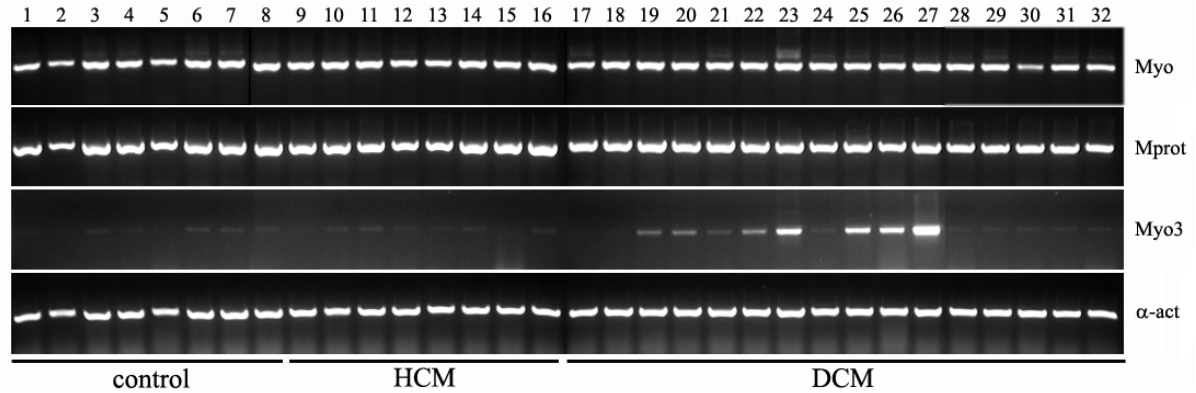
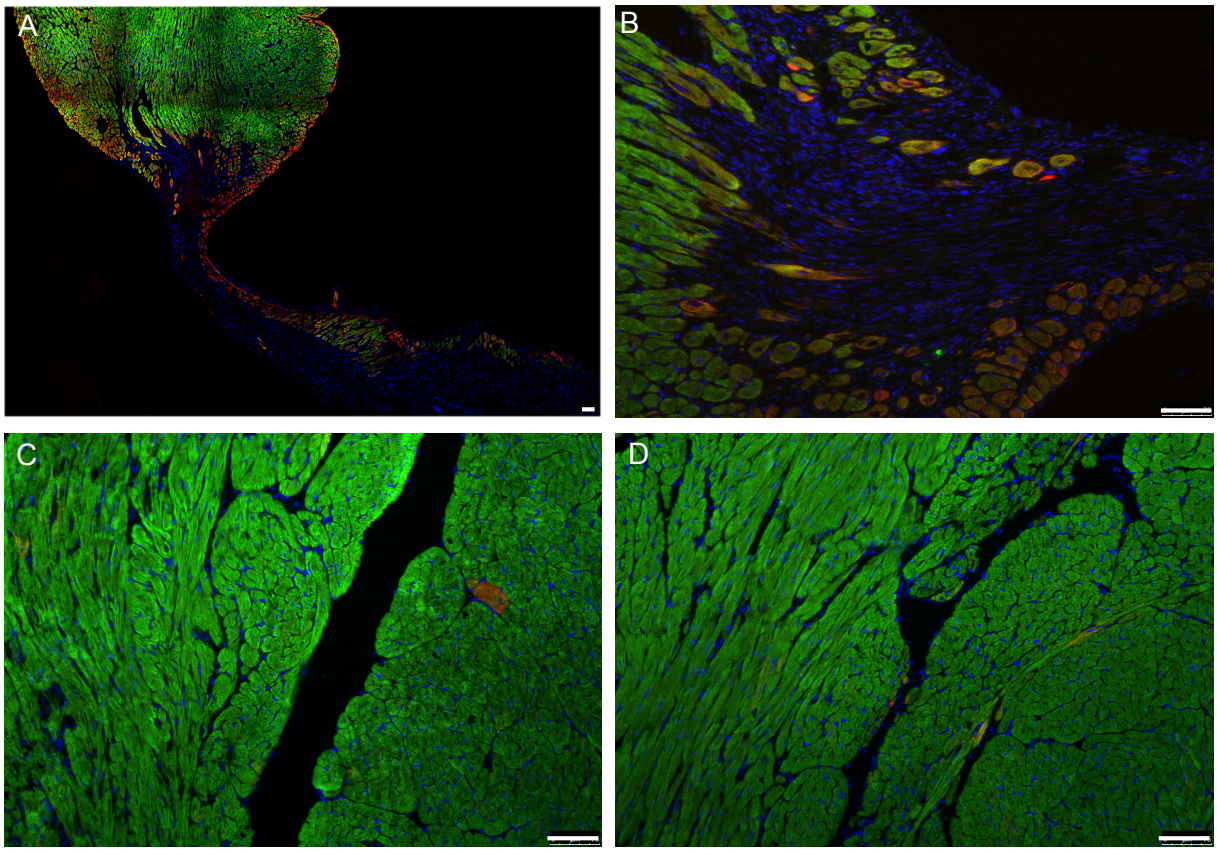
Supplementary Material

Figure S1



Expression of myomesin, M-protein and myomesin 3 genes in mouse cardiomyopathy models.
Total RNA extracted from mouse heart was used for RT-PCR analysis. Different sets of primers were used to detect myomesin (Myo), M-protein (Mprot), myomesin 3 (Myo3) and α-actinin as a control (α-act). *Lanes 1–8:* heart (left ventricle) extracts of control mice at the age of 2 weeks (lane 1), 1 month (lane 2), 1.5 months (lane 3), 2 months (lane 4) and 4 months (lanes 5-8). *Lanes 9-16:* heart (left ventricle) extracts of mouse models with a hypertrophic cardiomyopathy phenotype (age: 4 months; isoproterenol-treated mice (lanes 9-11) [3], angiotensinII overexpressing mice (lanes 12-13) [4], one-kidney-one-clip (1k1c) mouse hypertension model (14-16) [9]. *Lanes 17-32:* heart (left ventricle) extracts of mouse models which develop dilated cardiomyopathy: myosin binding protein C (MyBPC) transgenic mice (age: 1 month, lanes 17-18) [5], retinoic acid receptor (RAR) overexpressing mice (age: 2 months, lanes 19-20; age: 4 months, lane 21) [2],dystrophin knockout (mdx) mice (age: 4 months, lane 22), [6] tropomodulin-overexpressing transgenic (TOT) mice (age: 4 months, lane 23) [8], muscle LIM protein (MLP) knockout mice at the age of 2 weeks (lane 24), 1 month (lane 25), 2 months (lane 26), 4 months (lane 27) [1]and the cΔex3 β-catenin mice at the age of 2 weeks (lane 28), 1 month (lane 29), 2 months (lane 30) and 4 months (lanes 31-32). Myomesin and M-protein transcripts can be detected in all heart samples at similar levels, whereas the myomesin 3 transcript is upregulated in most of the dilated hearts with the highest levels in the MLP KO and TOT mice.

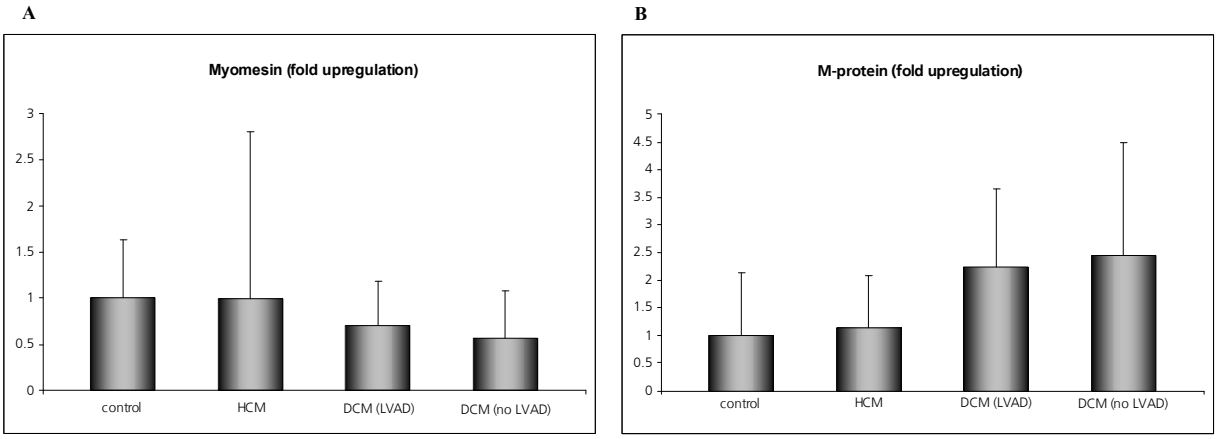
Figure S2



EH-myomesin is upregulated in the scar area of the mouse heart remodeled after myocardial infarction

Cryosections of mouse heart ventricles 4 weeks after induction of myocardial infarction due to left anterior descending artery (LAD) ligation (A, B) were stained with antibodies against M-protein (green), EH-myomesin (red) and with DAPI (blue). As controls, ventricular sections of sham operated mice (C) and of the non-infarcted region (D) of the LAD ligation mouse model have been used. EH-myomesin (red) is accumulated in the scar area of the heart remodeled after myocardial infarction (red in A and B) compared to control hearts (C) and non-infarcted region (D) whereas M-protein (green) shows a lower expression level in the infarcted part. In the infarcted region, many non-cardiomyocytes (e.g. fibroblasts) negative for M-protein or EH-myomesin demonstrate fibrotic tissue. Echocardiography showed a drastic reduction of heart function in the LAD ligation model (21.4 % ejection fraction) compared to the sham operated mice (51.5 % ejection fraction). The left ventricular mass / body weight ratio (mg/g) was clearly increased in the LAD ligation model (5,71) compared to sham controls (3.56). Age of the mice at date of echocardiography: 14 weeks. Scale bar: 75 µm.

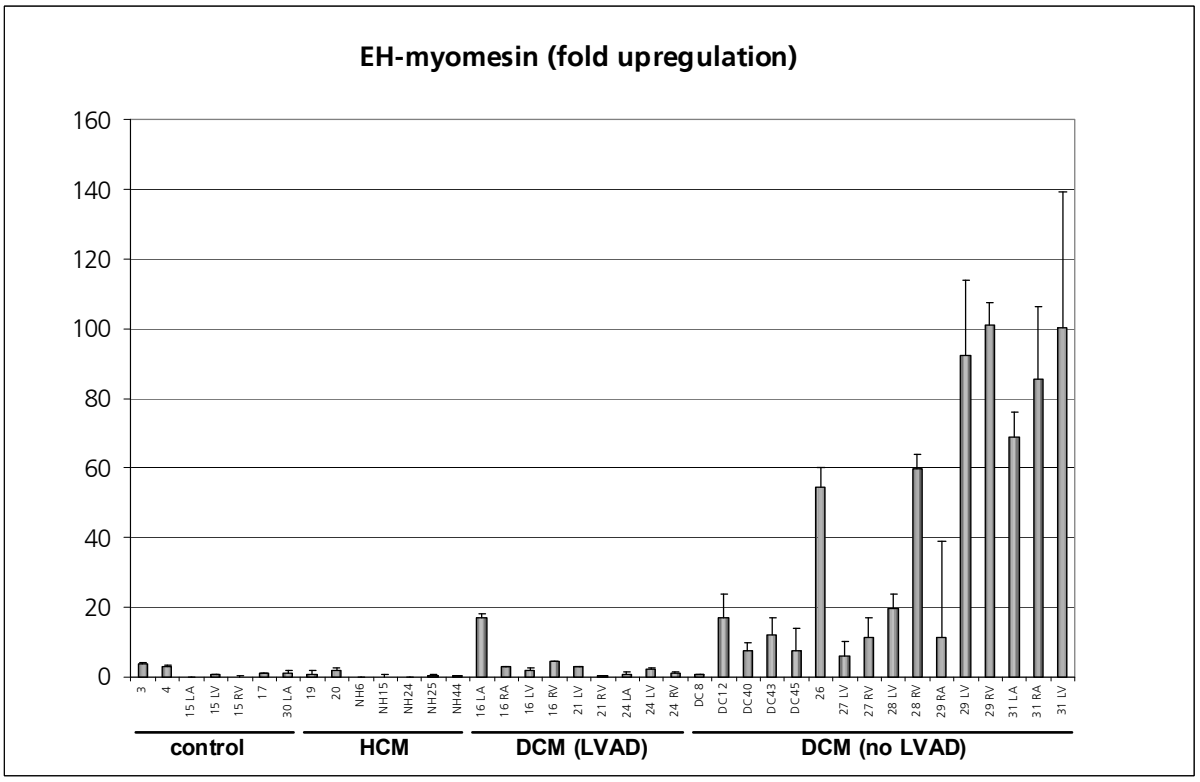
Figure S3



Total myomesin and M-protein expression is not altered in human patients suffering from DCM.

RT-qPCR analysis of human patients suffering from DCM or HCM using primers specific for myomesin (A) and M-protein (B). The total expression of these genes is not significantly changed in the hearts of patients suffering from DCM (N=13) compared to non-dilated control hearts (N=5) and hearts of patients suffering from HCM (N=7). α-actinin was used for normalization. Contr.=control, HCM=hypertrophic cardiomyopathy, DCM=dilated cardiomyopathy, LVAD=left ventricular assist device. Error bars represent the standard deviation.

Figure S4

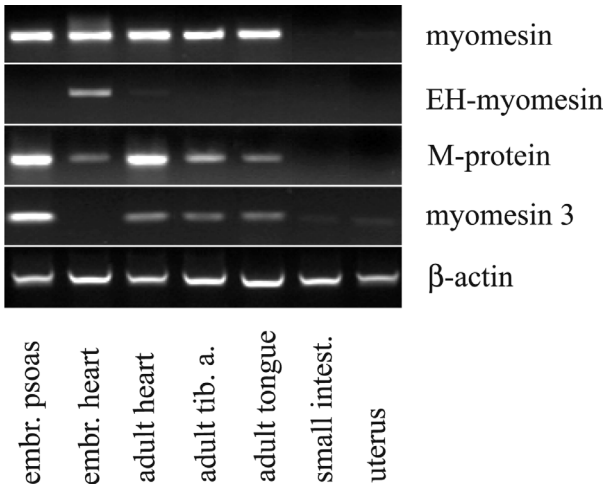


EH-myomesin is up-regulated in human DCM patients

RT-qPCR analysis of human patients suffering from DCM or HCM using primers specific for EH-myomesin. This isoform is clearly upregulated in the hearts of patients (N=10) suffering from DCM (right columns)

compared to non-dilated control hearts (N=5) and hearts of patients suffering from HCM (N=7). DCM patients under treatment with a left ventricular assist device (LVAD, N=3) show no significant re-expression of the EH-myomesin isoform. α-actinin was used for normalization. Contr.=control, HCM=hypertrophic cardiomyopathy, DCM=dilated cardiomyopathy, LVAD=left ventricular assist device, LV=left ventricle, RV=right ventricle, LA=left atrium, RA=right atrium. Error bars represent the standard deviation of triplicate measurements.

Figure S5



Expression of myomesin, M-protein and myomesin-3 genes in human tissues.

Different human muscle tissues were analysed by RT-PCR using primers specific for all myomesin family members. Myomesin can be detected in all types of human striated muscle but not in smooth muscle, in agreement to the RT-PCR analysis of mouse tissues [7]. A primer specific for the EH-segment of human was used to confirm the presence of EH-myomesin in the human embryonic heart. High levels of M-protein mRNA are found in embryonic skeletal muscle (psoas) and adult heart, in addition it can be found in adult m. tibialis anterior, tongue and traces in the embryonic heart. The myomesin-3 transcript can be detected not only in skeletal muscle with the highest level in the embryo (embryonic psoas), but also in the adult heart. In addition, adult human m. tibialis anterior and tongue show expression of myomesin-3 mRNA. In smooth muscle (small intestine and uterus), none of the myomesin genes is expressed. β-actin was used as loading control.

Table S1:

Primers used for RT-PCR

Primer Name	Species	Exon	Sequence	Product Size
Myomesin_fwd	Mouse	15	TGACCGTCGTAGGGGACAAACT	652 bp (EH-mm) 358 bp (mm)
Myomesin_rev	Mouse	18	TCAAGACAAGTGATGTCATAGG	
M-Protein_fwd	Mouse	4	CGGTCTCAAGCGGCTTCTTACG	466 bp
M-Protein_rev	Mouse	7	CCACCGCAGCATTGGTAGACAC	
Myomesin-3_fwd	Mouse	10	GCAGATACTCTACGCAGACCGC	446 bp
Myomesin-3_rev	Mouse	12	CCGTGACAACTGCTTCTGAGGC	
Actinin_fwd	Mouse	10	GGTGCAGGAGAAGTGCCAGCTG	400 bp
Actinin_rev	Mouse	12	CTCCTGCGCAATGGCAGCGATC	
Myomesin_ex34_fwd	Human	34	GAAGACAGTGGATCTCTCTGGA	247 bp
Myomesin_ex38_rev	Human	38	GAACTTGAGGTTGCAGTGGTC	
EH-Myomesin_fwd	Human	EH	GAGCGATGAGCCTGGTGGACTA	315 bp
Myomesin_ex18_rev	Human	18	AGAACCATTGAGTCACGAAAAC	
M-Protein_fwd	Human	3	CACAGAGAGCCTCCAGCCAGAC	287 bp
M-Protein_rev	Human	5	CCGCTCTTCAAATGTGTGTCTC	
Myomesin-3_fwd	Human	5	GCCGTCTGGGAGCACACCACGGT	149 bp
Myomesin-3_rev	Human	6	AGCAGCCCGTAGTTGTTGGTGAT	
Actinin_fwd	Human	13	GGAACGCTTACTCAGAAGAGGA	286 bp
Actinin_rev	Human	15	AGCTCTGAATCACCTTCTCCAC	

Abbreviations: fwd: forward; rev: reverse; EH embryonic heart; mm Myomesin; bp base pairs.

Table S2:

DCM mouse model	% EH-myomesin
β-catΔex3	436 ± 212
MLP KO	218 ± 54
RAR	355 ± 117
TOT	550

All 4 DCM mouse models tested by Western blot analysis show a clear accumulation of the EH-myomesin isoform in the heart ventricle compared to age-matched (2 months) control mice. Tested models: β-cateniņ Δexon3 (β-catΔex3), muscle LIM protein knockout mice (MLP KO), retinoic acid receptor overexpressing mice (RAR), tropomodulin-overexpressing transgenic mice (TOT).

References

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