SUPPLEMENTAL MATERIAL

Table S1. Echocardiography indices in the *sorbs2*$^{e8/-}$ heterozygous mice compared to WT controls at 4 weeks post-doxorubicin injection (20 mg/kg)

	WT siblings	*Sorbs2*$^{e8/-}$	P value
Mice number (n)	15	14	
HR (bpm)	356±63	350±74	0.829
IVSd (mm)	0.7±0.1	0.7±0.1	0.364
LVIDd (mm)	3.9±0.3	4.0±0.4	0.333
LVPWd (mm)	0.8±0.3	0.7±0.1	0.223
IVSs (mm)	1.1±0.1	1.1±0.1	0.518
LVIDs (mm)	2.8±0.3	2.9±0.4	0.348
LVPWs (mm)	1.1±0.1	1.1±0.1	0.882
LVEF (%,Cube)	63.1±5.6	63.5±5.8	0.843
LVEF (%, Teich)	61.7±5.6	61.9±5.8	0.955
LVFS (%)	28.4±3.7	28.7±3.8	0.827
LVd Mass (g)	0.7±0.0	0.7±0.0	0.743
LVs Mass (g)	0.7±0.0	0.7±0.0	0.808

HR, heart rate; bpm, beats per minute; IVSd, Interventricular septum thickness at end–diastole; LVIDd, left ventricular internal dimension at end-diastole; LVPWd, left ventricular internal dimension at end-diastole; IVSs, Interventricular septum thickness at end–systole; LVIDs, Left ventricular internal dimension at end-systole; LVPWs, Left ventricular posterior wall thickness at end–diastole; LVEF, left ventricular ejection fraction; LVFS, left ventricular fractional shortening; LVd, left ventricular at end-diastole; LVs, left ventricular at end-systole. Unpaired two-tailed student's *t*-test.

Table S2. Baselines of 59 ACM patients and 402 health control

	Patients with ACM (n=59)	Healthy Control (n=402)	p-value
Age, y	47.5 ± 13.7	62.8 ± 11.2	<0.001
Male, n (%)	37 (62.7)	265 (65.9)	0.628
Hypertension, n (%)	19 (32.2)	230 (57.2)	<0.001
Diabetes, n (%)	7 (11.9)	75 (18.7)	0.203
Alcohol, n (%)	12 (20.3)	91 (22.6)	0.692
LAIDd, mm	41.3 ± 13.1	33.4 ± 5.4	<0.001
LVIDd, mm	49.9 ± 10.2	46.8 ± 4.9	0.302
RAIDd, mm	47.1 ± 12.2	32.5 ± 6.7	<0.001
RVIDd, mm	50.1 ± 11.2	28.0 ± 5.2	<0.001
Maximum LV wall thickness, mm	9.7 ± 1.61	9.2 ± 0.7	0.421
Left ventricular eject fraction, %	53.5 ± 14.2	63.2 ± 7.6	<0.001
Implantable cardioverter-defbrillators, n (%)	15 (25.4)	0 (0.0)	<0.001

LAIDd, left atrial internal dimension at -diastole; LVIDd, left ventricular internal dimension at -diastole; RAIDd, right atrial internal dimension at diastole; RVIDd, right ventricular internal dimension at diastole. Unpaired two-tailed student's *t*-test.

Table S3. Diagnosis of the ACM patients carrying potential pathogenic variants in *SORBS2* gene according to 2019 HRS expert consensus statement

Genotype	Global and/or Regional Dysfunction and Structural Alterations	Tissue Characterization of Wall	Repolarization Abnormalities	Depolarization/ Conduction Abnormalities	Arrhythmias	Family History
c.679+1G>T	Major	NA	-	Minor	Major	NA
c.869+1C>G	Major	NA	Major	Minor	Major	Major

NA, data not available

Table S4. Clinical characteristics and genotypes of patients carrying potential pathogenic variants in the *SORBS2* gene

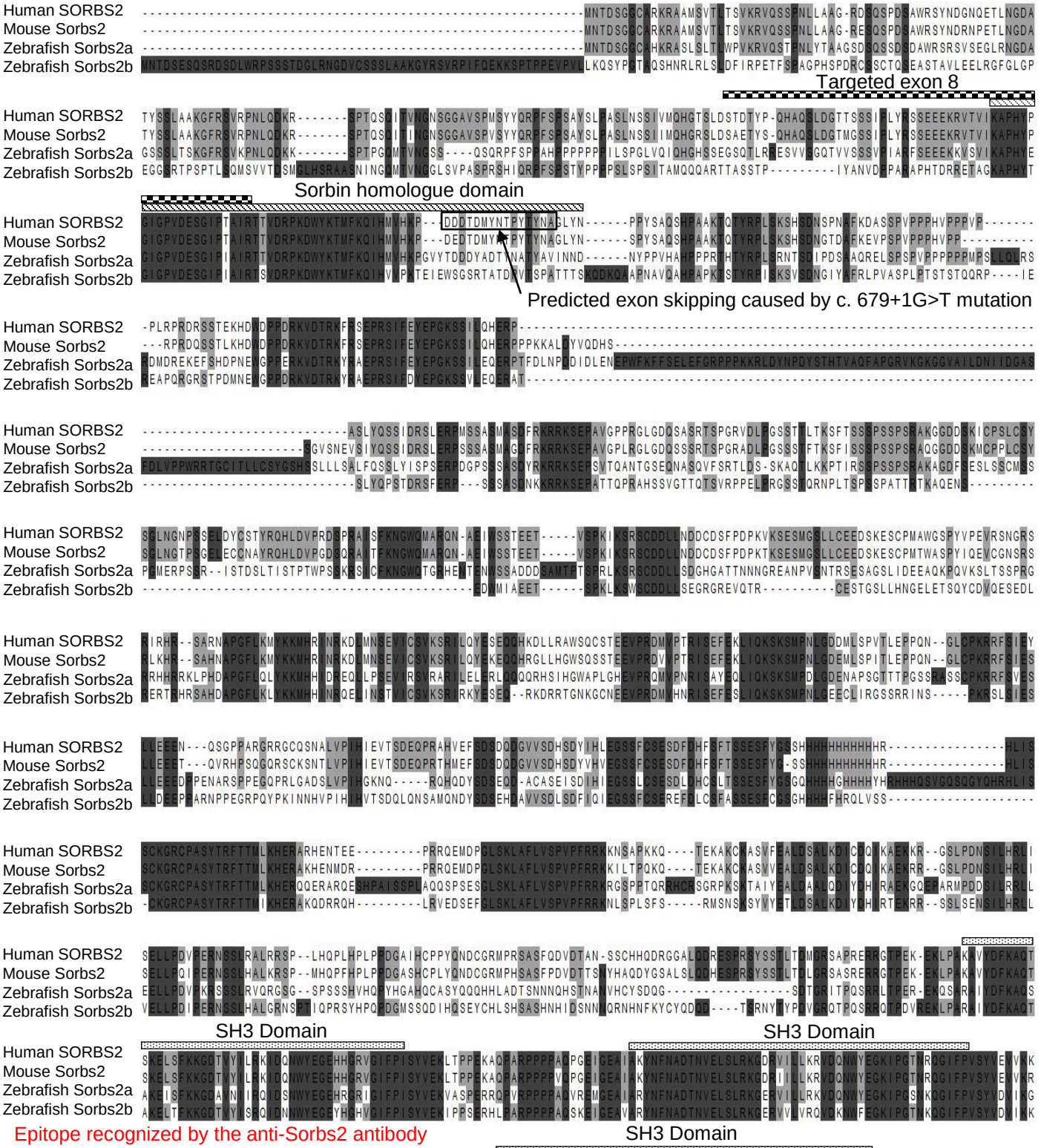
Gene	Genotype	Age y	LAIDd mm	LVIDd mm	RAIDd mm	RVIDd mm	LVEF %	Mutations for other known ACM genes
SORBS2	c.679+1G>T	43	32	50	33	45	68	None
SORBS2	c.869+1C>G	27	29	42	47	49	66	*PKP2*: c.2421C>A

RefSeq: *SORBS2*: NM_001270771.1; LAIDd, left atrial internal dimension at diastole; LVIDd, left ventricular internal dimension at diastole; RAIDd, right atrial internal dimension at diastole; RVIDd, right ventricular internal dimension at diastole; LVEF, left ventricular ejection fraction. ARVC, arrythmogenic right ventricle cardiomyopathy.

Table S5. Sanger sequencing primers of coding regions of the *SORBS2 and CTNNA3* gene

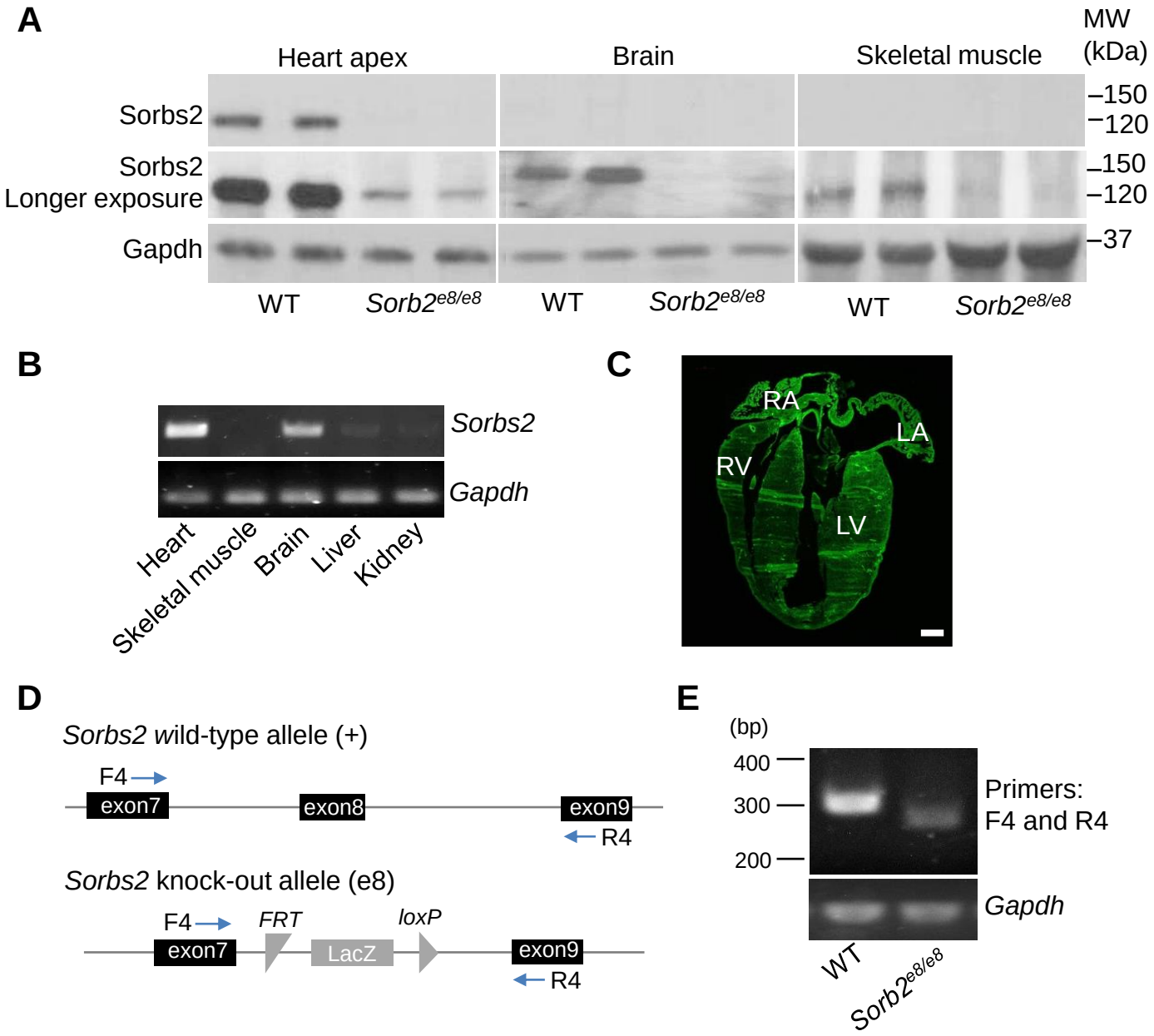
Name	Forward	Reverse
SORBS2-E4	5'- AAGAGTGGGGCACAAATACAAAG-3'	5'- AAACAAGCACAAAACGTCTCCAT-3'
SORBS2-E5	5'- AAACGTGAATAGTTATTAACACTCGC-3'	5'- GGTGCATTCCATGATTACTAATTCT-3'
SORBS2-E6E7	5'- GAAAGAGGCTGTAAGCAGTGGTC-3'	5'- TTGGCATGTTTGACATACCCA-3'
SORBS2-E8	5'- AGACACAGGTGGAGACATGGTCT-3'	5'- TACTCTCATGTGATTGGAAGTCTTTTA-3'
SORBS2-E9	5'- AGGAATGCACAAAGCCGTG-3'	5'- CTCTGTGTTACAGTTGGCATAAATATAC-3'
SORBS2-E10	5'- AACCGATGCAAAAGACGAATG-3'	5'- GCAAAGAGTCAGAACATTTTATCACA-3'
SORBS2-E11	5'- CCTTTTGTTATGCACGCTCTGA-3'	5'- TACGTTGCTTTTCTAACAAATTTCTG-3'
SORBS2-E12	5'- TGGCAGGTGCACTGATTCTTC-3'	5'- CAAAGATTTACTGTAATCAGGGCTTA-3'
SORBS2-E13	5'- AAATTGCTAATGGGTCTGAAATTC-3'	5'- CTCATGTGACTTTTGGAGCCAG-3'
SORBS2-E14	5'- TAAGGGCCATTTAACAACATTGA-3'	5'- CCAAGTAGAAATCAGGTCGCAT-3'
SORBS2-E15	5'- ATTCCAGACACTGTAGGTGAGAGC-3'	5'- GGTTTAGGAGTTACGCCATTGTC-3'
SORBS2-E16-Part1	5'- GGGAGTGATGAGATGTTGGAATG-3'	5'- ACATTGAAGTCACCAGCGATGA-3'
SORBS2-E16-Part2	5'- GTAAATCGAGTGTAGGAGGCCG-3'	5'- GAAGCTCCATGTGTGAACAATCA-3'
SORBS2-E17	5'- GAGTTCGTTACTGGTTTGGGATA-3'	5'- TGTGTTCGATAACGGTGTCTCTC-3'
SORBS2-E18	5'- GATCCTACTGCAATGCTTATCTCTTA-3'	5'- GTTTGATGATTCCCTGAGGTTTC-3'
SORBS2-E19E20	5'- ACCAGCAGGGAACCGTGAC-3'	5'- CAAGGATCATTCTTATCTAATCGGAC-3'
SORBS2-E21	5'- TTGTCAACCTAATAACCCGGTG-3'	5'- TCTGATGGCCTGAATGGACAT-3'
SORBS2-E22	5'- CGTGCAAAGCCATACATCTTACA-3'	5'- ACATTTGACCTTGCTTATTTTGCT-3'
SORBS2-E23	5'- GCCATCACAGCAAACCACG-3'	5'- ACAATTTTCATGGATATTCCTCGT-3'
SORBS2-E24	5'- TTGGAAACCGTAAGGCATGA-3'	5'- CACTGCTTATTTTGTCTGCCTATG-3'
CTNNA3-E2	5'-TTATTCTTCATTATTCATTTTTCCCAC-3'	5'-TCCTCTGAGGGCTATGGTTGAT_3'
CTNNA3-E3	5'-GCCTGGAGTGGGACTGAGC-3'	5'-TCTGGTCAACAGTAGGCTATTCGTA-3'
CTNNA3-E4	5'-GTGAAGCCAAGATGCACTAGGAT-3'	5'-GGCAGCAATTTTACTAGATGTTCCT-3'
CTNNA3-E5	5'-TTAGAAGATAAGTTTCCTCAGACCCA-3'	5'-GAGGACTGAACAGGCTTCTCATG-3'
CTNNA3-E6	5'-TGCTCTAAACGCCAACATGTG-3'	5'-ACATCTGCTCTACGGGGACCT-3'
CTNNA3-E7	5'-CTAGAAATTCCCAGGGTTGTTG-3'	5'-TGAACCAGCCGAGGCAGTA-3'
CTNNA3-E8	5'-CTGATAAGGCACACATTCTTTCTCT-3'	5'-TCTTAAGTGAAATGCTCATGAATCATA-3'
CTNNA3-E9	5'-AAGACAAGCCAATGTGGAGTGA-3'	5'-TAAGTATTGTGATTTTAGGAATTGTGC-3'
CTNNA3-E10	5'-AAGCAGAAGTTGCAGTCAGTCG-3'	5'-ACCAAGAGACTGTATAGGGTTTACTGAT-3'
CTNNA3-E11	5'-AATCCCTGACCTCAAGTAATCCAC-3'	5'-TTTACTGTTTTTCCTATTGTCGATGAT-3'
CTNNA3-E12	5'-CAATGTATGTCAAAAGAAGCAACAC-3'	5'-ATTTCCAATGTGCACTCTATCTGA-3'
CTNNA3-E13	5'-TTGTTTGTGACAGTAGATCTGACTTTG-3'	5'-TGAGAAATGATTGTATGGTCCCA-3'
CTNNA3-E14	5'-ACCCATTAGAGGCTGCCTAGAT-3'	5'-GAAATTGCTGGGTCATATGGTAAC-3'
CTNNA3-E15	5'-TGGCACTTGACACTCAGAGAATC-3'	5'-AAGGTGATGCATTACACTAAATTGAT-3'
CTNNA3-E16	5'-CTTCCTTATAATCGTCCATTACCTAGT-3'	5'-ATAGCCGTTCTTTGGGATGC-3'
CTNNA3-E17	5'-CGGAGTTCCATCTGTACACATTCT-3'	5'-ATTGTAAATGGTAGAAGCATATCAGAAG-3'
CTNNA3-E18	5'-ATTAGGTGCTGACCATACAGAAATG-3'	5'-GAGCAGCTTATTGTGACATTAATAGACTA-3'

Figure S1. Alignment of Sorbs2 protein sequences from human, mouse and zebrafish



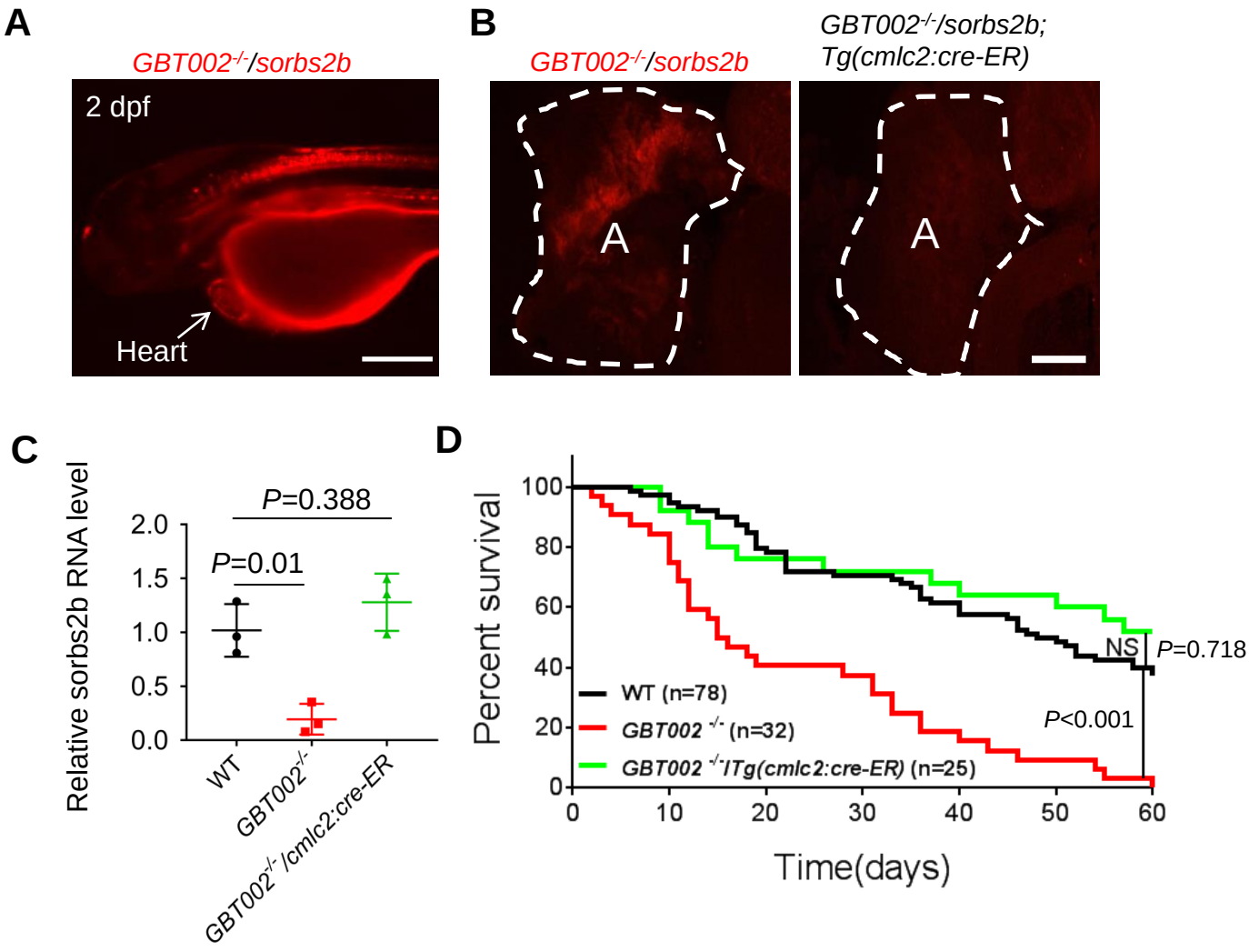
Sequences are more conserved in the N-terminal sorbin homologue domain and the C-terminal SH3 domain among 3 different species. The deletion in *Sorbs2* $^{e8/e8}$ mice removed N terminal sequences containing part of the sorbin homologue domain. The 15 amino acids that are encoded by exon 11 and disrupted by 679+1 G>T mutation are enclosed by a black rectangle frame. Epitope region recognized by the anti-Sorbs2 antibody used for Western blot and immunostaining in mouse tissues is indicated by a red solid line.

Figure S2. Targeted depletion of the exon 8 in the *Sorbs2*$^{e8/e8}$ mice



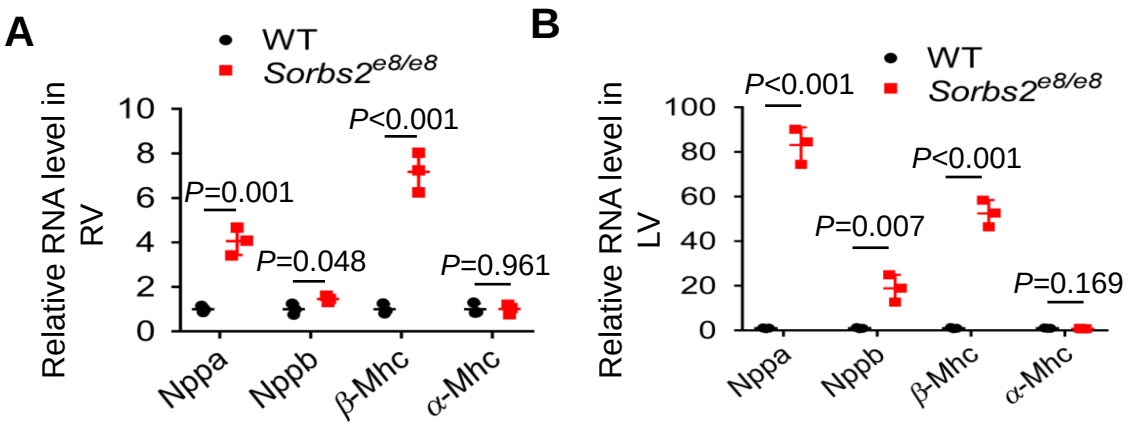
A, Western blotting analysis of Sorbs2 protein expression in the heart, brain and skeletal muscle of *Sorbs2*$^{e8/e8}$ and WT control mice. **B,** RT-PCR analysis of the *Sorbs2* transcript expression in different mice tissues. **C,** Shown are immunostaining of mice heart sections using an anti-Sorbs2 antibody. Sorbs2 is ubiquitously expressed in 4 cardiac chambers and septum. Scale bar: 50 μm. **D,** Schematic design of primers for RT-PCR to analyze the targeted deletion of exon 8 in the *Sorbs2*$^{e8/e8}$ mice. **E**, Shown are representative DNA gel images of RT-PCR to confirm the replacement of exon 8 with LacZ that leads to frameshift of Sorbs2 transcript in the *Sorbs2* $^{e8/e8}$ mice.

Figure S3. Myocardial expression of *sorbs2b* conveys the modifying effects of *GBT002*$^{-/-}$ on doxorubicin-induced cardiomyopathy (DIC) in zebrafish



A, Heart enriched expression of red fluorescent protein (RFP) reporter in the *GBT002/sorbs2b* mutant at 2 days post-fertilization (dpf). Scale bar, 200 μm. **B,** RFP expression in the *GBT002*$^{-/-}$ mutant heart, representing an RP2 insertion in the sorbs2b locus, was effectively excised in the *GBT002*$^{-/-}$*;Tg(cmlc2:cre-ER)* double fish after hydroxytamoxifen treatment. Scale bar: 200 μm. **C**, Quantitative RT-PCR results show that normal *sorbs2b* RNA is dramatically reduced in *GBT002*$^{-/-}$ fish compared to WT, but was largely restored in the *GBT002*$^{-/-}$*; Tg(cmlc2:cre-ER)* double fish heart. N=3. * : $p<0.05$. NS, not significant. One-way ANOVA. **D**, The survival rate is significantly lower in *GBT002*$^{-/-}$ mutant compared to WT fish after doxorubicin injection (20 μg/g) , which was largely rescued in the *GBT002*$^{-/-}$*; Tg(cmlc2:cre-ER)* double fish. N=25-78. NS, not significant. Log rank test.

Figure S4. Elevated expression of molecular markers for heart failure in the *Sorbs2*$^{e8/e8}$ mice



A-B, Quantitative RT-PCR analysis of heart failure markers in the RV (A) and LV (B) of the hearts of *Sorbs2*$^{e8/e8}$ mice compared to WT. RV, right ventricle. LV, left ventricle. N=3. Unpaired two-tailed Student's *t*-test.

Figure S5. Representative Immunostaining images of ICD proteins in the *Sorbs2*$^{e8/e8}$ and WT control mice mouse hearts

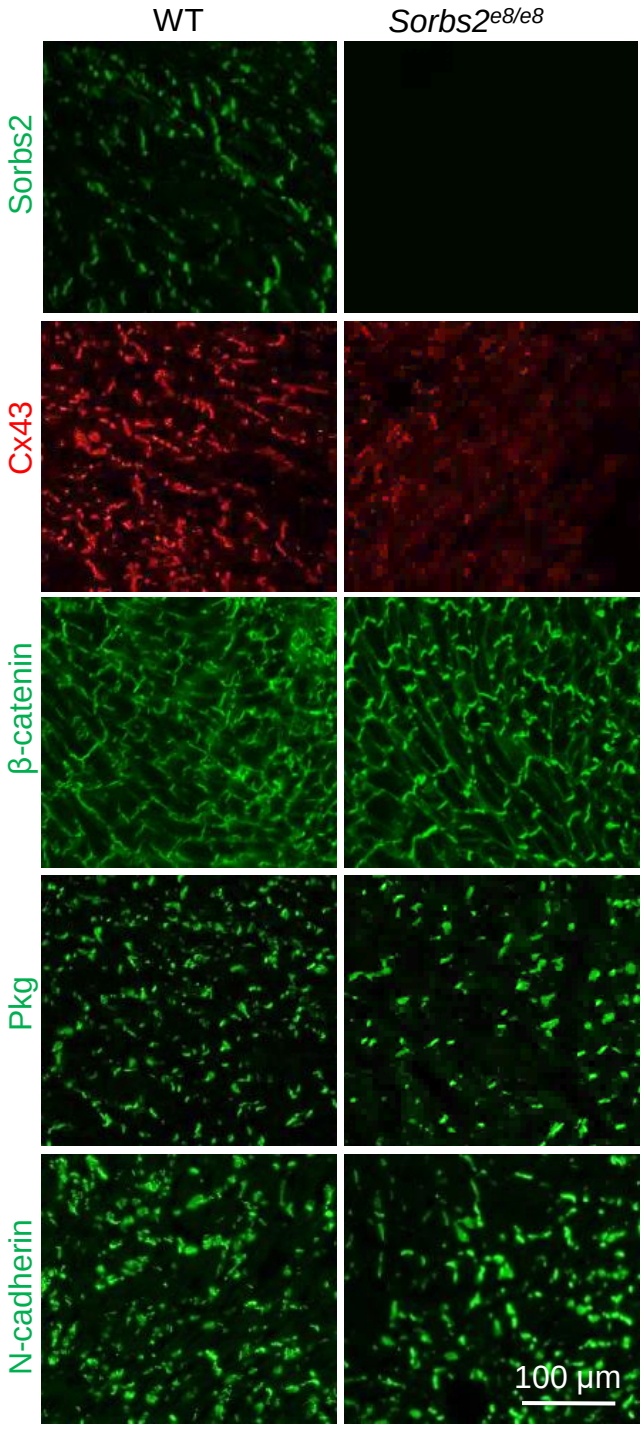
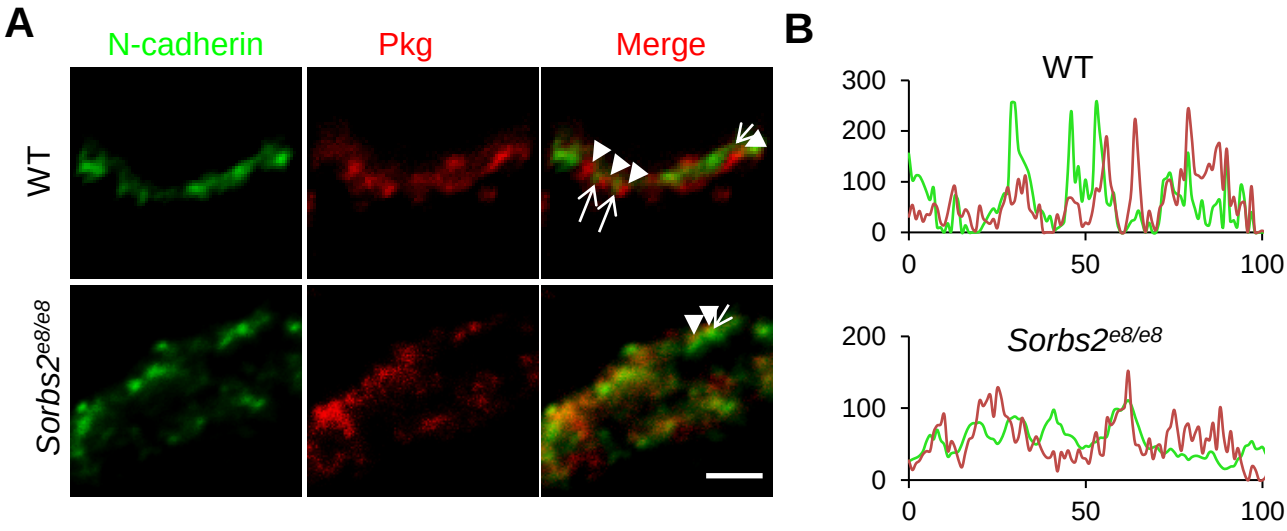


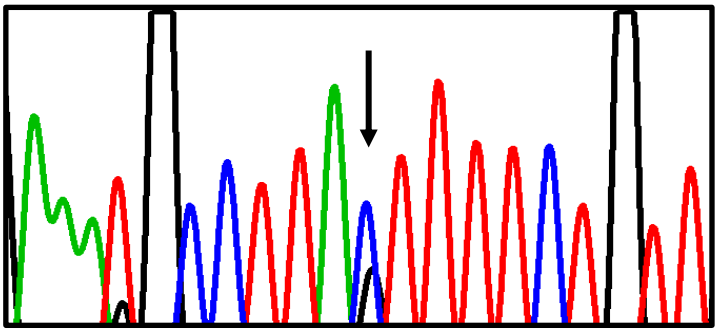
Figure S6. Altered expression patterns of N-cadherin in the *Sorbs2* $^{e8/e8}$ mice



A, Shown are images of mouse hearts after immunostaining using anti-N-cadherin antibodies (green, indicated by arrows) and anti-Pkg (red, indicated by arrow heads). The alternating expression pattern of Pkg and N-cadherin protein expression in WT mice is disturbed in *Sorbs2* $^{e8/e8}$ mice along ICD. Scale bar: 2 μm. LV: left ventricle, RV: right ventricle. **B,** Pseudo line analysis of images in **A**.

Figure S7. Identifications of *SORBS2* and other known ACM variants in an cohort of 59 ACM patients

A



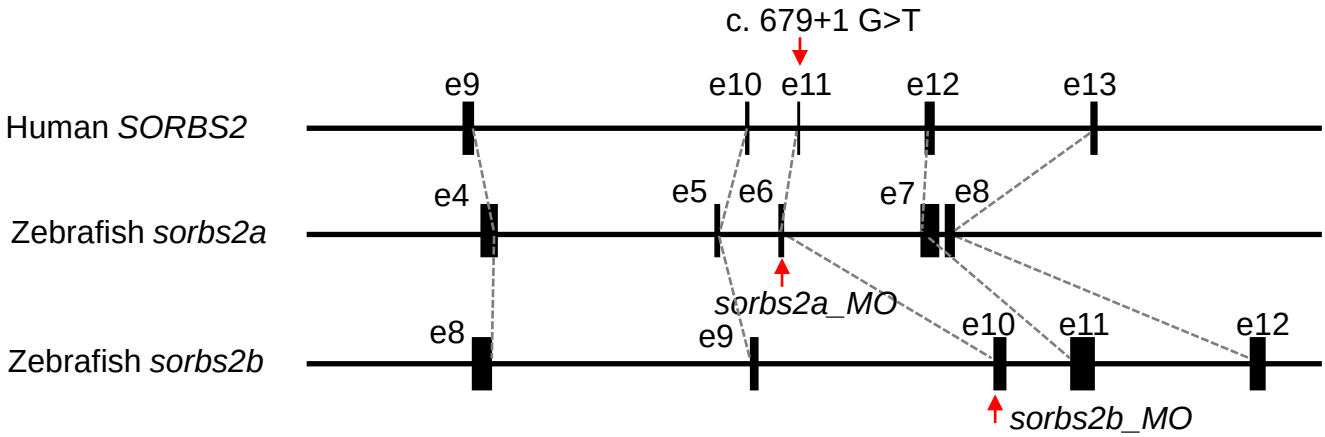
SORBS2 c.869+1C>G

B

No.	Gene	Official Full Name	Chr.	CDS n	Amplicons n	Target bp	Coverage %
1	*DES*	Desmin	2	9	17	1512	100
2	*DSC2*	Desmocollin 2	18	17	31	2606	89.0
3	*DSG2*	Desmoglein 2	18	15	36	3420	97.1
4	*DSP*	Desmoplakin	6	25	74	9364	99.7
5	*JUP*	Junction Plakoglobin	17	13	22	2381	100
6	*LMNA*	Lamin A/C	1	13	23	2249	100
7	*PKP2*	Plakophilin 2	12	14	28	2640	94.3
8	*PLN*	Phospholamban	6	1	10	1139	75.8
9	*RYR2*	Ryanodine Receptor 2	1	105	165	14830	99.5
10	*SCN5A*	Sodium Voltage-Gated Channel Alpha Subunit 5	3	30	62	6807	100
11	*TGFB3*	Transforming Growth Factor Beta 3	14	7	11	1316	100
12	*TMEM43*	Transmembrane Protein 43	3	12	16	1335	100
13	*TTN*	Titin	2	315	947	109511	99.1

A, Chromagraph illustrates c.869+1C>G mutation identified in the *SORBS2* gene from an ACM patient. **B,** List of an ACM gene panel that consists of 13 genes was sequenced by targeted sequencing.

Figure S8. Schematic illustration of corresponding exons among human *SORBS2* and its zebrafish orthologs *sorbs2a* and *sorbs2b* gene



The c.679+1G>T mutation is located in the exon 11 of *SORBS2* in human, corresponding to the exon 6 in *sorbs2a* and the exon 10 in *sorbs2b* gene in zebrafish.