Online Supplementary Material

The EP300/TP53 Pathway, A Suppressor of the Hippo and Canonical WNT Pathways, is Activated in Human Hearts with Arrhythmogenic Cardiomyopathy in the Absence of Overt Heart Failure

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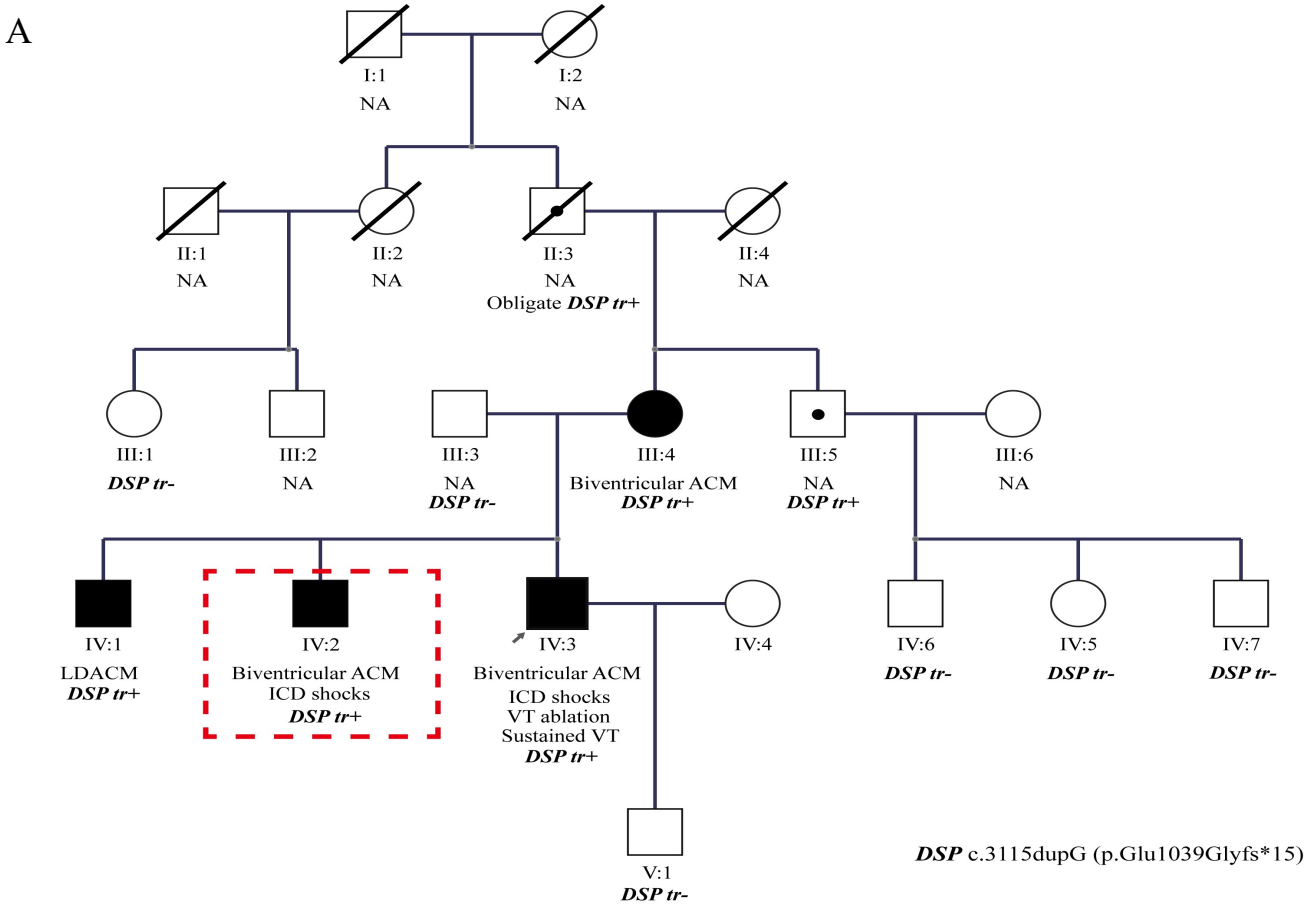
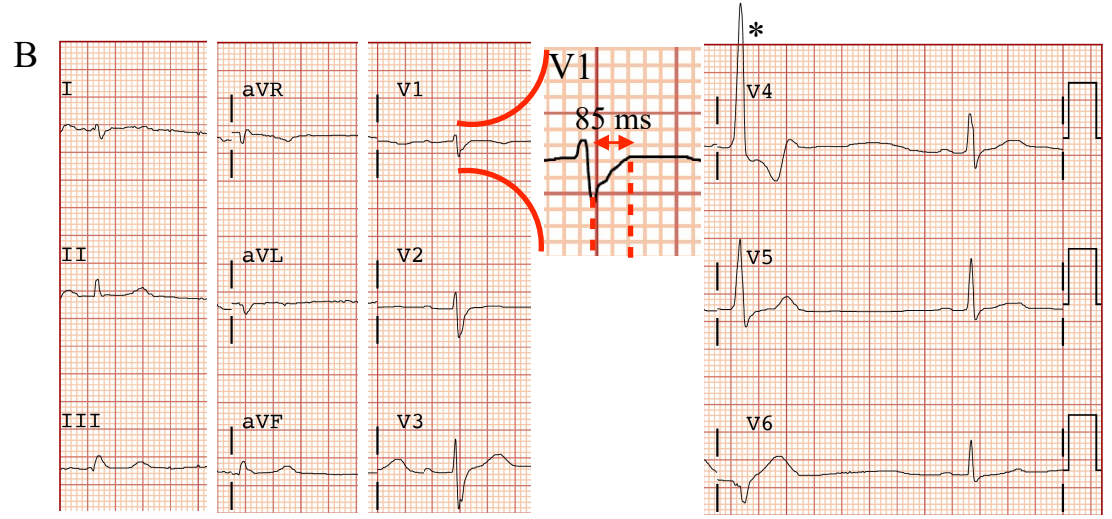
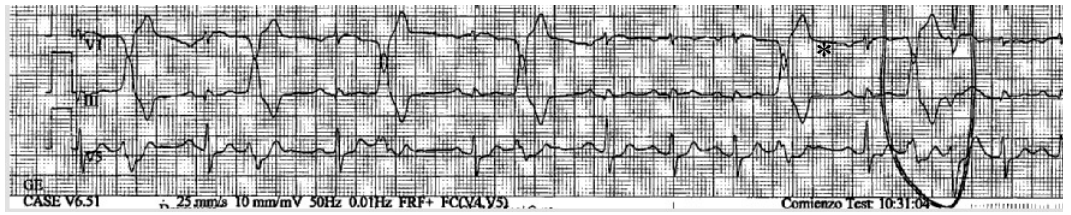
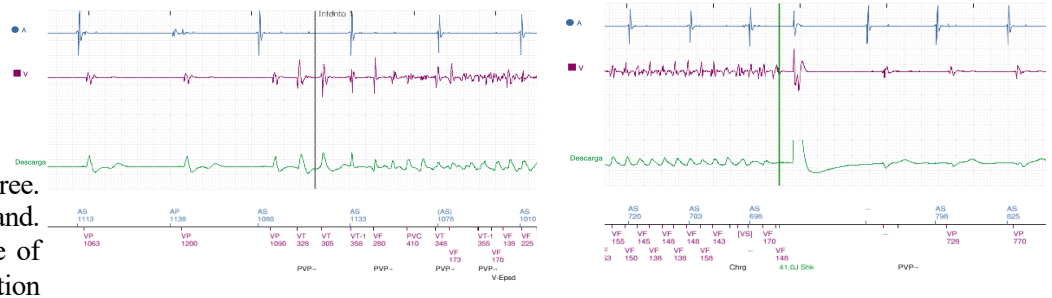
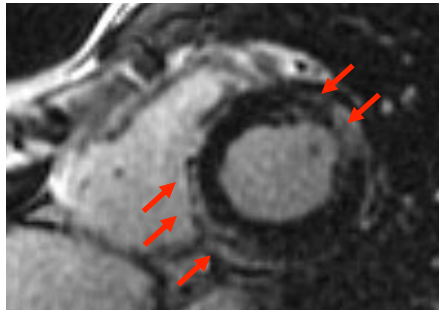
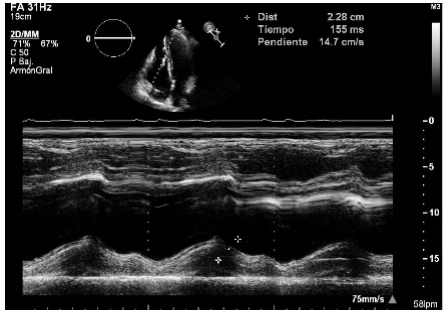








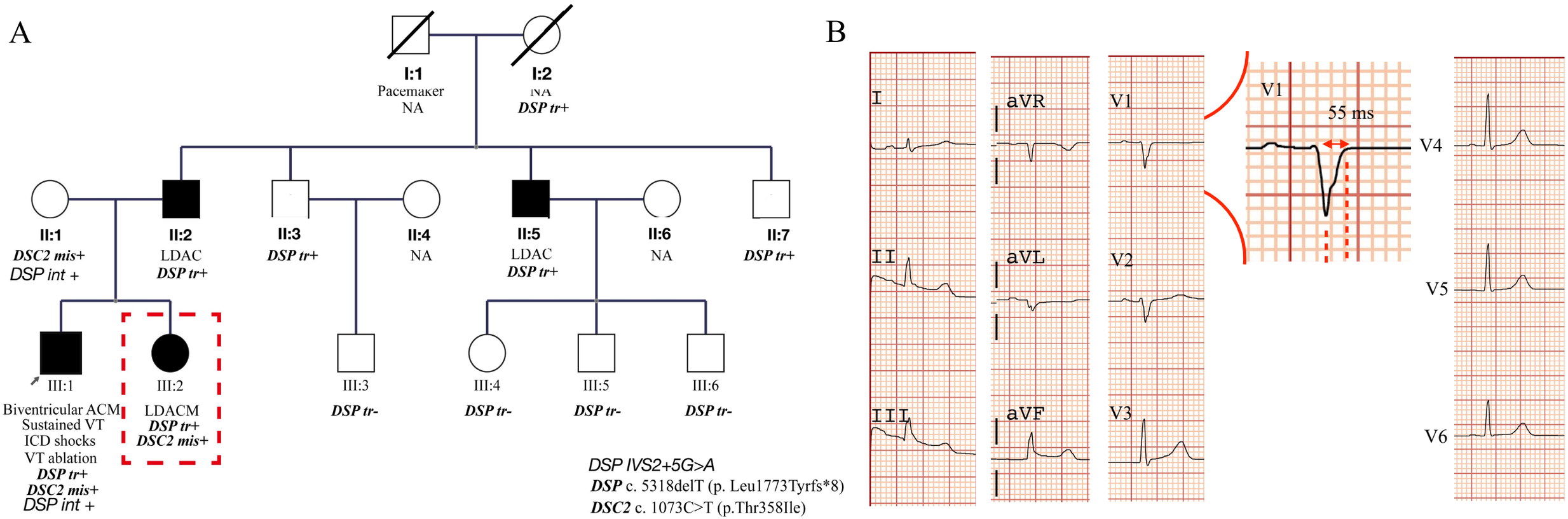




Online Figure 1: Phenotypic characteristics of patient 1 (discovery group)

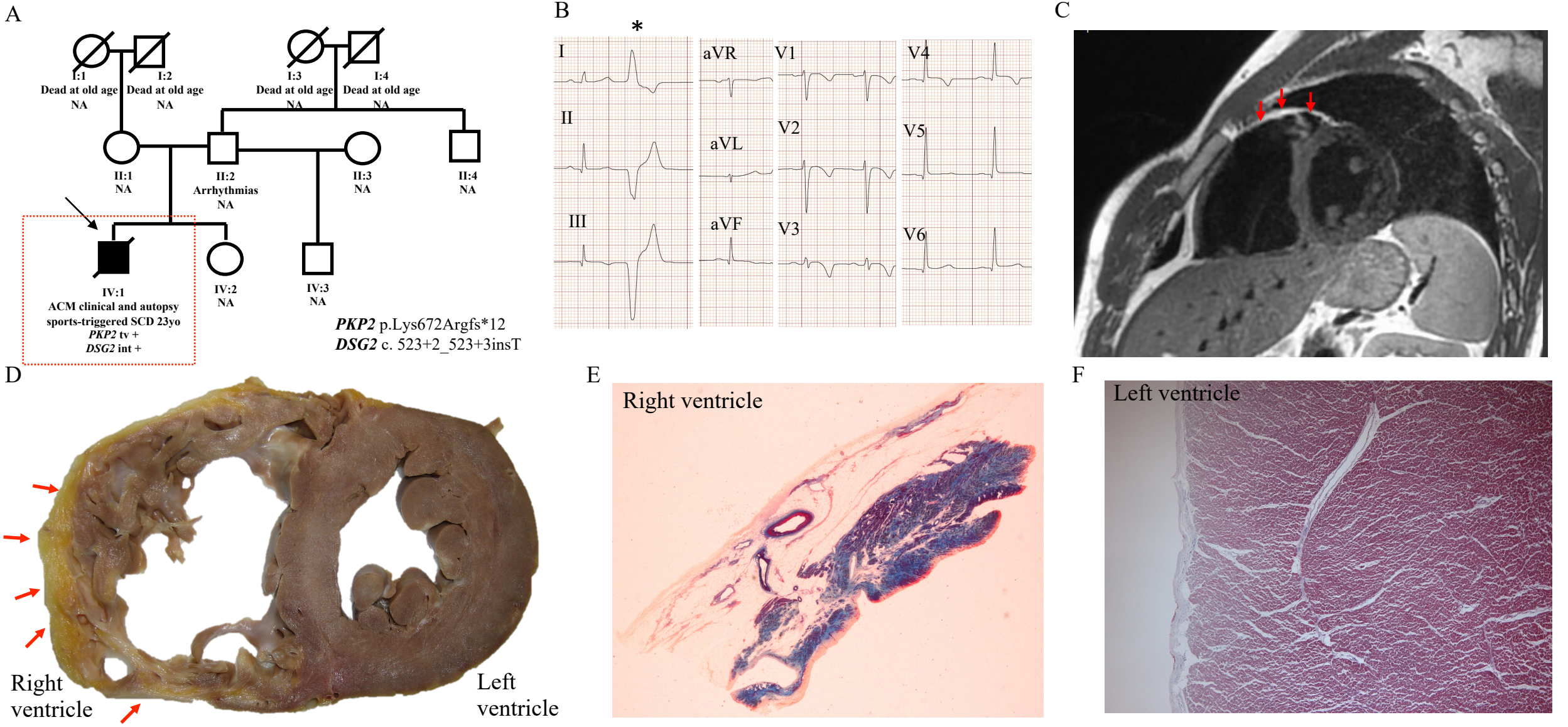
A. Pedigree. The patient position in the pedigree is highlighted in a dashed red square. The mutation is listed in the Pedigree. Squares and circles represent males and females. Crossed symbols denote deceased individuals. An arrow points to the proband. Solid black symbols reflect the ACM phenotype with structural abnormalities at the MRI. White symbols reflect absence of ACM at cardiac imaging or unknown cardiac phenotype. **B.** ECG showing low voltages (limb leads), terminal activation duration of QRS 85 ms, and a ventricular extrasystole (*). **C.** ECG tracing from the recovery period of an exercise test showing ventricular bigeminy originated at the right ventricular outflow tract and a polymorphic ventricular couplet. **D.** Recordings from an appropriate ICD therapy due to VF causing atrioventricular dissociation, terminated with a successful electric shock. **E**. CMR showing subepicardial LGE at the inferoseptum, inferior, and anterolateral left ventricular walls with a non dilated right ventricle. **F**. Echocardiogram showed preserved left and right ventricular systolic function, and normal TAPSE.

Abbreviations: NA: not available. *DSP tr*: desmoplakin truncating mutation; +:mutation is present; -:mutation is absent; ACM: arrhythmogenic cardiomyopathy; LDACM: left dominant arrhythmogenic cardiomyopathy; ICD: implanted cardioverter-defibrillator; VT: ventricular tachycardia; MRI: magnetic resonance imaging; ECG: electrocardiogram; VF: ventricular fibrillation; CMR: cardiac magnetic resonance; LGE: late gadolinium enhancement. TAPSE: tricuspid annular plane systolic excursion

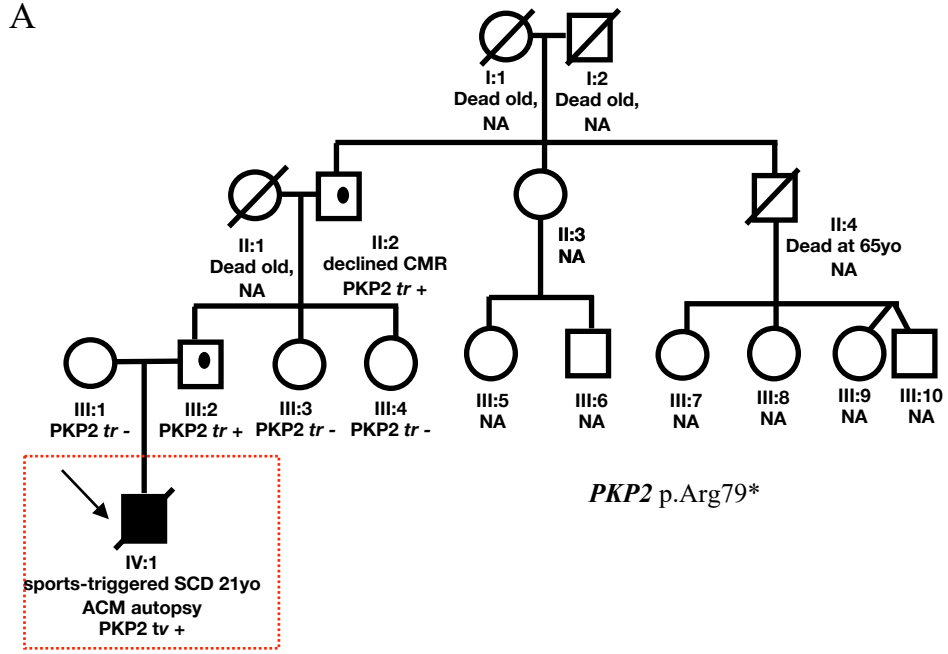


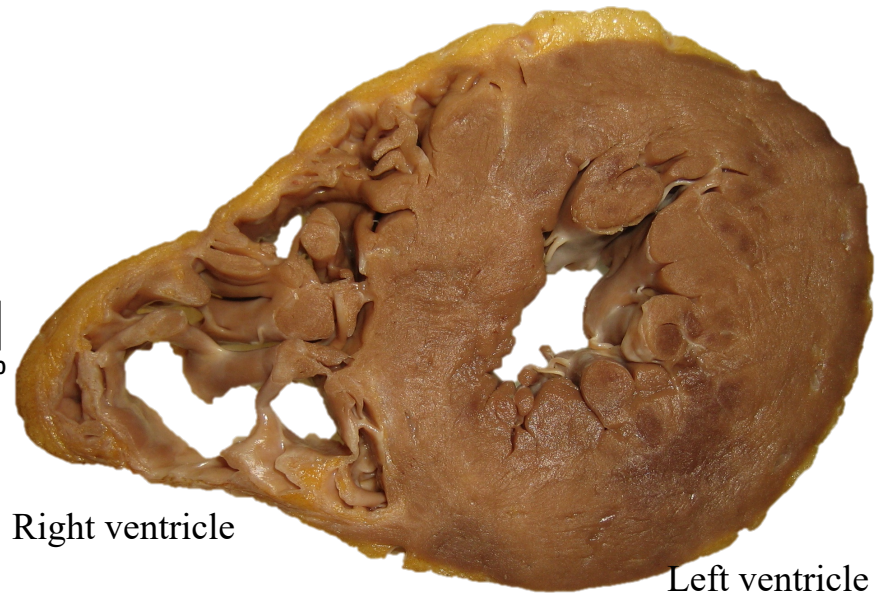
Online Figure 2: Phenotypic Characteristics of patient 2 (discovery group)
A. Pedigree. Symbols similar to those in Figure 1.
B. ECG showing low voltages (limb leads), and terminal activation duration of QRS 55 ms.
C. ECG tracing from a Holter monitor recording showing a non-sustained VT run.
D. Cardiac imaging showing widely extended subepicardial LGE at the left ventricle with non dilated right ventricle at the MRI (arrows).

Abbreviations: *DSP tr*: Desmoplakin truncating mutation; *DSC2 mis*: Desmocollin-2 missense mutation; *DSP* int: Desmoplakin intronic variant.
Other abbreviations are as in online Figure 1

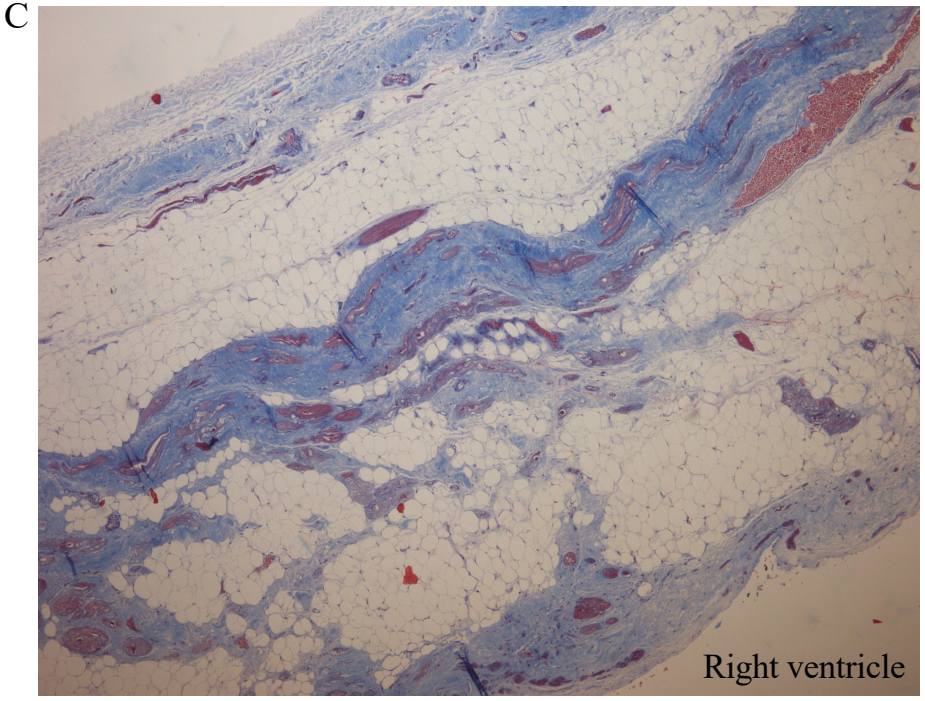


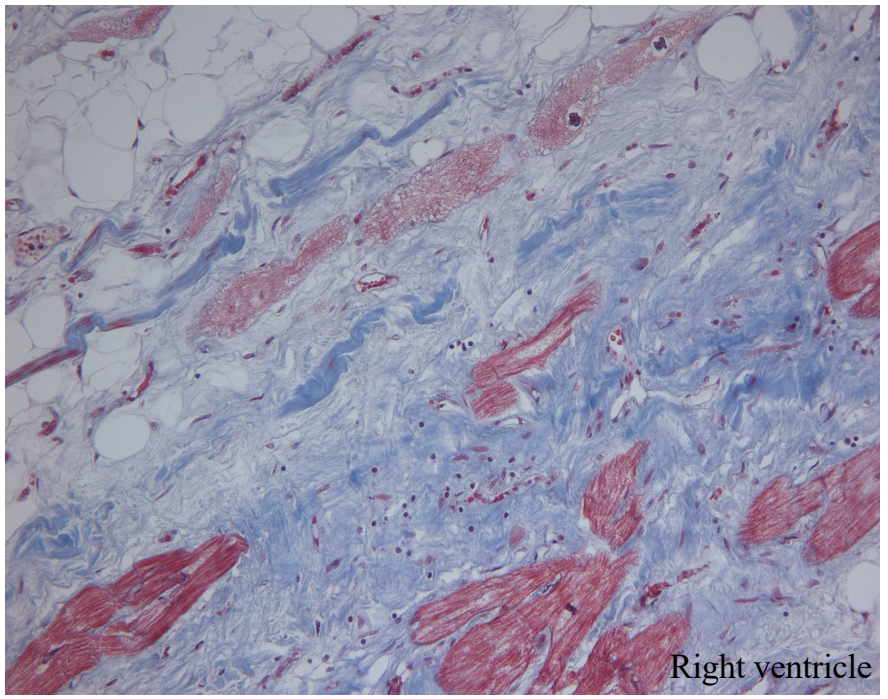
Online Figure 3. Phenotypic characteristics (replication group): A. Pedigree. The proband was presented with syncope and was diagnosed with ACM but continued sport activities. Symbols similar to Online Figure 1. **B.** 12-lead electrocardiogram showing a ventricular extrasystole (*) and negative T waves at V1-4. **C.** CMR showing evidence of fatty infiltration at the anterior wall of the right ventricle (arrows)**. D.** Cross-sectional view of the heart showing fatty infiltration (arrows), a dilated right ventricle and a normal left ventricle. **E and F.** Microscopic images of the right and left ventricles respectively, showing extensive fibrofatty infiltration of the right ventricle but not the left ventricle (Masson trichrome stained sections).
Abbreviations: *PKP2 tr:* Plakophillin-2 truncating mutation. DSG2 *int*: Desmoglein-2 intronic mutation. SCD: sudden cardiac death. Other abbreviations as in Online Figure 1 Legend.

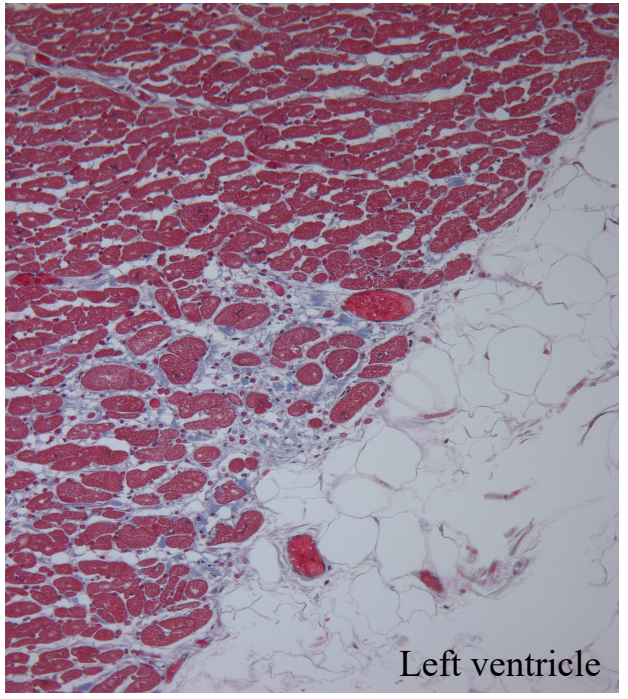


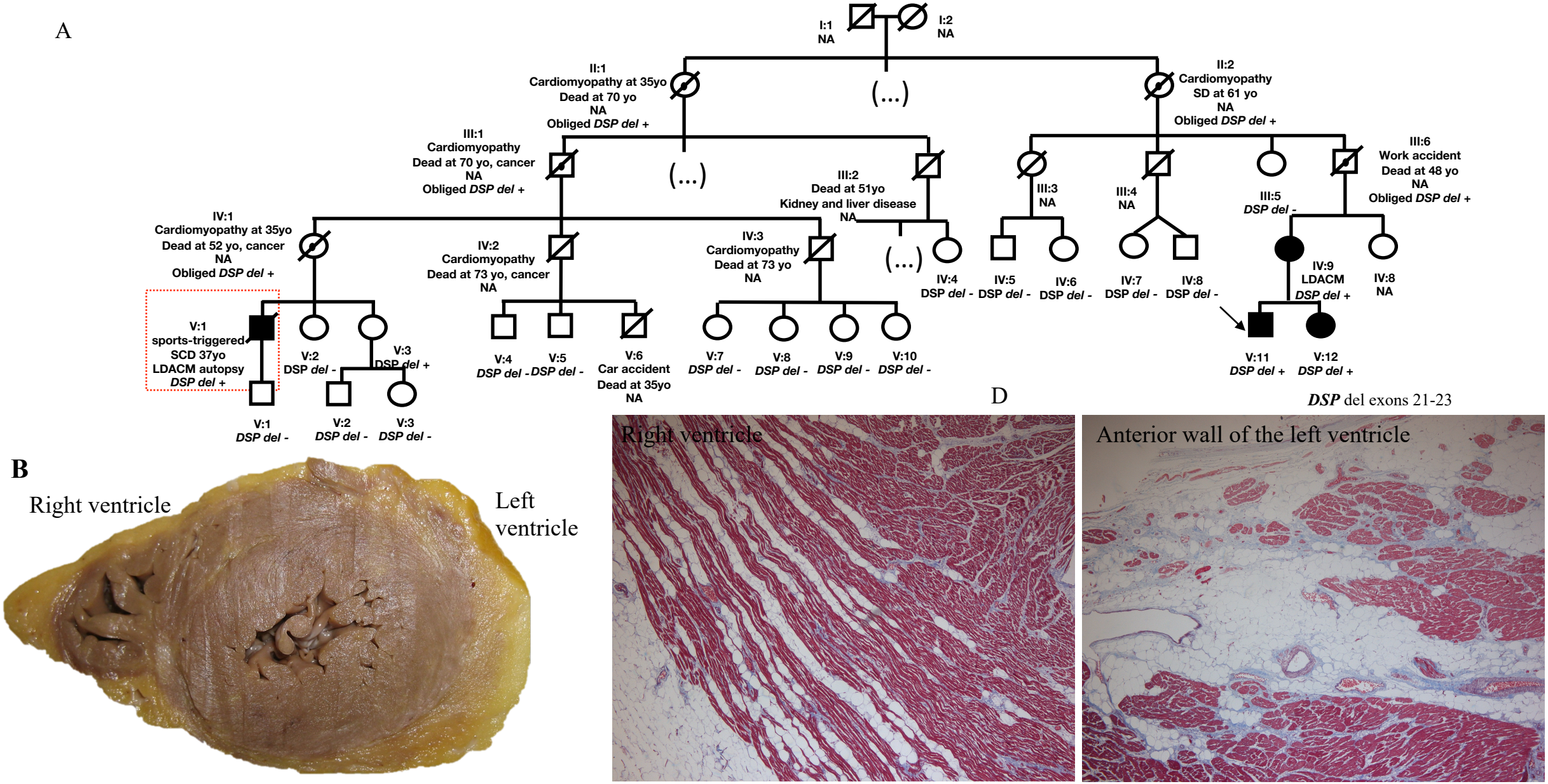


Online Figure 4: Phenotypic characteristics (validation population): A. Pedigree; The proband was a 21-year old apparently healthy male without a prior history of cardiovascular disease, who died suddenly during sport activities. **B**. Gross morphological cross section of the heart showing thinning of the right ventricular wall and fatty infiltration. The left ventricle appears normal. **C and D**. Low and high magnification fields showing extensive fibro-adipogenesis involving the right ventricle. **E.** Thin myocardial section from the left ventricle showing epicardial fat and mild interstitial fibrosis at the epicardium. Pathogenic variant p.Arg79Ter (c.235C>T) in the *PKP2* gene was identified.
Abbreviations as in online Figure 1



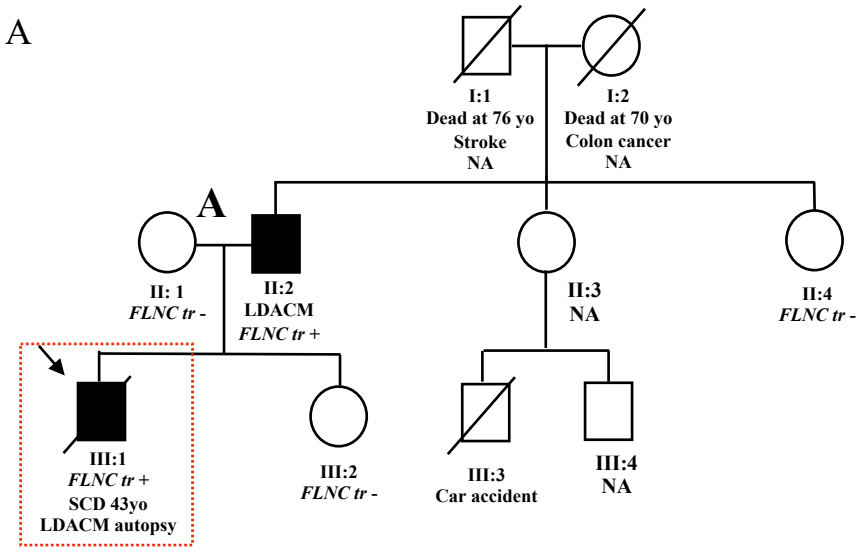






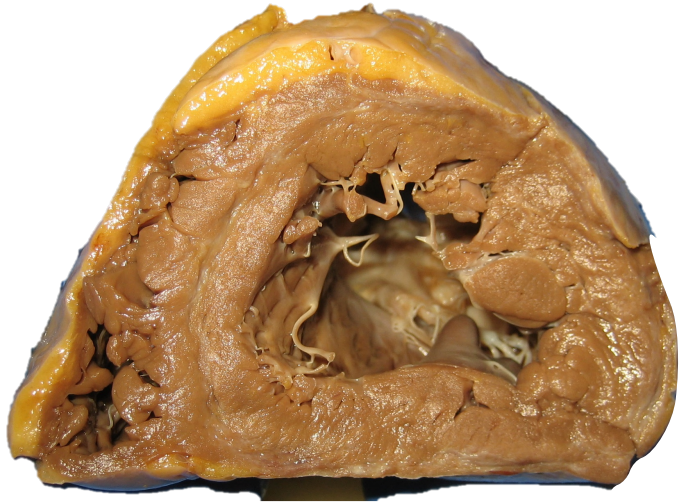
Online Figure 5. Phenotypic characteristics (validation group): A. Pedigree; The proband was a 37-year old male with a family history of arrhythmogenic cardiomyopathy who carried a deletion mutation in the *DSP* gene. **B.** Cross sectional view of the heart showing epicardial fat and a non-dilated ventricles. **C**. Masson trichrome-stained thin myocardial section from the right ventricle showing epicardial and interstitial adipocytes; **D.** Masson trichrome stained thin left ventricular section showing extensive fibro-adipogenesis. A large deletion involving exons 21-24 of the *DSP* gene was detected by Multiplex ligation-dependent probe amplification. Abbreviations as in Online Figures 1 and 3.

A



FLNC p.Arg1370Ter

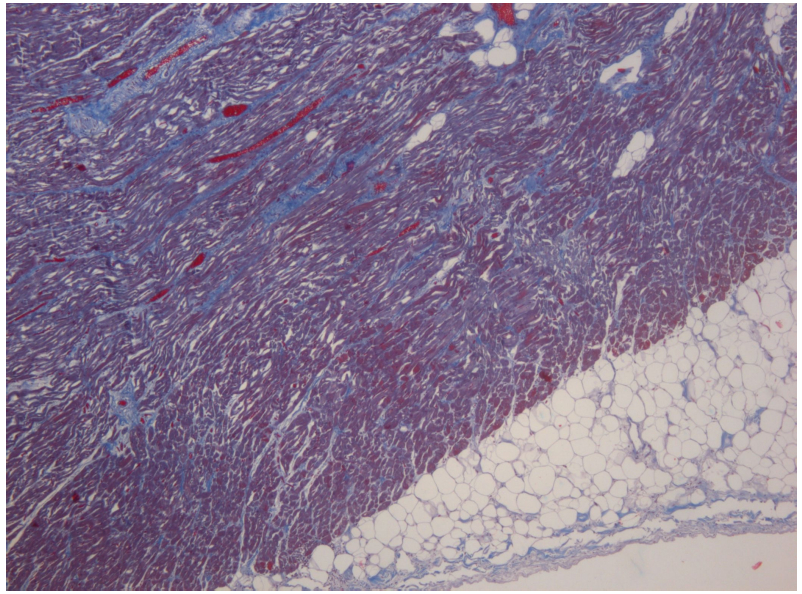
B



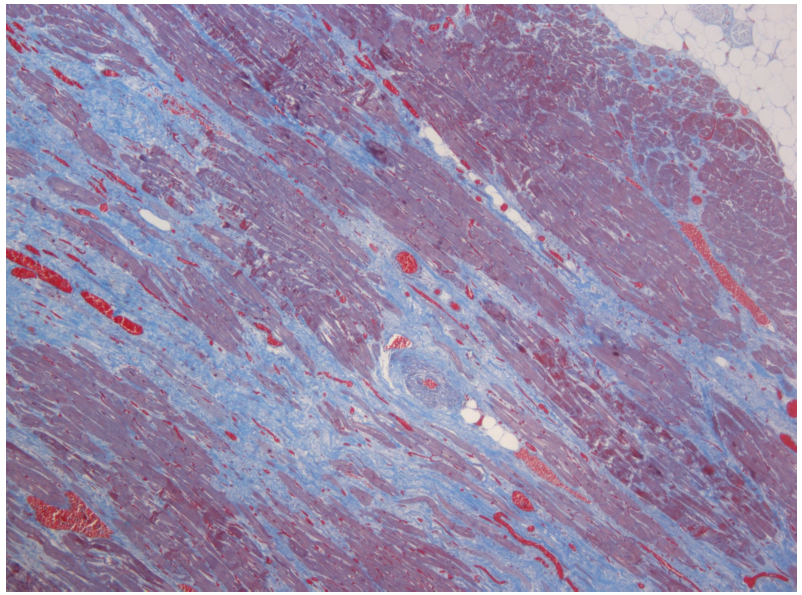
Right ventricle Left ventricle

Online Figure 6. Phenotypic characteristics (replication population): A. Pedigree. The proband was a 43-year old asymptomatic man with no prior history of cardiovascular disease, who died suddenly during routine activities. **B.** Gross cardiac cross section showing epicardial fat and a normal right and left ventricular cavity size. **C**. Masson trichrome stained thin myocardial section from the right ventricle showing epicardial fat. **D**. Masson trichrome stained thin myocardial section from the lateral wall of left ventricle showing extensive fibrosis. **E**. Thin myocardial section from the anterior wall of the left ventricle showing extensive fibro-adiposis. Genetic analysis led to identification of a truncating mutation p.Arg1370Ter (c.4108C>T) in the *FLNC* gene.
Abbreviations as in Online Figure1 1 and 3

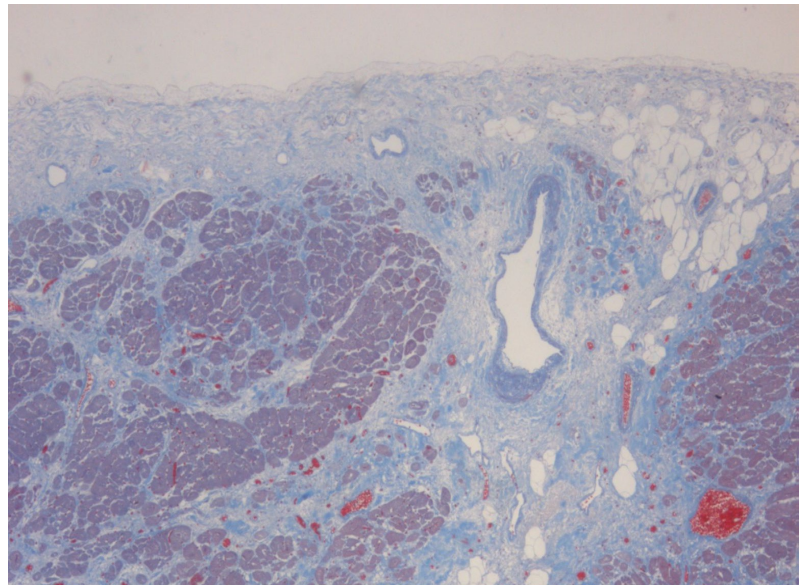
C Right ventricle

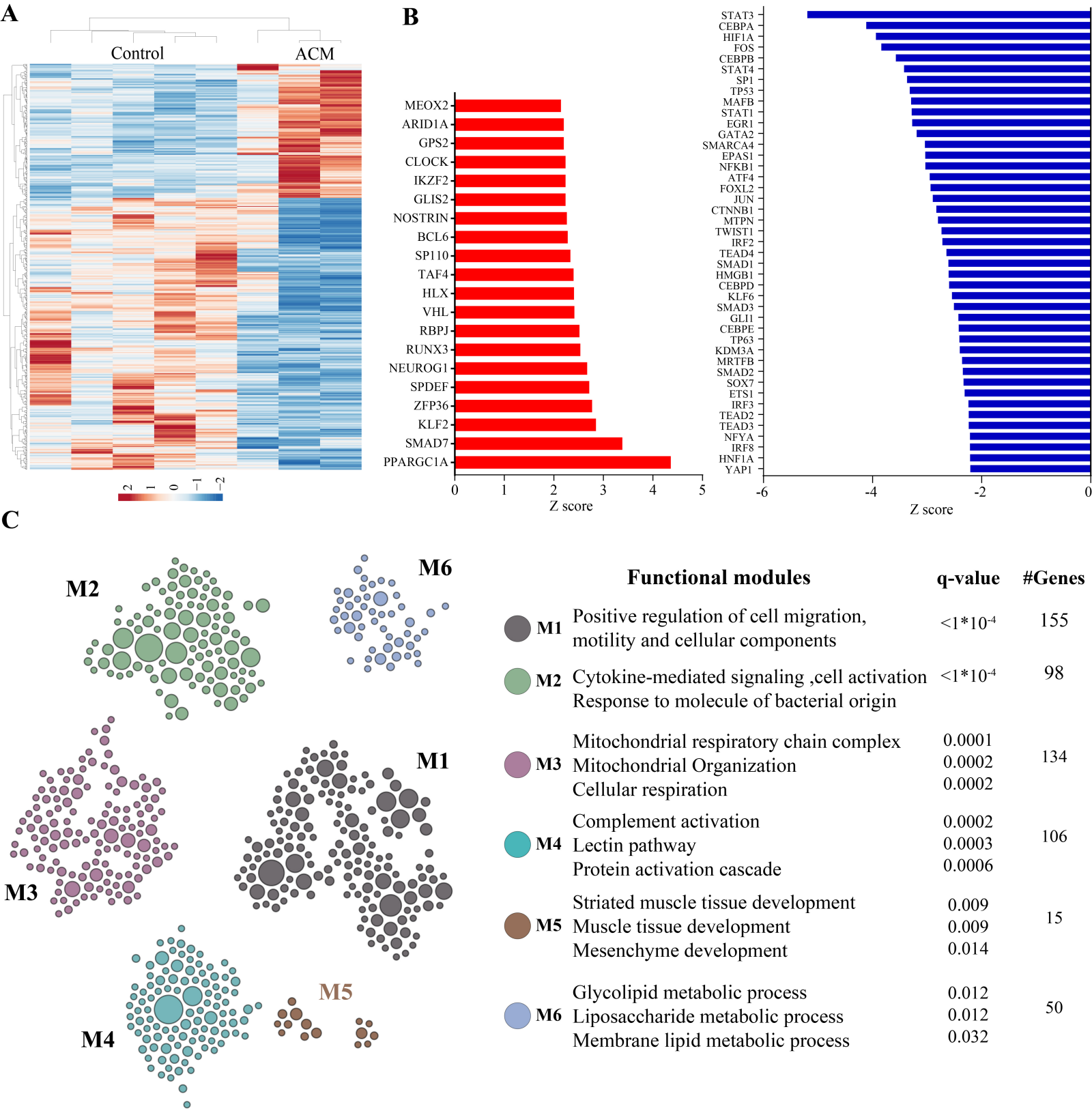


D Lateral wall of the left ventricle



E Anterior wall of the left ventricle





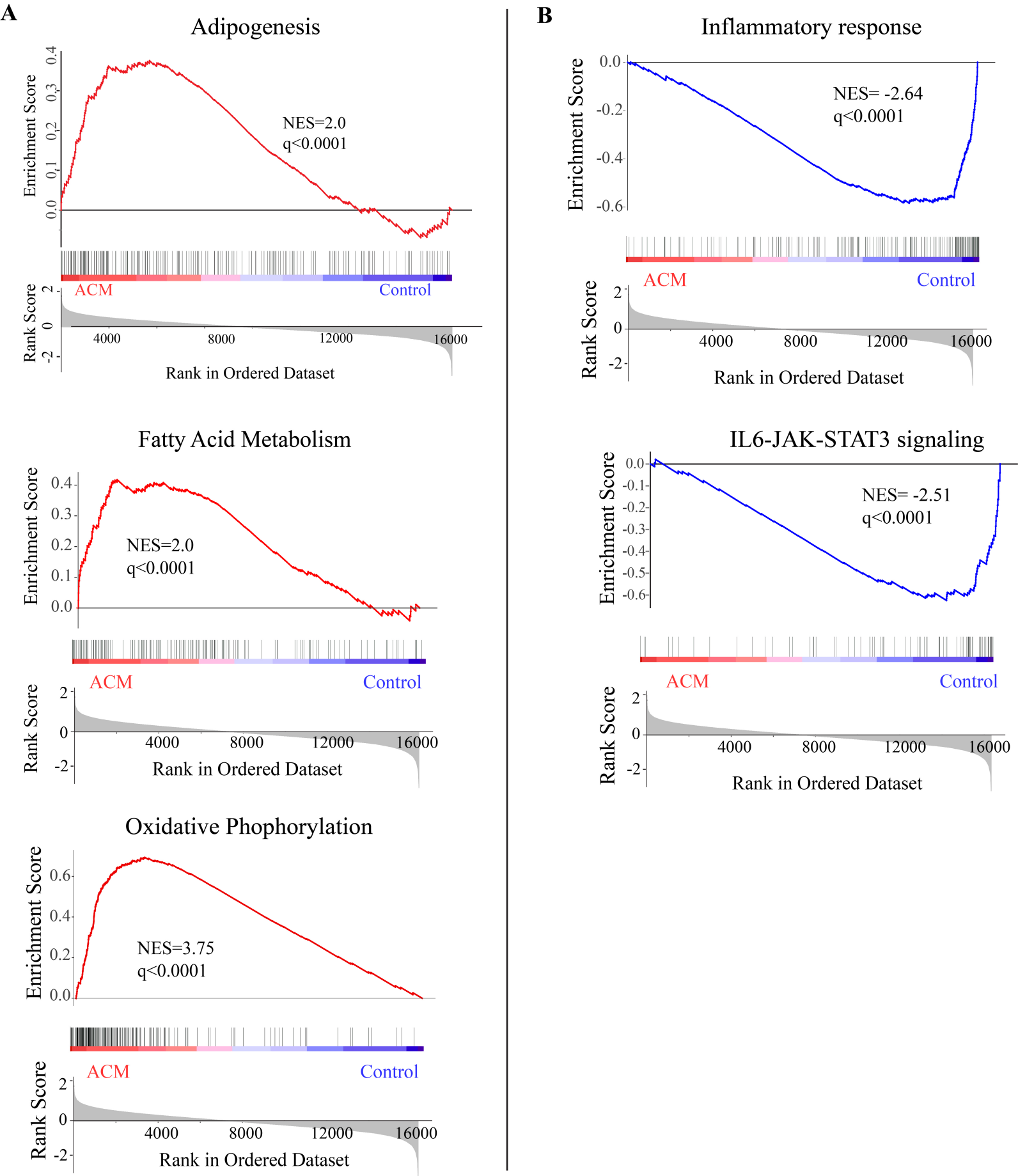
Functional modules		q-value	#Genes
M1	Positive regulation of cell migration, motility and cellular components	$<1*10^{-4}$	155
M2	Cytokine-mediated signaling ,cell activation Response to molecule of bacterial origin	$<1*10^{-4}$	98
M3	Mitochondrial respiratory chain complex Mitochondrial Organization Cellular respiration	0.0001 0.0002 0.0002	134
M4	Complement activation Lectin pathway Protein activation cascade	0.0002 0.0003 0.0006	106
M5	Striated muscle tissue development Muscle tissue development Mesenchyme development	0.009 0.009 0.014	15
M6	Glycolipid metabolic process Liposaccharide metabolic process Membrane lipid metabolic process	0.012 0.012 0.032	50

Online Figure 7. Differentially expressed genes (DEGs) coding for the secreted proteions (secretome) in the endomyocardial biopsy samples from patients with arrhythmogenic cardiomyopathy (ACM) caused by *DSP* truncation mutation.

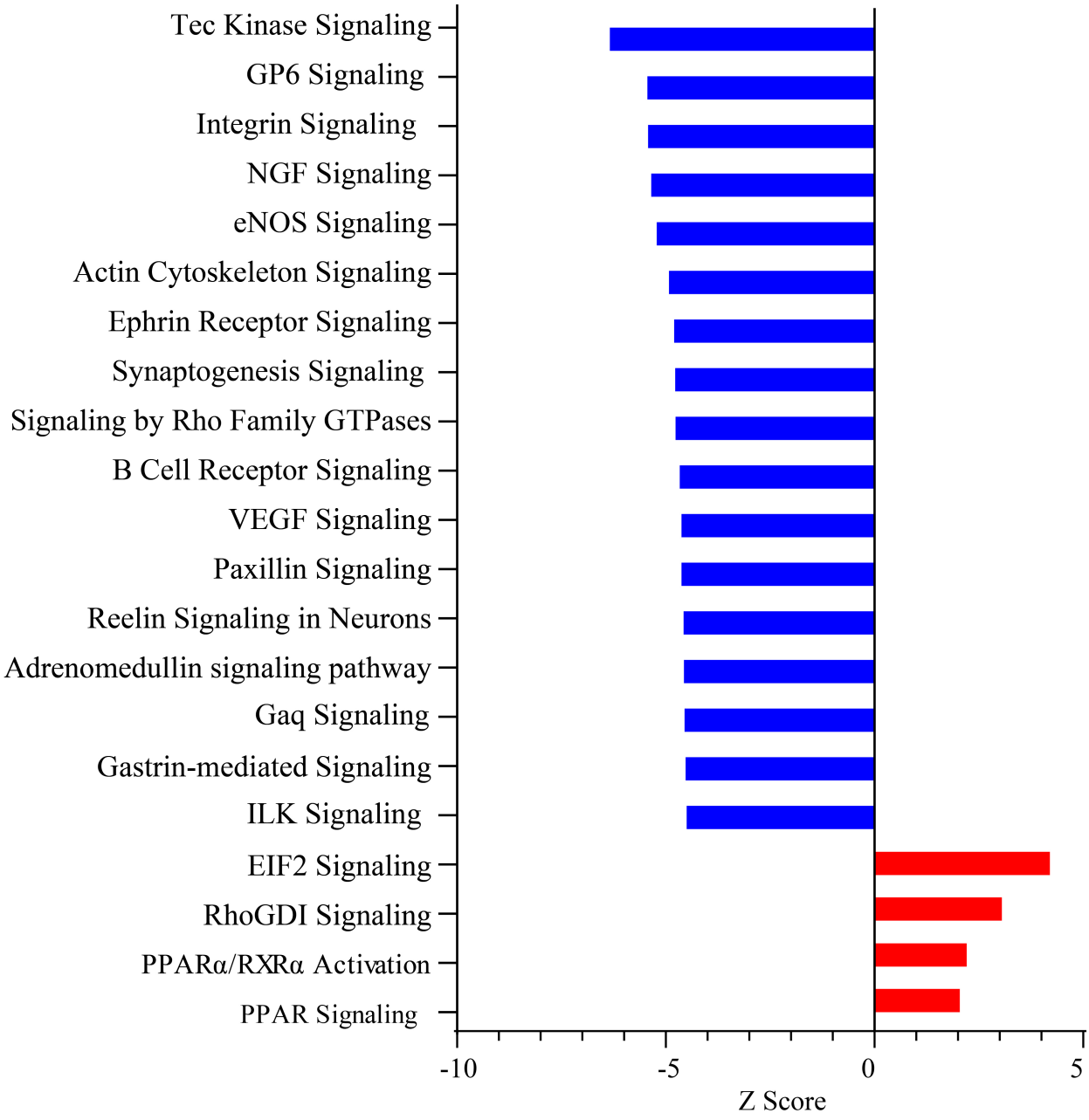
A. Heat map of the DEGs coding for the secretome.

B. Transcriptional regulators of the secretome, predicted to be activated (red) or suppressed (blue).

C. Clustering of the secretome based on their biological functions.



Online Figure 8. Additional dysregulated biological pathways. GSEA plots showing Hallmark signature for gene involved in adipogenesis, fatty acid metabolisn and oxidative phosphorylation are activated (A), whereas those in inflammatory response and IL6-JAK-STAT3 pathways are suppressed (B).



Online Figure 9. Canonical signal tranasduction pathways, as listed in the Ingenuity Pathway Analysis database, predicted to be dysregulated, based on the differentially expressed genes, in the heart in human ACM. Blue indicated suppressed and red activated pathways.

Online Table 1

Antibodies

Antibodies	Concentration	Supplier	Catalog number
YAP1	1:1000 (IB), 1:100 (IF)	Cell Signaling Technology	14074S
TEAD1	1:200 (IB), 1:200 (IF)	Santa Cruz	sc-376113
ANLN	1:1000 (IB), 1:100 (IF)	Invitrogen	PA5-84016
TAZ	1:1000 (IB), 1:200 (IF)	Cell Signaling Technology	72804S
GAPDH	1:1000 (IB)	Cell Signaling Technology	2118
KLF4	1:250 (IB), 1:50 (IF)	Santa Cruz	sc-166238
TCF7L2	1:500 (IB), 1:200 (IF)	Cell Signaling Technology	2569S
CDKN1A	1:100 (IB), 1:20 (IF)	BD Pharmingen	556431
CTNNB1	1:1000 (IB), 1:250 (IF)	Cell Signaling Technology	8480
DSP	1:200 (IF)	Abcam	ab71690
GJA1	1:200 (IF)	Sigma	C6219
Vimentin	1:250 (IF)	Abcam	ab92547
EP300	1:1000 (IB)	Cell Signaling Technology	sc-48343
Cleaved Caspase3	1:1000 (IB)	Cell Signaling Technology	9664
TP53	1:500 (IB),	Santa Cruz	6243
Acetyl-TP53 (K382)	1:1000 (IB)	Cell Signaling Technology	2525
p-TP53 (S15)	1: 1000 (IB)	Cell Signaling Technology	9284
Anti-mouse IgG HRP linked antibody	1:4000 (IB)	Cell Signaling Technology	7076
Anti-rabbit IgG HRP linked antibody	1:2000 (IB)	Cell Signaling Technology	7074
Goat anti-mouse IgG, Alexa Fluor 488	1:1000 (IF)	Invitrogen	A11029
Goat anti-Rabbit IgG, Alexa Fluor 488	1:1000 (IF)	Invitrogen	A11034
Goat anti-Rabbit IgG, Alexa Fluor 594	1:1000 (IF)	Invitrogen	A11012

Online Table 2

Baseline Characteristics of the Patients

Demographics			
Sex	Male	Female	Male
Age (years)	30	17	45
Height (cm)	187	170	175
Weight (kg)	78	54	70
BSA (m^2)	2.0	1.6	1.8
BMI (kg/m^2)	22.3	18.7	22.0
Proband	No	No	No
Family history of cardiomyopathy	Yes	Yes	Yes
Medical history			
Cardiac arrest	No	No	Yes
PVCs >500/24h	Yes	Yes	Yes
Non-sustained VT	No	Yes	Yes
Sustained VT/VF	No	No	Yes
Atrial fibrillation	No	No	No
Syncope	Yes	No	No
ICD implantation	Yes	Yes	Yes
Time to first ICD shock (total follow up years)	6 years (7 years)	No shock (10 years after ICD)	Yes (NA)
Radio frequency catheter ablation	No	No	No
Ischemic heart disease	No	No	No
Diabetes mellitus	No	No	No
Hypertension	No	No	No
Dyslipidemia	No	No	No
Ex-smoker & Current smoking	No	No	No
History and Physical examination			
Heart rate (bpm)	64	75	50
Systolic blood pressure (mmHg)	137	80	100
Diastolic blood pressure (mmHg)	82	50	60
Chest pain/tightness	No	No	No
Palpitations	Yes	Yes	Yes
NYHA Functional class	I	I	I
Medications			
Beta-blockers	Yes	No	Yes
Amiodarone	No	No	Yes
Flecainide	No	No	No
Sotalol	No	Yes	No
Other anti-arrhythmic drugs	No	No	No
RAAS drugs	Yes	No	Yes
ARVC Task force criteria			
Structural			
Major	No	No	No
Minor	No	No	No

Depolarization			
ε wave (major)	No	No	No
TAD≥ 55ms (minor)	Yes	Yes	No
Late Potentials on SAECG at 40 Hz filter (minor)	NA	NA	Yes
Repolarization			
T wave inversion V1-V3 with QRS duration <120 ms (major)	No	No	Yes
T wave inversion V1-V2 with QRS duration <120 ms (minor)	No	No	No
T wave inversion V4-V6 (minor)	No	No	Yes
T wave inversion V1-V4 with complete RBBB (major)	No	No	Yes
Arrhythmias			
VT: LBBB plus superior axis (major)	No	No	Yes
VT: LBBB plus inferior or unknown axis (minor)	No	No	No
PVCs count > 500/24h (minor)	Yes	Yes	Yes
Pathogenic variants			
Pathogenic ACM mutation carrier (major)	*DSP* c.3115dupG (p.Glu1039Glyfs*15)	*DSP* c. 5318delT (p. Leu1773Tyrfs*8)	*DSP* c.1339C>T (p. Gln447*)
Electrocardiography			
T-wave inversion in V5–V6±V4, I, aVL	No	No	No
T-wave inversion in II, III, aVF	No	No	Yes
T-wave inversion in V1–V6	No	No	No
Left axis deviation (QRS axis < -30°)	No	No	No
Leftward QRS axis (-30° < QRS axis < 0)	No	No	No
Echocardiography			
IVST (mm)	10	9	10
LVPWT (mm)	7	7	8
Maximum wall thickness (mm)	10	9	10
LVEDD (mm)	54	42	60
LVESD (mm)	37	29	48
LVEF (%)	58	70	43
RV dilatation	Non dilated RV	Non dilated RV	Non dilated RV
RVOT (mm)	29	NA	31
RV systolic function	Normal, FAC:50.7%, TAPSE: 27 mm	Normal RV function	Normal RV function
Cardiac magnetic resonance			
LVEDVi (ml/m^2)	91	81	109
LVEF (%)	63	63	46
LV LGE	Yes (subepicardial lateral, inferior, apical, and septal walls)	Yes (subepicardial and concentric)	Yes (extensive: global subepicardial, localized inferior,

			posterior, lateral, and anterior walls. Patchy: anterior septum at mid-apical level)
LV wall motion abnormalities	No	No	Yes (apex mainly)
RVEF (%)	45	56	58
RVEDVi, (ml/m^2)	91	71	NA
RV LGE	No	No	No
RV wall motion abnormalities	Yes (small dyskinetic areas at the RV free wall)	No	No

Abbreviations: BSA: body surface area; BMI: body mass index; PVCs: premature ventricular contractions; VT: ventricular tachycardia; VF: ventricular fibrillation; ICD: implantable cardioverter-defibrillator; NYHA: New York Heart Association; RAAS: renin–angiotensin–aldosterone system; TAD: terminal activation duration; SAECG: signal-averaged electrocardiography; RBBB, right bundle branch block; LBBB, left bundle branch block; IVST: interventricular septal thickness; LVPWT: left ventricular posterior wall; LVEDD: left ventricular end-diastolic diameter; LVESD: left ventricular end-systolic diameter; LVEF: left ventricular ejection fraction; RVEF: right ventricular ejection fraction; LV: left ventricle; RV: right ventricle; FAC: fractional area change; RVOT: right ventricular outflow tract; TAPSE: tricuspid annular plane systolic excursion; LVEDVi: left ventricular end-diastolic volume index; RVEDVi: right ventricular end-diastolic volume index; LGE: late gadolinium enhancement; SCD: Sudden cardiac death

Online Table 3

List of Mutations (Pathogenic Variants)

Tissue	Demographics			Mutation			
No.	**Source**	**Age**	**Sex**	**Gene**	**Reference transcript**	**DNA sequence change**	**Amino acid change**
1	Biopsy	30	Male	*DSP*	NM_004415.4	c.3115dupG	p.Glu1039Glyfs*15
2	Biopsy	17	Female	*DSP*	NM_004415.4	c.5318delT	p.Leu1773Tyrfs*8
				DSC2	NM_024422.6	c.1073C>T	p.Thr358Ile (rs139399951)
3	Biopsy	45	Male	*DSP*	NM_004415.4	c.1339C>T	p.Gln447*
4	Autopsy	37	Male	*DSP*	NM_004415.4	c.2878-46_6599del (Chr6:7577966-7584094)	Deletion of intron 21-exon 23
5	Autopsy	23	Male	*PKP2*	NM_001005242.3	c.2013delC	p.Lys672Argfs*12
				DSG2	NM_001943.5	c.523+2_523+3insT	
6	Autopsy	21	Male	*PKP2*	NM_001005242.3	c.235C>T	p.Arg79*
7	Autopsy	43	Male	*FLNC*	NM_001458.5	c.4108C>T	p.Arg1370*
8	Autopsy	52	Male	*TMEM43*	NM_024334.3	c.1073C>T	p.Ser358Leu