SUPPLEMENTARY DATA

SUPPLEMENTAL METHODS

Genetic sequencing

Samples underwent an automated extraction and purification process to obtain genomic DNA (QIAsymphony SP, Germany) according to the manufacturer's instructions. Library preparation was carried out using SureSelect Reagent kit (Agilent) for Illumina's paired-end multiplexed sequencing method, following the manufacturer's instructions. The enrichment of the regions of interest was performed by means of a SureSelect (Agilent) probe kit that selectively captures the coding zones and the flanking intronic areas of the selected genes. After the generation of clusters, the DNA libraries were sequenced on the Illumina HiSeq 1500 platform. The analyses of the sequencing data were performed using a proprietary bioinformatics pipeline to obtain a report of variants noted, along with their coverage and corresponding quality parameters.

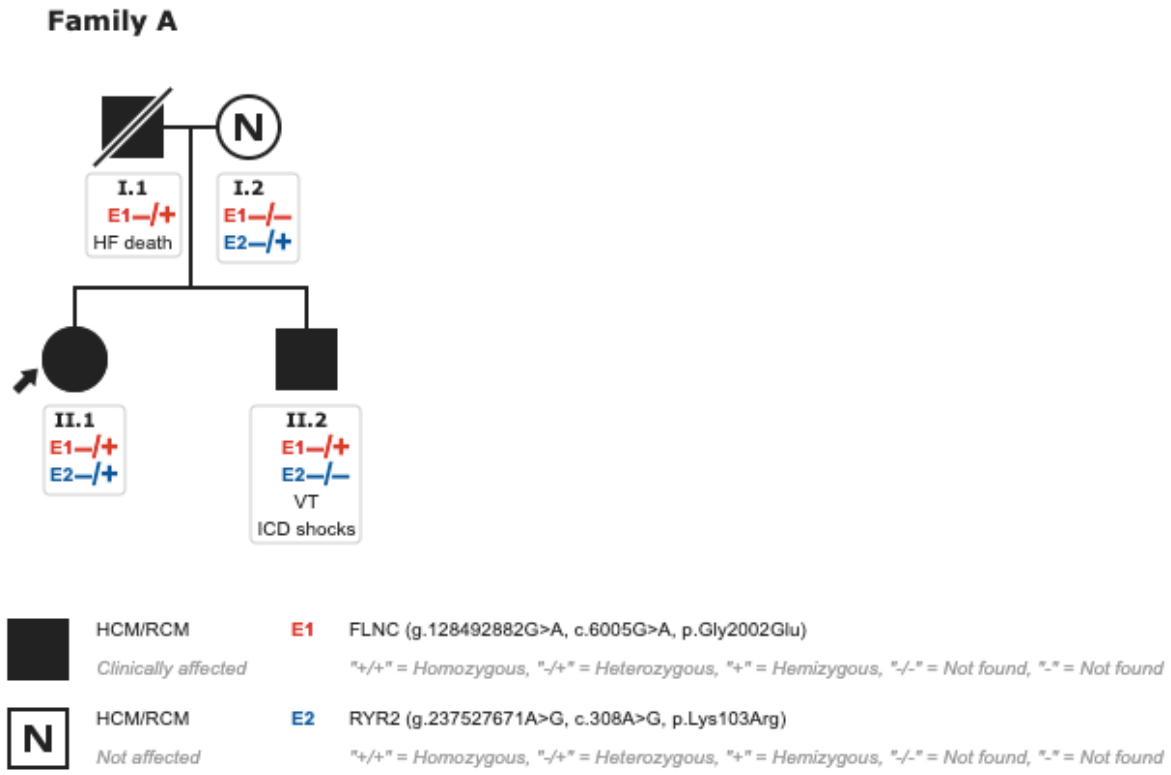
LOD score calculation

We calculated a 2-point logarithm of the odds (LOD) scores for 4 informative families with HCM (Figure 3 and table 4 of the supplementary data) by using the PARAMLINK package for R software, computed with the settings $\theta = 0$, phenocopy rate = 0.002 (prevalence of the disease in the general population) and indicated disease penetrance (0.95), a model commonly used for an autosomal dominant disease with relatively high penetrance. An indeterminate status was assigned to family members ≤ 45 years who did not meet the clinical criteria for HCM and to family members with confounding cardiac diagnoses. The phenocopy rate was adjusted to the prevalence of HCM, since the prevalence of RCM is not well established.

SUPPLEMENTAL RESULTS

Genetic analysis

In 2 families, distinct variants of unknown significance were identified. Family A exhibited the RYR2 p.Lys103Arg variant catalogued as a variant of unknown significance. The variant was identified in the proband and her mother, who did not exhibit catecholaminergic polymorphic ventricular tachycardia or HCM/RCM/LVHT phenotype. This variant is very rare in population databases, but no other criteria support a pathogenic or likely pathogenic interpretation.



In family B, which is a "de novo" presentation, we found MYBPC3_p.Gly596Arg and RYR2_p.Met4279Ile. Both were catalogued as variants of unknown significance. The RYR2 variant was only found in the proband and he did not show the CPVT phenotype. Regarding the MYBPC3 variant, it was also catalogued as a variant of unknown significance. This variant has a low frequency in the general population and it is suggested to be a variant of unknown significance or likely benign by the few submissions in ClinVar. The MYBPC3 was present in the mother but she did not manifest any cardiac phenotype. This information is added to the supplementary data.

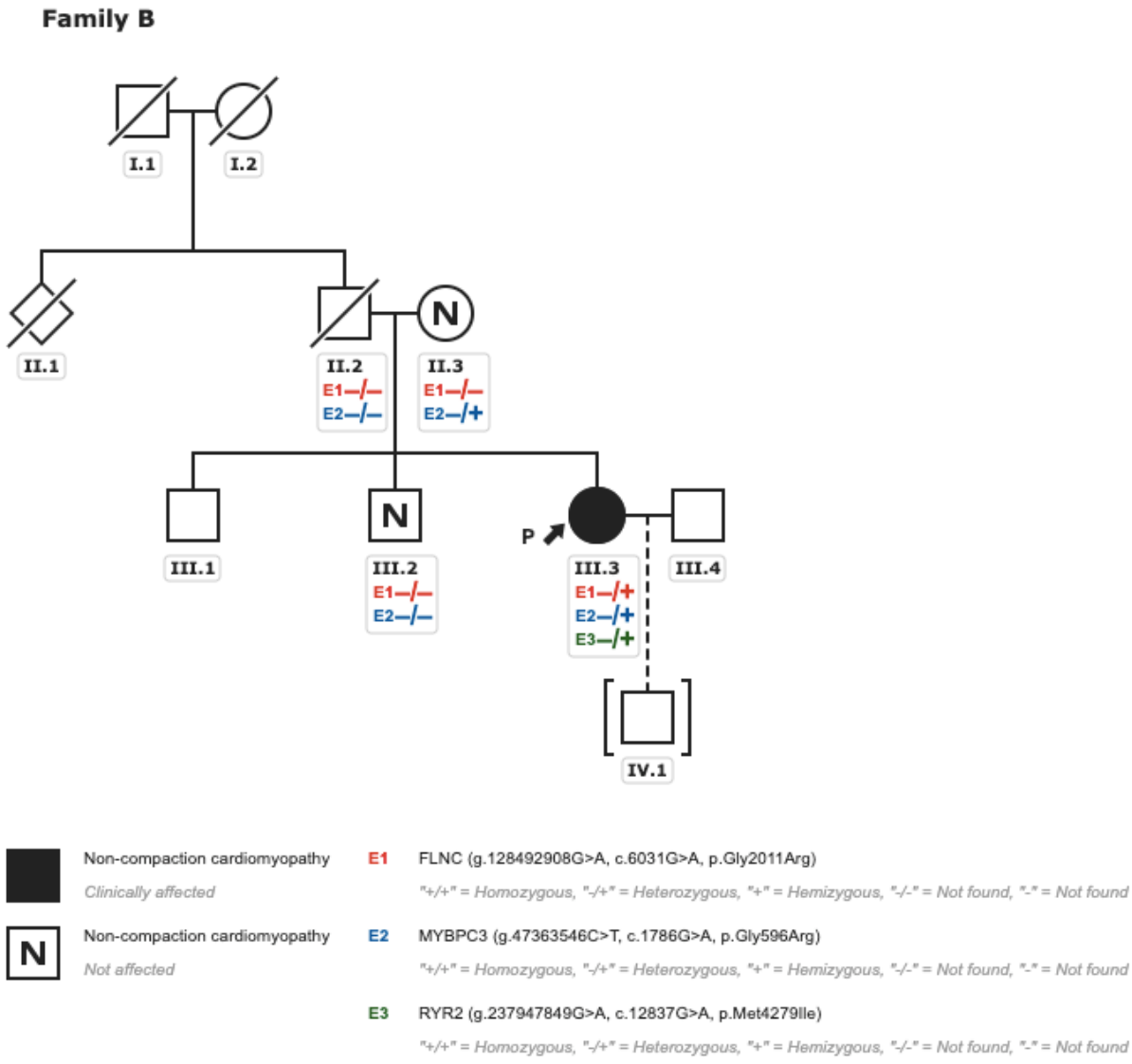


Table 1 of the supplementary data

Gene	Protein
A2ML1	alpha-2-macroglobulin-like protein 1
AARS2	Alanine-tRNA ligase, mitocondrial
ABCC9	ATP-binding cassette, subfamily C (CFTR/MRP), member 9
ACAD9	Acyl-CoA dehydrogenase family member 9, mitochondrial
ACADVL	Very long-chain specific acyl-CoA dehydrogenase, mitochondrial
ACTA1	Actin, alfa 1, skeletal muscle
ACTC1	Actin, alpha cardiac muscle 1
ACTN2	Alpha-actinin-2
AGK	Acylglycerol kinase, mitocondrial
AGL	Glycogen debranching enzyme
AGPAT2	1-acyl-sn-glycerol-3-phosphate acyltransferase beta
AKAP9	A-kinase anchor protein 9
AKT1	RAC-alpha serine/threonine-protein kinase
ALMS1	Alstrom syndrome protein 1
ALPK3	alpha-protein kinase 3
ANK2	Ankyrin 2
ANK3	Ankyrin-3
ANKRD1	Ankyrin repeat domain-containing protein 1
ANO5	Anoctamin-5
ATP5F1E	ATP synthase subunit epsilon, mitochondrial
ATPAF2	ATP synthase mitochondrial F1 complex assembly factor 2
BAG3	BAG family molecular chaperone regulator 3
BRAF	Serine/threonine-protein kinase B-raf
BSCL2	Seipin
C10orf71	Cardiac enriched FHL2-interacting protein
CACNA1C	Voltage-dependent L-type calcium channel subunit alpha-1C
CACNA1D	Voltage-dependent L-type calcium channel subunit alpha-1D
CACNA2D1	Voltage-dependent calcium channel subunit alpha-2/delta-1

CACNB2	Voltage-dependent L-type calcium channel subunit beta-2
CALM1	Calmodulin
CALM2	Calmodulin
CALM3	Calmodulin
CALR	Calreticulin
CALR3	Calreticulin 3
CAPN3	Calpain-3
CASQ2	Calsequestrin-2
CASZ1	Zinc finger protein castor homolog 1
CAV3	Caveolin 3
CAVIN1	Polymerase I and transcript release factor
CAVIN4	Caveolae-associated protein 4
CBL	E3 ubiquitin-protein ligase CBL
CDH2	Cadherin-2
CHRM2	Muscarinic acetylcholine receptor M2
COA5	Cytochrome C oxidase assembly factor 5
COA6	Cytochrome C oxidase assembly factor 6 homolog
COL7A1	Collagen alpha-1(VII) chain
COQ2	4-Hydroxybenzoate polyprenyltransferase, mitochondrial
COX15	Cytochrome C oxidase assembly protein COX15 homolog
COX6B1	Cytochrome C oxidase subunit 6B1
CRYAB	Alpha-crystallin B chain
CSNK1A1	Casein Kinase 1 Alpha 1
CSRP3	Cysteine and glycine-rich protein 3
CTNNA1	Catenin alpha-1
CTNNA3	Catenin alpha-3
CTNNB1	Catenin beta-1
DES	Desmin
DLD	Dihydrolipoyl dehydrogenase, mitochondrial
DMD	Dystrophin
DNAJC19	Mitochondrial import inner membrane translocase subunit TIM14
DNM1L	Dynamin-1-like protein

DOLK	Dolichol kinase
DSC2	Desmocollin 2
DSG2	Desmoglein 2
DSP	Desmoplakin
DTNA	Dystrobrevin alpha
ELAC2	Zinc phosphodiesterase ELAC protein 2
EMD	Emerin
EYA4	Eyes absent homolog 4
FAH	Fumarylacetoacetase
FBXO32	F-box only protein 32
FGF12	Fibroblast growth factor 12
FHL1	Four and a half LIM domains protein 1
FHL2	Four and a half LIM domains 2 (FHL-2)
FHOD3	FH1/FH2 domain-containing protein 3
FKRP	Fukutin-related protein
FKTN	Fukutin
FLNC	Filamin C
FOXD4	Forkhead box protein D4
FOXRED1	FAD-dependent oxidoreductase domain-containing protein 1
FXN	Frataxin, mitocondrial
GAA	Lysosomal alpha-glucosidase
GATA4	Transcription factor GATA-4
GATA5	Transcription factor GATA-5
GATA6	Transcription factor GATA-6
GATAD1	GATA zinc finger domain-containing protein 1
GFM1	Elongation factor G, mitocondrial
GJA1	Gap junction alpha-1 protein
GJA5	Gap junction alpha-5 protein
GLA	Alpha galactosidase A
GLB1	Beta-galactosidase
GNB2	Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-2
GNPTAB	N-acetylglucosamine-1-phosphotransferase subunits alpha/beta

GPD1L	Glycerol-3-phospate dehydrogenase 1-like protein
GREM2	Gremlin-2
GSK3B	Glycogen synthase kinase-3 beta
GUSB	Beta-glucuronidase
GYG1	Glycogenin-1
HCN4	Potassium/sodium hyperpolarization-activated cyclic nucleotide-gated channel 4
HFE	Hereditary hemochromatosis protein
HRAS	GTPase HRas
IDH2	Isocitrate dehydrogenase [NADP], mitochondrial
ILK	Integrin-linked protein kinase
IRX3	Iroquois-class homeodomain protein IRX-3
ISM2	Isthmin-2
JARID2	Jumonji (Jmj) A/T-rich interaction domain 2
JPH2	Junctophilin 2
JUP	Junction plakoglobin
KAT6B	Histone acetyltransferase KAT6B
KCNA5	Potassium voltage-gated channel subfamily A member 5
KCND2	Potassium voltage-gated channel subfamily D member 2
KCND3	Potassium voltage-gated channel subfamily D member 3
KCNE1	Potassium voltage-gated channel subfamily E member 1
KCNE2	Potassium voltage-gated channel subfamily E member 2
KCNE3	Potassium voltage-gated channel subfamily E member 3
KCNE5	Potassium voltage-gated channel subfamily E member 1-like protein
KCNH2	Potassium voltage-gated channel subfamily H member 2
KCNJ2	Inward rectifier potassium channel 2
KCNJ5	G protein-activated inward rectifier potassium channel 4
KCNJ8	ATP-sensitive inward rectifier potassium channel 8
KCNK17	Potassium channel subfamily K member 17
KCNK3	potassium channel subfamily K member 3
KCNQ1	Potassium voltage-gated channel subfamily KQT member 1
KLF10	Krueppel-like factor 10
KLHL24	Kelch-like protein 24

KRAS	GTPase KRas
LAMA2	Laminin subunit alpha-2
LAMA4	Laminin subunit alpha-4
LAMP2	Lysosome-associated membrane glycoprotein 2
LDB3	LIM domain-binding protein 3
LDLR	Low-density lipoprotein receptor
LIAS	Lipoyl synthase, mitocondrial
LMNA	Prelamin-A/C
LMOD2	Leiomodin-2
LZTR1	Leucine-zipper-like transcriptional regulator 1
MAP2K1	Dual specificity mitogen-activated protein kinase kinase 1
MAP2K2	Dual specificity mitogen-activated protein kinase kinase 2
MAP3K8	Mitogen-activated protein kinase kinase kinase 8
MEF2C	Myocyte-specific enhancer factor 2C
MIB1	E3 ubiquitin-protein ligase MIB1
MIR208A	MicroRNA 208ª
MIR208B	MicroRNA 208b
MLYCD	Malonyl-CoA decarboxylase, mitochondrial
MRPL3	39S Ribosomal protein L3, mitocondrial
MRPL44	39S Ribosomal protein L44, mitochondrial
MRPS22	28S Ribosomal protein S22, mitochondrial
MTO1	Protein MTO1 homolog, mitocondrial
MYBPC3	Myosin-binding protein C, cardiac-type
MYBPHL	Myosin-binding protein H-like
MYH11	Myosin-11
MYH6	Myosin-6
MYH7	Myosin-7
MYL2	Myosin regulatory light chain 2, ventricular/cardiac muscle isoform
MYL3	Myosin light chain 3
MYLK2	Myosin light chain kinase 2, skeletal/cardiac muscle
MYOM1	Myomesin-1
MYOT	Myotilin

MYOZ2	Myozenin-2
MYPN	Myopalladin
NEBL	Nebulette
NEXN	Nexilin
NF1	Neurofibromin
NKX2-5	Homeobox protein Nkx-2.5
NKX2-6	Homeobox protein Nkx-2.6
NNT	NAD(P) transhydrogenase, mitochondrial
NONO	Non-POU domain-containing octamer-binding protein
NOS1AP	Carboxyl-terminal PDZ ligand of neuronal nitric oxide synthase protein
NOTCH1	Neurogenic locus notch homolog protein 1
NPPA	Atrial natriuretic factor
NRAP	Nebulin-related anchoring protein
NRAS	GTPase NRas
OBSCN	Obscurin
OBSL1	Obscurin-like protein 1
OPA3	Optic atrophy 3 protein
PDHA1	Pyruvate dehydrogenase E1 component subunit alpha, somatic form, mitochondrial
PDLIM3	PDZ and LIM domain protein 3
PERP	p53 Apoptosis effector related to PMP-22
PHKA1	Phosphorylase b kinase regulatory subunit alpha, skeletal muscle isoform
PITX2	Pituitary homeobox 2
PKD2	Polycystin-2
PKP2	Plakophilin 2
PKP4	Plakophilin 4
PLN	Cardiac phospholamban
PMM2	Phosphomannomutase 2
PPA2	Inorganic pyrophosphatase 2, mitochondrial
PPCS	phosphopantothenate--cysteine ligase
PPP1CB	Serine/threonine-protein phosphatase PP1-beta catalytic subunit
PPP1R13L	relA-associated inhibitor
PRDM16	PR domain zinc finger protein 16

PRKAG2	5'-AMP-activated protein kinase subunit gamma-2
PSEN1	Presenilin-1
PSEN2	Presenilin-2
PTPN11	Tyrosine-protein phosphatase nonreceptor type 11
QRSL1	Glutamyl-tRNA(Gln) amidotransferase subunit A, mitochondrial
RAF1	RAF proto-oncogene serine/threonine-protein kinase
RANGRF	Ran guanine nucleotide release factor
RASA1	Ras GTPase-activating protein 1
RASA2	Ras GTPase-activating protein 2
RBM20	RNA-binding motif protein 20
RBM24	RNA-binding protein 24
RIT1	GTP-binding protein Rit1
RRAS	RAS related
RYR2	Ryanodine Receptor 2
SCN5A	Sodium Voltage-Gated Channel Alpha Subunit 5
SCO2	Synthesis Of Cytochrome C Oxidase 2
SDHA	Succinate Dehydrogenase Complex Flavoprotein Subunit A
SGCA	Sarcoglycan alpha
SGCB	Sarcoglycan beta
SGCD	Sarcoglycan delta
SGCG	Sarcoglycan gamma
SHOC2	SHOC2 leucine rich repeat scaffold protein
SLC22A5	Solute carrier family 22 member 5
SLC25A3	Solute carrier family 25 member 3
SLC25A4	Solute carrier family 25 member 4
SOS1	SOS Ras/Rac guanine nucleotide exchange factor 1
SOS2	SOS Ras/Rho guanine nucleotide exchange factor 2
SPEG	Striated muscle enriched protein kinase
SPRED1	Sprouty related EVH1 domain containing 1
SPRY1	Sprouty RTK signaling antagonist 1
SURF1	SURF1 cytochrome c oxidase assembly factor
SYNE1	Spectrin repeat containing nuclear envelope protein 1

SYNE2	Spectrin repeat containing nuclear envelope protein 2
SYNGAP1	Synaptic ras GTPase-activating protein 1
TAZ	Tafazzin, phospholipid-lysophospholipid transacylase
TBX20	T-Box transcription factor 20
TCAP	Titin-cap
TGFB3	Transforming growth factor beta 3
TMEM43	Transmembrane Protein 43
TMEM70	Transmembrane Protein 70
TMOD1	Tropomodulin 1
TNNC1	Troponin C1
TNNI3	Troponin I3
TNNI3K	TNNI3 interacting kinase
TNNT2	Troponin T2
TOR1AIP1	Torsin 1A interacting protein 1
TPM1	Tropomyosin 1
TRIM54	Tripartite motif containing 54
TRIM63	Tripartite motif containing 63
TSFM	Ts translation elongation factor, mitochondrial
TTN	Titin
TTR	Transthyretin
TXNRD2	Thioredoxin Reductase 2
VCL	Vinculin
WISP1	Cellular communication network factor 4
WT1	WT1 transcription factor
XK	X-linked kx blood group
ZBTB17	Zinc finger and BTB domain containing 17

Table 2 of the supplementary data

Antibodies	Pretreatment	Dilution Incubation time	Reference
Rabbit monoclonal antidesmin, clone Y66	Buffer EDTA pH8 25 min at 95 °C	1:75 PBS+tween20 Overnight at 4 °C	Abcam Cat. No: ab32362
Rabbit anti-collagen type I	Pepsin 10 min at 37ºC	1:500 PBS+tween20 1.5 h r/t	ACRIS Cat. No: R1038
Rabbit polyclonal anti-filamin C	Buffer citrate pH6 25 min at 95 °C 0.2% Triton X-100 (permeabilization)	1:100 PBS+tween20 Overnight at 4 °C	Abcam Cat. No: ABIN1714758
Rabbit polyclonal Anticonnexin 43/GJA1	Buffer citrate pH6 25 min at 95 °C	1:5000 PBS+tween20 1 h r/t	Abcam Cat. No: ab11370
Immpress antimouse IgG (peroxidase)	-	Ready-to-use 30 min	Vector Laboratories Cat. no.: MP 7402
Immpress antirabbit IgG (peroxidase)	-	Ready-to-use 30 min	Vector Laboratories Cat. No.: MP-7401

Table 3 of the supplementary data

Name	Sequence (5'-3')	Application
FLNC-P2301L-for	CAGTGAACTGAAAGAGGCTGCCGGGCAC	SDM
FLNC-P2301L-rev	GTGCCCGGCAGCCTCTTTCAGTTCACTG	SDM
FLNC-E2334K-for	CGGCGTGCCAGCCAAGTTCAGCATCTG	SDM
FLNC-E2334K-rev	CAGATGCTGAACTTGGCTGGCACGCCG	SDM
FLNC-E2340W-for	AGCATCTGGACCTGGGAGGCTGGC	SDM
FLNC-E2340W-rev	CGCCAGCCTCCCAGGTCCAGATGCT	SDM
CMV-for	CGCAAATGGGCGGTAGGCGTG	Sequencing
FLNC-c.536-for	CAACTTCAACCGTGACTGGCAG	Sequencing
FLNC-c.1065-for	GGCCAGAACATTGAACGCAGTC	Sequencing
FLNC-c.1659-for	TACGCCATCCCTCGCAGC	Sequencing
FLNC-c.2295-for	GCGGGTAAAGGTGTACGGC	Sequencing
FLNC-c.2813-for	GCAACATGGCAGTGACAGTGAC	Sequencing
FLNC-c.3346-for	CATGTGCTGTCAGCTACCTGC	Sequencing
FLNC-c.3863-for	GACGGCTCGTGTGCTCAAC	Sequencing
FLNC-c.4394-for	CACAGTGGACTGCAGTCAAGC	Sequencing
FLNC-c.5278-for	TGTGAGCTCCCCTTTCTGCAC	Sequencing
FLNC-c.5730-for	GAGTCATCACCTGTGATCTTGGC	Sequencing
FLNC-c.6244-for	CAGGCACGTGCTTGTCAGC	Sequencing
FLNC-c.6763-for	CCACGGTACTTGACAGCGAC	Sequencing
FLNC-c.7267-for	CTTGTCACTGTCCAGCTCAGAGAC	Sequencing
FLNC-c.7671-for	GCCTCGGACCAGTGACCTTG	Sequencing
M13-rev	CAGGAAACAGCTATGACC	Sequencing

SDM, Site-directed mutagenesis.

Table 4 of the supplementary data

Pedigree	*FLNC* variant	LOD score 95%
A	Gly2002Glu	0.1738
B	Gly2011Arg	0
C	Gly2320Arg	0.9325
D	Glu2334Lys	0
E	Arg2340Trp	0
F	Glu2334Lys	0
G	Pro2301Leu	0
H	Gly2011Arg	0.4367
I	Thr1823Ala	0.1661
J	Ser1955Leu	0.556
K	Ala2510Val	0.11
Combined LOD score		2.3725*

LOD score 95%, logarithm of the odds score calculated for a disease penetrance of 95%.

*Suggestive linkage ($P < .001$).

Table 5 of the supplementary data

Variant	Family	Fulfilled ACMG criteria	Classification
Gly2002Glu	A	PM2; PP3	Variant of uncertain significance
Gly2011Arg	B, H	PS2[a]; PM2; PP3	Likely pathogenic
Gly2320Arg	C	PM2; PP3; PP1[b]	Variant of uncertain significance
Glu2334Lys	D, F	PS2 [a]; PM2; PP3	Likely pathogenic
Arg2340Trp	E	PS2 [a]; PM2; PP3	Likely pathogenic
Pro2301Leu	G	PS2 [a]; PM2; PP3	Likely pathogenic
Thr1823Ala	I	PM2; PP3	Variant of uncertain significance
Ser1955Leu	J	PM2; PP3	Variant of uncertain significance
Ala2510Val	K	PM2; PP3	Variant of uncertain significance

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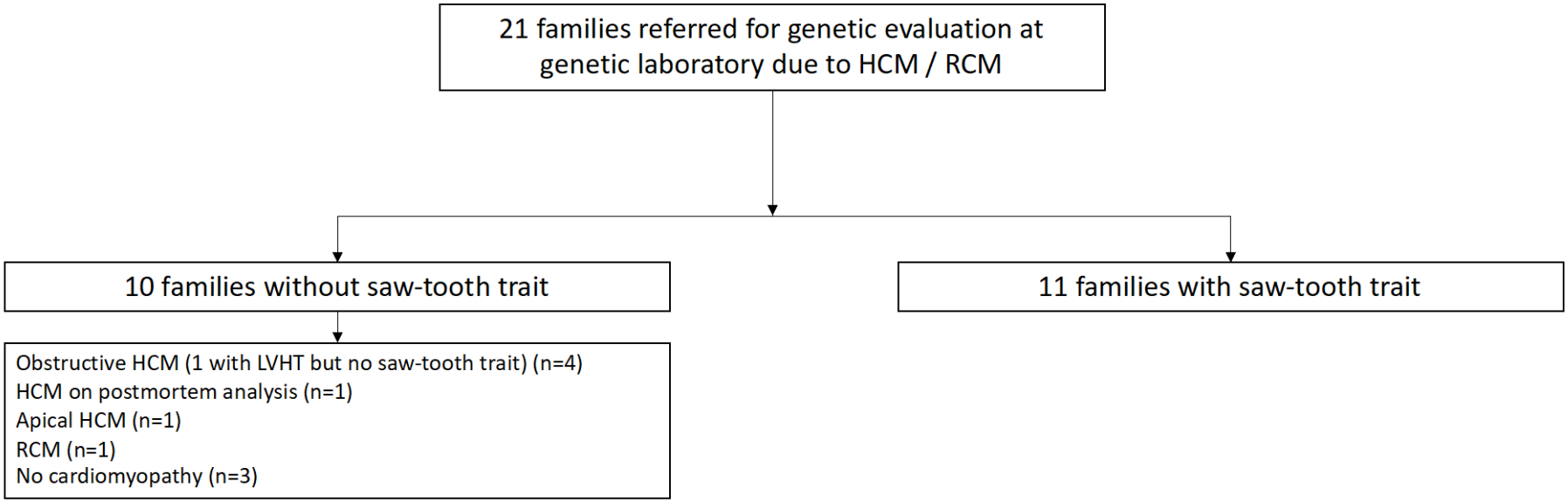
[a] PS2; strong; Confirmed paternity in 4 of the 5 families with *de novo* variants.
[b] PP1, supporting: LODs = 0.93, $P < .01$.

Table 6 of the supplementary data

FLNC variant	MutationTaster	DANN	FATHMM MKL coding
p.Gly2011Arg	1	0,999299	0,9902
Pro2301Leu	1	0,999	0,975
Glu2334Lys	1	0,999	0,978
Arg2340Trp	0,9999	0,998894	0,87934
Gly2320Arg	1	0,998	0,965
Gly2002Glu	1	0,998	0,992
Thr1823Ala	1	0,998	0,971
Ser1955Leu	1	0,999	0,980
Ala2510Val	1	0,999	0,966

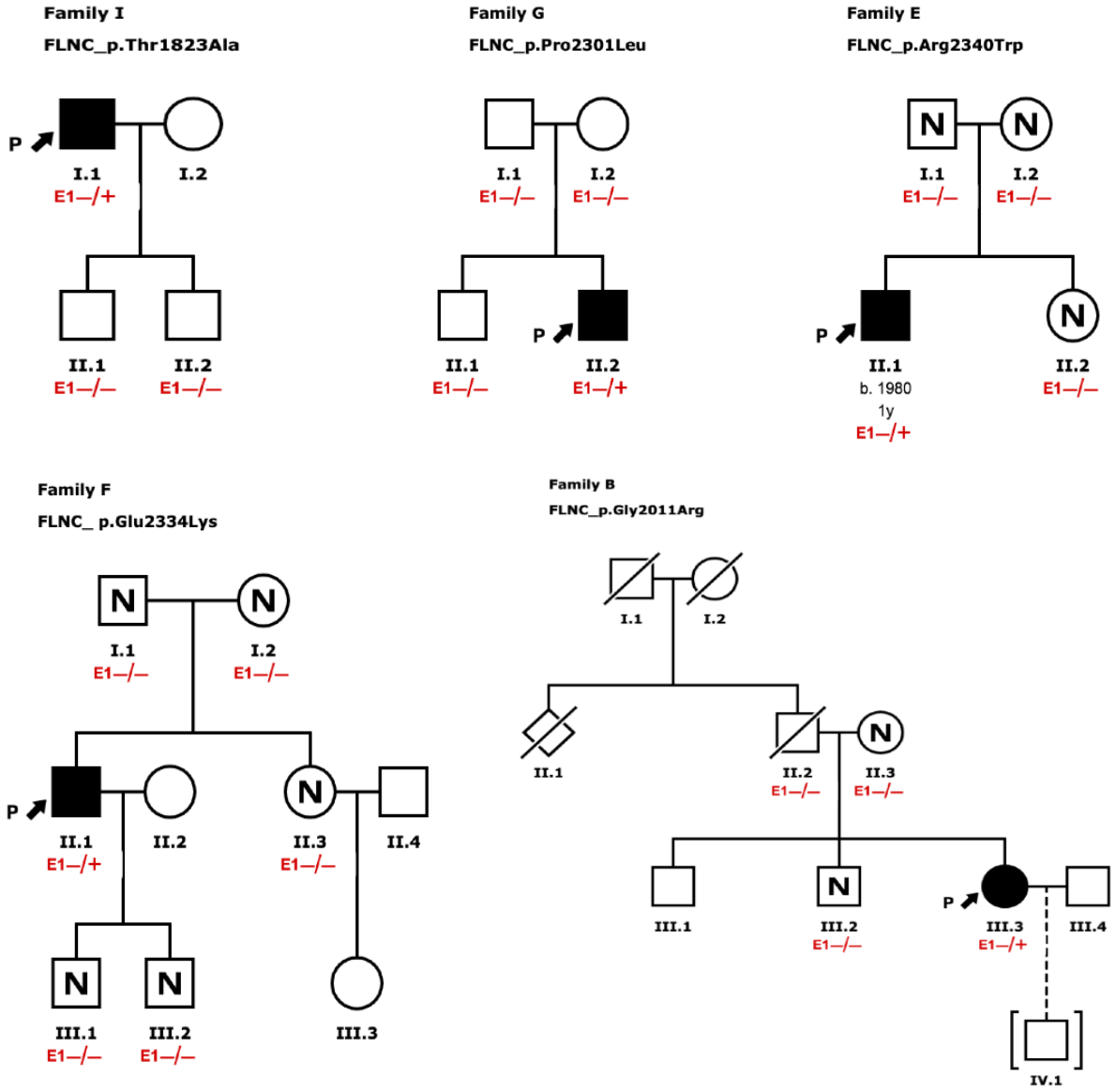
MutationTaster: ranges from 0 to 1. Values close to 1 indicate a high degree of prediction accuracy. DANN: the higher the score, the higher the potential pathogenicity of the variant. Values between 0 and 1. FATHMM: the higher the score, the higher the potential pathogenicity of the variant. Coding and noncoding variants are scored independently. Values between 0 and 1.

Figure 1 of thesupplementary data. Patients recruitment flowchart



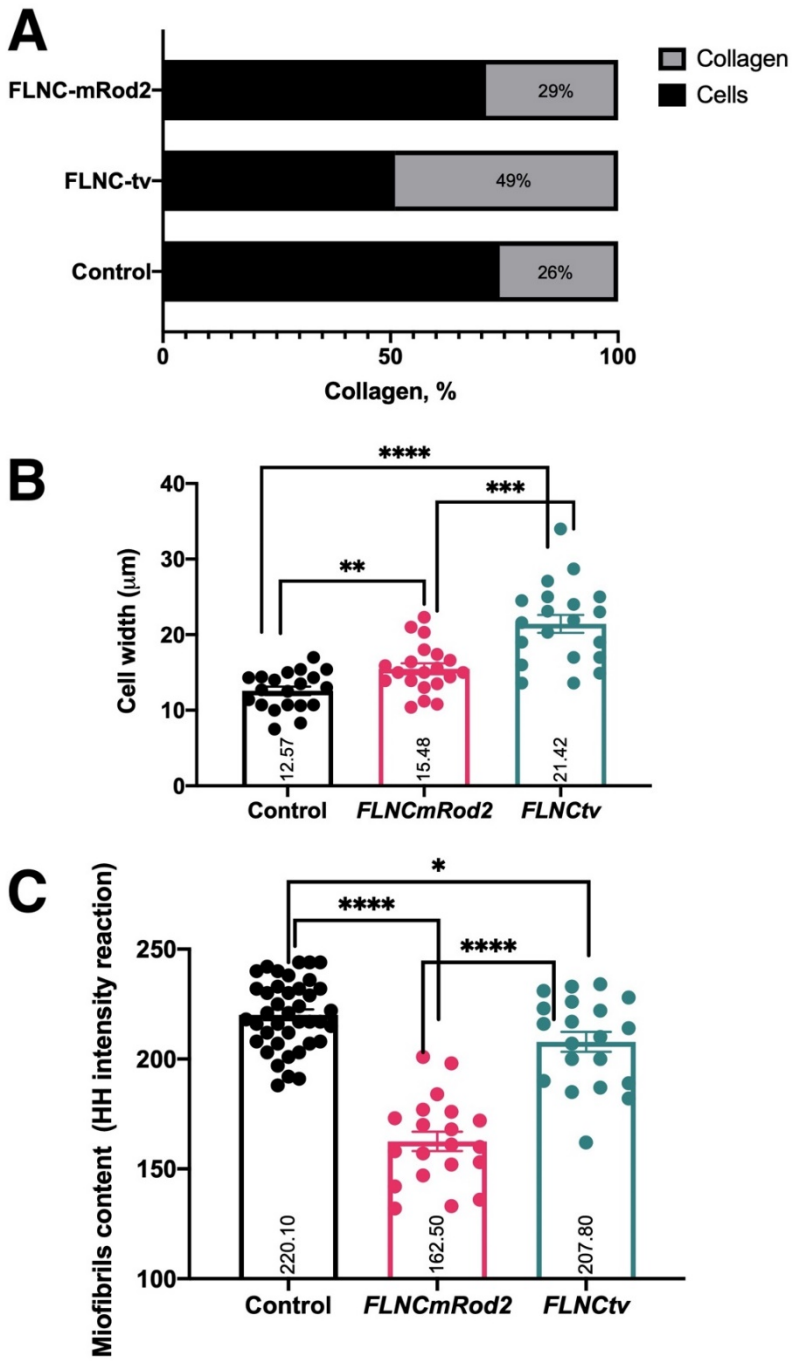
HCM, hypertrophic cardiomyopathy; LVHT, left ventricular hypertrabeculation; n, number; RCM, restrictive cardiomyopathy.

Figure 2 of the supplementary data. Family pedigrees of *de novo* variants in *FLNC*



Squares indicate males, circles indicate females, slashes indicate deceased individuals, black shading indicates cardiomyopathy phenotype, N indicates absence of phenotype. The arrows + P indicate the proband. Heterozygous carriers (E1 −/+) and noncarriers (E1 −/−).

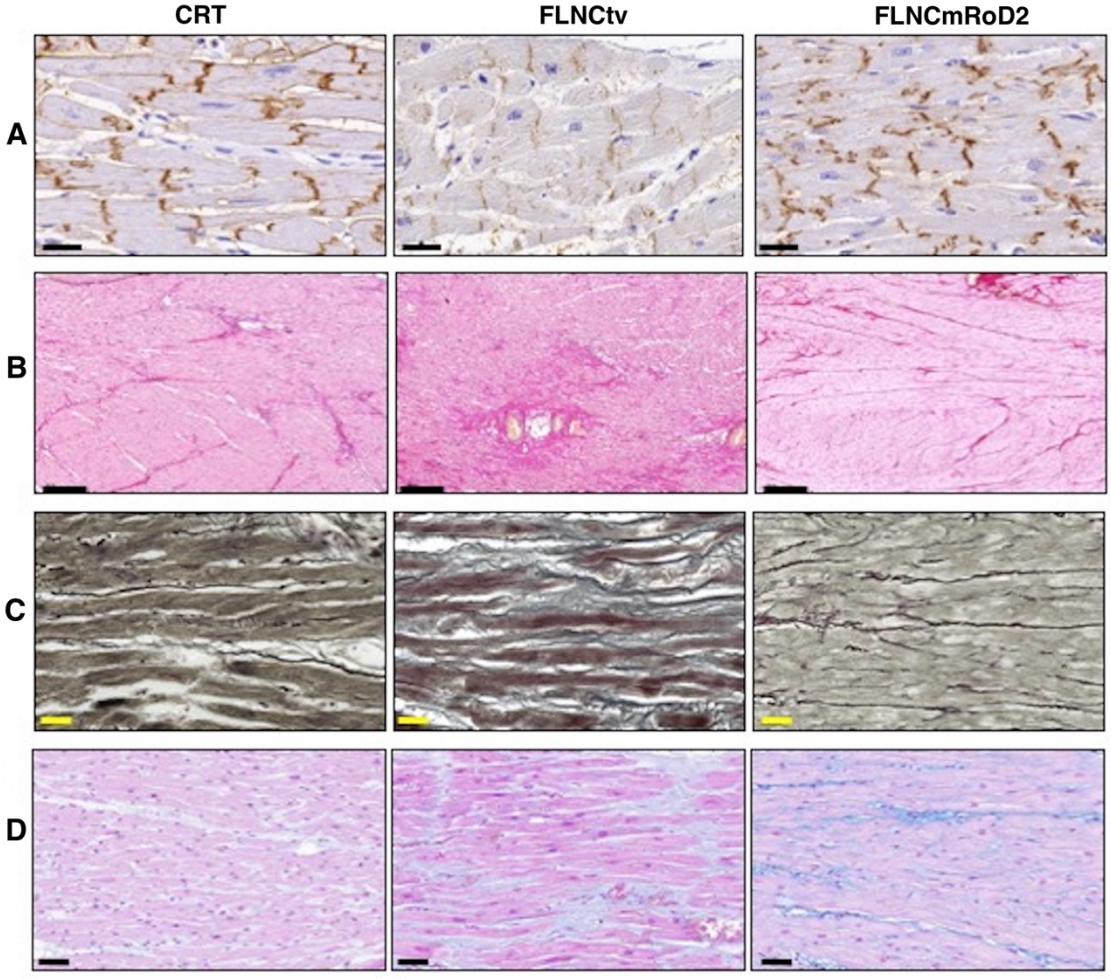
Figure 3 of the supplementary data. Quantification of collagen and myofibril content



A) Histogram representing collagen content observed in the 3 different conditions analyzed. Filamin C truncating variant (FLNCtv) presented more collagen than control and FLNC-mRod2.; B) Cell width quantification: FLNctv cells were significantly larger than control and FLNC-mRod2 cells. C) Myofibril content quantifications in the 3 different conditions showed that the control presented significantly more myofibrils than FLNC-tv and FLNC-mRod2, and FLNCmRod2 fewer than FLNC-tv.

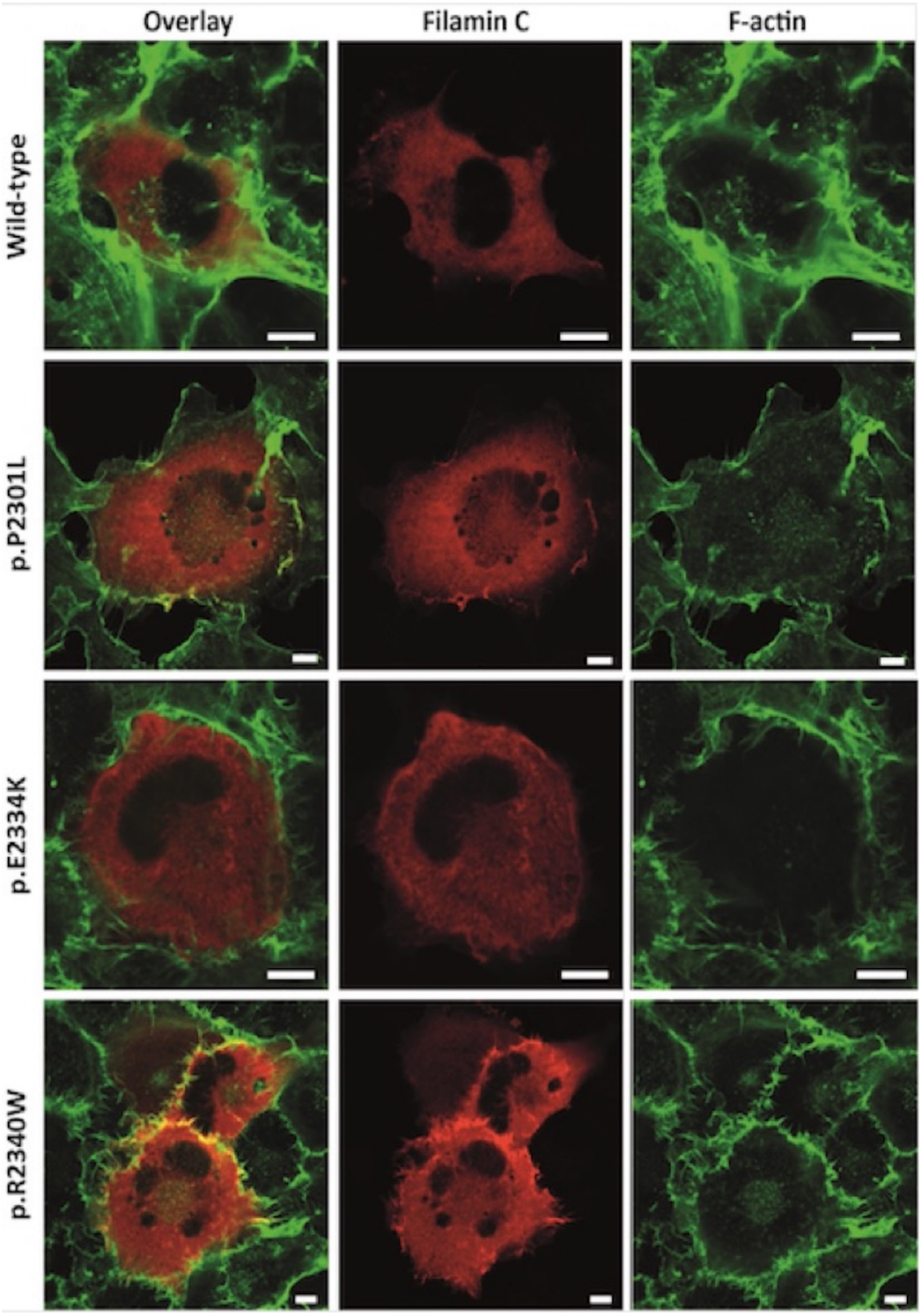
* $P < .05$; ** $P < .01$; *** = $P < .001$; **** $P < .0001$.

Figure 4 of the supplementary data. Comparison of remodeling of the collagen network and fibrotic response



A) Distribution pattern of CX-43 in the intercalary disc of cardiomyocytes; B) Histochemical identification of fibrillar collagens by Picrosirius staining (fibers stained in red), which clearly shows the increase of these fibers in genetically affected samples C) Gomori's metal reduction technique for the identification of reticular fibers (black fibers). D) Alcian blue histochemical staining for acid proteoglycans, which were considerably increased in genetically affected heart samples. Scale barr: 200 µm (A-B)and 20 µm (C-D).

Figure 5 of the supplementary data. Filamin C expression in transfected HT1080 cells



HT1080 cells were transfected with expression plasmids for wild-type and mutant filamin C (p.P2301L, p.E2334K and p.R2340W). c-Myc tagged filamin C is shown in red. F-actin (shown in green) was contained using phalloidin conjugated with Alexa-488. Representative cell images are shown. Scale bars = 10 µm.