iScience, Volume *23*

Supplemental Information

MCL-1 Inhibition by Selective BH3 Mimetics Disrupts Mitochondrial Dynamics Causing Loss of Viability and Functionality of Human Cardiomyocytes

Megan L. Rasmussen, Nilay Taneja, Abigail C. Neininger, Lili Wang, Gabriella L. Robertson, Stellan N. Riffle, Linzheng Shi, Bjorn C. Knollmann, Dylan T. Burnette, and Vivian Gama

Supplemental Information Titles and Legends

Figure S1: Corresponding to Figure 1. Analysis of oxygen consumption in hiPSC-CMs treated with MCL-1 inhibitor. (A, C) Oxygen consumption rate (OCR) was measured using the Seahorse Biosciences Mito Stress Test on an XFe96 analyzer. OCR after injection of FCCP was significantly reduced in cells treated with S63845 only (A) and S63845 +QVD (C) compared to vehicle controls (n = 3 independent experiments done in triplicate). *p < .05, **p <.01, ***p <.001, and ****p <.0001. Error bars indicate ±SEM. (B, D) ATP production was calculated from the corresponding OCR traces in panels A and C for each condition. ATP production was reduced in both conditions, with S63845 treatment alone resulting in significant impairment of ATP production compared to vehicle control (B). Graphs represent mean ±SEM and p-values were determined by student's t-test.

Figure S2: Corresponding to Figure 2. (A) hiPSC-CMs were plated on a CytoView MEA plate (Axion Biosystems) and treated with QVD and vehicle (DMSO), 0.5 μM AMG-176, or 0.5 μM AZD5991. Live-cell activity was recorded for 5 minutes and results were normalized to baseline recordings for each well. Beat period irregularity (A) was increased at 18 hrs in AZD-treated cells before cells stopped beating at 48 hrs, while DMSO and AMG had low levels of beat period irregularity overall. Spike amplitude mean (B) and spike slope mean (C) were both decreased by 20 hrs and completely reduced by 48 hrs in the AZD condition. Propagation consistency (D) and field potential duration (FPD) (E) were both significantly reduced in AZD-treated cells at 20 hrs in comparison to DMSO control and AMG-176. P-values were determined by two-way ANOVA and show significance as follows: * = DMSO +QVD vs. AZD5991 +QVD, # = AZD5991 +QVD vs. AMG-176 +QVD. One symbol indicates p <0.05, two symbols indicate p <0.01, three symbols indicate p <0.001, and four symbols indicate p <0.0001. Graphs represent mean ±SEM. (F) Schematic of MCL-1 knockdown experiments. hiPSC-CMs were plated from thaw in either the CytoView MEA plate or in a tissue culture-treated 96-well plate and fed regularly for 10 days,

followed by three rounds of siRNA transfection. Cells in the 96-well plate were lysed for Western blot following the third round of transfection. (G) Representative Western blot showing efficient knockdown of MCL-1 protein using siRNA. (H-J) Axion MEA recordings were taken as in panels A-E. Baseline recordings were taken at 0 days (pre-transfection), and subsequent recordings were taken at days 1, 3, 4, and 7 over the course of the knockdown paradigm depicted in panel F (n = 3 replicate experiments). MCL-1 knockdown resulted in higher beat period irregularity (H), higher maximum delay mean (I), and shorter FPD mean (J) compared to non-targeting control siRNA. *p <0.05, **p <0.01. Graphs indicate mean ±SD. (K) Vehicle-treated hiPSC-CMs have organized myofibril structure as shown by maximum intensity projections. hiPSC-CMs treated with 2 μM AMG-176 or 2 μM AZD5991 have myofibrils that are unorganized and poorly defined Z-lines when compared to controls. Scale: 5 μm. Representative images are shown for all panels. Quantification of myofibril phenotypes is shown (n > 20 cells per condition from 3 separate experiments). Error bars indicate ±SD.

Figure S3: Corresponding to Figure 3. (A) Representative Western blot showing cytosolic fraction and membrane fractions from hiPSC-CM protein lysates treated with vehicle or S63845 and QVD. Quantification of DRP-1 signal intensity normalized to either α-Tubulin for the cytosolic fraction (B) or VDAC for the membrane fraction (C). Western blots from 3 independent experiments were quantified and graphs represent mean ±SD. (D) Quantification for TOM20 band density from the experiment shown in Figure 3C. Graph represents mean ±SD. (E) Representative image of secondary PLA probe control sample. The area around the nucleus was avoided for all quantification purposes. Scale: 20 μm.

Figure S4: Corresponding to Figure 4. (A) Schematic of DRP-1 knockdown experiment followed by photo-conversion of mito-tdEos corresponding to Figure 4E-F. (B) Representative

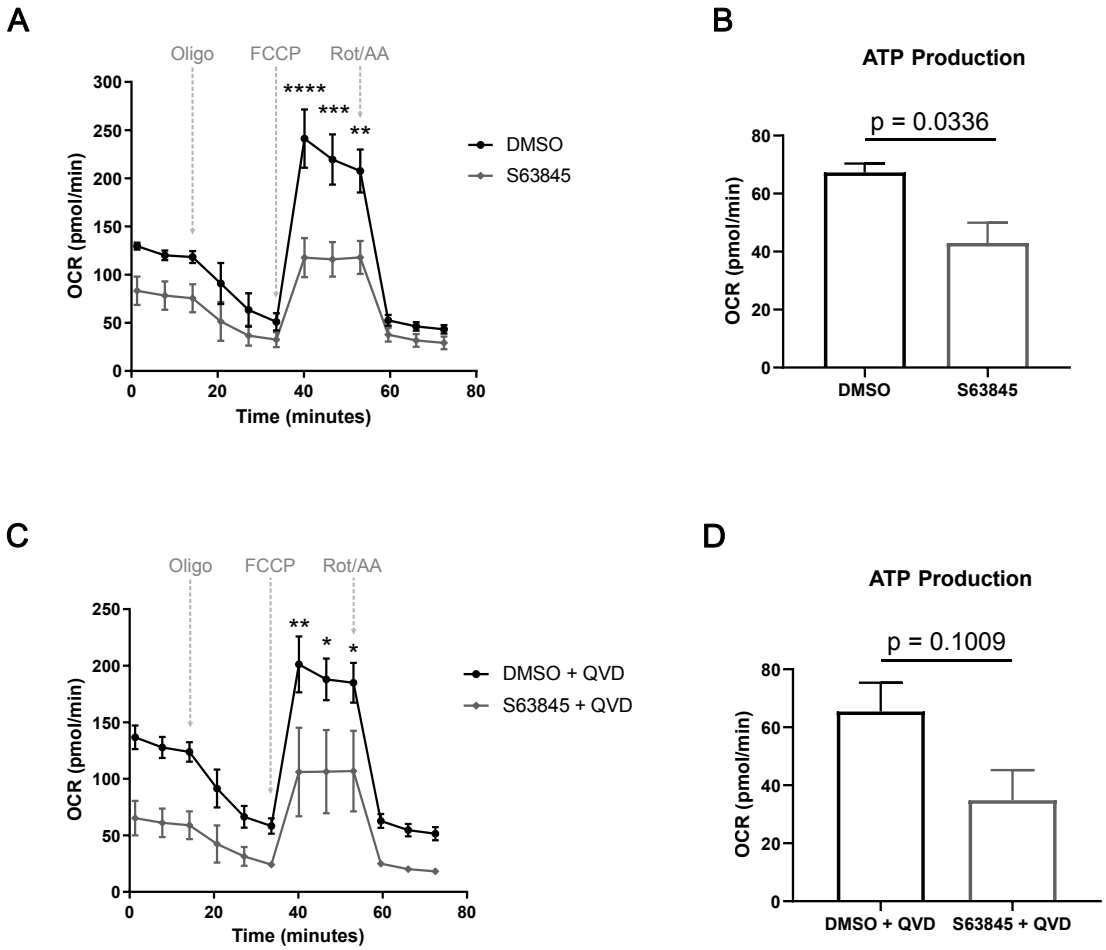
Western blot showing knockdown of DRP-1 compared to control siRNA corresponding to Figure 4E-F.

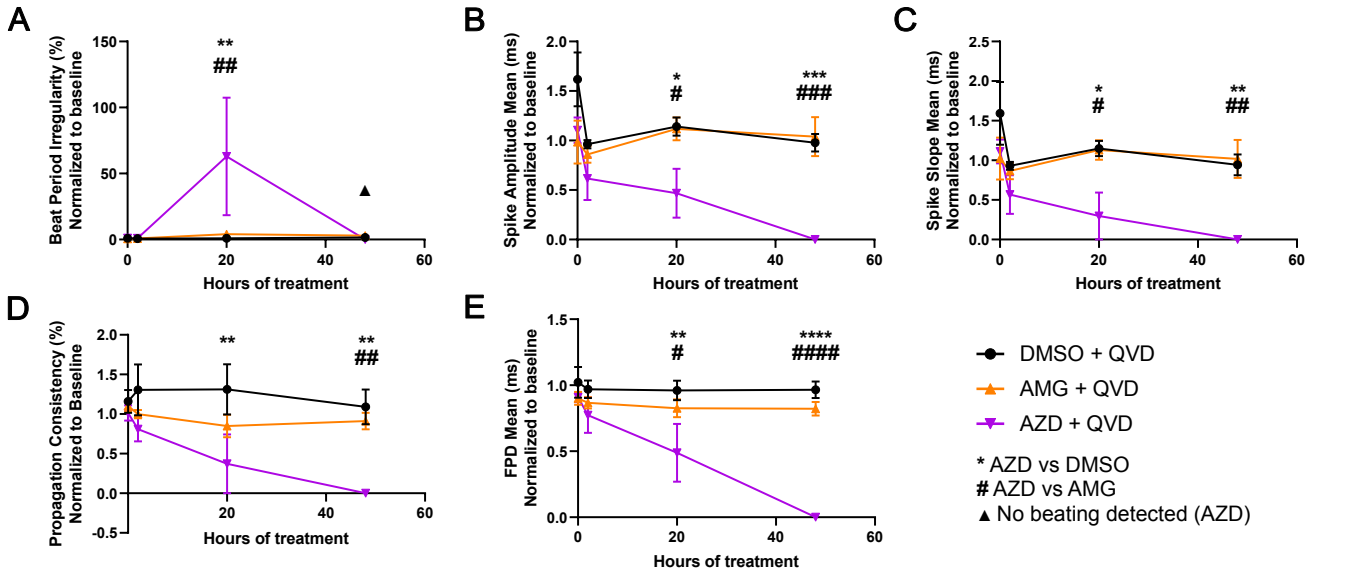
Figure S5: Corresponding to Figure 5. hiPSC-CMs were treated with S63845 at the indicated concentrations and either vehicle DMSO (A) or with QVD (B). Cell survival was measured over 4 days using an IncuCyte live cell imaging system. Representative images from each day of treatment were quantified and percentages of live cells are shown normalized to day 0 baseline. Graphs represent mean ±SEM and experiments were performed in 4 independent replicates. (C) hiPSC-CMs were treated with the indicated concentrations of AMG-176 or AZD5991 and vehicle DMSO (C) or QVD (D). Cell survival was measured and quantified as in panels A-B. Graphs represent mean ±SEM and experiments were performed in 3 independent replicates.

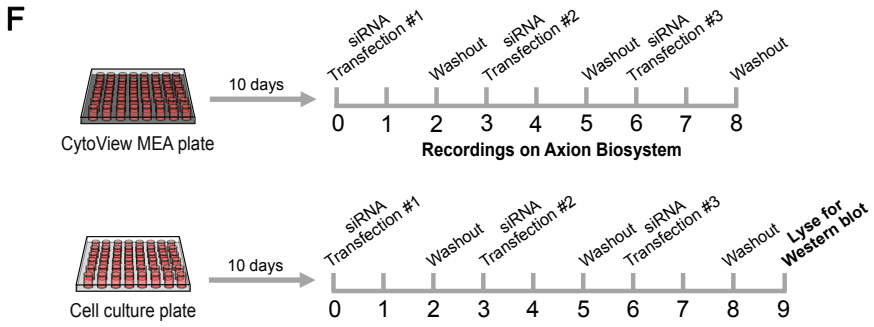
Figure S6: Corresponding to Figure 7. Chronic inhibition of MCL-1, but not BCL-2, results in cardiac activity defects. hiPSC-CMs were treated every 2 days with DMSO, 100 nM MCL-1*i* (S63845 - orange), 100 nM BCL-2*i* (ABT-199 - green), or both inhibitors (magenta) for 14 days. MEA plate was recorded 2 hours following each treatment for 5 minutes and results were normalized to baseline recording for each respective well, followed by normalization to DMSO (gray dotted line). Results of recordings for beat period mean (A) and field potential duration mean (B) are shown. P-values were calculated by two-way ANOVA and show significance as follows: * = DMSO vs. S63845, † = DMSO vs. Combination, # = S63845 vs. ABT-199, ‡ = S63845 vs. Combination, ● = ABT-199 vs. Combination. One symbol indicates $p < 0.05$, two symbols indicate $p < 0.01$, three symbols indicate $p < 0.001$, and four symbols indicate $p < 0.0001$. Error bars indicate ±SEM. (F-I) Mitochondria and F-actin were imaged at the end of the treatment paradigm in Figure 6A-E. Representative SIM images are shown of cells treated with DMSO (F), 100 nM S63845 (G), 100 nM ABT-199 (H), and 100 nM S63845 + 100 nM ABT-199 (Combination) (I). Scale: 10 μm.

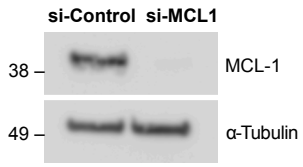
Figure S7: Corresponding to Figure 1- Figure 7. Main findings in the study.

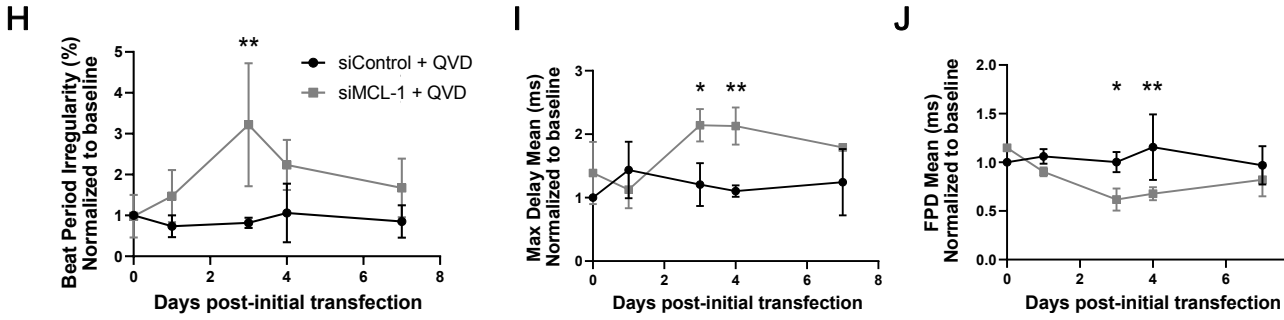
Supplemental Figure 1

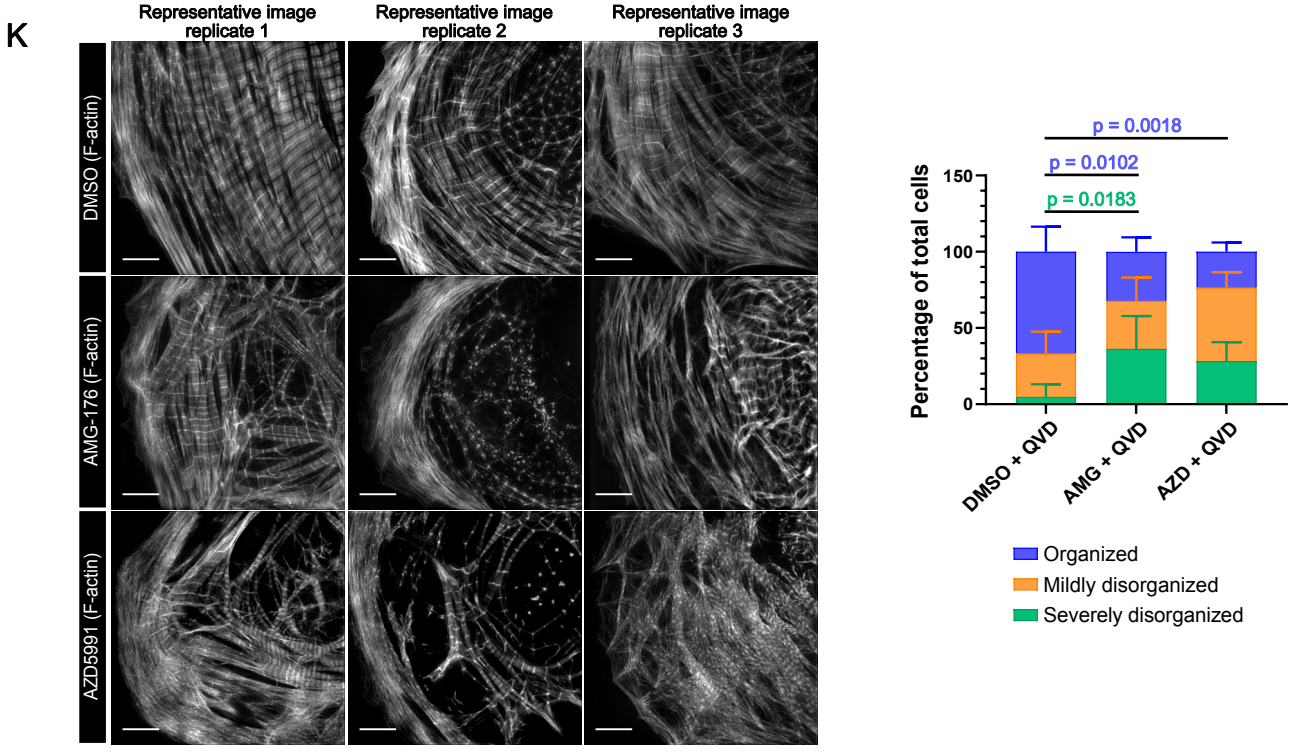



A
Beat Period Irregularity (%) Normalized to baseline
Hours of treatment
B
Spike Amplitude Mean (ms) Normalized to baseline
Hours of treatment
C
Spike Slope Mean (ms) Normalized to baseline
Hours of treatment
D
Propagation Consistency (%) Normalized to Baseline
Hours of treatment
E
FPD Mean (ms) Normalized to baseline
Hours of treatment
DMSO + QVD
AMG + QVD
AZD + QVD
* AZD vs DMSO
# AZD vs AMG
▲ No beating detected (AZD)


F
CytoView MEA plate
10 days
siRNA Transfection #1
Washout
siRNA Transfection #2
Washout
siRNA Transfection #3
Washout
Recordings on Axion Biosystem
Cell culture plate
Lyse for Western blot

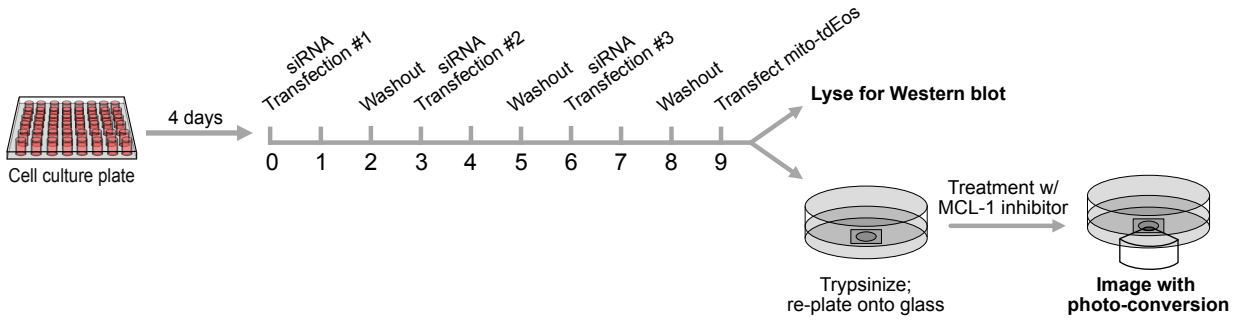

G
si-Control
si-MCL1
38
MCL-1
49
α-Tubulin


H
Beat Period Irregularity (%) Normalized to baseline
siControl + QVD
siMCL-1 + QVD
Days post-initial transfection
I
Max Delay Mean (ms) Normalized to baseline
Days post-initial transfection
J
FPD Mean (ms) Normalized to baseline
Days post-initial transfection

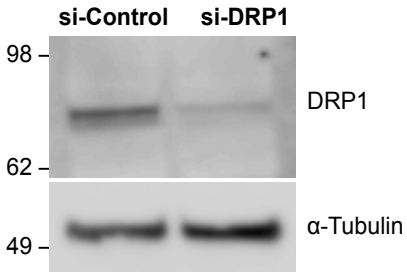

K
Representative image replicate 1
Representative image replicate 2
Representative image replicate 3
DMSO (F-actin)
AMG-176 (F-actin)
AZD5991 (F-actin)
p = 0.0018
p = 0.0102
p = 0.0183
Percentage of total cells
DMSO + QVD
AMG + QVD
AZD + QVD
Organized
Mildly disorganized
Severely disorganized

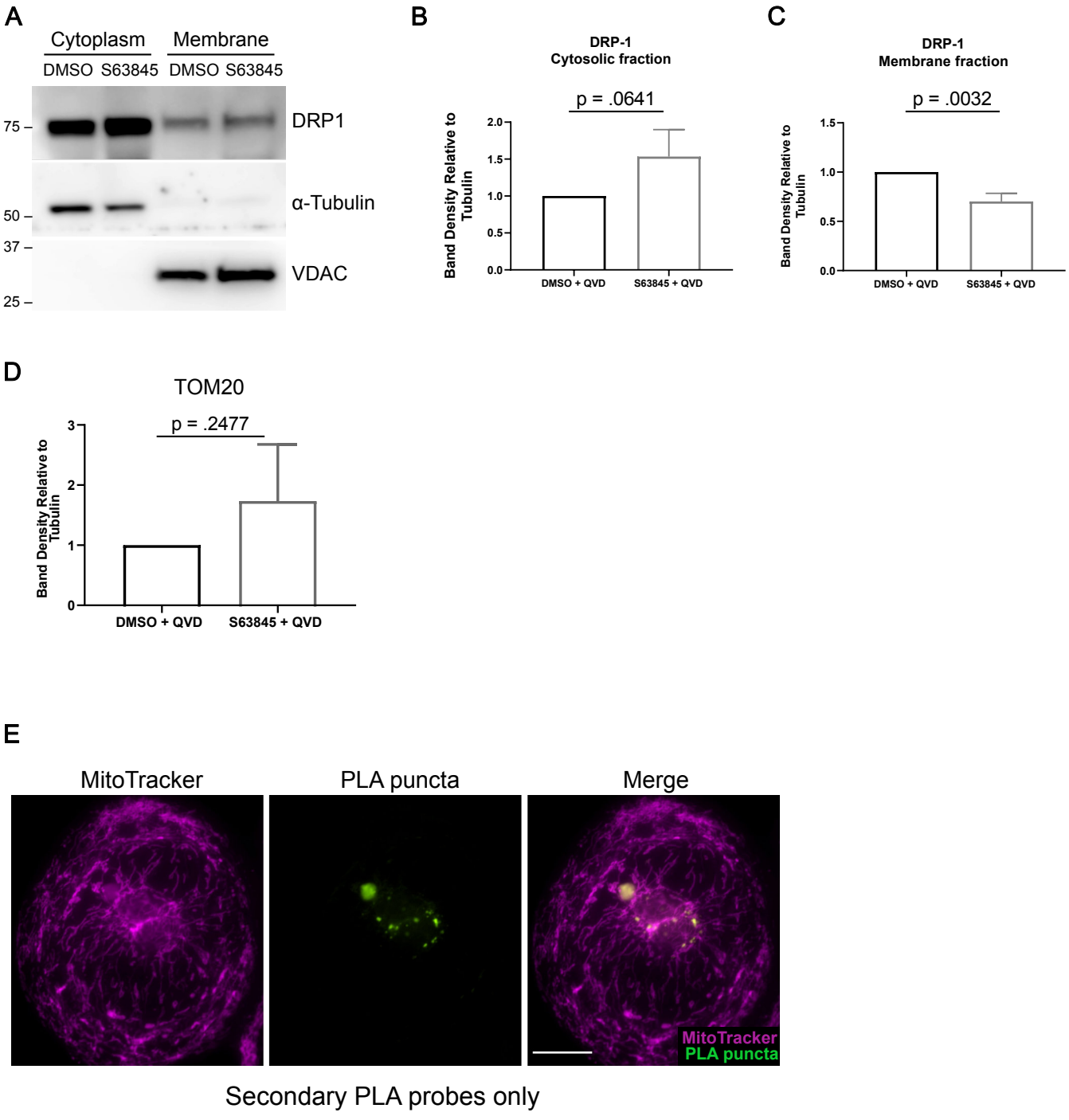
Supplemental Figure 4

A

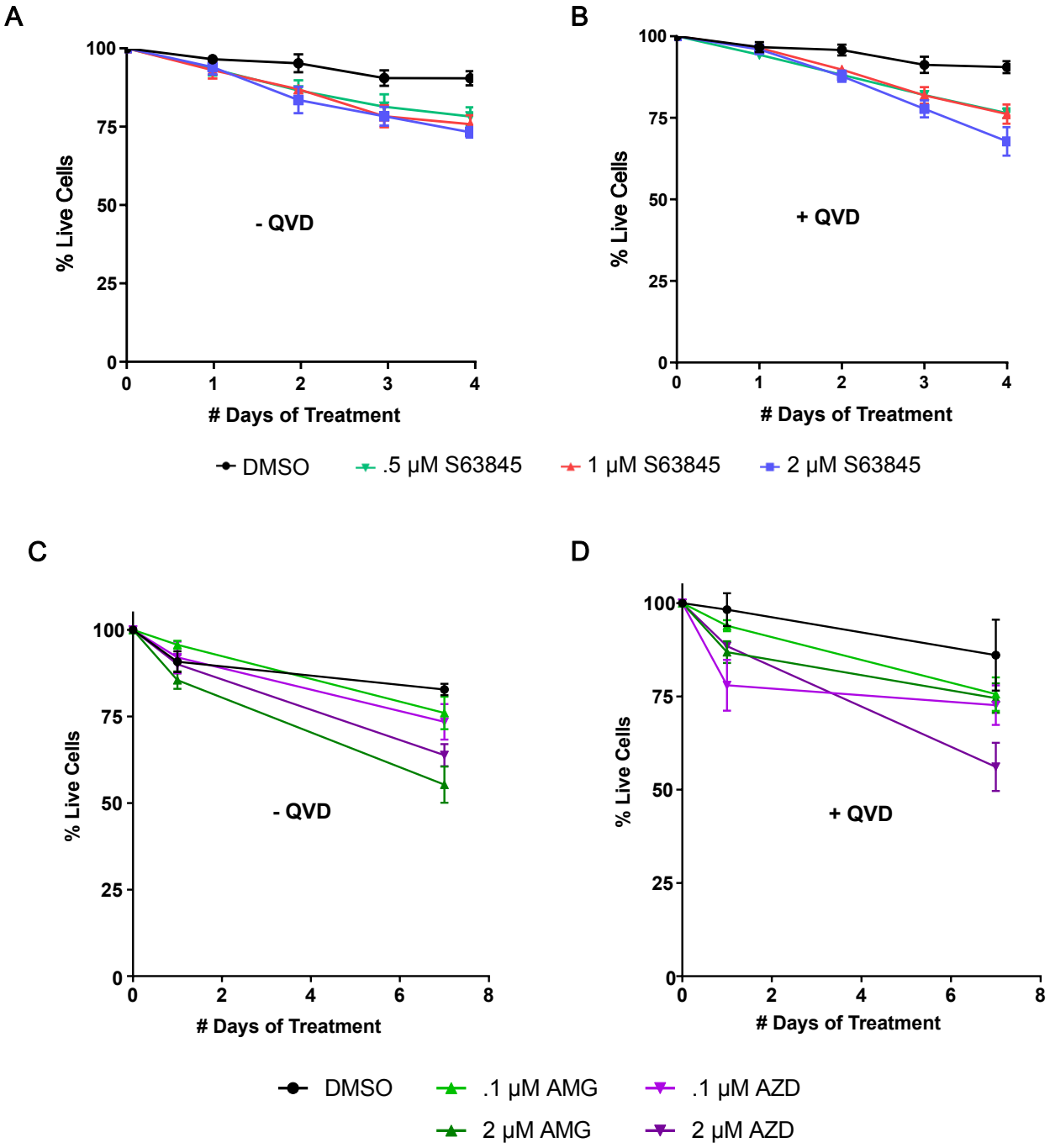


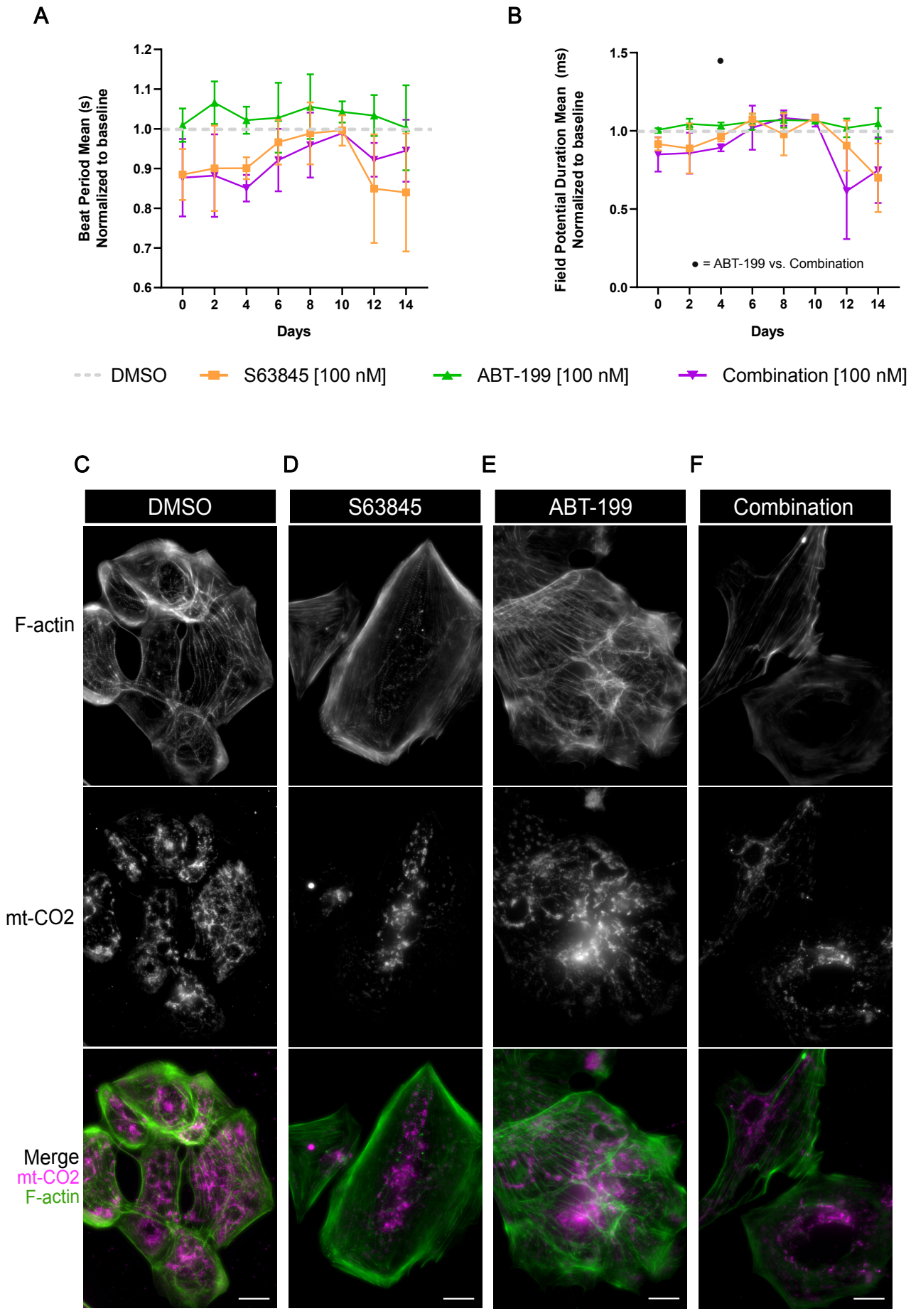
B



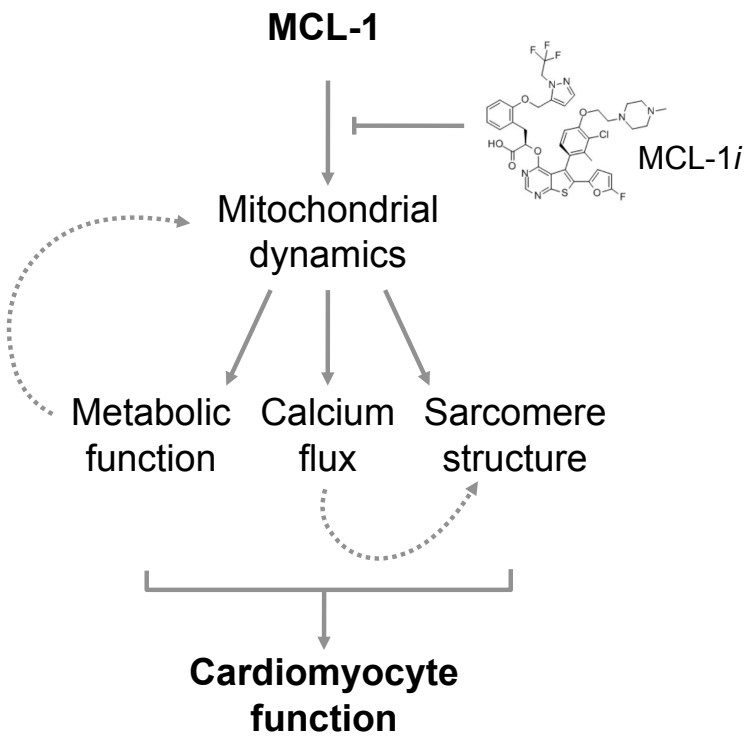

A
Cytoplasm
Membrane
DMSO S63845 DMSO S63845
75 –
50 –
37 –
25 –
DRP1
α-Tubulin
VDAC
B
DRP-1
Cytosolic fraction
p = .0641
Band Density Relative to Tubulin
2.0
1.5
1.0
0.5
0.0
DMSO + QVD
S63845 + QVD
C
DRP-1
Membrane fraction
p = .0032
Band Density Relative to Tubulin
1.5
1.0
0.5
0.0
DMSO + QVD
S63845 + QVD
D
TOM20
p = .2477
Band Density Relative to Tubulin
3
2
1
0
DMSO + QVD
S63845 + QVD
E
MitoTracker
PLA puncta
Merge
MitoTracker
PLA puncta
Secondary PLA probes only

Supplemental Figure 5




A
B
Beat Period Mean (s)
Normalized to baseline
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0
2
4
6
8
10
12
14
Days
Field Potential Duration Mean (ms)
Normalized to baseline
1.5
1.0
0.5
0.0
● = ABT-199 vs. Combination
DMSO
S63845 [100 nM]
ABT-199 [100 nM]
Combination [100 nM]
C
D
E
F
DMSO
S63845
ABT-199
Combination
F-actin
mt-CO2
Merge
mt-CO2
F-actin

Supplemental Figure 7



Materials and Methods

Cell Culture

Human induced pluripotent stem cell-derived cardiomyocytes (iCell Cardiomyocytes^2) were obtained from Cellular Dynamics International (#CMC-100-012-000.5). Cells were thawed according the manufacturer protocol in iCell Plating medium. Briefly, cells were thawed and plated on 0.1% gelatin at 50,000 cells/well in 96-well plates. Cells were maintained at 37°C and 5% CO_2 and fed every other day with iCell Cardiomyocyte Maintenance medium (Cellular Dynamics International #M1003). For knockdown experiments, wells were coated with 5 µg/mL fibronectin (Corning #354008) 1 hour prior to plating. For functional experiments using the Axion bioanalyzer, cells were plated on 50 µg/mL fibronectin in a 48-well CytoView MEA plate (Axion Biosystems #M768-tMEA-48B). For imaging experiments, cells were re-plated on glass-bottom 35 mm dishes (Cellvis #D35C4-20-1.5-N) coated with 10 µg/mL fibronectin. For live-cell imaging, cells were maintained at 37°C with 5% CO_2 in a stage top incubator (Tokai Hit). To account for batch effects, at least two different vials of iCell Cardiomyocytes^2 were used to replicate each experiment.

Human induced pluripotent stem cells (hiPSC) were generated from human fibroblasts as previously described (Wang et al., 2018). hiPSCs were maintained on growth factor-reduced Matrigel (Corning) coated plates in home-made E8 medium. hiPSCs were passaged every 4 days using 0.5 mM EDTA for 10 minutes at room temperature. hiPSCs were maintained at 37 °C at 5% CO_2 and received fresh medium daily.

hiPSC-derived cardiomyocytes (hiPSC-CMs) used in Figure 6 were generated using the small molecules CHIR 99021 (Selleck Chemicals) and IWR-1 (Sigma). Cardiac differentiation media were defined as M1 (RPMI 1640 with glucose with B27 minus insulin), M2 (RPMI 1640 minus glucose with B27 minus insulin), and M3 (RPMI 1640 with glucose with B27). hiPSCs were cultured until they reached 60% confluence, at which point cardiac differentiation was initiated (day 0). At day 0, hiPSCs were supplemented in M1 with 6 µM CHIR99021. On day 2, the media

was changed to M1. On day 3, cells were treated with 5 μM IWR-1 in M1. Metabolic selection was started at day 10 and cells were treated with M2 from day 10 to 16. On day 16, cells were transitioned to M3 with or without 0.1 uM triiodo-L-thyronine hormone (Sigma) and 1 uM Dexamethasone (Cayman) (Parikh et al., 2017). Media was changed every other day until day 30. For experiments in Figure 5, data was collected from at least three separate differentiations.

Cell Treatments

All treatments were added directly to cells in maintenance medium. The pan-caspase inhibitor Q-VD-OPh (SM Biochemicals #SMPH001) was added to cells at a concentration of 25 μM. The small molecule MCL-1 inhibitor derivative (S63845) was a gift from Joseph Opferman (St. Jude's Children Hospital). ABT-199 was purchased from Active Biochemicals (#A-1231). AMG-176 (#CT-AMG176) and AZD5991 (#CT-A5991) were purchased from ChemieTek. The necrosis inhibitor IM-54 (#SC222053A) was purchased from Santa Cruz Technologies and added to cells at a concentration of 10 μM. All stock solutions were prepared in DMSO. H_2O_2 solution (#H1009) was purchased from Sigma and added to cells at 10 μM in sterile water.

RNAi and Plasmid Transfection

Commercially available siRNAs targeting DRP-1 (DNM1L) (Thermo Fisher Scientific #AM51331, ID19561) and MCL-1 (Thermo Fisher Scientific #4390824, IDs8583) were used to generate transient knockdowns in hiPSC-CMs. Cells were seeded at 50,000 cells per well in a 96-well plate coated with 5 μg/mL fibronectin. Cells were transfected as per the manufacturer protocol using either TransIT-TKO Transfection Reagent (Mirus Bio #MIR2154) or RNAiMax (Thermo Fisher Scientific #13778075) in iCell maintenance media containing 25uM Q-VD-OPh (QVD). To increase knockdown efficiency, the transfection was repeated 48 and 96 hours later. Cells were left to recover for an additional 24 hours in fresh media containing 25uM QVD. Cells were lysed for Western blot or re-plated on glass-bottom 35 mm dishes and fixed for analysis by

immunofluorescence. Silencer Select Negative Control No. 1 (Thermo Fisher Scientific # 4390843) was used as a control.

Plasmids encoding mito-tdEos (Addgene #57644) or GCaMP5G (Addgene #31788) were transfected using ViaFect (Promega #E4981) as described in the manufacturer protocol. Cells were maintained until optimal transfection efficiency was reached before cells were imaged.

Immunofluorescence

For immunofluorescence, cells were fixed with 4% paraformaldehyde for 20 min and permeabilized in 1% Triton-X-100 for 10 min at room temperature. After blocking in 10% BSA, cells were treated with primary and secondary antibodies using standard methods. Cells were mounted in Vectashield (Vector Laboratories #H-1000) prior to imaging. Primary antibodies used include Alexa Fluor-488 Phalloidin (Thermo Fisher Scientific #A12379), mouse anti-mtCO2 (Abcam #ab110258), and mouse anti-Mitochondria (Abcam #ab92824). For Incucyte experiments, nuclei were visualized using NucLight Rapid Red Reagent (Essen Bioscience #4717). Alexa Fluor-488 (Thermo Fisher Scientific #A11008) and Alexa Fluor-568 (Thermo Fisher Scientific #A11011) were used as secondary antibodies. MitoTracker Red CMXRos (Thermo Fisher Scientific #M7512) added at 100 nM was used to visualize mitochondria in PLA experiments.

Membrane fractionation

Cells were lysed in 1X CHAPS (Sigma #C5070-5G) containing protease and phosphatase inhibitors for 20 mins on ice while vortexing, followed by centrifugation at 14,000 rpm for 20 mins. The supernatant was collected (cytosolic fraction) and the pellet was resuspended in the same volume of 1X CHAPS buffer containing protease and phosphatase inhibitors. Lysates were then prepared for gel electrophoresis and Western blot as described below.

Western blot

Gel samples were prepared by mixing cell lysates with LDS sample buffer (Life Technologies, #NP0007) and 2-Mercaptoethanol (BioRad #1610710) and boiled at 95°C for 5 minutes. Samples were resolved on 4-20% Mini-PROTEAN TGX precast gels (BioRad #4561096) and transferred onto PVDF membrane (BioRad #1620177). Antibodies used for Western blotting are as follows: DRP-1 (Cell Signaling Technologies #8570S), pDRP-1 S616 (Cell Signaling Technologies #4494), OPA1 (Cell Signaling Technologies #67589S), MCL-1 (Cell Signaling Technologies #94296S), TOM20 (Cell Signaling Technologies # 42406S), VDAC (Cell Signaling Technologies #4866T) and α-Tubulin (Sigma # 05-829).

Impedance assays

The Axion Biosystems analyzer was used to measure contractility and impedance in iPSC-CMs. Cells were plated on 48-well CytoView MEA plates and maintained for 10 days before treatment and recordings. Recordings were taken for 5 minutes approximately two hours after media change at 37°C and 5% CO_2. Cells were assayed using the standard cardiac analog mode setting with 12.5 kHz sampling frequency to measure spontaneous cardiac beating. The Axion instrument was controlled using Maestro Pro firmware version 1.5.3.4. Cardiac beat detector settings are as follows:

Beat Detection Threshold	300 µV
Min. Beat Period	250 ms
Max. Beat Period	5 s
Synchronized Beat Maximum Propagation Delay	30 ms
Minimum Active Channels Ratio	50.00%
Running Average Beat Count	10

Recordings for the hormone-matured hiPSC-CMs in Figure 6 were obtained using a

CardioExcyte96 instrument (Nanion Technologies). Cell preparation, data acquisition, and data processing were completed as described previously (Chavali et al., 2019).

Calcium imaging

Cells were transfected with GCaMP5G probe in 96-well plates and protein expression was allowed to stabilize for 48 hours. Cells were maintained on the microscope stage incubator for 30 minutes prior to imaging on a Nikon Eclipse Ti inverted microscope equipped with a 10X .30 NA objective. For each biological replicate, three technical replicates of a 10-second time-lapse recording were collected for each well. Baseline recordings were taken 24 hours prior to treatment. Subsequent recordings were taken 2 hours post treatment. Calcium intake was quantified in Fiji. Briefly, background fluorescence was first measured for intensity correction. A kymograph was then created using the Multiple Kymograph tool in Fiji using a 25-pixel thick line drawn across the entire cell. Calcium intake was measured as the ratio of the mean intensity of the brightest frame to the mean intensity of the darkest frame in the kymograph.

Plate-reader assays (CaspaseGlo 3/7 and CellTiter-Blue)

Cells were plated onto 1% gelatin-coated white round-bottomed 96-well plates at 20,000 cells/well. At least two duplicate wells were used per condition. At the end of inhibitor treatments, media was aspirated from the wells and a 1:1 ratio of CaspaseGlo 3/7 reagent (Promega #8091) and fresh media was added. The plates were incubated at room temperature for 1 hr in the dark and analyzed in a Promega GloMax luminometer according to manufacturer instructions. For the cell viability assay in Figure 5C, hiPSC-CMs were plated onto 1% gelatin-coated black clear-bottomed 96-well plates at 20,000 cells/well. Three replicate wells were used per condition. Cells were pre-treated with indicated inhibitors for 24 hours, followed by addition of fresh media and inhibitors or H_2O_2 for an additional 24 hours. 10% Triton-X-100 was added to untreated wells as a blank for normalization. CellTiter-Blue reagent was added to wells (20 µL) and plates were

incubated at 37°C and 5% CO_2 for 2 hrs. Plates were analyzed in a POLARstar Omega plate-reader (BMG LabTech) according to manufacturer instructions.

Seahorse Mito Stress Test

hiPSC-CMs were plated from thaw onto 1% gelatin-coated Seahorse XF96 V3 PS cell culture microplates 7 days before the assay at 20,000 cells per well. 24 hours prior to the assay, inhibitor treatments were added to triplicate wells in maintenance media. One hour prior to the assay, media was switched to XF DMEM media containing 1 mM pyruvate, 2 mM glutamine, and 10 mM glucose. Oxygen consumption rate (OCR) was measured sequentially after addition of 1.0 μM oligomycin, 0.5 μM FCCP, and 0.5 μM rotenone.

Proximity Ligation Assay (PLA)

Cells were plated onto fibronectin-coated 8-chamber MatTek glass slides (#CCS-8) at 10,000 cells/chamber. After treatments, cells were fixed in 4% PFA for 20 min and permeabilized in 1% Triton-100-X for 10 min at room temperature. Following fixation, the DuoLink proximity ligation assay (Sigma #DUO92014) was performed as per manufacturer protocol. The primary antibodies were incubated overnight at 4°C and are as follows: mouse anti-MCL-1 (Proteintech # 66026-1-Ig), rabbit anti-DRP-1 (Cell Signaling Technologies #8570S), rabbit anti-OPA1 (Cell Signaling Technologies #67589S), and control containing no primary antibody.

Photoconversion experiments

Mitochondrial network connectivity and fusion was assayed using photo-conversion of mitochondria tagged with mito-tdEos (Addgene #57644). Photo-conversion was performed on a Nikon Eclipse Ti inverted widefield microscope equipped with a 1.45 NA 100X Oil objective. Briefly, a stimulation region was closing down the field diaphragm and using the filter to shine 405 nm light for 6 seconds. Images for the converted (TxRed) and unconverted (FITC) were acquired

before and after stimulation. The TxRed image before stimulation was used to subtract background from the post-stimulation images, followed by thresholding and automated measurement in Fiji (Schindelin et al., 2012). The initial converted area immediately after stimulation was used as a measure of connectivity, while the spread of the converted signal after 20 minutes was used as a measure of fusion/motility. The initial converted area (TxRed channel) was normalized to the total unconverted area (FITC channel) to account for any initial variation in the total mitochondrial area.

Image acquisition

Super-resolution images for Figures 1 and 2 were acquired using a GE DeltaVision OMX microscope equipped with a 1.42 NA 60X Oil objective and a sCMOS camera. Super-resolution images for Figures 1, 2, 7, S2 and S6 were acquired using a Nikon SIM microscope equipped with a 1.49 NA 100x Oil objective an Andor DU-897 EMCCD camera. Images for Figures 4, S3 and S6 were acquired on a Nikon Eclipse Ti inverted widefield microscope equipped with a 1.45 NA 100X Oil or 1.40 NA 60X Oil objective. Image processing and quantification was performed using Fiji. Measurement of cell number to assay cell death was performed on a Incucyte S3 live cell imaging system (Essen Bioscience) equipped with a 10X objective. Images for the PLA experiments were acquired on a Nikon Eclipse Ti-E spinning disk confocal microscope equipped with a 1.40 NA 60X Oil objective and an Andor DU-897 EMCCD camera.

Statistical Analysis

All experiments were performed with a minimum of 3 biological replicates. Statistical significance was determined by unpaired, two-tailed Student’s t-test or by one- or two-way ANOVA as appropriate for each experiment. GraphPad Prism v8.1.2 was used for all statistical analysis and data visualization.

Error bars in all bar graphs represent standard error of the mean or standard deviation as described for each figure, while Tukey plots were represented with boxes (with median, Q1, Q3 percentiles), whiskers (minimum and maximum values within 1.5 times interquartile range) and solid circles (outliers). No outliers were removed from the analyses.

For MEA experiments, means from triplicate biological replicates (each with 2-3 technical replicate wells) for each measurement were plotted and significance was determined by two-way ANOVA. 20 hour data was excluded for two-way ANOVA in Figure 2B-C and E-F, since this time-point was not recorded for ABT-199; likewise, ABT-199 was excluded from analysis in Figure 2D.

For PLA experiments, images were quantified using Fiji. Briefly, background noise levels were subtracted, and number of puncta per ROI was normalized to mitochondrial area. ROIs in at least 5 cells per condition were quantified in three independent experiments.

Quantification of mitochondrial morphology was performed in NIS-Elements (Nikon); briefly, we segmented mitochondria in 3D and performed skeletonization of the resulting 3D mask. Skeleton major axis and sphericity measurements were exported into Excel, and the data was filtered and analyzed in MatLab. Quantification of actin organization was performed in a blinded fashion and percentages of each category are displayed. Cell viability measured using the Incucyte live cell imaging system was performed by automatic segmentation of nuclei in Fiji, followed by subtraction of dead cells as indicated by fragmented nuclei and rounded phenotype detected by phase contrast.

Key Resources Table

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Mouse anti-mitochondria [113-1]	Abcam	Cat#Ab92824 RRID:AB_10562769
Mouse anti-mtCO2	Abcam	Cat#Ab110258; RRID:AB_10887758
Alexa Fluor-488 Phalloidin	Thermo Fisher Scientific	Cat#A12379

Alexa Fluor-488	Thermo Fisher Scientific	Cat#A11008, RRID:AB_143165
Alexa Fluor-568	Thermo Fisher Scientific	Cat#A1101; RRID:AB_143157
Rabbit anti-pDRP-1 S616	Cell Signaling Technologies	Cat#3455S; RRID:AB_2085352
Rabbit anti-DRP-1	Cell Signaling Technologies	Cat#8570S; RRID:AB_10950498
Rabbit anti-pDRP-1 S616	Cell Signaling Technologies	Cat#4494; RRID:AB_11178659
Rabbit anti-OPA1	Cell Signaling Technologies	Cat#67589S; RRID:AB_2799728
Rabbit anti-MCL-1	Cell Signaling Technologies	Cat#94296S; RRID:AB_2722740
Rabbit anti-Tom20	Cell Signaling Technologies	Cat#42406S; RRID:AB_2687663
Rabbit anti-VDAC	Cell Signaling Technologies	Cat#4866T; RRID:AB_2272627
Mouse α-Tubulin	Millipore	Cat#05-829; RRID:AB_310035
Mouse anti-MCL-1	Proteintech	Cat#66026-1-Ig; RRID:AB_11041711
Chemicals, Peptides, and Recombinant Proteins		
Fibronectin	Corning	Cat#354008
CHIR 99012 GSK-3α and GSK-3β inhibitor	Selleck Chemicals	Cat#S1263
IWR-1 tankyrase inhibitor	Sigma	Cat#I0161
S63845 MCL-1 inhibitor derivative	Joseph Opferman, St. Jude's Children Hospital	N/A
Q-VD-OPh pan-caspase inhibitor	SM Biochemicals	Cat#SMPH001
ABT-199 BCL-2 inhibitor	Active Biochemicals	Cat#A-1231
TransIT-TKO Transfection Reagent	Mirus Bio	Cat#MIR2154
Lipofectamine RNAiMax Transfection Reagent	Thermo Fisher Scientific	Cat#13778075
Silencer Select Negative Control No. 1	Thermo Fisher Scientific	Cat#4390843
Viafect transfection reagent	Promega	Cat#E4981
AZD5991 MCL-1 inhibitor	ChemieTek	Cat#CT-A5991
AMG-176 MCL-1 inhibitor	ChemieTek	Cat#CT-AMG176
IM-54 necrosis inhibitor	Santa Cruz Biotechnology	Cat#SC222053A
Hydrogen peroxide solution	Sigma	Cat#H1009
Critical Commercial Assays		
DuoLink Proximity Ligation Assay	Sigma	Cat#DUO92014
Seahorse XF Cell Mito Stress Test Kit	Agilent	Cat#103015-100
Caspase-Glo 3/7 Assay	Promega	Cat#8091
CellTiter-Blue Cell Viability Assay	Promega	Cat#8081
Experimental Models: Cell Lines		
Human: iCell Cardiomyocytes[2], iPSC-derived cardiomyocytes	Cellular Dynamics International	Cat#CMC-100-012-000.5
Oligonucleotides		
siRNA targeting DRP-1	Thermo Fisher Scientific	Cat#AM51331 ID19561
siRNA targeting MCL-1	Thermo Fisher Scientific	Cat#4390824, IDs8583
Recombinant DNA		

Plasmid encoding mito-tdEos (tdEos-Mito-7)	Addgene Shtengel et al., 2009	Cat#57644; RRID:Addgene_57644
Plasmid encoding GCaMP (pCMV-GCaMP5G)	Addgene Akerboom et al., 2012	Cat#31788, RRID:Addgene_31788
Software and Algorithms		
Maestro Pro firmware version 1.5.3.4	Axion Biosystems	https://www.axionbiosystems.com/applications/cardiac-activity
Fiji	Schindelin et al., 2012	https://imagej.net/Fiji
GraphPad Prism v8.1.2	GraphPad	https://www.graphpad.com/scientific-software/prism/
NIS-Elements AR	Nikon	https://www.nikon.com/products/microscope-solutions/lineup/img_soft/nis-elements/
Other		
iCell Cardiomyocyte maintenance medium	Cellular Dynamics International	Cat#M1003
NucLight Rapid Red Reagent	Essen Bioscience	Cat#4717
MitoTracker Red CMXRos	Thermo Fisher Scientific	Cat#M7512
Incucyte S3 live cell imaging system	Essen Bioscience	Cat#4763
CytoView microelectrode array plate – 48 wells	Axion Biosystems	Cat#M768-IMEA-48B
CytoView MAESTRO Pro MEA system	Axion Biosystems	N/A
Promega GloMax luminometer	Promega	N/A
POLARstar Omega plate-reader	BMG LabTech	N/A
Seahorse XF calibrant	Agilent	Cat#100840-000
Seahorse XF96 V3 PS cell culture microplates	Agilent	Cat#101085-004
Seahorse XFe96 Extracellular flux assay kits	Agilent	Cat#102601-100
Seahorse XFe96 Analyzer	Agilent	N/A
Seahorse XF DMEM medium pH 7.4	Agilent	Cat#103575-100
Seahorse XF 1.0 M glucose solution	Agilent	Cat#103577-100
Seahorse XF 100mM pyruvate solution	Agilent	Cat#103578-100
Seahorse XF 200 mM glutamine solution	Agilent	Cat#103579-100

Supplemental Information References

Chavali, N.V., Kryshtal, D.O., Parikh, S.S., Wang, L., Glazer, A.M., Blackwell, D.J., Kroncke, B.M., Shoemaker, M.B., Knollmann, B.C., 2019. Patient-independent human induced pluripotent stem cell model: A new tool for rapid determination of genetic variant pathogenicity in long QT syndrome. Heart Rhythm 16, 1686–1695. https://doi.org/10.1016/j.hrthm.2019.04.031

Parikh, S.S., Blackwell, D.J., Gomez-Hurtado, N., Frisk, M., Wang, L., Kim, K., Dahl, C.P., Fiane, A., Tønnessen, T., Kryshtal, D.O., Louch, W.E., Knollmann, B.C., 2017. Thyroid and Glucocorticoid Hormones Promote Functional T-Tubule Development in Human-Induced Pluripotent Stem Cell–Derived Cardiomyocytes. Circ. Res. 121, 1323–1330. https://doi.org/10.1161/CIRCRESAHA.117.311920

Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch, S., Rueden, C., Saalfeld, S., Schmid, B., Tinevez, J.-Y., White, D.J., Hartenstein, V., Eliceiri, K., Tomancak, P., Cardona, A., 2012. Fiji: an open-source platform for biological-image analysis. Nat. Methods 9, 676–682. https://doi.org/10.1038/nmeth.2019

Wang, L., Kim, K., Parikh, S., Cadar, A.G., Bersell, K.R., He, H., Pinto, J.R., Kryshtal, D.O., Knollmann, B.C., 2018. Hypertrophic cardiomyopathy-linked mutation in troponin T causes myofibrillar disarray and pro-arrhythmic action potential changes in human iPSC cardiomyocytes. J. Mol. Cell. Cardiol. 114, 320–327. https://doi.org/10.1016/j.yjmcc.2017.12.002