Appendix

Disease modeling of an α-actinin 2 mutation guides clinical therapy in hypertrophic cardiomyopathy

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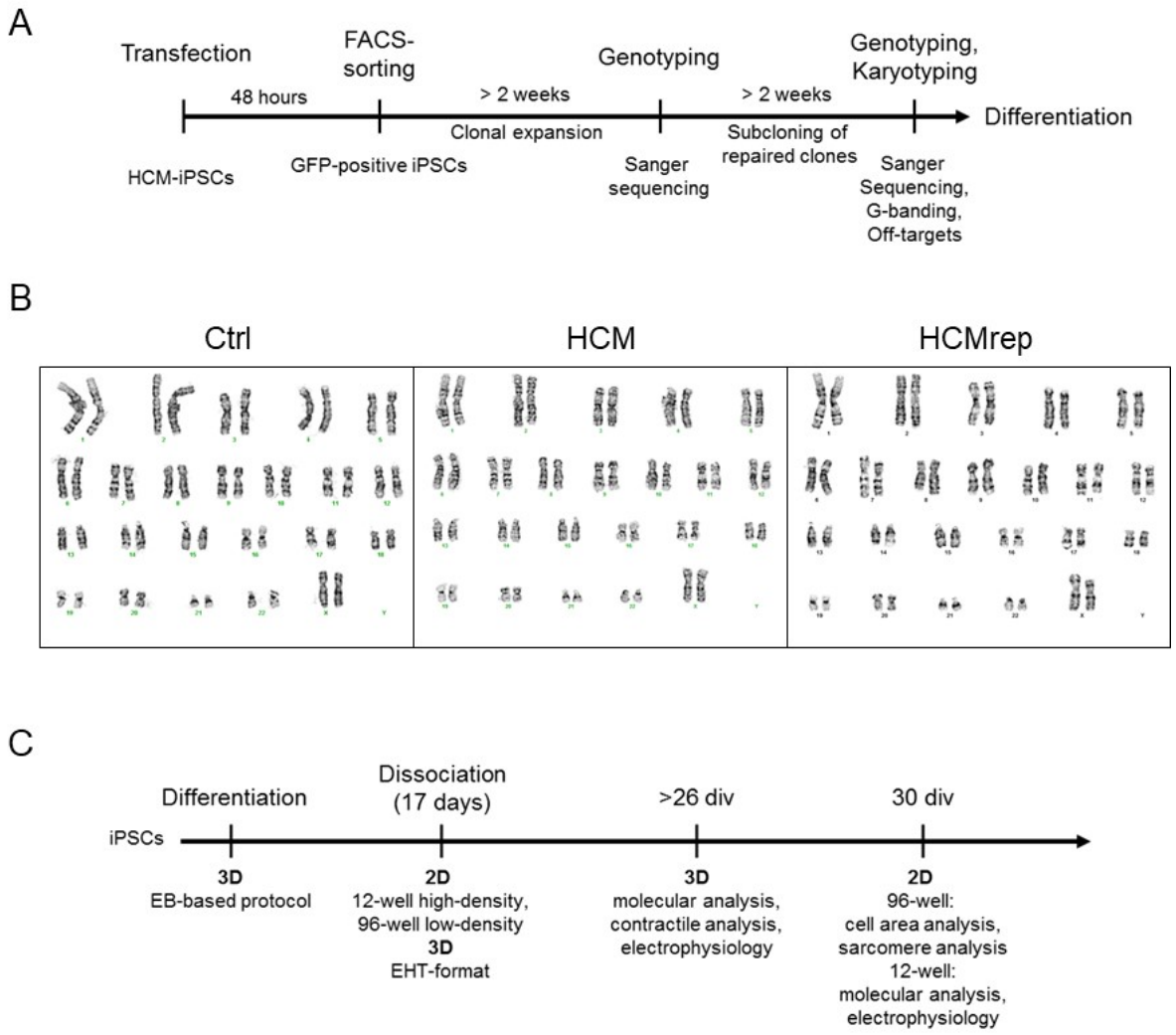
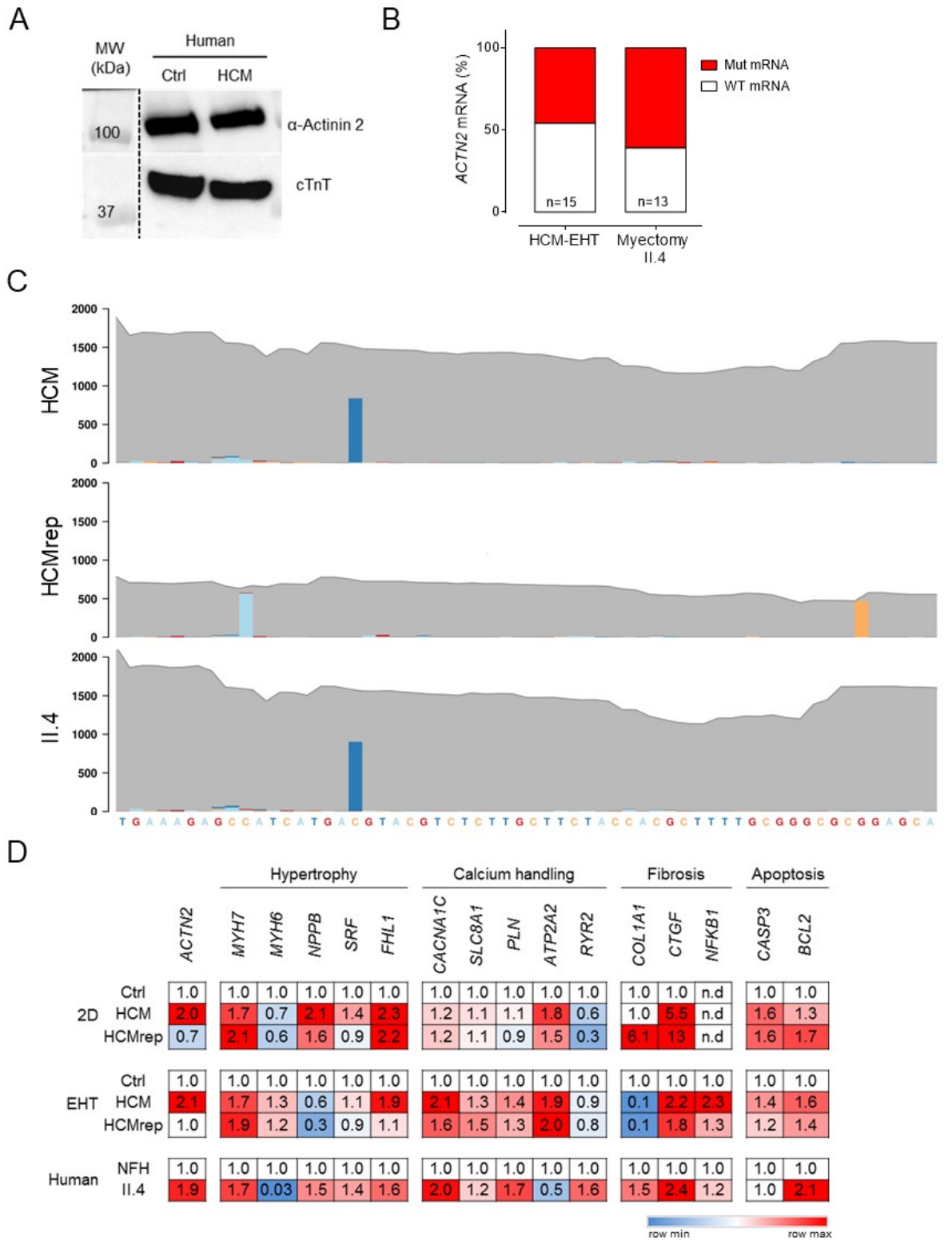
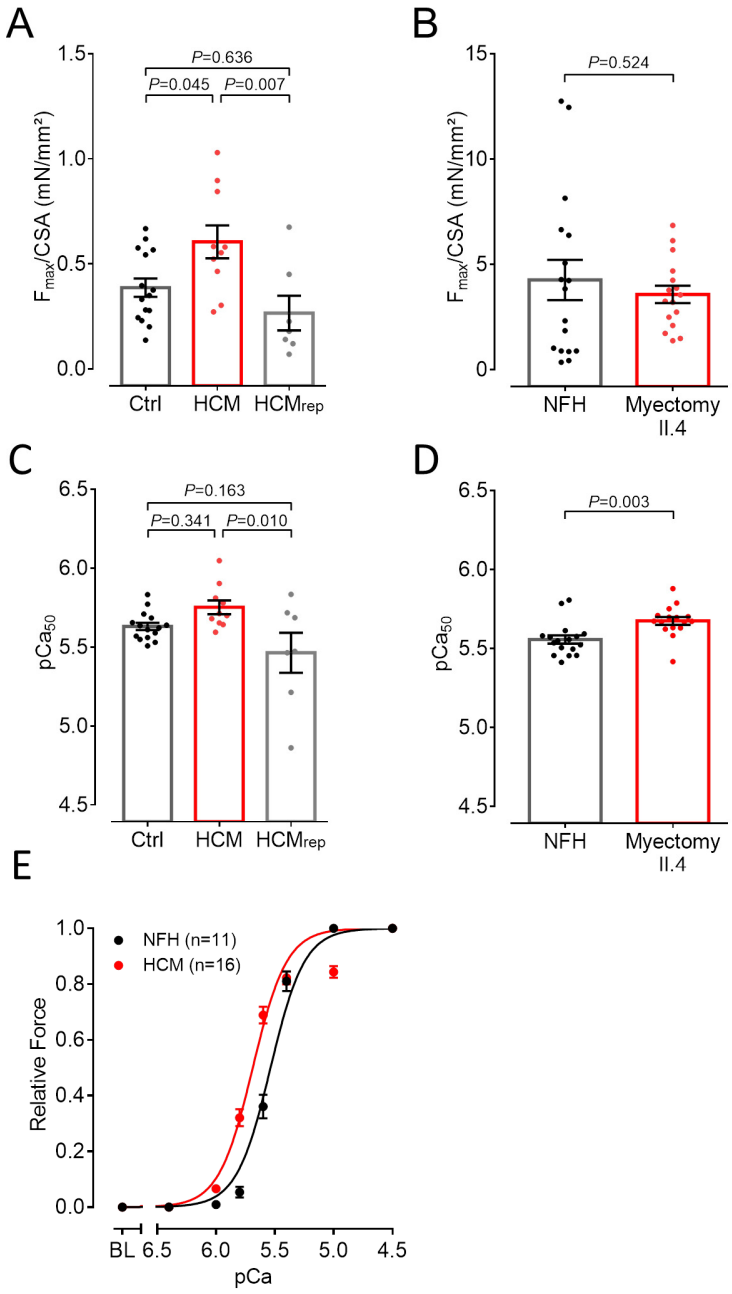


Appendix Figure S1. Generation of hiPSC lines and disease modeling protocol. (**A**) Protocol for generation of CRISPR/Cas9 isogenic control cell line HCMrep. Indicated are steps of quality assessment and approximate time needed for each step (iPSC, induced pluripotent stem cell). (**B**) G-banding results are depicted by these representative karyograms of the investigated hiPSC Ctrl- (passage 40), HCM- (passage 11) and HCMrep-line (passage 55). (**C**) Disease modeling approach for the production of hiPSC-CMs, models and experimental procedures that were used for this study (div, days *in vitro*; EB, embryoid body; EHT, engineered heart tissue).

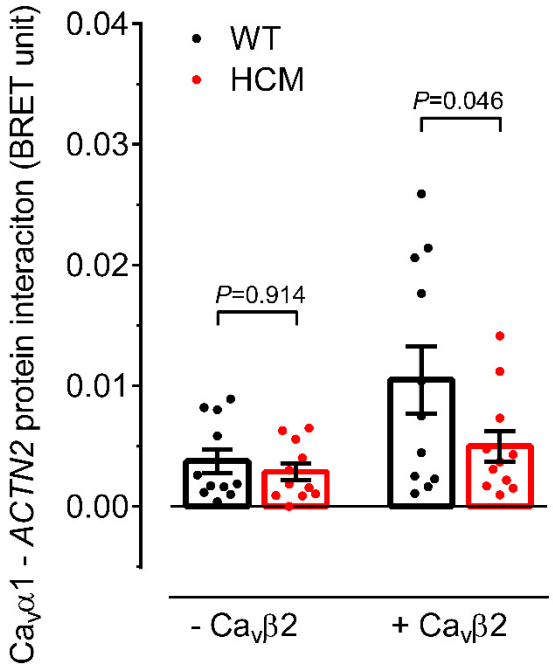


Appendix Figure S2. Molecular characterization of human tissue and hiPSC-derived CMs from 2D and 3D models. (**A**) Western blot analysis of human tissue samples stained with antibodies directed against α-actinin 2 and cardiac troponin T (cTnT; MW, molecular weight; kDa, kilodalton). (**B**) Quantification of mutant and wild-type mRNA in 3D-cultured hiPSC-derived engineered heart tissues and HCM-affected family member II.4. Transcripts were quantified by conversion of RNA to cDNA, subcloning of amplified *ACTN2* RT-PCR

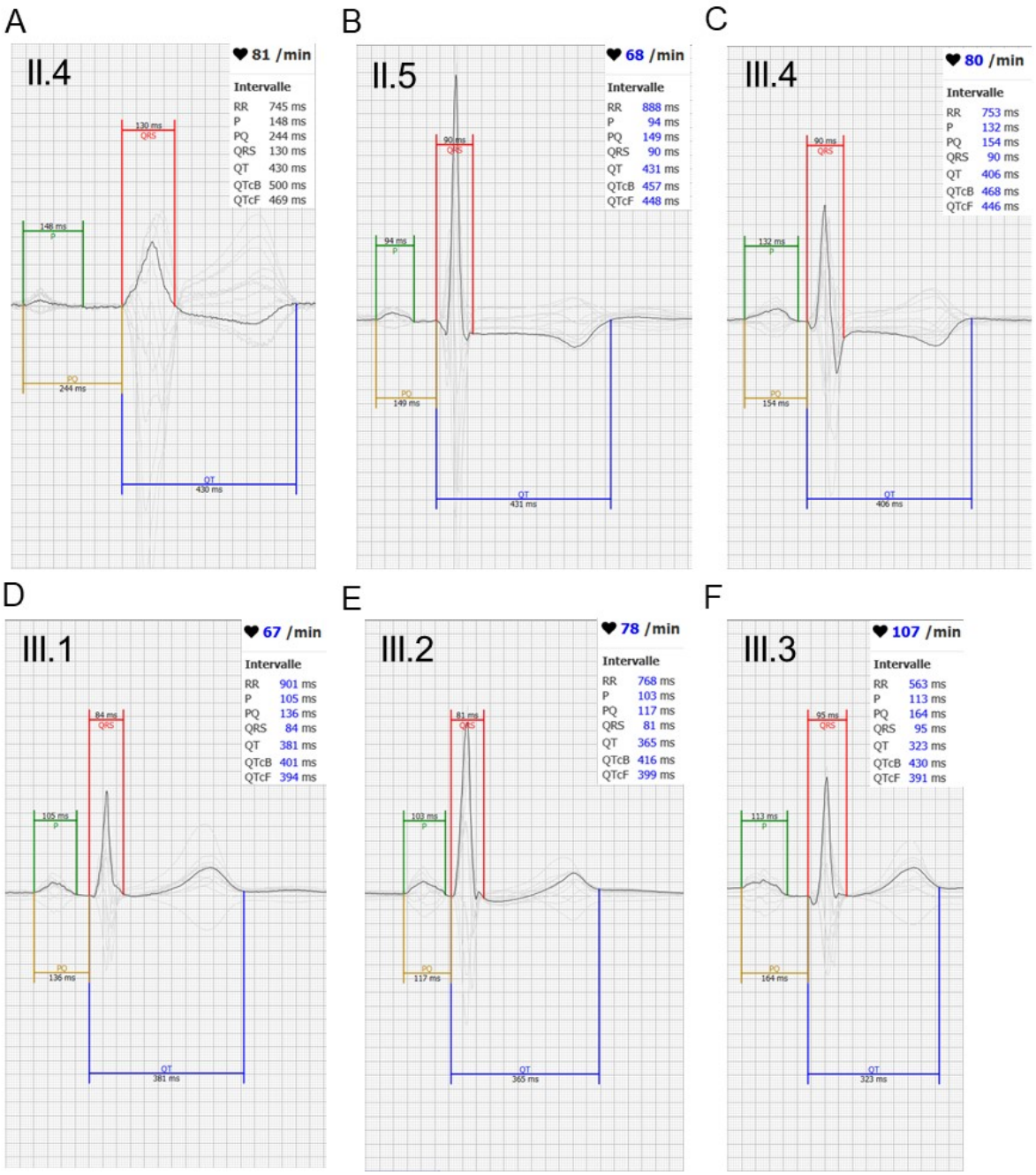
fragments and validation by sequencing (n=number of picked and sequenced clones; HCM-EHTs (n=15); HCM tissue (n=13); mut, mutant; WT, wild type). Data are expressed as the % of WT versus mutant mRNA transcripts. (**C**) RNA sequencing analysis revealed mismatches in the genomic range of chromosome 1 from 236.735.660 bp to 236.735.720 bp showing heterozygous C>T mutation of base 236.735.677 in ~ 50% of analyzed reads (blue bars out of grey zone) in both HCM-EHTs (n=7/6) and patient II.4 septal myectomy. CRISPR/Cas9 engineered isogenic control HCMrep-EHTs (n=7/3) showed no C>T transition at base 236.735.677, but the simultaneously introduced homozygous silent C>A and G>C mutations at bases 236.735.668 (light blue bar) and 236.735.714 (orange bar). (**D**) Gene expression analysis in hiPSC-CMs and -EHTs, performed with the nanoString nCounter® Elements technology. Data were normalized to housekeeping genes (*ABCF1*, *CLTC*, *GAPDH*, *ACTB)* and related to Ctrl (n=number of pooled samples/differentiations; 2D: Ctrl (n=16/3); HCM (n=15/3); HCMrep (n=18/3); EHTs: Ctrl (n=7/4); HCM (n=7/4); HCMrep (n=7/3)). Human samples were normalized to housekeeping genes (see method) and related to Ctrl (n=pool of 9 non-failing heart (NFH) tissues and n=1 for septal myectomy of patient II.4).



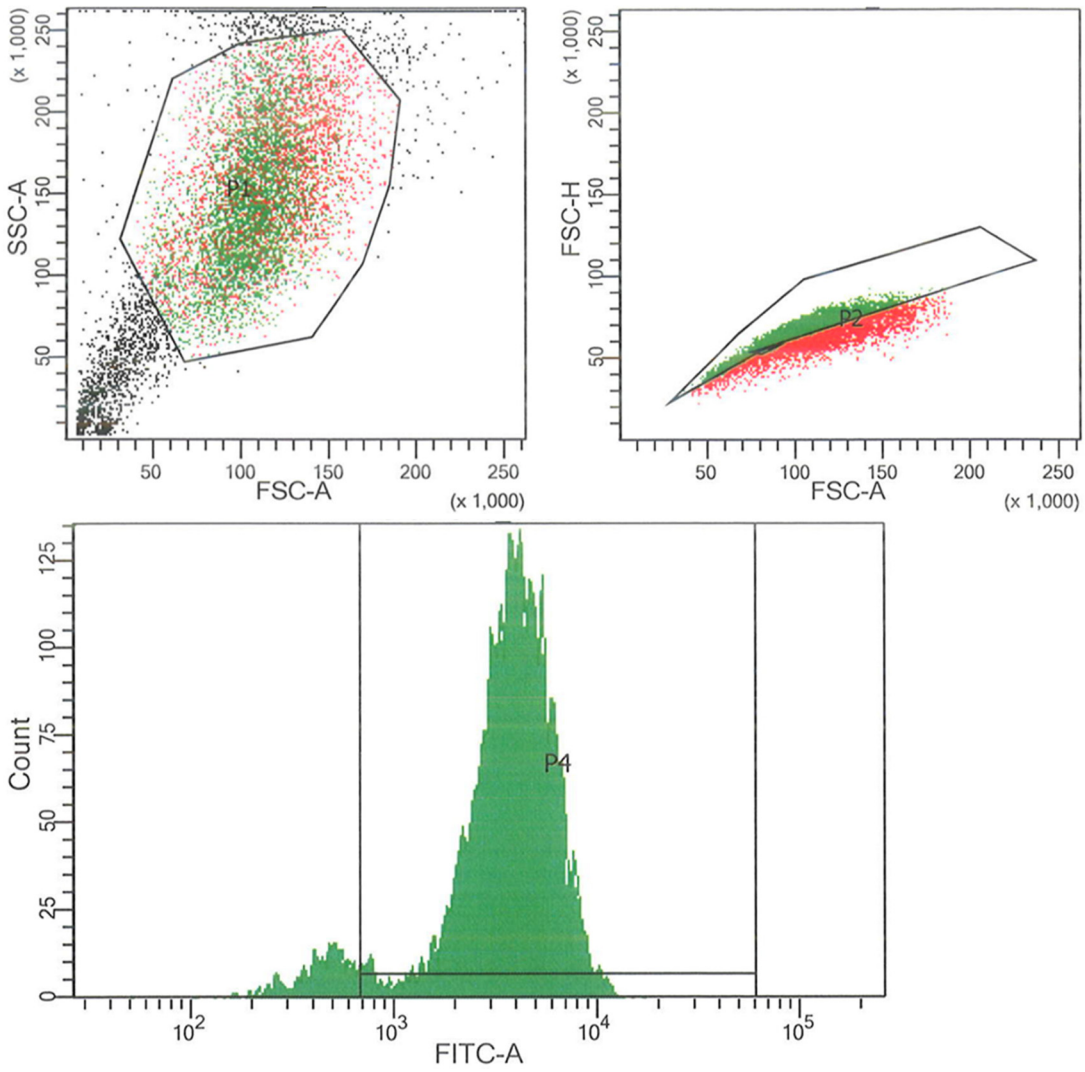
Appendix Figure S3. Force and calcium sensitivity measurements of 3D-cultured hiPSC-derived engineered heart tissues and HCM-affected family member II.4. (**A**) Functional parameters of maximal force (F_{max}) normalized to cross-sectional area (CSA) obtained from permeabilized EHT-strips (n=number of analyzed muscle strips; Ctrl (n=15), HCM (n=10), HCMrep (n=7)). Data are expressed as mean±SEM, one-way ANOVA plus Bonferroni's post-test. (**B**) Functional parameters of force obtained from muscle strips of non-failing human heart (NFH; n=15) and septal myectomy of index patient II.4 (n=16). Data are expressed as mean±SEM. Unpaired Student's *t*-test. (**C**) pCa_{50} values of EHT muscle strips from Ctrl (n=15), HCM (n=10), HCMrep (n=7). Data are expressed as mean±SEM, one-way ANOVA with Bonferroni´s post-test. (**D**) pCa_{50} values of non-failing heart (NFH) tissue (n=15), septal myectomy of index patient II.4 (n=16). Data are expressed as mean±SEM, unpaired Student's *t*-test. (**E**) pCa_{50} curve of non-failing heart (NFH) tissue (n=11), septal myectomy from patient II.4 (n=16)). Data are expressed as mean±SEM. Concentration response curves were fitted to the data points and force-pCa relationship comparison was done by using extra sum-of-squares F-test.



Appendix Figure S4. Validation of $Ca_v\alpha1.2$ and α-actinin 2 protein interaction by bioluminescence resonance energy transfer (BRET) assay. HL-1 transfected cells with α-actinin 2 wildtype (WT, n=11) and mutant (HCM, n=11) proteins were analyzed for their interaction with the $Ca_v\alpha1.2$ and $Ca_v\beta2$ domain. Data are expressed as mean±SEM, two-way ANOVA, followed by Sidak's multiple comparisons test.



Appendix Figure S5. Representative 12-lead surface ECG with averaged signal and overlay of all leads (in grey, apart from V5 in black) of the genotyped family sorted by positive *ACTN2* (**A**, patient II.4; **B**, patient II.5; **C**, patient III.4) and negative (**D**, patient III.1; **E**, patient III.2; **F**, patient III.3).



Population	#Events	%Parent	%Total
All Events	10,000	####	100.0
P1	7,105	71.0	71.0
P2	3,974	55.9	39.7
P4	3,684	92.7	36.8

Appendix Figure S6. Fluorescent activated cell sorting (FACS) analysis of cardiac troponin T (cTnT)-positive cells. Representative FACS-plots showing gating strategy for evaluation of cTnT-positive cells after cardiac differentiation. Fixed HCM-cardiomyocytes were stained with a directly labeled FITC cTnT antibody, quantified with the BD FACSCANTO II (BD Biosciences) and analyzed with the FACSDiva software (BD Biosciences).

Appendix Table S1. Analyzed HCM-associated genes

Gene	RefSeq Accession
ACTC1	NM_005159.4
ACTN2	NM_001103.2
ANKRD1	NM_014391.2
CSRP3	NM_003476.3
FHL1	NM_001159702.2; NM_001449.4
FLNC	NM_001458.4
GLA	NM_000169.2
LAMP2	NM_002294.2; NM_001122606.1; NM_013995.2
MYBPC3	NM_000256.3
MYH7	NM_000257.2
MYL2	NM_000432.3
MYL3	NM_000258.2
PLN	NM_002667.3
PRKAG2	NM_016203.3
TNNC1	NM_003280.2
TNNI3	NM_000363.4
TNNT2	NM_001276345.1
TPM1	NM_001018005.1 AY640414.1
TTR	NM_000371.3

Appendix Table S2. Analyzed off-targets in HCMrep by PCR.

NCBI accession number		off-target position
NG_029480	Exon	g.81,841-81,860
NM_016642	Exon	c.1037-1056
NG_013304	Exon	g.14,262-14,281
NG_029938	Exon	g.149,593-149,612
NM_000827	Exon	c.2942-2961
NG_009061	Exon	g.24,969-24,988
NM_025268	Exon	c.898-917
NC_018924	Intron	g.109,195,197-109,195,219
NC_018930	Intron	g.39,202,757-39,202,779
NC_018931	Intron	g.60,206,273-60,206,295
NC_018919	Intron	g.81,458,539-81,458,561
NC_018916	Intron	g.16,615,937-16,615,959
NC_018929	Intron	g.73,770,186-73,770,208

Appendix Table S3. Sequences of PCR primers

Primers	Forward (5´ to 3´)	Reverse (5´ to 3´)
ACTN2 genotyping	ggcccatgaaacacagaaat	agggccattcttcctcaagg
NG_029480	ggggtgtatggtgttcttgg	ggcaggaggacatggtttg
NM_016642	cttttgctttcctggtggct	gtctcctctggacagtctgc
NG_013304	ggaagagaagacactgggct	gactgagtgtgtgcagctgg
NG_029938	ccaaaggttcagagaagggc	cccggaagatgatggtgtct
NM_000827	tttgtttgatcccacagcaa	ggtctccatctgctccagtt
NG_009061	agggtgcttgagttgatcct	tgttggtggcagtggaca
NM_025268	ccgcagaagatgatgctgta	ggctgcagctccagtgatag
NC_018924	ttctgggttcaagccatcct	aagctcactgaaaggaaaggt
NC_018930	cttccagtccagagcaagtg	cagtcaaatcccagctctgc
NC_018931	tcagtttctacggccactgt	tgaaacctctctcttgccgt
NC_018919	agtgggttgctgcagagtaa	acaggtgtgagccatgtacc
NC_018916	tctgcactgtgtaggtcatgt	tgatgagaaaacgggaggca
NC_018929	gcaaggcatccacgaatagt	gctatttggggcactttggt
Allele-specific mRNA analysis	gccatggaaatcgctgagaa	atcctgttagccgctgtctc

Appendix Table S4. Acronyms and names of genes evaluated with the nanoString nCounter® Elements technology.

Gene	Acronym	Accession number (NCBI)
Alpha-Actinin 2	*ACTN2*	NM_001103.2
Myosin heavy chain 7	*MYH7*	NM_000257.2
Myosin heavy chain 6	*MYH6*	NM_002471.3
Natriuretic peptide B	*NPPB*	NM_002521.2
Serum response factor	*SRF*	NM_003131.3
Four-and-a-half-LIM-domains 1	*FHL1*	NM_001449.4
L-Type Ca^{2+} channel	*CACNA1C*	NM_199460.2
Natrium-Calcium Exchanger NCX	*SLC8A1*	NM_021097.1
Phospholamban	*PLN*	NM_002667.3
ATPase sarcoplasmic/endoplasmic reticulum Ca^{2+} transporting 2	*ATP2A2*	NM_001681.3
Ryanodine receptor 2	*RYR2*	NM_001035.2
Collagen type I alpha 1	*COL1A1*	NM_000088.3
Connective tissue growth factor	*CTGF*	NM_001901.2
Nuclear factor kappa B subunit 1	*NFKB1*	NM_003998.2
Caspase 3	*CASP3*	NM_032991.2
BCL2, apoptosis regulator	*BCL2*	NM_000657.2
ATP Binding Cassette Subfamily F Member 1	*ABCF1*	NM_001090.2
Clathrin Heavy Chain	*CLTC*	NM_004859.2
Glyceraldehyde 3-phosphate dehydrogenase	*GAPDH*	NM_002046.3
Beta-actin	*ACTB*	NM_001101.2
Phosphoglycerate kinase 1	*PGK1*	NM_000291.2
Tubulin Beta Class I	*TUBB*	NM_178014.3