

Supplementary Materials for

RNA-Binding-Motif 20-variants disrupt Ca²⁺-handling and metabolism in dilated and non-compaction cardiomyopathy stem cell models

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Supplementary Text

Material and methods

Cell staining

Fixation of the cells for IF and FLOW

IPSC or iPSC-CM were plated onto Geltrex-coated coverslips or 8-chambered coverslip system (Cellvis). For fixation, the cells were washed once with 1xPBS and incubated 20 min with 4 % Histofix solution (Roth). After Histofix was discarded, the cells were washed with 1x PBS and 1-2 mL Blocking solution (1% BSA in 1x PBS) was added. The cells were stored in Blocking solution at 4 °C until usage (up to 2 months).

For FLOW, one well with iPSC-CM was detached using 0.25 % trypsin/EDTA for 10 min at 37 °C, stopped with FCS (1:1) and transferred to a FCS centrifugation tube (all centrifugation steps at 200 g for 5 min). Cell pellets were washed once in 1x PBS and subsequently stored in Blocking buffer at 4 °C for up to one month.

Antibody stainings for IF and FLOW

For IF stainings, primary and secondary antibodies were diluted in Staining solution (0.1 % triton-x in 1 % BSA/PBS). Dilutions and all antibodies used are listed in Supplementary table 3. The secondary antibody dilutions ranged between 1:500 to 1:1000.

For FLOW- cTNT staining, the cells were incubated over night at 4 °C with the primary antibody cTNT in a 1:500 dilution in FACS buffer (1 % BSA/PBS with 0.1 % Triton-X). The next day, the cells were washed thrice with FACS buffer and subsequently incubated with the secondary antibody in a 1:1000 dilution in FACS buffer (Supplementary table 2). The cells were washed thrice again in FACS buffer und diluted in 200 µL 1x PBS for measurements using a FACS Canto II with the following settings: 10000 events, forward scatter 228 V, side scatter 440 V, 488 Alexa Fluor laser 390 V. The detection threshold was set according to the blank sample and cTNT positive cells were presented as cTNT positive cells in [%]. Cardiac differentiations with >85% positive for cardiac troponin T were used for further analysis.

For CM analysis of dissolved EHM, the fixed cells were centrifuged at 300 g, 5 min, 4° C and the ethanol supernatant aspirated. The cell pellet was resuspended in 0.5 mL blocking buffer and distributed into a U-shaped 96-multi well plate with one well used for an unstained IgG control and a second well for the antibody staining for each EHM sample. The antibody staining was sequentially performed in blocking buffer with an unspecific IgG or sarcomeric α -actinin primary antibody, followed by a double wash with PBS^{-/-} and a secondary antibody staining with anti-IgG and Hoechst 33342, each at RT for 1 h. The cell populations were analysed for viability and singularization, then assessed for the stained protein of interest using the BD LSR II flow cytometer and the FACSDiva software (BD Biosciences).

Gene analysis

RNA Isolation and cDNA synthesis

Total RNA was isolated as described in the SV Total RNA Isolation System (Promega) according to the manufacturer's instructions for "RNA Isolation from fibrous tissue". The DNase-digestion step was expanded to 30 min at RT. 100-300 ng of RNA was used in first-strand cDNA synthesis using the iScript cDNA Synthesis Kit (BioRad).

Quantitative PCR

Quantitative PCR was performed using 1x iQ™ SYBR® Green Supermix (Bio-Rad) with 5 ng cDNA and 500 nM primers (each forward and reverse) and run on the CFX real-time PCR detection system (Bio-Rad). Primers used are listed in Supplementary table 4.

Sequencing

Sanger Sequencing

To sequence the RBM20-exon 9 locus, gDNA was isolated using the QIAamp DNA Mini Kit (Qiagen) following the manufacturer's instructions. The gDNA was measured using a Nanodrop and adjusted to a value between 100 – 200 ng/μL. To amplify the exon 9 locus, a PCR was conducted with 2 μL cDNA in a PCR mix consisting of 1 μL forward and reverse primer (10 μM, Supplementary table 3), 5 μL Green buffer (5x) (Promega), 1.6 μL dNTP mix (10 mM) (Bioline), 0.1 μL Tag-polymerase (Promega) and 14.3 μL water (end volume 25 μL). The PCR was run with 56 °C annealing temperature with 30 cycles. The PCR product was sent for Sanger sequencing to Microsynth Seqlab (Göttingen).

Illumina Sequencing

RNA sequencing using poly(A) enrichment of the mRNA was performed for a total of 16 samples, comprising 3 LVNC, 5 resLVNC, 3 DCM1, and 5 resDCM1 samples. A median read depth of 76.52 million was achieved per pair-ended sequencing data, with a read length of 75 base pair. The reads were subjected to a quality check using the tool fastp^{1,2}. The reads were then aligned to human genome GRCh38 using the tool Subread^{3,4} using the splice-aware option. BAM files were then used as an input to the *featureCounts* tool of the Subread package, to count the number of reads aligning to the gene features as defined in NCBI RefSeq annotation for hg38 (build 38.2). For the counting, only uniquely mapping fragments were considered. Read counts were imported in R package *DESeq2*⁵ for normalization of sequencing depth and analysis of the differentially expressed genes between groups. Wald tests were used to calculate log2 fold changes and associated p-values, which were adjusted for multiple testing using the Benjamini-Hochberg procedure. Differentially expressed genes with an adjusted p-value < 0.05 were considered significant. DEXSeq was used for the analysis of differential exon usage, focusing on variations in exon-level expression patterns between groups. The exon counts were normalized, and generalized linear models (GLMs) were employed to test for significant differences in exon usage across conditions⁶. To account for hidden batch effects, Surrogate Variable Analysis (SVA) was performed. The normalized counts for a total of 28,395 genes are visualized as a volcano plot. Gene Ontology (GO) term enrichment analysis was performed on the differentially expressed genes identified through DESeq2 using Cytoscape with ClueGo plugin (settings: Ontologie KEGG, pathway with p-value < 0.05).

Karyotyping

Karyotyping was performed at Life & Brain (Bonn, Germany) via array-based genome-wide genotyping utilizing the Illumina BeadArray. Data was analyzed in GenomeStudio v2.0 (Illumina) with the cnvPartition 3.2.0 plug-in.

Western Blot

Protein lysis

Cell pellets were lysed in lysis buffer (20 mM Tris-HCl [pH 7.4], 200 mM NaCl, 20 mM NaF, 1 % Igepal, 1mM Na₃VO₄, 1mM DTT, 1x PhosStop, 1x cOmplete, EDTA-free protease inhibitor). The solution was incubated 30 min on ice, and subsequently centrifuged and the supernatant collected. Protein concentration was determined with the Pierce BCA protein assay kit according to manufacturer's instructions.

SDS-electrophoresis, blotting and imaging

20 µg protein were denatured in Laemmli buffer at 37 °C for 30 min and separated in SDS-polyacrylamide gels (4-15 % Mini-Protean precast Gel; BioRad) with constant 15-20 mA/gel. Next, the proteins were transferred to either 0.45 µm nitrocellulose membrane (RBM20) or methanol activated 0.45 µm PVDF (PLN, PLN-S16p, PLN-T17p) membranes using a semi-dry transfer system (BioRad). Membranes were blocked in 5 % milk or 5 % BSA depending on the antibody (Supplementary table 2). Primary antibodies were diluted in 1 % milk or 1 % BSA (Supplementary table 2) and incubated over night at 4 °C. HRP-coupled secondary antibodies (Supplementary table 2) were incubated for 1 h at room temperature. For imaging with the ChemiDoc XRS+ imaging system (BioRad), the membranes were incubated for 5 min with Immobilon chemiluminescent HRP substrate (Millipore) and imaged with increasing exposure time.

Microscopy

For morphology and ALP stainings, the Axio Observer. A1 (Zeiss) was used. Immunofluorescence stainings were imaged with Axio Observer. Z1 (Zeiss) or the LAS X THUNDER (Leica). For live cell imaging of FURA-2-AM calcium imaging, an Olympus Motoc AE31 microscope was used with an IonOptix epifluorescence acquisition system. For live cell imaging of Fluo-4-AM calcium imaging, the LSM710 confocal system (Zeiss) was used. For live cell imaging of TMRM/Mitotracker measurements the LAS X SP5 (Leica) was used.

dSTORM

7000 iPSC-CM were plated onto an eight-well chamber coverslip system (Cellvis). Cells were fixated 7 days later and stained with 1:500 RBM20 primary antibody and 1:500 Alexa-647 secondary antibody and a nucleus staining (DAPI). Instead of mounting solution, 500 µL 1x PBS was added to each well. The dSTORM imaging was performed using an Olympus IX-71 inverted microscope equipped with an APON 60xOTIRF (NA 1.49) oil immersion objective and a nosepiece stage (IX2-NPS, Olympus). Alexa Fluor 647 was excited using a 639 nm laser (Genesis MX639-1000, Coherent) at an intensity of approximately 2 kW/cm² to induce photoswitching. The imaging buffer consisted of PBS supplemented with 100 mM cysteamine hydrochloride (Sigma-Aldrich), pH 7.4, 4 % glucose, 8 units/ml glucose oxidase and 160 units/ml catalase. The excitation light

was filtered through a 642/10 nm bandpass filter (Semrock) and focused on the back focal plane of the objective. A lens system and mirror, positioned on a linear translation stage, enabled switching between different illumination modes (EPI, HILO, and TIRF). Fluorescence emission was collected by the same objective and transmitted through a beam splitter (FF410/504/582/669-Di01, Semrock). The emitted light was further filtered using a bandpass filter (Brightline HC 679/41, Semrock) and projected onto an electron-multiplying CCD camera (iXon Ultra 897, Andor). Additional lenses in the detection path resulted in a final pixel size of 128.9 nm. A total of 15,000 frames were acquired at a frame rate of 67 Hz (15 ms exposure time). Image reconstruction was performed using rapidSTORM 3.3⁷, applying a threshold to exclude signals with fewer than 700 photons from the localization data. Further analysis was conducted using the software LOCAN⁸. To investigate RBM20 density within the nucleus, a region of interest (ROI) encompassing the nucleus was selected for each dSTORM image. For analyzing RBM20 in the cytoplasm, an extended region around the nuclear ROI, with a distance of 2 μm , was chosen. For cluster analysis the localizations within these ROIs were grouped using a DBSCAN (Density-Based Spatial Clustering of Applications with Noise)⁹ algorithm with parameters set to $\epsilon = 20$ and $\text{minPoints} = 3$ ¹⁰. A cluster was defined as a group of localizations with a minimum of three localizations, originating from one or a few secondary antibodies. Cluster densities in the nucleus and cytoplasm, as well as the ratio of these two densities (nucleus density : cytoplasm density) per cell were calculated.

Electron microscopy

3D spheroids were washed once with 1 $\times$ PBS and fixed overnight at 4 °C in isosmotic Karnovsky's fixative. On the following day, the fixative was replaced with 0.1 M cacodylate buffer (pH 7.4). Samples were embedded in 4% low melting agarose then stained with 1% osmium tetroxide and 1% uranyl acetate, dehydrated through a graded ethanol series, followed by acetone, and embedded in Durcupan resin using a laboratory microwave. Ultrathin sections (70 nm) were mounted on copper grids, post-stained with 3% lead citrate, and imaged using a Talos L120C transmission electron microscope (Thermo Scientific) operated at 120 kV and equipped with a 4 K $\times$ 4 K Ceta CMOS camera (Thermo Scientific). The analysis and visualization were performed in IMOD software.

Live cell imaging

Calcium kinetics with Fluo-4-AM

2.5 – 3 $\times 10^5$ iPSC-CM were plated onto Geltrex-coated 24 mm round coverslips and imaged 7-9 days later. Cells were loaded with 1.5 mL Tyrode solution (140 mM NaCl, 5.4 mM KCl, 1 mM MgCl_2 , 1.8 mM CaCl_2 , 10 mM Na-HEPES, and 10 mM glucose, pH=7.4) containing 2.5 μM Fluo-4-AM and 0.025 % Pluronic (both from Thermo Fischer Scientific). Image acquisition was performed at RT with a Zeiss LSM 710 confocal microscope and a 63x 1.4 oil objective in line scan mode (512 pixels, 45.5 μm (zoom factor 3), 1057.7 Hz, 12 bit and 20,000 cycles at maximal speed). The cells were continually paced at 0.25 Hz by placing a customized electrode into the measuring set-up. Calcium analysis was done as described by us previously for calcium kinetics and spark analysis with ImageJ Sparkmaster plugin¹¹. For the spark analysis the 3D (width*height*amplitude) sum of a defined area during diastole was calculated. Drug treatments were conducted by directly

diluting the compound into the measuring solution with the appropriate concentrations of Iso (1 μ M), verapamil (30 nM) and metoprolol (5 μ M). The drugs were incubated for 15 min before the subsequent measurement.

Calcium content with FURA-2-AM

3 – 4x10⁴ iPSC-CMs were digested onto Geltrex – coated 35 mm round Fluoro dishes (World Precision Instruments) and imaged 7-9 days later. Cells were loaded with 5 μ M FURA-2-AM dye was dissolved in 1 ml Tyrode solution (140 mM NaCl, 5.4 mM KCl, 1 mM MgCl₂, 1.25 mM CaCl₂, 10 mM Na-HEPES, and 10 mM glucose, pH=7.4) for 15 min at 37 °C followed by incubation with Tyrode solution for 15 min at 37 °C. Finally, the Tyrode solution was refreshed before measuring the cells at RT. The FURA-2 loaded cells were excited at 340 and 380 nm and the emissions were measured at 510 nm. Data is shown as ratios of 340/380 nm with subtracted background values. For every dish, 3-4 single cells were measured by recording Ca²⁺ transients with increased pacing: 0.25, 0.5, and 1.0 Hz. The parameters were analyzed using the IonOptix software. For AIP treatment, 1 μ M AIP was directly diluted into the measuring solution after the basal measurements and incubated for 15 min.

Mitochondrial calcium with mtPericam

For mitochondrial calcium measurements, 3x10⁵ iPSC-CM were seeded on 35 mm dishes (MatTek) and transfected 5 days later with ratiometric Pericam Mt3.1 (mtPericam)¹² with the Lipofectamine LTX kit (Thermo Fisher) mixing 2 μ g mtPericam-plasmid, 300 μ L OptiMEM, 12 μ L lipofectamine LTX reagent and 1 μ g lipofectamine PLUS reagent. This mixture was incubated 5 min at RT and subsequently added to the iPSC-CM in 1.7 mL Cardio Culture medium supplemented with 1x Pen/strep. After 6 h, at 37 °C medium was exchanged for Cardio Culture medium. 48 h after transfection, the iPSC-CM were imaged using the SP8 confocal microscope (Leica). To conduct measurements, the medium was exchanged to Tyrode solution (140 mM NaCl, 5.4 mM KCl, 1 mM MgCl₂, 1.8 mM CaCl₂, 10 mM Na-HEPES, and 10 mM glucose, pH=7.4) and the cells were paced at 0.5 Hz. Ca²⁺-bound and Ca²⁺-unbound mtPericam was visualized with excitations of 488 nm (14%) and 405 nm (8%), respectively, in a sequential scan and emissions were captured with photomultiplier tubes set to 500-550 nm. Images were analyzed using ImageJ determining the mean pixel intensity for each picture. Afterwards, the ratio for the mean intensities were determined for Ca²⁺-bound divided by the Ca²⁺-unbound values.

TMRM measurement

2.5 – 3 x 10⁵ iPSC-CM were plated onto Geltrex-coated 24 mm round coverslips and imaged 7-9 days later. Cells were stained with 2.5 nM TMRM (Thermo Fisher Scientific) and 100 nM Mitotracker Green (Thermo Fisher Scientific) in Cardio Culture medium for 1 h at 37 °C. After 1 h, the solution was replaced with Cardio culture medium with 2.5 nM TMRM and images were acquired on the confocal system SP5 from Leica. For analysis, the mean value pixel intensity for the TMRM and Mitotracker channel were calculated using ImageJ and subsequently the ratio of TMRM/Mitotracker was used for quantification.

Contractility measurements

Contractility measurements were performed using the IonOptix Calcium and Contractility System in combination with an inverted microscope equipped with a 40x objective. Contractility as a parameter for systolic function was quantified with pixel intensity and pixel correlation algorithms, which detect changes in image intensity patterns (from a brightfield image) within a defined region of interest over time relative to a reference frame. Seven days prior to the experiments, 3 – 5 spheroids were digested and seeded onto Geltrex-coated 35 mm dishes (MatTek). Measurements were recorded in IonWizard 6.3 software at 34 – 36 °C, analyzing 10-20 cells at a pacing frequency of 0.5 Hz. Cells were sequentially perfused with Tyrode solutions (140 mM NaCl, 5.4 mM KCl, 1 mM MgCl₂, 10 mM HEPES, 10 mM glucose, pH=7.4) containing increasing CaCl₂ concentrations (0.6 mM, 1.2 mM and 1.8 mM) with 5 min intervals between each step to ensure complete solution exchange and contractility was recorded for 17 s per cell. Data analysis to calculate the contractility peak height was performed using CytoSolver 2.2.2 and Microsoft Excel.

Fig. S1

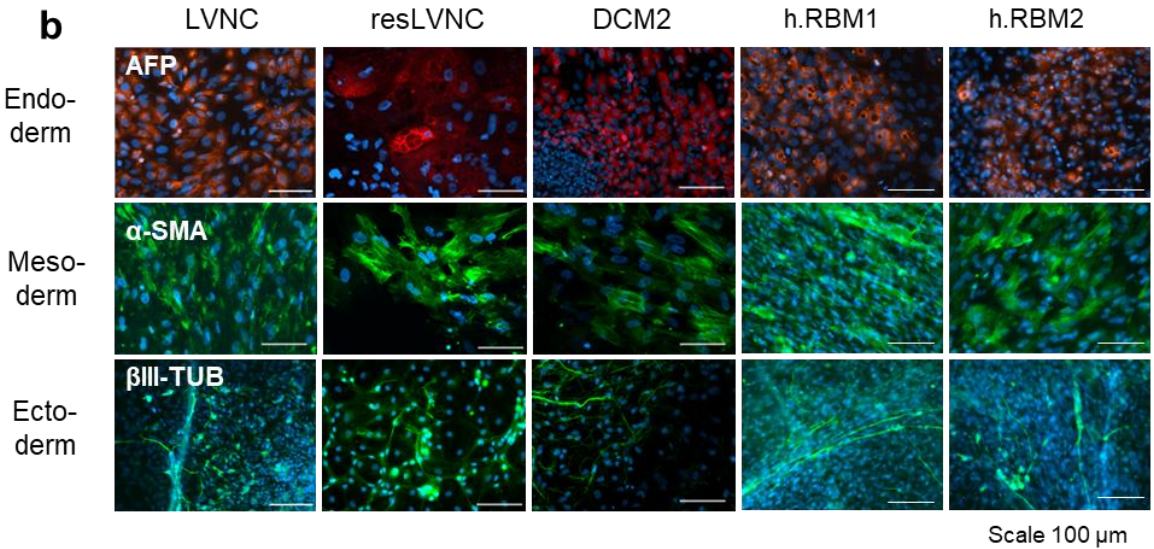
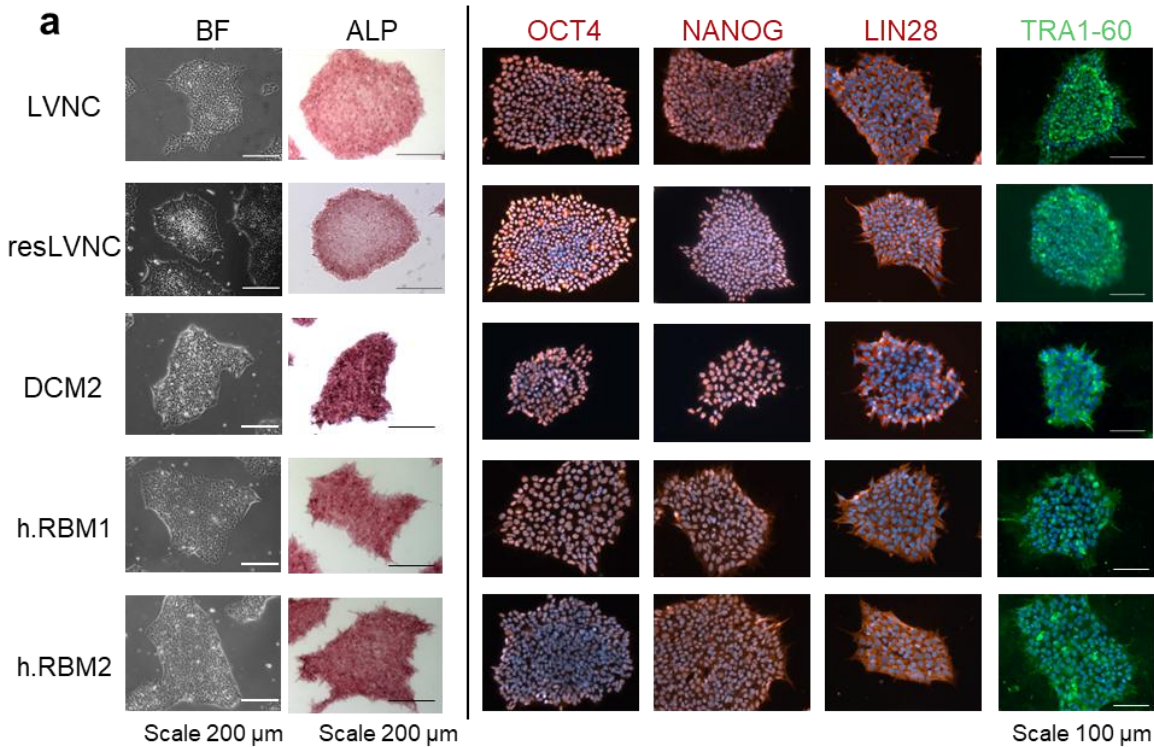


Fig. S1: All iPSC lines show full pluripotency.

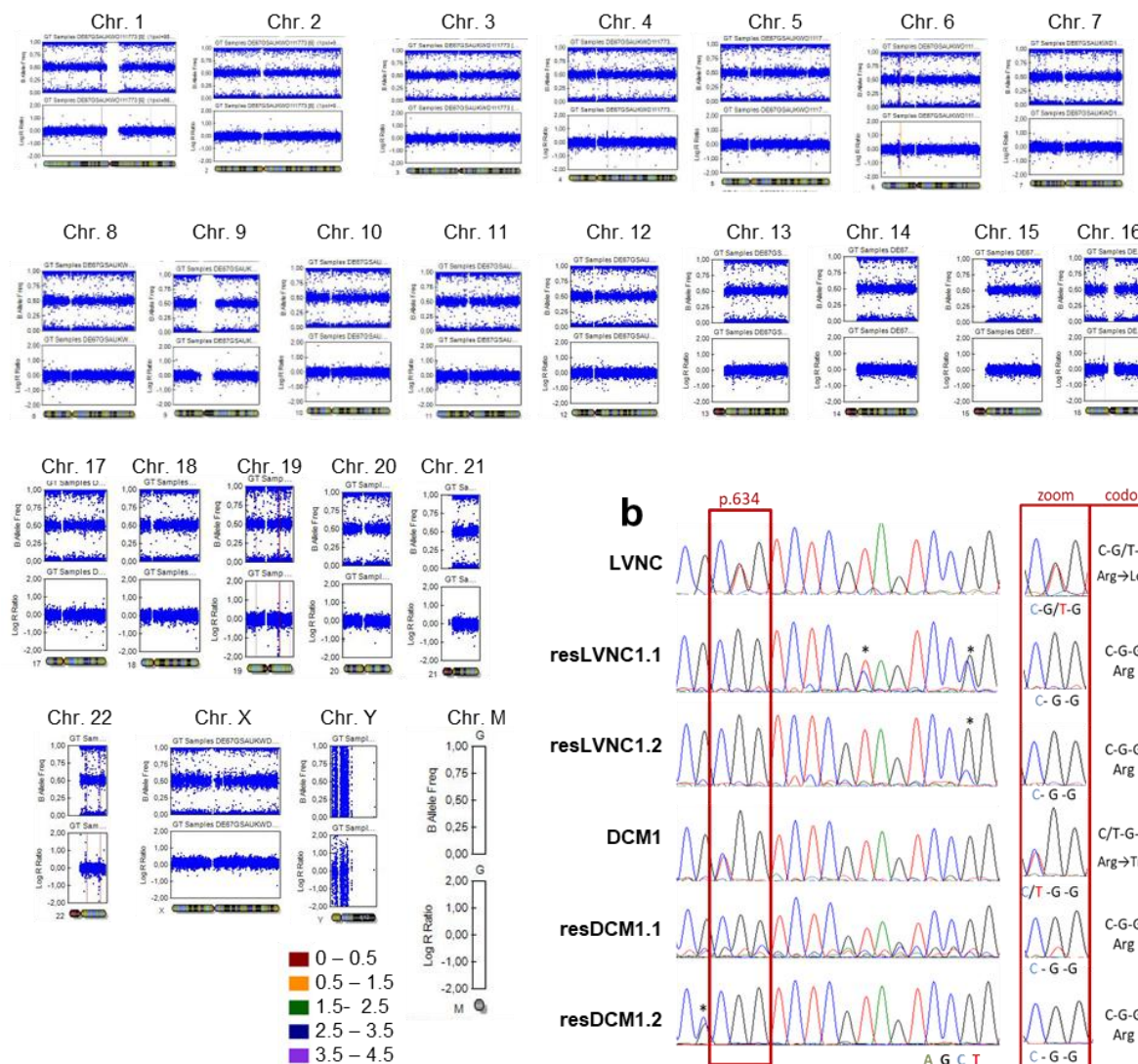
Generated iPSC lines from LVNC-family: LVNC II.3 and from the DCM family: DCM2 III.10, h.RBM1 II.3 and h.RBM2 III.3. h = healthy

a: Brightfield and immunofluorescence stainings of iPSC. All iPSC lines are positive for stem cell morphology, ALP activity, and stem cell marker expression OCT4, NANOG, LIN28, and TRA1-60. Brightness and contrast have been enhanced. BF: Brightfield; ALP: alkaline phosphatase.

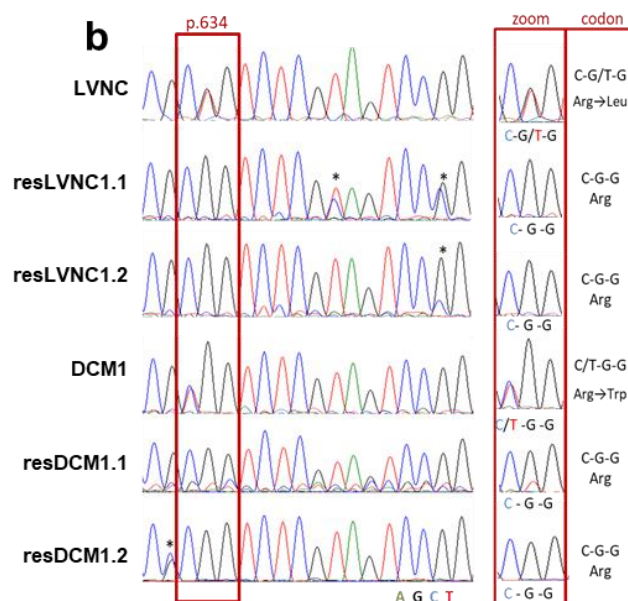
b: All iPSC lines differentiate into cells from all three germ layers stained with the appropriate antibodies against endoderm (AFP: alpha-1-fetoprotein), mesoderm (α -SMA: alpha smooth muscle) and ectoderm (β III-TUB: tubulin beta 3 class III). Brightness and contrast have been enhanced.

Fig. S2

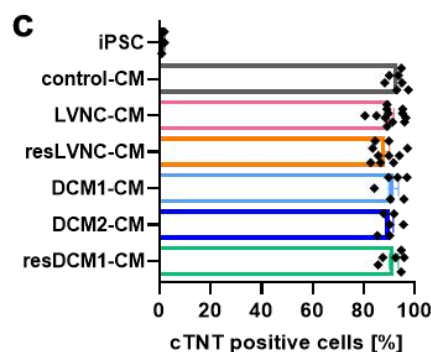
a



b



c



d

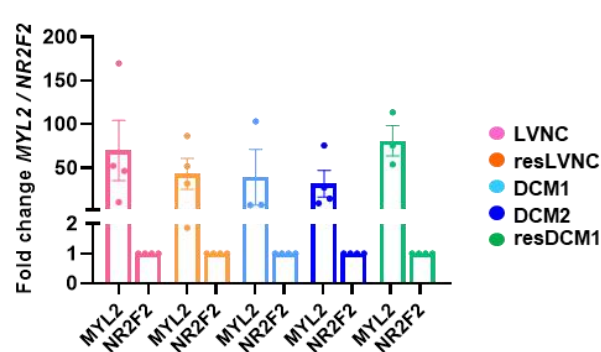


Fig. S2: Genomic integrity and differentiation into iPSC-CM of LVNC- and DCM-affected family members with RBM20 mutations.

a: All iPSC lines retain genetic integrity and a normal karyotype (XX or XY, 46). Exemplary karyotype shown for resLVNC iPSC (XX, 46). Graphs show B Allele Frequency and Log R Ratio for each chromosome. Chr. = chromosome

b: Respective Sanger sequencing results of the RBM20 locus exon 9. The zoom visualizes position c.1900-1902, which encodes the amino acid number 634 within the RBM20 gene. Wt-RBM20 encodes C-G-G for p.R634 as an arginine (Arg), whereas LVNC harbors a heterozygous missense mutation C-G/T-G leading to p.R634L (leucine (Leu)) and DCM with C/T-G-G leading to p.R634W (tryptophane (Trp)).

c: FLOW cytometry analysis of 60-90 days-old patient and control iPSC-CM stained with cardiac Troponin T (cTNT) antibody. Each dot represents one cardiac differentiation. Undifferentiated iPSC served as negative control.

d: QPCR analysis of ventricular marker *MYL2* and atrial marker *NR2F2*. Both genes were normalized to the housekeeper *18s* and subsequently normalized to *NR2F2* as a value of one to show the fold change of *MYL2* in relation to the atrial *NR2F2* in every sample. Each dot represents one cardiac differentiation.

Fig. S3

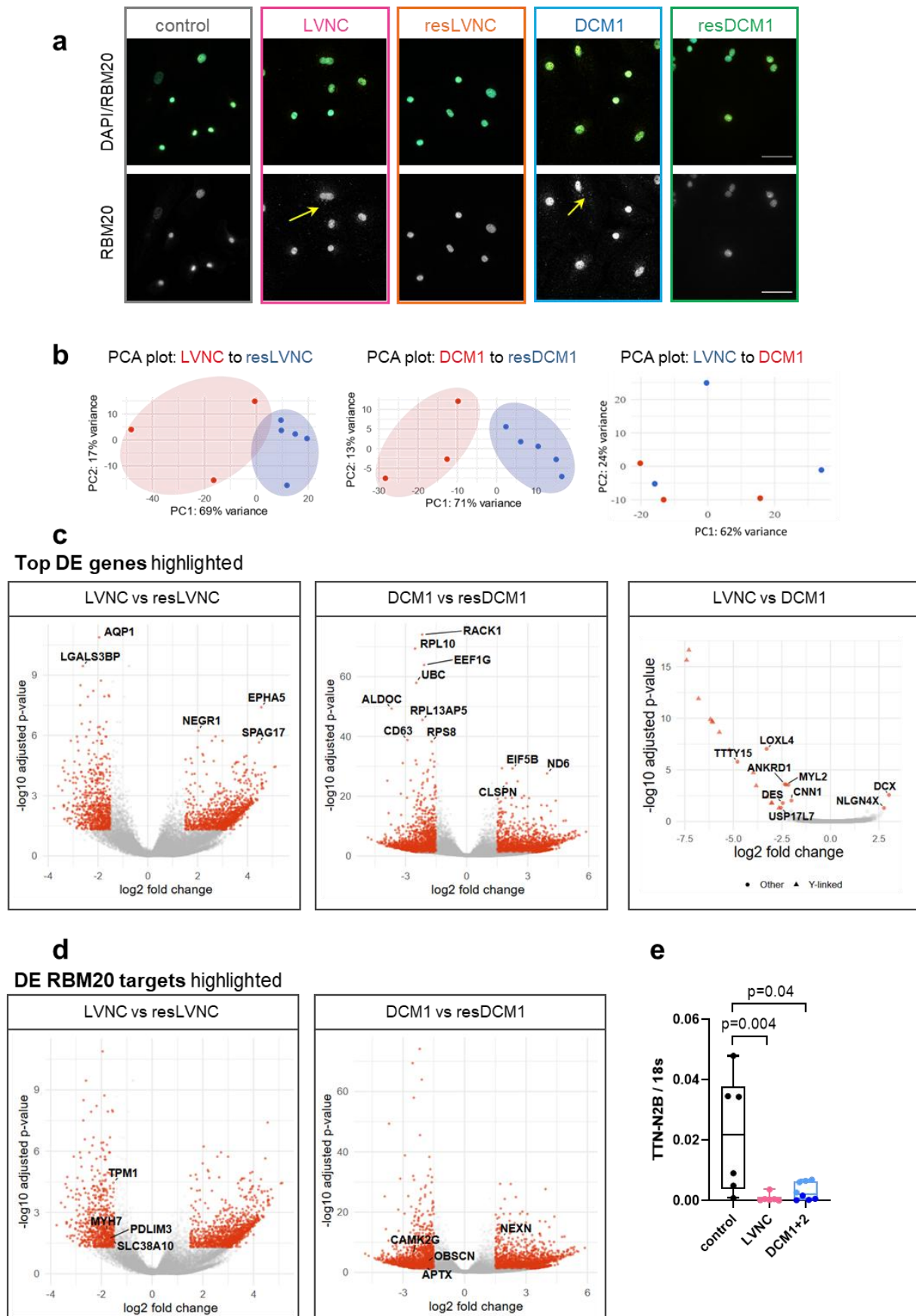


Fig. S3: RBM20-dependent mis-localization and *TTN* splicing.

a: RBM20 accumulates in the cytoplasm. Representative immunofluorescence stainings of control-, LVNC-, resLVNC-, DCM1- and resDCM1-CM. Yellow arrows marks exemplary RBM20 cytoplasmatic localization. Antibody staining against RBM20 (yellow) with counterstaining of the nucleus (cyan). Scale bars: 50 μ m.

b: The figure shows Principal Component Analysis (PCA) plots generated before differential expression analysis that highlights clusters as expected (patient vs rescue). The PCA plot for LVNC vs DCM1 shows some overlap between the two groups. This suggests that the gene expression profiles of LVNC and DCM1 are more similar to each other than compared to their isogenic controls.

c, d: Differential gene expression analysis:

c: Volcano plots showing DE genes with key markers labeled in comparison of LVNC vs resLVNC, DCM1 vs resDCM1, and LVNC vs DCM. The $-\log_{10}$ adjusted p-value is plotted against the $\log_2$ fold change for each comparison.

d: Volcano plots highlighting DE genes that are targets of RBM20 in the same comparison as shown in c. Specific RBM20 target genes are labeled.

DE = Differential (gene) expression, vs = versus

e: QPCR analysis of RBM20 splice target *TTN* as *N2B* isoform normalized to *18s*. Data is shown as box plots, whereas every dot represents one differentiation experiment. P-values by Mann-Whitney test Volcano plots highlighting DE genes that are targets of RBM20 in the same comparison as shown in D. Specific RBM20 target genes are labeled.

DE = Differential (gene) expression, vs = versus

Fig. S4

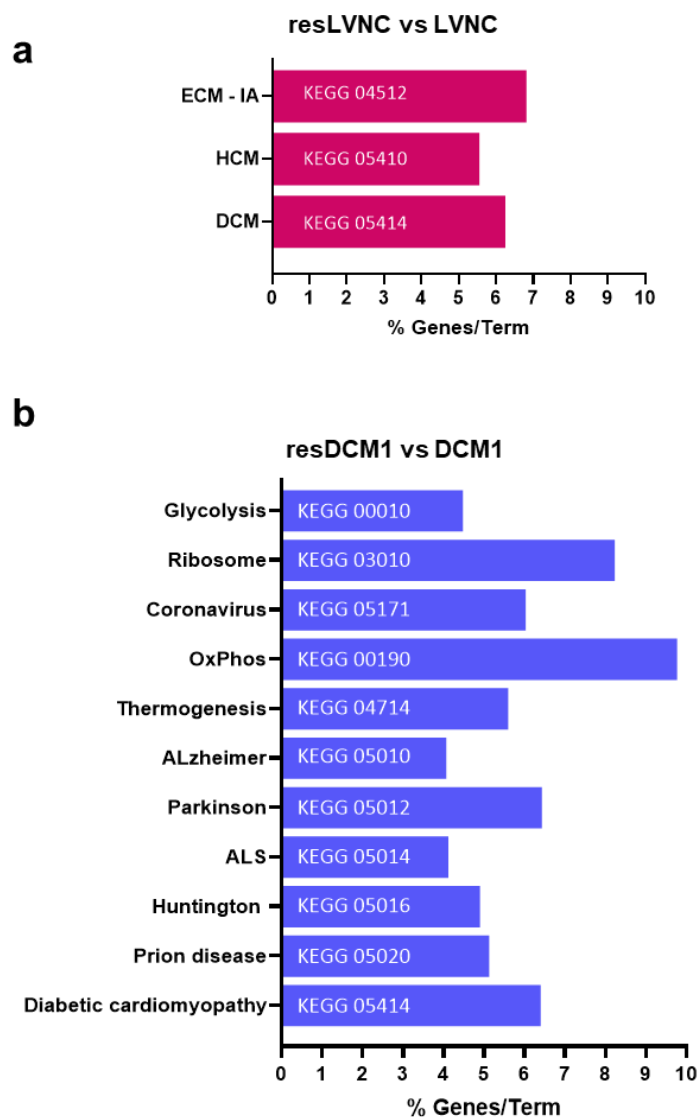


Fig. S4: KEGG GO-term hits ($pV < 0.05$) for the Top100 differentially expressed genes.

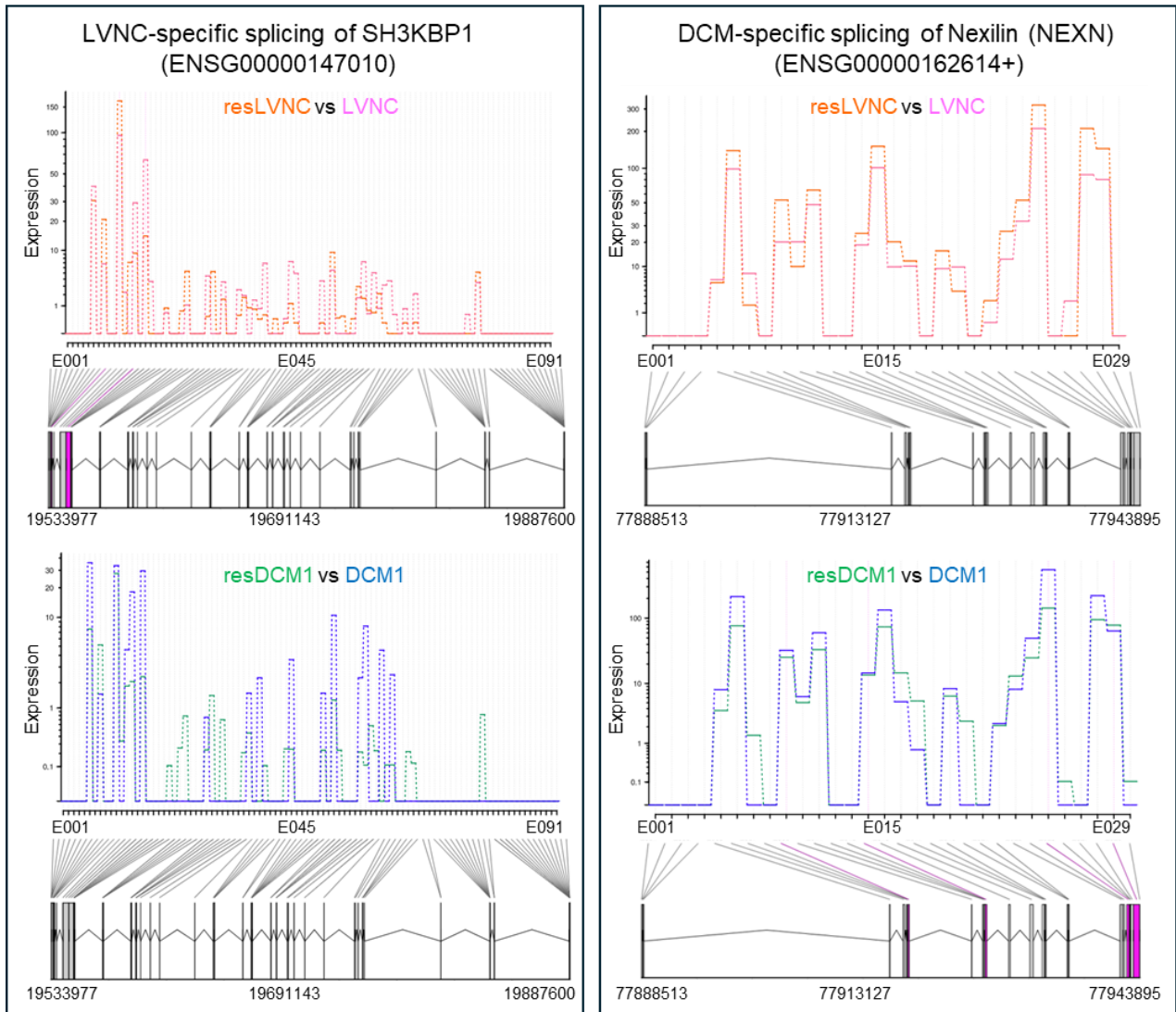
a: ResLVNC vs LVNC.

b: ResDCM1 vs DCM1.

IA: interaction, HCM: hypertrophic cardiomyopathy, DCM: dilated cardiomyopathy, OxPhos: oxidative phosphorylation, ALS: amyotrophic lateral sclerosis

Fig. S5

a



b

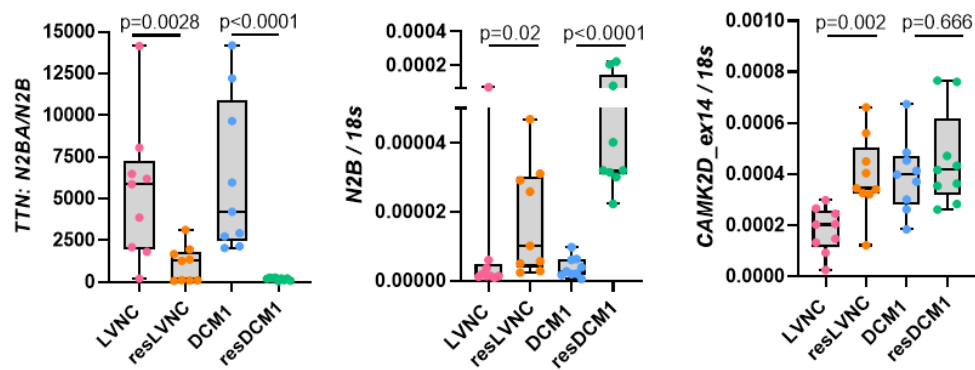


Fig. S5: Splice graphs with exonic bin usage of LVNC and DCM-specific targets.

a: Graphs represent the differential exon expression profiles. The y-axis represents normalized read counts of exons (exon usage), and the x-axis shows individual exons within a gene. The lower panels show the gene structure, with the bars below the x-axis representing exons, and the lines between the bars representing introns. The numbers at the bottom are genomic locations of the gene. Purple bars mark exons with significantly altered usage (FDR-adjusted p-value < 0.05) in the DEX analysis of isogenic versus patient cell lines.

b: Analysis of *TTN* and *CAMK2D* splicing in 3D-spheroid matured iPSC-CM. *TTN* splicing for the isoforms N2B and N2BA and *CAMK2D* splicing for exon 14. Data is shown as box plots, where each dot represents independently cultured spheroids. P-values are determined with Mann-Whitney test LVNC vs resLVNC and DCM1 vs resDCM1.

Fig. S6

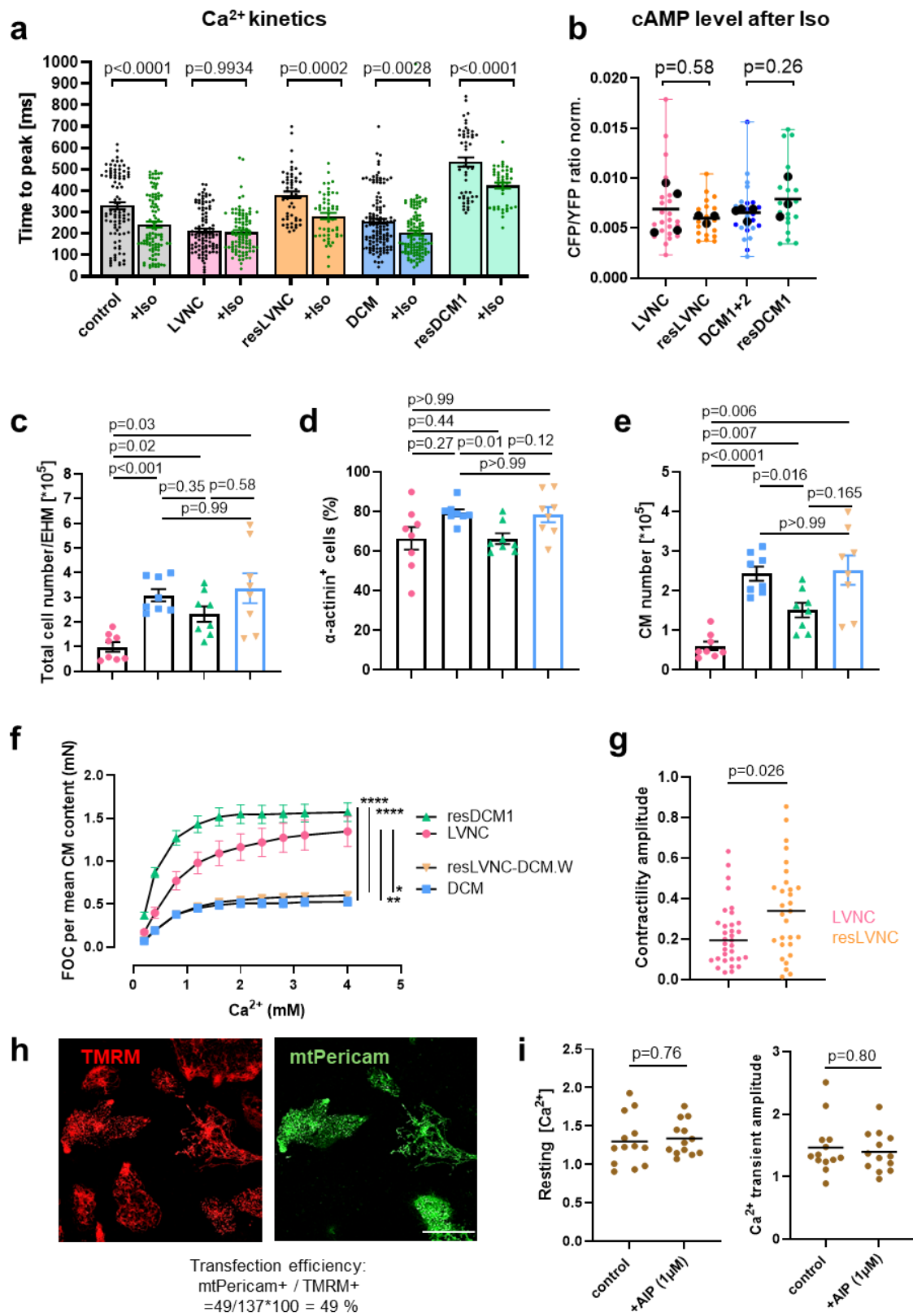


Fig. S6: LVNC- and DCM-CM show differential Ca^{2+} handling pathologies.

a: Ca^{2+} transient rise time (time to peak) time at basal (untreated) condition and after 15 min Isoprenaline (Iso, 1 μM) treatment with Fluo-4-AM. LVNC-CM showed decreased basal rise time levels and no measurable reaction to Iso. Data is presented as bar graph with mean $\pm$ SEM showing each measured cell as a single dot with [number of differentiations/analyzed cells] for basal: control [6/94], LVNC [6/101], resLVNC [3/55], DCM [7/126] and resDCM1 [3/51] and Iso stimulated: control [6/101], LVNC [6/97], resLVNC [3/53], DCM [7/119] and resDCM1 [3/44]. P-values by Two-way ANOVA Sidak's multiple comparison test Basal vs Iso.

b: cAMP changes after Iso treatment among the iPSC-CM lines. Quantification of maximal FRET response to IBMX after Isoprenaline (Iso) stimulation. P-value was calculated by nested t-test.

c-e: Number of cells from digested EHM following organ bath measurements: [number of differentiations/analyzed EHM] for LVNC [3/8], DCM1 [3/8], resDCM1 [3/8], and resLVNC-DCM1.W [3/8]. P-value by Brown-Forsythe and Welch ANOVA with Dunnett's multiple comparisons test.

c: Total cell number per EHM measured using Casy cell counter. LVNC-EHM show decreased total and iPSC-CM (CM) number compared to all other analyzed EHM.

d: Alpha-actinin-positive cells (%) after digestion of EHM measured via Flow Cytometry.

e: Proportion of CM calculated by total cell number and a-actinin positive cells.

f: FOC per CM normalized to viable iPSC-CM. The resDCM1- and LVNC-EHM show increased FOC compared to DCM1 and resLVNC-DCM.W line. P-values were calculated by Two-way RM ANOVA with Geisser-Greenhouse correction. P-value (column factor) for resDCM1 vs DCM1; resDCM1 vs resLVNC-DCM.W; LVNC vs resLVNC-DCM.W; LVNC vs DCM (significant values are marked * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$) and LVNC vs resDCM1; DCM1 vs resLVNC-DCM.W (not significant). 8 EHM were analyzed for each line.

g: Contractility measurements using microscopy with edge-detection algorithm in LVNC- and resLVNC-spheroid isolated iPSC-CM. As a parameter for systolic function, the contractility peak amplitude was calculated. Data is presented as scatter plot showing single cell measurements from $n=2$ spheroid-experiments for LVNC [$n=33$] and resLVNC [$n=24$]. P-value was determined by Mann Whitney test.

h: Transfection efficiency for mtPericam. mtPericam was transfected into iPSC-CM and measured 2-4 days afterwards. For transfection efficiency and localization verification iPSC-CM were counterstained with TMRM. Brightness and contrast has been enhanced for visualization purposes. Scale: 50 μm

i: Treatment of control-CM with a CAMK2D-inhibitor (AIP) and its effect on diastolic/resting Ca^{2+} and Ca^{2+} transient amplitude. After basal measurements, cells were treated with AIP (1 μM) for 15 min. Data shows all single cell measurements ($n=13$) as scatter plot with mean. P-values were calculated by Mann Whitney test.

Fig. S7

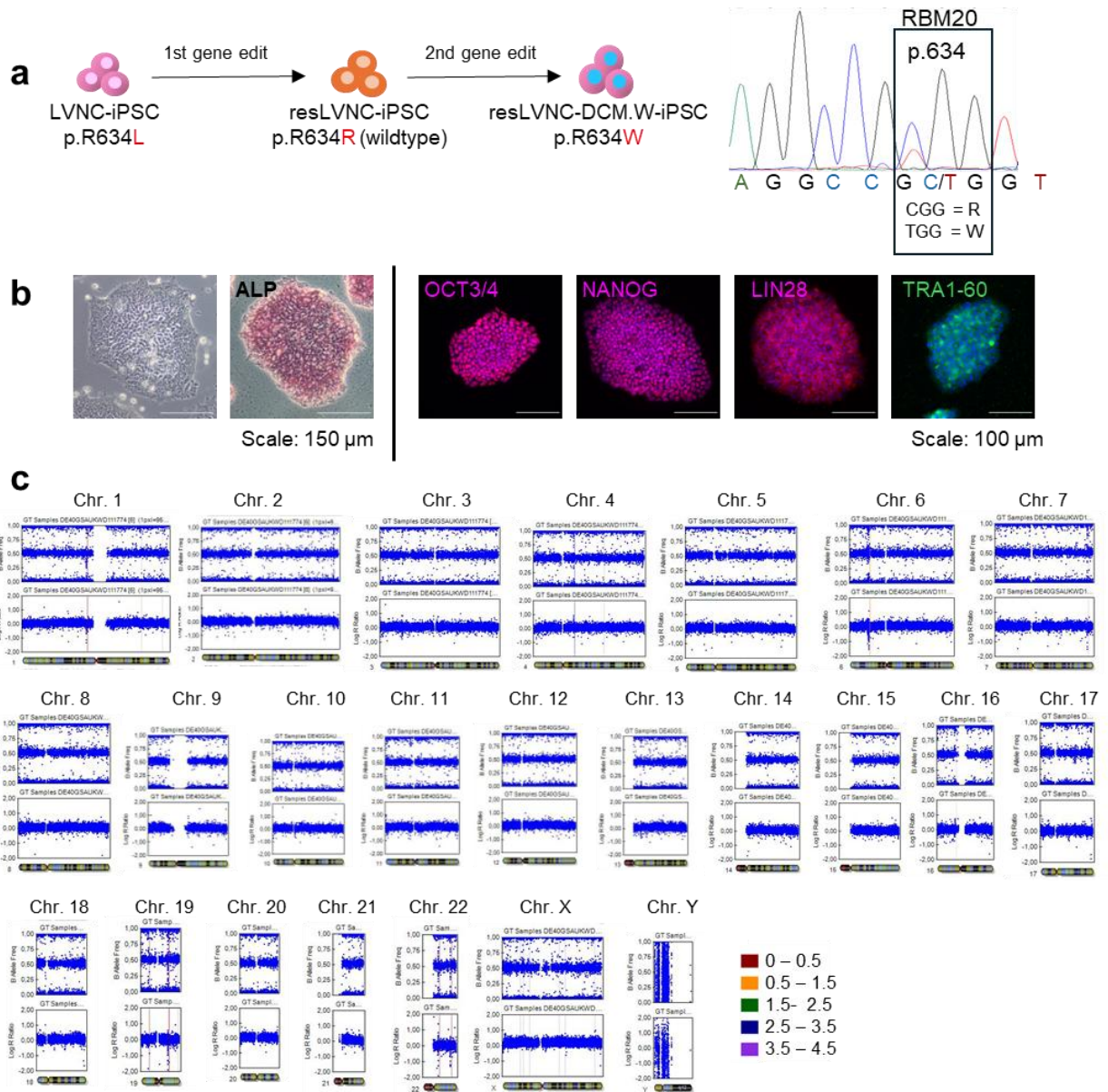


Fig. S7: Characterization of resLVNC-DCM.W-iPSC.

a: The resLVNC-DCM.W iPSC line is the LVNC patient background with the DCM mutation introduced.

b: Brightfield and immunofluorescence stainings of iPSC. The resLVNC-DCM.W-line is positive for stem cell morphology, ALP activity and stem cell markers OCT4, NANOG, LIN28 and TRA1-60. Brightness and contrast has been enhanced. BF: Brightfield; ALP: alkaline phosphatase.

c: Molecular karyotyping of resLVNC-DCM.W iPSC showing B Allele Frequency and Log R Ratio for each chromosome. Cells retain a normal karyotype (46, XX). Chr. = chromosome

Supplemental tables

Table S1: Patient data from the latest follow-up visit

	LVNC	DCM1	DCM2
Age at last visit	69	57	53
Gender	female	male	male
Age of diagnosis	39	43	38
NYHA	II	I	I
hsTnT	9	7	5
Nt-ProBNP	65	95	50
GFR	55	74	106
Hypertension	No	No	No
Hyerlipidemia	Yes	Yes	No
DM-II	No	Yes	No
Smoking	No	No	No
Coronary artery disease	No	No	No
atrial fibrillation	No	No	No
Ventricular tachycardia	Yes	Yes	No
LGE in MRI	Yes	Yes	Yes
LVEF in MRI	46	48	48
ACE/AT1	Yes	Yes	Yes
MRA	Yes	Yes	Yes
Betablocker	Yes	Yes	Yes
SGLT2-inhibitor	No	Yes	No
ICD	Yes (55years old)	No	No

Table S2: Summary of generated iPSC-lines from donor samples.

Patient donor	sample	Reprogramming	Intern-ID of clones
Healthy-1 from DCM II.3	Skin/Fibroblasts	Sendai	S3RBM7 S3RBM8
Healthy -2 from DCM III.3	Skin/Fibroblasts	Sendai	S4RBM2 S4RBM6
LVNC: II.3	Skin/Fibroblasts	Plasmids	P6RBM1 P6RBM2
DCM1: III.8	Blood/PMBC	Sendai	S9RBM2 S9RBM4
DCM2: III.10	Skin/Fibroblasts	Sendai	S10RBM3 S10RBM5
ResLVNC1	Rescue in P6RBM1	-	6cr11 6cr20 6cr21 6cr29
ResDCM1	Rescue in S9RBM2	-	9cr35 9cr49
LVNC-DCM.W	634W mutation into 6cr29	-	6cr634W_clon20

Table S3: List of antibodies used in this study.

Marker	Antibody	Dilution	ID and company
Pluripotency marker	IF: goat anti-OCT4	1:40	R and D; AF1759 RRID AB_354975
	IF: mouse anti-SOX2	1:200	R and D; MAB2018 RRID AB_358009
	IF: goat anti-LIN28	1:300	R and D; AF3757 RRID AB_2234537
	IF: goat anti-NANOG	1:50	R and D; AF1997 RRID AB_355097
	IF: mouse anti-TRA1-60	1:200	Abcam, ab16288 RRID AB_778563
	IF: mouse anti-SSEA4	1:200	Abcam, ab16287 RRID AB_778073
Germlayer marker	IF: rabbit anti-AFP	1:500	Dako, A0008 RRID AB_2650473
	IF: mouse anti- α -SMA	1:3000	Sigma, A2547 RRID AB_476701
	IF: mouse anti- β -III-TUBULIN	1:2000	Covance, MMS-435P RRID AB_2313773
Sarcomeric marker	IF: mouse anti- α -actinin	1:1000	Sigma, A7811 RRID: AB_476766
	IF: rabbit anti-Titin	1:750	MyoMedix, TTN-9 RRID:AB_2734750
	FLOW: mouse anti-cTNT	1:500	Thermo Fisher, MS295PABX RRID:AB_61810
Cardiac function	IF: rabbit anti-RBM20	1:500	Myomedix, RBM20-1
	Western: mouse anti-PLN	1:5000 in milk	Thermo Fisher, MA3-922 RRID:AB_2252716
	Western: rabbit anti-PLN-S16p	1:5000 in milk	Badrilla, A010-12AP RRID:AB_2617047
	Western: rabbit anti-PLN-T17p	1:5000 in milk	Badrilla, A010-13 RRID:AB_2617048
	Western: rabbit anti-RBM20	1:750 In BSA	Myomedix, RBM20-1
Secondary antibody	IF: AF488 donkey anti-rabbit IgG	1:1000	Invitrogen, A31572 RRID AB_162543
	IF: AF555 donkey anti-goat IgG	1:1000	Invitrogen, A21432 RRID AB_141788
	IF: AF647 donkey anti-rabbit	1:500	Invitrogen, A31573 RRID:AB_2536183
	IF and FLOW: AF488 donkey anti-mouse IgG	1:1000	Invitrogen, A21202 RRID AB_141607

	IF: Cy3 goat anti-mouse IgG + IgM	1:300	Jackson Immuno, 115–165-068 RRID AB_2338686
	Western: ECL Mouse IgG HRP-Linked 1ml	1:10000	Th. Geyer, NA931 RRID:AB_772210
	Western: ECL Rabbit IgG HRP-Linked 1ml	1:10000	Th. Geyer, NA934 RRID:AB_772206
Nucleus staining	IF: Hoechst	1:5000	Sigma, 33258

Table S4: Primer list.

Gene	Forward 5' -> 3'	Reverse 5' -> 3'
18s	ACCCGTTGAACCCCATTCGTGA	GCCTCACTAAACCATCCAATCGG
GAPDH	AGAGGCAGGGATGATGTTCT	TCTGCTGATGCCCCCATGTT
RBM20 – exon9	GAGTGACACAGTTACATGCAC	GTG GGACCTCGGGGAGA
RBM20	CCTCCACTTGCCGCATATCTGT	AGACCAGGCATTTCTGAGCGTG
MYL2	CGGAGAGGTTTTCCAAGGAGGA	CTCTTCTCCGTGGGTGATGATG
NR2F	CCGACCGGGTGGTCGCCTTTATGGA	CGGCTGGTTGGGGTACTGGCTCCTA
TTN-N2B	CCAATGAGTATGGCAGTGTCA	TACGTTCCGGAAGTAATTTGC
TTN-N2BA	GCCACACTAACTGTGACAGAGG	GGCTGCCTTACCCACAAAAG
RYR2-24bp	GTCACAGGATCCCAACGCAG	CTTTGCTGGCACTGATTGTCTG
RYR2	CTTGAGGTTGGCTTTCTGCCAG	TGTGCCAGCAAAGAGAGGAGAC
LDB3-exon5	TCAAAGCGTCCCATTCCTATC	CGGGAGAA GCAGGGCTAAA
LDB3	ACCTCGTGGTGGCCATTG	GTGGAGATGGGAATGGGACG
CAMk2D- exon14	CCATCTTGACAACTATGCTGGCT	GAACACTCGAACTGGACTTCCT
CAMk2D	ACACGGTGACTCCTGAAGCCAA	GTCTCCTGTCTGTGCATCATGG
TRDN-exon9	GTCCATGGGGATTTAAAACCAGG	CTTCAAGGGCAGGTGATGC
TRDN	GGAGGACAAAGAGAAAGCAGCTG	AGGTGGAATGGCTGGGCTTTGT
IMMT-exon5-6	AAACAGCCTGCCTCACAAC	TCCTTCAATGCACCCTCCAC
IMMT	CAGGCTGTCAATGCACACTCCA	CATCTACTGCCTTTCTGCGTTCC

Table S5: Top 100 differentially expressed genes resLVNC vs LVNC.

ENTREZID	SYMBOL	GENENAME	Log2 Fold Change	Raw P-value	Adjusted P-value
358	AQP1	aquaporin 1 (Colton blood group)	-1.951±0.238	6.32 × 10 ⁻¹⁶	1.30 × 10 ⁻¹¹
3959	LGALS3BP	galectin 3 binding protein	-2.607±0.338	4.99 × 10 ⁻¹⁴	3.52 × 10 ⁻¹⁰
102724652	LOC102724652	crystallin alpha A2	-0.756±0.804	6.79 × 10 ⁻¹⁴	3.52 × 10 ⁻¹⁰
1409	CRYAA	crystallin alpha A	-0.754±0.804	6.83 × 10 ⁻¹⁴	3.52 × 10 ⁻¹⁰
112	ADCY6	adenylate cyclase 6	-1.882±0.256	4.54 × 10 ⁻¹³	1.87 × 10 ⁻⁹
26001	RNF167	ring finger protein 167	-1.402±0.193	6.01 × 10 ⁻¹³	2.07 × 10 ⁻⁹
80023	NRSN2	neurensin 2	-2.705±0.373	1.09 × 10 ⁻¹²	3.20 × 10 ⁻⁹
3949	LDLR	low density lipoprotein receptor	-2.188±0.308	2.37 × 10 ⁻¹²	6.10 × 10 ⁻⁹
37	ACADVL	acyl-CoA dehydrogenase very long chain	-1.572±0.224	4.33 × 10 ⁻¹²	9.93 × 10 ⁻⁹
6840	SVIL	supervillin	-0.945±0.137	6.11 × 10 ⁻¹²	1.15 × 10 ⁻⁸
8048	CSRP3	cysteine and glycine rich protein 3	-1.743±0.250	6.04 × 10 ⁻¹²	1.15 × 10 ⁻⁸
6604	SMARCD3	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily d, member 3	-1.552±0.223	6.73 × 10 ⁻¹²	1.16 × 10 ⁻⁸
11180	WDR6	WD repeat domain 6	-2.405±0.342	7.39 × 10 ⁻¹²	1.17 × 10 ⁻⁸
327	APEH	acylaminoacyl-peptide hydrolase	-2.713±0.395	2.12 × 10 ⁻¹¹	3.12 × 10 ⁻⁸
2044	EPHA5	EPH receptor A5	4.556±0.641	2.87 × 10 ⁻¹¹	3.94 × 10 ⁻⁸
7284	TUFM	Tu translation elongation factor, mitochondrial	-2.311±0.357	1.81 × 10 ⁻¹⁰	2.12 × 10 ⁻⁷
6711	SPTBN1	spectrin beta, non-erythrocytic 1	-1.223±0.189	1.85 × 10 ⁻¹⁰	2.12 × 10 ⁻⁷
10221	TRIB1	tribbles pseudokinase 1	-1.661±0.257	1.71 × 10 ⁻¹⁰	2.12 × 10 ⁻⁷
1523	CUX1	cut like homeobox 1	-1.108±0.173	2.05 × 10 ⁻¹⁰	2.22 × 10 ⁻⁷
4240	MFG8	milk fat globule EGF and factor V/VIII domain containing	-2.654±0.407	2.56 × 10 ⁻¹⁰	2.64 × 10 ⁻⁷
7057	THBS1	thrombospondin 1	-1.927±0.302	3.81 × 10 ⁻¹⁰	3.74 × 10 ⁻⁷
286336	FAM78A	family with sequence similarity 78 member A	-1.999±0.314	4.17 × 10 ⁻¹⁰	3.91 × 10 ⁻⁷

ENTREZID	SYMBOL	GENENAME	Log2 Fold Change	Raw P-value	Adjusted P-value
25805	BAMBI	BMP and activin membrane bound inhibitor	-1.781±0.282	5.41 × 10 ⁻¹⁰	4.85 × 10 ⁻⁷
163702	IFNLR1	interferon lambda receptor 1	-2.356±0.375	6.12 × 10 ⁻¹⁰	5.26 × 10 ⁻⁷
257194	NEGR1	neuronal growth regulator 1	2.033±0.326	7.14 × 10 ⁻¹⁰	5.89 × 10 ⁻⁷
3693	ITGB5	integrin subunit beta 5	-1.973±0.318	9.20 × 10 ⁻¹⁰	7.30 × 10 ⁻⁷
30845	EHD3	EH domain containing 3	-1.924±0.310	1.16 × 10 ⁻⁹	8.86 × 10 ⁻⁷
196385	DNAH10	dynein axonemal heavy chain 10	2.718±0.433	1.60 × 10 ⁻⁹	1.14 × 10 ⁻⁶
593	BCKDHA	branched chain keto acid dehydrogenase E1 subunit alpha	-2.845±0.453	1.60 × 10 ⁻⁹	1.14 × 10 ⁻⁶
826	CAPNS1	calpain small subunit 1	-1.693±0.275	1.66 × 10 ⁻⁹	1.14 × 10 ⁻⁶
146691	TOM1L2	target of myb1 like 2 membrane trafficking protein	-1.667±0.273	1.79 × 10 ⁻⁹	1.15 × 10 ⁻⁶
58498	MYL7	myosin light chain 7	-2.473±0.395	1.77 × 10 ⁻⁹	1.15 × 10 ⁻⁶
27122	DKK3	dickkopf WNT signaling pathway inhibitor 3	-1.347±0.223	2.83 × 10 ⁻⁹	1.77 × 10 ⁻⁶
6506	SLC1A2	solute carrier family 1 member 2	2.574±0.421	3.13 × 10 ⁻⁹	1.85 × 10 ⁻⁶
2896	GRN	granulin precursor	-3.089±0.500	3.06 × 10 ⁻⁹	1.85 × 10 ⁻⁶
3679	ITGA7	integrin subunit alpha 7	-2.036±0.338	3.27 × 10 ⁻⁹	1.85 × 10 ⁻⁶
65997	RASL11B	RAS like family 11 member B	-1.859±0.310	3.32 × 10 ⁻⁹	1.85 × 10 ⁻⁶
55089	SLC38A4	solute carrier family 38 member 4	2.996±0.496	3.42 × 10 ⁻⁹	1.86 × 10 ⁻⁶
1917	EEF1A2	eukaryotic translation elongation factor 1 alpha 2	-2.815±0.462	3.92 × 10 ⁻⁹	2.07 × 10 ⁻⁶
200162	SPAG17	sperm associated antigen 17	4.466±0.686	4.44 × 10 ⁻⁹	2.26 × 10 ⁻⁶
57658	CALCOCO1	calcium binding and coiled-coil domain 1	-1.204±0.203	4.48 × 10 ⁻⁹	2.26 × 10 ⁻⁶
4607	MYBPC3	myosin binding protein C3	-2.193±0.362	4.73 × 10 ⁻⁹	2.32 × 10 ⁻⁶
7436	VLDLR	very low density lipoprotein receptor	-1.059±0.180	5.31 × 10 ⁻⁹	2.55 × 10 ⁻⁶
4192	MDK	midkine	-1.866±0.311	5.51 × 10 ⁻⁹	2.55 × 10 ⁻⁶
57158	JPH2	junctional protein 2	-1.832±0.308	5.56 × 10 ⁻⁹	2.55 × 10 ⁻⁶
3213	HOXB3	homeobox B3	1.997±0.340	8.83 × 10 ⁻⁹	3.96 × 10 ⁻⁶

ENTREZID	SYMBOL	GENENAME	Log2 Fold Change	Raw P-value	Adjusted P-value
1284	COL4A2	collagen type IV alpha 2 chain	-2.368±0.402	1.25 × 10 ⁻⁸	5.47 × 10 ⁻⁶
65018	PINK1	PTEN induced kinase 1	-2.786±0.467	1.32 × 10 ⁻⁸	5.67 × 10 ⁻⁶
146862	UNC45B	unc-45 myosin chaperone B	-1.374±0.238	1.37 × 10 ⁻⁸	5.75 × 10 ⁻⁶
2975	GTF3C1	general transcription factor IIIC subunit 1	-1.249±0.219	1.67 × 10 ⁻⁸	6.90 × 10 ⁻⁶
94005	PIGS	phosphatidylinositol glycan anchor biosynthesis class S	-1.589±0.281	2.15 × 10 ⁻⁸	8.40 × 10 ⁻⁶
4924	NUCB1	nucleobindin 1	-2.381±0.411	2.16 × 10 ⁻⁸	8.40 × 10 ⁻⁶
7074	TIAM1	TIAM Rac1 associated GEF 1	2.195±0.382	2.10 × 10 ⁻⁸	8.40 × 10 ⁻⁶
30008	EFEMP2	EGF containing fibulin extracellular matrix protein 2	-2.143±0.377	2.26 × 10 ⁻⁸	8.65 × 10 ⁻⁶
4258	MGST2	microsomal glutathione S-transferase 2	-1.423±0.252	2.41 × 10 ⁻⁸	9.05 × 10 ⁻⁶
9844	ELMO1	engulfment and cell motility 1	2.221±0.387	2.66 × 10 ⁻⁸	9.78 × 10 ⁻⁶
777	CACNA1E	calcium voltage-gated channel subunit alpha1 E	3.955±0.659	2.86 × 10 ⁻⁸	1.04 × 10 ⁻⁵
147906	DACT3	dishevelled binding antagonist of beta catenin 3	-1.751±0.313	3.14 × 10 ⁻⁸	1.12 × 10 ⁻⁵
23467	NPTXR	neuronal pentraxin receptor	-1.689±0.300	3.25 × 10 ⁻⁸	1.14 × 10 ⁻⁵
8828	NRP2	neuropilin 2	-1.377±0.247	3.37 × 10 ⁻⁸	1.16 × 10 ⁻⁵
9348	NDST3	N-deacetylase and N-sulfotransferase 3	3.872±0.646	3.45 × 10 ⁻⁸	1.17 × 10 ⁻⁵
3913	LAMB2	laminin subunit beta 2	-2.071±0.363	3.66 × 10 ⁻⁸	1.22 × 10 ⁻⁵
115123	MARCHF3	membrane associated ring-CH-type finger 3	-1.640±0.298	4.21 × 10 ⁻⁸	1.38 × 10 ⁻⁵
79915	ATAD5	ATPase family AAA domain containing 5	1.722±0.310	4.37 × 10 ⁻⁸	1.41 × 10 ⁻⁵
3673	ITGA2	integrin subunit alpha 2	-1.838±0.338	4.48 × 10 ⁻⁸	1.42 × 10 ⁻⁵
8566	PDXK	pyridoxal kinase	-1.529±0.276	4.65 × 10 ⁻⁸	1.45 × 10 ⁻⁵
5156	PDGFRA	platelet derived growth factor receptor alpha	-1.898±0.345	4.81 × 10 ⁻⁸	1.48 × 10 ⁻⁵
5922	RASA2	RAS p21 protein activator 2	1.515±0.277	5.15 × 10 ⁻⁸	1.56 × 10 ⁻⁵
23038	WDTC1	WD and tetratricopeptide repeats 1	-2.258±0.412	5.70 × 10 ⁻⁸	1.70 × 10 ⁻⁵
10723	SLC12A7	solute carrier family 12 member 7	-3.345±0.569	6.20 × 10 ⁻⁸	1.83 × 10 ⁻⁵

ENTREZID	SYMBOL	GENENAME	Log2 Fold Change	Raw P-value	Adjusted P-value
51555	PEX5L	peroxisomal biogenesis factor 5 like	2.645±0.470	6.50 × 10 ⁻⁸	1.89 × 10 ⁻⁵
10622	POLR3G	RNA polymerase III subunit G	2.255±0.408	7.05 × 10 ⁻⁸	2.02 × 10 ⁻⁵
2621	GAS6	growth arrest specific 6	-2.942±0.521	7.15 × 10 ⁻⁸	2.02 × 10 ⁻⁵
8626	TP63	tumor protein p63	3.631±0.619	7.61 × 10 ⁻⁸	2.12 × 10 ⁻⁵
219595	FOLH1B	folate hydrolase 1B (pseudogene)	3.548±0.623	7.96 × 10 ⁻⁸	2.19 × 10 ⁻⁵
2846	LPAR4	lysophosphatidic acid receptor 4	3.554±0.617	8.07 × 10 ⁻⁸	2.19 × 10 ⁻⁵
6900	CNTN2	contactin 2	4.292±0.734	8.81 × 10 ⁻⁸	2.36 × 10 ⁻⁵
672	BRCA1	BRCA1 DNA repair associated	1.768±0.328	8.93 × 10 ⁻⁸	2.36 × 10 ⁻⁵
5339	PLEC	plectin	-2.342±0.423	9.48 × 10 ⁻⁸	2.48 × 10 ⁻⁵
9469	CHST3	carbohydrate sulfotransferase 3	-1.829±0.338	9.69 × 10 ⁻⁸	2.50 × 10 ⁻⁵
10690	FUT9	fucosyltransferase 9	3.495±0.619	1.03 × 10 ⁻⁷	2.61 × 10 ⁻⁵
29106	SCG3	secretogranin III	3.862±0.668	1.09 × 10 ⁻⁷	2.74 × 10 ⁻⁵
25844	YIPF3	Yip1 domain family member 3	-1.948±0.361	1.12 × 10 ⁻⁷	2.79 × 10 ⁻⁵
5819	NECTIN2	nectin cell adhesion molecule 2	-2.739±0.501	1.17 × 10 ⁻⁷	2.86 × 10 ⁻⁵
389	RHOC	ras homolog family member C	-2.059±0.378	1.20 × 10 ⁻⁷	2.89 × 10 ⁻⁵
5315	PKM	pyruvate kinase M1/2	-1.854±0.340	1.19 × 10 ⁻⁷	2.89 × 10 ⁻⁵
2137	EXTL3	exostosin like glycosyltransferase 3	-1.296±0.243	1.33 × 10 ⁻⁷	3.16 × 10 ⁻⁵
7168	TPM1	tropomyosin 1	-1.529±0.283	1.49 × 10 ⁻⁷	3.49 × 10 ⁻⁵
105375661	NA	NA	5.129±0.864	1.54 × 10 ⁻⁷	3.58 × 10 ⁻⁵
23385	NCSTN	nicastatin	-1.005±0.190	1.60 × 10 ⁻⁷	3.66 × 10 ⁻⁵
84457	PHYHIPL	phytanoyl-CoA 2-hydroxylase interacting protein like	3.390±0.599	1.63 × 10 ⁻⁷	3.69 × 10 ⁻⁵
58476	TP53INP2	tumor protein p53 inducible nuclear protein 2	-1.388±0.262	1.65 × 10 ⁻⁷	3.71 × 10 ⁻⁵
5454	POU3F2	POU class 3 homeobox 2	5.074±0.839	1.69 × 10 ⁻⁷	3.75 × 10 ⁻⁵
91304	TMEM259	transmembrane protein 259	-2.016±0.375	1.73 × 10 ⁻⁷	3.79 × 10 ⁻⁵

ENTREZID	SYMBOL	GENENAME	Log2 Fold Change	Raw P-value	Adjusted P-value
56981	PRDM11	PR/SET domain 11	-1.270±0.242	1.78 × 10 ⁻⁷	3.87 × 10 ⁻⁵
339123	JMJD8	jumonji domain containing 8	-3.411±0.598	1.88 × 10 ⁻⁷	4.03 × 10 ⁻⁵
558	AXL	AXL receptor tyrosine kinase	-1.991±0.379	1.91 × 10 ⁻⁷	4.07 × 10 ⁻⁵
7005	TEAD3	TEA domain transcription factor 3	-2.006±0.377	2.07 × 10 ⁻⁷	4.35 × 10 ⁻⁵
3636	INPPL1	inositol polyphosphate phosphatase like 1	-1.666±0.313	2.40 × 10 ⁻⁷	5.00 × 10 ⁻⁵
780	DDR1	discoidin domain receptor tyrosine kinase 1	-2.286±0.426	2.52 × 10 ⁻⁷	5.09 × 10 ⁻⁵

Table S6: Top 100 differentially expressed genes resDCM1 vs DCM1.

ENTREZID	SYMBOL	GENENAME	Log2 Fold Change	Raw P-value	Adjusted P-value
10399	RACK1	receptor for activated C kinase 1	-2.185±0.116	4.01 × 10 ⁻⁷⁹	7.99 × 10 ⁻⁷⁵
6134	RPL10	ribosomal protein L10	-2.533±0.139	4.09 × 10 ⁻⁷⁴	4.07 × 10 ⁻⁷⁰
1937	EEF1G	eukaryotic translation elongation factor 1 gamma	-2.088±0.119	1.82 × 10 ⁻⁶⁸	1.21 × 10 ⁻⁶⁴
7316	UBC	ubiquitin C	-2.474±0.148	2.34 × 10 ⁻⁶²	1.16 × 10 ⁻⁵⁸
230	ALDOC	aldolase, fructose-bisphosphate C	-3.683±0.237	1.21 × 10 ⁻⁵³	4.81 × 10 ⁻⁵⁰
728658	RPL13AP5	ribosomal protein L13a pseudogene 5	-2.172±0.146	9.01 × 10 ⁻⁵⁰	2.99 × 10 ⁻⁴⁶
967	CD63	CD63 molecule	-2.905±0.209	5.61 × 10 ⁻⁴³	1.60 × 10 ⁻³⁹
6202	RPS8	ribosomal protein S8	-1.717±0.125	2.06 × 10 ⁻⁴²	5.13 × 10 ⁻³⁹
6143	RPL19	ribosomal protein L19	-1.668±0.129	2.88 × 10 ⁻³⁸	6.38 × 10 ⁻³⁵
4637	MYL6	myosin light chain 6	-1.778±0.141	4.23 × 10 ⁻³⁶	8.43 × 10 ⁻³³
6208	RPS14	ribosomal protein S14	-2.504±0.197	5.80 × 10 ⁻³⁶	1.05 × 10 ⁻³²
498	ATP5F1A	ATP synthase F1 subunit alpha	-1.588±0.128	1.62 × 10 ⁻³⁵	2.68 × 10 ⁻³²
3959	LGALS3BP	galectin 3 binding protein	-3.136±0.251	5.36 × 10 ⁻³⁵	8.21 × 10 ⁻³²
1642	DDB1	damage specific DNA binding protein 1	-1.419±0.115	1.11 × 10 ⁻³⁴	1.58 × 10 ⁻³¹
5213	PFKM	phosphofructokinase, muscle	-2.462±0.200	1.52 × 10 ⁻³⁴	2.01 × 10 ⁻³¹
10939	AFG3L2	AFG3 like matrix AAA peptidase subunit 2	-1.598±0.132	1.05 × 10 ⁻³³	1.31 × 10 ⁻³⁰
800	CALD1	caldesmon 1	1.738±0.145	3.72 × 10 ⁻³³	4.35 × 10 ⁻³⁰
9669	EIF5B	eukaryotic translation initiation factor 5B	2.253±0.188	4.53 × 10 ⁻³³	5.01 × 10 ⁻³⁰
4720	NDUFS2	NADH:ubiquinone oxidoreductase core subunit S2	-1.894±0.159	1.54 × 10 ⁻³²	1.62 × 10 ⁻²⁹
3945	LDHB	lactate dehydrogenase B	-1.929±0.164	6.91 × 10 ⁻³²	6.88 × 10 ⁻²⁹
6130	RPL7A	ribosomal protein L7a	-2.125±0.181	2.28 × 10 ⁻³¹	2.16 × 10 ⁻²⁸
4541	ND6	NADH dehydrogenase subunit 6	3.950±0.337	2.64 × 10 ⁻³¹	2.39 × 10 ⁻²⁸
5250	SLC25A3	solute carrier family 25 member 3	-1.654±0.145	4.05 × 10 ⁻³⁰	3.51 × 10 ⁻²⁷

ENTREZID	SYMBOL	GENENAME	Log2 Fold Change	Raw P-value	Adjusted P-value
7415	VCP	valosin containing protein	-1.926±0.171	2.06 × 10 ⁻²⁹	1.71 × 10 ⁻²⁶
6132	RPL8	ribosomal protein L8	-2.745±0.243	6.39 × 10 ⁻²⁹	5.09 × 10 ⁻²⁶
6415	SELENOW	selenoprotein W	-3.895±0.343	8.37 × 10 ⁻²⁹	6.41 × 10 ⁻²⁶
6122	RPL3	ribosomal protein L3	-1.926±0.173	1.10 × 10 ⁻²⁸	8.10 × 10 ⁻²⁶
374393	FAM111B	FAM111 trypsin like peptidase B	2.504±0.226	1.42 × 10 ⁻²⁸	1.01 × 10 ⁻²⁵
388524	RPSA2	ribosomal protein SA 2	-2.141±0.194	4.01 × 10 ⁻²⁸	2.75 × 10 ⁻²⁵
63967	CLSPN	claspin	1.961±0.179	7.10 × 10 ⁻²⁸	4.56 × 10 ⁻²⁵
4678	NASP	nuclear autoantigenic sperm protein	1.249±0.114	7.03 × 10 ⁻²⁸	4.56 × 10 ⁻²⁵
9500	MAGED1	MAGE family member D1	-2.188±0.201	1.90 × 10 ⁻²⁷	1.18 × 10 ⁻²⁴
1499	CTNNB1	catenin beta 1	-1.549±0.143	3.73 × 10 ⁻²⁷	2.25 × 10 ⁻²⁴
6175	RPLP0	ribosomal protein lateral stalk subunit P0	-1.525±0.142	1.17 × 10 ⁻²⁶	6.83 × 10 ⁻²⁴
2170	FABP3	fatty acid binding protein 3	-1.571±0.148	2.26 × 10 ⁻²⁶	1.29 × 10 ⁻²³
653513	LOC653513	phosphodiesterase 4D interacting protein-like	-2.169±0.204	3.28 × 10 ⁻²⁶	1.82 × 10 ⁻²³
23326	USP22	ubiquitin specific peptidase 22	-1.220±0.117	2.20 × 10 ⁻²⁵	1.19 × 10 ⁻²²
3921	RPSA	ribosomal protein SA	-2.601±0.247	3.17 × 10 ⁻²⁵	1.66 × 10 ⁻²²
1340	COX6B1	cytochrome c oxidase subunit 6B1	-1.512±0.146	3.53 × 10 ⁻²⁵	1.80 × 10 ⁻²²
506	ATP5F1B	ATP synthase F1 subunit beta	-1.563±0.152	1.11 × 10 ⁻²⁴	5.55 × 10 ⁻²²
84081	NSRP1	nuclear speckle splicing regulatory protein 1	1.765±0.172	1.27 × 10 ⁻²⁴	6.15 × 10 ⁻²²
1345	COX6C	cytochrome c oxidase subunit 6C	-1.428±0.139	1.60 × 10 ⁻²⁴	7.60 × 10 ⁻²²
5660	PSAP	prosaposin	-2.357±0.230	1.99 × 10 ⁻²⁴	9.23 × 10 ⁻²²
25824	PRDX5	peroxiredoxin 5	-3.232±0.308	3.36 × 10 ⁻²⁴	1.52 × 10 ⁻²¹
118	ADD1	adducin 1	-1.488±0.147	4.45 × 10 ⁻²⁴	1.97 × 10 ⁻²¹
10574	CCT7	chaperonin containing TCP1 subunit 7	-1.571±0.155	6.99 × 10 ⁻²⁴	3.03 × 10 ⁻²¹
517	ATP5MC2	ATP synthase membrane subunit c locus 2	-2.211±0.219	9.40 × 10 ⁻²⁴	3.99 × 10 ⁻²¹

ENTREZID	SYMBOL	GENENAME	Log2 Fold Change	Raw P-value	Adjusted P-value
1666	DECR1	2,4-dienoyl-CoA reductase 1	-1.791±0.178	1.06 × 10 ⁻²³	4.39 × 10 ⁻²¹
5858	PZP	PZP alpha-2-macroglobulin like	2.820±0.281	1.71 × 10 ⁻²³	6.93 × 10 ⁻²¹
23107	MRPS27	mitochondrial ribosomal protein S27	-2.372±0.236	2.62 × 10 ⁻²³	1.05 × 10 ⁻²⁰
3032	HADHB	hydroxyacyl-CoA dehydrogenase trifunctional multienzyme complex subunit beta	-1.385±0.140	6.07 × 10 ⁻²³	2.37 × 10 ⁻²⁰
309	ANXA6	annexin A6	-1.379±0.140	8.71 × 10 ⁻²³	3.34 × 10 ⁻²⁰
3006	H1-2	H1.2 linker histone, cluster member	-2.927±0.292	8.89 × 10 ⁻²³	3.34 × 10 ⁻²⁰
4538	ND4	NADH dehydrogenase subunit 4	2.711±0.275	9.77 × 10 ⁻²³	3.61 × 10 ⁻²⁰
10541	ANP32B	acidic nuclear phosphoprotein 32 family member B	2.222±0.227	1.09 × 10 ⁻²²	3.95 × 10 ⁻²⁰
5430	POLR2A	RNA polymerase II subunit A	1.908±0.195	1.12 × 10 ⁻²²	3.99 × 10 ⁻²⁰
23521	RPL13A	ribosomal protein L13a	-1.510±0.153	1.20 × 10 ⁻²²	4.19 × 10 ⁻²⁰
150082	LCA5L	lebercilin LCA5 like	3.067±0.311	1.30 × 10 ⁻²²	4.46 × 10 ⁻²⁰
284459	ZNF875	zinc finger protein 875	-2.032±0.206	1.43 × 10 ⁻²²	4.82 × 10 ⁻²⁰
10106	CTDSP2	CTD small phosphatase 2	-1.443±0.148	1.80 × 10 ⁻²²	5.96 × 10 ⁻²⁰
195828	ZNF367	zinc finger protein 367	1.524±0.157	2.30 × 10 ⁻²²	7.51 × 10 ⁻²⁰
37	ACADVL	acyl-CoA dehydrogenase very long chain	-1.642±0.169	2.55 × 10 ⁻²²	8.21 × 10 ⁻²⁰
6136	RPL12	ribosomal protein L12	-1.723±0.177	2.75 × 10 ⁻²²	8.68 × 10 ⁻²⁰
29896	TRA2A	transformer 2 alpha homolog	1.381±0.143	3.66 × 10 ⁻²²	1.14 × 10 ⁻¹⁹
2137	EXTL3	exostosin like glycosyltransferase 3	-2.093±0.216	4.63 × 10 ⁻²²	1.42 × 10 ⁻¹⁹
8367	H4C5	H4 clustered histone 5	-2.407±0.246	4.89 × 10 ⁻²²	1.48 × 10 ⁻¹⁹
389247	ING2-DT	ING2 divergent transcript	4.380±0.451	1.07 × 10 ⁻²¹	3.17 × 10 ⁻¹⁹
2030	SLC29A1	solute carrier family 29 member 1 (Augustine blood group)	-2.442±0.253	1.28 × 10 ⁻²¹	3.75 × 10 ⁻¹⁹
4190	MDH1	malate dehydrogenase 1	-1.312±0.137	1.44 × 10 ⁻²¹	4.16 × 10 ⁻¹⁹
6774	STAT3	signal transducer and activator of transcription 3	-1.334±0.140	1.71 × 10 ⁻²¹	4.87 × 10 ⁻¹⁹
7314	UBB	ubiquitin B	-1.216±0.128	1.87 × 10 ⁻²¹	5.24 × 10 ⁻¹⁹

ENTREZID	SYMBOL	GENENAME	Log2 Fold Change	Raw P-value	Adjusted P-value
5702	PSMC3	proteasome 26S subunit, ATPase 3	-2.243±0.234	2.05 × 10 ⁻²¹	5.68 × 10 ⁻¹⁹
54892	NCAPG2	non-SMC condensin II complex subunit G2	1.680±0.176	2.13 × 10 ⁻²¹	5.81 × 10 ⁻¹⁹
10476	ATP5PD	ATP synthase peripheral stalk subunit d	-2.236±0.235	4.50 × 10 ⁻²¹	1.21 × 10 ⁻¹⁸
55215	FANCI	FA complementation group I	1.595±0.169	5.33 × 10 ⁻²¹	1.42 × 10 ⁻¹⁸
1072	CFL1	cofilin 1	-1.465±0.156	9.57 × 10 ⁻²¹	2.51 × 10 ⁻¹⁸
3073	HEXA	hexosaminidase subunit alpha	-2.262±0.238	1.60 × 10 ⁻²⁰	4.15 × 10 ⁻¹⁸
5518	PPP2R1A	protein phosphatase 2 scaffold subunit Aalpha	-2.462±0.265	3.05 × 10 ⁻²⁰	7.78 × 10 ⁻¹⁸
284361	EMC10	ER membrane protein complex subunit 10	2.196±0.239	3.19 × 10 ⁻²⁰	8.04 × 10 ⁻¹⁸
161497	STRC	stereocilin	3.670±0.394	4.00 × 10 ⁻²⁰	9.83 × 10 ⁻¹⁸
1327	COX4I1	cytochrome c oxidase subunit 4I1	-2.252±0.244	3.95 × 10 ⁻²⁰	9.83 × 10 ⁻¹⁸
105378510	NA	NA	3.944±0.427	5.93 × 10 ⁻²⁰	1.42 × 10 ⁻¹⁷
9168	TMSB10	thymosin beta 10	-1.659±0.181	5.97 × 10 ⁻²⁰	1.42 × 10 ⁻¹⁷
27000	DNAJC2	DnaJ heat shock protein family (Hsp40) member C2	1.434±0.157	5.90 × 10 ⁻²⁰	1.42 × 10 ⁻¹⁷
4509	ATP8	ATP synthase F0 subunit 8	2.997±0.329	1.22 × 10 ⁻¹⁹	2.85 × 10 ⁻¹⁷
8031	NCOA4	nuclear receptor coactivator 4	-1.605±0.177	1.59 × 10 ⁻¹⁹	3.68 × 10 ⁻¹⁷
4539	ND4L	NADH dehydrogenase subunit 4L	2.440±0.269	1.67 × 10 ⁻¹⁹	3.83 × 10 ⁻¹⁷
6567	SLC16A2	solute carrier family 16 member 2	-2.574±0.283	2.21 × 10 ⁻¹⁹	5.00 × 10 ⁻¹⁷
10916	MAGED2	MAGE family member D2	-1.939±0.215	2.78 × 10 ⁻¹⁹	6.22 × 10 ⁻¹⁷
220869	ZNG1E	Zn regulated GTPase metalloprotein activator 1E	1.305±0.146	3.15 × 10 ⁻¹⁹	6.97 × 10 ⁻¹⁷
6155	RPL27	ribosomal protein L27	-1.251±0.140	4.26 × 10 ⁻¹⁹	9.33 × 10 ⁻¹⁷
4540	ND5	NADH dehydrogenase subunit 5	2.769±0.308	4.59 × 10 ⁻¹⁹	9.94 × 10 ⁻¹⁷
2934	GSN	gelsolin	-1.993±0.223	5.23 × 10 ⁻¹⁹	1.12 × 10 ⁻¹⁶
343099	CCDC18	coiled-coil domain containing 18	1.693±0.190	6.03 × 10 ⁻¹⁹	1.28 × 10 ⁻¹⁶
6141	RPL18	ribosomal protein L18	-2.350±0.260	6.74 × 10 ⁻¹⁹	1.41 × 10 ⁻¹⁶

ENTREZID	SYMBOL	GENENAME	Log2 Fold Change	Raw P-value	Adjusted P-value
8508	NIPSNAP1	nipsnap homolog 1	-2.286±0.254	6.85 × 10 ⁻¹⁹	1.42 × 10 ⁻¹⁶
6185	RPN2	ribophorin II	-1.588±0.178	6.96 × 10 ⁻¹⁹	1.43 × 10 ⁻¹⁶
284695	ZNF326	zinc finger protein 326	1.614±0.182	8.01 × 10 ⁻¹⁹	1.63 × 10 ⁻¹⁶
1938	EEF2	eukaryotic translation elongation factor 2	-0.928±0.105	9.17 × 10 ⁻¹⁹	1.85 × 10 ⁻¹⁶
8899	PRPF4B	pre-mRNA processing factor 4B	1.401±0.159	9.67 × 10 ⁻¹⁹	1.93 × 10 ⁻¹⁶

Table S7: Top 24 differentially expressed genes LVNC vs DCM1.

ENTREZID	SYMBOL	GENENAME	Log2 Fold Change	Raw P-value	Adjusted P-value
8287	USP9Y	ubiquitin specific peptidase 9 Y-linked	-7.291±0.597	1.28 × 10 ⁻²¹	2.44 × 10 ⁻¹⁷
8653	DDX3Y	DEAD-box helicase 3 Y-linked	-7.411±0.607	2.42 × 10 ⁻²⁰	2.30 × 10 ⁻¹⁶
7404	UTY	ubiquitously transcribed tetratricopeptide repeat containing, Y-linked	-6.797±0.650	1.94 × 10 ⁻¹⁶	1.23 × 10 ⁻¹²
6192	RPS4Y1	ribosomal protein S4 Y-linked 1	-6.183±0.666	2.64 × 10 ⁻¹⁴	1.26 × 10 ⁻¹⁰
8284	KDM5D	lysine demethylase 5D	-6.096±0.671	4.93 × 10 ⁻¹⁴	1.87 × 10 ⁻¹⁰
9086	EIF1AY	eukaryotic translation initiation factor 1A Y-linked	-6.069±0.676	7.59 × 10 ⁻¹⁴	2.41 × 10 ⁻¹⁰
7544	ZFY	zinc finger protein Y-linked	-5.719±0.685	8.24 × 10 ⁻¹³	2.24 × 10 ⁻⁹
84171	LOXL4	lysyl oxidase like 4	-3.285±0.494	3.87 × 10 ⁻¹¹	9.21 × 10 ⁻⁸
246126	TXLNGY	taxilin gamma Y-linked (pseudogene)	-5.205±0.704	5.54 × 10 ⁻¹¹	1.17 × 10 ⁻⁷
64595	TTY15	testis expressed transcript, Y-linked 15	-4.786±0.723	8.51 × 10 ⁻¹⁰	1.62 × 10 ⁻⁶
22829	NLGN4Y	neuroligin 4 Y-linked	-3.967±0.672	1.08 × 10 ⁻⁸	1.86 × 10 ⁻⁵
27063	ANKRD1	ankyrin repeat domain 1	-2.349±0.445	1.52 × 10 ⁻⁷	2.41 × 10 ⁻⁴
4633	MYL2	myosin light chain 2	-2.291±0.437	1.82 × 10 ⁻⁷	2.67 × 10 ⁻⁴
347273	CAVIN4	caveolae associated protein 4	-2.168±0.416	2.07 × 10 ⁻⁷	2.82 × 10 ⁻⁴
378951	RBM1Y1J	RNA binding motif protein Y-linked family 1 member J	-3.824±0.754	2.70 × 10 ⁻⁷	3.42 × 10 ⁻⁴
1641	DCX	doublecortin	3.018±0.662	2.24 × 10 ⁻⁶	2.66 × 10 ⁻³
5940	RBM1Y1A1	RNA binding motif protein Y-linked family 1 member A1	-3.279±0.765	3.71 × 10 ⁻⁶	4.15 × 10 ⁻³
1264	CNN1	calponin 1	-2.009±0.449	8.88 × 10 ⁻⁶	9.39 × 10 ⁻³
1674	DES	desmin	-2.452±0.575	1.73 × 10 ⁻⁵	1.62 × 10 ⁻²
728403	TSPY8	testis specific protein Y-linked 8	-3.043±0.768	1.71 × 10 ⁻⁵	1.62 × 10 ⁻²
378949	RBM1Y1D	RNA binding motif protein Y-linked family 1 member D	-3.011±0.768	1.79 × 10 ⁻⁵	1.62 × 10 ⁻²
392197	USP17L7	ubiquitin specific peptidase 17 like family member 7	-2.554±0.764	5.49 × 10 ⁻⁵	4.75 × 10 ⁻²
57502	NLGN4X	neuroligin 4 X-linked	2.761±0.719	6.12 × 10 ⁻⁵	4.92 × 10 ⁻²

ENTREZID	SYMBOL	GENENAME	Log2 Fold Change	Raw P-value	Adjusted P-value
100289087	TSPY10	testis specific protein Y-linked 10	-2.649±0.769	6.20×10^{-5}	4.92×10^{-2}

Table S8: KEGG GOterm hits for Top100 differentially expressed genes LVNC vs resLVNC.

ID	Term	Ontology Source	% Associate No. Genes	No. Of Genes	Associated Genes Found
KEGG:04512	ECM-receptor interaction	KEGG_13.05.2021	6.82	6	COL4A2, ITGA2, ITGA7, ITGB5, LAMB2, THBS1
KEGG:05410	Hypertrophic cardiomyopathy	KEGG_13.05.2021	5.56	5	ITGA2, ITGA7, ITGB5, MYBPC3, TPM1
KEGG:05414	Dilated cardiomyopathy	KEGG_13.05.2021	6.25	6	ADCY6, ITGA2, ITGA7, ITGB5, MYBPC3, TPM1

Table S9: KEGG GOterm hits for Top100 differentially expressed genes DCM1 vs resDCM1.

ID	Term	Ontology Source	% Associate No. Genes	No. Of Genes	Associated Genes Found
KEGG:00010	Glycolysis/ Gluconeogenesis	KEGG_13.05.2021	4.48	3	ALDOC, LDHB, PFKM
KEGG:03010	Ribosome	KEGG_13.05.2021	8.23	13	RPL10, RPL12, RPL13A, RPL18, RPL19, RPL27, RPL3, RPL7A, RPL8, RPLP0, RPS14, RPS8, RPSA
KEGG:05171	Coronavirus	KEGG_13.05.2021	6.03	14	RPL10, RPL12, RPL13A, RPL18, RPL19, RPL27, RPL3, RPL7A, RPL8, RPLP0, RPS14, RPS8, RPSA, STAT3
KEGG:00190	Oxidative Phosphorylation	KEGG_13.05.2021	9.77	13	ATP5F1A, ATP5F1B, ATP5MC2, ATP5PD, ATP8, COX4I1, COX6B1, COX6C, ND4, ND4L, ND5, ND6, NDUFS2
KEGG:04714	Thermogenesis	KEGG_13.05.2021	5.60	13	ATP5F1A, ATP5F1B, ATP5MC2, ATP5PD, ATP8, COX4I1, COX6B1, COX6C, ND4, ND4L, ND5, ND6, NDUFS2
KEGG:05010	Alzheimer	KEGG_13.05.2021	4.07	15	ATP5F1A, ATP5F1B, ATP5MC2, ATP5PD, ATP8, COX4I1, COX6B1, COX6C, CTNNB1, ND4, ND4L, ND5, ND6, NDUFS2, PSMC3
KEGG:05012	Parkinson	KEGG_13.05.2021	6.43	16	ATP5F1A, ATP5F1B, ATP5MC2, ATP5PD, ATP8, COX4I1, COX6B1, COX6C, ND4, ND4L, ND5, ND6, NDUFS2, PSMC3, UBB, UBC
KEGG:05014	Amyotrophic lateral sclerosis	KEGG_13.05.2021	4.12	15	ATP5F1A, ATP5F1B, ATP5MC2, ATP5PD, ATP8, COX4I1, COX6B1, COX6C, ND4, ND4L, ND5, ND6, NDUFS2, PSMC3, VCP
KEGG:05016	Huntington	KEGG_13.05.2021	4.9	15	ATP5F1A, ATP5F1B, ATP5MC2, ATP5PD, ATP8, COX4I1, COX6B1, COX6C, ND4, ND4L, ND5, ND6,

					NDUFS2, POLR2A, PSMC3
KEGG:05020	Prion disease	KEGG_13.05.2021	5.13	14	ATP5F1A, ATP5F1B, ATP5MC2, ATP5PD, ATP8, COX4I1, COX6B1, COX6C, ND4, ND4L, ND5, ND6, NDUFS2, PSMC3
KEGG:05415	Diabetic cardiomyopathy	KEGG_13.05.2021	6.4	13	ATP5F1A, ATP5F1B, ATP5MC2, ATP5PD, ATP8, COX4I1, COX6B1, COX6C, ND4, ND4L, ND5, ND6, NDUFS2

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