Supplemental Material

Table S1. Customized classification of pathogenic variants based on the recommendations of the ACMG guideline.

Classification	Major criteria	Supporting criteria
Pathogenic	1.Widely reported variant with conclusive evidence of genotype-phenotype association and with consensus about its pathogenicity. 2.Demonstrated co-segregation with a phenotype (＞10 meiosis) 3.Co-segregation in at least 2 families (≤10 meiosis), or present in at least 5 probands with the same phenotype and meeting at least 2 supporting criteria.	A. Protein-truncating variant in a gene where loss of function is a proven pathogenic mechanism B. Functional studies that supporting pathogenicity. C. De novo presentation in the setting of a novel disease in the family (maternity and paternity confirmed) D. Missense variant that generates the same amino-acid change as a previously reported pathogenic variant. E. Variant with very low frequency/absent in the control population (MAF＜0.001%).
Likely pathogenic	1.Protein-trucating variant with very low frequency/absent in the control population (MAF＜0.001%) that affects a gene where loss of function is not an established pathogenic mechanism or that does not meet criteria to be considered pathogenic. 2.Missense variant/in-frame insertion or deletion in a non-repetitive region of a gene which does not meet criteria to be considered pathogenic, but that meets at least 3 supporting criteria.	A. Variant with very low allelic frequency/absent in the control population (MAF＜0.001%). B. De novo presentation in the setting of a novel disease in the family (maternity and paternity unconfirmed). C. Patient's phenotype or family history suggests that disease could be explained by mutations in the gene (gene with well-established phenotype-genotype association). D. Bioinformatics predictors agree that it would be deleterious. E. Located in a mutational hot-spot, functional domain, or relevant region of the codified protein. F. Reported in at least 2 unrelated individuals that presented the same phenotype.

Variants of uncertain significance	1.Variants with contradictory information about their pathogenicity 2.Variant that do not meet criteria for being included in another classification category	
Likely benign	1.Variant allele frequency in control populations is higher than the expected for diseases or has a MAF＞0.01%. 2.Absence of variant co-segregation with the phenotype in at least one family. 3.Meeting at least 2 supporting criteria.	A. Missense variant in a gene where only variants causing protein truncation have shown association with disease. B. Functional study showing that the variant does not affect the structure or function of the encoded protein. C. Bioinformatics predictors agree that the variant would not alter the function of the protein (including splicing variants outside the consensus region of the gene). D. In-frame insertion/deletions in a repetitive gene region without known function. E. Presence of the variant in homozygosis in control population.
Benign	1.MAF＞1% in any of the control population databases. 2.Previously reported in the literature with well-established evidence of consensus about its non-disease-causing classification, and with no contradictory data. 3.Absence of co-segregation with the disease in at least 2 reported families. 4.Meeting at least 2 supporting criteria.	A. Variant allele frequency in controls population is higher than expected for disease or has a MAF＞0.01% B. Absence of co-segregation of the variant with the phenotype in at least 1 family. C. Functional study showing that the variant does not affect the structure of function of the encoded protein. D. Presence of the variant in healthy unaffected subjects at an age at which the disease should be full penetrant (variant must be in homozygosis in recessively inherited disease, or in hemizygosis in X-linked diseases).

ACMG[19], American College of Medical Genetics and Genomics; MAF, minor allele frequency.

Table S2. The variants of *FHOD3* detected in HCM patients and controls.

Transcript effect (NM_ 001281740.1)	Protein (NP_001268669.1)	Type	Variant classification[#]	dsSNP	*FHOD3* domain	CADD	SIFT	Polyphen	GnomAD[&] MAF%	ExAC[*] MAF%	In-house MAF%	Phenotype (n)
c.274C>T	p.Arg92Trp	Missense	VUS	rs759696197	GBD/FH3	27.1	0.005	0.967	0.0032	0.0051	0.0284	HCM (1)
c.562C>T	p.Arg188Cys	Missense	VUS	rs143579901	GBD/FH3	24.2	0.001	0.015	0.0032	0	0.0284	HCM (1)
c.566A>G	p.Asn189Ser	Missense	LB	rs747688287	GBD/FH3	17.74	0.49	0.001	0.0008	0.0008	0.0852	HCM (1); control (2)
c.595A>G	p.Ile199Val	Missense	B	rs61735987	GBD/FH3	17.31	0.31	0.002	1.6533	3.2533	7.2414	HCM (136); control (112)
c.646G>A	p.Val216Ile	Missense	VUS	rs551483382	GBD/FH3	26.6	0.004	0.758	0.0084	0.0084	0.0284	HCM (1)
c.776C>T	p.Thr259Met	Missense	VUS	rs770013602	GBD/FH3	26.2	0.008	0.414	0.0096	0.0165	0.0284	HCM (1)
c.796A>G	p.Met266Val	Missense	VUS	Novel	GBD/FH3	24.1	0.187	0.21	0	0	0.0284	Control (1)
c.958G>T	p.Val320Leu	Missense	LB	rs571359036	GBD/FH3	22.1	0.247	0.047	0.0062	0.0092	0.1420	HCM (1); control (4)
c.1004C>G	p.Pro335Arg	Missense	B	rs117005081	GBD/FH3	23.0	0.058	0.159	1.2641	2.9346	0.7098	HCM (16); control (9)
c.1007G>A	p.Ser336Asn	Missense	VUS	Novel	GBD/FH3	13.15	NA	NA	0	0	0.0284	HCM (1)
c.1063C>T	p.Arg355Trp	Missense	VUS	Novel	GBD/FH3	25.9	0.001	0.471	0	0	0.0284	HCM (1)
c.1097C>T	p.Ser366Leu	Missense	VUS	rs747730516	GBD/FH3	27.4	0.001	0.982	0.0032	0.0041	0.0568	HCM (2)
c.1157C>T	p.Pro386Leu	Missense	VUS	Novel	GBD/FH3	24.7	NA	NA	0	0	0.0284	Control (1)
c.1189C>T	p.Arg397Cys	Missense	VUS	rs760874847	GBD/FH3	20.7	0.091	0.001	0	0	0.0568	HCM (2)
c.1286+2delT	NA	Spicing	P	Novel	Ex				0	0	0.1136	HCM (4)
c.1297G>A	p.Ala433Thr	Missense	B	rs62083981	Ex	0.945	NA	NA	2.3784	3.4027	0.1136	HCM (3); control (1)
c.1309C>T	p.Gln437Ter	Nonsense	LP	Novel	Ex	35			0	0	0.0284	HCM (1)
c.1364C>T	p.Ser455Leu	Missense	B	rs2848901	Ex	16.74	NA	NA	27.0205	38.1965	43.7923	HCM (486); control (343)
c.1411G>A	p.Gly471Arg	Missense	B	rs72895597	Ex	1.256	NA	NA	12.8884	10.1348	0.9938	HCM (11); control (24)
c.1552G>A	p.Val518Met	Missense	VUS	Novel	Ex	25.7	NA	NA	0	0	0.0284	HCM (1)

c.1580C>T	p.Ser527Phe	Missense	VUS	Novel	Ex	25.5	NA	NA	0	0	0.0284	HCM (1)
c.1640A>C	p.Glu547Ala	Missense	VUS	Novel	Ex	23.7	NA	NA	0	0	0.0284	HCM (1)
c.1703G>T	p.Arg568Leu	Missense	VUS	Novel	Ex	20.9	NA	NA	0	0	0.0284	HCM (1)
c.1733T>A	p.Phe578Tyr	Missense	VUS	Novel		19.2	NA	NA	0	0	0.0284	Control (1)
c.1754C>A	p.Ser585Tyr	Missense	LB	rs200702049		21.5	0.005	0.348	0.0046	0.0091	0.2271	HCM (5); control (3)
c.1844C>T	p.Pro615Leu	Missense	LB	rs199579476		2.072	1.0	0.0	0.0024	0.0016	0.0284	Control (1)
c.1912C>T	p.Arg638Trp	Missense	LB	rs141148037	CC	26.4	0.0	0.995	0.0478	0.0561	0.1136	HCM (4)
c.1924G>A	p.Glu642Lys	Missense	VUS	Novel	CC	26.2	0.005	0.979	0	0	0.0284	HCM (1)
c.2077C>T	p.Arg693Trp	Missense	VUS	rs533572045	DID	29.1	0.0	0.292	0.0064	0	0.0284	Control (1)
c.2078G>A	p.Arg693Gln	Missense	VUS	rs148866621	DID	23.2	0.083	0.001	0.0096	0.0087	0.0284	HCM (1)
c.2090G>A	p.Arg697Gln	Missense	VUS	rs553341694	DID	23.2	0.006	0.811	0.0065	0.0095	0.0568	HCM (2)
c.2129C>G	p.Ala710Gly	Missense	B	rs61735993	DID	22.2	0.057	0.197	13.873	13.5774	7.2961	HCM (118); control (102)
c.2260G>A	p.Glu754Lys	Missense	LB	rs139884505	DID	15.4	0.462	0.002	0.1721	0.1285	0.0284	HCM (1)
c.2321A>G	p.Gln774Arg	Missense	B	rs61735994	DID	6.648	0.423	0.0	2.2748	2.8685	0.0852	Control (3)
c.2429G>T	p.Gly810Val	Missense	VUS	Novel	DID	22.7	0.02	0.022	0	0	0.0284	HCM (1)
c.2464G>A	p.Val822Phe	Missense	LB	Novel	DID	12.27	NA	NA	0	0	0.0852	Control (3)
c.2584G>A	p.Asp862Asn	Missense	VUS	rs544119818	DID	21.1	0.671	0.002	0.0032	0.0043	0.0568	HCM (2)
c.2746T>G	p.Ser916Ala	Missense	VUS	Novel	DID	20.8	0.091	0.711	0	0	0.0568	HCM (1); control (1)
c.2824G>C	p.Glu942Gln	Missense	VUS	rs779000457	DID	27.1	0.016	0.986	0.0025	0.0035	0.0284	HCM (1)
c.2837G>A	p.Ser946Asn	Missense	VUS	Novel	DID	15.46	0.247	0.058	0	0	0.0284	HCM (1)
c.2915C>T	p.Pro972Leu	Missense	LB	rs551904999	DID	11.00	0.332	0.002	0.0064	0	0.0568	Control (2)
c.2954C>A	p.Ala985Asp	Missense	VUS	Novel	DID	25.9	0.003	0.996	0	0	0.0284	Control (1)
c.3005A>T	p.Glu1002Val	Missense	VUS	Novel	DID	26.9	0.003	0.408	0	0	0.0284	HCM (1)

c.3187G>A	p.Ala1063Thr	Missense	LB	rs560946106	FH2	13	0.515	0.003	0.0064	0.0041	0.0568	HCM (2)
c.3412T>C	p.Ser1138Pro	Missense	VUS	Novel	FH2	23.1	0.071	0.101	0	0	0.0284	HCM (1)
c.3478G>A	p.Ala1160Thr	Missense	VUS	rs746707013	FH2	24.6	0.004	0.777	0.0032	0	0.0284	Control (1)
c.3587C>G	p.Thr1196Arg	Missense	VUS	Novel	FH2	25.2	0.01	0.999	0	0	0.0284	Control (1)
c.3601G>A	p.Asp1201Asn	Missense	LB	rs554487359	FH2	24	0.023	0.493	0.0127	0.0034	0.0284	HCM (1)
c.3624G>C	p.Gln1208His	Missense	VUS	Novel	FH2	24.2	0.001	0.909	0	0	0.0284	HCM (1)
c.3796A>G	p.Ile1266Val	Missense	VUS	Novel	FH2	25.2	0.276	0.028	0	0	0.0284	HCM (1)
c.3976G>A	p.Val1326Ile	Missense	B	rs2303510	FH2	25.6	0.143	0.145	33.5504	34.1531	25.454	HCM (435); control (374)
c.4270T>A	p.Tyr1424Asn	Missense	VUS	rs753641918	FH2	25.1	0.314	0.003	0.0012	0.0017	0.0568	HCM (1); control (1)
c.4519G>A	p.Ala1507Thr	Missense	LB	rs574765321		25.9	0.052	0.946	0.0255	0.04	0.0852	HCM (1); control (2)
c.4586C>T	p.Pro1529Leu	Missense	VUS	Novel		18.4	0.342	0.052	0	0	0.0284	Control (1)
c.4667A>G	p.Asn1556Ser	Missense	LB	rs139930679		14.11	0.617	0.002	0.0223	0.0091	0.1136	HCM (2); control (2)
c.4702C>T	p.Arg1568Cys	Missense	VUS	rs770836110	DAD	28.1	0.187	0.917	0.0016	0.0008	0.0284	Control (1)
c.4708G>A	p.Val1570Ile	Missense	LB	rs201824593	DAD	27.2	0.005	0.991	0.0939	0.0064	0.0284	HCM (1)
c.4787T>C	p.Leu1596Ser	Missense	VUS	Novel	DAD	24.1	0.0	0.994	0	0	0.0284	Control (1)

#Determined according to criteria in Table S1. P, pathogenic; LP, likely pathogenic; VUS, variants of uncertain significance; LB, likely benign; B, benign.

&GnomAD: (the Genome Aggregation) https://gnomad.broadinstitute.org/ *ExAC: (Exome Aggregation Consortium) http://exac.broadinstitute.org

CADD, Combined Annotation Dependent Depletion score (phred); v1.3 (August, 2015)[21]; SIFT, SIFT (sorting intolerant from tolerant) algorithm; computed from ENSEMBL 55 (September, 2014)[22]; POLYPHEN, Polymorphism Phenotyping (v2; September 2014)[23].

HCM, hypertrophic cardiomyopathy; MAF, minor allele frequency; GBD/FH3, GTPase-binding domain/formin homology 3 domain; Ex, exclusively cardiac isoform; CC, coiled-coiled; FH2, formin homology 2 domain; DAD, diaphanous auto-inhibitory domain.

Table S3. Primer used for Sanger sequencing confirmation of *FHOD3* candidate variants.

	Forward primer	Reverse primer
FHOD3-E3	5' ATTTTCCCAACATGGTCAAGC 3'	5' CAGAAGAACCTCATCTACCCC 3'
FHOD3-E6	5' TTGGTGCCTTAATTGCATC 3'	5' CATTTATACTGTAACGGCTTG 3'
FHOD3-E7	5' CGATTCAGCACATACTCGTGTT 3'	5' CCTCTCCCAGGTAAGCTCAT 3'
FHOD3-E8	5' TGCCATCACTGGATACGTC 3'	5' TTCCAAATAAGCCCACAAGCA 3'
FHOD3-E10	5' GGGCAATCCTGAAATGCAGTCAC 3'	5' AAATCCACCGAGATGTTTGGC 3'
FH0D3-E11	5' CTCTTTTCCTGGCTTTGTCT 3'	5' AGTTCTCTAATGAAAACATGCTC 3'
FHOD3-E12	5' ACCTCCTTGCCCTCTATAAGTCT 3'	5' CTGTGTTCTCCTCCCCGAGT 3'
FHOD3-E13	5' CTGTGTTCTCCTCCCCGAGT 3'	5' GAGTTCTGATTTGCACACC 3'
FHOD3-E15P1	5' ATCTGAAACAAGAAGACCCGAG 3'	5' AGTTGTAAAGTCACATGCCTT 3'
FHOD3-E15P2	5' CCTGGAATACTTCTATAACTCCC 3'	5' GCCCAAGAATACATGAGTCCC 3'
MYH7-E17&18	5' CTCACACCCTACCTCCCCACAC 3'	5' GAGGTCCTGTTCCCAGGGCGGT 3'
FHOD3-E17	5' TGTGTGATGCTGCCATTTCCC 3'	5' AGTTGCTGTCTCAGTATTAGCCT 3'
FHOD3-E18	5' CCCTTCACAGCATTGCCTCGAT 3'	5' CCACACTCCTTGTCCCCAGACA 3'
FHOD3-E19P1	5' TGAGCCCAATGACAAGGTCCC 3'	5' AATCTCTTCAGCCCTACCCAAC 3'
FHOD3-E19P2	5' AGTCACTCCCATGTGTCAGGC 3'	5' ACTCTGTCTTCGGCTGCACC 3'
FHOD3-E21	5' CTTGCCTAGAATGTCCTATGTGT 3'	5' TCAATTCACCCTCCGTACCCT 3'
FHOD3-E22	5' GTCCAGAGCCCTTGTCACC 3'	5' CTACAGGATGAGGGATGGGG 3'
FHOD3-E28	5' AGCCCTCTGGATCTATCACTAGC 3'	5' CAACGTCAACAGCCAACCCT 3'

Table S4. Demographic and Clinical characteristics of the subjects in the discovery study.

Variable	HCM cases	Non-HCM controls	*P*-value*
Sample size	1000	761	
Age at enrolment, year	47.9 ± 14.6	47.7 ± 14.8	0.453
Male, n (%)	645 (64.5)	521 (68.5)	0.07
BMI, kg/m^2	25.5 ± 3.7	24.9 ± 3.4	0.001
Echocardiography			
MVT, mm	22.6 ± 5.8	9.8 ± 4.2	＜0.001
Left atrial, mm	41.7 ± 7.2	26.1 ± 4.2	＜0.001
LVEDD, mm	44.0 ± 6.3	42.2 ± 5.2	＜0.001
Ejection fraction, %	67.5 ± 8.2	65.4 ± 7.7	0.25

Continuous variables were presented as mean ± standard deviation; the categorical variable sex was presented as number (n) and percentage (%).

*Continuous variables were compared by Student's *t*-test; the categorical variable sex was compared by chi-squared test.

HCM, hypertrophic cardiomyopathy; BMI, body mass index; MVT, maximum left ventricular wall thickness; LVEDD, left ventricular end-diastolic diameter.

Table S5. LOD score indicating linkage between the truncating variant c.1286+2delT of *FHOD3* and hypertrophy cardiomyopathy in Pedigrees.

Pedigree ID	Number of individuals genotyped	Number of carriers	LOD score 95%	LOD score 80%
A	3	1	NA	NA
B	7	3	1.141	0.966
C	4	3	0.301	0.301
D	3	2	0	0
Combined LOD score			1.441	1.267

LOD, Logarithm of the odds.

LOD score 95%: Logarithms of the odds score calculated for a disease penetrance of 95%.

LOD score 80%: Logarithms of the odds score calculated for a disease penetrance of 80%.

Table S6. Demographic and Clinical characteristics of HCM patients with *FHOD3* candidate variants.

Variable	Male	Female	*P*-value*
Sample size	24	9	
Age at enrolment, year	45.2 ± 18.1	45.3 ± 15.4	0.981
Age of diagnosis, year	40.5 ± 14.7	38.9 ± 14.9	0.787
BMI, kg/m^2	24.6 ± 3.0	24.4 ± 2.6	0.874
Family history of SCD, n (%)	3 (12.5)	1 (11.1)	0.913
Echocardiography			
MVT, mm	23.9 ± 7.4	22.7 ± 5.5	0.646
Left atrial, mm	43.6 ± 7.0	41.4 ± 6.7	0.430
LVEDD, mm	46.9 ± 7.4	43.4 ± 6.7	0.217
Ejection fraction, %	64.0 ± 12.2	72.3 ± 4.5	0.059
Outflow tract obstruction, n (%)	12 (50.0)	7 (77.8)	0.150

Continuous variables were presented as mean ± standard deviation; the categorical variable sex was presented as number (n) and percentage (%).

*Continuous variables were compared by Student's *t*-test; the categorical variables were compared by chi-squared test.

HCM, hypertrophic cardiomyopathy; BMI, body mass index; MVT, maximum left ventricular wall thickness; LVEDD, left ventricular end-diastolic diameter.

Table S7. Univariable and multivariable Cox regression analysis of the association between *FHOD3* candidate variants and SCD in patients with hypertrophy cardiomyopathy.

Variants	Crude HR (95% CI)	Crude *P*-value	Adjusted HR (95% CI)	Adjusted *P*-value
FHOD3 variants	7.245 (2.541-17.363)	<0.001	6.791 (2.268-17.353)	0.001
LVEDD	1.033 (0.971-1.087)	0.295	1.042 (0.975-1.107)	0.228
Family history of SCD	3.133 (1.256-7.050)	0.016	3.517 (1.382-8.167)	0.010
MVT	1.102 (1.035-1.168)	0.003	1.094 (1.028-1.159)	0.005
Left atrial diameter	1.037 (0.984-1.088)	0.167	1.011 (0.954-1.068)	0.688

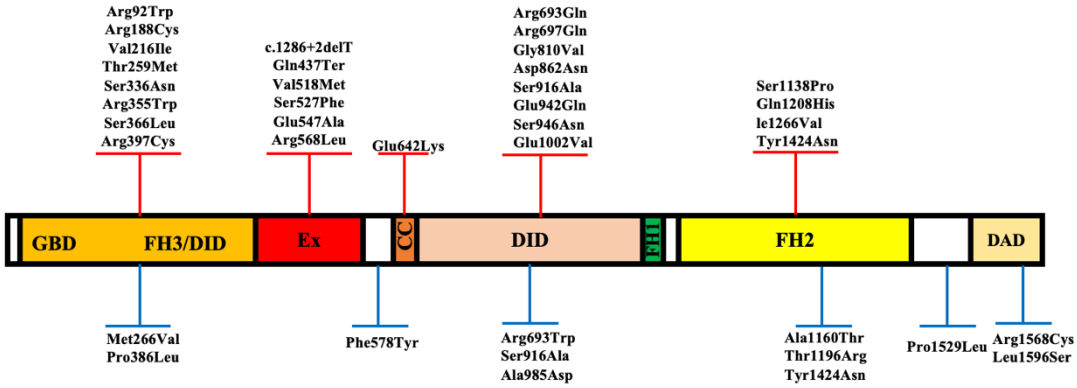
SCD, sudden cardiac death; HR, hazard ratio; CI, confidence interval; MWT, maximal wall thickness; LVEDD, left ventricular end-diastolic diameter.

Table S8. Univariable and multivariable Cox regression analysis of the association between *FHOD3* candidate variants and all-cause death in patients with hypertrophy cardiomyopathy.

Variants	Crude HR (95% CI)	Crude *P*-value	Adjusted HR (95% CI)	Adjusted *P*-value
FHOD3 variants	3.352 (1.224-7.459)	0.022	3.020 (1.090-6.852)	0.035
LVEDD	1.054 (1.014-1.090)	0.010	1.051 (1.007-1.092)	0.025
Family history of SCD	1.813 (0.842-3.519)	0.121	1.762 (0.811-3.458)	0.143
MVT	1.020 (0.971-1.070)	0.425	1.026 (0.974-1.078)	0.318
Left atrial diameter	1.047 (1.010-1.083)	0.014	1.029 (0.989-1.067)	0.155

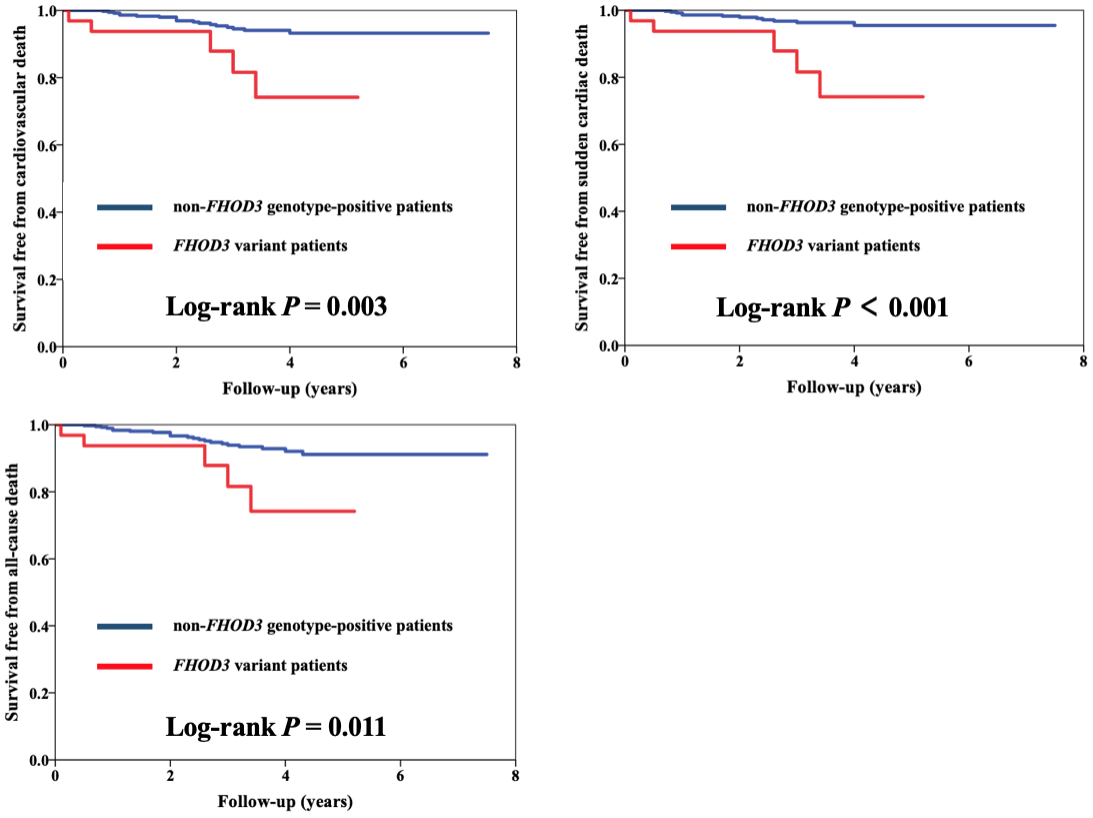
SCD, sudden cardiac death; HR, hazard ratio; CI, confidence interval; MWT, maximal wall thickness; LVEDD, left ventricular end-diastolic diameter

Figure S1. The schematic of FHOD3 protein and the distribution of candidate variants.



The schematic structure of FHOD3 protein is quoted from the paper by Ochoa et al[15]. The distribution of *FHOD3* candidate variants identified in patients with hypertrophy cardiomyopathy (up) and controls (down) were displayed. Ex, exclusively cardiac isoform; CC, coiled-coil region; DAD, diaphanous auto-regulatory domain; DID, diaphanous inhibitory domain; FH, formin homology domain; GBD, GTPase-binding domain.

Figure S2. Cumulative Kaplan–Meier analysis showing that *FHOD3* candidate variants were associated with a higher risk of outcomes than non-*FHOD3* genotype-positive patients (A-C).



A, cardiovascular death; B; sudden cardiac death, C; all-cause death. *P* values were calculated using the log-rank test.