohistochemistry. Comparison of adult left ventricular tissue stained as outlined in the methods section compared to a slide stained without primary antibodies demonstrates that the Cronos titin signal is barely above background autofluorescence of the sample. Scale bars = 5μm.



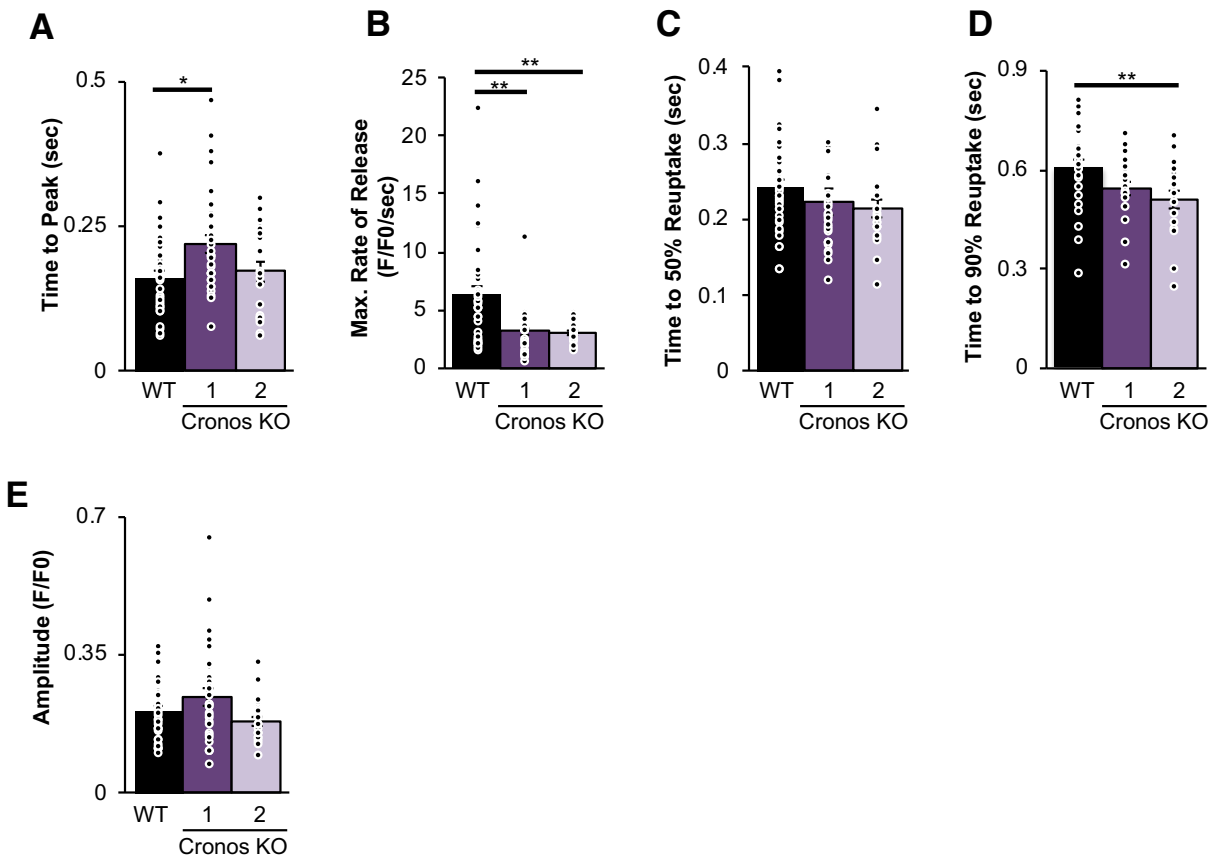
Supplemental Figure 9. Titin epitope staining of Cronos KO CMs. Staining for Z-disk, I-band, and M-line titin indicates that these domains are still expressed and correctly integrated into sarcomeres in Cronos KO CMs. Scale bars = 20μm.



Supplemental Figure 10: **Additional titin protein gels and western blots.** Coomassie stain and additional western blots of samples show in Cronos western blot in Figure 6B. A schematic showing the region of titin each antibody targets is shown in Figure S2C.



Supplemental Figure 11. EHT measurements of Cronos KO CMs. Video measurements of EHTs seeded with Cronos KO CMs while pacing at 1.5Hz indicate that (A) passive force and (B) passive tension are not significantly different compared to WT. (C) Maximum twitch velocity is significantly decreased in Cronos KO EHTs, while (D) time to peak is significantly faster in one of the Cronos KO lines measured. Time to (E) 50% and (F) 90% relaxation were not significantly different in Cronos KO tissues compared to WT. Analysis of immunofluorescent images of EHTs revealed that (H) sarcomere length was not significantly different between Cronos KO samples and WT controls. For (A)-(G): WT: n=23; Cronos KO-1: n=12; Cronos KO-2: n=6;. For (H): WT: n=6; Cronos KO-1: n=5; Cronos KO-2: n=5. *: adjusted p-value<0.05; **: adjusted p-value<0.01. Nominal P-values were calculated using ANOVAs. Adjusted p-values were calculated using Tukey's post-hoc test and are reported in this figure.



Supplemental Figure 12: Single-cell calcium transients of Cronos KO CMs. Single-cell calcium transient measurements indicate Cronos KO CMs have (A) a longer time to peak of transient and (B) significantly lower maximum rate of release of calcium. Reuptake kinetics are slightly faster at (C) 50% and (D) 90%. (E) The amplitude of calcium transients is unchanged. WT: n=35; Cronos KO-1: n=32; Cronos KO-2: n=22. Error bars indicate standard error. *: adjusted p-value<0.05; **: adjusted p-value<0.01. Nominal P-values were calculated using ANOVAs. Adjusted p-values were calculated using Tukey's post-hoc test and are reported in this figure.

Supplemental Videos

Video S1: WT CM expressing mCherry-α-actinin imaged every 30 minutes for 12 hours. Scale bar = 50μm.

Video S2: TTN-$Z^{-/-}$-1 CM expressing mCherry-α-actinin imaged every 30 minutes for 12 hours. Scale bar = 50μm.

Supplemental References

1. Kreitzer FR, Salomonis N, Sheehan A, Huang M, Park JS, Spindler MJ, Lizarraga P, Weiss WA, So P, Conklin BR. A robust method to derive functional neural crest cells from human pluripotent stem cells. *Am J Stem Cells*. 2013;2:119–131.
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