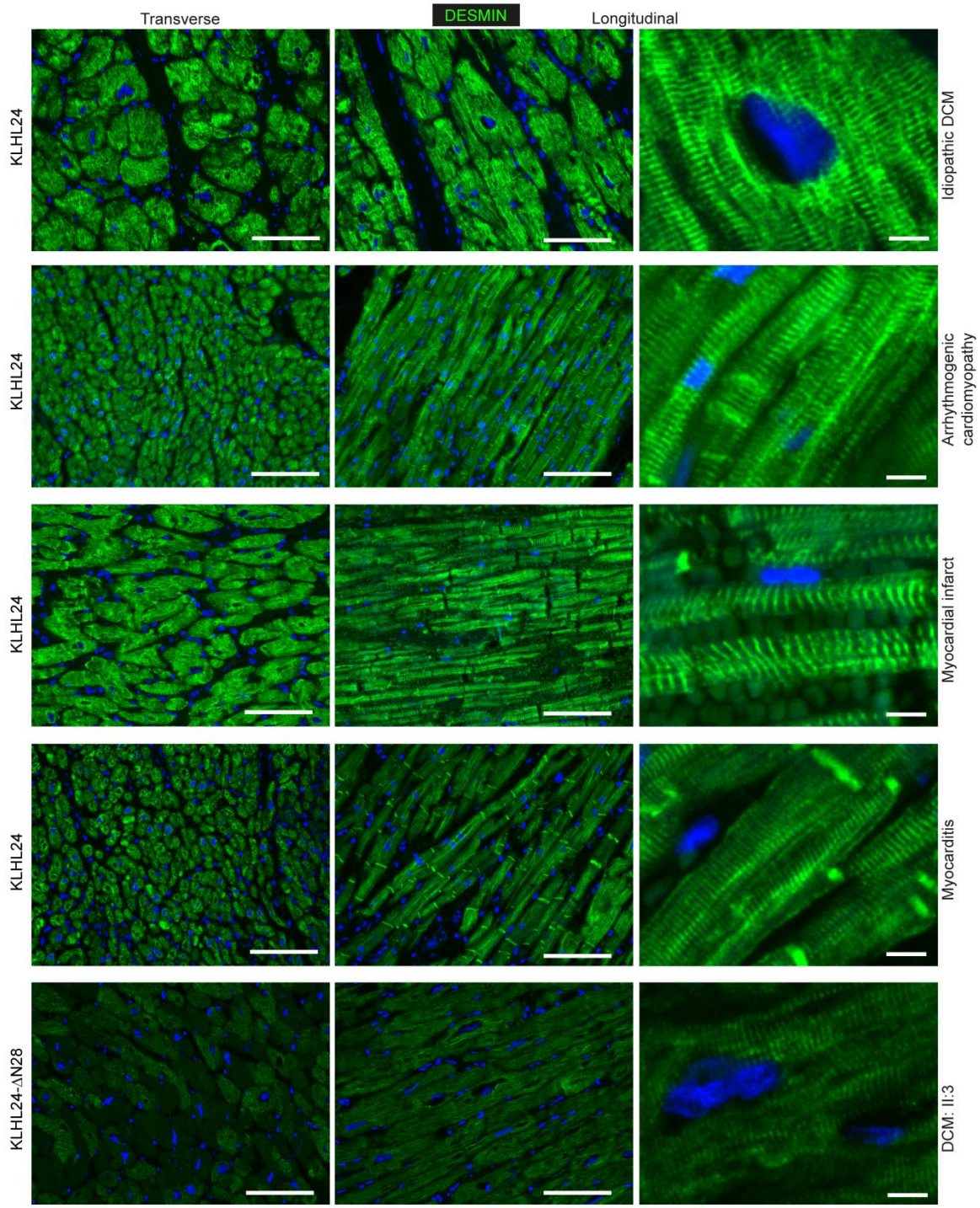
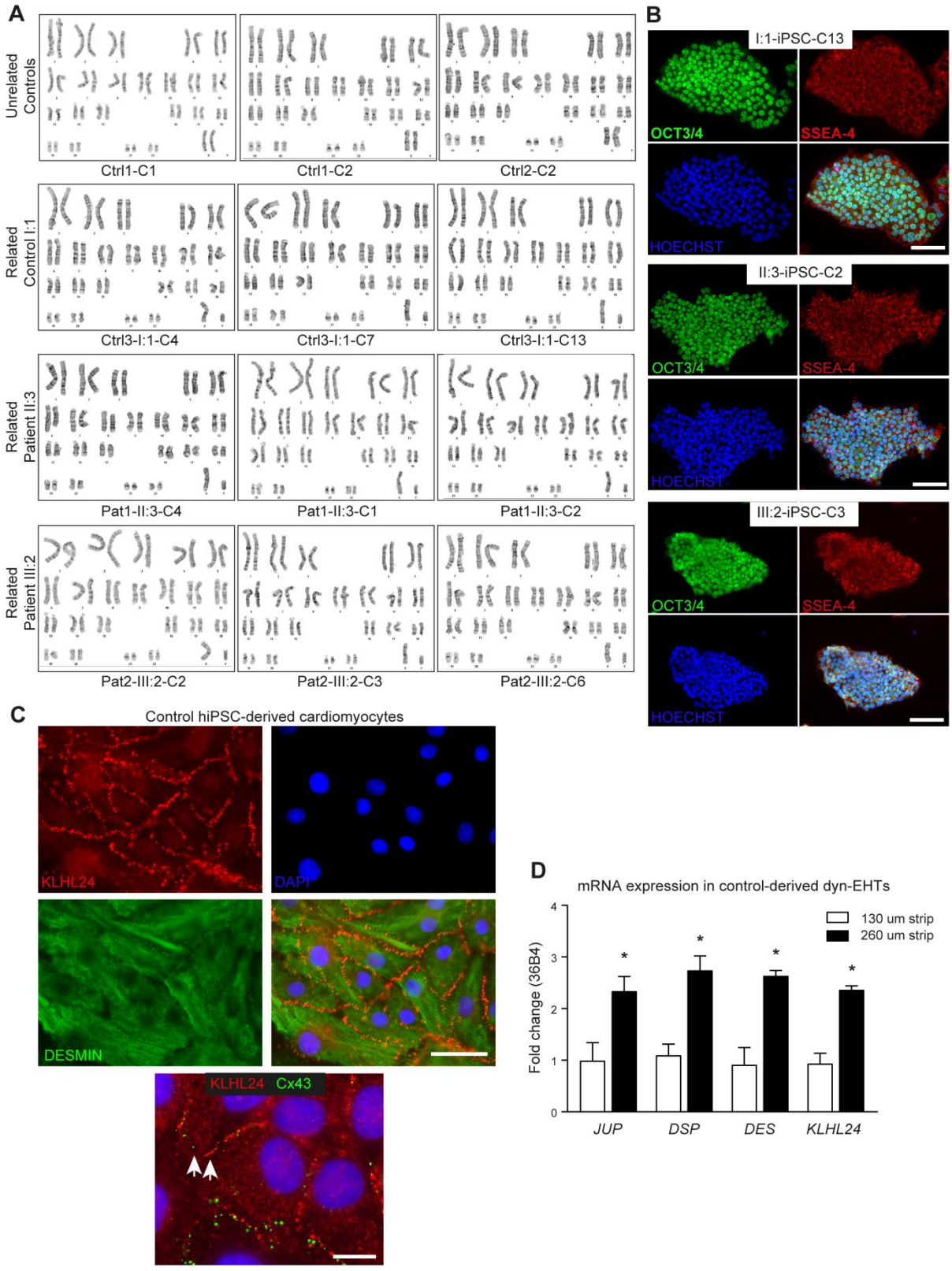
SUPPLEMENTAL FIGURES AND FIGURE LEGENDS

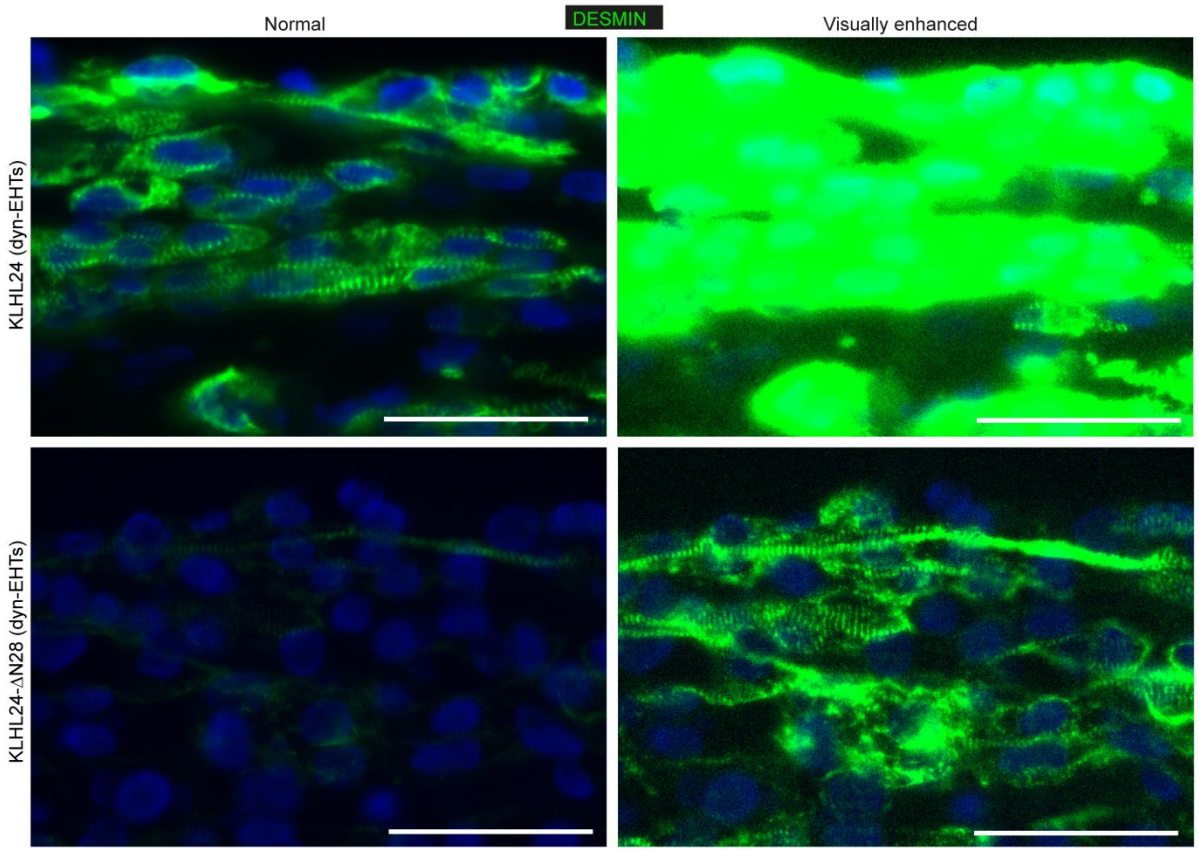


Supplemental Figure 1: Overview of all explanted heart stainings with desmin. IFA of desmin in cardiomyocytes of human explanted hearts with heart failure of different etiologies including patient II:3, both in the transverse (left) and the longitudinal (middle) direction. Part of these images are also depicted in Figure 2A. These scales bars are 50 µm. On the right, a zoom-in of the longitudinal section is depicted, showing the sarcomeric striations of desmin. These scale bars are 5 µm.

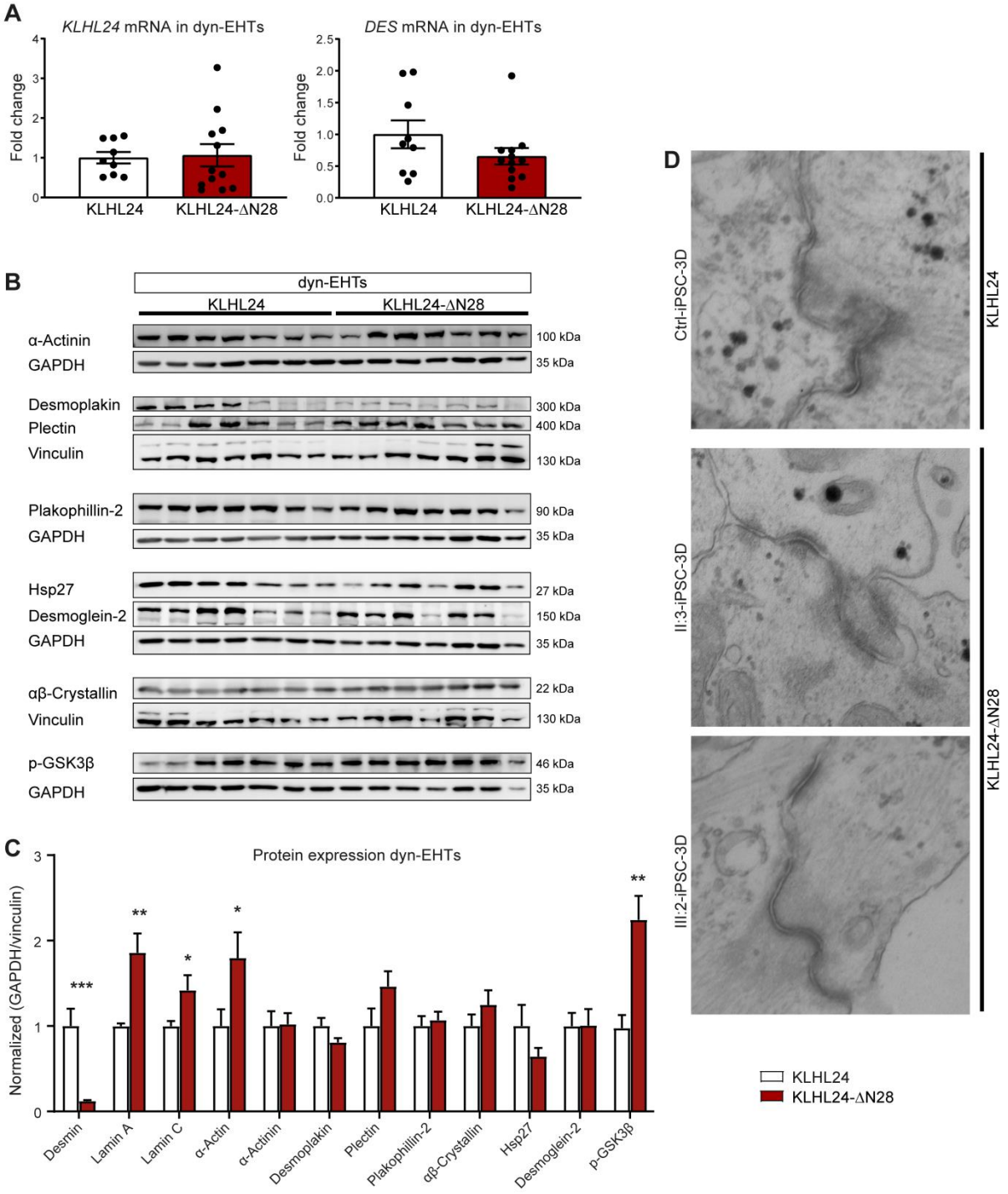


Supplemental Figure 2: Validation op hiPSC lines and KLHL24 expression in control cardiomyocytes/dyn-EHTs A) Karyotype of all hiPSC lines used in this study. "C" stands for different clonal lines. **B)** Representative IFA of pluripotent markers, OCT3/4 and SSEA-4 in hiPSC lines generated of the three

family members included in this study. Scale bars are 50 µm. **C)** IFA shows co-labeling of KLHL24, desmin and Cx43 in 2D cultured control cardiomyocytes. Scale bars are 50 µm. The arrows point towards their localization at the cell periphery where the scale bar is 10 µm. **D)** mRNA expression of genes encoding plakoglobin (*JUP*), desmoplakin (*DSP*), desmin (*DES*) and KLHL24 (*KLHL24*) in control-derived dyn-EHTs loaded with 260 vs. 130 µm strips. n=3 tissues/group; *p<0.05 (Mann Whitney U test compared to 130 µm strip dyn-EHTs).



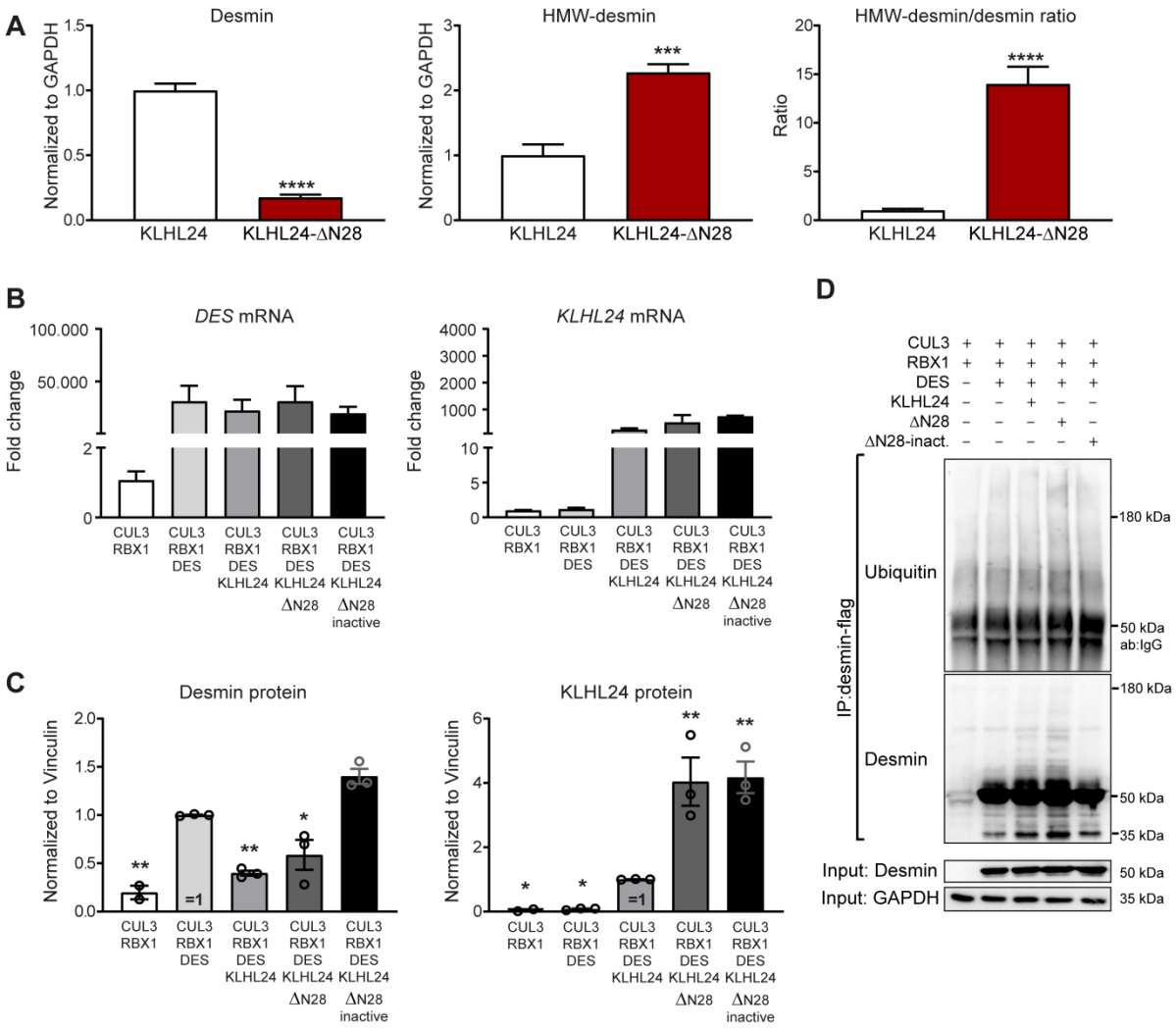
Supplemental Figure 3: Desmin network assessment in dyn-EHTs. IFA labeling of desmin in dyn-EHTs. The left panel depicts zoom-in images of the desmin network where the upper image is representative for control and the lower image for patient-derived dyn-EHTs. The right panel shows these same images, using the same settings, but with visual enhancements (increased contrast and brightness). Now in patient-derived tissues, the entire desmin network can be better observed and although it is 10-fold less extensive, the structure seems to be well preserved. Scale bars are 50 µm.



Supplemental Figure 4: Analysis of patient-derived dyn-EHTs.

A) mRNA expression of *KLHL24* and *DES* in patient compared to control-derived dyn-EHTs. (n=9 of control vs. n=12 for patient-derived dyn-EHTs). **B)** Western blots of several cardiac markers, binding partners of desmin and other desminopathy-associated proteins in dyn-EHTs. **C)** Quantified protein levels of blots depicted in panel B and Figure 2, where some blots of panel B are derived from the same gels as Figure 2 (n=7 of control and patient-derived dyn-EHTs). *p<0.05; **p<0.01; ***p<0.001; (unpaired *T*-test, between patient and control-

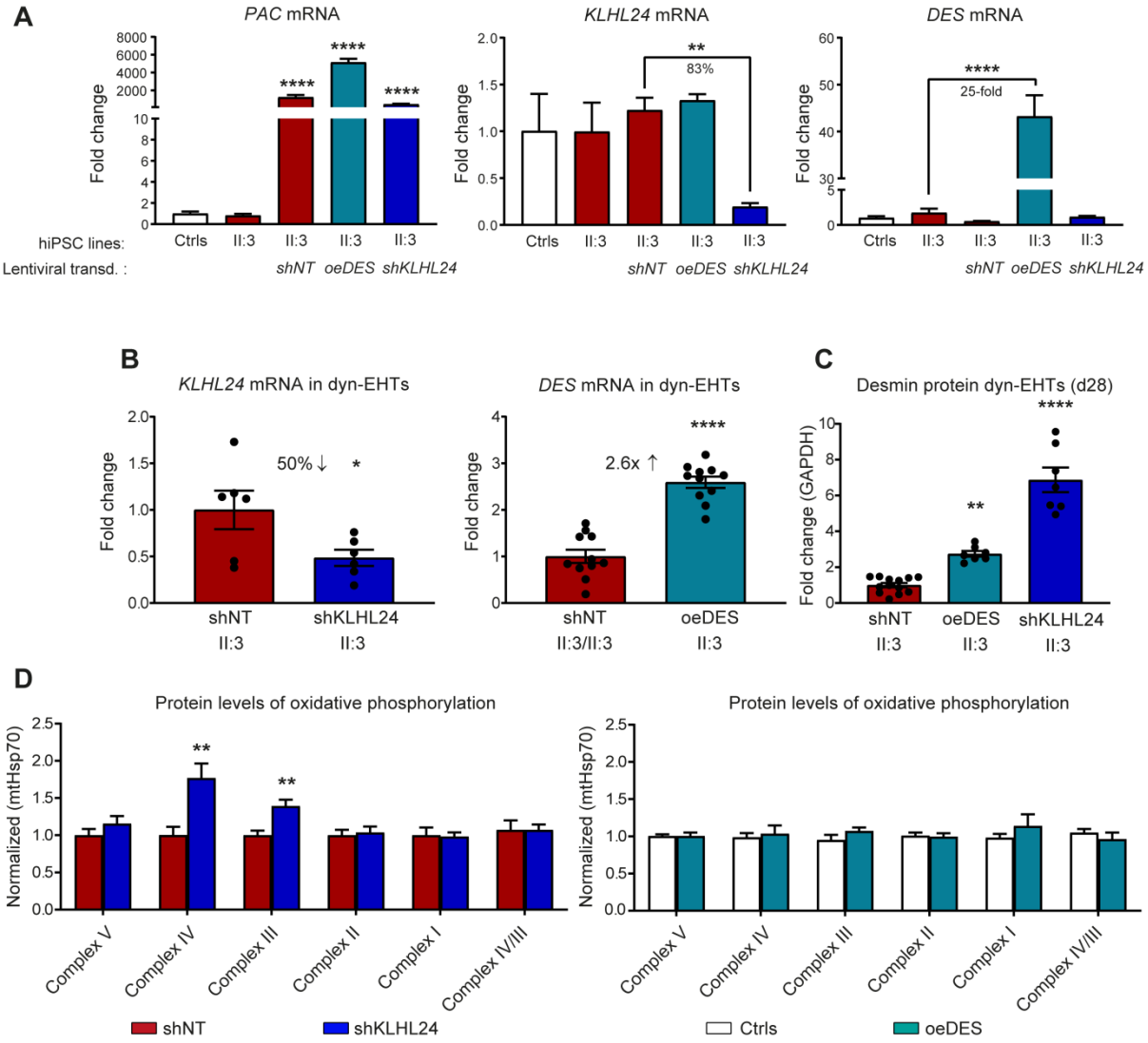
derived dyn-EHTs). **D)** Overview TEM images of control and patient-derived dyn-EHTs at day 28, emphasizing normally preserved intercalated disc structures.



Supplemental Figure 5: Desmin ubiquitination quantification in dyn-EHTs and additional data from HEK293A transfection studies.

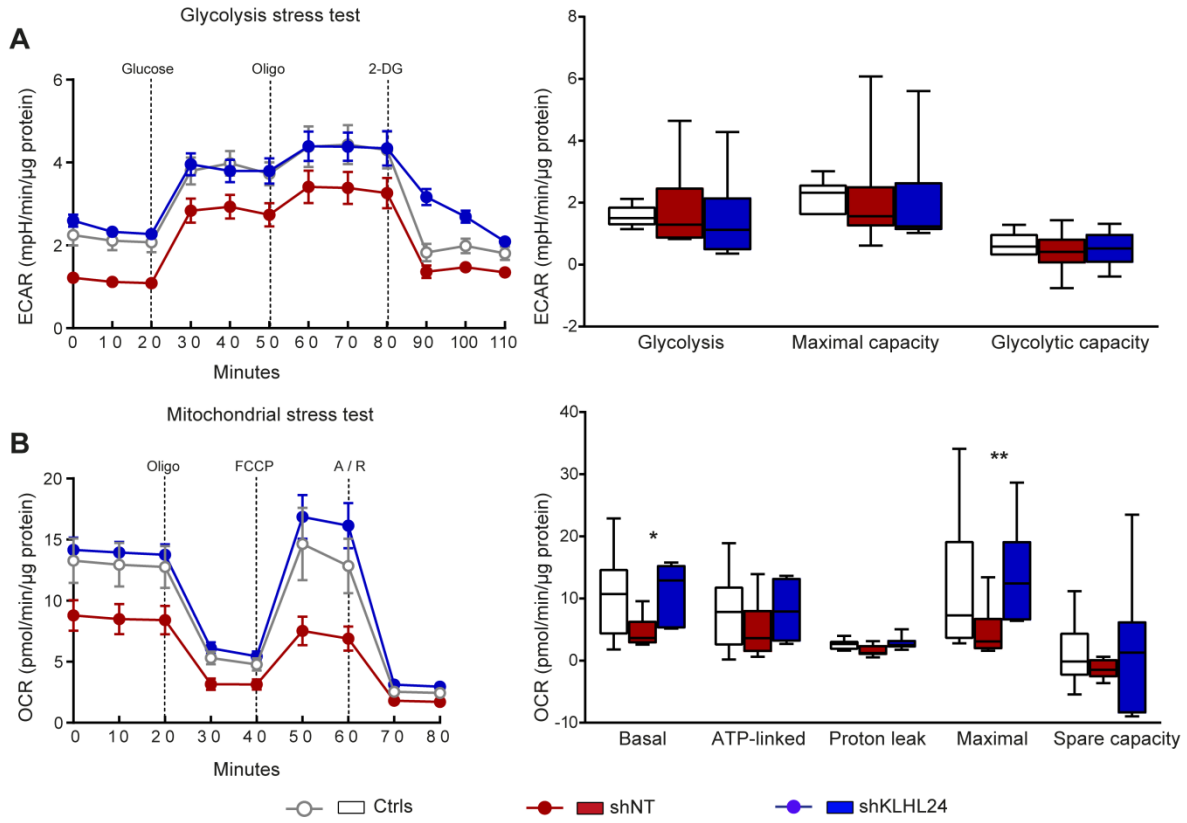
A) Quantification of desmin and high molecular weight (HMW) desmin protein levels, including their ratio, in dyn-EHTs. n=7/group; ***p<0.001 (Mann Whitney U test, compared to control-derived dyn-EHTs); ****p<0.0001 (Mann Whitney U test, compared to control-derived dyn-EHTs). **B)** mRNA expression of *DES* and *KLHL24*, 48 hours post-transfection of HEK-cells (n=3 experiments), (desmin:KLHL24> 1:1 transfection ratio). **C)** Quantified protein levels of desmin and KLHL24, 48 hours post-transfection of HEK-cells (n=3 experiments), (desmin:KLHL24> 1:1 transfection ratio). Differences in transfection efficiency were normalized by setting the baseline of each experiment at 1. ("=1" in graph represents the baseline); *p<0.05 (1-way ANOVA on desmin protein, compared to co-transfection of RBX1, CUL3 and DES);**p<0.01 (compared to co-transfection of RBX1, CUL3 and DES); *p<0.05 (1-way ANOVA on KLHL24 protein, compared to co-transfection of RBX1, CUL3, DES and KLHL24);**p<0.01 (compared to co-transfection of RBX1, CUL3, DES

and KLHL24). **D)** Separate western blots of desmin (Y66; rabbit) and ubiquitin (FK2; mouse) on desmin immunoprecipitation fractions of transfected HEK293A cells, using a flag-tagged desmin plasmid (IP antibody anti-flag M2; mouse), (desmin:KLHL24> 5:1 transfection ratio). The input levels used for the IP are depicted below on a separate gel, containing desmin and GAPDH. ab IgG> refers to the heavy-chain band of to the antibody used for immunoprecipitation.



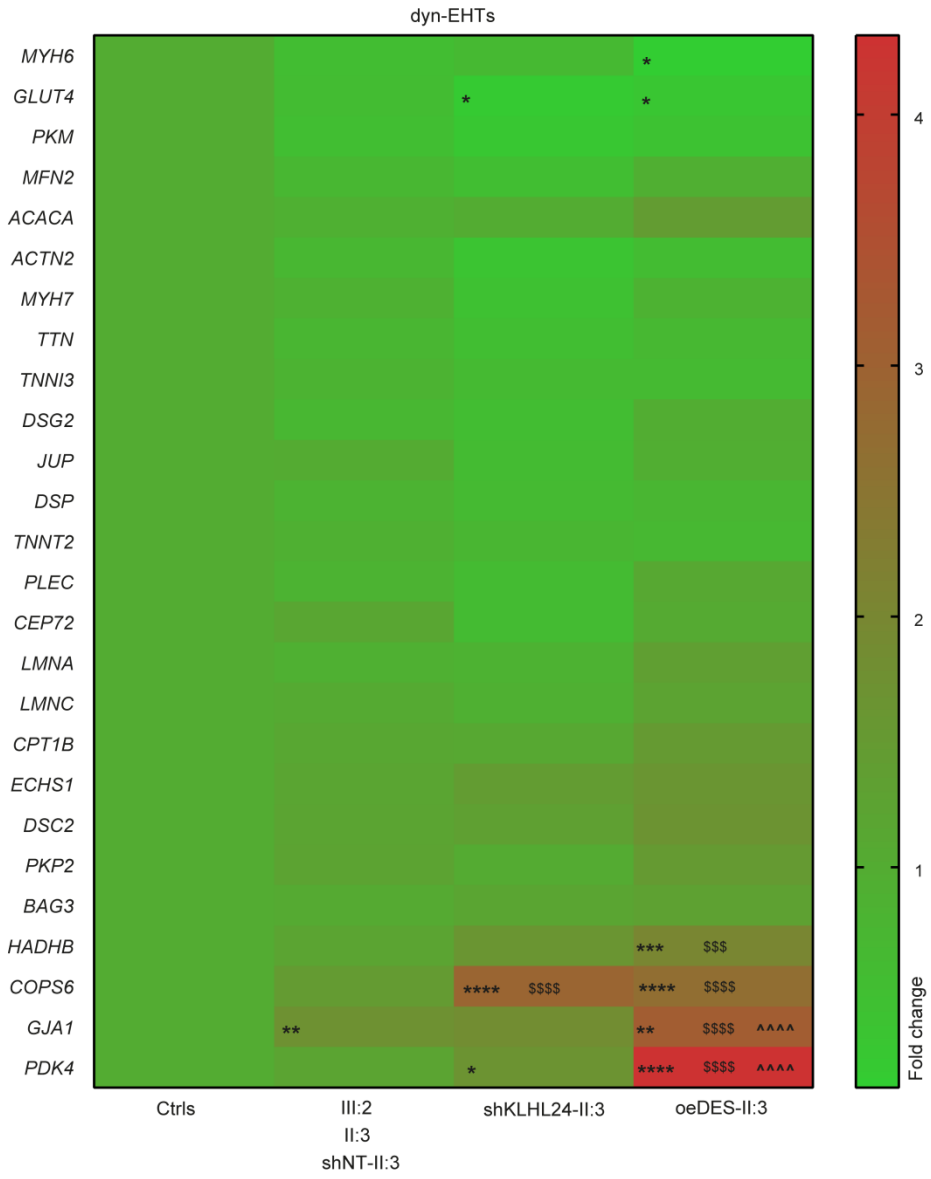
Supplemental Figure 6: Quantified mRNA and protein levels in both rescue models

A) mRNA expression of *PAC* (puromycin), *KLHL24* and *DES* in hiPSC after lentiviral transduction and puromycin selection. (n=3/group); ****p<0.0001(1-way ANOVA of *PAC* expression, compared to both control and non-transduced II:3 hiPSC); **p<0.01 (Unpaired *T*-test, shKLHL24 vs. shNT-II:3-hiPSC); ****p<0.0001 (Unpaired *T*-test, oeDES-II:3 vs. non-transduced II:3-hiPSC); **B)** mRNA expression of *KLHL24* and *DES* in dyn-EHTs at day 28. n=6 for II:3 and 6 for shNT-II:3 group; n=6 for shKLHL24-II:3 group; n=11 for oeDES-II:3 group *p<0.05 (Unpaired *T*-test); ****p<0.0001 (Unpaired *T*-test). **C)** Desmin protein levels measured in dyn-EHTs at day 28. n=18 for shNT-II:3, II:3 and III:2 tissues combined, n=7 for oeDES and n=6 for shKLHL24-II:3 tissues; **p<0.01 (1-way ANOVA compared to shNT;II:3;III:2 patient-derived dyn-EHTs); ****p<0.0001 (compared to shNT;II-3;III:2 patient-derived dyn-EHTs). **D)** Quantified protein levels of OXPHOS complexes I-V in dyn-EHTs at day 28. n=7/group; **p<0.01 (Unpaired *T*-test).



Supplemental Figure 7: Seahorse analysis of patient-derived cardiomyocytes with RNAi of *KLHL24*.

A) The graph on the left shows the extracellular acidification rate (ECAR) of cardiomyocytes measured during the glycolysis stress test, depicted as collective data (n=7). The Ctrl reference is I:2-derived. The bar graph on the right represents the different fractions summarized. **B)** The left graph shows the oxygen consumption rate (OCR) measured during the mitochondrial stress test, depicted as collective data (n=7). The Ctrl reference is familial I:2-derived. The right graph represents the different fractions summarized. *p<0.05 (2-way ANOVA, post-hoc Uncorrected Fishers LSD test shNT vs. shKLHL24 condition); **p<0.01 (shNT and shKLHL24 condition).



Supplemental Figure 8: Heatmap of mRNA expression in dyn-EHTs

Heatmap containing mRNA expression of genes related to desmosomal proteins, sarcomeric proteins, metabolic enzymes and several desmin-related/binding proteins, measured in the different dyn-EHT groups. Statistical analysis performed using 2-way ANOVA, corrected for multiple comparisons using FDR Benjamini and Hochberg method. (*p<0.05; **p<0.01; ***p<0.001; ****p<0.0001 compared to Ctrls group); ($$$p<0.001 ; $$$$p<0.0001 compared to III:3; II:2; shNT group); (^^^^p<0.0001 compared to shKLHL24 group). n=9 for Ctrl group; n=18 for III:3; II:2; shNT-II:3 group; n=6 for shKLHL24-II:3 group; n=7 for oeDES-II:3 group.

Tabel S1: Immunolabeling				
Protein	**Clone or Cat. no.**	**Clonality**	**Supplier**	**Usage**
Actin	AC-15	monoclonal	Merck	WB
a-Actinin	EA-53	monoclonal	Sigma	WB
Actin	Phalloidin-rodamine	NA	Invitrogen	IF
cardiac Troponin T	ab45932	polyclonal	Abcam	IHC-P
Connexin 43	4E6.2	monoclonal	Sigma	IF
Desmin (epitope before the C-terminus)	Y-20	polyclonal	Santa Cruz	WB, IF, IHC-P
Desmin	DE-R-11	monoclonal	Abcam	IHC-P
Desmin (raised against whole protein)	RD301	monoclonal	Santa Cruz	IP
Desmin (C-terminus)	Y-66	Recombinant monoclonal	Abcam	WB
Ubiquitin (all)	FK2	Monoclonal	Merck	WB
Desmoglein 2	10G11	monoclonal	Progen	WB
Desmoplakin I&II	DP2.15	monoclonal	Abcam	WB
Desmoplakin-I (ROD)	DP2.17	monoclonal	Fitzgerald	WB, IF
FLAG-tag	M2	monoclonal	Merck	WB, IP
GAPDH	10R-G109a	monoclonal	Fitzgerald	WB
GSK3B (Ser9)	D3A4	monoclonal	Cell signalling	WB
HSP27	G3.1	monoclonal	Abcam	WB
KLHL24	ab104089	polyclonal	Abcam	IF
Lamin A/C	N18	polyclonal	Santa Cruz	WB
mtHSP70	JG1	monoclonal	Thermo Fisher	WB
OCT3/4	H-134	polyclonal	Santa Cruz	IF
OXPHOS (I-V)	ab110413	moAb Cocktail	Abcam	WB
-CI	ab110242	monoclonal	Abcam	WB
-CII	ab14714	monoclonal	Abcam	WB
-CIII	ab14745	monoclonal	Abcam	WB
-CIV	ab14705	monoclonal	Abcam	WB
-CV	ab14748	monoclonal	Abcam	WB
Plakoglobin	15F11	monoclonal	Abcam	WB, IF
Plakophillin 2	PP2/62/86/150	monoclonal	Progen	WB
Plectin	10F6	monoclonal	Santa Cruz	WB
SSEA-4	MC813	monoclonal	Invitrogen	IF
Vinculin	SPM227	monoclonal	Abcam	WB

1

Table S2: RT-PCR		
Gene	**Forward '5 - '3**	**Reverse '3 - '5**
ACACA	AGAGGGAACATCCCTACGCT	CGAAAAGAGACCATTCCGCC
ACTN2	AAGCACAAGCCACCCAAG	GCAGCGTGTTGAAGTTGATCT
BAG3	CCCATCGAGAAACTGCACCT	CCACCTCTTTGCGGATCACT
CEP72	CGACTCCAAAGAGAGCGTCC	CCACTCGCACTCTGCAATGA
COPS6	GTCCTCTACGACCGACAAGG	CCAACCAGTGTGGTGCCTAA
CPT1B	CTCCTTTCCTTGCTGAGGTG	TCTCGCCTGCAATCATGTAG
DES	CTGAGCAAAGGGGTTCTGAG	ACTTCATGCTGCTGCTGTGT
DSC2	CGGAGATTGTTGCGGTTGA	GGAAAGACGTGCTGCTGTATCA
DSG2	TCCACTATGCCACCAACCAC	GCTGGAGCATACACCCTCTC
DSP	CAGTGGTGTCAGCGATGATGT	TGACGCTGGATATGGTGGAA
ECHS1	ATCTATGCCGGTGAGAAGGC	GACAAGACCTGCTTGCTTGG
GAPDH	GCACCGTCAAGGCTGAGAAC	GTGGTGAAGACGCCAGTGGA
GJA1	GGAATGCAAGAGAGGTTGAAAG	GGCATTTGGAGAAACTGGTAGA
GLUT4	TAGGCTCCGAAGATGGGGAA	CCCAGCCACGTCTCATTGTA
HADHB	CCCAGCTGTCCAGACCAAAA	TCCGATGCAACAAACCCGTA
JUP	AGTAGCCACGATGGAGGTGA	AGGTGTATGTCTGCTGCCAC
KLHL24	GACTAGGCCACGCAGGTC	TCCAACTCGCTCACATCCTC
KLHL24-tr6-1453-1711	ATGCAGTCTGTGCTCTAAGG	CCATCATAGCCACCGACAAG
KLHL24-tr6-1692-1827	GTTGTCGGTGGCTATGATGG	GTTTGCCTACACAGCTAGTC
LMNA	GCTCTTCTGCCTCCAGTGTC	ACATGATGCTGCAGTTCTGG
LMNB	CTCAGTGACTGTGGTTGAGGA	AGTGCAGGCTCGGCCTC
MFN2	ATGCATCCCCACTTAAGCAC	CCAGAGGGCAGAACTTTGTC
MYH6	GATAGAGAGACTCCTGCGGC	TCGGTCATCTTGGTGCTTCC
MYH7	CGAAGGGCTTGAATGAGGAGT	TCCTCCCAAGGAGCTGTTAC
PDK4	CCTTTGGCTGGTTTTGGTTA	CCTGCTTGGGATACACCAGT
PKM	GTGGGGCCATAATCGTCCTC	GACGAGCTGTCTGGGGATTC
PKP2	GCAAATGGTTTGCTCGATTT	GGCTGGTAATCTGCAATGGT
PLEC	GCGCCTACTCCAAGTACCTC	GCTGTAGTAGCCCTTGGTGG
PPIA	ACTTCACACGCCATAATG	ACCCGTATGCTTTAGGAT
TNNI3	CCAACTACCGCGCTTATGC	CTCGCTCCAGCTCTTGCTTT
TNNT2	TGGAGGCAGAGAAGTTCGAC	CCTGTTTCGGAGAACATTGAT
TTN	CGTCAGAACCTCACGGTCAA	GGGGCGGGGTTTCATCTTTA

1