SUPPLEMENTAL MATERIAL

A Premature Termination Codon Mutation of MYBPC3 Causes Hypertrophic Cardiomyopathy via Chronic Activation of Nonsense-Mediated Decay

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SUPPLEMENTAL METHODS

Genome editing with clustered regularly interspaced short palindromic repeats/Cas9 (CRISPR/Cas9). Genome editing with CRISPR/Streptococcus pyogenes Cas9 (spCas9) was performed by transfection of the vector pSpCas9(BB)-2A-GFP (PX458; a gift from Feng Zhang; Addgene plasmid # 48138) after ligation of two annealed reverse complementary guide DNA oligos for site-specific DNA double strand break initiation. The guide DNA oligos were designed using a web-based tool (*crispr.mit.edu*) and chosen based on a high score for on-target binding and the lowest off-target score. Single-stranded oligo-DNAs (ssODN) were designed for homologous recombination to either correct or mutate the specific locus as published before.[1] Co-transfection of iPSCs with the CRISPR/Cas9 vector and ssODN was performed using the Lipofectamine 3000 Reagent (Thermo Fisher Scientific). 1 μg of CRISPR/Cas9 vector and 4 μg of ssODN were used for each well of a 6-well plate with 40% confluent iPSCs. 24-48 hr after transfection, cells were dissociated into single cells using TrypLE express 1x (Thermo Fisher Scientific) and sorted by flow cytometry for GFP expression. Positive cells were collected and seeded with 1,000 cells per well of a 6-well plate for single cell clone expansion. Seven to ten days after seeding, individual clones were picked for single clone expansion in one well of a 48-well plate and for DNA preparation. Genome edited lines were screened for non-homologous end joining (NHEJ) in top ranked off-target sites predicted by *crispr.mit.edu* using PCR and Sanger sequencing (Supplemental Table S1)[2].

MYBPC3_943 guide: CCGGCTGCTTTTCTGAGTGC; DNA-oligo: TGCACAAAGGGGCAC TCACGCAGGATCTCCTGCACTGTCACCGGCTCCGTGGTGGTAACAGGGGCTCCAGGC CCTGCCATATTGTGTGCCCGCACTCGGAAAAGCAGCCGGGCCCCCGTGGGCAGGT.

MYBPC3_1073 guide: GTCAGTCACCCGGGAGATCC; DNA-oligo GGTCCCTGGAGCCAG

TGACCCCCTGCTCACTGTCAGGAGGCGTGGTGACCCAACTGGGTCTGTCTCCCGCAG ACAAGCCAAGTCCTCCCCAGGATCTCcGGGTGACTGACGCCTGGGGTCTTAATGT.

Genome editing with transcription activator-like effector nucleases (TALEN). TALEN pair vectors were designed and constructed using the rapid TALEN assembly system as previously described.[3] Five hundred base-pair fragments of wild-type MYBPC3 Exon 27 and adjacent intronic sequences were synthesized as GeneArt String DNA fragments (Life Technologies) to make left and right homologous arms, and cloned into the PB-MV1Puro-TK vector (Transposagen), as previously described.[3,4] Two silent mutations in the homologous arms were inserted to avoid re-cleavage of the genomic sequence. Both TALEN pair and targeting vectors were delivered into iPSCs by nucleofection using the P3 Primary Cell 4D-Nucleofector X Kit (Lonza). Afterwards, cells with the correct targeting vector integration were selected by puromycin (Life Technologies) and genotyped. To excise the selection cassette, transient expression of piggyBac™ transposase was performed by transfection of excising piggyBac™ transposase mRNA (Transposagen) using Lipofectamine MessengerMAX™ (LifeTechnologies). After negative selection using ganciclovir (Sigma Aldrich), the established clones were genotyped by PCR and bidirectional direct sequencing. Genome edited lines were screened for NHEJ in top ranked off-target sites predicted the bioinformatics tool PROGNOS [6] using PCR and Sanger sequencing (Supplemental Table S2).

Genotype screening. DNA preparation was performed using QuickExtract solution (Epicenter). Site-specific PCR was run with PrimeSTAR® GXL DNA Polymerase (Clontech). PCR products were run on a 1% agarose gel and stained with Midori-green DNA stain (Bulldog-Bio) for

specificity. Positive clones were sent for Sanger sequencing to evaluate successful homologous recombination.

RNA isolation, cDNA generation and quantitative PCR (qPCR). Total RNA was isolated using the miRNeasy Mini kit (QIAGEN) or Direct-zol™ RNA MiniPrep Kit (Zymo Research) according to the manufacturer's protocols. 400 ng of RNA was used for synthesizing cDNA with the iScript cDNA Synthesis kit (Bio-Rad). 6 ng of the cDNA was used per reaction to quantify gene expression using TaqMan probes and TaqMan Universal PCR Master Mix (Thermo Fisher Scientific) or iQ Sybr Green master mix (Bio-Rad) with specific primers (Supplemental Table S3).

Cell size measurement. Single iPSC-CMs were seeded into 10 wells of a 96-well plate for each differentiation batch with 500 cells/well for 3-6 differentiation batches per iPSC line. Seven days after seeding, cells were stained with Calcein AM (Thermo Fisher Scientific) live cell dye for 30 min at 2 μM and subsequently imaged using a Cytation 5 Imaging Reader (Biotek). Data analysis was performed using the Cytation 5 software to evaluate cell size. For each well, the average cell size was calculated and used for further downstream data analysis.

Single cell micropatterning. iPSC-CMs were micro-patterned as single cells on a 12 kPa polyacrylamide hydrogel at a 7:1 length-to-width ratio recapitulating the adult CM-like morphology.[7] Polyacrylamide hydrogel was applied on 22x22 mm coverslips and solidified with UV-activation. After UV-activation of the hydrogel surface with Sulfo-sanpah (Thermo Fisher Scientific), we used a stencil-based microfabrication approach to apply Matrigel (Corning, 1:20 dilution in PBS) to achieve defined patterns with a 7:1 length-to-width ratio. Each coverslip was

placed in one well of a 6-well plate and washed with PBS (Invitrogen). Dissociated iPSC-CMs were seeded as single cell suspension in RPMI+B27 media with $7x10^5$ cells/well. Analysis was performed at least 7 days after seeding.

Immunofluorescence staining. For immunofluorescent staining, cells were fixed with 4% poly-formamide (PFA)/PBS for 10 min, permeabilized with 0.1% Triton X-100 for 15 min, and blocked with 8% goat serum. The following primary antibodies were used: cardiac troponin T (cTNNT2; Abcam), sarcomeric α-actinin (αSA; Sigma Aldrich), MYBPC3 (Abcam), and DAPI (Molecular Probes). Alexa-fluor 488 and 594 goat anti-rabbit and anti-mouse labeled secondary antibodies were used and cells were imaged with a confocal microscope (Carl Zeiss, LSM 710 Meta, Göttingen, Germany) at 20x to 63x objectives. Images of micro-patterned iPSC-CMs were analyzed with ImageJ to obtain the sarcomeric length. Fluorescence intensity of either MYBPC3 or sarcomeric α-actinin (αSA) was evaluated longitudinally throughout single myofibers analyzed using plot profile (ImageJ). Sarcomeric length was calculated by peak-to-peak detection (for sarcomeric αSA) and detection of lowest points of intensity for MYBPC3 (20-100 sarcomeres were evaluated per cell).

Ca^{2+} imaging. For ratiometric calcium imaging, dissociated iPSC-CMs were seeded on Matrigel-coated coverslips (CS-24/50, thickness 1 mm, Warner Instruments). After 7 days, 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 37°C temperature, followed by washing with Tyrode's solution. iPSC-CMs were field-stimulated at 0.5 Hz at 37 °C. Single cell Ca^{2+} imaging was conducted on a Nikon Eclipse Ti-E inverted microscope mounted with 40× oil immersion

objective (0.95 NA). Fura-2 was excited at 340 nm and 380 nm wavelength using a Lambda DG-4 ultra-high-speed wavelength switcher (Sutter Instrument), and the emission of Fura-2 was collected over 510 nm wavelength.

Ca^{2+} analysis. Raw data exported from Nikon NIS Elements were analyzed using a custom Python script (https://github.com/GeorgeMcMullen/CalciPy) built specifically to automate processing of ratiometric calcium imaging data. The script iterates over the captured cell lines, calculates ratios between rows, subtracts background noise (which is also captured), and uses look-ahead peak detection to determine local maxima and minima. Because the exported data are interpolated, the script uses the detected peaks to calculate rise and decay times and to identify which combination of rows presents the correct 340/380 ratio. A centered window moving average is applied to the decay data only (each individual set of maximum to minimum), slightly smoothing the signal to obtain more consistent initial values for curve fitting. Python's curve fitting functions found in SciPy are then used with an exponential decay function to establish the signal's parameters, most significantly rate constant, and Tau. The original signal and fitted curve results are then plotted and exported as PDFs for easy visual review. In addition, calculated results for rate constant, Tau, decay time, amplitude, diastolic, beat rate, beat variation, and velocity are all outputted to a comma separated value text file (CSV) for further analysis. The script can be configured with different peak detection look-ahead windows, amplitude windows, background noise reduction, smoothing filters and more, and has shown consistent results even when it is run against noisy or arrhythmic signals.

Western blot. For western blot analysis, iPSC-CMs were lysed using RIPA buffer (Thermo Fisher Scientific) with 10 µl/1x10^4 cells. Protein yields from each sample were estimated with bicinchoninic acid assays (Thermo Pierce). For loading, 11 µl lysate was mixed with 4x loading buffer and 10x reducing agent (Life Technologies), and 15 µl were loaded per well on 4-12% Mini or Midi PROTEAN® TGX Stain-Free™ Precast Gels (BioRad). Subsequently, proteins were transferred to PVDF membranes using a Trans-Blot® Turbo™ semidry Transfer System (BioRad). The membranes were then blocked in blotting grade blocker non-fat dry milk (BioRad) and incubated with primary antibody overnight at 4 °C or for 1 hr at room temperature. Blots were incubated with the appropriate secondary antibodies (IRDye® 800CW Goat anti-Rabbit and 680RD Goat anti mouse; Licor) for 1 hr at room temperature, washed, and visualized using an Odyssey CLx imaging system (Licor). Primary antibodies used were mouse anti-MYBPC3 (sc-137180, E-7, Santa Cruz; raised against amino acids 1-120 mapping at the N-terminus of MYBPC3 of human origin), rabbit anti-MYBPC3 (ab108522, Abcam; raised against amino acids 100-200 at the N-terminus within human MYBPC3; AP12436a, Abgent raised against amino acids 189-218 from the N-terminal region of human MYBPC3), mouse anti-alpha sarcomeric actin (αSA; A7811, Sigma Aldrich), rabbit anti-αSA (ab68167, Abcam), mouse-anti-GAPDH (MA5-15738; Thermo Fisher), and mouse anti-mono-ubiquitinylated and poly-ubiquitinylated conjugates (BML-PW8810, EnzoLifesciences).

Capillary nano-immuno assay. Capillary nano-immuno assay (cNIA) was performed on a Peggy Sue® system (ProteinSimple; Bio-Techne). 12–230 kilodalton (kDa) size assays were carried out as follows: each analysis cycle was composed of 12 capillaries, including a ladder and 11 samples. Six microliters of protein samples were denatured with a Fluorescent Master Mix® containing

fluorescent molecular weight markers and 200 mM dithiotreitol, according to ProteinSimple recommendations. The mixtures were heated at 95 °C for 5 min. Primary anti-MYBPC3 antibody (Abgent) was diluted with antibody diluent to obtain a final dilution of 1/50th. Samples, blocking reagent, primary antibodies, HRP-conjugated second antibodies, chemiluminescent substrate, separation, and stacking matrices were dispensed to designated wells in a 384-well plate. The plate was then loaded on the Peggy Sue® platform to perform the different steps of separation electrophoresis and immunodetection automatically. Briefly, the proteins were separated inside a capillary per sample for 40 min at 250 volts and fixed on the surface of these capillaries. The incubations of primary antibody and secondary antibody lasted 120 min and 30 min, respectively. Finally, the chemiluminescent revelation was performed incubating capillaries with a mix of luminol and peroxide. The determination of sizes, areas, heights, and signal to noise (S/N) ratios of MYBPC3 were automatically calculated on Compass for Simple Western® software, version 3.1.7.

Mass spectrometry. Proteins were extracted from iPSC-CMs after homogenization and sonication in RIPA buffer (Thermo Fisher Scientific). Protein digestion was performed as previously described.[8] Briefly, protein yields from each sample were estimated with bicinchoninic acid assays (Thermo Pierce); 100 µg of proteins were then digested from each sample on 10 kDa molecular-weight cutoff polyethersulfone filters (Nanosep; Pall Life Sciences). The protein samples were washed with 8 M urea and the buffer was exchanged on-filter with triethylammonium bicarbonate (50 mM, 500 µl), after which the protein samples underwent reduction with tris(2-carboxyethyl)phosphine (10 mM, 70 °C, 5 min) and alkylation with iodoacetamide (18 mM, ambient temperature, 30 min). Proteins were digested on-filter (16 hr, 37

°C) with sequencing-grade modified trypsin/Lys-C mix (50:1, Promega). Proteolysis was terminated and peptides were eluted by incubation with 20 µl of 10% trifluoroacetic acid (Pierce) (30 min, 37 °C) followed by centrifugation (13,000×g, ambient temperature, 15 min). The digested peptides were quantified using a Pierce colorimetric peptide quantification kit, then labeled with 10-plex tandem mass tags (Thermo) at room temperature with 600 rpm shaking for 2 hr. Labeled peptides were quenched with 5% hydroxylamine following the manufacturer's protocol. Labeled peptides were fractionated into 6 fractions using pH-10 reversed-phase spin columns (Pierce), then analyzed by liquid chromatography-tandem mass spectrometry to identify protein species and quantify relative abundance in each sample. On-line second-dimension (low-pH) reversed-phase chromatography was performed using a single Easy-nLA 1000 nano-UPLC system on an EasySpray C18 column (PepMap, 3-µm particle, 100-Å pore; 75 µm x 150 mm; Thermo) in 180-min reversed-phase gradient. Mass spectrometry was performed using a Thermo Orbitrap Fusion high-resolution mass spectrometer, coupled online to the nano-UPLC through a Thermo EasySpray interface. Mass spectrometry signals were acquired in fourier-transform (FT)/FT/FT mode using conventional parameters. Briefly, each FT MS1 survey scan was acquired at 120,000 resolving power in profile mode, followed by FT MS2 scan on automatically determined maximal number of ions with monoisotopic peak selection at 35% collision-induced dissociation energy, at 60 ms injection time, and at 30,000 resolution for peptide identification. This was followed by FT MS3 scan with multi-notch isolation (n=5) at 65% collision energy at 60,000 resolution in the low-mass range for tandem mass tag reporter ion quantification. Dynamic exclusion of 90 seconds was used.

Acquired mass spectra were centroided and converted to .ms1, .ms2, and .ms3 formats using RawConverter (v.1.1.0).[9] Database search was performed using the modified SEQUEST

algorithm implemented in ProLuCID (v.1.3)[10] against a reverse-decoyed protein sequence database (UniProt Reference Proteome Homo sapiens, reviewed, accessed 09/20/2016, 20,183 forward entries and 20,183 decoy entries).[11] Static cysteine carboxyamidomethylation (C +57.021464 Da; Unimod accession #4) modification, peptide N-terminal and lysine sixplex Tandem Mass Tag (N-term/K +229.162932 Da; Unimod accession #737), and up to three of the variable methionine oxidation (M +15.994915 Da; Unimod accession #35) or asparagine deamidation (N +0.984016 Da; Unimod accession #7) were searched. Tryptic, semi-tryptic, and non-tryptic peptides within a 10-ppm parent mass window surrounding the candidate precursor mass were searched. Peptide ions from up to 3 isotopic peaks with fragment mass tolerance of 600 ppm were allowed. Protein inference was performed by DTASelect v.2.0,[12] requiring ≤1% global peptide false discovery rate and 2 unique peptides per protein for the protein to be considered. Modified or non-tryptic peptides were subjected to separate statistical filters to limit false discovery using the –modstat and –trypstat options; 10-plex tandem mass tag-based peptide quantification was performed at MS3 spectra using Census 2.[13] Tag intensity was normalized to bridging reporter ions and the ratios of which were log-transformed for across-sample comparison. Statistical analysis of differential expression was performed using limma (v.3.34.3) in R/Bioconductor (v.3.6).[14, 15]

Proteasome inhibition. For proteasome inhibition, 943hom iPSC-CMs were treated with Bortezomib (52 μM), Carfilzomib (280 nM), Epoxomyin (1 μM), MG132 (100μM), and DMSO (all Sigma Aldrich) for 24 and 48 hr. Samples were washed and lysed in RIPA buffer (Thermo Fisher).

Supplemental Tables

Supplemental Table S1. SpCRISPR/Cas9 genome edited lines

1073cor

Sequence	Score	Mismatches	Locus
TCTCCGGGTGACTGACGCCTGGG	2.3	3MMs [1:2:3]	chr11:-47354835
CTGCCTGGTGACTGACACCTTGG	0.7	3MMs [3:6:17]	chr6:+32939737
CACACGGATGAGTGACGCCTGAG	0.7	4MMs [2:4:8:12]	chr10:+105527026
CTGCTGGGGGACTGACCCCTCGG	0.4	4MMs [3:5:9:17]	chr1:-43199442
TTCCCGGGTCCCTGACACCTCAG	0.3	4MMs [1:10:11:17]	chr12:+7033795
CACCCAGGAGCCTGACGCCTGAG	0.3	4MMs [2:6:9:11]	chr21:-45788981
CTTTCGGGTGAGTGACCCCTCAG	0.3	4MMs [3:4:12:17]	chr8:-145058691
CCCCCGGGTGACTGAGGCCACAG	0.3	3MMs [2:16:20]	chr6:+166864497
CTCCTGGATGACTGACTCCAGGG	0.3	4MMs [5:8:17:20]	chr12:-113400758
CTCAGGGGTCACTGAGGCCTGAG	0.2	4MMs [4:5:10:16]	chr10:+102896704

943cor2

Sequence	Score	Mismatches	Locus
AGGAAGGCACACAATATGGCTGG	1.3	4MMs [1:2:4:5]	chr8:-106589093
CTCTGGGCTCACAATATGGCTAG	0.8	4MMs [1:3:4:9]	chr11:-27761712
GGGGGGGCACACAACATGGCGGG	0.8	3MMs [2:4:15]	chr8:-26370884
CAGAGCGCACACAATATGGCAGG	0.8	4MMs [1:2:4:6]	chr10:-107063716
TTTCGGGGACAAAATATGGCAGG	0.7	4MMs [1:3:8:12]	chr11:+42857939
TTGCTGGCAAACAATATGGGAGG	0.7	4MMs [1:5:10:20]	chrX:+33815597
GCGCCGCCGCACAATATGGCGAG	0.6	4MMs [2:5:7:9]	chr1:-185014679
GTGTGGCCACACAACATGGCAGG	0.5	3MMs [4:7:15]	chr8:+73118295
GTGGTGACACACAATATGGAGAG	0.5	4MMs [4:5:7:20]	chr2:+66019247

GTGAGGGGACAGAATAGGGCGGG	0.3	4MMs [4:8:12:17]	chr9:+31363107

204hom

Sequence	Score	Mismatches	Locus
CGGGGTGCTCGTCCGAGTGCAGG	0.7	4MMs [2:5:10:11]	chr9:+140400453
GCGGCTGCGGCTCCGAGTGCTGG	0.5	4MMs [1:9:10:11]	chr1:-161049375
AGGGCTTCTTTTCCGACTGCGGG	0.4	4MMs [1:2:7:17]	chr20:+61513582
CCAGCTGCTTTTCCAAGTACTGG	0.3	3MMs [3:15:19]	chr1:+19492194
CCATCTGCTTTCCCGAGTACAAG	0.2	4MMs [3:4:12:19]	chr22:+23465402
CCGGCCACTTACCCGAGTGCAGG	0.2	4MMs [6:7:11:12]	chr8:-104153270
GGGGCTGCTTTTCCAAGTACGGG	0.1	4MMs [1:2:15:19]	chr19:+18313256
CCGGGTCCTTCTCCGAGAGCCGG	0.1	4MMs [5:7:11:18]	chr20:-10654609
TCTGCTGCTTTTCCGGCTGCTGG	0.1	4MMs [1:3:16:17]	chr8:-143625569
CAGTCTGCTTTTCAGATTGCAGG	0.1	4MMs [2:4:14:17]	chr1:-24287593

Supplemental Table S2. TALEN genome edited lines

Ctrl943

PCR Primers	Amplicon (bp)	TALEN Score	Locus
Fw: CGTTCTGGGCAGAGCATTCTGG RV: GTGTCAGTGGTGACACAGCC	328	93.61	chr11:+47356661
Fw: GCTTGGAAGCTGAGTCATGCAC RV: CAGGATGGGGAAGGAGGAAAG	408	92.98	chr15:+54124229
Fw: GTGTCATCAAAGGTGGGCCTC RV: CAGTGACCTGCCCGAGTCAA	328	85.80	chr2:+217170301
Fw: CTGGGAATTAGGTGACCTTCCTG RV: CAGGGTTCCCTGCAAAGCTC	326	85.47	chr2:+113959568
Fw: GCATTCTGCAAGCTTCTGCATCTG RV: GGCCTAGAGATAGCAAACCCG	349	84.88	chr18:+5960524
Fw: CCACTCCATCTCATGGAAGGAGAA RV: TGCTGAGGACAGCAAAAGGGC	326	84.26	chr6:+73133139
Fw: CACCTCAACTGTAGGGTTACCCTT RV: GCATAAACACTGCTTCAGTTTCTCACAGTC	414	83.65	chr19:+48168294
Fw: TCTGTGGAGGGCAGAGGAAC RV: AGAGAACCGGAGCTTTGCCTTCTT	329	83.29	chr5:+1118500
Fw: CCATGCATCATGCTCTATACACATGCAG RV: GCCATTCAGGAAGGGAAGAAAGC	379	82.93	chr3:+120570976
Fw: GGACAACTGGAGGGTGAGCA RV: AAGCCAGGGACAGCCTGAAG	348	82.67	chr22:+21384891

943cor

PCR Primers	Amplicon (bp)	TALEN Score	Locus
Fw: GCTTGGAAGCTGAGTCATGCAC RV: CAGGATGGGGAAGGAGGAAAG	408	92.98	chr15:+54124229
Fw: TACACGGCCGCACCTTTCTG RV: AGCTAGGCTGGTCGTCTGATG	329	90.19	chr9:+94839652
Fw: GCAGATGAGCATAAGCATCTGACC RV: GGTTGTGACTGGCAAGGATGAAG	485	89.63	chr4:+150922237
Fw: CTCCGGCTCTAACGCTAAGAC RV: GTTGGCAAGGACAATCAGCCC	336	86.64	chr2:+217170301
Fw: GTGTCATCAAAGGTGGGCCTC RV: CAGTGACCTGCCCGAGTCAA	328	85.80	chr2:+85107237
Fw: CTGGGAATTAGGTGACCTTCCTG RV: GGCCTAGAGATAGCAAACCCG	326	85.47	chr2:+113959568
Fw: GCATTCTGCAAGCTTCTGCATCTG RV: GGCCTAGAGATAGCAAACCCG	349	84.88	chr18:+5960524
Fw: GCGGAGTTAAGAATGTCAGGGC RV: CACACCCCTCTGCTATTTGCTG	341	84.31	chr10:+97363677
Fw: CCACTCCATCTCATGGAAGGAGAA RV: TGCTGAGGACAGCAAAAGGGC	326	84.26	chr19:+48168294
Fw: CCTTTACATTTTTGCTCCCCCTGC RV: CCTTGCTAGCCAGGAGAGAG	452	83.84	chr6:+73133139

Supplemental Table S3. qPCR primer

Gene	Forward	reverse
ACTA1	AGGTCATCACCATCGGCAACGA	GCTGTTGTAGGTGGTCTCGTGA
ACTB	CACCATTGGCAATGAGCGGTTC	AGGTCTTTGCGGATGTCCACGT
ADRB1	TTCCTGCCCATCCTCATGCACT	GTAGAAGGAGACTACGGACGAG
ARID5A	TGGCAAGCAGAACGGAATCCAG	CTTGTAGAGGCTGACCAGGAAG
ATP2A2	GGACTTTGAAGGCGTGGATTGTG	CTCAGCAAGGACTGGTTTTCGG
ATP2B2	CATCCTCAACGAACTCACCTGC	GATATTGTCGCCAGTGACCATGC
CASQ1	CTGGTAGACTCTGAGAAGGATGC	GCAGAAACTCCACGATGGTGTC
CSRP3	CACTGCGAAGTTTGGAGAGTCC	AGCGGAAACAGGTCTTGTGCCA
FBXO32	CACTGGTCCAAAGAGTCGGCAA	GCACAAAGGCAGGTCAGTGAAG
FZD2	TCGTGTACCTGTTCATCGGCAC	CTGTGTAGAGCACGGAGAAGAC
FZD6	GGCAGTGTATCTGAAAGTGCGC	GATGTGGAACCTTTGAGGCTGC
GAPDH	GTCTCCTCTGACTTCAACAGCG	ACCACCCTGTTGCTGTAGCCAA
GNAO1	CTATGACCAGGTGCTCCACGAA	GGAAGAGAATGATGGAGGTATCG
HSPB1	CTGACGGTCAAGACCAAGGATG	GTGTATTTCCGCGTGAAGCACC
HSPB6	GCCACTTTTCGGTGCTGCTAGA	GCGCGACGAATCCGTGCTCAT
HSPB7	GAGACGCCTATGAGTTTGCGGT	GGCACTTGTGAGCGAAGGTGTT
IGF1	CTCTTCAGTTCGTGTGTGGAGAC	CAGCCTCCTTAGATCACAGCTC
IGF2	TGGCATCGTTGAGGAGTGCTGT	ACGGGGTATCTGGGGAAGTTGT
IL6ST	CACCCTGTATCACAGACTGGCA	TTCAGGGCTTCCTGGTCCATCA
IRS1	AGTCTGTCGTCCAGTAGCACCA	ACTGGAGCCATACTCATCCGAG
JAK2	CCAGATGGAAACTGTTCGCTCAG	GAGGTTGGTACATCAGAAACACC
MAP2K1	GGTGTTCAAGGTCTCCCACAAG	CCACGATGTACGGAGAGTTGCA

MAP3K7	CAGAGCAACTCTGCCACCAGTA	CATTTGTGGCAGGAACTTGCTCC
MDM2	TGTTTGGCGTGCCAAGCTTCTC	CACAGATGTACCTGAGTCCGATG
MYBPC3	AACCTGTCAGCCAAGCTCCACT	CCACAATGGTGTCTGGTATGCG
NPPB	TCTGGCTGCTTTGGGAGGAAGA	CCTTGTGGAATCAGAAGCAGGTG
PLCB4	CTGACCAAGAGGAGGAAGCTCA	CCTGCTCATCTTCTACAGTGACC
POLR2A	GAGAGCGTTGAGTTCCAGAACC	TGGATGTGTGCGTTGCTCAGCA
PPP3CA	GCCCTGATGAACCAACAGTTCC	GCAGGTGGTTCTTTGAATCGGTC
QRFPR	CCTGCTCATCACCTTCTTCTGC	GTCACAACAGCGGTAGACTGGA
RRAS	CTGCTGGTGTTCGCCATTAACG	GATCTGCCTTGTTCCCGACCAA
TBP	TGTATCCACAGTGAATCTTGGTTG	GGTTCGTGGCTCTCTTATCCTC
TCAP	GGAAGGATCTGACACTGTCCAC	ATGCCCATCCGCATCATCAGCC
TNFRSF12A	TCTGAGCCTGACCTTCGTGCTG	GGCACATTGTCACTGGATCAGC
TNNT2	AAGAGGCAGACTGAGCGGGAAA	AGATGCTCTGCCACAGCTCCTT
UPF1	AACGAGCACCAAGGCATTGGCT	GGCTGCTTTGATAGTGCCTTCG

Supplemental Table S4. List of significantly regulated genes. Uploaded as separate Excel File in the Online Supplement.

SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure S1: HCM patients and genome editing of iPSCs. **(A)** Patient characteristics of included HCM patients with mutations in *MYBPC3* leading to a premature termination codon (PTC). Two unrelated patients were included with a p.R943x mutation and one patient with a R1073P_Fsx4 mutation. One healthy line was used to introduce a heterozygous p.R943x mutation. **(B)** Locations of PTC mutations in *MYBPC3* DNA and protein resulting in premature stop codons (PTC). **(C)** TALEN-based strategy in the example to introduce the R.943x mutation in a healthy (ctrl) iPSC line using a PiggyBac vector system. **(D)** CRISPR/Cas9 based strategy. Guide-RNA targeted sequence is highlighted in green, the protospacer adjacent motif (PAM) is highlighted in red, and the sequence of the single-stranded DNA oligo is highlighted in yellow. **(E)** Sanger sequencing traces of ctrl iPSCs and p.R943x mutated isogenic iPSCs (ctrl943). Point mutation is highlighted in blue, and the silent mutation after PiggyBac insertion is highlighted in red. **(F)** Sanger sequencing traces of patient 1073het iPSCs and the CRISPR/Cas9 corrected iPSCs (1073cor). Het, heterozygous; hom, homozygous; cor, corrected; SCVI, Stanford Cardiovascular Institute; LVOT, left ventricular outflow tract; ICD, implantable cardioverter defibrillator; m, male; f, female; nsVT, non-sustainable ventricular tachycardia; SCD, sudden cardiac death; SAM, systolic anterior movement; PB, piggyback.

Supplemental Figure S2: Comparison of sarcomeric structures and cell size in isogenic iPSC-CMs. **(A)** Micro-patterned iPSC-CMs (ctrl, ctrl943) stained with MYBPC3 (red), alpha sarcomeric actin (αSA; green), and DAPI (scale bar = 20 μm). **(B)** Representative blots of fluorescence intensity longitudinally throughout a myofiber analyzed using plot profile (ImageJ), showing MYBPC3 expression in micro-patterned iPSC-CMs (ctrl, ctrl943). **(C)** Quantification of

sarcomeric length based on MYBPC3 expression in micro-patterned iPSC-CMs (n=5/6 cells, 15-100 sarcomeres per cell). **(D-E)** Cell size measurement of unpatterned iPSC-CMs in isogenic comparisons (n=4-6 differentiation batches). **(D)** ctrl vs. ctrl943 and **(E)** 943cor2 vs. 943het2, and 1073cor vs. 1073het (AU, arbitrary units).

Supplemental Figure S3: Evaluation of contractility in HCM iPSC-CMs. (A) Assessment of contraction using an iPSC-CM monolayer vector-based imaging approach. The relative differences of disease (ctrl943) vs healthy (ctrl) were calculated for each respective experiment (n=5). **(B)** iPSC-CMs (ctrl, ctrl943) were treated with dexamethasone (Dex), triiodothyronine (T3), and insulin-like growth factor 1 (IGF1) for 72 hr prior to contractility analysis (n=2 differentiation batches each). **(C)** Evaluation of isoproterenol (Iso), phenylephrine (PE), and endothlin-1 (ET1)/IGF1 treatment on contractility in healthy (943cor, ctrl) and HCM (943het, ctrl943) iPSC-CMs (n=2 differentiation batches, respectively). **(D)** Evaluation of the effects of 25 nM digoxin (Dig) for 3 days and 1 nM for 7 days on the contractility of healthy (943cor, ctrl) and HCM (943het, ctrl943) iPSC-CMs (n=2 differentiation batches, respectively). **(E)** Representative engineered heart tissues (EHTs) on racks in a 24-well plate. **(F)** Evaluation of force generation in μN in all isogenic lines (n=2-4 differentiation batches with 2-3 EHTs each). (* $p<0.05$; ** $p<0.01$).

Supplemental Figure S4: Evaluation of calcium handling in HCM iPSC-CMs. (A,C,E) Fura2 calcium indicator ratiometric analysis of calcium handling in iPSC-CMs of **(A)** the healthy (ctrl) and the isogenic introduced iPSC line (ctrl943), **(C)** iPSC-CMs of the second patient with a p.R943x mutation (943het2) and corrected (943cor2), and **(E)** iPSC-CMs of the patient with a p.R1073P_Fsx4 mutation (1073het) and corrected (1073cor). (**B,D,F**) mRNA expression of

ATP2A2. (**B**) ctrl and ctrl943, (**D**) 943cor2 and 943het2, and (**F**) 1073cor and 1073het (n=70-160 cells from n=2-4 differentiation batches each; * $p<0.05$; ** $p<0.01$).

Supplemental Figure S5: MYBPC3 expression in HCM iPSC-CMs. (A,C) Total *MYBPC3* mRNA expression evaluated by qPCR (n=4 differentiation batches each). (**B,D)** Allelic expression of mutant mRNA (mut.) and wild type (WT) within total *MYBPC3* mRNA in HCM iPSC-CMs analyzed using **(B)** RNA-seq (ctrl943) or **(D)** Droplet Digital PCR (943het2 and 1073het; fractions are presented in % of total *MYBPC3* mRNA, n=3 differentiation batches each). **(E,F)** Western blot analysis of MYBPC3 protein levels and quantification of MYBPC3 protein expression normalized to alpha sarcomeric actin (αSA) in isogenic iPSC-CMs. **(E)** ctrl vs. ctrl943. **(F)** 943cor2 vs. 943het2 and 1073cor vs. 1073het (# $p<0.1$; * $p<0.05$; ** $p<0.01$).

Supplemental Figure S6: MYBPC3 expression in HCM iPSC-CMs. (A-C) Western blot analysis of MYBPC3 protein levels and quantification of MYBPC3 protein expression normalized to GAPDH in isogenic iPSC-CMs comparing **(A)** 943cor, 943het and 943hom, **(B)** ctrl and ctrl943, and **(C)** 1073cor and 1073het. **(D)** Western blot analysis of EHTs from all isogenic lines and quantification of MYBPC3 (MYBPC3 protein expression normalized to α-sarcomeric actin; αSA).

Supplemental Figure S7: Detection of truncated MYBPC3 peptides. (A) Distribution of MYBPC3 peptides along the full-length protein in mass spectrometry and ratios of MYBPC3 peptides before and after the point of mutation in isogenic comparisons (left panel: 943cor vs. 943het; right panel: ctrl vs. ctrl943; n=4 differentiation batches each). **(B)** Detection of full length (150 kDa) and truncated (125 kDa) MYBPC3 in lysates of HEK293 cells transfected with healthy

and mutated (p.R943x) cDNA vectors with the Abcam MYBPC3 antibody (protein lysate from 943het iPSC-CMs served as control). **(C)** Treatment of homozygous (943hom) iPSC-CMs with different proteasome inhibitors for 24 and 48 hr. Blot is stained with a mono-/polyubiquitin antibody. **(D)** Western blot of samples after proteasome inhibition in 943hom iPSC-CMs (protein lysate from 943cor iPSC-CMs serving as control). **(E)** Representative DMSO and proteasome inhibitor treated 943hom iPSC-CM lysates evaluated on a nanofluidic-immuno assay (NIA) with diluted 943corr iPSC-CM-derived protein lysate spiked in for full length MYBPC3. Full length MYBPC3 is detected at around 158 kDa and predicted truncated MYBPC3 is detectable at 125 kDa after proteasome inhibition with Carfilzomib and Epoxomycin. **(F)** Samples without full length MYBPC3 spiked in. An unspecific peak is detectable at 146 kDa and predicted truncated MYBPC3 is detectable at around 125 kDa after proteasome inhibition with Bortezomib, Carfilzomib, and Epoxomycin. **(G)** Western blot analysis of 943cor and 943het (n=1, and n=3 differentiation batches, respectively, top panel), and 943hom (n=3; 2 untreated 943cor lysates serving as control for antibody staining of the membrane, bottom panel) after NMD inhibition. Quantification of MYBPC3 protein expression normalized to αSA in 943het iPSC-CMs (αSA, α-sarcomeric actin; siSCR, siRNA scramble control; siUPF1, siRNA against UPF1).

Supplemental Figure S8: Distribution of dysregulated genes in HCM iPSC-CMs. (A) Principal component analysis (PCA) of RNA-sequencing samples (ctrl, ctrl943, 943cor, 943het). **(B)** Significantly differentially up- and down-regulated pathways in HCM (ctrl943, 943het) vs. health (ctrl, 943cor) iPSC-CMs identified with Ingenuity Pathway Analysis (QIAGEN Bioinformatics) ranked by the individual z-score.

Supplemental Figure S9: Distribution of dysregulated genes in HCM iPSC-CMs. (A) Simplified scheme of the respective genes identified as gene signature involved in various cardiac signaling pathways based on literature search. Genes upregulated in HCM (ctrl943, 943het) vs. health (ctrl, 943cor) are presented in blue, genes downregulated in HCM (ctrl943, 943het) vs. health (ctrl, 943cor) are presented in red. **(B)** Hierarchical clustering of the relative expression of the gene signature in 1073cor vs. 1073het iPSC-CMs (expression analysis by qPCR; n=3 differentiation batches, respectively). **(C)** Correlation of the gene signatures of the isogenic pair generated from the patient line with the p.R1073P_Fsx4 mutation (1073cor vs. 1073het) and p.R943x mutated iPSC-CMs (943cor vs. 943het, ctrl vs. ctrl93; n=3 differentiation batches, respectively; $r=0.88$; $p<0.001$).

Supplemental Figure S10: NMD inhibition. (A) mRNA expression of *UPF1* and *MYBPC3* in 943het iPSC-CMs treated with siUPF1 vs. siSCR (scramble control; n=3). **(B)** Correlation of the gene signature after siUPF1 mediated NMD inhibition in 943cor iPSC-CMs and the gene signature in 943het iPSC-CMs (n=2-3 differentiation batches, respectively). **(C)** Ratiometric analysis of calcium handling after NMD inhibition in 943cor iPSC-CMs vs. controls (n=100-120 cells in 3 differentiation batches, respectively; siSCR – siRNA scramble control; siUPF1 – siRNA against UPF1; * $p<0.05$).

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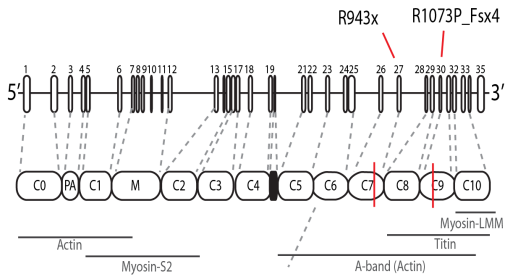
Figures

Supplemental Figure S1

A

Line Genotype	943het +/-	943cor +/+	943hom -/-	1073het +/-	1073cor +/+	943het2 +/-	943cor2 +/+	ctrl +/+	ctrl943 +/-
SCVI Biobank ID -Genome editing	204	204 corrected	204 homo- zygous	373	373 corrected	502	502 corrected	113	113- mutated (p.R943x)
gender	m			m		f		m	
Variant	p.Arg943x; c.2827C>T			p.Arg1073 ProFsx4, c.3217dupC		p.Arg943x; c.2827C>T		healthy	p.Arg943x; c.2827C>T
Family history	+++			(+)		++		-	
Hypertrophy	23 mm			20 mm		15 mm		-	
LVOT gradient	+			(+)		SAM		-	
Arrhythmia	-			nsVT		SCD		-	
ICD	+			+		+ (2x)		-	
Syncope	(2)			-		+++		-	
MRI abnorm	+			+		+		-	
symptomatic	-			-		Chest pain		-	

B



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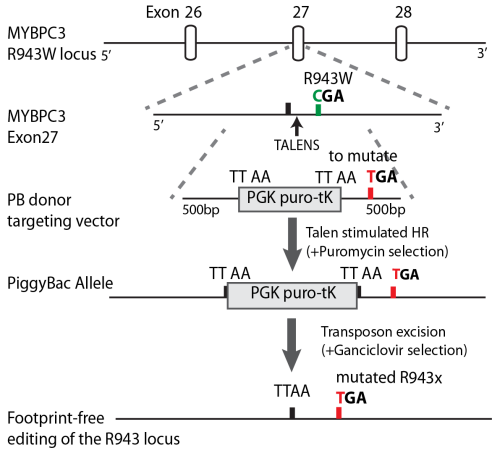

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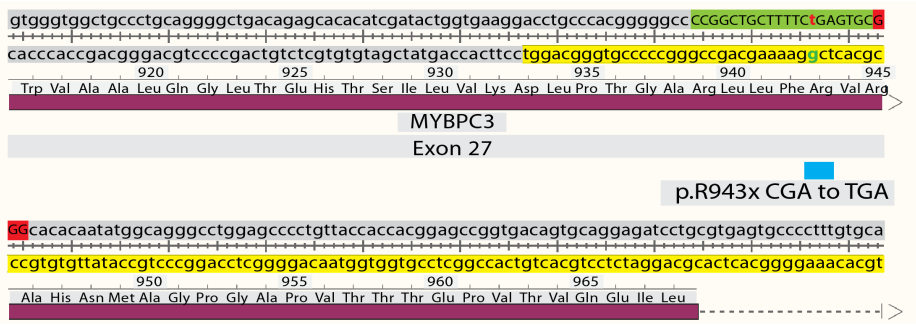

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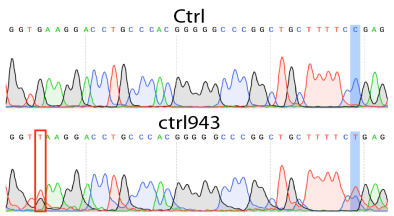

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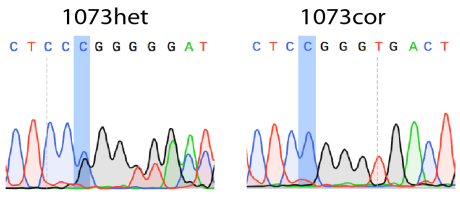

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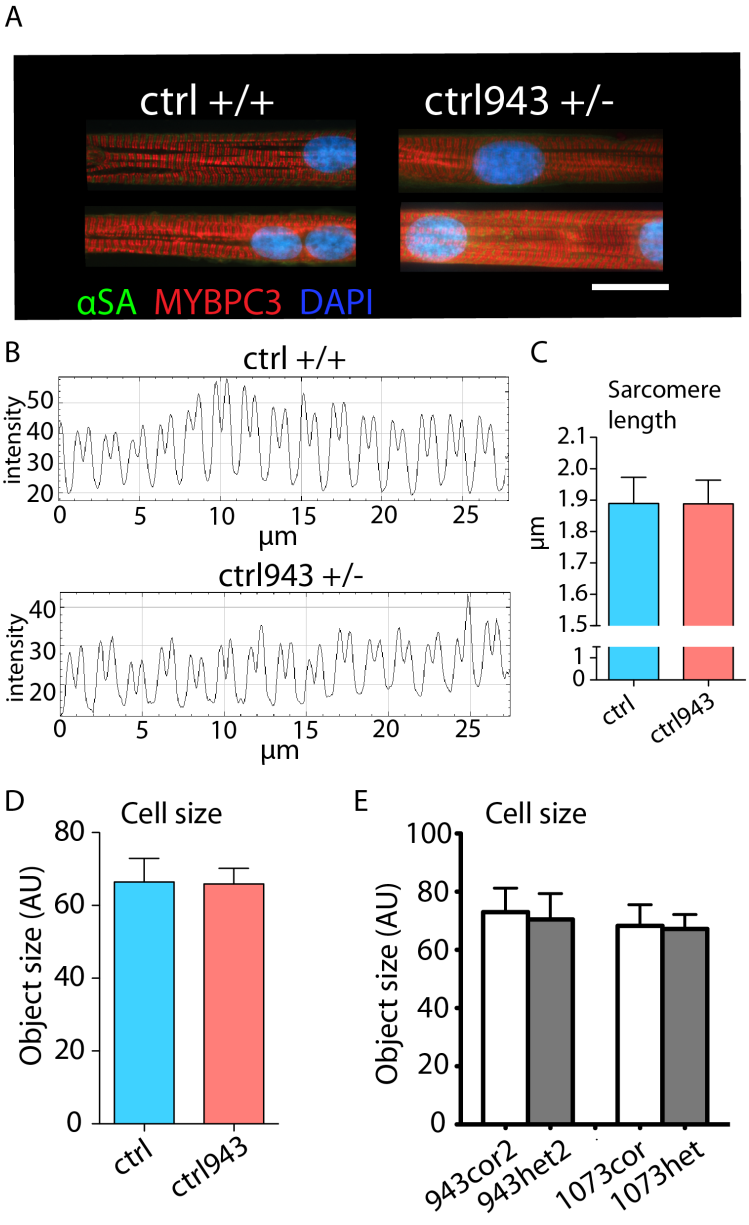

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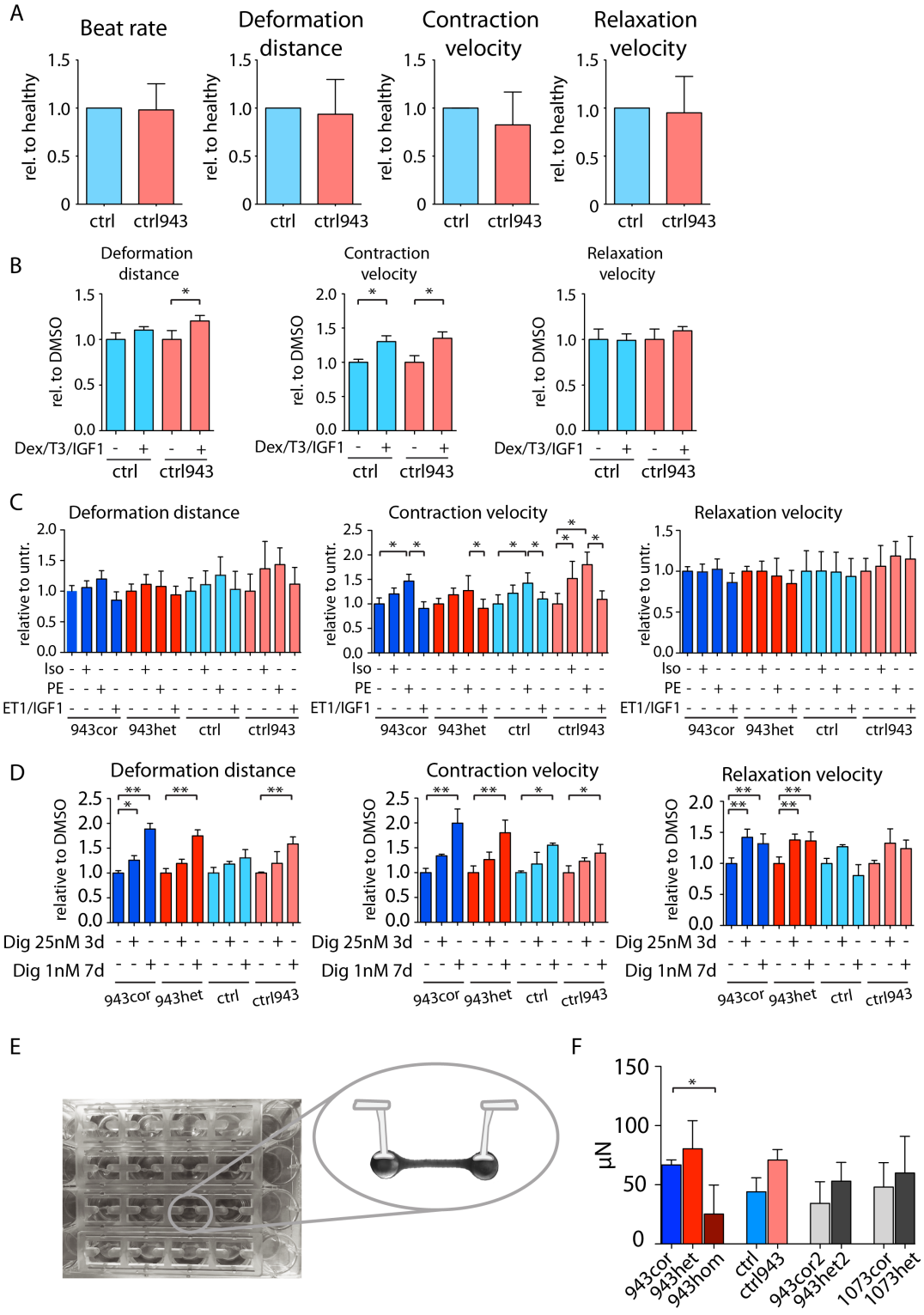

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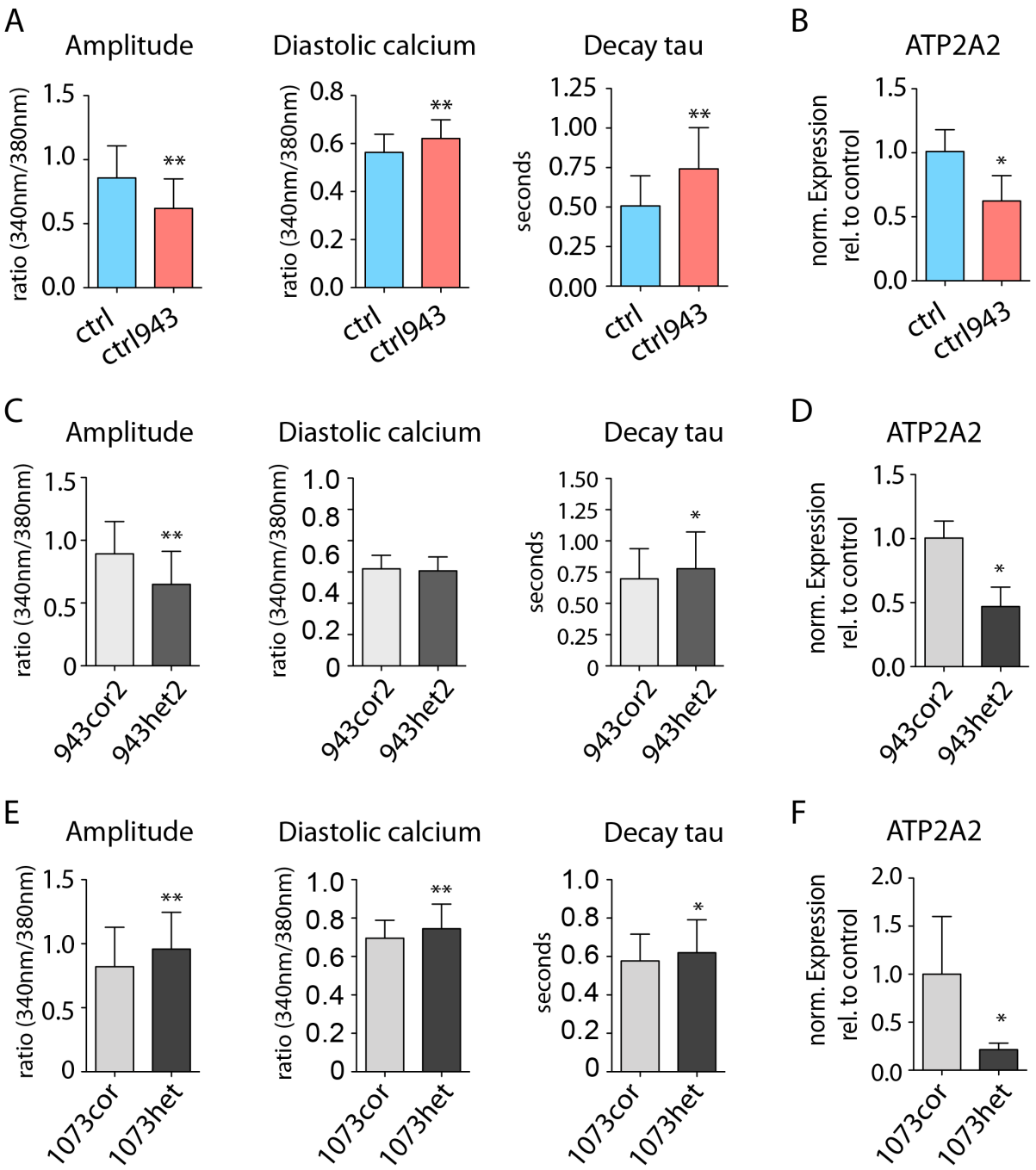

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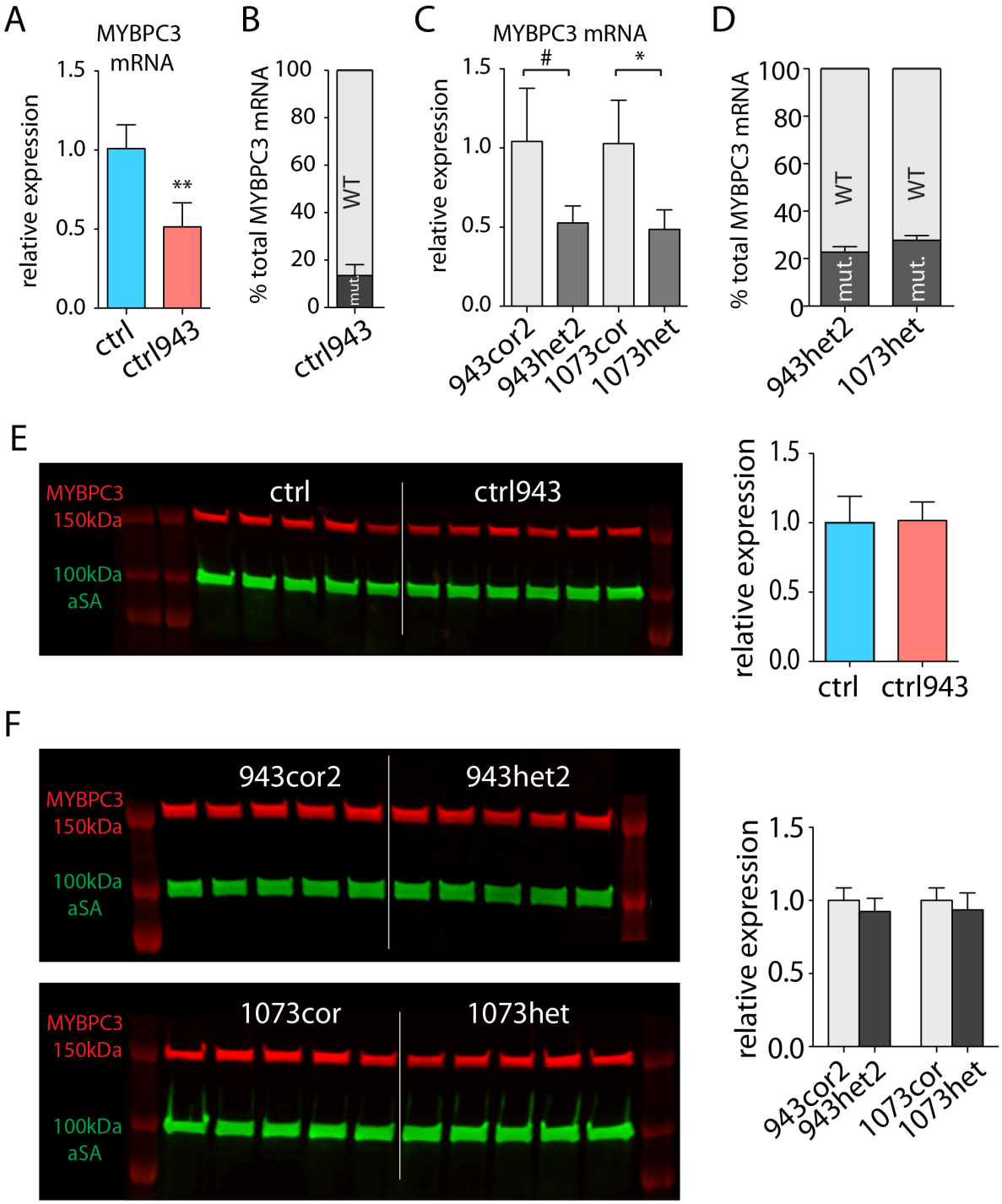
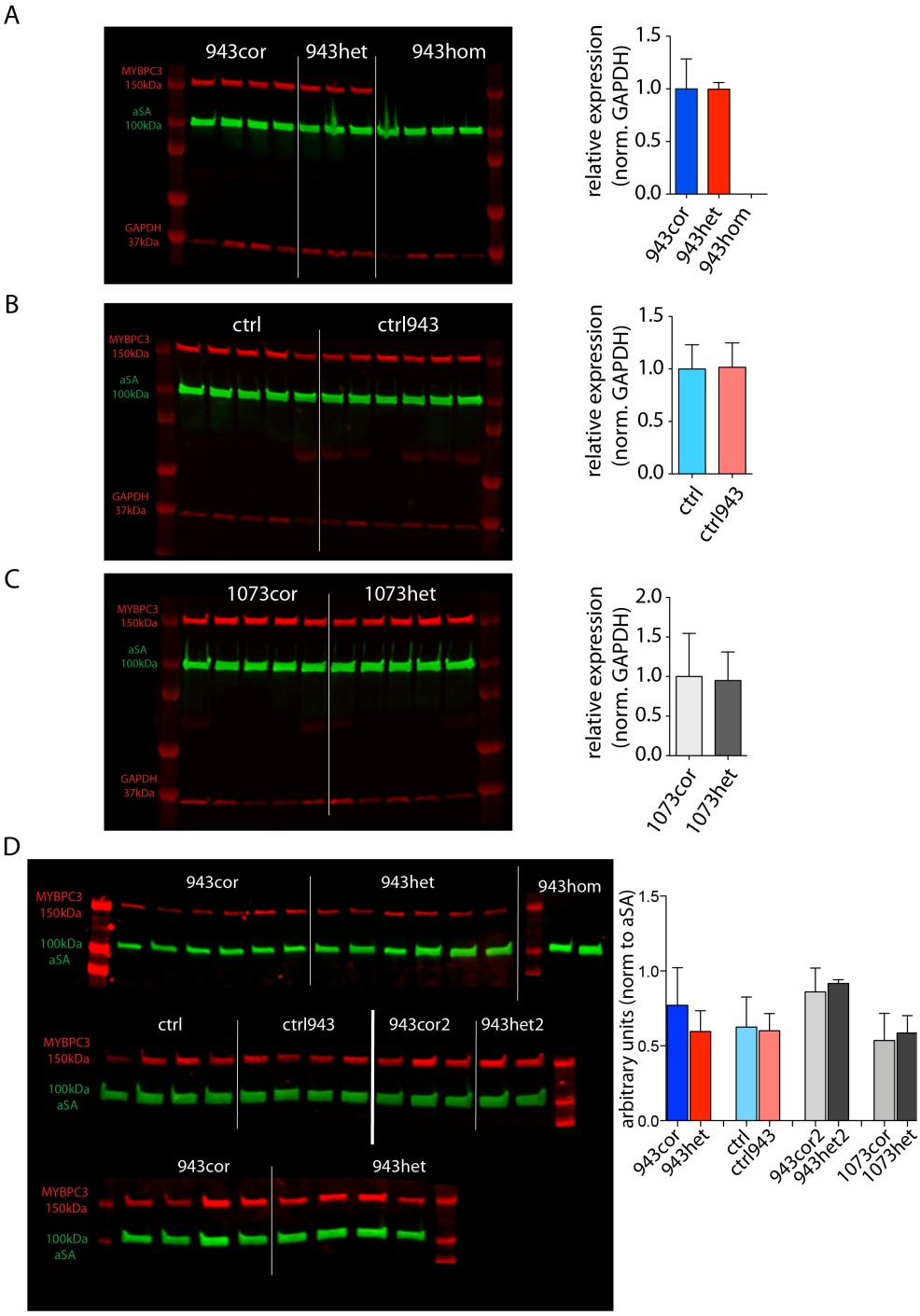


Supplemental Figure S6



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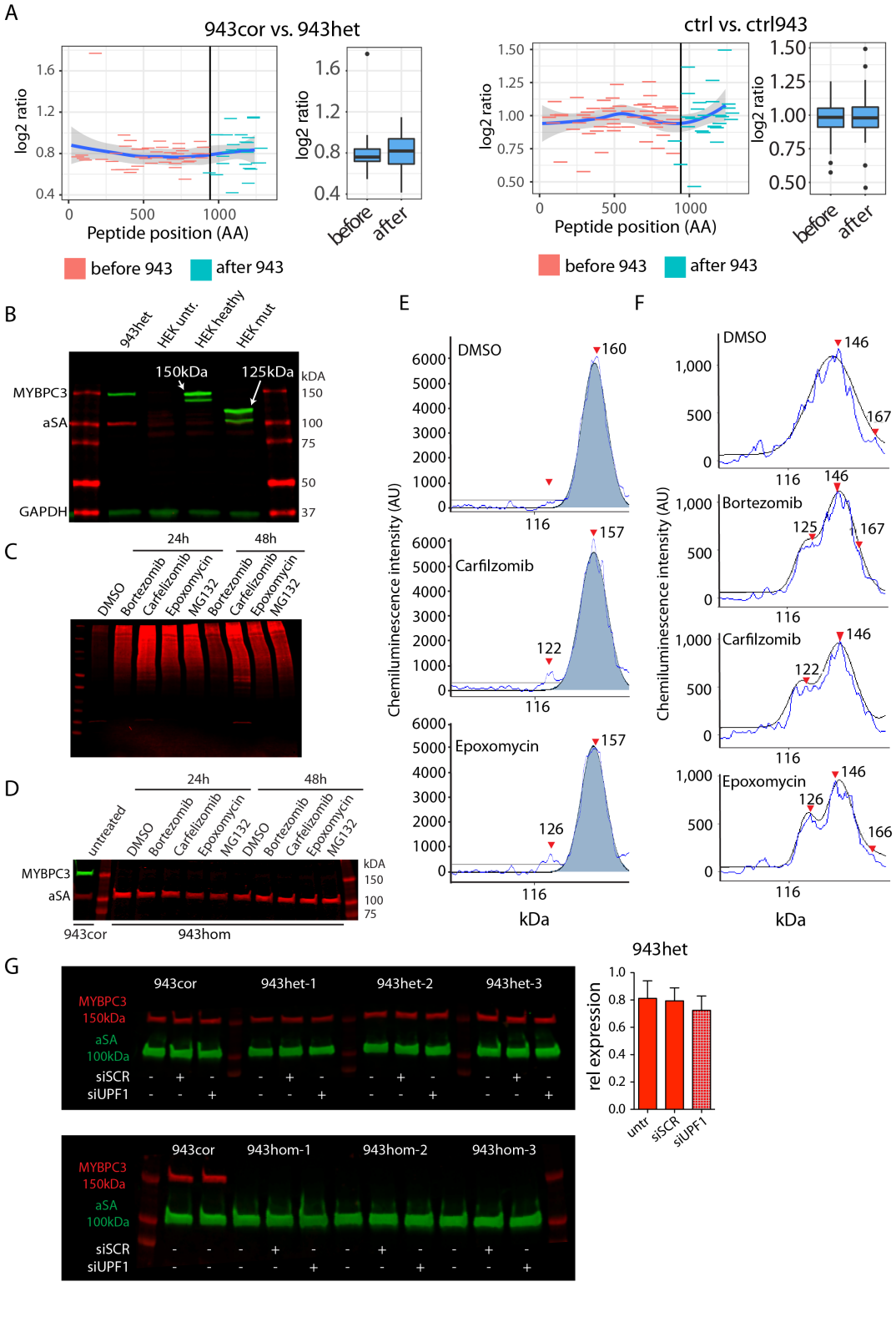

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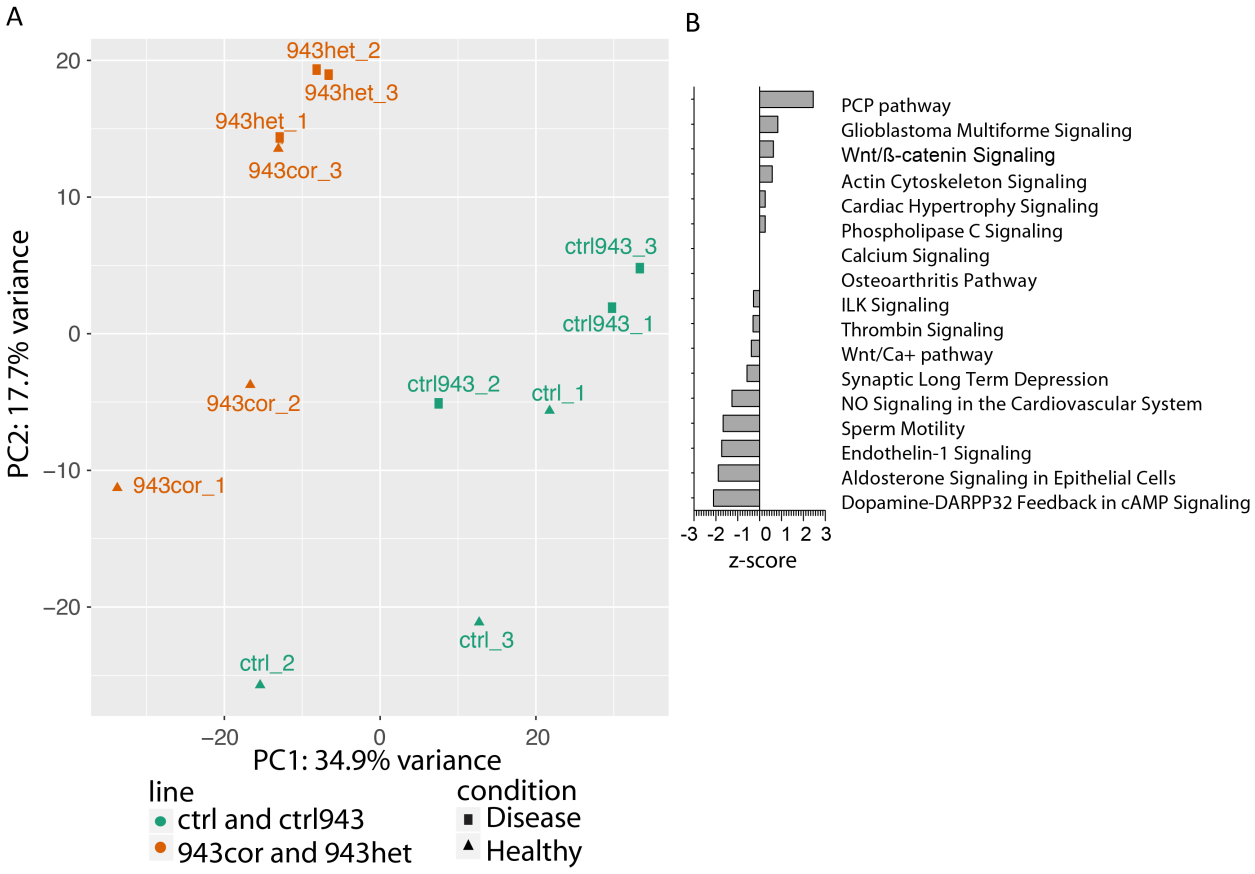
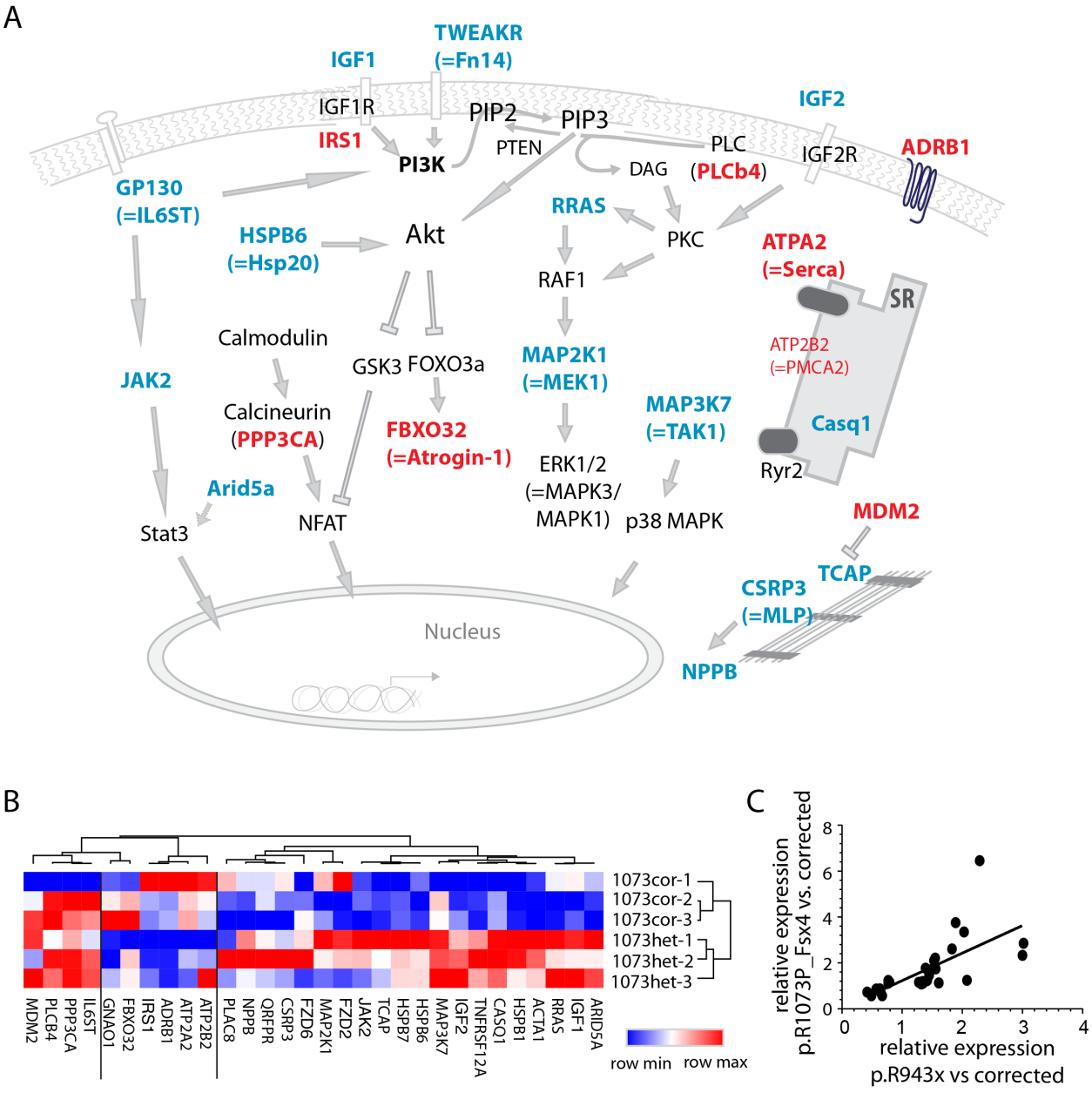


Supplemental Figure S9



Supplemental Figure S10