# SUPPLEMENTAL MATERIAL

## The Unfolded Protein Response as a Compensatory Mechanism and Potential Therapeutic Target in PLN R14del Cardiomyopathy

Dries A.M. Feyen, PhD[1,2] *; Isaac Perea-Gil, PhD[1,3]*; Renee G.C. Maas, MS[4]; Magdalena Harakalova, MD, PhD[4]; Alexandra A. Gavidia, BS[3]; Jennifer Arthur Ataam, PhD[1,3]; Ting-Hsuan Wu, MS[3]; Aryan Vink, MD, PhD[5]; Jiayi Pei, PhD[4]; Nirmal Vadgama, PhD[1,3]; Albert J. Suurmeijer, MD, PhD[6]; Wouter P. te Rijdt, MD, PhD[7,8]; Michelle Vu, BS[1,2]; Prashila L. Amatya, BS[1,2]; Maricela Prado, MS[3]; Yuan Zhang, BS[3]; Logan Dunkenberger, BS[3]; Joost P.G. Sluijter, PhD[4]; Karim Sallam, MD[1,2]; Folkert W. Asselbergs, MD, PhD[4, 9, 10]; Mark Mercola, PhD[1,2]; Ioannis Karakikes, PhD[1,3†]

## Supplemental Methods

**Human iPSC reprogramming and culture.** Peripheral blood mononuclear cells (PBMCs) were reprogrammed to hiPSCs using the CytoTune™-iPS 2.0 Sendai Reprogramming Kit (Thermo Fisher Scientific) according to the manufacturer's instructions with modifications. PBMCs were expanded in StemPro-34 SFM media (Life technologies) supplemented with cytokines: SCF (100 ng/mL), FLT-3 (100 ng/mL), IL-3 (20 ng/mL), IL-6 (20 ng/mL) and EPO (2 U/mL). After six days, 2 x$10^5$ PBMCs were transduced with the three CytoTune® 2.0 reprogramming vectors in one well of a 24-well plate in 0.5 mL in complete StemPro-34 SFM. Twenty-four hours post-transduction the PBMCs were pelleted by centrifugation, resuspended in fresh complete StemPro-34 SFM and plated in one Matrigel-coated well of a 24-well plate. Three days later the media was replaced with StemPro-34 SFM without cytokines and cultured for an additional 3 days. The cells were gradually transitioned into E8 stem cell medium in a step-wise manner over 5-6 days by replacing 20% of the media each day. Stem cell-like colonies were manually picked about two weeks post-transduction and expanded in E8 stem cell media (Life Technologies) on plates coated with human ESC-qualified Matrigel (BD Biosciences) under hypoxic conditions (5% $O_2$, 5% $CO_2$) at 37 ºC. Cells were dissociated with Gentle Cell Dissociation Reagent (StemCell Technologies) with E8 medium supplemented with 2.5 μM Y-27632 (SelleckChem).

**Immunofluorescence analysis of pluripotency markers.** The hiPSCs were seeded in Matrigel-coated well in a 96-plate (Greiner Bio-One) and cultured for 4-5 days. The cells were washed with PBS, fixed with 4% paraformaldehyde. After permeabilizing with 0.3% Triton-X diluted in PBS supplemented with 2% BSA, 2% FBS, the cells were incubated with primary antibodies (Human Pluripotent Stem Cell Immunocytochemistry Kit, R&D System; Tra-1, Millipore) overnight at 4 ºC. After washing, cells were incubated with secondary antibodies Alex Fluor 488/555 / Hoechst 33342 (Invitrogen). Images were taken on an IC200 Kinetic Imaging Cytometer (Vala Sciences; 20x 0.75 N.A.) and further processed using ImageJ software (National Institutes of Health).

**Karyotyping.** The hiPSCs were snap-frozen in liquid nitrogen and genomic DNA (gDNA) was extracted using the Blood and Tissue DNA extraction kit (Qiagen). SNP karyotyping analysis of >713,014 SNPs was performed using Genome-Wide HumanOmniExpress-24 BeadChips v1.1 on a HiScan sequencing platform per the manufacturer's directions (Illumina) and analyzed using the KaryoStudio v1.4 software (Illumina).

**Cardiomyocyte differentiation.** Differentiation towards cardiomyocytes was carried out following a small molecule Wnt-activation/inhibition protocol previously described[37,38]. Briefly, hiPSCs were first treated with CHIR99021 (4-6 μM; Tocris) in RPMI/B27 without insulin (Life Technologies) for 72 hours, and then with IWR (3 μM; Selleck Chemicals) for another 48 hours. The media was then replaced with RPMI/B27 with insulin (Life Technologies) and refreshed every 2 days. Spontaneously beating cells was typically observed 8-10 days post-differentiation. On day 13 post-differentiation, hiPSC-CMs were metabolically selected in RPMI-B27 without D-glucose (Life Technologies) supplemented with 0.2% Sodium DL-lactate (Sigma) for 96 hours. Differentiated hiPSC-CMs were maintained with RPMI/B27 medium. All experiments were conducted by using hiPSC-CMs between 35 and 50 days after culture in maturation media[39] (2D baseline contractility assessment, siRNA UPR experiment, XBP-1 splicing reporter) or RPMI/B27 (scRNA-seq, BiX experiments).

**CRISPR-Cas9 genome editing**. Genome editing was performed in hiPSCs to either correct or introduce the PLN R14del mutation in hiPSCs by CRISPR-Cas9-mediated homology-directed repair (HDR) as described[38]. Briefly, the gRNA with the highest specificity score (gRNA: TTGAGGCATTTCAATGGTTG) was cloned into pSpCas9(BB)-2A-GFP (PX458; a gift from Feng Zhang; Addgene plasmid #48138). The PX458 vector (0.5 μg) and single-stranded oligodeoxynucleotide donor template (ssODN, 4.0 μg) were co-transfected in hiPSCs using 10 μL Lipofectamine Stemfect (Thermo Fisher Scientific). Twenty-four hours after transfection, the cells were dissociated with 1X TrypLE Express (Thermo Fisher Scientific) and GFP+ cells were sorted

by FACS. Single-cell colonies were screened by PCR (PLN_Fw: AGGAGAGAAAGAGAGACAGACA; PLN_Rv: TCACTGTCACATATTAACCACCA) and Sanger sequencing to verify the insertion or correction of the PLN R14del mutation. The top eight ranking off-target sites predicted by the COSMID tool[40] were also assessed (**Tables II-V in the Supplement**). The following sequence was used as HDR donor templates:

>PLN R14del ssODN
CTCGACCACTTAAAACTTCAGACTTCCTGTCCTGCTGGTATCATGGAGAAAGTCCAATACCT
CACTCGCTCAGCTATAAGAGCATCAACCATTGAAATGCCTCAACAAGCACGTCAAAAGCTA
C

>PLN WT ssODN
CTCGACCACTTAAAACTTCAGACTTCCTGTCCTGCTGGTATCATGGAGAAAGTCCAATACCT
CACTCGCTCAGCTATAAGAAGAGCATCAACCATTGAAATGCCTCAACAAGCACGTCAAAAG
C

The primers and ssODNs were synthesized by Integrated DNA Technologies (IDT).

**Single-Cell RNA-seq library construction and sequencing.** At 45 days after differentiation, cells were dissociated by incubation with 10x TrypLE solution for 10 min at 37 $^{o}$C. Cells were filtered through a 40 μm cell strainer (BD Falcon), centrifuged at 100*g* for 3 min, and resuspended in PBS supplemented with 0.1% BSA. Single-cell encapsulation, cDNA generation, and preamplification as well as library preparation were performed using the Chromium Single Cell 3′ v2 reagent kit according to the instruction manual. Briefly, about 5,000 cells per sample were encapsulated into microdroplets and the barcoded complementary DNAs (cDNAs) were combined and amplified for library preparation according to the manufacturer's protocol (10x genomics). Libraries were sequenced on the NextSeq 500 sequencing system with a target of 40000 to 50000 reads per cell (Illumina).

**Processing and analysis of scRNA-seq data.** The raw FASTQ files were processed with the Cellranger software v1.3.0 (10x Genomics) for demultiplexing, mapping to the hg19, and quality control. The absolute unique molecular identifier (UMI) counts were quantified per gene per cell

to generate a gene-barcode matrix for each sample. These sparse matrices were aggregated and preprocessed using SAVER[41] to impute missing data prior to downstream analysis. The Seurat package 2.0 implemented in R was used to perform normalization, unbiased clustering, t-distributed stochastic neighbor embedding (t-SNE) visualization, and differential gene expression analysis as described in the tutorials (http://satijalab.org/seurat/)[42]. Briefly, the aggregated cell count matrix was first normalized by dividing the number of UMI for each transcript by the total UMI for the cell, multiplying by 10,000, and log-transformed. Highly variable genes were selected using the FindVariableGenes function in Seurat and used as input for principal component analysis (PCA). Based on the first 20 principal components, cell clusters were identified based on their PCA score. To visualize cells in a high-dimensional space, two-dimensional projections created by t-SNE. To assign identities to these subpopulations, we cross-referenced their marker genes with known cardiac subtype markers from the literature. To detect differentially expressed genes between wild-type and PLN R14del iPSC-CM sub-populations, we performed pairwise comparisons using the non-parametric Wilcoxon rank-sum test through the *FindMarkers* function. An adjusted *P*-value (Bonferroni correction) cut-off $< 2 \times 10^{-6}$ was used to identify differentially expressed genes.

**Three-dimensional Engineered Heart Tissues (3D-EHTs).** The 3D-EHTs were generated in agarose casting molds using solid silicone racks (EHT Technologies) as described with modifications[43]. Briefly, about $1x10^6$ iPSC-CMs were suspended in a fibrin hydrogel (100 µL total) composed of 10 µL Matrigel (Corning), 5 mg/mL bovine fibrinogen (2.53 µL of 200 mg/mL fibrinogen reconstituted in 0.9% NaCl) and supplemented with 0.1 mg/mL aprotinin (Sigma Aldrich) and 3 U/mL thrombin (Sigma Aldrich). Once polymerized, the silicone racks with the newly formed fibrin gels were transferred to a new 24-well plate and cultured for 3-4 weeks in culture medium consisting of DMEM:RPMI media (1:1) supplemented with 0.25% dialyzed fetal bovine serum (JR Scientific), 0.5x B27 supplement (Gibco), 5% knock-out serum replacement (Gibco),

1% penicillin/streptomycin (Gibco), and 33 µg/mL aprotinin. The culture medium was refreshed every 3-4 days. Videos of the deflecting posts were recorded at 75 frames/s using the SI8000 Cell Motion Imaging System (Sony), at baseline and 72 hours following BiX treatment. The video recordings were processed by MuscleMotion[44] to quantify the contraction amplitudes. Absolute force values were derived from calibrated measurements of post displacement considering an elastic modulus of 1.7 MPa, a post radius of 0.5 mm and a distance between posts (length) of 10 mm.[37]

**High-throughput contractility analysis.** The hiPSC-CMs were plated on Matrigel-coated surfaces at a density of 20,000 cells per well in a 384-well plate (Greiner Bio-One) in 100 µL of replating media (RPMI-B27, 10% knock out serum replacement). The replating media was gradually transitioned to RPMI/B27 by replacing 50% of the media every 2 days for 6-8 days prior to analysis. For siRNA-mediated knockdown, hiPSC-CMs were transfected with siRNAs (Silencer Select, Ambion) with 0.2 µL Lipofectamine RNAiMax (Invitrogen) 20 nM final concentration. Prior to analysis, the iPSC-CMs cells were loaded with Tetramethylrhodamine methyl ester dye (TMRM, 400 nM) and fluorescent time-lapse images were acquired automatically using the IC200 KIC instrument (Vala Sciences) at an acquisition frequency of 100 Hz for a duration of 10 s using a 20x objective (0.75 NA). The contractility image analysis was performed using custom particle image velocity software as previously described[45].

**XBP1-splicing reporter / AAV production.** The F-*XBP1* and F-*XBP1*ΔDBD[7] were a kind gift from Dr. Miura (University of Tokyo). The two XBP1 splicing reporters were cloned into the pAAV-CMV vector (Takara). HEK293T cells were co-transfected (Lipofectamine 2000, Thermo) with pAAV-F-XBP1 (or pAAV-F-*XBP1*ΔDBD), pRC2, and pHelper (Takara). ). After 3 days the cells were collected and the AAV particles extracted from the cell pellets using the AAVpro extraction solutions (Takara). The AAV2 particles were incubated at 37 °C for 30 minutes with Cryonase Cold-active Nuclease (2 U/µl) and purified with a resin-based approach according to

manufacturer's instructions (AAVpro purification kit, Takara). The purity was evaluated by the SDS-PAGE and the amount of viral genome was quantified using a real-time qPCR assay using the ITR sequence of AAV2 as a target according to the manufacturer's protocol (Takara). The hiPSC-CMs were seeded in a 384-well at 20,000 cells per well and infected with 5 x $10^3$ viral genomes per cell. Fluorescent images were acquired with the IC200 KIC (Vala Sciences, San Diego, CA). For quantification, nuclear expression (F-*XBP1*) was calculated as a percentage of positive nuclei to total nuclei (Hoechst 33342, Invitrogen), and cytoplasmic expression (F-*XBP1*ΔDBD) was calculated as integrated fluorescent density.

**Human heart tissue immunohistochemistry.** Human heart tissue was obtained from the local tissue biobanks of the Departments of Pathology from the University Medical Center Utrecht and the University Medical Center Groningen, The Netherlands. Three explanted hearts from heart failure patients carrying the heterozygous pathogenic PLN R14del variant and autopsy heart tissue from three sudden death patients with the PLN R14del variant expected to harbor PLN aggregates in cardiomyocytes were included. Immunohistochemistry was also performed on explanted hearts from patients with arrhythmogenic cardiomyopathy (ARVC) negative for the pathogenic PLN R14del variant, ischemic cardiomyopathy (ICM), and control autopsy hearts (genetic variants are shown in **Table I in the Supplement**). Tissue sections (3 μm) of formalin-fixed and paraffin-embedded (FFPE) myocardium were stained for PLN and different UPR sensors using immunohistochemistry. Sections were manually stained for PLN (PLN ser-10 Bradilla, A010-10AP, 1:200), BiP (GRP78/BiP, Proteintech, 66574-1-Ig, 1:200), PDI (PDI, Proteintech, 66422-1-Ig, 1:200) using antigen retrieval solution pH9 (PLN, BiP) and pH6 (Hsp70, PDI) and incubation overnight at 4 °C. For each heart three hotspot areas were selected: outer- and inner compact myocardium and trabecular myocardium. In each hotspot 250 cells were counted using 400X magnification. Positive cardiomyocytes were averaged as a mean score per patient. The PLN aggregates were examined by their characteristic features as described

previously[13]. Specifically, the size, shape, and localization were used to identify and characterize the aggresomes. BiP and PDI positive cells were scored according to dark red staining that was localized perinuclear or more diffuse in the cytosol. All cases were examined by two independent observers and guided by a certified pathologist.

**Protein expression analysis.** The hiPSC-CMs were washed with cold PBS and lysed in RIPA buffer (Thermo Fisher) supplemented with 1X Protease and Phosphatase Inhibitor Cocktail (Sigma) for 30 minutes on ice. For BiX experiments, hiPSC-CMs were treated with 0.1 μM BiX or vehicle control (DMSO) for 72 hours prior to cell lysis. Protein quantification was performed using the BCA method according to manufacturer's protocol (Pierce), and an equal amount of protein (10-20 μg) was loaded on a precast 4-20% polyacrylamide gel (Bio-Rad), followed by blotting onto a PVDF membrane using the Trans-blot Turbo system (Bio-Rad). Membranes were blocked in 5% BSA in TBS for 1 hour at room temperature. After blocking, the membranes were incubated with primary antibodies overnight while shaking at 4 ºC. After incubation with anti-mouse or anti-rabbit horseradish-coupled secondary antibody, the bands were visualized with FluroChem E (Protein Simple) imager. The antibodies used for the Western Blot analyses are shown in **Table VI in the Supplement**.

**Intracellular Calcium analysis.** For ratiometric calcium imaging, dissociated hiPSC-CMs were seeded on Matrigel-coated 35 mm dishes with a 20 mm coverglass bottom (Matek). After 7 days, the cells were treated with BiX (0.1 μM) or DMSO. Seventy-two hours later, the cells were loaded with 5 μM Fura-2AM (Thermo Fisher Scientific) with 0.02% Pluronic F-127 (Thermo Fisher Scientific) in Tyrode's solution for 10 min at room temperature. Following two washes in Tyrode's solution, $Ca^{2+}$ traces were acquired using the MultiCell HTS system (Ionoptix). The cells were electrically paced at 1.0 Hz at 37 °C. Calcium transient analysis was performed using the IonWizard software (Ionoptix).

**Patient RNA sequencing for UPR genes.** Human cardiac tissue collection was approved by the Biobank Research Ethics Committee, University Medical Center Utrecht, Utrecht, the Netherlands (protocol number WARB 12/387). Written informed consent was obtained or in certain cases waived by the ethics committee when obtaining informed consent was not possible due to the death of the individual. Heart samples collected at autopsy or transplantation were obtained from a homogeneous cohort of patients who all carried the same pathogenic PLN R14del mutation (n = 6). Four control hearts obtained from rejected organ donors (n = 3) or from autopsy (n =1) were used as a reference. RNA was isolated using ISOLATE II RNA Mini Kit (Bioline) according to the manufacturers' instructions with minor adjustments. After the selection of mRNA, libraries were prepared using the NEXTflexTM Rapid RNA-seq Kit (Bio Scientific). Libraries were sequenced on the Nextseq500 platform (Illumina), producing single-end reads of 75bp. Reads were aligned to the human reference genome GRCh37 using STAR v2.4.2a[46]. Picard's AddOrReplaceReadGroups v1.98 (http://broadinstitute.github.io/picard/) was used to add read groups to the BAM files, which were sorted with Sambamba v0.4.5[47] and transcript abundances were quantified with HTSeq-count v0.6.1p1[48] using the union mode. Subsequently, reads per kilobase per million mapped reads (RPKMs) were calculated with edgeR's RPKM function[49].

**hiPSC-CM RNAseq.** Total RNA was isolated from PLN R14del hiPSC-CMs at 72h post-treatment with BiX (0.1 μM) or DMSO control. Sequencing libraries were generated using the NEBNext Ultra Directional RNA Library Prep Kit for Illumina. Clustering of the index-coded samples was performed on a cBot Cluster Generation System using TruSeq PE Cluster Kit v3-cBot-HS (Illumina). Sequencing was carried out on an Illumina HiSeq platform. For each sample 30 to 40 million 150-base paired-end reads were acquired, and data were analyzed using the ENCODE-DCC RNA-seq pipeline (https://github.com/ENCODE-DCC/rna-seq-pipeline). Briefly, reads were aligned to the human genome (GRCh38) using STAR, version 2.5.1b, and gene and transcript quantifications were performed by RSEM (1.2.31). Differential expression analysis was performed

in R version 3.4 using the DESeq2 package[50]. Heat maps of gene expression were generated using the online tool Morpheus.

## Supplemental Tables

**Table I:** Cohort used in the histopathological experiments.

| Case | Phenotype | cDNA sequence | Protein sequence | Average Age per group | % Female/Male per group |
|---|---|---|---|---|---|
| 1 | PLN | c.40_42delAGA | p.(Arg14del) | | |
| 2 | PLN | c.40_42delAGA | p.(Arg14del) | | |
| 3 | PLN | c.40_42delAGA | p.(Arg14del) | 45.8 | 50% F/ 50% M |
| 4 | PLN | c.40_42delAGA | p.(Arg14del) | | |
| 5 | PLN | c.40_42delAGA | p.(Arg14del) | | |
| 6 | PLN | c.40_42delAGA | p.(Arg14del) | | |
| 7 | ARVC | c.1705A>T | p.K569(DSP) | | |
| 8 | ARVC | c.397C>T | p.Gln133(PKP2) | 52.3 | 0% F/ 100% M |
| 9 | ARVC | c.1705A>T | p.K569(DSP) | | |
| 10 | ICM | - | - | | |
| 11 | ICM | - | - | 65 | 33% F/ 66% M |
| 12 | ICM | - | - | | |
| 13 | Control | - | - | | |
| 14 | Control | - | - | 51 | 100% F/ 0% M |
| 15 | Control | - | - | | |

Abbreviations: PLN (NM_002667.4) = phospholamban, DSP (NM_004415.3) = desmoplakin, PKP2 (NM_004572.3) = plakophilin 2. ACM = arrhythmogenic cardiomyopathy, ICM = ischemic cardiomyopathy.

**Table II:** Primers designed to amplify regions corresponding to the top eight ranking predicted CRISPR/Cas9 off-targets of the gRNA used for genomic editing.

| Chr Position | Strand | Cut site | PCR Primer Forward | PCR Primer Reverse |
|---|---|---|---|---|
| chr1:225739235-225739257 | + | 225739251 | AGGAAAACATTTCTGCCACCCAC C | CCCTTCCCTTTACAAACTGTAGCC |
| chr1:242736904-242736925 | + | 242736919 | GACCTTTTGATGATCCATTCCCGC | CCATGCACCTAGTTGTGGGTTTC |
| chr11:30291992-30292013 | - | 30291998 | CAGCAGCTTGTTCCAACTGCC | GCCCCATTTCTTCACACTGTGATC |
| chr7:80359634-80359656 | + | 80359650 | ACGACGCTGAAATTATGAAGTGC CATCC | GTCACTTACGTTGACCTGGCTTTG |
| chr11:118161983-118162004 | + | 118161998 | AGGCTCGTGGTGGAGGTTGA | TATTGGGTGGTGCCGGTGAC |
| chr2:161606365-161606386 | - | 161606371 | GCCTGAAAGGCAGGATTCTTACA C | TCTGTCACAAGGGCTCCTTGG |
| chr15:36300817-36300838 | - | 36300823 | CCCTGGCACATGTTCTAATCACTC | CCTCCCACTGCTTTCTAGCTTTC |
| chr5:137204544-137204566 | - | 137204550 | CCAATATCAACATGTACAAGACA GTTGCCATTC | GCCTATTTTGTTTCATCTGGTATAA GCCAATACTG |

**Table III:** Sanger sequencing of amplified regions corresponding to the top eight ranking predicted CRISPR/Cas9 off-targets of the gRNA used for genomic editing in the healthy donor hiPSC line

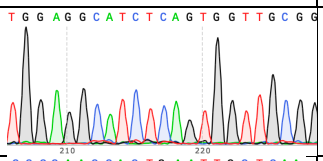
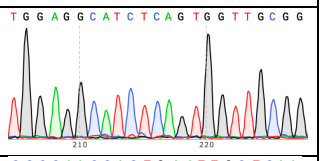
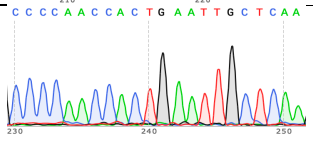
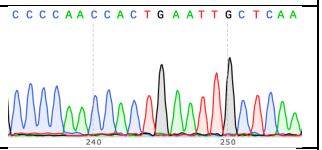
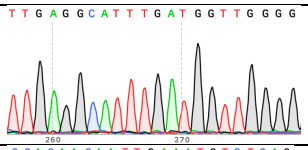
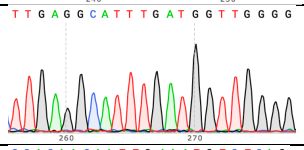
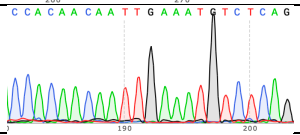
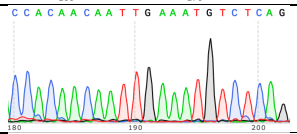
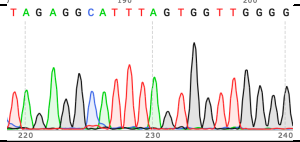
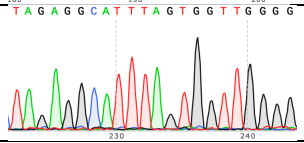
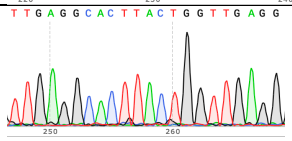
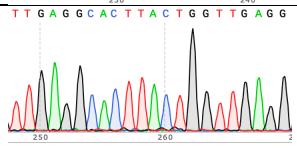
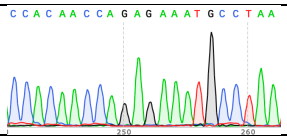
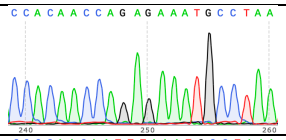
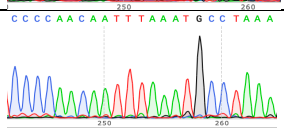
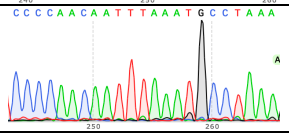
| # | Chr Position | Cut site | Mismatch | In Silico Hit | Healthy donor | Healthy donor introduced |
|---|---|---|---|---|---|---|
| 1 | chr1:225739235-225739257 | 225739251 | 3 | TGGAGGCATCTCAGTGGTTGCGG |  |  |
| 2 | chr1:242736904-242736925 | 242736919 | 2 | TTGAGCAATTCAGTGGTTGGGG |  |  |
| 3 | chr11:30291992-30292013 | 30291998 | 1 | TTGAGGCATTTGATGGTTGGGG |  |  |
| 4 | chr7:80359634-80359656 | 80359650 | 3 | CTGAGACATTTCAATTGTTGTGG |  |  |
| 5 | chr11:118161983-118162004 | 118161998 | 2 | TAGAGGCATTTAGTGGTTGGGG |  |  |
| 6 | chr2:161606365-161606386 | 161606371 | 2 | TTGAGGCACTTACTGGTTGAGG |  |  |
| 7 | chr15:36300817-36300838 | 36300823 | 2 | TTAGGCATTTCTCTGGTTGTGG |  |  |
| 8 | chr5:137204544-137204566 | 137204550 | 3 | TTTAGGCATTTAAATTGTTGGGG |  |  |

**Table IV:** Sanger sequencing of amplified regions corresponding to the top eight ranking predicted CRISPR/Cas9 off-targets of the gRNA in the patient (1) hiPSC line.

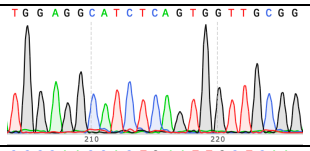
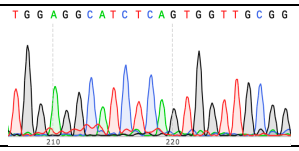
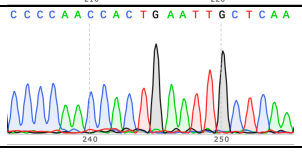
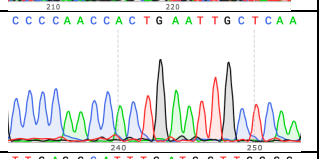
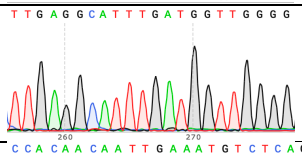
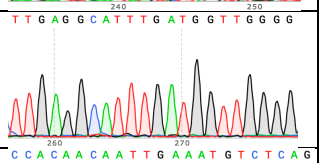
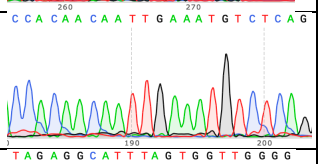
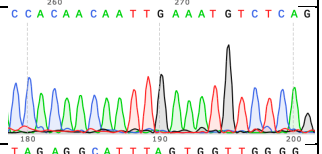
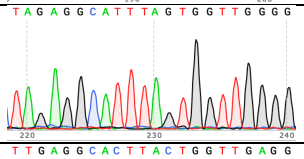
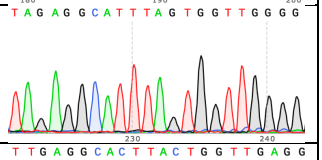
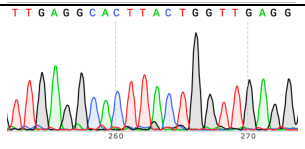
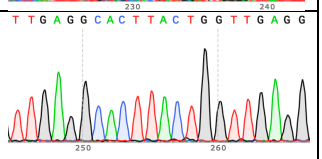
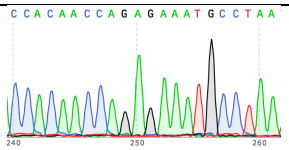
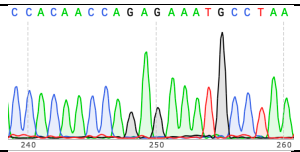
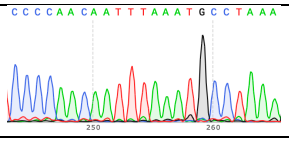
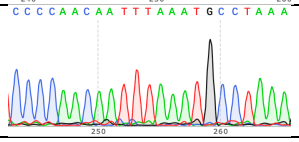
| # | Chr Position | Cut site | Mismatch | In Silico Hit | Patient 1 | Patient 1 corrected |
|---|---|---|---|---|---|---|
| 1 | chr1:225739235-225739257 | 225739251 | 3 | TGGAGGCATCTCAGTGGTTGCGG |  |  |
| 2 | chr1:242736904-242736925 | 242736919 | 2 | TTGAGCAATTCAGTGGTTGGGG |  |  |
| 3 | chr11:30291992-30292013 | 30291998 | 1 | TTGAGGCATTTGATGGTTGGGG |  |  |
| 4 | chr7:80359634-80359656 | 80359650 | 3 | CTGAGACATTTCAATTGTTGTGG |  |  |
| 5 | chr11:118161983-118162004 | 118161998 | 2 | TAGAGGCATTTAGTGGTTGGGG |  |  |
| 6 | chr2:161606365-161606386 | 161606371 | 2 | TTGAGGCACTTACTGGTTGAGG |  |  |
| 7 | chr15:36300817-36300838 | 36300823 | 2 | TTAGGCATTTCTCTGGTTGTGG |  |  |
| 8 | chr5:137204544-137204566 | 137204550 | 3 | TTTAGGCATTTAAATTGTTGGGG |  |  |

**Table V:** Sanger sequencing of amplified regions corresponding to the top eight ranking predicted CRISPR/Cas9 off-targets of the gRNA the patient (2) hiPSC line.

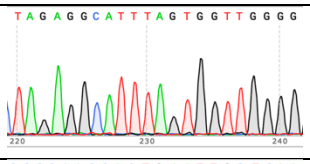
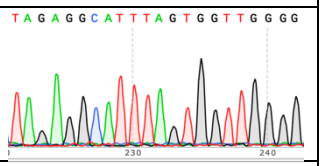
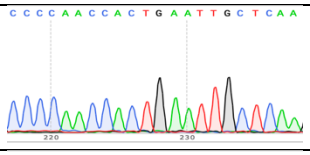
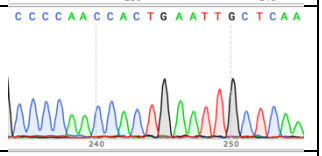
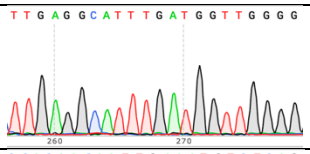
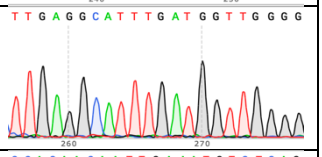
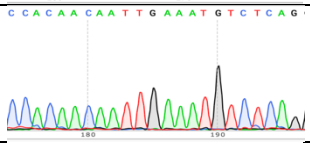
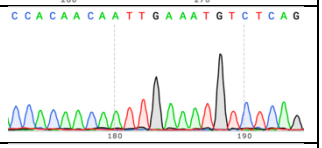
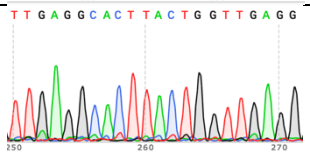
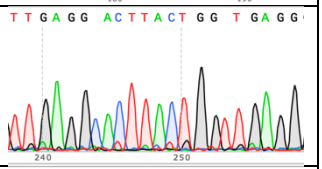
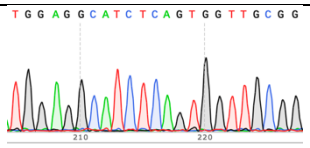
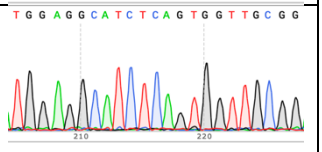
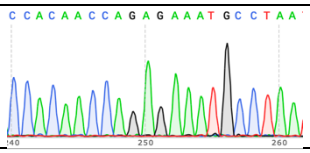
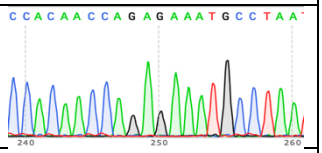
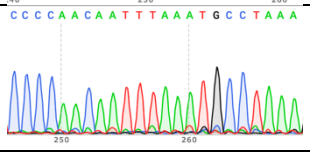
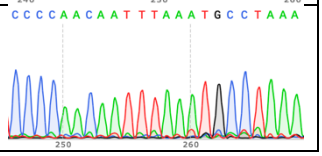
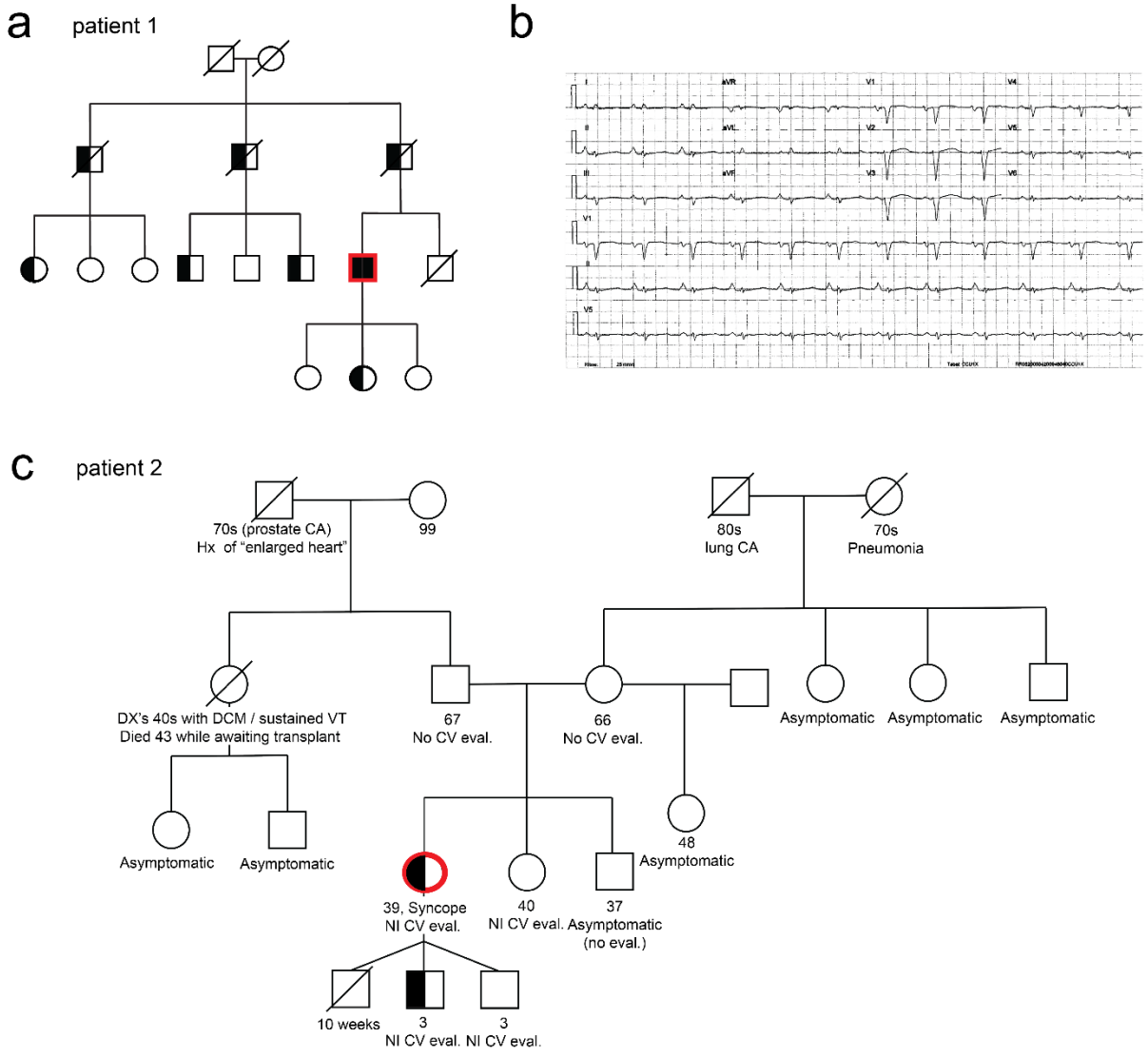
| # | Chr Position | Cut site | Mismatch | In Silico Hit | Patient 2 | Patient 2 corrected |
|---|---|---|---|---|---|---|
| 1 | chr1:225739235-225739257 | 225739251 | 3 | TGGAGGCATCTCAGTGGTTGCGG |  |  |
| 2 | chr1:242736904-242736925 | 242736919 | 2 | TTGAGCAATTCAGTGGTTGGGG |  |  |
| 3 | chr11:30291992-30292013 | 30291998 | 1 | TTGAGGCATTTGATGGTTGGGG |  |  |
| 4 | chr7:80359634-80359656 | 80359650 | 3 | CTGAGACATTTCAATTGTTGTGG |  |  |
| 5 | chr11:118161983-118162004 | 118161998 | 2 | TAGAGGCATTTAGTGGTTGGGG |  |  |
| 6 | chr2:161606365-161606386 | 161606371 | 2 | TTGAGGCACTTACTGGTTGAGG |  |  |
| 7 | chr15:36300817-36300838 | 36300823 | 2 | TTAGGCATTTCTCTGGTTGTGG |  |  |
| 8 | chr5:137204544-137204566 | 137204550 | 3 | TTTAGGCATTTAAATTGTTGGGG |  |  |

**Table VI:** Antibodies used in the protein expression analysis.

| Antibody | Catalog # | Species | Manufacturer | Dilution |
|---|---|---|---|---|
| Anti-MYBPC3 | ab108522 | Rabbit mAb | Abcam | 1/5000 |
| Anti-Cardiac Troponin T | ab45932 | Rabbit pAb | Abcam | 1/10000 |
| Anti-Calsequestrin | ab3516 | Rabbit pAb | Abcam | 1/5000 |
| Anti-Troponin I | 4002S | Rabbit pAb | Cell Signaling | 1/1000 |
| Anti-NCX1 | 79350S | Rabbit mAb | Cell Signaling | 1/1000 |
| Anti-Ryanodine Receptor | MA3-916 | Mouse mAb | Thermofisher | 1/2000 |
| Anti-Tropomyosin | T2780 | Mouse mAb | Sigma Aldrich | 1/2000 |
| Anti-Phospholamban | A010-14 | Mouse mAb | Bradilla | 1/5000 |
| Anti-Phospholamban (Ser16) | 07-052 | Rabbit pAb | Millipore | 1/1000 |
| Anti-SERCA2 | NB300-581 | Mouse mAb | Novusbio | 1/1000 |
| Anti-GAPDH | MA5-15738 | Mouse mAb | Thermofisher | 1/2000 |
| Anti-BiP | #3177 | Rabbit mAb | Cell Signaling | 1/1000 |
| Anti-PDI | #3501 | Rabbit mAb | Cell Signaling | 1/1000 |
| Anti-HSP90 | 13492 | Mouse mAb | Abcam | 1/2000 |

## Supplemental Figures

**Figure I. Pedigree and clinical presentation of recruited PLN-R14del patients. a**, Pedigree of the first




R14del family recruited in this study. Half-filled squares (male) and circles (female) represent individuals carrying the specific heterozygous R14 deletion mutation. Filled box represents affected carriers and the red box indicates a patient (52-year-old) donated for hiPSC line generation. The proband's father died suddenly at a young age without obvious causes. **b**, The ECG from the R14del patient showed a significantly prolonged QTc (443 ms) with low voltages in the extremity leads. The patient was implanted with an implantable cardioverter-defibrillator and underwent HTx when ejection fraction dropped under 25%. **c**, Pedigree of the second R14del family recruited in this study. The red circle indicates the patient (39-year-old) that donated blood for hiPSC line generation. The patient has a family history of heart disease

on her paternal side. Hx, history; Dx, diagnosis; CA, cancer; VT, ventricular tachycardia; CV, cardiovascular; NI, negative.

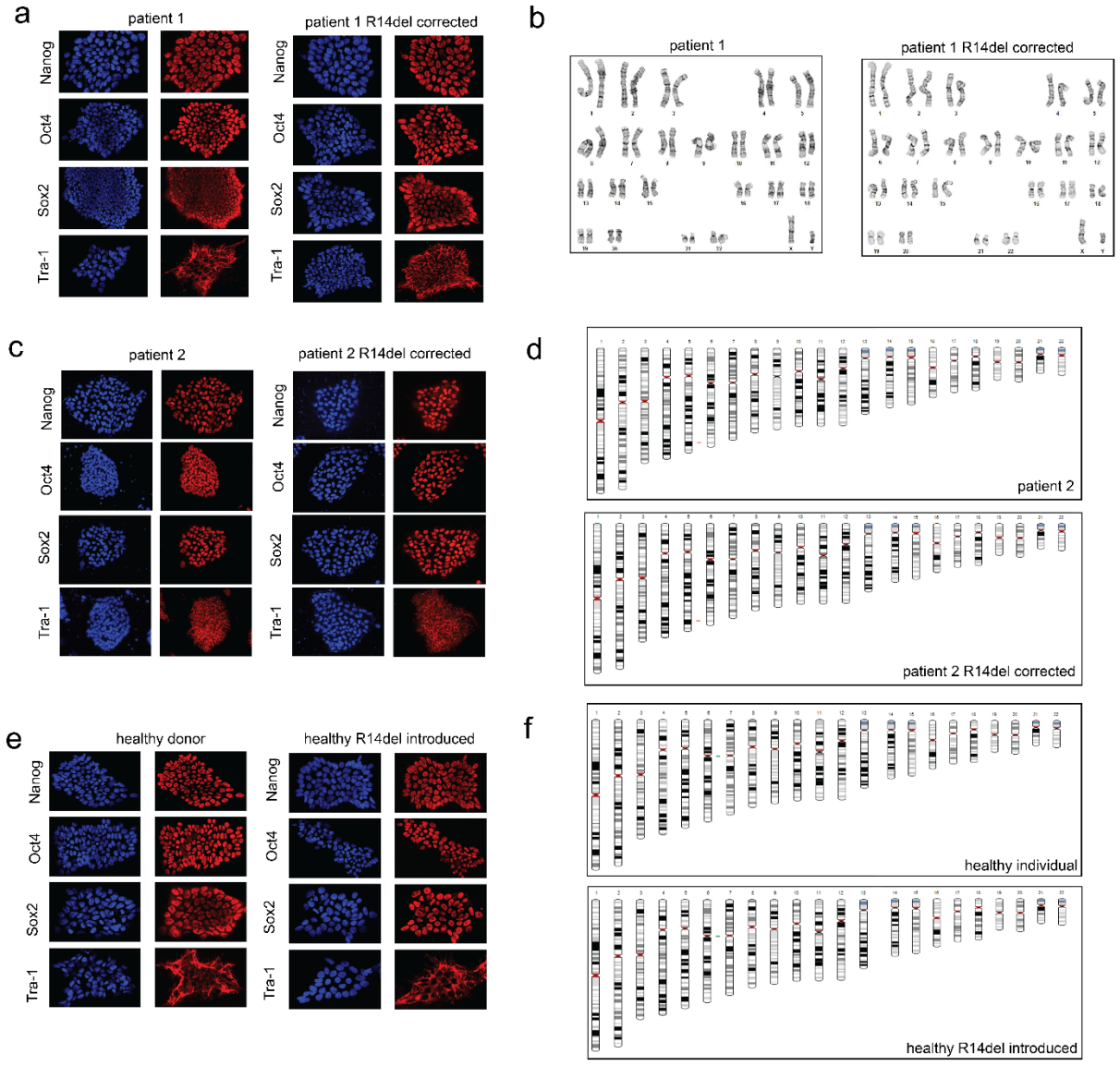



**Figure II. Pluripotency markers and karyotyping of iPSC-lines. a,c,e,** Immunostaining (red; Nanog, Oct-4, Sox-2, and Tra-1 / blue; Hoechst) of pluripotency markers in all three lines and isogenic derivatives. **b,** G-banded karyotype of patient 1 and isogenic corrected lines showed normal karyotype with no clonal abnormalities detected. **d,f,** Single nucleotide polymorphism (SNP) karyotyping preformed on second patient and healthy donor hiPSC-lines detected no genomic abnormalities.

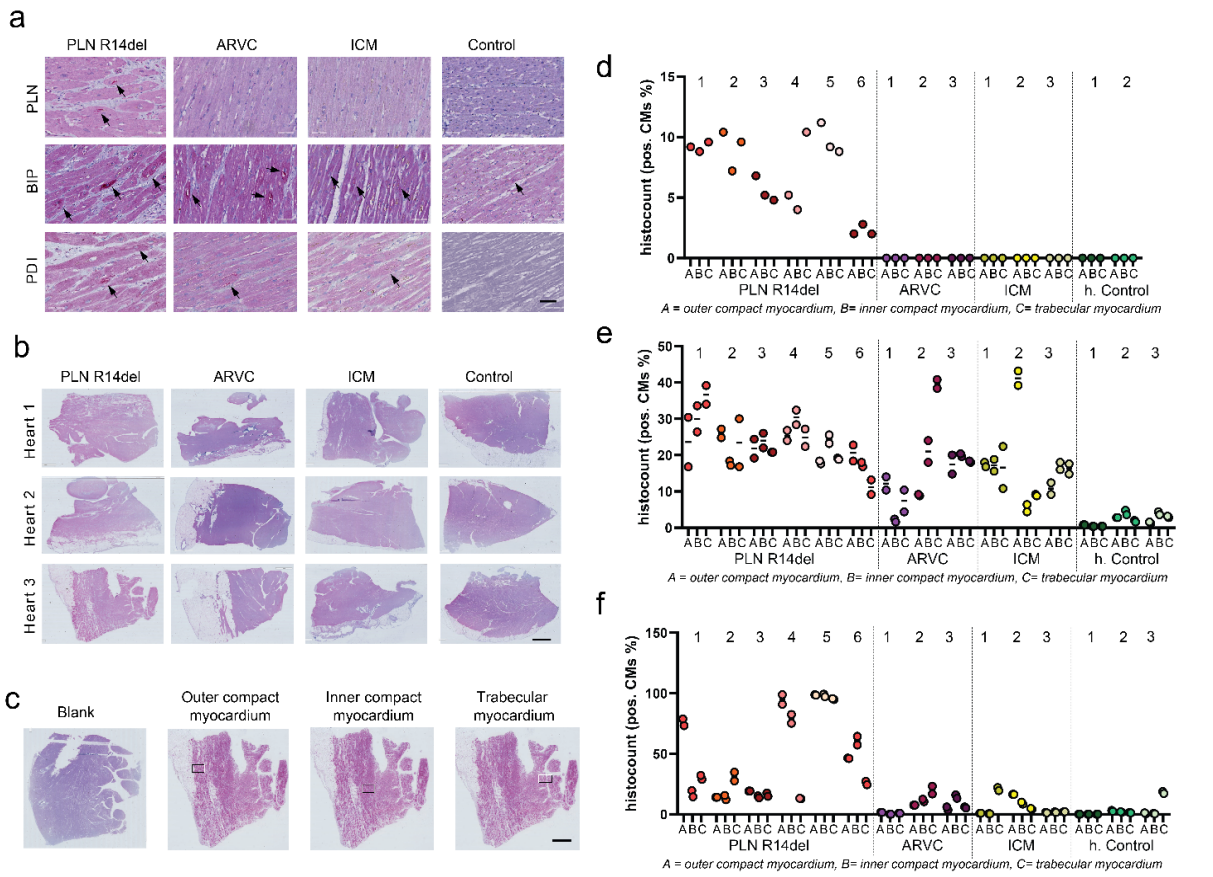



**Figure III. Histopathological analysis of UPR activation in the cardiac tissue of PLN-R14del patients.** **a,** Lower magnification histological sections of human myocardium from patients with PLN R14del, ACM, and ICM versus control hearts stained with PLN, BIP, or PDI. Depicted are representative images from the scanned sections of each group. Scale bar = 70 μm**. b,** Whole histological sections of human myocardium from three patients with PLN R14del, ACM, and ICM versus control hearts. Scale bar = 4 mm. **c**, Histological sections of human myocardium of a patient carrying the PLN R14del mutation depicting individual scoring spots (outer compact, inner compact, and trabecular myocardium). In each spot, 250 nuclei were counted for PLN aggregates, and for each heart, the average of the regions was used as a quantification for PLN expression. **d-f**, Immunolabeling scoring across all the patients (numbered) in different myocardial regions (outer compact myocardium, A; inner compact myocardium, B; trabecular myocardium, C).

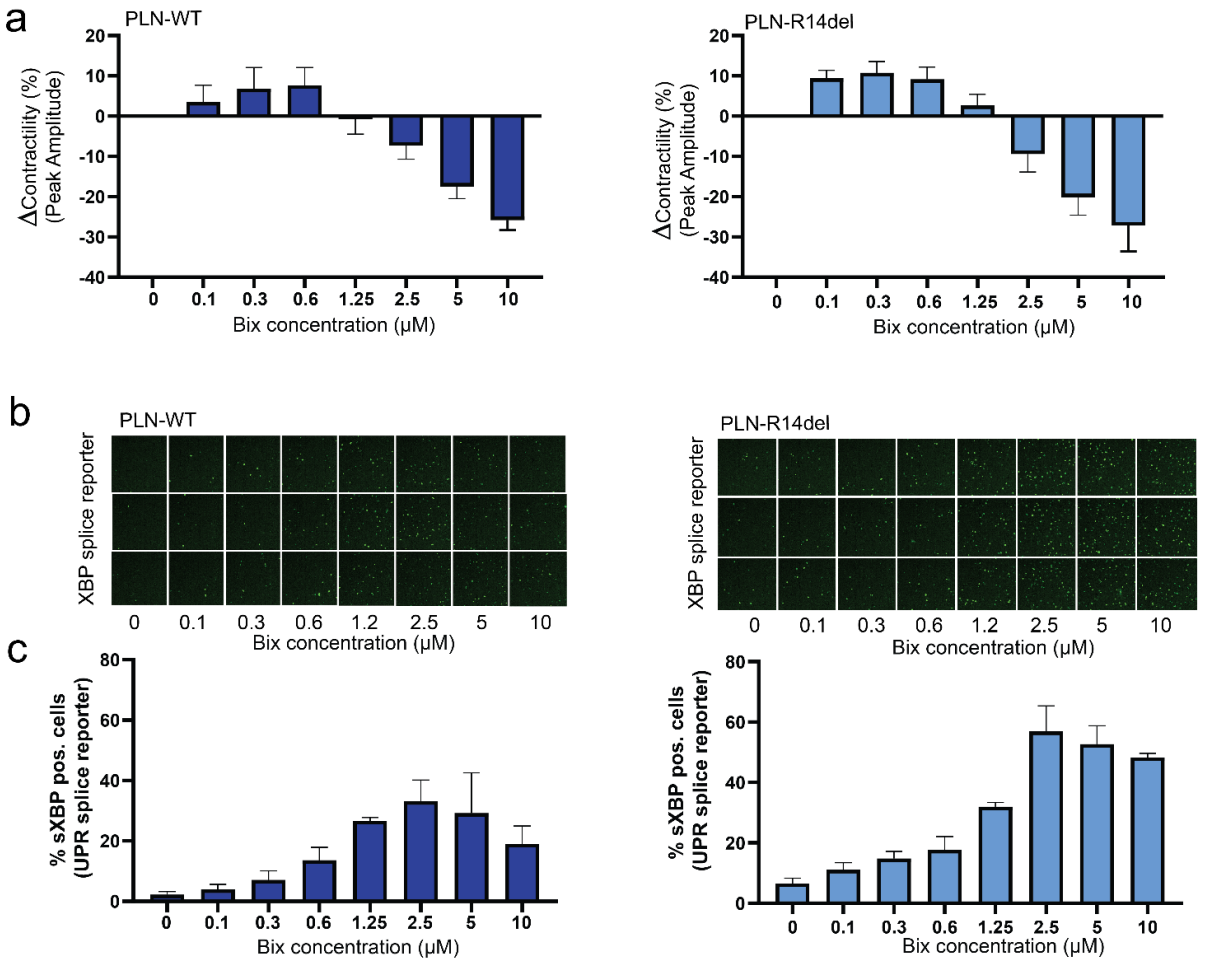



**Figure IV. Dose response curve for effect of BiX on contractility and XBP splicing in hiPSC-CMs. a,** 2-D contractility assessment in PLN-WT (left) and R14del (right) hiPSC-CMs treated with escalating dose of BiX. Contractility is represented as percent change from vehicle control (DMSO) control per batch (3 batches, n=4-6 wells each). **b**, Representative fluorescent images of nuclear expression of F-*XBP1*-venus in iPSC-CMs treated with escalating dose of BiX. **c**, Quantification of XBP splicing in iPSC-CMs treated with escalating dose of BiX. XBP splicing represented as percentage of F-*XBP1*-venus positive nuclei to total nuclei (2 batches, n=6 wells each).

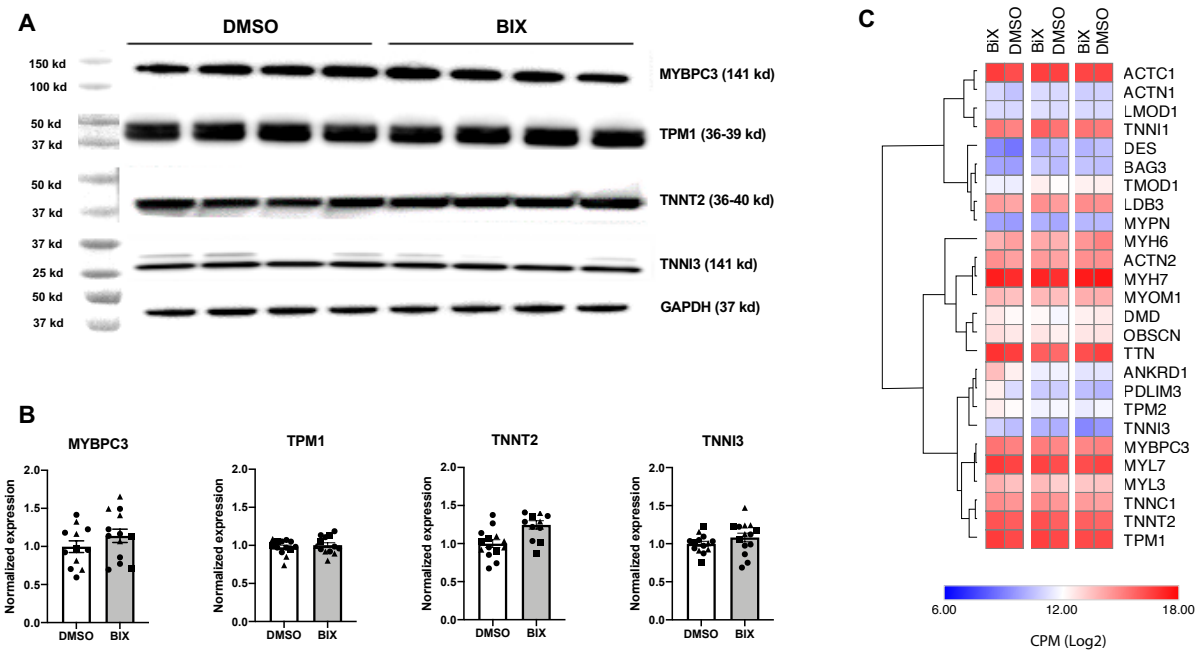



**Figure V. The effect of BiX treatment on myofilament expression in PLN R14del hiPSC-CMs**. **a-b,** Western blot analysis and quantification of myofilament proteins in PLN R14del hiPSC-CMs treated with 0.1 µM BiX or vehicle control (DMSO) for 72 h. Data presented the mean ± SEM of PLN R14del hiPSC-CMs derived from three hiPSC lines (n=4-8 replicates per line). Circles (●), squares (■), triangles (▲), represent the three PLN R14 hiPSC mutant lines. Protein expression levels were normalized to GAPDH loading control and expressed as relative to respective DMSO-treated hiPSC-CMs. **c,** Heat map showing the mRNA expression levels of myofilament genes in PLN R14del hiPSC-CMs treated with 0.1 µM BiX or DMSO control for 72 h. CPM, transcript counts per million reads.

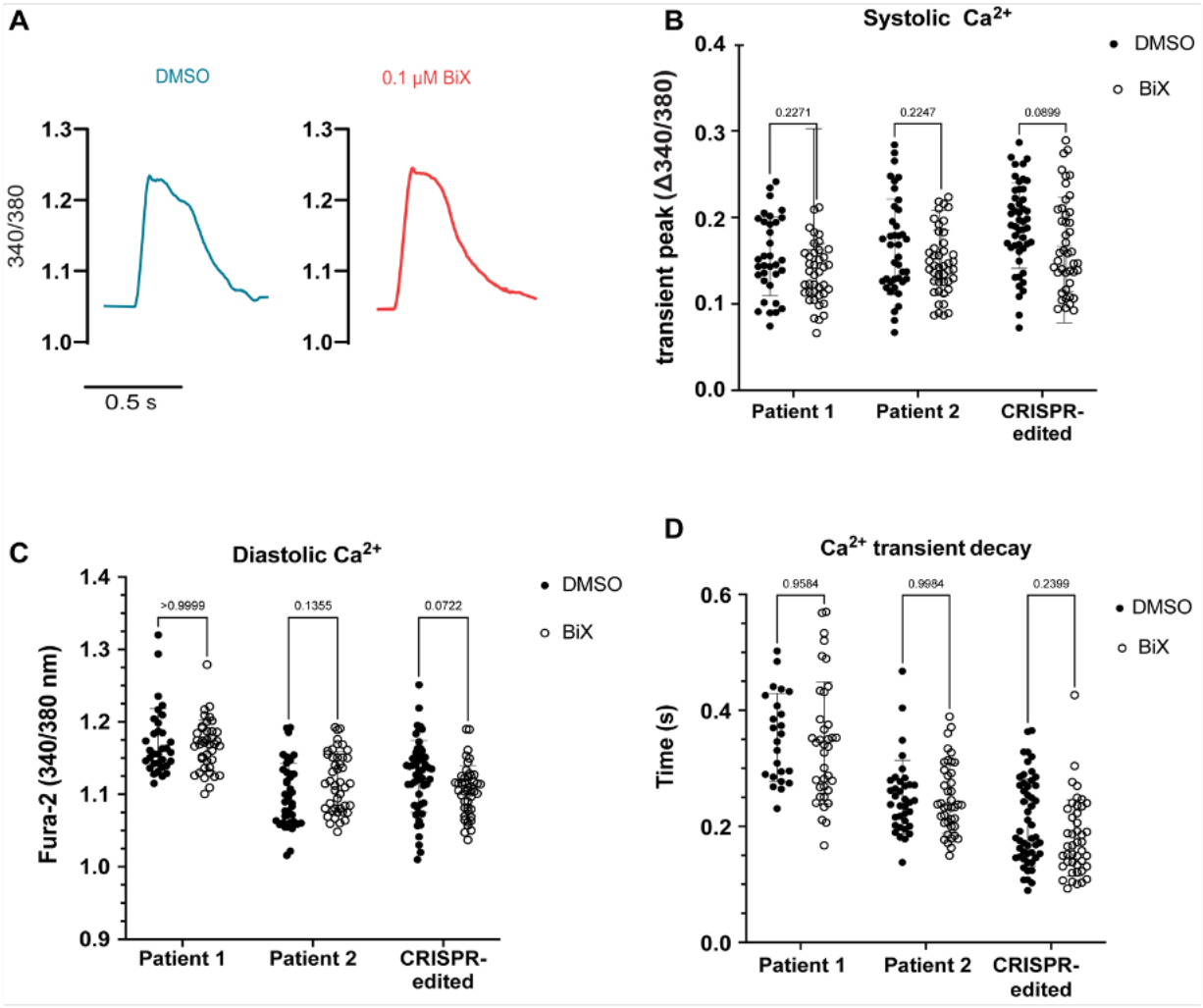



**Figure VI. The effect of BiX treatment on calcium transient properties of PLN R14del hiPSC-CMs. a,** Representative intracellular calcium traces of BiX- and vehicle control (DMSO)-treated hiPSC-CMs. **b-d,** Quantification of calcium transient parameters as indicated. Data represent mean ± SD; DMSO: n=34, n=40, and n=49; BiX: n=40 , n=44, and n=44 Patient 1, Patient 2, and CRISPR-edited hiPSC-CMs, respectively.

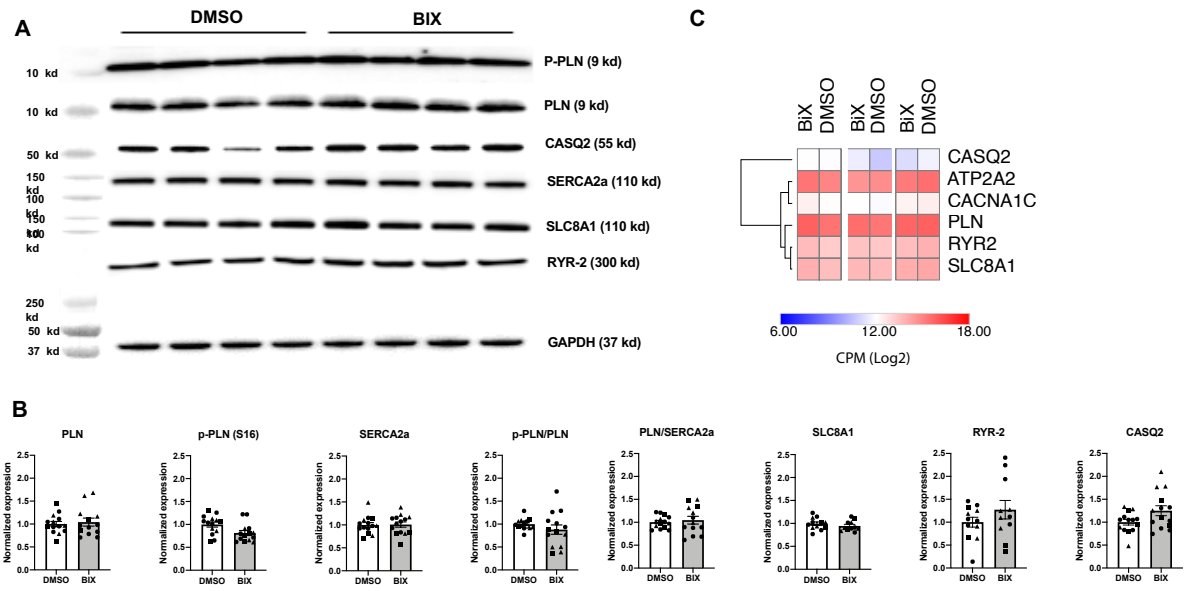



**Figure VII. The effect of BiX treatment on calcium handling protein expression in PLN R14del hiPSC-CMs**. **a-b,** Western blot analysis and quantification of calcium handling protein expression in PLN R14del hiPSC-CMs treated with 0.1 mM BiX or DMSO control for 72 h. Data presented the mean ± SEM of PLN R14del hiPSC-CMs derived from 3 hiPSC lines (n = 4-8 replicates per line). Circles (●), squares (■), triangles (▲), represent the 3 hiPSC PLN R14del mutant lines. Protein expression levels were normalized to GAPDH loading control and expressed as relative to the respective DMSO-treated hiPSC-CMs. **c,** Heat map showing the mRNA expression levels of calcium handling genes in R14del hiPSC-CMs treated with 0.1 μM BiX or DMSO control for 72 h. CPM, transcript counts per million reads.