Supplemental Material

Detailed Methods

Myocardial samples

Left ventricular (LV) tissue from the interventricular septum (IVS) was obtained from hypertrophic cardiomyopathy (HCM) patients harboring thick and thin filament gene mutations during myectomy surgery to relieve LV outflow obstruction. Our study included patients carrying heterozygous mutations in *MYBPC3* (n=21; $MYBPC3_{mut}$), *MYH7* (n=6; $MYH7_{mut}$), *TNNI3* (n=2; $TNNI3_{mut}$) and *TPM1* (n=1; $TPM1_{mut}$). The $MYBPC3_{mut}$ group consisted of patients with truncating (n=17) and missense (n=4) mutations. Data for these two $MYBPC3_{mut}$ groups are presented separately. IVS tissue was also obtained during heart transplantation surgery from 1 end-stage failing HCM patient carrying a homozygous *TNNT2* mutation ($TNNT2_{mut}$). IVS myectomy tissue from 7 HCM patients in whom no mutation was found after screening of 8 genes (sarcomere mutation-negative HCM; HCM_{smn}) and cardiac tissue from 12 non-failing donors served as controls. Donors (age range from 14 to 65 years; mean 39±5 years; 9/3 male/female, respectively) had no history of cardiac abnormalities, normal ECG and normal ventricular function on echocardiography within 24 hours of heart transplantation. Tissue was collected in cardioplegic solution and immediately frozen and stored in liquid nitrogen. Samples were obtained after written informed consent and the study protocol was approved by the local ethical committees.

Isometric force measurements

Small cardiac tissue samples were thawed in relaxing solution (5.95 mM Na_2ATP, 6.04 mM $MgCl_2$, 2 mM EGTA, 139.6 mM KCl, 10 mM Imidazole, pH 7.0) and cardiomyocytes were mechanically isolated by tissue disruption. Cardiomyocytes were chemically permeabilized by incubation for 5 minutes in relaxing solution containing 0.5% (v/v) Triton-X100 and glued between a force transducer and a piezoelectric motor.[1] Isometric force measurements were performed at maximal and submaximal [Ca^{2+}] (ranging from 1 to 30 µmol/L) and sarcomere lengths of 1.8 and 2.2 µm (Online Figures IA and IB). Average sarcomere lengths were determined by means of a spatial Fourier transformation as described previously.[2] Passive force (F_{pas}) was determined by shortening the myocyte in a relaxing solution (10^{-9} µmol/L) by 30% of its length. Maximal developed force (F_{max}) was determined by activating the cardiomyocyte at saturating [Ca^{2+}] (30 µmol/L), generating a total force value (F_{total}). F_{max} was obtained by subtracting F_{pas} from F_{total} (i.e. $F_{max}=F_{total}-F_{pas}$). Maximal tension (in kN/m^2) was calculated as F_{max} normalized to cross-sectional area of the cardiomyocytes. Force-Ca^{2+} relations were fit to a modified Hill equation and myofilament Ca^{2+}-sensitivity was denoted as EC_{50} ([Ca^{2+}] at which half of F_{max} was reached). The length-dependent increase in myofilament Ca^{2+}-sensitivity upon an increase in sarcomere length is based on the difference in EC_{50} at sarcomere lengths of 1.8 and 2.2 µm (ΔEC_{50}). Additional force measurements were performed following exogenous PKA treatment of cells for 40 minutes at 20ºC in relaxing solution containing the catalytic subunit of PKA (100 U/incubation, Sigma).

Exchange of recombinant human wild-type troponin complex in single cardiac cells

Preparation of recombinant human wild-type troponin complex

Expression of cDNA encoding human wild-type cardiac troponin subunits (cTnC, myc-tag labeled cTnT (cTnT-myc), cTnI), purification and reconstitution were performed as described previously.[3]

Troponin exchange protocol

Single cardiomyocytes from the $TNNT2_{mut}$ heart and one of the $TNNI3_{mut}$ hearts were mechanically isolated by tissue disruption in ice-cold rigor solution (132 mM NaCl, 5 mM KCl, 1

mM $MgCl_2$, 10 mM Tris, 5 mM EGTA, 1 mM NaAzide, pH 7.1). Cardiomyocytes were chemically permeabilized by incubation for 5 minutes in rigor solution containing 0.5% (v/v) Triton-X100. After permeabilization, cells were washed twice with rigor solution followed by washing in exchange solution (10 mM imidazole, 200 mM KCl, 5 mM $MgCl_2$, 2.5 mM EGTA, 1 mM DTT) (pH 6.9). Subsequently, single cardiomyocytes were incubated overnight at 4°C in exchange solution containing the appropriate concentration of recombinant human troponin complex (0.25, 0.5 and 1.0 mg/mL) with the addition of 4 mM $CaCl_2$, 4 mM DTT, 5 µl/mL protease inhibitor cocktail (PIC, Sigma, P8340) and 10 µl/mL phosphatase inhibitor cocktail 2 and 3 (PhIC, Sigma, P2850, P5726) (pH 6.9). The next day, cells were washed twice in rigor solution followed by washing in relaxing solution. Our previous study showed a homogenous distribution of recombinant troponin complex in cardiomyocytes using this exchange protocol.[4]

Determination of troponin exchange percentage

Half of the cardiomyocyte suspension was used for isometric force measurements, whereas the other half was used to analyze troponin exchange percentage. This half was treated with 2D-clean-up kit (GE Healthcare), homogenized in sample buffer (15% glycerol, 62.5 mM Tris (pH 6.8), 1% (w/v) SDS and 2% (w/v) DTT) and protein concentration was measured with *RCDC* Protein Assay kit II (Biorad) as described previously.[3]

To determine the degree of exchange of endogenous mutant troponin by recombinant wild-type cardiac troponin Western blotting was performed. Recombinant wild-type cTnT was labeled with a myc-tag, which allowed differentiation between endogenous and recombinant cardiac troponin complex. Proteins were separated on a 1D SDS-PAGE and blotted onto a nitrocellulose membrane. A specific monoclonal antibody was used against cTnT (Clone JLT-12, Sigma) to detect endogenous and recombinant cTnT by chemiluminescence (ECL, Amersham Biosciences) as described previously.[3]

Myofilament protein phosphorylation

SYPRO Ruby and ProQ-Diamond staining of gradient gels

Myofilament protein phosphorylation levels in HCM and donor myocardium and in PKA-treated samples (100 U/mL relaxing solution) were analyzed on 4-15% pre-cast Tris-HCl gels (BioRad) and stained with SYPRO Ruby and ProQ-Diamond phosphostain as described previously.[5] Phosphorylation of cMyBP-C and cTnI was normalized to SYPRO-stained cMyBP-C and cTnI, respectively. Protein phosphorylation values were normalized to the values found in untreated donors, which were set to 1.

Western blot analysis of cMyBP-C phosphorylation at PKA sites

Phosphorylation of the cMyBP-C PKA sites Ser275 and Ser284 was assessed using phosphospecific antibodies in Western blots.[1]

Phos-Tag acrylamide gels

Phos-Tag™ acrylamide gels were performed to visualize phosphorylated cTnI species using alkoxide-bridged dication manganese (Mn^{2+}) complex as phosphate-binding tag (Phos-tag) molecule. Mn^{2+}-Phos-Tag molecules specifically bind phosphorylated proteins and as a result, their migration speed is highly reduced. Non-phosphorylated and phosphorylated cTnI species were separated in 1D PAGE with polyacrylamide-bound Mn^{2+}-Phos-Tag, transferred to Western blots and probed with anti-cTnI monoclonal antibody (8I-7 Spectral Diagnostics).[6]

Data analysis

Data analysis and statistics were performed using Prism version 4.0 (Graphpad Software, Inc., La Jolla, CA) and SPSS version 15.0 (IBM, Armonk, NY). Data are presented as mean±SEM of all single cardiomyocytes per patient group (8 groups, i.e. the 6 HCM sarcomere mutation positive groups (HCM_{mut}), HCM_{smn} and non-failing donor). To take into account the repeated sample assessments within patient/donor groups multilevel analysis was performed. Comparison between all groups was performed for Ca^{2+}-sensitivity at 2.2 µm sarcomere length

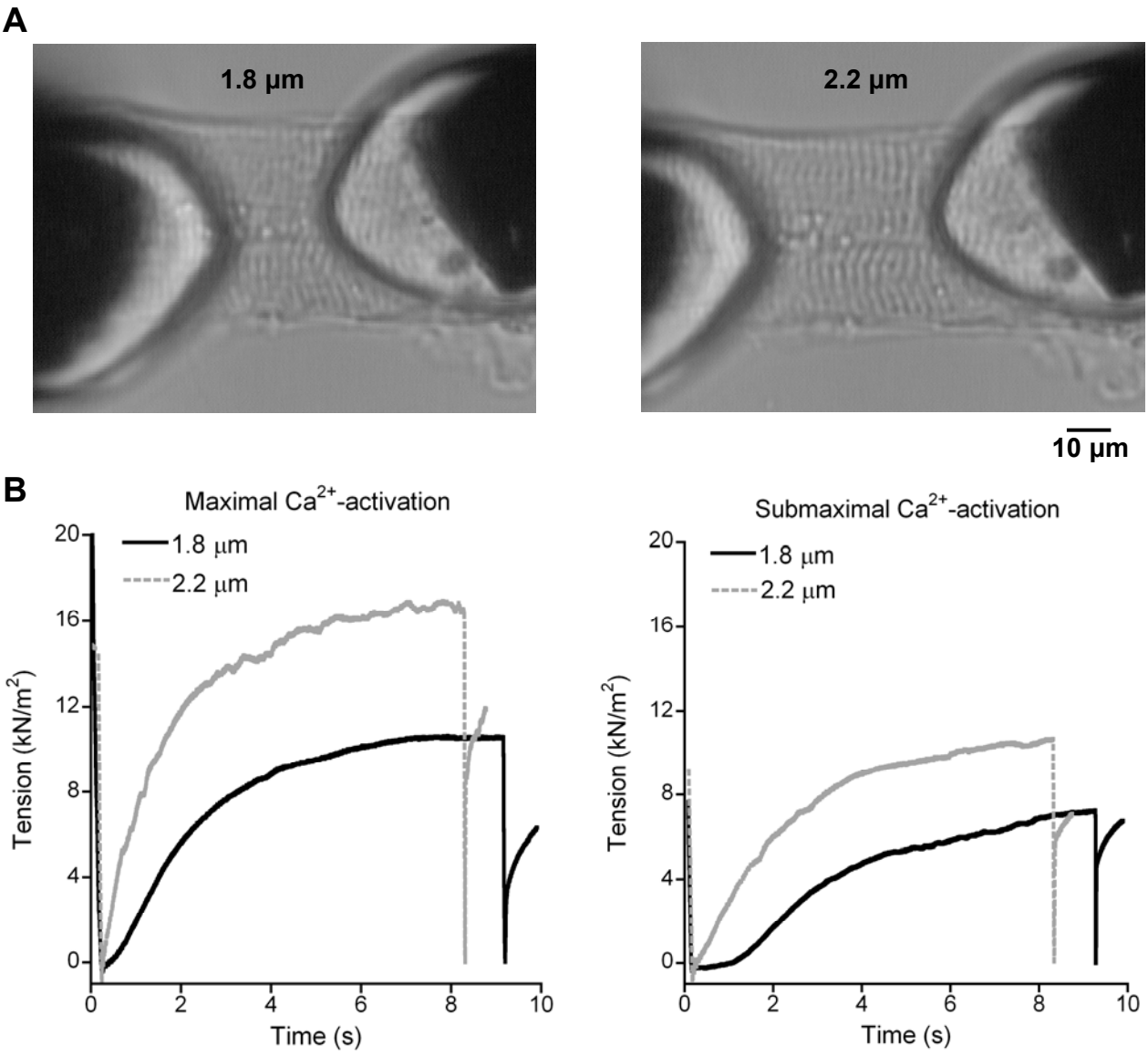
and length-dependent activation of cardiomyocytes before and after PKA. Paired-group comparisons were performed for F_{max} at 1.8 and 2.2 µm sarcomere length before and after PKA.

All data was tested for normality using the Shapiro-Wilk Test. Normality was assumed when $p>0.05$ and the variances were equal. When assumption of normality was violated the data set was logarithmic-transformed and normality re-tested.[7] Detailed information on statistical analyses of the data presented in Figures 1-2 and 5 and Table 2 of the manuscript are presented in the Online Tables III to X. To take differences in group size into account multilevel analysis was performed (Online Tables III to VII and X to XI). Two data sets were expressed as logarithmic-transformed groups (Online Tables III and V). To check for paired-group differences on F_{max} between sarcomere lengths of non-treated (Online Table VIII) or treated cardiomyocytes with PKA (Online Table IX), paired-samples t-Test was conducted. $p<0.05$ was considered significant for both tests and 95% confidence intervals (CI) were calculated to gain insight into the range of the mean differences between and among groups. In case of logarithmic transformed data, a set of 95% CIs was calculated by taking the exponential of the natural logarithmic values (Online Tables III and V). The CIs reflect ratios stating that the mean difference between two groups can be [x to y] time higher or lower than the group of comparison.

Exact significance levels (p values) and 95% confidence intervals are given in the tables below.

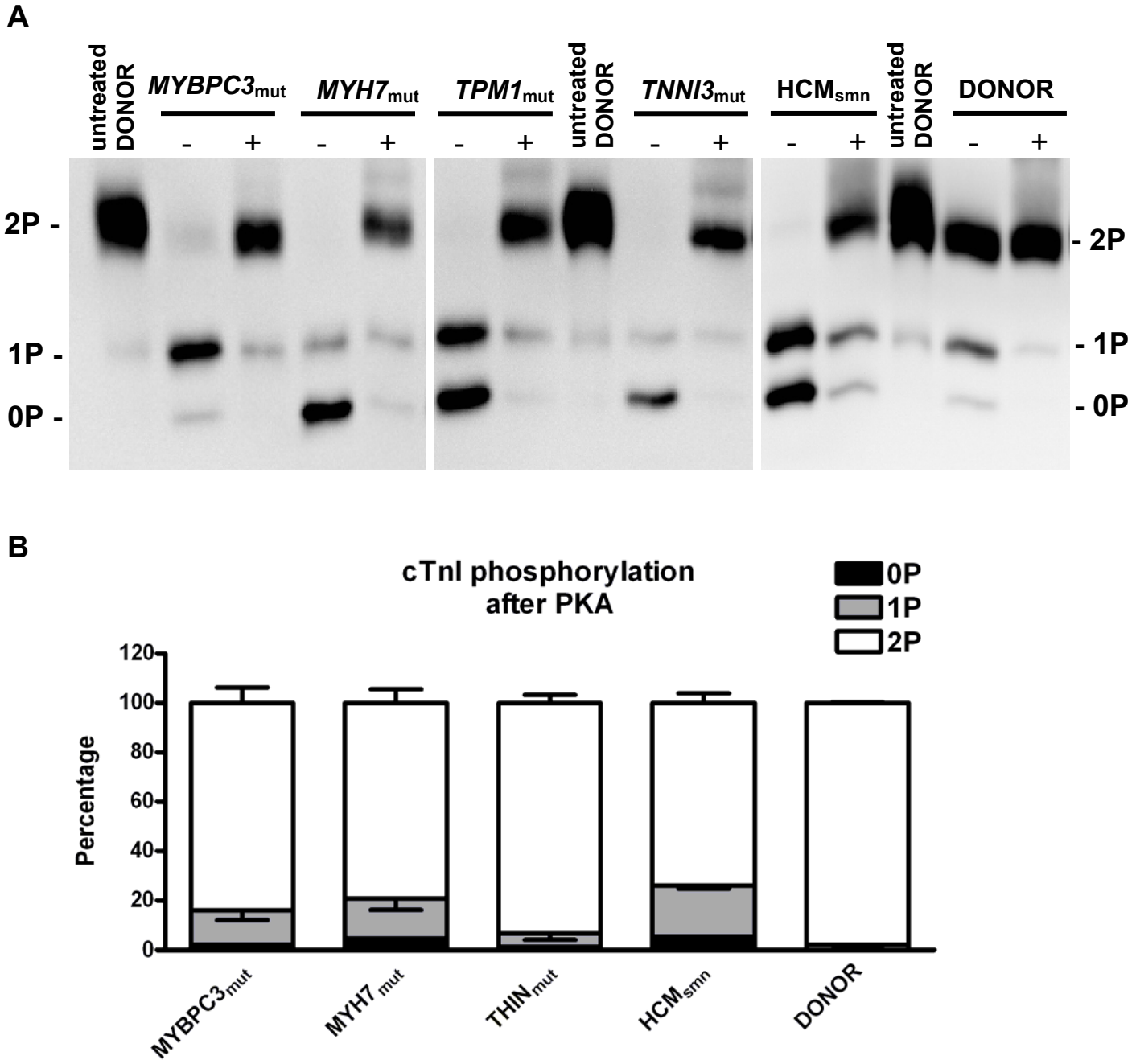
Supplemental Figures

Online Figure I



Online Figure I. Isometric force measurements in a single cardiomyocyte. A. Triton-permeabilized single cardiomyocyte isolated from a *TPM1*$_{mut}$ heart at a short (1.8 µm; left) and long (2.2 µm; right) sarcomere length. **B.** Force recordings of a single cardiomyocyte isolated from a *TPM1*$_{mut}$ heart at 1.8 µm (left) and 2.2 µm (right) during maximal and submaximal Ca^{2+}-activation.

Online Figure II



Online Figure II. Phosphorylation forms of cTnI. A. Heart samples were incubated without (-) and with (+) PKA (2 *MYBPC3*$_{mut}$, 2 *MYH7*$_{mut}$, 3 samples with thin filament gene mutations (THIN$_{mut}$), 2 HCM$_{smn}$ and 2 donor samples) and separated on a Phos-Tag acrylamide gel to visualize the distribution of un- (0P), mono- (1P) and bis- (2P) phosphorylated forms of cTnI. PKA clearly increased phosphorylation of cTnI in all HCM samples. Untreated donor samples were included as reference. **B.** After PKA treatment cTnI phosphorylation shifted to the phosphorylation pattern present in donor myocardium, although the percentage of bisphosphorylated cTnI was still lower compared to donor.

Supplemental Tables

Online Table I. Effect of PKA on F_{max} at a sarcomere length of 2.2 μm.

	F_{max} (kN/m^2)		Number of samples/cells
Sample	**before PKA** (2.2 μm)	**after PKA** (2.2 μm)	
truncating			
MYBPC3$_{mut}$	22.8±2.6*	21.0±2.4*	N=14; n=33
missense			
MYBPC3$_{mut}$	14.2±2.2*	14.5±1.4*	N=1; n= 4
MYH7$_{mut}$	15.0±1.6*	15.0±1.5*	N=6; n=21
TNNT2$_{mut}$	15.0±3.6*	14.7±2.6*	N=1; n=5
TNNI3$_{mut}$	19.0±2.3*	18.3±2.2*	N=2; n=12
TPM1$_{mut}$	16.1±1.1*	17.7±3.0*	N=1; n=5
HCM$_{smn}$	22.4±2.6*	20.2±2.2*	N=7; n=19
DONOR	30.9±2.9	29.7±2.7	N=8; n=22

$p<0.05$ was considered significant; * vs donor; N= number of samples; n= number of cardiomyocytes.

Online Table II. Mean force characteristics for individual HCM mutations.

		Effect PKA					Effect sarcomere length							
Mutation	**Type**	**EC_{50}**	**EC_{50} after PKA**	**N/n**	**ΔEC_{50}**	**N/n**	**F_{max} 1.8 µm**	**F_{max} 2.2 µm**	**ΔEC_{50}**	**N/n**	**F_{max} 1.8 µm after PKA**	**F_{max} 2.2 µm after PKA**	**ΔEC_{50} after PKA**	**N/n**
***MYBPC3*$_{mut}$**	**Truncating**													
c.927-2A>G **(1-3)**	splice site	2.18±0.15	2.96±0.11	3/14	0.89±0.36	3/5	16.4±3.5	31.0±6.0	0.35±0.04	2/6	31.6±3.6	40.6±4.8	0.92±0.10	2/7
c.1458-1G>C **(4)**	splice site	2.96±0.32	3.94±0.14	1/3	0.72±0.20	1/2								
c.2373duplicationG **(5-13)**	insertion	2.38±0.09	3.18±0.07	9/43	0.94±0.15	8/18	22.3±1.8	33.0±3.0	0.49±0.06	9/31	24.4±2.2	35.7±4.7	0.95±0.10	2/7
c.2864.2865delCT **(14-16)**	deletion	3.20±0.28	4.58±0.46	2/8	1.01±0.17	2/5	8.4±2.4	11.7±3.2	0.38±0.09	2/6				
c.3407.3409del **(17)**	deletion	1.99±0.18	2.09±0.20	1/5	0.39±0.03	1/2	19.8±3.0	30.9±4.9	0.31±0.12	1/4	24.9±0.8	35.5±1.7	0.60±0.05	1/5
***MYBPC3*$_{mut}$**	**Missense**													
p.E258K **(18,19)**	missense	1.80±0.14	3.74±1.08	2/6			12.0±2.4	13.1±2.6	0.07±0.14	2/6	11.7±0.9	13.8±2.6	0.19±0.19	2/6
p.G531R **(20)**	missense	1.59±0.15	3.09±0.42	1/3			16.7±1.8	20.6±3.4	0.39±0.04	1/3	11.0±4.0	14.1±4.1	0.26±0.12	1/3
p.R597Q **(21)**	missense	1.66±0.06	3.52±0.29	1/7	1.46±0.21	1/4	11.3±2.4	16.2±3.4	0.32±0.32	1/3	11.1±0.7	13.7±1.1	0.39±0.06	1/3
***MYH7*$_{mut}$**														
p.R403Q **(1)**	missense	1.65±0.11	3.05±0.14	1/6	1.54±0.10	1/3	4.6±0.4	5.7±0.6	0.34±0.19	1/3	7.3±2.1	8.5±2.2	0.36±0.38	1/3
p.V606M **(2)**	missense	1.27±0.13	3.07±0.40	1/12	2.16±0.42	1/5	11.6±1.6	17.3±2.4	0.26±0.08	1/7	24.1±3.5	29.2±6.3	0.71±0.33	1/4
p.S782R **(3)**	missense	3.59±0.77	3.00±0.36	1/6	-0.03±0.31	1/3	13.2±2.1	12.8±2.4	-0.03±0.18	1/3	22.2±10.1	23.8±.8.8	-0.01±0.10	1/3
p.R787H **(4)**	missense	1.91±0.13	2.79±0.16	1/10	0.86±0.14	1/5	28.6±5.9	34.8±5.5	0.22±0.08	1/5	19.4±2.0	25.0±3.6	0.25±0.06	1/5
p.T1377M **(5,6)**	missense	1.60±0.06	2.77±0.40	2/17	1.17±0.41	2/5	14.8±1.6	18.6±1.7	0.37±0.07	2/14				
***TNNT2*$_{mut}$**														
p.K280N **(1)**	missense	2.44±0.08	2.56±0.18	1/5	0.15±0.21	1/5	21.5±3.2	24.4±6.0	0.35±0.16	1/6	13.7±2.1	15.2±2.4	0.25±0.19	1/4
***TNNI3*$_{mut}$**														
p.R145W **(1,2)**	missense	2.68±0.14	3.95±0.25	2/12	0.99±0.26	2/12	9.8±2.5	10.1±2.8	0.29±0.15	2/8	8.4±1.4	9.5±1.9	0.28±0.19	2/10
***TPM1*$_{mut}$**														
p.M281T **(1)**	missense	2.05±0.13	3.18±0.26	1/5	1.08±0.24	1/5	10.7±2.1	17.0±3.0	0.30±0.14	1/6	9.1±2.3	9.6±1.7	-0.13±0.09	1/4

Numbers between brackets indicate the samples as shown in Table 1 of the manuscript. N= number of samples; n= number of cardiomyocytes; Mean average of each mutation group. F_{max}: maximal developed force given in kN/m^2. EC_{50}: Ca^{2+}-sensitivity given in µmol/L. ΔEC_{50}: PKA-mediated and length-dependent change in myofilament Ca^{2+}-sensitivity.

Detailed Statistics

Online Table III (Figure 1A). Differences in Ca^{2+}-sensitivity (EC_{50}) at sarcomere length of 2.2 µm.

Samples	p value	95% CI (%)
MYBPC3$_{mut}$ (truncating) vs donor	*p<0.0001	-0.15 to -0.07
MYBPC3$_{mut}$ (missense) vs donor	*p<0.0001	-0.32 to -0.18
MYH7$_{mut}$ vs donor	*p<0.0001	-0.32 to -0.23
TNNT2$_{mut}$ vs donor	*p=0.001	-0.15 to -0.04
TNNI3$_{mut}$ vs donor	*p=0.002	-0.13 to -0.28
TPM1$_{mut}$ vs donor	*p<0.0001	-0.25 to -0.11
HCM_{smn} vs donor	*p=0.001	-0.14 to 0.04

Multilevel analysis. p<0.05 was considered significant; *indicates significant result.

Online Table IV (Figure 1B). Differences in PKA-mediated change in myofilament Ca^{2+}-sensitivity (ΔEC_{50}) at sarcomere length of 2.2 µm.

Samples	p value	95% CI (µmol/L)
MYBPC3$_{mut}$ (truncating) vs donor	*p=0.025	0.05 to 0.77
MYBPC3$_{mut}$ (missense) vs donor	*p=0.008	0.27 to 1.71
MYH7$_{mut}$ vs donor	*p=0.008	0.13 to 0.87
TNNT2$_{mut}$ vs donor	p=0.341	-0.97 to 0.34
TNNI3$_{mut}$ vs donor	*p=0.037	0.03 to 1.00
TPM1$_{mut}$ vs donor	p=0.095	-0.12 to 1.33
HCM_{smn} vs donor	p=0.218	-0.15 to 0.67
MYBPC3$_{mut}$ (truncating) vs *TNNT2*$_{mut}$	§p=0.026	0.09 to 1.37
MYBPC3$_{mut}$ (missense) vs *TNNT2*$_{mut}$	§p=0.005	0.41 to 2.12
MYH7$_{mut}$ vs *TNNT2*$_{mut}$	§p=0.014	0.17 to 1.47
TNNI3$_{mut}$ vs *TNNT2*$_{mut}$	§p=0.024	0.11 to 1.55
TPM1$_{mut}$ vs *TNNT2*$_{mut}$	§p=0.042	0.03 to 1.82
HCM_{smn} vs *TNNT2*$_{mut}$	p=0.093	-0.10 to 1.24

Multilevel analysis. p<0.05 was considered significant; * and §indicate significant results.

Online Table V (Figure 1C). Differences in Ca^{2+}-sensitivity (EC_{50}) after PKA treatment at sarcomere length of 2.2 µm.

Samples	p value	95% CI (%)
MYBPC3$_{mut}$ (truncating) vs donor	p=0.419	-0.07 to 0.03
MYBPC3$_{mut}$ (missense) vs donor	p=0.328	-0.10 to 0.03
MYH7$_{mut}$ vs donor	*p=0.023	-0.12 to -0.01
TNNT2$_{mut}$ vs donor	*p=0.005	-0.20 to -0.04
TNNI3$_{mut}$ vs donor	*p=0.042	0.00 to 0.12
TPM1$_{mut}$ vs donor	p=0.561	-0.11 to 0.06
HCM_{smn} vs donor	p=0.264	-0.10 to 0.02
MYBPC3$_{mut}$ (truncating) vs HCM_{smn}	p=0.643	-0.04 to 0.06
MYBPC3$_{mut}$ (missense) vs HCM_{smn}	p=0.968	-0.06 to 0.06
MYH7$_{mut}$ vs HCM_{smn}	p=0.213	-0.08 to 0.02
TNNT2$_{mut}$ vs HCM_{smn}	#p=0.033	-0.16 to -0.01
TNNI3$_{mut}$ vs HCM_{smn}	#p=0.001	0.04 to 0.15
TPM1$_{mut}$ vs HCM_{smn}	p=0.867	-0.10 to 0.10

Multilevel analysis. p<0.05 was considered significant; *and #indicate significant results.

Online Table VI (Figures 2C and 2D). Differences in length-dependent changes in myofilament Ca^{2+}-sensitivity (ΔEC_{50}) of non-treated (**C**) and PKA pre-treated (**D**) cardiomyocytes.

C.	Samples	p value	95% CI (µmol/L)
	MYBPC3$_{mut}$ (truncating) vs donor	*p<0.0001	-0.48 to -0.18
	MYBPC3$_{mut}$ (missense) vs donor	*p<0.0001	-0.75 to -0.34
	MYH7$_{mut}$ vs donor	*p<0.0001	-0.64 to -0.33
	TNNT2$_{mut}$ vs donor	*p=0.003	-0.70 to -0.15
	TNNI3$_{mut}$ vs donor	*p<0.0001	-0.72 to -0.23
	TPM1$_{mut}$ vs donor	*p=0.001	-0.75 to -0.19
	HCM_{smn} vs donor	*p<0.0001	-0.55 to -0.23
D.	**Samples**	**p value**	**95% CI (µmol/L)**
	MYBPC3$_{mut}$ (truncating) vs donor	p=0.971	-0.30 to 0.31
	MYBPC3$_{mut}$ (missense) vs donor	*p<0.0001	-1.01 to -0.33
	MYH7$_{mut}$ vs donor	*p=0.004	-0.80 to -0.16
	TNNT2$_{mut}$ vs donor	*p=0.021	-1.05 to -0.09
	TNNI3$_{mut}$ vs donor	*p=0.003	-0.91 to -0.19
	TPM1$_{mut}$ vs donor	*p<0.0001	-1.44 to -0.48
	HCM_{smn} vs donor	p=0.797	-0.30 to 0.39
	MYBPC3$_{mut}$ (truncating) vs HCM_{smn}	p=0.803	-0.34 to 0.27
	MYBPC3$_{mut}$ (missense) vs HCM_{smn}	#p<0.0001	-1.05 to -0.37
	MYH7$_{mut}$ vs HCM_{smn}	#p=0.002	-0.85 to -0.20
	TNNT2$_{mut}$ vs HCM_{smn}	#p=0.013	-1.10 to -0.13
	TNNI3$_{mut}$ vs HCM_{smn}	#p=0.001	-0.95 to -0.23
	TPM1$_{mut}$ vs HCM_{smn}	#p<0.0001	-1.49 to -0.52

Multilevel analysis. p<0.05 was considered significant; *and #indicate significant results.

Online Table VII (Figures 5B to 5E). Differences in length-dependent changes in myofilament Ca^{2+}-sensitivity (ΔEC_{50}) of non-treated (**B** and **D**) and PKA pre-treated (**C** and **E**) cardiomyocytes.

B.	**Samples**	**p value**	**95% CI (µmol/L)**
	38% *TNNT2*$_{mut}$ vs *TNNT2*$_{mut}$	p=0.560	-0.53 to 0.29
	22% *TNNT2*$_{mut}$ vs *TNNT2*$_{mut}$	p=0.050	-0.74 to 0.00
	14% *TNNT2*$_{mut}$ vs *TNNT2*$_{mut}$	p=0.500	-0.50 to 0.25
	TNNT2$_{mut}$ vs Donor	*p=0.002	0.17 to 0.68
	38% *TNNT2*$_{mut}$ vs Donor	*p=0.003	-0.89 to -0.19
	22% *TNNT2*$_{mut}$ vs Donor	*p<0.0001	-1.10 to -0.49
	14% *TNNT2*$_{mut}$ vs Donor	*p=0.001	-0.85 to -0.24
C.	**Samples**	**p value**	**95% CI (µmol/L)**
	38% *TNNT2*$_{mut}$ vs *TNNT2*$_{mut}$	p=0.790	-0.43 to 0.55
	22% *TNNT2*$_{mut}$ vs *TNNT2*$_{mut}$	p=0.070	-0.03 to 0.94
	14% *TNNT2*$_{mut}$ vs *TNNT2*$_{mut}$	§p=0.020	0.09 to 0.99
	TNNT2$_{mut}$ vs Donor	*p=0.004	0.20 to 0.94
	38% *TNNT2*$_{mut}$ vs Donor	*p=0.020	-0.92 to -0.10
	22% *TNNT2*$_{mut}$ vs Donor	p=0.570	-0.53 to 0.30
	14% *TNNT2*$_{mut}$ vs Donor	p=0.870	-0.40 to 0.34
D.	**Samples**	**p value**	**95% CI (µmol/L)**
	TNNI3$_{mut}$ vs Donor	*p=0.001	-0.73 to -0.22
	TNNI3$_{mut}$ + 90% vs Donor	*p<0.0001	-1.04 to -0.35
E.	**Samples**	**p value**	**95% CI (µmol/L)**
	TNNI3$_{mut}$ + 90% vs *TNNI3*$_{mut}$	§p=0.007	0.17 to 0.93
	TNNI3$_{mut}$ vs Donor	*p=0.007	-0.93 to -0.17
	TNNI3$_{mut}$ + 90% vs Donor	p=0.530	-0.33 to 0.62

Multilevel analysis. p<0.05 was considered significant; *and §indicate significant results.

Online Table VIII (Table 2). Effect of sarcomere length increase on F_{max}.

	Samples	**p value**	**95% CI (kN/m^2)**
F_{max} (1.8 vs 2.2 µm)	**truncating**		
	MYBPC3$_{mut}$	**p<0.0001	-12.67 to -7.73
	missense		
	MYBPC3$_{mut}$	**p<0.0001	-4.18 to -0.82
	MYH7$_{mut}$	**p<0.0001	-6.14 to -1.42
	TNNT2$_{mut}$	p=0.530	-14.28 to 8.33
	TNNI3$_{mut}$	p=0.190	-0.11 to 0.45
	TPM1$_{mut}$	**p=0.008	-10.15 to -2.54
	HCM$_{smn}$	**p<0.0001	-14.17 to -7.72
	Donor	**p=0.001	-11.81 to -3.57

Paired samples t-Test. p<0.05 was considered significant; **indicates significant result.

Online Table IX (Table 2). Effect of sarcomere length increase on F_{max} in PKA pre-treated cardiomyocytes.

	Samples	p value	95% CI (kN/m^2)
F_{max} (1.8 vs 2.2 µm)	**truncating**		
	MYBPC3$_{mut}$	**p<0.0001	-12.10 to -6.61
	missense		
	MYBPC3$_{mut}$	**p=0.0028	-4.73 to -0.18
	MYH7$_{mut}$	**p=0.004	-6.14 to -1.42
	TNNT2$_{mut}$	p=0.35	-5.78 to 2.80
	TNNI3$_{mut}$	p=0.18	-2.88 to 0.63
	TPM1$_{mut}$	p=0.48	-2.68 to 1.52
	HCM$_{smn}$	**p<0.0001	-14.39 to -9.07
	Donor	**p<0.0001	-13.45 to -6.51

Paired samples t-Test. p<0.05 was considered significant; **indicates significant result.

Online Table X (Table 2). Differences in length-dependent changes in maximal force (ΔF_{max}) of non-treated (**A**) and PKA pre-treated (**B**) cardiomyocytes.

A.	**Samples**	**p value**	**95% CI (kN/m^2)**
	MYBPC3$_{mut}$ (truncating) vs donor	p=0.458	-0.24 to 5.21
	MYBPC3$_{mut}$ (missense) vs donor	*p=0.023	-11.69 to -0.87
	MYH7$_{mut}$ vs donor	*p=0.021	-8.95 to -0.73
	TNNT2$_{mut}$ vs donor	p=0.119	-13.12 to 1.51
	TNNI3$_{mut}$ vs donor	*p=0.011	-14.99 to -1.99
	TPM1$_{mut}$ vs donor	p=0.513	-9.74 to 4.89
	HCM$_{smn}$ vs donor	p=0.523	-2.85 to 5.58
B.	**Samples**	**p value**	**95% CI (kN/m^2)**
	MYBPC3$_{mut}$ (truncating) vs donor	p=0.711	-3.94 to 2.70
	MYBPC3$_{mut}$ (missense) vs donor	*p<0.0001	-11.24 to -3.81
	MYH7$_{mut}$ vs donor	*p=0.001	-9.72 to -2.67
	TNNT2$_{mut}$ vs donor	*p=0.002	-13.74 to -3.23
	TNNI3$_{mut}$ vs donor	*p<0.0001	-12.95 to -5.35
	TPM1$_{mut}$ vs donor	*p=0.001	-14.65 to -4.15
	HCM$_{smn}$ vs donor	p=0.277	-1.67 to 5.76

Multilevel analysis. p<0.05 was considered significant; * indicates significant results.

Online Table XI (Online Table I). Effect of PKA on F_{max} at a sarcomere length of 2.2 µm before (**A**) and after PKA treated (**B**) cardiomyocytes.

A.	**Samples**	**p value**	**95% CI (kN/m^2)**
	MYBPC3$_{mut}$ (truncating) vs donor	*p=0.015	-14.40 to -1.61
	MYBPC3$_{mut}$ (missense) vs donor	*p=0.010	-29.29 to -4.03
	MYH7$_{mut}$ vs donor	*p<0.0001	-22.94 to -8.76
	TNNT2$_{mut}$ vs donor	*p=0.008	-27.30 to -4.27
	TNNI3$_{mut}$ vs donor	*p=0.007	-20.38 to -3.22
	TPM1$_{mut}$ vs donor	*p=0.013	-26.22 to -3.20
	HCM$_{smn}$ vs donor	*p=0.025	-15.83 to -1.06
B.	**Samples**	**p value**	**95% CI (kN/m^2)**
	MYBPC3$_{mut}$ (truncating) vs donor	*p=0.005	-14.60 to 2.70
	MYBPC3$_{mut}$ (missense) vs donor	*p=0.037	-24.23 to -0.74
	MYH7$_{mut}$ vs donor	*p<0.0001	-21.14 to -7.95
	TNNT2$_{mut}$ vs donor	*p=0.007	-25.49 to -4.08
	TNNI3$_{mut}$ vs donor	*p=0.006	-19.17 to -3.21
	TPM1$_{mut}$ vs donor	*p=0.019	-23.56 to -2.15
	HCM$_{smn}$ vs donor	*p=0.008	-16.18 to -2.44

Multilevel analysis. $p<0.05$ was considered significant; * indicates significant results.

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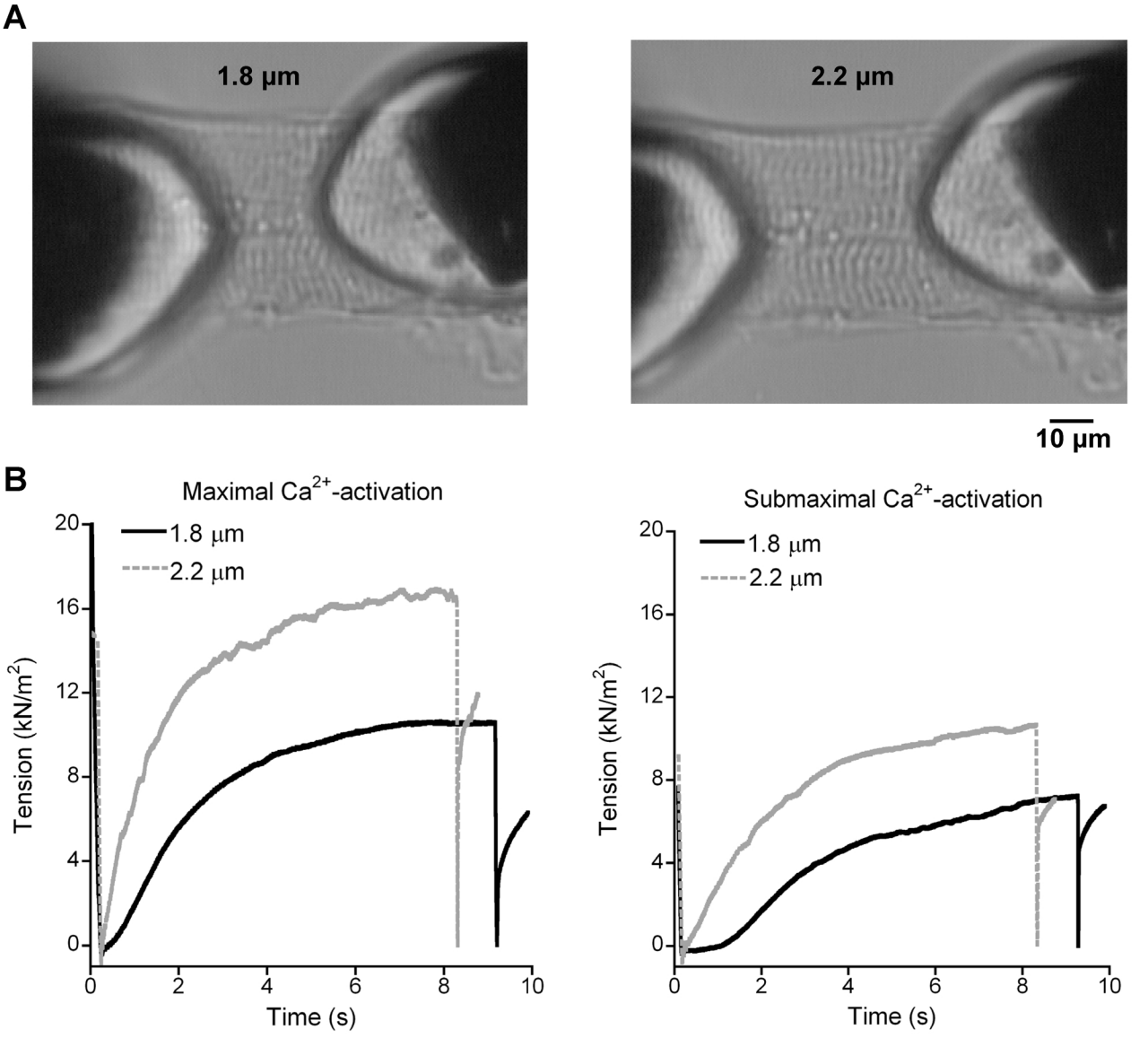
A
1.8 µm
2.2 µm
10 µm
B
Maximal Ca2+-activation
1.8 µm
2.2 µm
Tension (kN/m2)
Time (s)
Submaximal Ca2+-activation
1.8 µm
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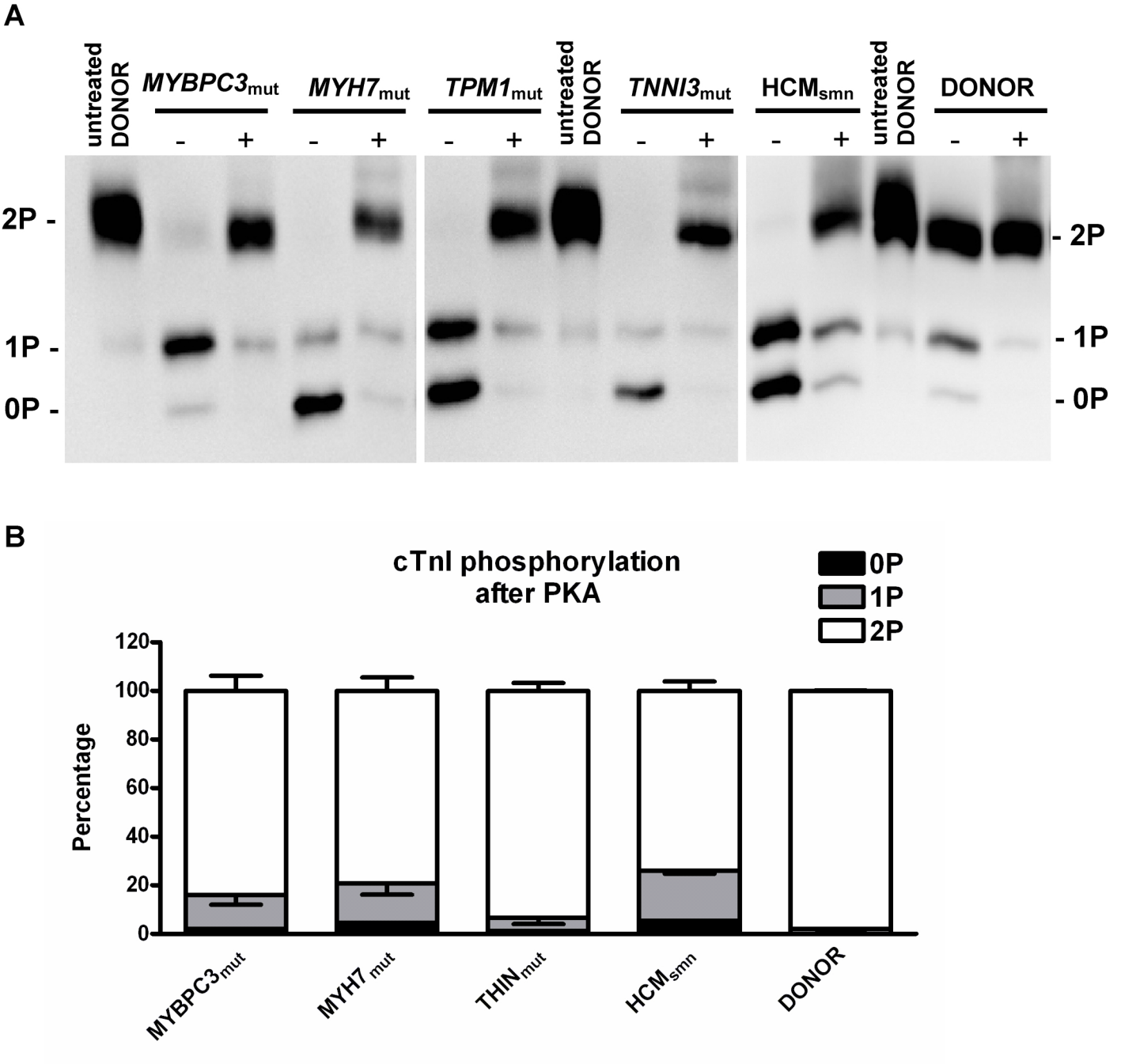
Tension (kN/m2)
Time (s)


A
untreated DONOR
MYBPC3mut
MYH7mut
TPM1mut
untreated DONOR
TNNI3mut
HCMsmn
untreated DONOR
DONOR
- + - + - + - + - + - +
2P -
1P -
0P -
- 2P
- 1P
- 0P
B
cTnI phosphorylation after PKA
0P
1P
2P
Percentage
120
100
80
60
40
20
0
MYBPC3mut
MYH7 mut
THINmut
HCMsmn
DONOR