Supplemental Material

Figure S1

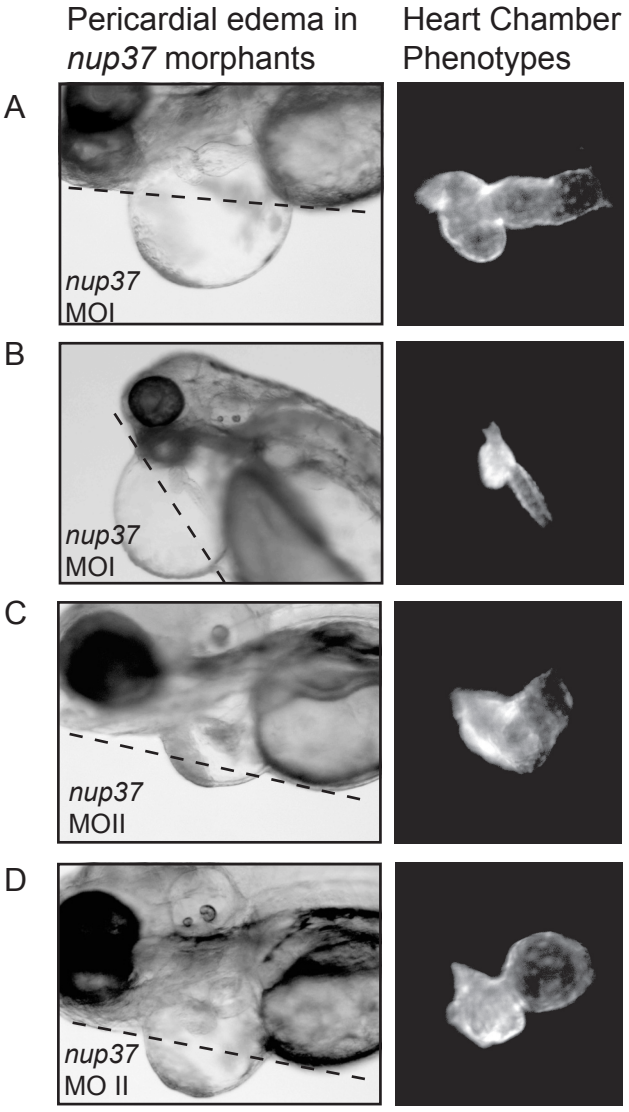
Pericardial edema in *nup37* morphants
Heart Chamber Phenotypes
A
nup37 MOI
B
nup37 MOI
C
nup37 MOII
D
nup37 MO II

Figure S2

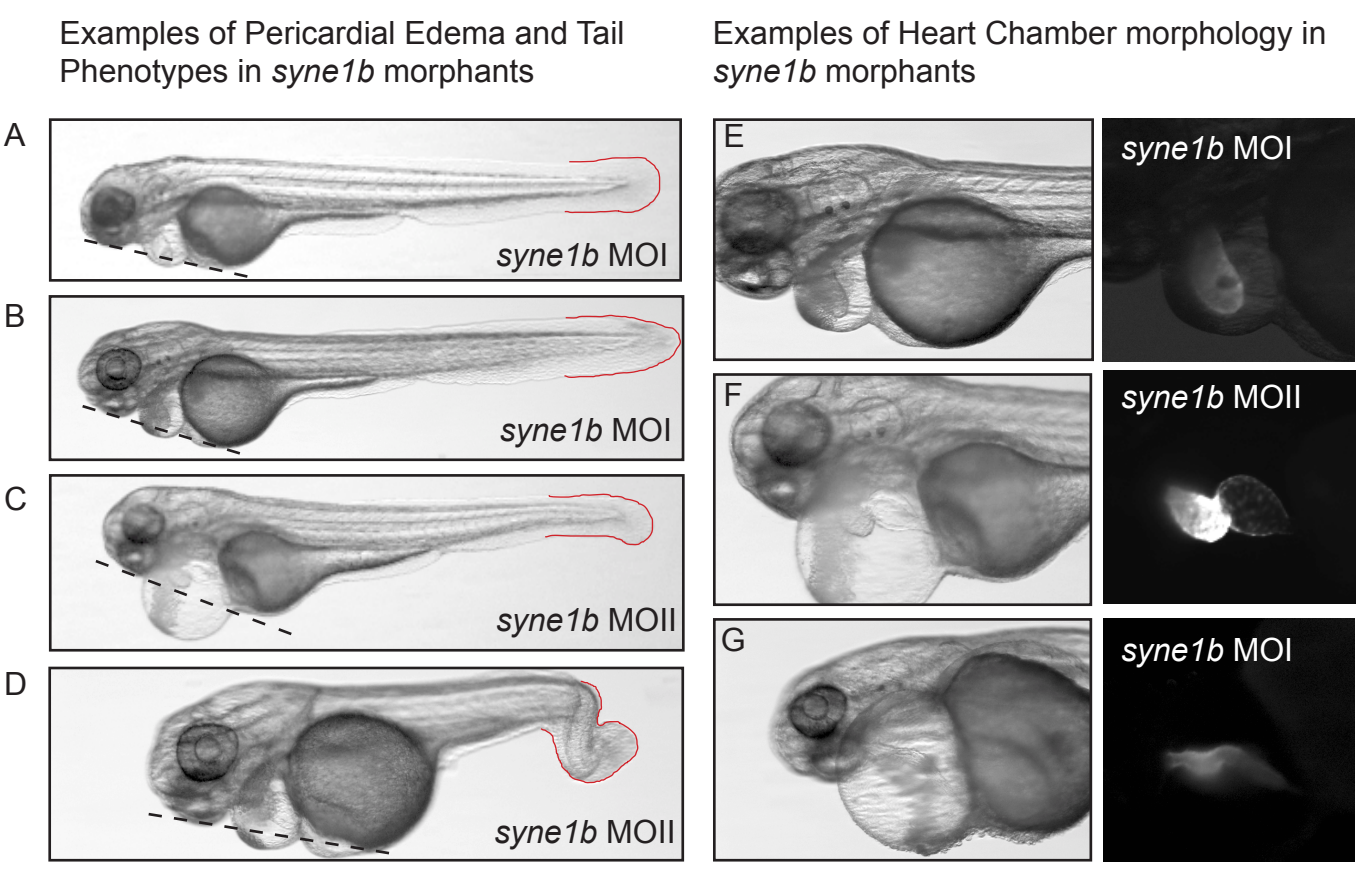
Examples of Pericardial Edema and Tail Phenotypes in *syne1b* morphants
A
syne1b MOI
B
syne1b MOI
C
syne1b MOII
D
syne1b MOII
Examples of Heart Chamber morphology in *syne1b* morphants
E
syne1b MOI
F
syne1b MOII
G
syne1b MOI

Table S1: Genes on CVD Diagnostic Lists

Gene	Description	Arrhythmia Dx List	Cardiomyopathy Dx List	MVP / TAA Dx List
ABCC9	ATP-binding cassette sub-family C member 9	1	1	
ACADS	Short-chain specific acyl-CoA dehydrogenase, mitochondrial		1	
ACADVL	Very long-chain specific acyl-CoA dehydrogenase, mitochondrial		1	
ACTA2	Actin, aortic smooth muscle			1
ACTC1	Actin, alpha cardiac muscle 1		1	
ACTN2	Alpha-actinin-2		1	
AGK	Acylglycerol kinase, mitochondrial		1	
AGL	Glycogen debranching enzyme		1	
AKAP9	A-kinase anchor protein 9	1		
ALMS1	Alstrom syndrome protein 1		1	
ANK2	Ankyrin-2	1		
ANKRD1	Ankyrin repeat domain-containing protein 1		1	
APOA1	Apolipoprotein A-I		1	
ARSB	Arylsulfatase B		1	
BAG3	BAG family molecular chaperone regulator 3		1	
CACNA1C	Voltage-dependent L-type calcium channel subunit alpha-1C	1		
CACNB2	Voltage-dependent L-type calcium channel subunit beta-2	1		
CASQ2	Calsequestrin-2	1	1	
CAV3	Caveolin-3	1	1	

CBS	Cystathionine beta-synthase			1
COL3A1	Collagen alpha-1(III) chain			1
CRYAB	Alpha-crystallin B chain		1	
CSRP3	Cysteine and glycine-rich protein 3		1	
CTF1	Cardiotrophin-1		1	
DES	Desmin		1	
DMD	Dystrophin		1	
DNAJC19	Mitochondrial import inner membrane translocase subunit TIM14		1	
DSC2	Desmocollin-2	1	1	
DSG2	Desmoglein-2	1	1	
DSP	Desmoplakin	1	1	
DTNA	Dystrobrevin alpha		1	
EMD	Emerin		1	
EYA4	Eyes absent homolog 4		1	
FBN1	Fibrillin-1			1
FBN2	Fibrillin-2			1
FHL2	Four and a half LIM domains protein 2		1	
FKTN	Fukutin		1	
GAA	Lysosomal alpha-glucosidase		1	
GBE1	1,4-alpha-glucan-branching enzyme		1	
GJA5	Gap junction alpha-5 protein	1		
GLA	Alpha-galactosidase A		1	
GLB1	Beta-galactosidase		1	
GNPTAB	N-acetylglucosamine-1-phosphotransferase subunits alpha/beta		1	
GPD1L	Glycerol-3-phosphate dehydrogenase 1-like protein	1		

GYS1	Glycogen [starch] synthase, muscle		1	
HFE2	Hemojuvelin		1	
HRAS	GTPase Hras		1	
JUP	Junction plakoglobin	1	1	
KCNA5	Potassium voltage-gated channel subfamily A member 5	1		
KCNE1	Potassium voltage-gated channel subfamily E member 1			
KCNE2	Potassium voltage-gated channel subfamily E member 2	1		
KCNE3	Potassium voltage-gated channel subfamily E member 3	1		
KCNH2	Potassium voltage-gated channel subfamily H member 2	1		
KCNJ2	Inward rectifier potassium channel 2	1		
KCNQ1	Potassium voltage-gated channel subfamily KQT member 1	1		
LAMA4	Laminin subunit alpha-4		1	
LAMP2	Lysosome-associated membrane glycoprotein 2	1	1	
LDB3	LIM domain-binding protein 3		1	
LMNA	Prelamin-A/C		1	
MYBPC3	Myosin-binding protein C, cardiac-type		1	
MYH11	Myosin-11			1
MYH6	Myosin-6		1	
MYH7	Myosin-7		1	

MYL2	Myosin regulatory light chain 2, ventricular/cardiac muscle isoform		1	
MYL3	Myosin light chain 3		1	
MYLK	Myosin light chain kinase, smooth muscle			1
MYLK2	Myosin light chain kinase 2, skeletal/cardiac muscle		1	
MYOZ2	Myozenin-2		1	
MYPN	Myopalladin		1	
NEXN	Nexilin		1	
NPPA	Natriuretic peptides A	1		
PKP2	Plakophilin-2	1	1	
PLN	Cardiac phospholamban		1	
PRKAG2	5'-AMP-activated protein kinase subunit gamma-2		1	
PSEN1	Presenilin-1		1	
PSEN2	Presenilin-2		1	
RBM20	RNA-binding protein 20		1	
RYR2	Ryanodine receptor 2	1	1	
SCN1B	Sodium channel subunit beta-1	1		
SCN3B	Sodium channel subunit beta-3	1		
SCN4B	Sodium channel subunit beta-4;SCN4B	1		
SCN5A	Sodium channel protein type 5 subunit alpha	1	1	
SGCD	Delta-sarcoglycan		1	
SLC22A5	Solute carrier family 22 member 5		1	

SLC25A20	Mitochondrial carnitine/acylcarnitine carrier protein		1	
SLC25A4	ADP/ATP translocase 1		1	
SLC2A10	Solute carrier family 2, facilitated glucose transporter member 10			1
SMAD3	Mothers against decapentaplegic homolog 3			1
SNTA1	Alpha-1-syntrophin	1		
TAZ	Tafazzin		1	
TCAP	Telethonin		1	
TGFB2	Transforming growth factor beta-2			1
TGFB3	Transforming growth factor beta-3	1	1	1
TGFBR1	TGF-beta receptor type-1			1
TGFBR2	TGF-beta receptor type-2			1
TMEM43	Transmembrane protein 43	1	1	
TMPO	Lamina-associated polypeptide 2, isoform alpha		1	
TNNC1	Troponin C, slow skeletal and cardiac muscles		1	
TNNI3	Troponin I, cardiac muscle		1	
TNNT2	Troponin T, cardiac muscle		1	
TPM1	Tropomyosin alpha-1 chain		1	
TTN	Titin		1	
TTR	Transthyretin		1	
VCL	Vinculin		1	

Table S2: Patients, Phenotypes, and Results Returned

Case	Phenotype	Age	Sex	Ethnicity	Dx Lists Run	Dx List Results	Variants returned	Previous genetic testing
Arrhythmia								
NCG_00096	Afib; suspected short QT syndrome	22	M	AA	Arrhythmia CM	Neg Neg	none	none
NCG_00334	LQT	60	F	Caucasian	Arrhythmia	Neg	none	none
NCG_00345	LQT	25	F	AA	Arrhythmia	Pos	KCNH2: NM_000238.3:c.2927delA, NP_000229.1:p.Asp976fs (LP)	none
NCG_00574	DCM; LQT	50	F	AA	Arrhythmia CM	Uncertain Uncertain	SCN5A: NM_198056.2:c.86C>T, NP_932173.1:p.Ala29Val (VUS)	none
NCG_00680	HCM; LQT; Afib	75	F	Caucasian	CM Arrhythmia	Pos Uncertain significance	MYH7: NM_000257.2:c.1750G>A, NP_000248.2:p.Gly584Ser (LP) KCNH2: NM_000238.3:c.1039C>T, NP_000229.1:p.Pro347Ser (VUS)	none
NCG_00682	HCM; Afib	59	M	Caucasian	CM Arrhythmia	Pos Neg	MYBPC3: NM_000256.3:c.1624G>C, NP_000247.2:p.Glu542Gln (KP)	none
NCG_00683	HCM; LQT; rheumatic valvular disease	63	F	Caucasian	CM Arrhythmia TAAD	Neg Neg Neg	none	none
NCG_00804	DCM; history of sustained VT	63	M	AA	CM Arrhythmia	Uncertain Neg	TTN: NM_001267550.1:c.92677A>G NP_001254479.1:p.Lys30893Glu (VUS)	none
Cardiomyopathy								
NCG_00017	DCM; HF	37	M	AA	CM	Uncertain; 2 variants found in DCM	ACTN2: NM_001103.2:c.2323C>T, NP_001094.1:p.His775Tyr (VUS) ACTN2: NM_001103.2:c.2567C>T,	none

						disease gene	NP_001094.1:p.Pro856Leu (VUS)	
NCG_00020	DCM; HF	49	M	AA	CM	Neg	none	none
NCG_00024	DCM (giant cell myocarditis)	18	M	AA	CM	Neg	none	GeneDx negative
NCG_00061	HCM	42	M	Caucasian	CM	Uncertain	CSRP3: NM_003476.4:c.10T>C, NP_003467.1:p.Trp4Arg (VUS)	none
NCG_00071	DCM; HF	66	M	Caucasian	CM	Uncertain	TTN: NM_001267550.1:c.102932C>G NP_001254479.1:p.Ser34311* (VUS)	none
NCG_00145	DCM; HF	49	F	Caucasian	CM	Neg	none	none
NCG_00208	DCM; HF	63	F	AA	CM	Uncertain	TTN: NM_001267550.1:c.102523C>T, NP_001254479.1:p.Arg34175* (VUS)	none
NCG_00352	DCM; HF	58	M	Caucasian	CM	Uncertain	MY6: NM_002471.3:c.3428G>A, NP_002462.2:p.Arg1143Gln (VUS)	none
NCG_00362	Early-onset CM; HF	23	F	Caucasian	CM	Uncertain	TNNT2: NM_000364.3:c.421C>T, NP_000355.2:p.Arg141Trp (VUS)	none
NCG_00370	HCM	29	M	Caucasian	CM	Pos	Homozygous DSG2: NM_001943.3:c.674_678delTTACC, NP_001934.2:p.Thr226fs (LP) LAMA4: NM_001105206.2:c.4583G>A, NP_001098676.2:p.Arg1528His (VUS) LDB3: NM_001080114.1:c.331A>G, NP_001073583.1:p.Asn111Asp (VUS)	none
NCG_00522	DCM	40	M	Caucasian	CM	Pos	BAG3: NM_004281.3:c.946C>T, NP_004272.2:p.Gln316*	none
NCG_00534	DCM	37	F	AA	CM	Neg	none	none
NCG_00536	DCM	37	F	AA	CM	Pos	TTN: NM_001267550.1:c.87295_87296insT, NP_001254479.1:p.Asn29099fs	none

NCG_00537	DCM	46	M	AA	CM	Uncertain	DSP: NM_004415.2:c.2683T>C, NP_004406.2:p.Tyr895His	none
NCG_00544	DCM	50	F	AA	CM	Uncertain	MYH6: NM_002471.3:c.5095C>T, NP_002462.2:p.Arg1699Trp	none
NCG_00561	LVNC	47	M	Caucasian	CM	Uncertain	TTN: NM_001267550.1:c.13058delC, NP_001254479.1:p.Pro4353fs (VUS) TTN: NM_001267550.1:c.23965C>T, NP_001254479.1:p.Arg7989Cys (VUS) TTN: NM_001267550.1:c.52139A>T, NP_001254479.1:p.Asp17380Val (VUS)	none
NCG_00611	DCM	26	M	AA	CM	Uncertain	VCL: NM_003373.3:c.1856C>T, NP_003364.1:p.Ala619Val NM_014000.2:c.1856C>T, NP_054706.1:p.Ala619Val (VUS)	none
NCG_00615	DCM	26	F	AA	CM	Uncertain	TTN: NM_001267550.1:c.80338C>T, NP_001254479.1:p.Pro26780Ser (VUS)	none
NCG_00632	DCM	28	M	Caucasian	CM	Pos	TTN: NM_001267550.1:c.94855C>T, NP_001254479.1:p.Arg31619* (LP)	none
NCG_00644	DCM	41	M	AA	CM	Neg	none	none
NCG_00645	DCM	38	F	AA	CM	Neg	none	none
NCG_00661	DCM	60	M	AA	CM	Uncertain	MYPN: NM_032578.3:c.3583G>A, NP_115967.2:p.Val1195Met (VUS) TTN: NM_001267550.1:c.87623A>T, NP_001254479.1:p.Tyr29208Phe (VUS)	none
NCG_00673	DCM	47	M	AA	CM	Neg	none	none
NCG_00678	HCM	58	F	AA	CM	Uncertain	TTN: NM_001267550.1:c.93367G>C, NP_001254479.1:p.Val31123Leu (VUS) TTN: NM_001267550.1:c.72181A>G,	none

							NP_001254479.1:p.Met24061Val (VUS)	
NCG_00687	HCM	60	M	Caucasian	CM	Uncertain	MYL2: NM_000432.3:c.433G>A, NP_000423.2:p.Asp145Asn (VUS)	none
NCG_00689	HCM; mild LVH	17	M	Caucasian	CM	Pos	MYBPC3: NM_000256.3:c.350delC, NP_000247.2:p.Pro117fs (LP)	none
NCG_00700	HCM with conduction system disease	23	F	Caucasian	CM	Pos	DES: NM_001927.3:c.1360C>T, NP_001918.3:p.Arg454Trp (LP)	none
NCG_00719	early onset DCM	29	F	AA	CM	Neg	none	none
NCG_00732	CM; carnitine deficiency	10	F	Caucasian	CM	Pos	SLC22A5: NM_003060.3:c.62_64delTCT, NP_003051.1:p.Ile21_Phe22delinsIle (LP) SLC22A5: NM_003060.3:c.939T>A, NP_003051.1:p.Phe313Leu (VUS)	none
NCG_00758	DCM	45	F	AA	CM	Uncertain	TTN: NM_001267550.1:c.62467C>T, NP_001254479.1:p.Arg20823Cys (VUS)	none
NCG_00759	DCM	52	M	AA	CM	Neg	none	none
NCG_00788	ASH variant of HCM and adrenal myelolipoma	56	F	Caucasian	CM	Neg	none	none
NCG_00805	HCM	62	F	AA	CM	Uncertain	NEXN: NM_144573.3:c.987_989delAGA, NP_653174.3:p.Ala329_Glu330delinsAla (VUS)	none
NCG_00838	HCM	35	M	Caucasian	CM	Pos	MYBPC3: NM_000256.3:c.1624G>C, NP_000247.2:p.Glu542Gln (KP)	none

Thoracic Aneurysm and Dissection								
NCG_00099	Thoracic aortic aneurysm	27	F	Caucasian	TAAD	Neg	none	Neg for: TGFBR1; MYH11; ACTA2; exons 1-7 of TGFBR2
NCG_00157	Thoracic aortic aneurysm	44	M	Caucasian	TAAD	Uncertain	FBN2: NM_001999.3:c.4312G>A, NP_001990.2:p.Glu1438Lys (VUS)	Neg TGFBR1 and TGFBR2 sequencing
NCG_00261	MVP	16	F	Caucasian	TAAD	Neg	none	none
NCG_00354	MVP;TAA	59	M	Caucasian	TAAD	Neg	none	Neg for: COL3A1; FBN1
NCG_00435	Thoracic and Ascending Aortic Aneurysms	60	F	Caucasian	TAAD	Neg	none	none
NCG_00484	Aortic dissection	75	M	Caucasian	TAAD	Neg	none	none
NCG_00540	Bicuspid aortic valve and ascending aortic aneurysm	4	F	Caucasian	TAAD	Neg	none	none
NCG_00721	Aortic aneurysm	43	F	Caucasian	TAAD	Pos	FBN1: NM_000138.4:c.3902G>T, NP_000129.3:p.Gly1301Val (LP)	Unclear based on pt. report
NCG_00787	Near total occlusion of left internal carotid artery	2	M	Caucasian	TAAD	Neg	none	none
NCG_00865	MVP	46	M	Caucasian	TAAD	Neg	none	Neg for: COL3A1; COL5A1;

								COL5A2
NCG_00973	TAAD, Dilated aortic root	18	F	AA	TAAD	Neg	none	none
NCG_00985	TAAD	56	F	Caucasian	TAAD	Neg	none	none
NCG_00998	TAAD	51	M	Caucasian	TAAD	Neg	none	none

Abbreviations: Dx: Diagnostic; Afib: Atrial fibrillation; LQT: long QT; AA: African American; CM: cardiomyopathy; DCM: dilated cardiomyopathy; HCM: hypertrophic cardiomyopathy; TAAD: thoracic aneurysm and aortic dissection; MVP: mitral valve prolapse; HF: heart failure; VT: ventricular tachycardia; LVNC: left ventricular non-compaction; Neg: negative; Pos: positive. If a likely pathogenic (LP) or known pathogenic (KP) variant was identified, co-existing VUS that could not be excluded as benign in genes that fit the patient's phenotype were reported back to participants and discussed in a nuanced manner (like all VUS) as likely to be at best contributory, given the co-existence of a pathogenic variant.

Table S3: No Difference in Distribution or Number of Deleterious TTN MS variants in CVD cases vs. controls

Location	AA start	AA end	Cases CADD > 20	Controls CADD > 20	Cases CADD > 15	Controls CADD > 15	Cases CADD > 13	Controls CADD > 13	Cases CADD > 10	Controls CADD > 10	Totals (Cases)	Totals (Controls)
Z-band	1	831	1	4	1	5	1	5	1	5	1	5
Near-Z	832	2169	0	2	0	5	0	6	0	6	0	6
I-band	2170	15655	1	24	4	38	4	48	5	55	7	64
A-band	15656	33588	2	48	7	80	8	92	9	99	10	101
M-band	33589	35992	1	5	2	6	3	10	3	10	4	10
Expected Values for Chi-Square Goodness of Fit Test												
No. of cases	54	**Expected**	0.48		0.60		0.60		0.60		0.60	
No. of controls	451		0.24		0.60		0.72		0.72		0.72	
			2.87		4.54		5.73		6.57		7.65	
			5.73		9.56		10.99		11.83		12.07	
			0.60		0.72		1.19		1.19		1.19	
Goodness of Fit Test p-values for each CADD score threshold (none are significant at 0.05)												
Chi-sq			0.32		0.42		0.28		0.31		0.09	

Table S4: Median percent of bases in CVD genes covered at <8x

ABCC9 0	*ACADS* 0	*ACADVL* 0.44	*ACTA2* 0	*ACTC1* 0	*ACTN2* 0	*AGK* 0.57	*AGL* 0
ALMS1 .11	*ANK2* 0.07	*ANKRD1* 0	*APOA1* 0	*ARSB* 1.96	*BAG3* 0	*CACNA1C* 0.2	*AKAP9* 0.55
CACNB2 0	*CASQ2* 0	*CAV3* 0	*CBS* 1.45	*COL3A1* 1.23	*COL5A1* 2.14	*CRYAB* 0	*CSRP3* 0
CTF1 18.08	*DES* 0	*DMD* 0.07	*DNAJC19* 0	*DSC2* 0.22	*DSG2* 0	*DSP* 0.17	*DTNA* 0
EFEMP2 0	*ELN* 0.07	*EMD* 0	*EYA4* 0	*FBN1* 0	*FBN2* 0.01	*FHL2* 0	*FKTN* 0.51
FLNA 0.05	*GAA* 0	*GBE1* 0	*GJA5* 0	*GLA* 0.51	*GLB1* 9.15	*GNPTAB* 0	*GPD1L* 0
GYS1 4.16	*HAMP* 0	*HFE2* 0	*HRAS* 0	*JUP* 0.04	*KCNA5* 0	*KCNE1* 0	*KCNE2* 0
KCNE3 0	*KCNH2* 4.6	*KCNJ2* 0	*KCNQ1* 6.7	*LAMA4* 0	*LAMP2* 7.69	*LDB3* 3.18	*LMNA* 5.51
MED12 2.4	*MFAP5* 0	*MYBPC3* 1.18	*MYH11* 0	*MYH6* 4.64	*MYH7* 4.06	*MYL2* 0	*MYL3* 0
MYLK 0.3	*MYLK2* 0	*MYOZ2* 0	*MYPN* 0	*NEXN* 1.63	*NOTCH1* 1.52	*NPPA* 0	*PKP2* 7.26
PLN 2.02	*PRKAG2* 0	*PRKG1* 0	*PSEN1* 0	*PSEN2* 0	*RBM20* 0.49	*RYR2* 0.15	*SCN1B* 3.47
SCN3B 0	*SCN4B* 3.35	*SCN5A* 0	*SGCD* 0	*SKI* 1.42	*SLC22A5* 0	*SLC25A20* 0	*SLC25A* 4
SLC2A10 0	*SMAD3* 0.31	*SNTA1* 11.92	*TAZ* 0	*TCAP* 0	*TGFB2* 0	*TGFB3* 0	*TGFBR1* 6.42
TGFBR2 0	*TMEM43* 0	*TMPO* 0.86	*TNNC1* 0	*TNNI3* 0.16	*TNNT2* 0	*TPM1* 0	*TTN* 2.11
TTR 0	*VCL* 0.79						

Video S1: Optical video recording of heartbeat in Tg zebrafish injected with 7ng morpholino targeting znup37

Video S2: Optical video recording of heartbeat in zebrafish injected with 8ng morpholino targeting zsyne1b