Supplementary Table 1. Primers used for PCR amplification and sequencing of the myopalladin gene.

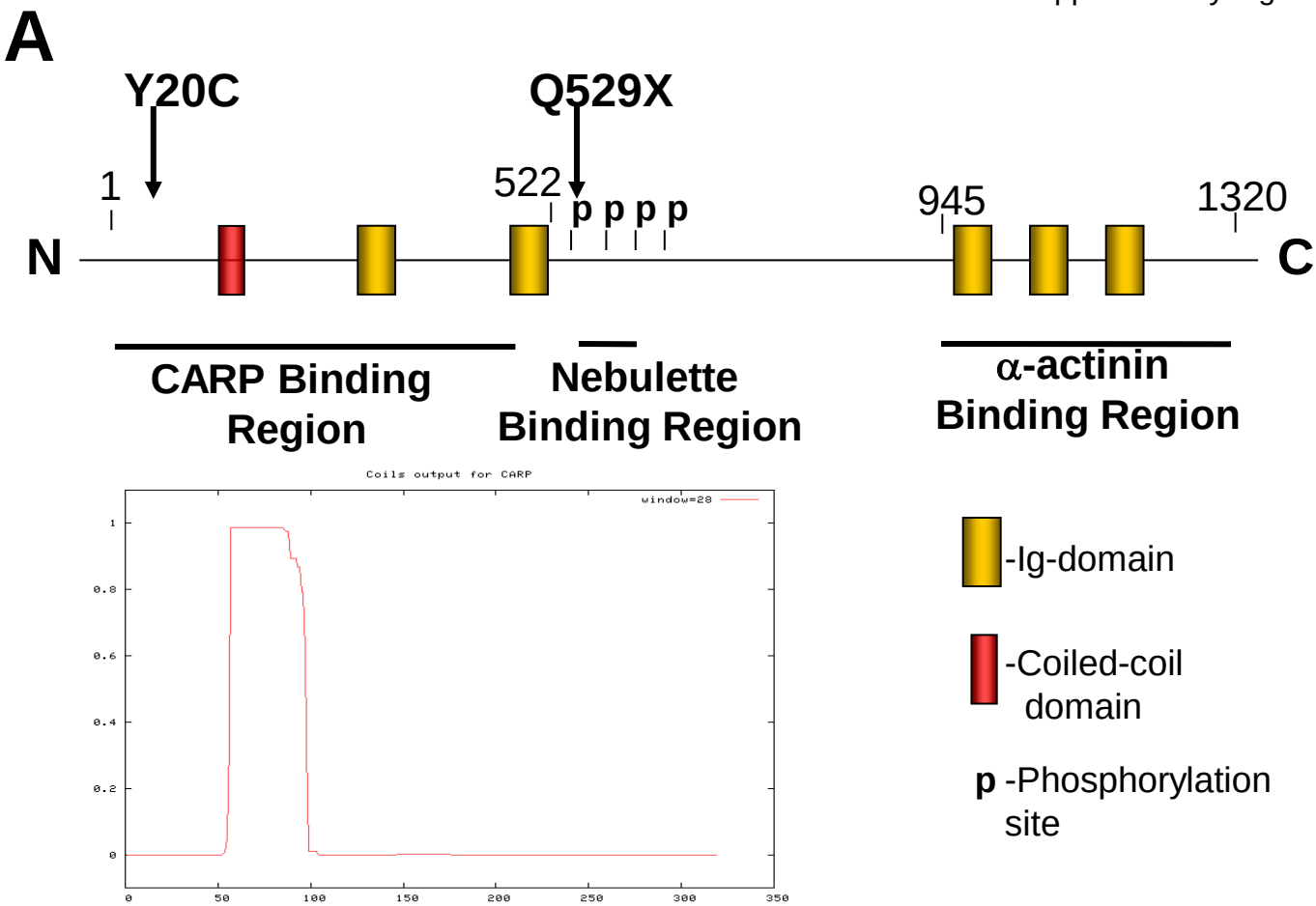
Exon	Forward primer sequence 5'->3'	Reverse primer sequence 5'->3'	Annealing temperature
2	CGAGTCTGCAGTGCTATAAT	AAATGATAATACTTGGGAGG	54°C
3	GCTCATTTAAGAGAATATCT	AGGCCCTCAATAAATATTTA	54°C
4	TACTGAGTTCATTTATAAGA	GTAACCAATTAATCATTCTC	52°C
5	GCAGTGATGCCATTACTAGT	ATGGTCATGGTCACCATGAC	50°C
6	TAAACTTAGAATACATCAGTT	ATCTTGTATCCTATAACTTT	54°C
7	TTTTTCTAAAATTCAACACC	AAAATAAGTGTGTACAATAA	58°C
8	TGAAATTCTATAGGAAGCTT	ATCTCAGTGTAACTTCATTA	60°C
9	TTGTAGAATCATCATCTGAT	GGAATGGAAACACAAAATCT	60°C
10	AACACTTTCCCATTTGTGCA	GTTCCATTACTTTTAAGGAG	58°C
11	GAAAGGTGAGGCCAATAATA	CAAGCTATTCAATGTGCACA	58°C
12	GATTTCTAGGCCTTTTTACC	GAGGACTGAATCAAGCAAAA	68°C
13	CCTCAATTGTACTGATGGAC	AGGCACTGTCTATAGCTTCA	60°C
14	CAGACCCTAGTTCATTAACC	AGGTCTCATTCACAATGACA	60°C
15	GGTGTTCTGGTCCAGAAATT	TACTTTGGTGCTCACGATAG	52°C
16	ATGCCTATTATCATAAGTTT	CCACAAAACAAATACAAATA	58°C
17	GATAAAGAATTCAGCCATCA	CTCTTTGGGTAATGACTTTG	56°C
18	AGCAAGACTCTGTCTCAAAA	ATAGCAGCAGACTTATTTGG	58°C
19	TCCTGGAACCCTAAATTTGA	TGCCTGACCCATTTATCTTT	60°C
20	TGAGGACAGAATGCACCTCT	CTTGGAAACCACCAAGTCTG	60°C

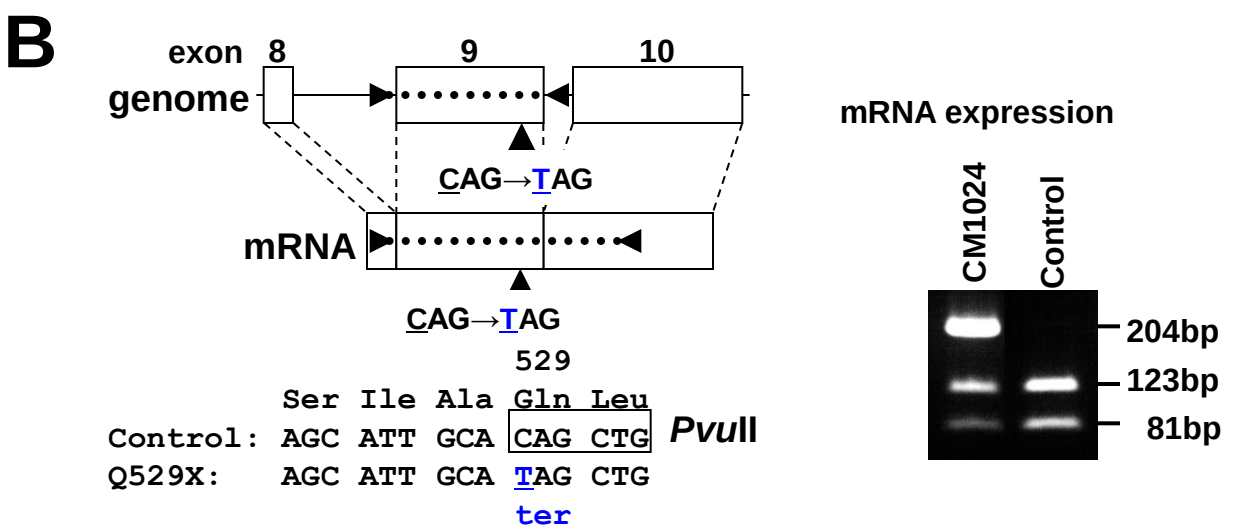
Comments: All PCR reactions were performed at 40 cycles using Platinum®*Taq* DNA Polymerase (Invitrogen) according to the manufacturer's manuals.

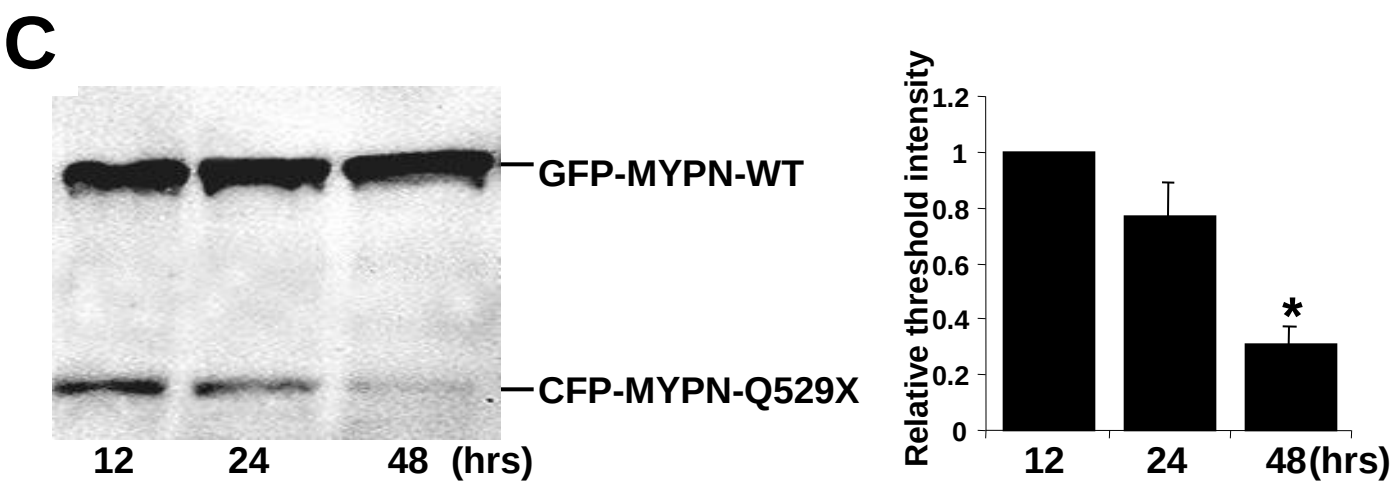
Supplementary Table 2. Distribution of myopalladin genotypes in patients and control cohort.

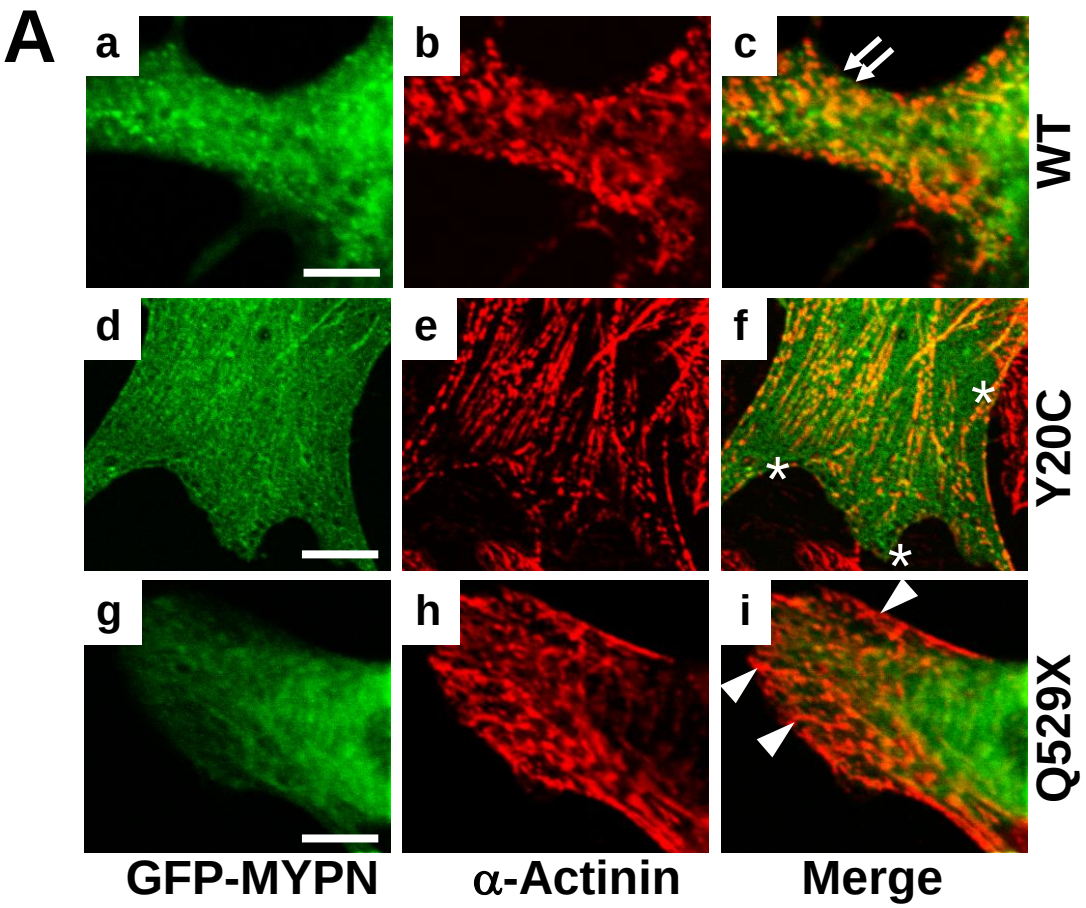
	Location	Nucleotide change	Amino acid change Codon	HCM patients (n=484	DCM patients (n=348)	RCM patients (n=68)	Control (n=1020)	dbSNP
1	Exon2	GCC GCG	p.A155A	0	0	0	1	rs142867001
2		CCA CCG	p.P281P	0	0	0	1	rs74143022
3	Exon 4	AAT AAC	p.N376N	1	0	0	0	synonymous
4		GGC GGU	p.G368G	1	0	0	0	rs144764983
5	Exon 5	GTC GCC	p.V393A	7	3	0	12	rs11596653
6	Exon 6	CAG CAA	p.Q417Q	5	0	0	-	rs10997948
7	Exon 7	GAG AAG	p.E467K	1	0	0	3	rs74143030
8		ACT ACC	p.T473T	1	0	0	3	synonymous
9	Exon 10	TCT TCC	p.549SS	3	0	0	-	rs2673794
10		GAG AAG	p.E614K	3	0	0	3	rs143338091
11		ACC ACA	p.T623T	1	16	0	35	rs1854624
12		CCC CCT	p.P625P	6	12	0	-	rs2673793
13		TTC TTG	p.F628L	6	9	0	7	rs10823148
14	Exon 11	ACC AGC	p.N691S	7	7	0	6	rs10997975
15		AGT AAT	p.S707N	7	0	0	-	rs7916821
16		GCC GCA	p.A721A	3	0	0	-	rs71584491
17		AGG AGC	p.S803R	4	0	0	2	rs3814182
18		GGA AGA	p.G804R	1	0	0	2	rs62620248
21	Exon 13	CGG CAG	p.R955Q	0	2	0	3	rs149887823
22	Exon 16	CCG CCA	p.P1073P	1	0	0	3	synonymous
23	Exon 17	CCA ACA	p.P1135T	0	4	0	6	rs7079481
24		CGC CGT	p.R1139R	2	0	0	3	synonymous
25	Exon 20	GTG GGG	p.V1306G	0	1	0	3	

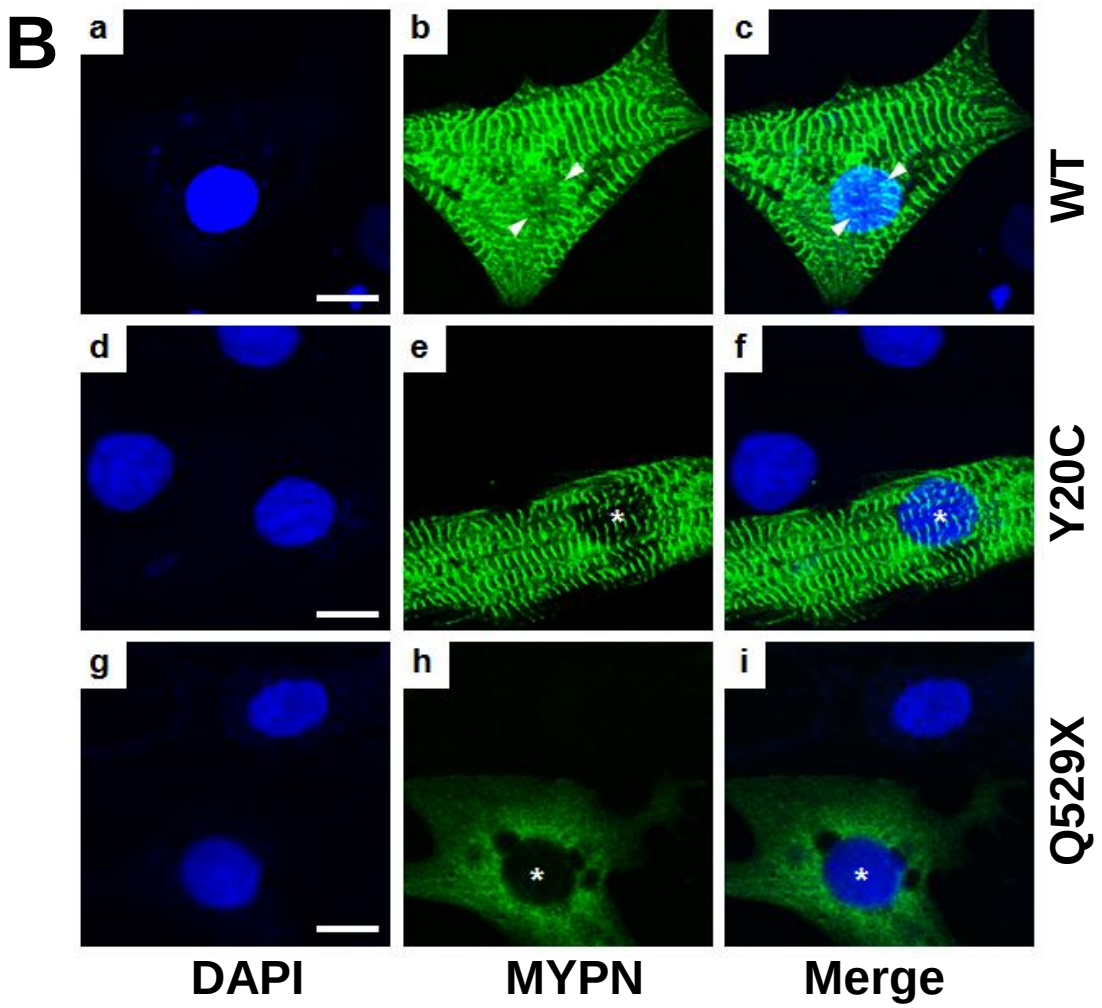
Comments: Genetic variants described in Table 1 are not included in the supplementary table. Data is updated as November 08, 2011.


A
Y20C
Q529X
1
522
p p p p
945
1320
N
C
CARP Binding Region
Nebulette Binding Region
α-actinin Binding Region
Coils output for CARP
window=28
-Ig-domain
-Coiled-coil domain
p -Phosphorylation site


B
exon 8 9 10
genome
CAG→TAG
mRNA
CAG→TAG
529
Ser Ile Ala Gln Leu
Control: AGC ATT GCA CAG CTG
Q529X: AGC ATT GCA TAG CTG
ter
PvuII
mRNA expression
CM1024
Control
204bp
123bp
81bp


C
GFP-MYPN-WT
CFP-MYPN-Q529X
12 24 48 (hrs)
Relative threshold intensity
1.2
1
0.8
0.6
0.4
0.2
0
*
12 24 48(hrs)


A
a
b
c
WT
d
e
f
Y20C
g
h
i
Q529X
GFP-MYPN
α-Actinin
Merge


B
a
b
c
WT
d
e
f
Y20C
g
h
i
Q529X
DAPI
MYPN
Merge

Legends to Supplementary Figures.

Supplementary Figure 1.

A. Structure of MYPN protein and functional regions. Y20C is located at the CARP-binding domain of the N-terminal of MYPN upstream to coiled-coil region, while Q529X truncates last three Ig-domains of MYPN. Lower panel demonstrates predicted coils for CARP affinity.

B. *Pvu*II digestion pattern of RT-PCR products from paraffin-embedded myocardium of the RCM patient CM1024 and control. Forward and reverse primers for PCR analysis were designed in exon 8 and 10, respectively. Mutant allele was expressed at a similar level as the normal allele and no abnormal exon-skipping was observed (left panel).

C. Expression of GFP-MYPN-WT and CFP-MYPN-Q529X. Proteins were extracted from HeLa cells at 12, 24, and 48 h after the transfection with equal amount of each MYPN construct, and detected with anti-Living Colors antibody followed by secondary antibody. Western blot of showed progressive decrease of CFP-MYPN-Q529X compared to GFP-MYPN-WT (left panel). Data from repetitive experiments are shown on right. Relative amount of CFP-MYPN-Q529X to GFP-MYPN-WT at 12 h after the transfection was set to 1.0 and those at 24 h and 48 h after the transfection was calculated by assuming that the amount of GFP-MYPN was not changed during the experimental course. Data are represented as mean ± S.D. *, $P<0.001$.

Supplementary Figure 2.

A. Co-localization of MYPN and α-actinin in immature NRCs. NRCs transfected with GFP-WT-MYPN (a-c), GFP-Y20C-MYPN (d-f), or GFP-Q529X-MYPN (g-i) were immunostained with anti-α-actinin antibody (b, e, and h). Merged images of GFP and anti-α-actinin in red are shown (c, f, and i). Sarcomeric α-actinin is co-localized with GFP-WT-MYPN (c, white arrows) in the Z bodies at the spreading ends of the NRCs, while GFP-Y20C-MYPN demonstrates diffuse localization and lack of co-localization with α-actinin (f, asterisks). Decreased GFP-Q529X-MYPN expression at the periphery of NRCs is also noted (i, white arrowheads). Scale bars indicate 5μm.

B. Nuclear localization of MYPN in neonatal rat cardiomyocytes. Nuclei of NRCs transfected with GFP-WT-MYPN (a-c), GFP-Y20C-MYPN (d-f), or GFP-Q529X-MYPN (g-i) are stained with DAPI (a, d, and g). GFP signals linked to MYPN are not observed in the nuclei with GFP-Y20C-MYPN and GFP-Q529X-MYPN (asterisks), while GFP-WT-MYPN is observed at the Z-discs and nucleus (arrowheads). Bars=10μm.