SI Appendix

Materials and Methods

Experimental model and subject details

The human subjects portion of this study was reviewed and approved by the Colorado Multiple Institutions Review Board (COMIRB #06-0452). Human hearts from healthy donors and patients with Danon Disease were obtained from a tissue bank maintained by the Division of Cardiology at the University of Colorado (COMIRB #01-568). All patients were followed by the University of Colorado Heart Failure Program and offered participation in the research protocol. All research involving animals complied with protocols approved by the Institutional Animal Care and Use Committee (IACUC) of University of Colorado.

Generation and characterization of hiPSCs

Reprogramming of human skin fibroblast cells derived hiPSCs was performed following modification of a previous protocol (1). Briefly, human fibroblast cells were infected with retrovirus produced in L293 cells transfected with pMXs plasmid DNA (encoding Oct3/4, Sox2, Klf4, and c-Myc, respectively): pUMVC: pCMV-VSV-G (8:8:1) together with FuGENE6 (Promega) according to the manufacturer's protocol. After the infection, media was replaced by FP medium (DMEM/high glucose with 10% FBS, and 50 units and 50 mg/mL Penicillin and Streptomycin). Cells were replated to a 10 cm dish containing 2.4 million of mitomycin C-inactive SNL cells at day 5 and maintained with human ES medium. After 2 to 3 weeks, hiPSC colonies with ES-like morphology became visible and were picked at around day 30. After the hiPSC lines were established, cells were maintained in 12-well-plate coated with MatriGel (Corning) and fed with fresh mTeSR1 daily. When the cell confluence reached 70-85%, cells were dissociated with Gentle Cell Dissociation Reagent (STEMCELL Technologies) and split into a MatriGel coated dish with mTeSR1 (STEMCELL Technologies) supplemented with 10 μM Y-27632 (Enzo Life Sciences). After 24h, media were replaced with mTeSR1.

To generate non-integrating iPSC lines, healthy male adult human dermal fibroblasts (Lonza, CC2511) were infected with CytoTune™-iPS 2.0 Sendai Reprogramming Kit (Thermo Fisher Scientifics) according to the manufacturer's instruction. Colonies were isolated 2 weeks after infection and passed in mTeSR1 for at least 10 passages until cells were clear of Sendai virus before used. hiPSC lines including CUSO-2, *LAMP-2A* KO, *LAMP-2B* KO and *LAMP-2C* KO were generated by the Sendai viral method.

To characterize the pluripotency of the generated hiPSCs, cells were replated to a 24-well-plate coated with MatriGel and fed with mTeSR1. After 3 days, cells were fixed with 4% PFA at room temperature for 20 min, and then blocked and permeabilized with PBS plus 10% horse serum (GEMINI) and 0.2% Triton at room temperature for 1 hour. Then, cells were stained with antibodies against SSEA-3 (Developmental Studies

Hybridoma Bank, 1:10), SSEA-4 (Developmental Studies Hybridoma Bank, 1:10), TRA-2-49 (Developmental Studies Hybridoma Bank, 1:20), TRA-1-81 (Cell Signaling Technology; 1:20) or Nanog (Cell Signaling Technology, 1:100) at room temperature for 1 hour, followed by secondary antibody staining at room temperature for 1 hour. The karyotype tests of hiPSCs were performed by Children's Hospital Los Angeles.

To assess the differentiation potential of the generated hiPSC lines, the hiPSC colonies were detached via scratching with a cell lifter. The whole colonies were replated to a 60 mm non-tissue culture dish and cultured with Human ES medium without bFGF (STEMCELL Technologies) overnight. The embryoid bodies were harvested and replated to a 0.1% gelatin-coated tissue culture plate and maintained in Human ES medium without bFGF. Then, the cells were fixed and stained with ectoderm marker Tuj1 (Biolegend, 1:200), mesoderm marker SMA (Santa Cruz, 1:500) and endoderm α1 fetoprotein (R and D Systems, 1:100), respectively.

Differentiation of hiPSCs into CMs and culture of hiPSC-CMs

At the time cells reached ~85% confluence, hiPSCs were dissociated as single cells with Accumax (STEMCELL Technologies) at 37°C for 5 min and replated to a 24-well plate pre-coated with MatriGel. Cells were fed with 1 mL of mTeSR1 per well for 4 days before induction. At day 0, medium was changed to RPMI1640 (Life Technologies) with B27 supplement minus insulin (Life Technologies) plus 8 μM ChIR99021 (Cayman) (2). On day 1 (24h), medium was changed to RPMI1640 with B27 supplement minus insulin. On day 3 (72h), media was replaced with combined medium (made by combining 0.5 mL of old medium with 0.5 mL fresh RPMI1640/B27 supplement minus insulin, supplemented with 5 μM IWP2 (Thermo Fisher Scientifics)). On day 5, media was replaced with fresh RPMI1640/B27 supplement minus insulin. On day 7, media was replaced with RPMI1640/B27 supplement (Life Technologies). Cells were then maintained in RPMI1640/B27 medium with medium changed every 3 days. Contracting cells were observed starting on day 7. On day 20, hiPSC-CMs were enriched by lactate selection, using glucose free DMEM supplemented with 4 mM lactate, for 5 days (3). To quantify the percentage of hiPSC-CMs in the total cell population, cells were fixed and stained with cTnT (Thermo Scientific, 1:400) followed by flow cytometry analysis.

CRISPR/Cas9-mediated genome editing in hiPSCs

SgRNAs for gene specific targeting were designed using the online tool on http://crispr.mit.edu website. The targeting RNP complex, composed of synthetic Alt-R CRISPR-Cas9 crRNAs (IDT), Alt-R CRISPR-Cas9 tracrRNA (IDT), Alt-R Cas9 Electroporation Enhancer (IDT) and Alt-R S.p. Cas9 Nuclease 3NLS (IDT), was delivered into hiPSCs by electroporation with the Amaxa human stem cell nucleofector starter kit (Lonza). After electroporation, single cell clones were collected and cultured for genotyping by PCR to confirm the targeting event. The genomic sequence of positive

clone of edited hiPSCs was determined by Sanger sequencing. The sequences for sgRNAs and genotyping PCR primers used in this study were listed in **Tables S2** and **S3**.

Analysis of glycogen and vacuole accumulation

For glycogen accumulation, Periodic Acid-Schiff (PAS) staining was performed using the Periodic Acid-Schiff Kit (Sigma). In brief, cells were fixed in 4% formaldehyde for 2 min, washed with 1xTBST, stained for 5 min with periodic acid, and washed 3 times with DPBS. Cells were then stained with Schiff's reagent for 15 min, washed 3 times with DPBS, counterstained with hematoxylin solution for 90 sec, and washed 3 times with DPBS before imaging.

For vacuole accumulation, cells were fixed in 2% Paraformaldehyde/2.5% Glutaraldehyde at room temperature for 1 hour. Fixation solution was then removed and replaced with 0.1 M Cacodylate buffer (Electron Microscopy Sciences). Further sample preparation and electron microscopy imaging was performed by the Electron Microscopy Center in the University of Colorado School of Medicine.

TUNEL assay

To assess cell death, TUNEL staining was performed using the *In Situ* Cell Death Detection Kit TMR red (Roche) according to the manufacturer's manual. In brief, cells were fixed with 4% PFA for 1 hour at room temperature and permeabilized using 0.1% Triton X-100 in 0.1% sodium citrate for 2 min on ice. Cells were then incubated in the labeling enzyme solution in a 37°C-humidified chamber for 1 hour. This was followed by co-immunostaining with the cardiac marker cTnT (Thermo Scientific, 1:400) before imaging. Cells treated with DNase I (Sigma) or pretreated with 10 μM H_2O_2 (Sigma) for 24 hours were used as positive controls.

Starvation assays

For starvation, cells were washed with DPBS three times and incubated in starvation medium at 37°C for 1, 2 or 4 hours. For CMs, glucose-free DMEM (gibco) was used as starvation medium; for non-CMs, the composition of the starvation medium is as previously described (4). To block autophagy flux, 400 nM of bafilomycin A1 (Cayman) was added to the starvation medium.

Plasmids and cloning

HA-ATG14 (5), FLAG-STX17, FLAG-SNAP29 and FLAG-VAMP8 (6) plasmids were purchased from Addgene. Human LAMP-2B cDNA clone was purchased from Origene. The C-terminus Myc-DDK tag was removed by digesting the plasmid with EcoRV and FseI, and then ligated with a DNA linker containing a stop codon. The resulting tag-free LAMP-2B cDNA plasmid was then used for this study. LAMP-2BΔCCD plasmid was generated by removing the cytosolic coiled coil domains (RRKSYAGYQTL) using the Q5 Site-Directed Mutagenesis Kit (NEB) according to manufacturer's instructions. LAMP-2A cDNA was PCR amplified from a reverse transcripted cDNA library from total RNA isolated from HEK293 cells and subcloned into the same backbone as the tag-free LAMP-

2B cDNA plasmid by SgfI and SacII. The sequences for the DNA linker and cloning primers were listed in Table S3.

siRNA treatments

Negative control and gene-specific siRNAs were purchased from Dharmacon. siRNAs were delivered into the target cells with Lipofectamine RNAiMAX (Invitrogen, for non-CMs) or Lipofectamine 3000 (Invitrogen, for CMs) reagents according to the manufacturer's protocols. Cells were incubated with siRNA for 48 hours before switching to regular culture medium for recovery. Cells were lysed at 72-hours post infection for downstream protein analysis.

Immunostaining and microscopy imaging

Cells were fixed in 2% paraformaldehyde for 30 min at room temperature and then washed 3 times with DPBS. The cells were permeabilized in 0.5% Triton X-100 for 20 min at room temperature. After blocking in DPBS containing 10% horse serum for 30 min at room temperature, cells were incubated with primary antibodies against α-actinin (Sigma, 1:500), cTnT (Thermo Scientific, 1:400), cTnI (PhosphoSolutions, 1:500), MYL2 (Proteintech, 1:200), HA (UBPBio, 1:500), FLAG (Gallus Immunotech, 1:500), and/or LC3B (Sigma, 1:250) in 10% horse serum in DPBS for 1 hour at room temperature. Cells were washed 3 times with DPBS and then incubated with secondary antibodies (Molecular Probes Alexa Fluor 488, 1:400 or Alexa Fluor 555, 1:1,000; Abcam Alexa Fluor 647, 1:400) and Hoechst (Molecular Probes, 1:5,000) for 30 min at room temperature. Cells were washed 3 times with DPBS before imaging by either EVOS FL Cell Imaging System (Life Technologies) or Olympus FV1000 FCS/RICS confocal microscope. For confocal microscopy, images were acquired using a 60x or 100x oil-immersion objective lens, and different fluorescent channels were captured individually and merged using Fluoview software (Olympus).

For co-staining with LysoTracker, live hiPSC-CMs were incubated with 100 nM LysoTracker Red DND-99 (Molecular Probes) in culture medium for 30 min at 37°C before fixation (7).

Immunoblotting and gene expression analysis

For immunoblotting, cells were washed with ice-cold DPBS twice before lysing in ice-cold lysis buffer (150 mM NaCl, 50 mM Tris-Cl pH 7.4, 1 mM EDTA, 1% Triton, with Complete mini tablet (Roche), and 1 mM phenylmethylsulphonyl fluoride freshly added before use). 10 μg of lysate per sample was loaded for Western blot analysis. The primary antibodies used include the following: anti-OPA1 (BD Biosciences, 1:1,000), anti-Pink1 (Novus Biologicals, 1:1,000), anti-VDAC (Cell signaling, 1:1,000), anti-LC3B (Sigma, 1:1,000), anti-LAMP-1 (Santa Cruz, 1:500), anti-LAMP2 (Developmental Studies Hybridoma Bank, 1:1,000), anti-STX17 (Sigma, 1:500), anti-VAMP8 (Sigma, 1:1,000), anti-ATG14 (Cell signaling, 1:1,000), anti-HA (UBPBio, 1:1,000), anti-HA (Rockland, 1:4,000), anti-FLAG (UBPBio, 1:1,000), anti-FLAG M2-Peroxidase (Sigma, 1:5000) and anti-GAPDH (Ambion,

1:5,000). The secondary antibodies used include the following: Goat Anti-Mouse IgG (Southern Biotech, 1:2,000) and Goat Anti-Rabbit IgG (Life Technologies, 1:2,000). For separation of MYH6 and MYH7, cell lysates were run on a modified 6% SDS-PAGE (separating acrylamide/bis ratio 1:100; resolving gel buffer pH 9.0; running gel buffer pH 8.2; β-mercaptoethanol 600 µl/L inner gel buffer). Gels were run overnight at 4°C and stained with BioSafe Coomassie Blue protein stain (Bio-Rad).

For gene expression analysis, total RNA was extracted with TRizol reagent (Invitrogen) and the RNeasy Plus Universal Mini Kit (Qiagen). cDNA was synthesized with the Superscript III First-Strand Synthesis System (Invitrogen). qPCR was performed using the SYBR Green PCR Master Mix (Applied Biosystems). GAPDH was used as an internal control. The primers for qPCR are listed in Table S3.

Transcriptome analysis

Total RNA from hiPSC-CMs was extracted with TRIzol reagent (Invitrogen) and purified with the RNeasy Plus Universal Mini Kit (Qiagen). The sequencing libraries preparation and RNA-seq were performed by the Genomics and Microarray Core Facility at the University of Colorado Anschutz Medical Campus by using a HiSeq 4000 sequencing system (Illumina). FastQC was used for the raw RNA-seq reads for the control of sequence quality, GC content, the presence of adaptor sequences and duplicated reads and ensure homogeneity of sequencing reads between samples. The reads passed quality control were aligned to NCBI GRCh38 human reference genome with Bowtie2 (8). The read count per gene was generated from the aligned sequencing reads using HTSeq 0.9.1 (9) with GTF file for NCBI GRCh38. DESeq2 (10) was used perform normalization of read counts across samples and differential expression analysis between Danon and control samples. The differentially expressed genes with > 1.5 fold expression change were used in the heatmap. Gene ontology analysis was performed using the web-based gene set analysis toolkit (http://webgestalt.org). For RNA-sequencing of adult mouse CMs, ventricular CMs were isolated from adult mouse hearts using a Langendorff perfusion system. Total RNA was isolated using Trizol reagent (Ambion) and genomic DNA was removed with the TURBO DNase I kit (Ambion). RNA concentration and quality was assessed with the Agilent Bioanalzyer (Agilent, Santa Clara, CA). Library construction was prepared using the TruSeq RNA sample Preparation Kit (Illumina) and sequenced using an Illumina HiSeq2500 (Illumina, Santa Clara, CA) with a read depth of 20-25 million reads at 1x100bp. RNA-sequencing read densities (and sashimi plots) were generated using the Integrative Genomics Viewer (http://software.broadinstitute.org/software/igv/).

All RNA-seq data have been deposited in the Gene Expression Omnibus (GEO). Accession numbers for those experiments reported in this paper are GSE71405 for NMCMs (11), GSE102792 for adult mouse cardiomyocytes and GSE108429 for hiPSC-CMs.

Generation of adenovirus and infection

Adenovirus including Ad-LacZ, Ad-GFP, Ad-LAMP-2A, Ad-LAMP-2B, Ad-HA-ATG14, Ad-FLAG-VAMP8 and Ad-mRFP-EGFP-LC3 were generated using the ViraPower Adenoviral Gateway Expression Kit (Invitrogen). In brief, genes of interested in this study were cloned to pENTR 2B (Invitrogen) to create the expression clones in the pAd/CMV/V5-DEST plasmid using Gateway® Technology (Invitrogen). Then, PacI-digested pAd-DEST expression plasmids were transfected to 293A cells using Lipofectamine 3000 (Invitrogen) reagent. Crude viral lysate were collected 7-10 days post-transfection. For virus amplification, a 10 cm plate of 293A cells was infected with 500 µL of crude viral stock, and the amplified virus was harvested 2 days after infection. Plaque assays were performed to determine the titer of an adenoviral stock using the Seaplaque Agarose (Lonza). MOI of 5 was used for CM infection.

Transfection

Cell transfection was performed using Polyethylenimine (PEI, Sigma) or Lipofectamine 3000 (Invitrogen) reagents according to protocols provided by manufacturers. Cells were harvested 48 hours post transfection for immunoblotting or immunoprecipitation.

Co-immunoprecipitation assays

Cells were lysed in ice-cold lysis buffer (150 mM NaCl, 50 mM Tris-Cl pH 7.4, 1 mM EDTA, 1% Triton, with Complete mini tablet (Roche), and 1 mM phenylmethylsulphonyl fluoride freshly added before use). Whole-cell lysates (input) were collect after removal of cell debris by centrifugation. 500 µg of lysate per sample was then incubated with indicated antibody and Dynabeads Protein G (Invitrogen) overnight at 4°C to pull down protein complexes. Normal mouse IgG (Santa Cruz) was used to replace antibody in negative control samples. Beads were washed 4 times with ice-cold lysis buffer and boiled in 1x SDS loading buffer for 15 min to elute proteins. Eluted proteins were subjected to immunoblotting as described in "**Immunoblotting and gene expression analysis"**.

Analysis of mitochondrial morphology and membrane potential

Live hiPSC-CMs were incubated with 200 nM MitoTracker Orange (for abundance, Molecular Probes) or 100 nM Tetramethylrhodamine (TMRM) (for membrane potential, Molecular Probes) in culture medium for 20 min or 30 min at 37°C respectively. Cells were washed with DPBS and counterstained with 1 µg/mL DAPI (EMD Millipore) before being analyzed by flow cytometry. Fluoresce intensities of DAPI negative cells were analyzed by the FACS Caliber (BD Sciences) and FlowJo software. Mitochondrial morphology was examined using Olympus FV1000 FCS/RICS confocal microscope.

Measurement of mitochondrial function

For mitochondrial function assays, Seahorse XF Cell Mito Stress tests were performed according to manufacturer's instructions. In brief, 60,000 hiPSC-CMs were seeded in 0.1% gelatin-coated Seahorse assay wells. Culture medium was changed to Seahorse XF Cell Mito Stress Test kit assay medium (Seahorses Biosciences) with indicated supplements on the day of assay and incubated for 1 hour in a 37°C non-CO2 incubator. Oxygen

consumption rate (OCR) was measured using a Seahorses Biosciences extracellular flux analyzer with the Cell Mito Stress Kit (Seahorses Biosciences) and normalized to total cell number. Mitochondrial function metrics were calculated based on changes of OCR after addition of oligomycin (2.5 μM), FCCP (1 μM), or antimycin (2.5 μM)/rotenone (2.5 μM) as directed in the Cell Mito Stress Kit manual.

Measurement of cellular ATP

Cellular ATP levels were measured by the ATP Bioluminescence Assay Kit HS II (Roche) according to manufacturer's instructions. Briefly, 200,000 cells per sample were collected and lysed in the cell lysis reagent provided with the assay kit. The luminescence generated by incubating cell lysates with luciferase reagent was detected by a GloMax Multi Detection System (Promega) and the readout was normalized to cell number. The ATP level of each sample was then calculated based on the comparison to an ATP standard curve.

Measurement of ROS

Live hiPSC-CMs were stained with 5 μM MitoSox (Molecular Probes) in culture medium for 10 min at 37°C. Cells were washed with DPBS and counterstained with 1 μg/mL DAPI (EMD Millipore) before being analyzed by flow cytometry. Fluoresce intensities of DAPI negative cells were analyzed by the FACS Caliber (BD Sciences) and FlowJo software.

Measurement of autophagic flux in hiPSC-CMs using mRFP-EGFP-LC3

hiPSC-CMs were infected with Ad-mRFP-EGFP-LC3 at MOI of 5. Four days later, half of cells were washed with DPBS 3 times and treated with starved medium for 4 hours. Cells were incubated in DPBS for confocal imaging using Olympus FV1000 FCS/RICS confocal microscope. Images were acquired using a 60x water-immersion objective lens, and different fluorescent channels were captured individually and merged using Fluoview software (Olympus).

Isolation of mitochondrial

Mitochondrial isolation from hiPSC-CMs was performed as previously described (12). In brief, about 2 million cells per sample were lysed in an ice-cold mitochondrial isolation buffer (MIB) containing: 200 mM mannitol, 70 mM sucrose, 5 mM HEPES and 1 mM EGTA, pH 7.5, and Complete mini tablet (Roche). Cell lysate was passed through a 1 mL syringe with a 26 1/2 G needle 20 times. Lysate was then centrifuged at 600g for 10 min at 4 °C. The supernatant was transferred to a new 1.5 ml tube and centrifuged again at 14,000g for 15 min at 4 °C. The supernatant was collected in a separate tube as cytosolic fraction. Then, the pellet containing the mitochondria fraction was resuspended in fresh MIB buffer and centrifuged again at 14,000g for 15 min at 4°C. The washed mitochondrial pellet was resuspended in MIB and directly used for protein quantification and immunoblotting.

Measurement of human heart myofibril mechanics

Human hearts from patients with Danon Disease were obtained from a tissue bank maintained by the Division of Cardiology at the University of Colorado. Hearts were collected at the time of orthotropic cardiac transplantation. Control hearts were obtained from unused donor hearts that could not be used for transplantation.

Myofibril mechanics were quantified using the fast solution switching technique as described previously (13). Frozen LV sections were skinned in 0.5% Triton-X in rigor solution (132 mM NaCl, 5 mM KCl, 1 mM MgCl2, 10 mM Tris, 5 mM EGTA, pH 7.1) containing protease inhibitors (10 μM leupeptin, 5 μM pepstatin, 200 μM PMSF and 10 μM E64), as well as 500 μM NaN3 and 500 μM DTT at 4°C overnight. Skinned LVs were washed in fresh rigor solution and homogenized (Tissue-Tearor, Thomas Scientific) in relaxing solution (pCa 9.0) containing protease inhibitors. Myofibril suspensions were transferred to a temperature controlled chamber (15°C) containing relaxing solution. Myofibril bundles were mounted between two micro-tools. One tool was connected to a motor that could produce rapid length changes (Mad City Labs). The second tool was a calibrated cantilevered force probe (6-8 μm/μN; frequency response 2-5 KHz). Myofibrils were set 5-10% above slack myofibril length. Average sarcomere lengths and myofibril diameters were measured using ImageJ software. Mounted myofibrils were activated and relaxed by rapidly translating the interface between two flowing streams of solutions of different pCa. Maximal tension generation was collected and analyzed using customized LabView software. Maximal tension (mN/mm2) represents maximal tension generated at full calcium activation (pCa 4.5).

Measurement of CM contractility by micropost arrays

hiPSC-CMs on day 50 were shipped to University of Washington. One week later, CMs were seeded on microposts for experiments. Micropost experiments were blindedly conducted in Sniadecki Lab. Arrays of microposts were used to calculate the twitch force of individual cells following previously established protocols (14). Briefly, arrays of polydimethylsiloxane (PDMS) microposts with bending stiffness k_{post} = 56.5 nN/μm were fabricated on glass coverslides (25 mm circle no. 2, VWR) through a soft lithography process. The spacing between posts was 6 μm. The tips of the microposts were coated with mouse laminin (Life Technologies) via microcontact printing, and the hiPSC-CMs were seeded onto the microposts in Attoflour® viewing chambers (Life Technologies) at a density of approximately 75,000 cells per cm^2 in RPMI medium with B27 supplement and 10% fetal bovine serum. The following day, the media was removed and replaced with serum-free RPMI medium, which was exchanged every other day. Once the cells resumed beating (typically 3 to 5 days after seeding), contractions of individual cells were imaged (at a minimum of 70 FPS) using a Hamamatsu ORCA-Flash2.8 Scientific CMOS camera fitted on a Nikon Eclipse Ti upright microscope using a 60x water immersion objective. Prior to imaging, the cell culture media was replaced with a tyrode buffer containing 1.8 mM Ca^{2+}, and a live cell chamber was used to maintain the cells at 37 °C

throughout the imaging process. A custom-written matlab code was used to track the deflection, Δ_{post}, of each post underneath a cell, and to calculate force, $F_{post} = k_{post} \times \Delta_{post}$. The reported values of twitch force were normalized by cell size by summing the twitch force at each post and dividing by the number of posts underneath an individual cell. The spread area of individual CMs on microposts were calculated using the NIS-Elements area tool by hand-tracing the cell outline from the first frame of the video.

Quantification and statistical analysis

Statistical significance was calculated using paired Student's *t* tests. $P<0.05$ was considered a significant difference. For quantification of Western blotting, gel images were quantified using densitometry analysis in Image J (NIH), and normalized to GAPDH. Data was collected from three independent experiments, normalized to a single control condition for each experiment. Statistical analysis of quantified gels was conducted in Graphpad Prism (Graphpad Software Inc.), using Student's *t*-test. P-values <0.05 were considered significant. For the micropost experiments, statistical analysis was performed on data sets where the average twitch force of each cell was considered as an individual data point. The data sets consisted of 3 or more independent differentiations for each cell line, with 20-25 cells from each batch. Parametric statistics were performed using a one-way ANOVA ("anova1" function in MATLAB) with a Bonferroni post-hoc test (using the "multcompare" function in MATLAB) to determine statistical difference between groups. When comparing the mean values of the maximal tension of myofibrils between control and patients with Danon disease, a one-way ANOVA function in GraphPad with Tukey's multiple comparison test was used. The resulting p-value < 0.05 was considered a significant difference between group means.

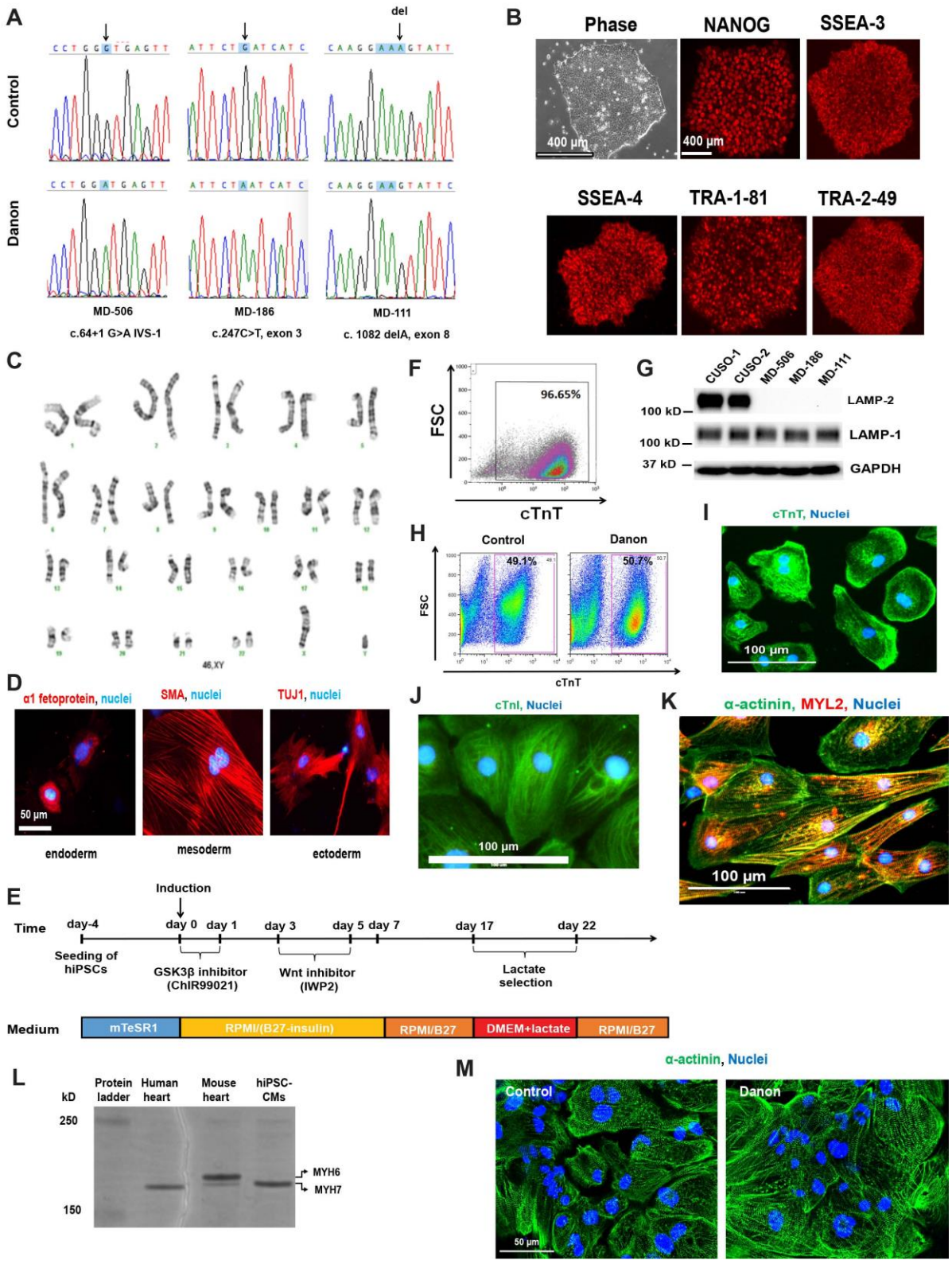


Fig. S1. Generation of hiPSCs and hiPSC-CMs.

(**A**) Mutations in the *LAMP-2* gene in patients with Danon disease confirmed by DNA-sequencing. Arrows indicate mutation sites relative to control genomic sequence.
(**B**) Representative hiPSC-colony morphology and expression of pluripotency markers NANOG, SSEA-3, SSEA-4, TRA-1-81 and TRA-2-49 in hiPSCs.
(**C**) Representative karyotype image. hiPSCs used in this study had normal 46, XY karyotype.
(**D**) hiPSCs used in this study were able to differentiate into derivatives of three germlayers: endoderm, mesoderm and ectoderm.
(**E**) Schematic indicating the protocol and timeline for cardiac differentiation of hiPSCs and enrichment of CMs.
(**F**) Enrichment of CMs derived from hiPSCs (hiPSC-CMs) for this study by lactate selection. Flow cytometry analysis of $cTnT^+$ CMs in culture was performed 30 days post-induction shown in (**E**).
(**G**) Immunoblotting analysis of LAMP-2 and LAMP-1 expression in control and Danon hiPSC-CMs. Control and Danon hiPSC-CMs expressed comparable levels of LAMP-1, a lysosomal marker.
(**H**) Differentiation of control and Danon hiPSCs into CMs. Before lactate selection, flow cytometry analysis of $cTnT^+$ CMs in culture was performed 10 days post-induction shown in (**E**).
(**I-K**) Representative immunofluorescence images of hiPSC-CMs stained for cTnT (green; **I**), cTnI (green; **J**), α-actinin (green; **K**) and MYL2 (red; **K**).
(**L**) Expression of MYH6 and MYH7 in mouse hearts, human hearts and hiPSC-CMs.
(**M**) Representative confocal images of control and Danon hiPSC-CMs stained for α-actinin.

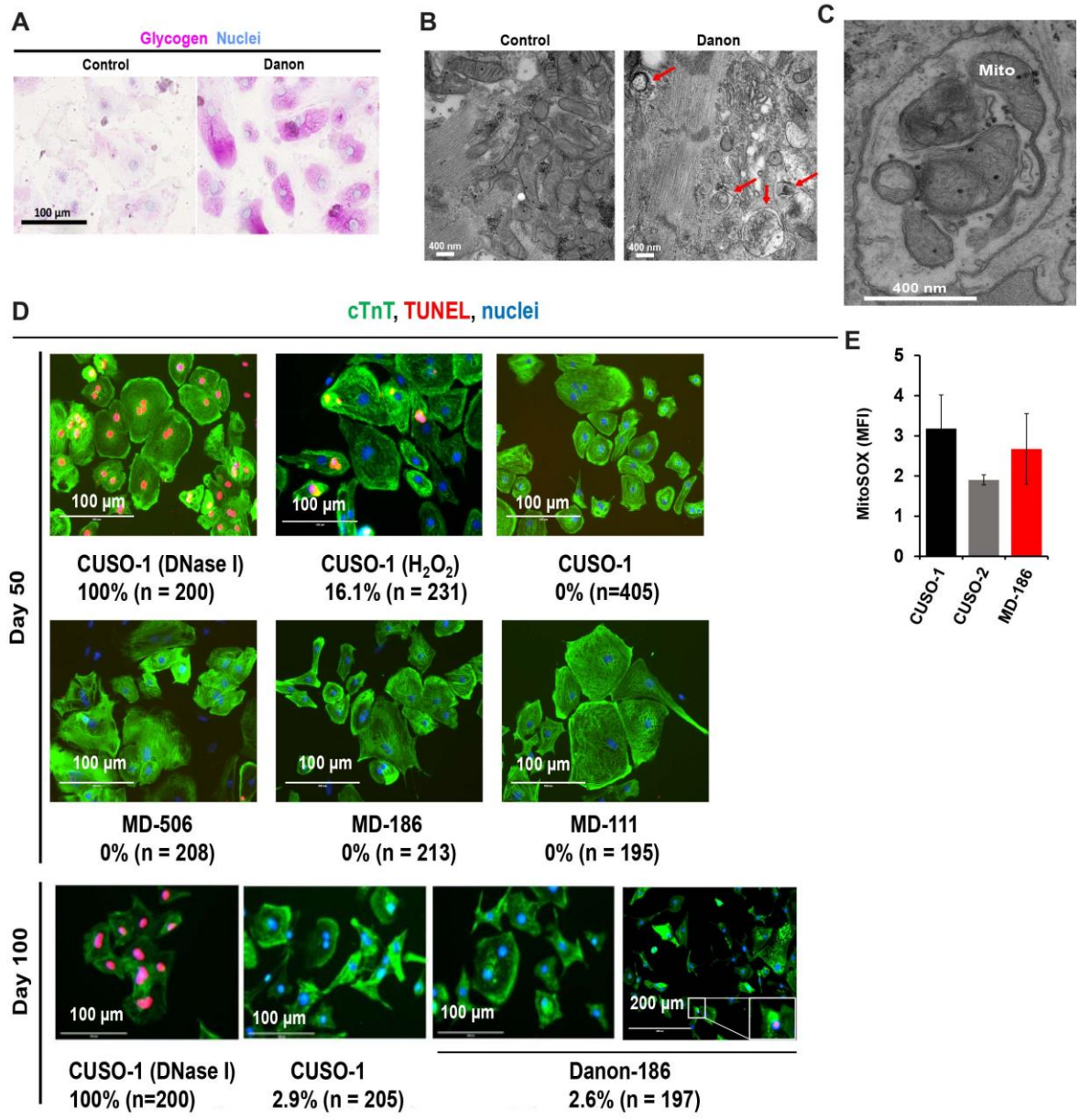


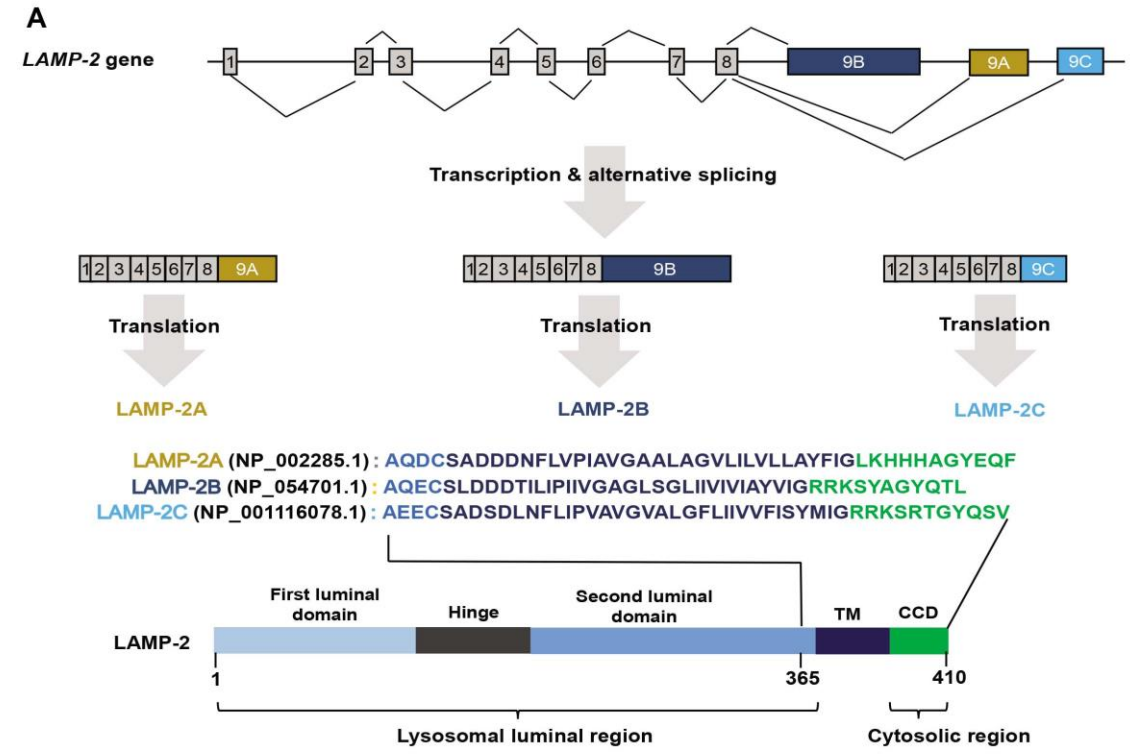
Fig. S2. Glycogen, vacuoles, and apoptosis in Danon hiPSC-CMs.

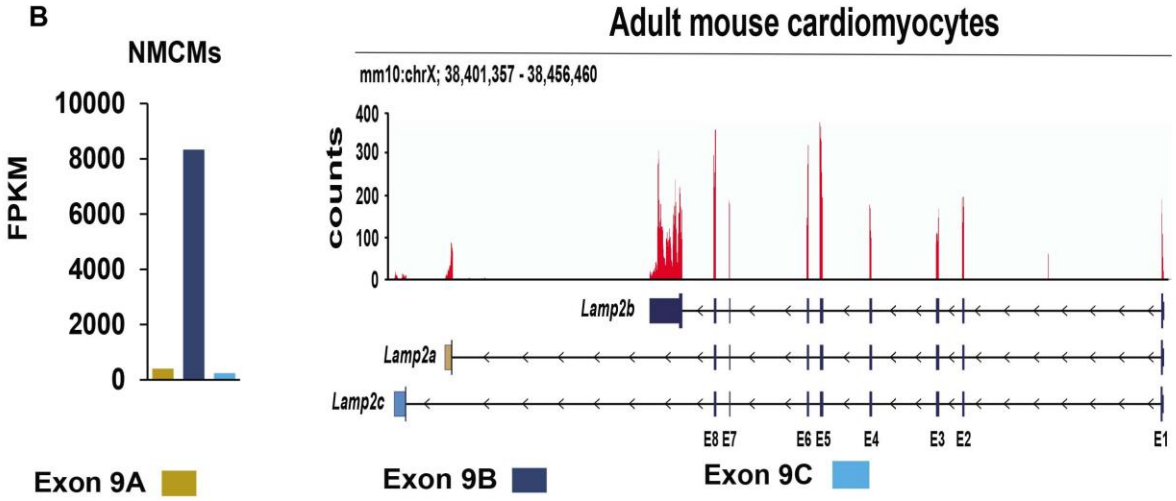
(**A**) Detection of glycogen in hiPSC-CMs by Periodic Acid–Schiff (PAS) staining.

(**B-C**) Representative transmission electron microscopy images of hiPSC-CMs. Red arrows point to vacuoles. A double-membrane autophagic vacuole containing mitochondria (Mito) from a Danon hiPSC-CM is shown in **C**.

(**D**) Representative fluorescence images of hiPSC-CMs immunostained for cTnT (green), TUNEL (red) and DNA (blue) on day 50 and day 100 post-induction. Percentages of

TUNEL^{+} CMs and total numbers of examined cells from three independent experiments for each line are shown below the image. hiPSC-CMs were treated with DNase I or 10 μM of H_2O_2 for 24 hours as positive controls.
(**E**) Cellular ROS were assessed by MitoSOX intensity. Live hiPSC-CMs were incubated with MitoSOX followed by flow cytometry analysis. Three independent repeats were performed. Data are presented as mean fluorescence intensity (MFI) ± s.d..


A
LAMP-2 gene
1
2
3
4
5
6
7
8
9B
9A
9C
Transcription & alternative splicing
1 2 3 4 5 6 7 8 9A
1 2 3 4 5 6 7 8 9B
1 2 3 4 5 6 7 8 9C
Translation
Translation
Translation
LAMP-2A
LAMP-2B
LAMP-2C
LAMP-2A (NP_002285.1) : AQDCSADDDNFLVPIAVGAALAGVLILVLLAYFIGLKHHHAGYEQF
LAMP-2B (NP_054701.1) : AQECSLDDDTILIPIIVGAGLSGLIIVIVIAYVIGRRKSYAGYQTL
LAMP-2C (NP_001116078.1) : AEECSADSDLNFLIPVAVGVALGFLIIVVFISYMIGRRKSRTGYQSV
First luminal domain
Hinge
Second luminal domain
TM
CCD
LAMP-2
1
365
410
Lysosomal luminal region
Cytosolic region


B
NMCMs
FPKM
10000
8000
6000
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2000
0
Adult mouse cardiomyocytes
mm10:chrX; 38,401,357 - 38,456,460
counts
400
300
200
100
0
Lamp2b
Lamp2a
Lamp2c
E8 E7
E6 E5
E4
E3 E2
E1
Exon 9A
Exon 9B
Exon 9C

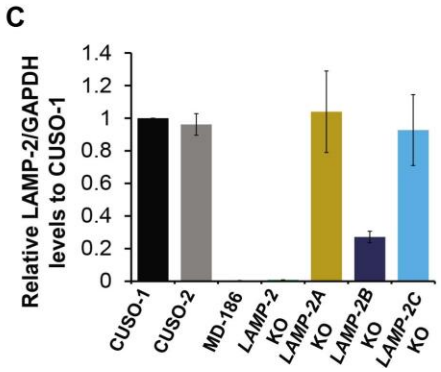
C
Relative LAMP-2/GAPDH levels to CUSO-1
1.4
1.2
1
0.8
0.6
0.4
0.2
0
CUSO-1
CUSO-2
MD-186
LAMP-2 KO
LAMP-2A KO
LAMP-2B KO
LAMP-2C KO

Fig. S3. LAMP-2B is the predominant LAMP-2 isoform expressed in mouse CMs.
(**A**) The *LAMP-2* gene encodes its three isoforms, LAMP-2A, LAMP-2B, and LAMP-2C, through alternative splicing. The three isoforms have identical lysosomal luminal domains, but distinct amino acid sequences for transmembrane (TM) domain and cytosolic CCD.
(**B**) Expression of the three LAMP-2 isoforms in mouse CMs. In adult mouse CMs, read densities (red) were plotted along *Lamp2* using the Integrative Genomics Viewer (IGV) and quantified as counts. RNA-seq data have been deposited in the Gene Expression Omnibus (GEO). Accession numbers are GSE71405 for NMCMs (11), and GSE102792 for adult mouse cardiomyocytes. FPKM, fragments per kilobase of exon per million reads. NMCMs, neonatal mouse ventricular CMs.
(**C**) Quantification of LAMP-2 protein in indicated hiPSC-CMs from three independent experiments shown in **Fig. 1*B***. Data are presented as mean ± s.d.

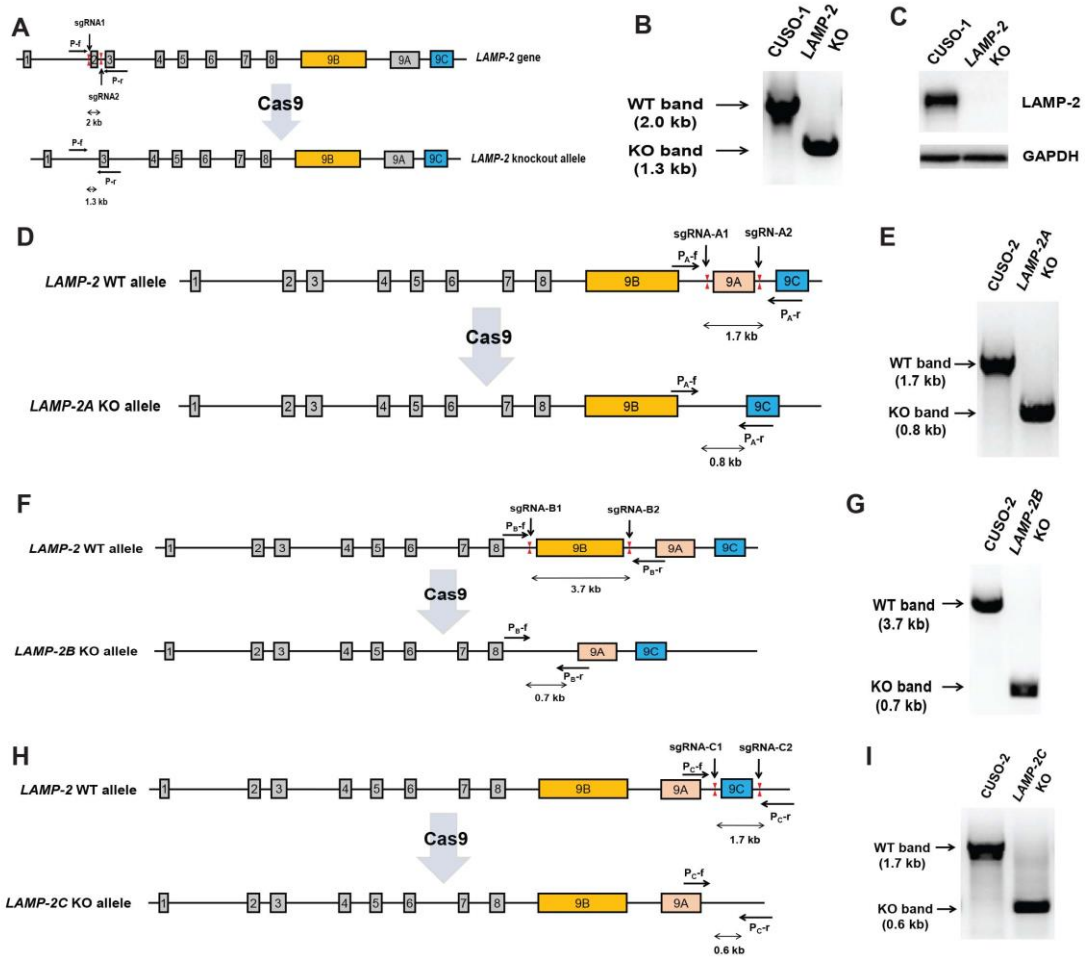

Fig. S4. Generation of hiPSCs to delete *LAMP-2*, *LAMP-2A*, *LAMP-2B* and *LAMP-2C*, respectively, using CRISPR/Cas9 technology.

Genome editing and PCR genotyping strategy for deletion of LAMP-2 (**A** and **B**), *LAMP-2A* (**D** and **E**), *LAMP-2B* (**F** and **G**) and *LAMP-2C* (**H** and **I**) are shown.

(**C**) Immunoblotting analysis of LAMP-2 protein in *LAMP-2* KO and isogenic control hiPSC-CMs.

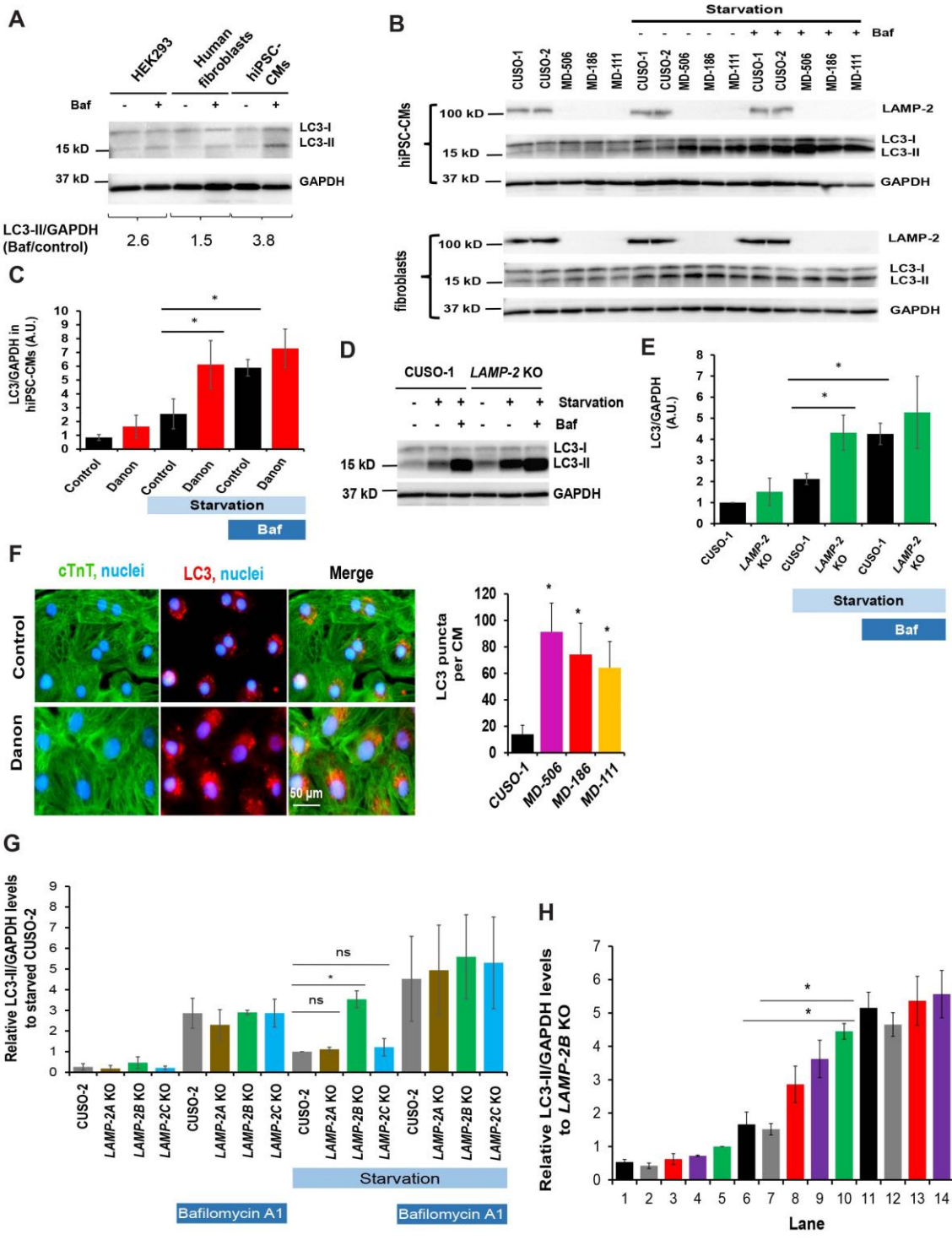
A
HEK293
Human fibroblasts
hiPSC-CMs
Baf
LC3-I
LC3-II
15 kD
37 kD
GAPDH
LC3-II/GAPDH (Baf/control)
2.6
1.5
3.8
B
Starvation
Baf
CUSO-1
CUSO-2
MD-506
MD-186
MD-111
hiPSC-CMs
fibroblasts
100 kD
LAMP-2
C
LC3/GAPDH in hiPSC-CMs (A.U.)
Control
Danon
Starvation
Baf
D
CUSO-1
LAMP-2 KO
E
LC3/GAPDH (A.U.)
F
cTnT, nuclei
LC3, nuclei
Merge
Control
Danon
50 µm
LC3 puncta per CM
G
Relative LC3-II/GAPDH levels to starved CUSO-2
LAMP-2A KO
LAMP-2B KO
LAMP-2C KO
ns
Bafilomycin A1
H
Relative LC3-II/GAPDH levels to LAMP-2B KO
Lane

Fig. S5. Accumulation of LC3-II levels in LAMP-2 deficient hiPSC-CMs.

(**A**) Cells were cultured in regular medium with or without 400 nM of bafilomycin A_1 (Baf) for 2 hours followed by immunoblotting analysis for LC3-I, LC3-II and GAPDH as a loading control. Fold-change of LC3-II/GAPDH caused by bafilomycin A_1 treatment for each type of cells was quantified.

(**B-C**) Autophagic flux in control and Danon hiPSC-CMs, and human skin fibroblasts under regular and starvation conditions was assayed. Cells were cultured in either regular or starvation medium with or without 400 nM of bafilomycin A_1 (Baf) for 4 hours followed by immunoblotting analysis. Densitometry quantification of LC3-II/GAPDH for hiPSC-CMs from three independent experiments is shown in (**C**), with data being normalized to CUSO-1 under regular conditions in each experiment, and combined into groups as indicated. Data from 3 independent experimental repeats are presented as mean ± s.d. * P<0.05 as assessed by Student's *t*-test.

(**D-E**) Autophagic flux in control and *LAMP-2* KO hiPSC-CMs was assayed as in (**B**). Data in (**E**) were normalized to CUSO-1 under regular conditions in each experiment, and are presented as mean ± s.d. * P<0.05 as assessed by Student's *t*-test. Three independent experimental repeats were conducted.

(**F**) Representative immunofluorescence images of hiPSC-CMs stained for cTnT (green) and LC3 (red). hiPSC-CMs were cultured under starvation conditions for 4 hours before they were fixed for immunostaining. LC3 puncta in each CM were counted. Twenty to thirty cells for each group were analyzed. n=3. Student's *t*-test. *P < 0.001. Data are presented as mean + s.d.

(**G**) Densitometry quantification of LC3-II/GAPDH from three independent experiments shown in **Fig. 2*A***. Data are presented as mean ± s.d. * P<0.05 as assessed by Student's *t*-test. ns, not significant.

(**H**) Densitometry quantification of LC3-II/GAPDH from three independent experiments shown in **Fig. 2*B***. Data are presented as mean ± s.d. * P<0.05 as assessed by Student's *t*-test.

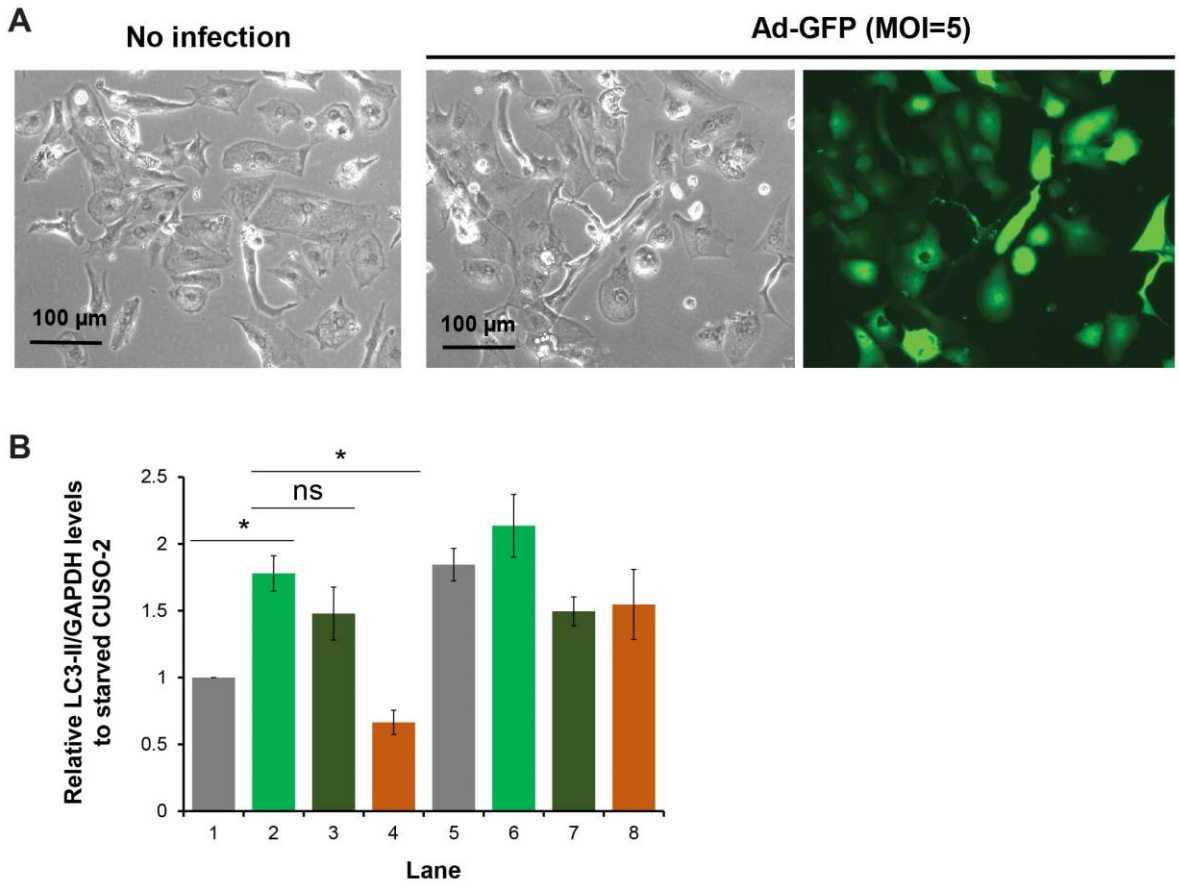

Fig. S6. Overexpression of LAMP-2B in hiPSC-CMs using an adenoviral vector.
(**A**) hiPSC-CMs were infected with adenovirus carrying green fluorescent protein (GFP) at an MOI (multiplicity of infection) of 5. Cells were imaged 3 days post-infection.
(**B**) Densitometry quantification of LC3-II/GAPDH from three independent experiments shown in **Fig. 2*C***. Data are presented as mean ± s.d. * P<0.05 as assessed by Student's *t*-test. ns, not significant.

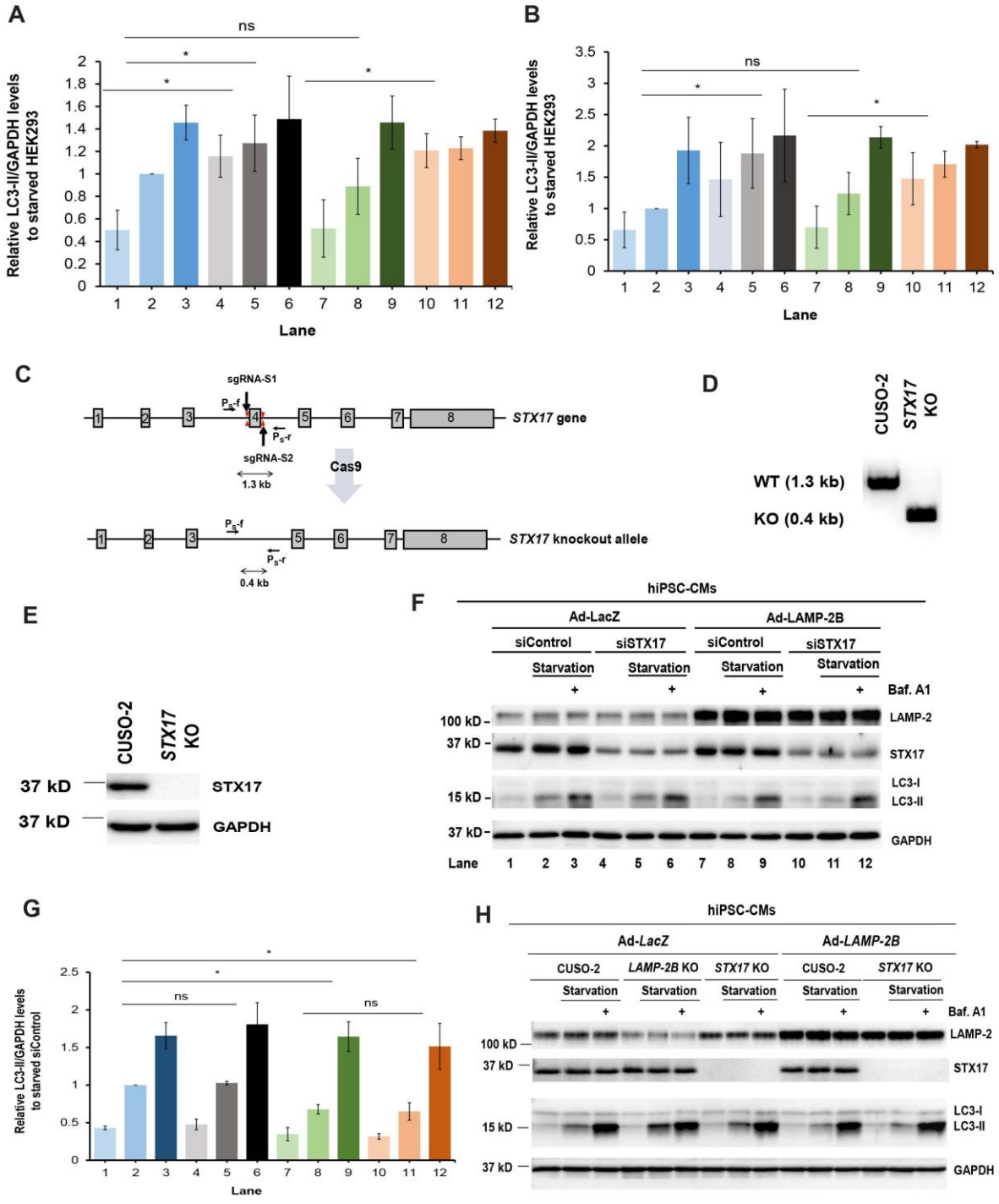

Fig. S7. The CCD is required for LAMP-2B to promote autophagosome-lysosome fusion, independently of STX17.

(A) Quantification of LC3-II/GAPDH from three independent experiments shown in **Fig. 4*A*** with data being normalized to siControl-treated cells under starved conditions in each

experiment. Data are presented as mean ± s.d. * P<0.05 as assessed by Student's *t*-test. ns, not significant.

(B) Quantification of LC3-II/GAPDH from three independent experiments shown in **Fig. 4*D*** with data being normalized to siControl-treated cells under starved conditions in each experiment. Data are presented as mean ± s.d. * P<0.05 as assessed by Student's *t*-test. ns, not significant.

(C) Genome editing strategy for knockout of *STX17* in hiPSCs using the CRISPR/Cas9 technology.

(D) PCR genotyping for deletion of *STX17*. PCR primers are shown in (**C**).

(**E**) Immunoblotting analysis of STX17 protein in *STX17* KO and isogenic control hiPSCs.

(F-G) Immunoblotting analysis of indicated proteins in hiPSC-CMs. hiPSC-CMs infected with adenovirus carrying LacZ (Ad-LacZ) or LAMP-2B (Ad-LAMP-2B) were treated with siRNAs against luciferase or STX17. Quantification of LC3-II/GAPDH from three independent experiments shown in (**G**) with data being normalized to siControl-treated hiPSC-CMs under starved conditions in each experiment. Data are presented as mean ± s.d. * P<0.05 as assessed by Student's *t*-test. ns, not significant.

(**H**) Immunoblotting analysis of indicated proteins in control, *LAMP-2B* KO, *STX17* KO hiPSC-CMs. hiPSC-CMs infected with adenovirus carrying LacZ (Ad-LacZ) or LAMP-2B (Ad-LAMP-2B). Quantification of LC3-II/GAPDH is shown in (**Fig. 4*F***).

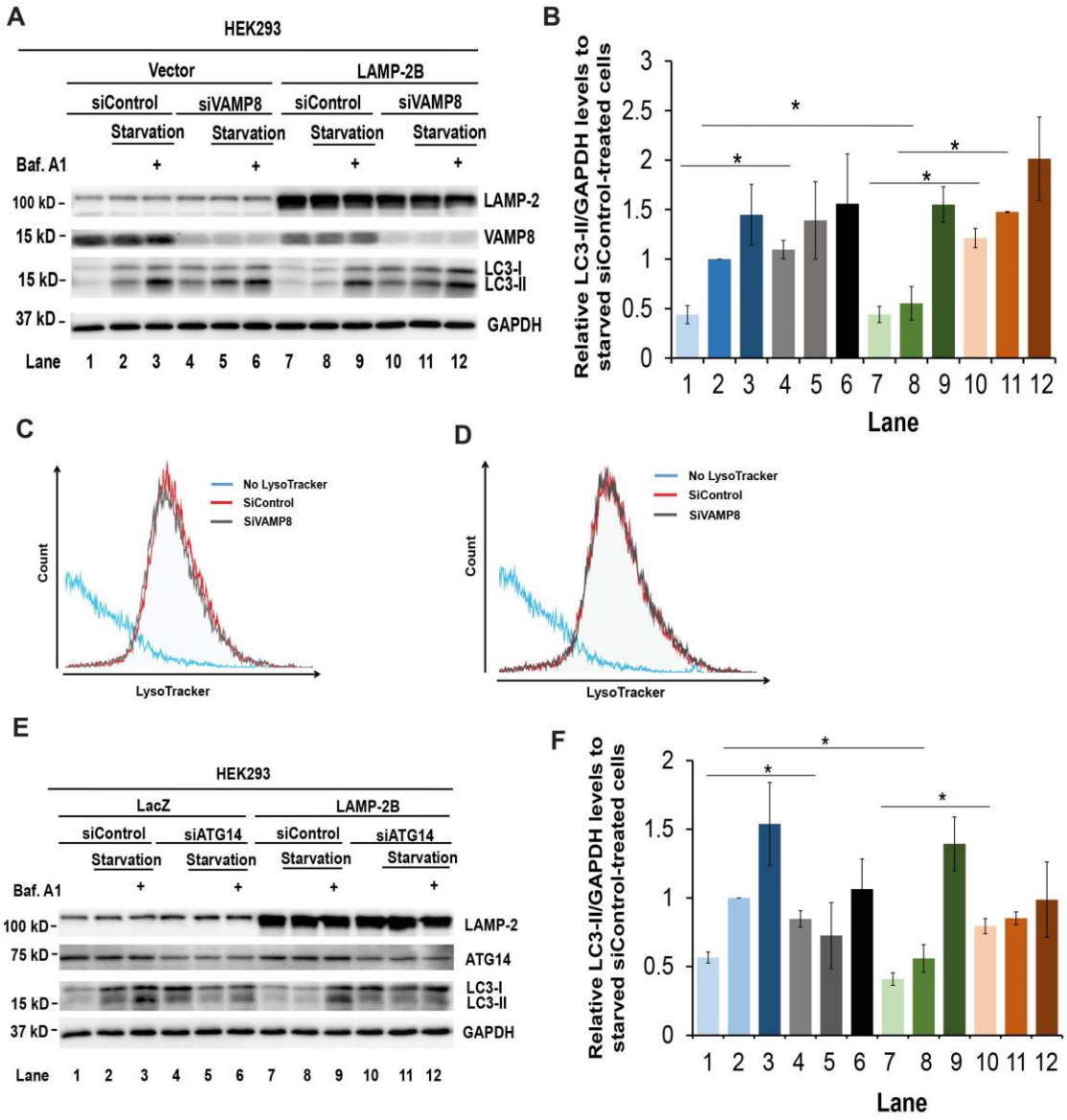

Fig. S8. VAMP8 and ATG14 are required for LAMP-2B to promote fusion between autophagosomes and endosomes/lysosomes.

(**A**-**B**) Immunoblotting analysis of indicated proteins in HEK293. HEK293 cells were transfected with empty vector or LAMP-2B, then were treated with siRNAs against luciferase or VAMP8. Three days later, cells were cultured in regular or starvation medium for 4 hours with or without 400 nM of bafilomycin A_1 (Baf. A_1). Western blotting was performed with anti-LAMP-2, anti-LC3, anti-VAMP8, and anti-GAPDH antibodies. Quantification of LC3-II/GAPDH from three independent experiments is shown in (**B**), with data being normalized to siControl-treated cells under starved conditions in each experiment. Data are presented as mean ± s.d. * $P<0.05$ as assessed by Student's *t*-test.

(**C-D**) Knockdown of VAMP8 in hiPSC-CMs did not affect lysosome abundance. hiPSC-CMs were treated with siRNAs against luciferase as control and against VAMP8. Three days later, hiPSC-CMs were cultured in regular (**C**) or starvation (**D**) medium for 4 hours. Cells were incubated with LysoTracker followed by flow cytometry.
(**E-F**) Immunoblotting analysis of indicated proteins in HEK293 cells. HEK293 cells were transfected with empty vector or LAMP-2B, then were treated with siRNAs against luciferase or ATG14. Autophagic flux was assayed as described in (**A**). Quantification of LC3-II/GAPDH from three independent experiments is shown in (**F**), with data being normalized to siControl-treated cells under starved conditions in each experiment. Data are presented as mean ± s.d. * $P<0.05$ as assessed by Student's *t*-test.

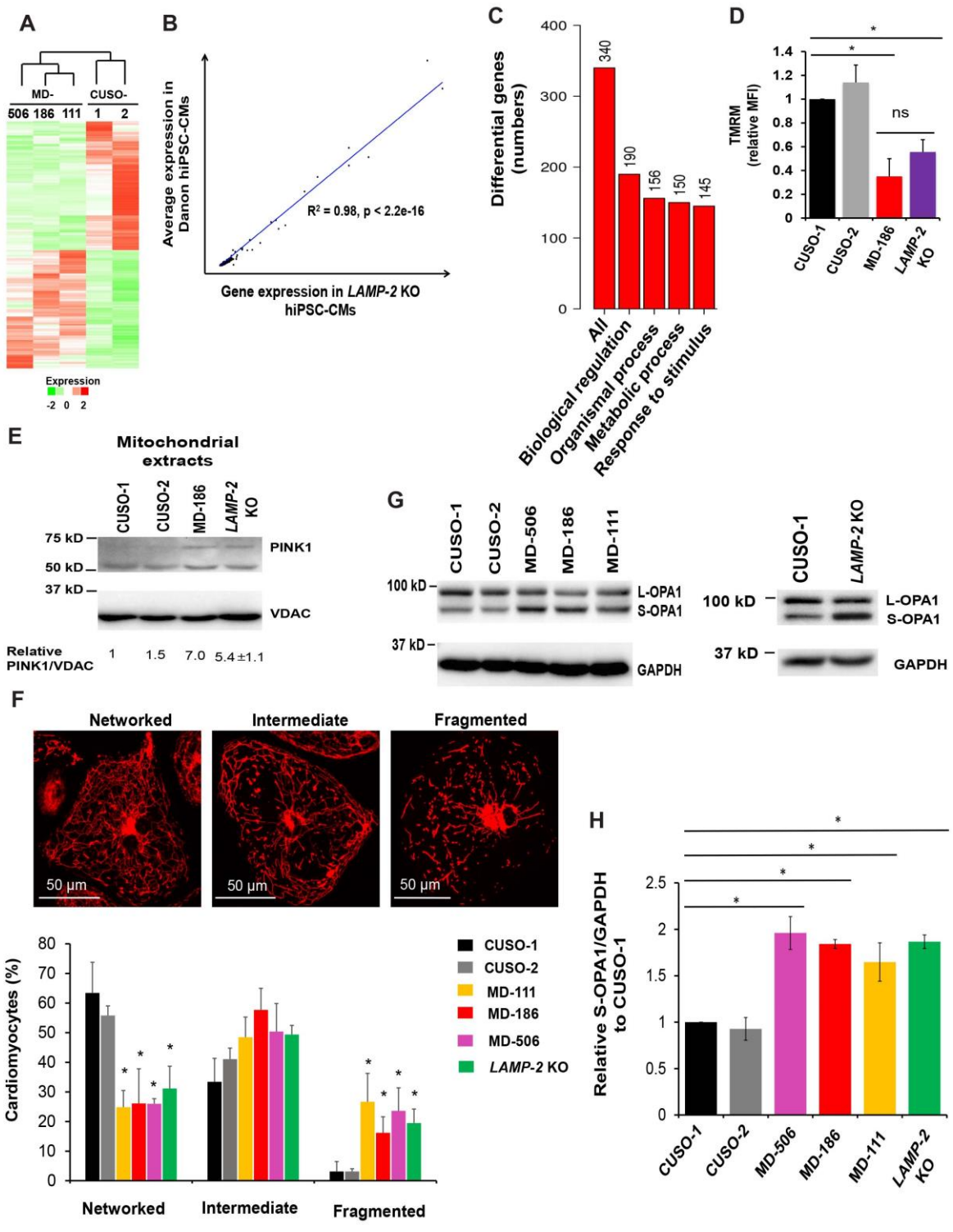
A
MD-
CUSO-
506 186 111
1 2
Expression
-2 0 2
B
Average expression in Danon hiPSC-CMs
R² = 0.98, p < 2.2e-16
Gene expression in LAMP-2 KO hiPSC-CMs
C
Differential genes (numbers)
340
190
156
150
145
All
Biological regulation
Organismal process
Metabolic process
Response to stimulus
D
TMRM (relative MFI)
ns
CUSO-1
CUSO-2
MD-186
LAMP-2 KO
E
Mitochondrial extracts
CUSO-1
CUSO-2
MD-186
LAMP-2 KO
75 kD
50 kD
37 kD
PINK1
VDAC
Relative PINK1/VDAC
1
1.5
7.0
5.4 ±1.1
G
CUSO-1
CUSO-2
MD-506
MD-186
MD-111
100 kD
L-OPA1
S-OPA1
37 kD
GAPDH
CUSO-1
LAMP-2 KO
F
Networked
Intermediate
Fragmented
50 µm
Cardiomyocytes (%)
CUSO-1
CUSO-2
MD-111
MD-186
MD-506
LAMP-2 KO
Networked
Intermediate
Fragmented
H
Relative S-OPA1/GAPDH to CUSO-1
CUSO-1
CUSO-2
MD-506
MD-186
MD-111
LAMP-2 KO

Fig. S9. Accumulation of fragmented mitochondria in LAMP-2 deficient hiPSC-CMs.
(**A**) Dendrogram cluster and heat map of 420 differentially expressed genes in control versus Danon hiPSC-CMs identified by RNA-seq. RNA-seq data have been deposited in the Gene Expression Omnibus (GEO) with an accession number of GSE108429.
(**B**) Correlation analysis of gene expression in three Danon hiPSC-CM lines and *LAMP-2* KO hiPSC-CMs.
(**C**) Gene ontology (GO) analysis showing biological processes associated with genes with known functions differentially expressed in control and Danon hiPSC-CMs.
(**D**) TMRM fluorescence intensity of live hiPSC-CMs was analyzed by flow cytometry. n=3. Student's *t*-test. **P* < 0.05. ns, not significant. Data are presented as mean + s.d.
(**E**) PINK1 protein was analyzed via immunoblotting on isolated mitochondria from hiPSC-CMs. The outer mitochondrial membrane protein voltage dependent anion channel 1 (VDAC) was used as a loading control. Relative PINK1/VDAC levels to CUSO-1 are shown underneath the VDAC blot. Three independent experiments for CUSO-1 and *LAMP-2* KO were conducted.
(**F**) Mitochondrial morphology was categorized in a blinded manner in hiPSC-CMs based on confocal images. Networked morphology in a CM is classified as a mixture of > 60% fused and < 40% fragmented mitochondria. Intermediate morphology is classified as a mixture of 30% - 60% fused and 70% - 40% fragmented mitochondria. Fragmented morphology is classified as a mixture of < 30% fused and > 70% fragmented mitochondria. More than 70 CMs for each hiPSC-CM lines were categorized per experiment. Three independent experiments were conducted. Data are presented as mean + s.d. Student's *t*-test. **P* < 0.05.
(**G-H**) Immunoblotting analysis of L-OPA1 and S-OPA1 in control, Danon and *LAMP-2* KO hiPSC-CMs. Densitometry quantification of S-OPA1/GAPDH from three independent experiments is shown in (**H**), with data being normalized to CUSO-1 in each experiment. Data from 3 independent experimental repeats are presented as mean ± s.d. * P<0.05 as assessed by Student's *t*-test.

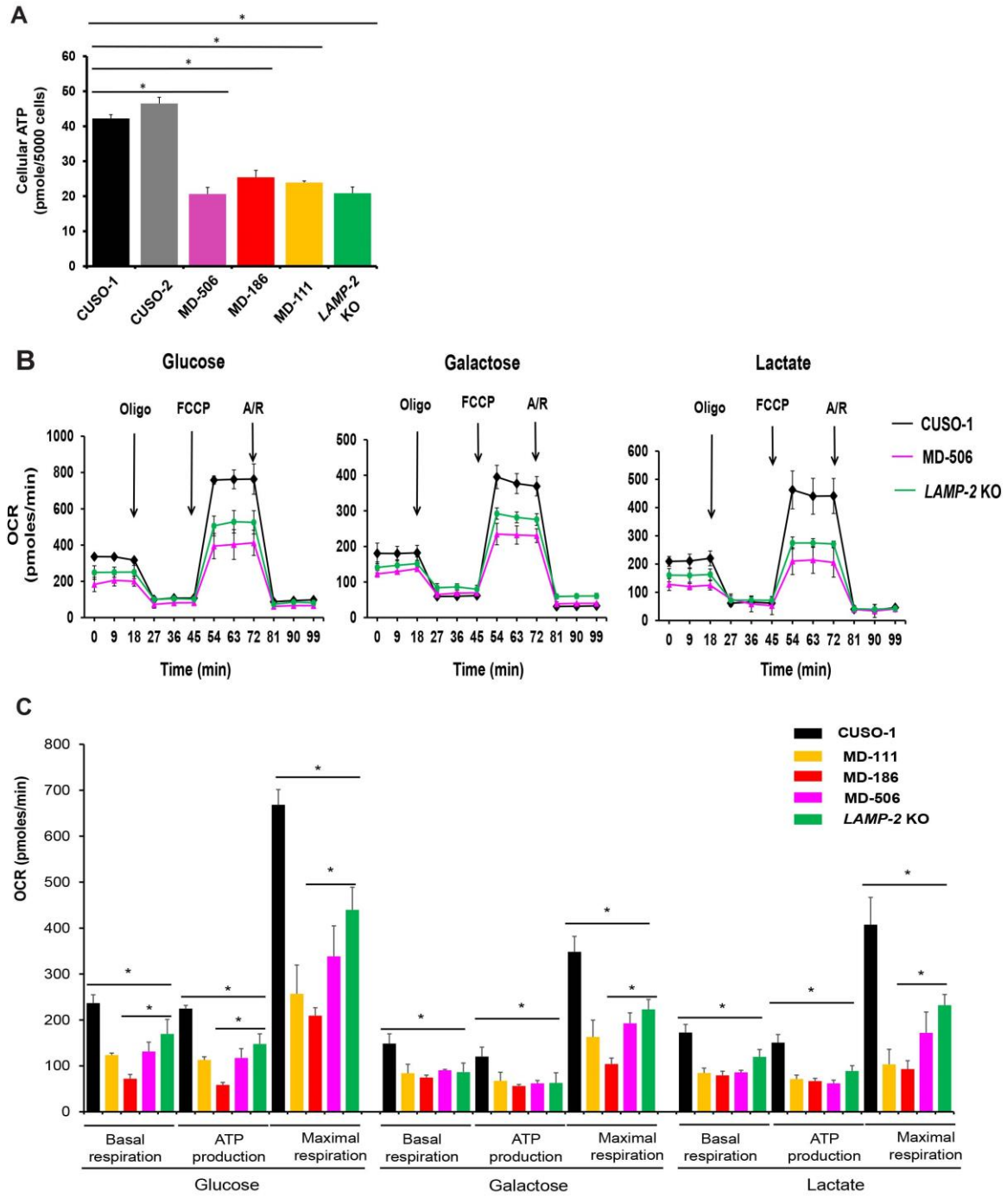

Fig. S10. LAMP-2 deficiency is sufficient to cause mitochondrial dysfunction in hiPSC-CMs.

(**A**) ATP levels in two control lines (CUSO-1 and CUSO-2), three Danon lines (MD-111, MD-186, and MD-506) and the *LAMP-2* KO line were summarized. n=3, Student's *t*-test, **P* < 0.001. Data are presented as mean + s.d.

(**B-C**) OCR of control, Danon and *LAMP-2* KO hiPSC-CMs in glucose, galactose or lactate was measured using the Seahorse XF Cell Mito Stress Test Kit. Activity of specific mitochondrial components was measured by OCR after sequentially adding oligomycin (Oligo) to inhibit mitochondrial F_1F_0 ATP synthase, an uncoupling agent (FCCP) to disrupt the mitochondrial membrane potential, and antimycin A plus rotenone (A/R) to inhibit mitochondrial complex I and complex III. Representative time course data for control, Danon and *LAMP-2* KO hiPSC-CMs are shown in (**B**). Data are expressed as mean ± s.d. Statistical analysis of OCR is shown in (**C**). n=4. Data are presented as mean + s.d. Student's *t*-test, **P* < 0.01.

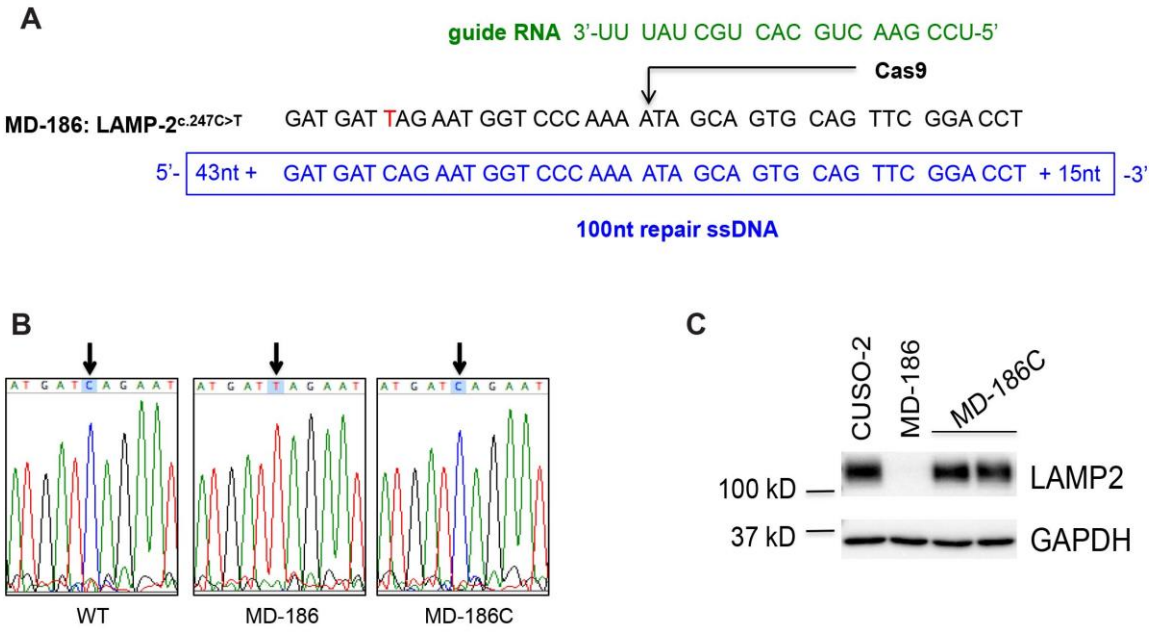

Fig. S11. Targeted correction of the *LAMP-2* mutation c.247C>T in MD-186.
(**A**) Schematic of gene correction using the CRISPR/Cas9 approach. The *LAMP-2* genomic locus contains the mutation c.247C>T in red. The guide RNA and the repair oligonucleotide are shown.
(**B**) The point mutation and corrected sequence were determined via Sanger sequencing.
(**C**) Immunoblotting analysis of LAMP-2 expression in control, Danon MD-186 and corrected MD-186C hiPSCs.

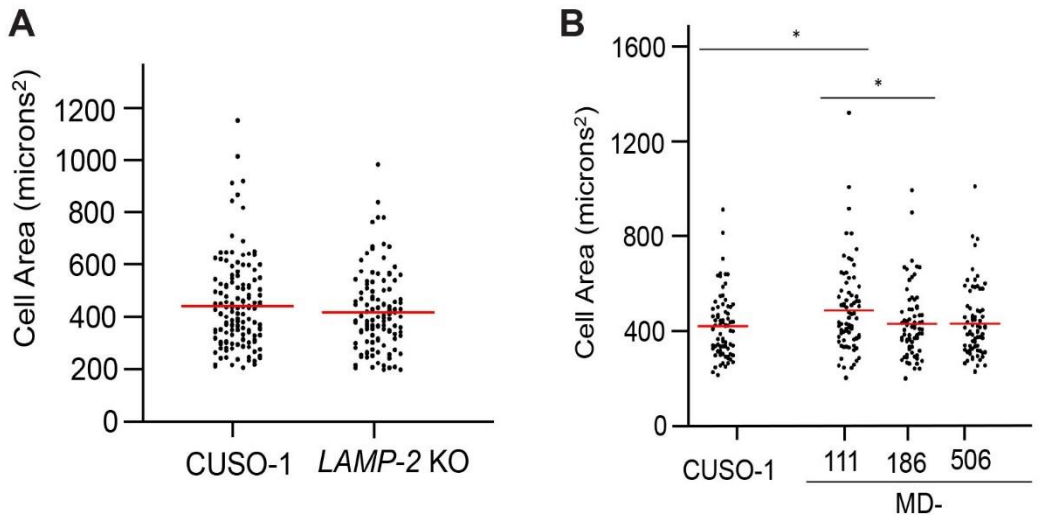

Fig. S12. Adhesion of hiPSC-CMs to microposts. Micropost-based contractile assays were performed under a blinded condition. More than 3 independent experiments (with ~25 cells each) were performed for each hiPSC-CM lines on day 60. The spread area of individual hiPSC-CMs on microposts were measured, which is shown in (**A**) for CUSO-1 and *LAMP-2* KO hiPSC-CMs, in (**B**) for CUSO-1 and three Danon hiPSC-CM lines. Each data point represents an individual cell. Lines represent average force per post for each group. Statistical analysis was performed using a 1-way ANOVA with a Bonferroni post-hoc test. P-values or statistically significant differences are specified, otherwise pairwise statistics are not significant. *, $p < 0.05$.

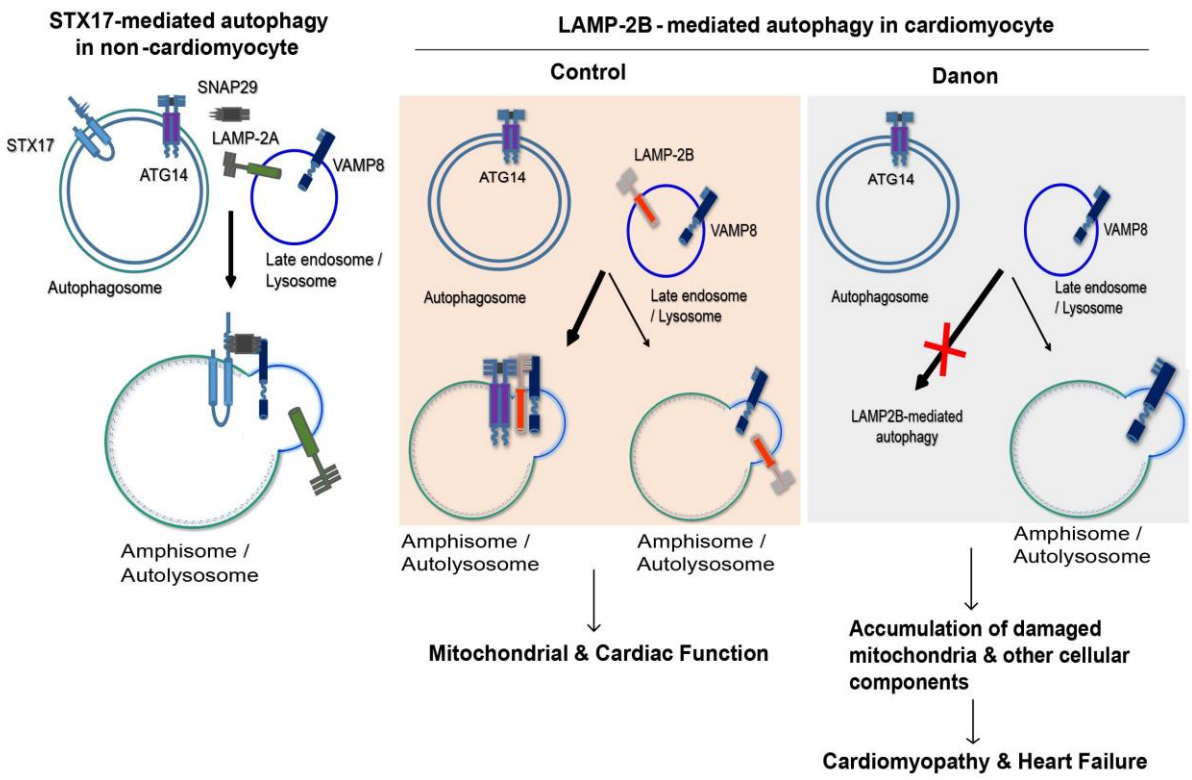

Fig. S13. LAMP-2B-mediated fusion between autophagosomes and late endosomes/lysosomes in human CMs. In non-CMs in which LAMP-2A is the major isoform of LAMP-2, STX17 is essential for autophagic fusion by interacting with SNAP29 and VAMP8. ATG14 promotes STX17-mediated fusion. In CMs wherein LAMP-2B is predominantly expressed, LAMP-2B promotes autophagic fusion by interacting with VAMP8 and ATG14 via its cytosolic CCD, in a STX17-independent manner. STX17 is not essential for autophagy in CMs. Mutations in the *LAMP-2* gene cause *LAMP-2B* deficiency in patients with Danon disease. The *LAMP-2B* deficiency impairs autophagic fusion, leading to accumulation of damaged cellular components that causes cardiomyopathy and heart failure in patients with Danon disease.

Table S1. Clinical features of patients in this study.

Patient ID			CUSO-1	CUSO-2	MD-111	MD-186	MD-506
Symptom onset in years			NA	NA	14	15	7
Heart	Heart disease		No	No	Yes	Yes	Yes
	ECHO	AE	NA	NA	18	25	11
		Findings	NA	NA	Severe LV hypertrophy,; IVSd: 1.87cm, LVPWd: 1.80cm; LVIDd: 5.62-cm; EF 30%	Moderate LV hypertrophy; IVSd: 1.10cm; LVPWd: 1.0cm; LVIDd: 5.5cm EF 30%;	Mild LV hypertrophy; EF normal
	EKG	AE	NA	NA	18	25	11
		Findings	NA	NA	WPW, LVH(v)	WPW	WPW, arrhythmias
Heart transplant (age in years)			NA	NA	Yes (19)	Yes (27)	No (15)
Skeletal muscle			Normal	Normal	Muscle weakness	Muscle weakness	Muscle weakness
Cognition			Normal	Normal	Mild cognitive difficulties	Require special education	Require special education
Eye			Normal	Normal	Blurry vision	Pigmentary retinopathy	Mild astigmatism

ECHO: Echocardiography; EKG: Electrocardiography; AE: age of examination in years; IVSd: End-diastolic interventricular septal thickness; End-diastolic LVPWd: Left ventricular posterior wall thickness; LVIDd: End-diastolic left ventricular intraventricular diameter; WPW: Wolff-Parkinson White syndrome; EF: ejection fraction. NA: not applicable.

Table S2. SgRNAs for CRISPR/Cas9-mediated genome editing.

SgRNA ID	Target exon	Target region (PAM is underlined)	20-nt sgRNA sequence
SgRNA1	*LAMP-2* exon2	AGAAGTTTTACACCCCTACC AGG	AGAAGUUUUACACCCCUAC C
SgRNA2	*LAMP-2* exon2	ATGATCTGAAGACGACTATA TGG	AUGAUCUGAAGACGACUAU A
SgRNA-A1	*LAMP-2* exon 9A	ACTTCCTAACACGCATATTT TGG	ACUUCCUAACACGCAUAUU U
SgRNA-A2	*LAMP-2* exon 9A	TTGGGTCTGTATCATCCCTA GGG	UUGGGUCUGUAUCAUCCCU A
SgRNA-B1	*LAMP-2* exon 9B	CAACTTCAAGTAACTAAGAC AGG	CAACUUCAAGUAACUAAGAC
SgRNA-B2	*LAMP-2* exon 9B	GGCCTCGATTGATGCTAGG CAGG	GGCCUCGAUUGAUGCUAGG C
SgRNA-C1	*LAMP-2* exon 9C	GCAAGCGCAATTCTCTATTT TGG	GCAAGCGCAAUUCUCUAUU U
SgRNA-C2	*LAMP-2* exon 9C	TGGAACACCTGTATGGGTT ATGG	UGGAACACCUGUAUGGGUU A
SgRNA-S1	*STX17* exon 4	ATATAGTGTAACCATTGAGC AGG	AUAUAGUGUAACCAUUGAG C
SgRNA-S2	*STX17* exon 4	GTCCAAGGATTCAGCATATT GGG	GUCCAAGGAUUCAGCAUAU U
SgRNA-for correction	*LAMP-2* exon 3	TCCGAACTGCACTGCTATTT TGG	UCCGAACUGCACUGCUAUU U
Repair ssDNA for correction	*LAMP-2* exon 3	TTCAGACCATGGCACTGTG ACATATAATGGAAGCATTTG TGGGGATGATCAGAATGGT CCCAAAATAGCAGTGCAGT TCGGACCTGGCTTTTCCTG GATT	

Table S3. PCR primers.

Primer ID	Sequence	Note
hLAMP2A_qPCR_f	GTTCAGCCTTTCAATGTGACAC	
hLAMP2A_qPCR_r	CACTAGAATAAGTACTCCTGCCAAG	
hLAMP2B_qPCR_f	GGTTCAGCCTTTCAATGTGAC	
hLAMP2B_qPCR_r	TGAAAGACCAGCACCAACTAT	
hLAMP2C_qPCR_f	CCCCTGGGAAGTTCTTATATGTG	
hLAMP2C_qPCR_r	AAAGTTGAGGTCAGAGTCAGC	
hGAPDH_qPCR_f	CTCTGGTA AGTGGATATTGTTGCC	
hGAPDH_qPCR_r	AGAGATGATGACCCTTTTGGCTCC	
Linker_sense	ATCAGACTCTGTAATCCGG	LAMP-2B
Linker_antisense	ATTACAGAGTCTGAT	
Q5_hLAMP2BΔCCD_f	TAATCCGGCCGCGGTCAT	LAMP-2BΔCCD
Q5_hLAMP2BΔCCD_r	GCCAATTACGTAAGCAATCACTATAACG	
hLAMP-2A_SgfI_f	GCGATCGCCATGGTGTGCTTCCGCCTCTTC	LAMP-2A
hLAMP-2A_SacII_r	CCGCGGCTAAAATTGCTCATATCCAGCATGATG	
P-f	CGTTTGGGAAGACATCCGTG	Genotyping of *LAMP-2* KO
P-r	CCAGTGTTGTAGGAAAATGAGACGC	
P_A-f	GCACTTTGTGTTGCCTACTCCTACC	Genotyping of *LAMP-2A* KO
P_A-r	ACTGCCTCCCTTCTGAGATTGC	
P_B-f	CCACTGAGAGGCTAATCTGGCTATG	Genotyping of *LAMP-2B* KO
P_B-r	TCACTGGTTCCCTAACTGACTATGC	
P_C-f	TGGTTCGGTGGAATGGACTG	Genotyping of *LAMP-2C* KO
P_C-r	TCACATAGGGAATGGGAGAGTGC	
P_S-f	GGCAGCATGTGAAGAAATAGGC	Genotyping of *STX17* KO
P_S-r	TCCCTGAAGTCCCACTCCATAAGC	
P_{MD-186}-f	CCAGAGGAGTGGTATGTAGCAGAAG	Genotyping of MD-186C
P_{MD-186}-r	CCAGTGTTGTAGGAAAATGAGACGC	

Movie S1. Spontaneous contraction of monolayer hiPSC-CMs.

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