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LAP2α loss impairs heart function and stress response in mice

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Abstract

Rationale—Lamina-Associated Polypeptide 2α (LAP2α) is a mammalian chromatin-binding protein that interacts with a fraction of A-type lamins in the nuclear interior. As mutations in lamins and LAP2α lead to cardiac disorders in humans, we hypothesized that these factors may play important roles in heart development and adult tissue homeostasis.

Objective—We asked whether the presence of LAP2α was required for normal cardiac function.

Methods and results—To study the molecular mechanisms of the disease, we analyzed heart structure and function in complete and conditional *Lap2α*$^{-/-}$ mice as well as *Lap2α*$^{-/-}$*/Mdx* mutants. Unlike conditional deletion of LAP2α in late embryonic striated muscle, its complete knockout caused systolic dysfunction in young mice, accompanied by sporadic fibrosis in old animals, as well as deregulation of major cardiac transcription factors GATA4 and MEF2c. Activation of compensatory pathways, including downregulation of β-adrenergic receptor signaling, resulted in reduced responsiveness of the myocardium to chronic β-adrenergic stimulation and stalled the progression of LAP2α-deficient hearts from hypertrophy towards cardiac failure. Dystrophin deficiency in an *Mdx* background resulted in a transient rescue of the *Lap2α*$^{-/-}$ phenotype.

Conclusion—Our data suggest a novel role of LAP2α in the maintenance of cardiac function under normal and stress conditions.

Keywords

Lamins; LAP2α; dilated cardiomyopathy; β-adrenergic receptors

Introduction

Dilated cardiomyopathy (DCM) is a primary myocardial disease characterized by dilation and impaired contraction of one or both heart ventricles. One of the genes most frequently involved in the development of DCM is *LMNA*, which encodes the nuclear intermediate filament proteins lamin A and lamin C[1]. Mutations in lamin A/C cause the most severe forms of DCM, posing

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Conflicts of interest
None.

a high risk of heart failure in symptomatic patients[1]. Besides heart muscle disease, mutations in *LMNA* cause a variety of pathological conditions in skeletal muscle, skin, nerve, bone and adipose tissue, known as laminopathies[2], emphasizing the importance of the search for molecular disease mechanisms.

Recently, research on laminopathies has focused on lamin A/C-interacting proteins, whose mutations have been linked to a similar spectrum of human disorders[3]. One of the best studied lamin A/C-binding partners is Lamina Associated Polypeptide 2α (LAP2α), an unusual splice variant of the mammalian *LAP2* gene[4]. All LAP2 proteins (α, β, γ, δ, ε, ζ) share a common chromatin-binding structural motif called the LEM (LAP2-Emerin-MAN1) domain at their N-terminus. The C-terminus of most LAP2 variants comprises a transmembrane region, which targets them to the inner nuclear membrane, where they serve mainly structural roles[5]. LAP2α lacks the common LAP2 transmembrane domain and possesses an additional chromatin-binding region at its C-terminal end, which mediates targeting to the nuclear interior[4]. In the nucleoplasm LAP2α specifically interacts with a fraction of lamin A/C via its unique C-terminal tail[6]. Together, LAP2α and lamin A/C influence various nuclear processes, such as epigenetic chromatin regulation, gene expression and signal transduction[7]. In particular, LAP2α-lamin A/C complexes have been found to control the balance between proliferation and differentiation of early progenitor cells in regenerative tissues by affecting the E2F/retinoblastoma pathway[8].

Interestingly, a mutation in LAP2α (c. 2068C>T, p. R690C), which lowers its binding affinity for lamin A/C *in vitro*, has also been linked to DCM[9]. As changes in both LAP2α and lamin A/C cause pathological heart conditions, we hypothesized that an intact LAP2α-lamin A/C complex may be necessary for proper cardiac function and that mislocalization or absence of one of the components would lead to disease.

Most *Lmna* transgenic mice generated so far show complex phenotypes, including various stages of heart failure[10, 11]. To see whether the presence of LAP2α is also important for normal cardiac output, we analyzed heart structure and function in previously generated *Lap2α*$^{-/-}$ mice[8]. Here we describe a new mouse model of cardiomyopathy and provide novel insights into mechanisms governing heart development and tissue homeostasis.

Materials and Methods

Mice

Lap2α$^{-/-}$ mice were kept on a mixed *Mus musculus* C57BL/6×129 genetic background[8]. Compound mutant *Lap2α*$^{-/-}$*/Mdx* animals were obtained by crossing *Lap2α*$^{-/-}$ mice with the *Mdx* line[12]. Generation of conditional LAP2α knockout mice is described in Online Figure I. All histological and physiological analyses were done by observers blinded for the genotype, as well as treatment of the animal. Mice aged 2 days were considered as newborn, 10 weeks as young and 10–12 months as old.

Mice were kept and handled in accordance with the procedures outlined in the Guide for the Care and Use of Laboratory Animals. Experiments were performed according to permissions from Austrian authorities.

Echocardiography

Mice were anesthetized with 5% isoflurane/O_2 and maintained on 2% mixture during the experiment. Trans-thoracic echocardiography was performed using Vingmed Vivid Five (GE Technology) equipped with a 10-MHz linear transducer[13].

Gravimetric, histological and biochemical analyses

were performed according to standard procedures described in more detail in the Online Supplement.

Isoproterenol (ISO)-induced heart failure model

Mice anesthesia and echocardiography were performed as described above. Alzet® mini-osmotic pumps (1007D), filled with 15 mg/kg/day isoproterenol/PBS (Sigma Aldrich), were implanted according to manufacturer's instructions. 6 old male *Lap2α*$^{+/+}$ and 7 *Lap2α*$^{-/-}$ mice were infused with ISO for 7 days and 2 littermate couples received only PBS (sham).

Statistical analysis

Experimental outliers were identified using Grubb's test and excluded from further analyses. One mouse was removed from the initial echocardiography data (FS% = 20.76, $FS\%_{mean}$ = 35.54, SD = 5.39, n = 12) and one littermate pair from the MEF2c mRNA expression analysis (KO/WT = 4.82, KO/WT_{mean} = 1.80, SD = 1.74, n = 5). Data were analyzed either by paired Student's t-test, one-way ANOVA or two-way ANOVA (followed by Boniferroni post hoc test for multiple comparisons) where appropriate, using Microsoft Excel XP. $P<0.05$ was considered significant. Presented values are means ± standard error (SE).

Results

LAP2α-deficient mice used in this study were generated by Cre recombinase-mediated excision of the LAP2α-specific exon 4 of the *Lap2* gene in the germline[8]. To confirm the absence of the gene product, we performed western blot, semi-quantitative PCR (semiqPCR) and immunofluorescence analyses of *Lap2α*$^{-/-}$ heart muscle tissue. LAP2α was detectable neither at an mRNA nor a protein level, while the expression, as well as localization of lamin A/C and other LAP2 isoforms were not significantly altered (Figure 1, Online Figure II).

Absence of LAP2α causes ventricular systolic dysfunction in mice

The symptoms in LAP2α- and most lamin A/C-linked human cardiomyopathies appear predominantly in adults[5, 9]. In mouse lamin A/C-linked laminopathy models, however, heart defects develop in young animals and cause early mortality[10, 11]. As *Lap2α*$^{-/-}$ mice are grossly indistinguishable from their WT littermates and have a normal life expectancy[8], we analyzed heart function in young, as well as old animals. Echocardiography in 10 weeks old *Lap2α*$^{-/-}$ mice revealed ventricular systolic dysfunction, characterized by significantly decreased left ventricular fractional shortening (FS%) and ejection fraction (EF%) values. Moreover, enlarged left atria (LA) in male LAP2α-deficient mice emphasized the defective cardiac phenotype, indicating a possible left ventricular and left atrial volume overload (Figure 2, Online Table I). The FS% and EF% values remained similarly depressed in old *Lap2α*$^{-/-}$ male mice (aged 10–12 months), suggesting that the functional defect was not progressive. Interestingly, cardiac parameters in *Lap2α*$^{-/-}$ females appeared largely comparable to WT at both ages (Online Table I).

Old *Lap2α*$^{-/-}$ mice present only sporadic cases of cardiac fibrosis

To see whether the functional defect in *Lap2α*$^{-/-}$ hearts was accompanied by structural changes, we performed gravimetric and histological analyses. *Lap2α*$^{-/-}$ hearts had normal morphology and similar heart weight/body weight (HW/BW) indexes compared to WT littermates in newborn, young and old animals (data not shown). Furthermore, no overt histological pathologies were detectable in young *Lap2α*$^{-/-}$ mice (Figure 3A). Old *Lap2α*$^{-/-}$ hearts, however, showed high phenotypic variability in the degree of fibrosis, as assessed by the extent of interstitial collagen deposition in the left ventricle. Although the overall difference

in the extent of cardiac fibrosis between *Lap2α*$^{-/-}$ and WT mice was found to be statistically insignificant (Online Figure III), out of 11 *Lap2α*$^{-/-}$ tested mice, 18% developed extensive subendocardial fibrosis of the left ventricle (Figure 3B). In addition to fibrosis, one mouse presented regions of extremely thin, transparent myocardium which collapsed in the absence of internal blood pressure (Figure 3C). In contrast, WT animals did not exhibit signs of increased fibrosis at any age.

De-regulated expression of major cardiac transcription factors in male LAP2α-deficient mice

In an attempt to identify the molecular mechanisms leading to systolic dysfunction in *Lap2α*$^{-/-}$ mice, we analyzed the expression of several markers connected to myocardial remodeling. Two major cardiac transcription factors, GATA4 and MEF2c, as well as their downstream targets – brain natriuretic peptide (BNP) and proteins involved in Serum Response Factor (SRF)-mediated transcription of immediate early and muscle-specific genes, Myocardin A and Striated Muscle Activator of Rho Signaling (STARS)[14–18] – showed deregulated expression patterns in male *Lap2α*$^{-/-}$ mice (Figure 4A, B). Whereas MEF2c expression was repressed in newborn *Lap2α*$^{-/-}$ hearts and reached WT levels by the age of 10 weeks, GATA4, Myocardin A and STARS were down-regulated only in old hearts. In addition, consistent with the reported cooperative regulation of BNP expression by both GATA4 and MEF2c[18], BNP mRNA levels were significantly lower in LAP2α-deficient hearts compared to WT at all ages (Figure 4A). The expression of other MEF2c and GATA4 targets, such as α- and β-myosin heavy chain and atrial natriuretic factor (ANF)[19] was not significantly changed in *Lap2α*$^{-/-}$ mice (data not shown). The expression of the aforementioned factors in female *Lap2α*$^{-/-}$ hearts was comparable to WT (data not shown). Our data show that loss of LAP2α affects the expression of GATA4 and MEF2c, as well as some of their downstream targets, which in turn may influence the expression of a plethora of genes involved in cardiovascular development and stress-induced hypertrophic growth.

Since old *Lap2α*$^{-/-}$ mice showed occasional fibrosis, we analyzed the expression of known fibrotic markers in WT and LAP2α-deficient hearts (Figure 4C). Interestingly, loss of LAP2α caused a downregulation of connective tissue growth factor (CTGF), an inducer of fibroblast proliferation and extracellular matrix synthesis[20], but affected only mildly the expression of its activator, the transforming growth factor β_2 (TGFβ_2). Altogether, our data demonstrate that loss of LAP2α in mice leads to deregulated expression of genes involved in cardiac remodeling and fibrosis.

Lap2α$^{-/-}$ mice show a blunted response to chronic isoproterenol infusion

GATA4 and MEF2c play important roles during cardiomyocyte hypertrophy[21, 22]. To see whether their deregulation in *Lap2α*$^{-/-}$ hearts affects their response to cardiac hypertrophic stimuli, we subjected *Lap2α*$^{-/-}$ mice to chronic infusion of the β-adrenergic agonist isoproterenol (ISO) for 7 days. To detect ISO-induced changes in heart function and structure, we performed echocardiography before and after the treatment, as well as gravimetric and histological analyses at the end of the experiment. As shown by the HW/BW indexes, ISO administration caused a similar degree of cardiac hypertrophy in *Lap2α*$^{+/+}$ and *Lap2α*$^{-/-}$ mice (Figure 5A), indicating that the hypertrophic growth response is not grossly affected by the loss of LAP2α. Similarly, the extent of subendocardial fibrosis caused by chronic ISO infusion[23] was comparable in *Lap2α*$^{+/+}$ and *Lap2α*$^{-/-}$ mice (Online Figure IV).

In addition to hypertrophy, ISO treatment caused dilation of left heart ventricles, as shown by echocardiography (Figure 5B, Online Table II). In WT animals, systolic and diastolic left ventricular diameters (LVD) and the corresponding ventricular volumes exhibited a significant increase, indicating the progression from cardiac hypertrophy towards heart failure. In contrast, left ventricles of *Lap2α*$^{-/-}$ mice were already slightly enlarged at the baseline and showed only

milder additional dilation upon ISO treatment, reaching end point sizes similar to WT. HW/BW indexes and echocardiography parameters of sham (PBS) treated animals were not altered by the procedure (Online Table II). These data point to a blunted cardiac stress response in LAP2α-deficient mice.

FS values in treated animals were only slightly affected, showing that chronic ISO infusion did not significantly impair left ventricular systolic function either in *Lap2α*$^{+/+}$ or *Lap2α*$^{-/-}$ mice (Figure 5B, Online Table II). This is in agreement with previous studies[23].

The preserved basal systolic function in ISO-induced cardiac hypertrophy has been associated with downregulation of β-adrenergic receptor (β-AR)-mediated inotropic responses[23]. An ISO-induced downregulation of β-AR has also been shown to cause a blunted reactivity to its subsequent re-administration[23]. Therefore, we hypothesized that the reduced responsiveness of *Lap2α*$^{-/-}$ myocardium to ISO might also be a consequence of similar de-sensitization of the β-AR signaling pathway. To test this, we analyzed the expression of β_2-AR in LAP2α-deficient and WT mice at baseline, as well as after ISO treatment. As expected, *Lap2α*$^{-/-}$ hearts showed lower baseline expression levels of β_2-AR mRNA and protein in young and old mice (Figure 5C, Online Figure V). Importantly, the difference in relative β_2-AR mRNA levels in untreated LAP2α knockout versus WT hearts increased with age (Figure 5C), suggesting that the downregulation of β-AR signaling in *Lap2α*$^{-/-}$ mice is an aging-dependent phenomenon and may be a consequence of heart function impairment. ISO treatment caused an additional downregulation of β_2-AR mRNA in *Lap2α*$^{-/-}$ hearts, albeit not as extensive as in the WT situation (~71% in WT vs. ~39% in *Lap2α*$^{-/-}$), resulting in comparable end-point expression levels in both genotypes (Figure 5C).

Development of β-AR-mediated hypertrophy is associated with the activation of the fetal cardiac transcriptional program[23], including the expression of embryonic transcription factors ANF and GATA4[24]. In accordance with this, ANF and GATA4 mRNA levels were upregulated in ISO-treated hearts of both *Lap2α*$^{+/+}$ and *Lap2α*$^{-/-}$ mice (Figure 5D). However, consistent with the reduced β-AR levels in old *Lap2α*$^{-/-}$ mice, the increase in ISO-induced ANF expression was significantly lower in LAP2α-deficient myocardium compared to WT. Relative GATA4 levels were also substantially lower in *Lap2α*$^{-/-}$ than in WT hearts after ISO treatment, although the ISO-mediated increase of GATA4 expression was similar in both genotypes. Overall, these results suggest that loss of LAP2α affects cardiac-specific transcription and progression of ISO-induced hypertrophy.

Absence of dystrophin delays the onset of ventricular dysfunction in *Lap2α*$^{-/-}$/*Mdx* mice

Since baseline systolic dysfunction in *Lap2α*$^{-/-}$ mice was not progressive and did not affect their life span, we thought that the effect of LAP2α loss might be augmented in a cardiomyopathy background. Therefore, we crossed *Lap2α*$^{-/-}$ mice with *Mdx* mutants[12], which develop skeletal muscle dystrophy and DCM at an advanced age due to the absence of dystrophin[25, 26].

The homozygous *Lap2α*$^{-/-}$/*Mdx* mutant animals were born according to Mendelian ratios and appeared grossly indistinguishable from their *Mdx* littermates. Unexpectedly, echocardiography in young *Lap2α*$^{-/-}$/*Mdx* mice showed normal heart function, with FS values similar to WT and *Mdx* (Figure 6, Online Table III). This suggests that the absence of dystrophin can pre-sensitize the heart and turn on compensatory pathways leading to a transient rescue of the *Lap2α*$^{-/-}$ phenotype. Nevertheless, old compound mutant animals, as well as *Mdx* mice, developed similar heart function defects as *Lap2α*$^{-/-}$ mice (Figure 6, Online Table I, III).

LAP2α is dispensable in late-embryonic and adult cardiomyocytes

LAP2α is highly expressed in proliferating tissues[27, 28]. Since the vast majority of adult cardiomyocytes persists in a post-mitotic stage[29], and only low levels of LAP2α are present in nuclei throughout adult myocardium[9] (Figure 1), we thought that LAP2α may exhibit its major activity during early cardiomyocyte proliferation and myocardial growth, when its expression is clearly detectable (Online Figure VI). To address this question, we generated conditional knockout mice by crossing *Lap2α*$^{fl\Delta Neo/fl\Delta Neo}$ animals with mice expressing Cre recombinase under the control of striated muscle-specific muscle creatine kinase (*Mck*) promoter[30] (Online Figure I). In this system, the expression of Cre recombinase and the consequent deletion of LAP2α are turned on in heart and skeletal muscle during later stages of mouse embryonic development and striated muscle differentiation. *Lap2α*$^{fl\Delta Neo/fl\Delta Neo}$*/Mck-Cre*$^{+}$ mice were born at Mendelian ratios and did not demonstrate any overt phenotype. LAP2α mRNA and protein levels were significantly reduced in *Lap2α*$^{fl\Delta Neo/fl\Delta Neo}$*/Mck-Cre*$^{+}$ hearts, indicating an efficient recombination in the myocardium (Figure 7A). In contrast, other tissues like spleen, which do not express MCK[31], had normal levels of LAP2α (our unpublished data). Echocardiography in *Lap2α*$^{fl\Delta Neo/fl\Delta Neo}$*/Mck-Cre*$^{+}$ mice showed normal heart function (Figure 7B, Online Table IV) and histological, as well as morphometric analyses, did not reveal any pathological heart phenotype (Figure 7C and data not shown). In accordance, the expression levels of MEF2c and GATA4 mRNA were similar in *Lap2α*$^{fl\Delta Neo/fl\Delta Neo}$*/Mck-Cre*$^{+}$ and *Lap2α*$^{+/+}$/*Mck-Cre*$^{+}$ hearts (data not shown). Thus, LAP2α expression in late-embryonic and adult cardiomyocytes is dispensable for normal heart function, but may be important during earlier stages of heart development (before E13.5 when the *Mck* promoter becomes active) or in non-striated muscle cells of the heart.

Discussion

In this study we describe a new mouse model of cardiomyopathy caused by the absence of LAP2α, a major binding partner of lamin A/C in the nucleoplasm[6]. Together with previous studies linking lamin A/C and lamin-binding inner nuclear membrane proteins, emerin and nesprin, to congenital heart disorders[1, 3, 9, 32], our data suggest a major role of Atype lamin complexes in normal heart function.

A mutation in the α-specific exon 4 of the human *LAP2* gene has previously been linked to familial DCM[9]. Here we show that the absence of LAP2α in mice also leads to a heart disease. Interestingly, only male *Lap2α*$^{-/-}$ mice showed a heart defect, whereas female animals exhibited normal cardiac function implicating gender-specific factors in the development of the disease. Similar gender-related phenotype variations were described in other cardiomyopathy mouse models[33], including mice carrying the H222P-*Lmna* mutation[34]. Although the exact background of this phenomenon is still unknown, gender based differences in cardiac dysfunction were linked to the activity of steroid hormones[35]. At present no data about the risk of heart failure in male versus female carriers of LAP2α mutations in humans are available.

In an attempt to disclose the molecular pathways of the disease, we analyzed the expression of factors involved in the development and remodeling of the myocardium. *Lap2α*$^{-/-}$ mice showed deregulated expression of major heart transcription factors, MEF2c[14] and GATA4[17], as well as some of their downstream targets, at different life stages. Both GATA4 and MEF2c play essential roles in embryonic heart development and hypertrophic growth[14, 17, 21, 22]. Since MEF2c is required for normal heart development (see review[14]), its downregulation in the absence of LAP2α might compromise the early stages of cardiac development in *Lap2α*$^{-/-}$ mice.

The appearance of systolic dysfunction in young *Lap2α*$^{-/-}$ mice and the delayed decrease in GATA4 levels only in old mice suggests that this deregulation may be a consequence rather than the cause of the disease. Despite lower GATA4 expression levels, *Lap2α*$^{-/-}$ hearts were able to undergo hypertrophic growth. In support of our data, mice with reduced GATA4 levels (G4D mice) show a similar heart function defect at baseline, as well as the ability to grow under hypertrophic conditions[21]. Accordingly, G4D mice develop fibrosis only after pressure overload as a consequence of increased stress-induced cardiomyocyte death[21].

GATA4 and MEF2c synergistically activate the expression of BNP[18], a cardiac hormone involved in the regulation of blood pressure and fluid-electrolyte balance, which also plays a role in the inhibition of cardiac fibroblast proliferation and extracellular matrix production[36]. Decreased levels of BNP in the absence of LAP2α could potentially explain the observed occurrence of cardiac fibrosis in *Lap2α*$^{-/-}$ mice. Interestingly, BNP-deficient mice (*Nppb*$^{-/-}$), which exhibit normal heart morphology and hypertrophic growth after ventricular pressure overload, show a higher incidence of fibrosis (~50%) in male vs. female mice at the age of 15 weeks[37].

The blunted response of LAP2α-deficient hearts to chronic isoproterenol infusion points to the existence of compensatory pathways activated in response to changes in cardiac function in *Lap2α*$^{-/-}$ mice. The attenuated hypertrophic growth and lack of fibrosis observed in β-AR knockout mice after pressure overload[38] indicate that the downregulation of β-AR signaling found in *Lap2α*$^{-/-}$ hearts might be a part of this process.

The variability in the extent of fibrosis at baseline, as well as after chronic ISO infusion, might be explained by the observed deregulated expression of pro- (CTGF) and anti-fibrotic (BNP) factors in *Lap2α*$^{-/-}$ mice. The observed downregulation of CTGF in old LAP2α-deficient hearts might be a part of an extensive compensatory mechanism activated in response to loss of LAP2α to protect the myocardium from further tissue deterioration and loss of function.

Since LAP2α is highly expressed in proliferating tissues and only weakly in post-mitotic tissues[9, 27, 28], such as heart muscle[29], we hypothesized that LAP2α might be required during cardiac development and/or postnatal myocardial remodeling, mediated by putative cardiac stem cells[39]. Therefore, we generated conditional knockout mice which lose LAP2α during later stages of embryonic development and adult striated muscle differentiation[30]. *Lap2α*$^{fl\Delta Neo/fl\Delta Neo}$*/Mck-Cre*$^{+}$ mice showed normal heart function and normal levels of GATA4 and MEF2c, supporting the model according to which the defect in complete knockout mice might arise during early embryonic development and at early stages of muscle differentiation, before the *Mck* promoter becomes active, and/or it might be a consequence of LAP2α loss from heart stem- and non-striated muscle cells.

We have previously shown that LAP2α retains a sub-fraction of the nuclear lamin A/C pool inside the nucleoplasm in proliferating skin fibroblasts and intestinal cells. In contrast, nucleoplasmic lamin A/C is lost in non-dividing differentiated cells[8], implicating the nucleoplasmic pool of lamin A/C in regulation of the transition from proliferating to the differentiated state. The localization of lamin A/C has also been shown to change in cardiomyocytes during aging and development, going from being mainly nucleoplasmic to the nuclear periphery[40]. The elongated shape and different orientation of cardiomyocyte nuclei within heart tissue, however, precluded the detection of potential changes in lamin A/C localization in LAP2α-deficient tissue. In view of the lack of heart defects in muscle-specific conditional LAP2α knockout mice, there is a possibility that a potential mislocalization of lamin A/C in LAP2α-deficient cardiomyocyte precursor or non-striated muscle cells may be the primary cause of the *Lap2α*$^{-/-}$ cardiac defect. Alternatively lack of LAP2α may change the function, rather than the localization of lamin A/C, such as binding to epigenetic modifiers and

components of different signaling cascades[7], which in turn may lead to cardiomyopathy. This model is consistent with previous reports that have linked lamin A/C-related cardiomyopathies to changes in signaling pathways, such as TGFβ, PI3-kinase and MAPK[41], which influence MEF2c and GATA4 expression during development and hypertrophic growth[42, 43] and are also affected by β-AR signaling[23].

In summary, we show that the absence of LAP2α causes a baseline ventricular systolic dysfunction in male mice and activates compensatory pathways that prevent further decline of heart function under chronic stress conditions. The origins of these defects, which could lie in impaired proliferation and/or differentiation of early embryonic cardiomyocytes, resident cardiac stem cells or non-muscle cardiac tissue, remain to be discovered.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Non-standard Abbreviations and Acronyms

ANF	Atrial Natriuretic Factor
β-AR	β-Adrenergic Receptor
BNP	Brain Natriuretic Peptide
CTGF	Connective Tissue Growth Factor
DCM	Dilated Cardiomyopathy
EF%	Ejection Fraction
EDVI	End-Diastolic Left Ventricular Volume Index
ESVI	End-Systolic Left Ventricular Volume Index
FS%	Fractional Shortening
GAPDH	Glyceraldehyde 3-Phosphate Dehydrogenase
HPRT	Hypoxanthine-Guanine Phosphoribosyltransferase
HW/BW	Heart Weight/Body Weight
ISO	Isoproterenol
IVS	Interventricular Septum
LA	Left Atrium
LAP2α	Lamina-Associated Polypeptide 2α
LEM	LAP2-Emerin-MAN
LVD	Left Ventricular Diameter

LVPW	Left Ventricular Posterior Wall Thickness
MAPK	Mitogen Activated Protein Kinase
MEF2c	Myocyte Enhancer Factor 2c
PI3-kinase	Phosphoinositide 3-kinase
SE	Standard Error, SRF, Serum Response Factor
STARS	Striated Muscle Activator of Rho Signaling
TGFβ	Transforming Growth Factor β
WT	Wild Type

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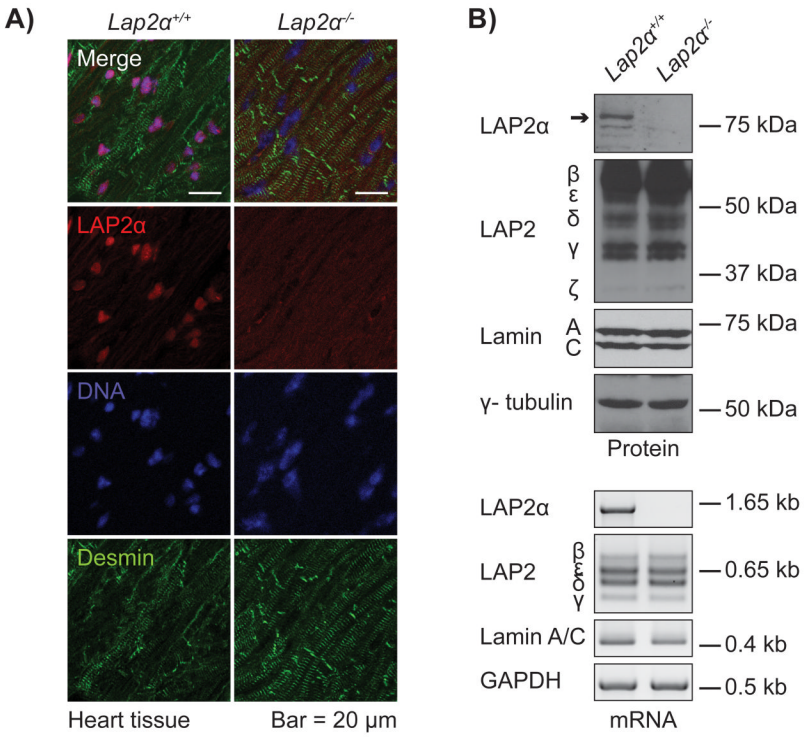

Figure 1. LAP2α is absent from cardiac tissue of *Lap2α*$^{-/-}$ mice

A) Immunofluorescence analysis of *Lap2α*$^{+/+}$ and *Lap2α*$^{-/-}$ heart tissue. **B)** LAP2α mRNA and protein are absent from heart muscle tissue of *Lap2α*$^{-/-}$ mice, while the expression of lamin A/C and alternative LAP2 splice variants remains unaltered. (Western blot and semiqRT-PCR analyses; samples were normalized for endogenous γ-tubulin and Glyceraldehyde 3-phosphate Dehydrogenase (GAPDH) content respectively; n = 3 male + 3 female littermate pairs).

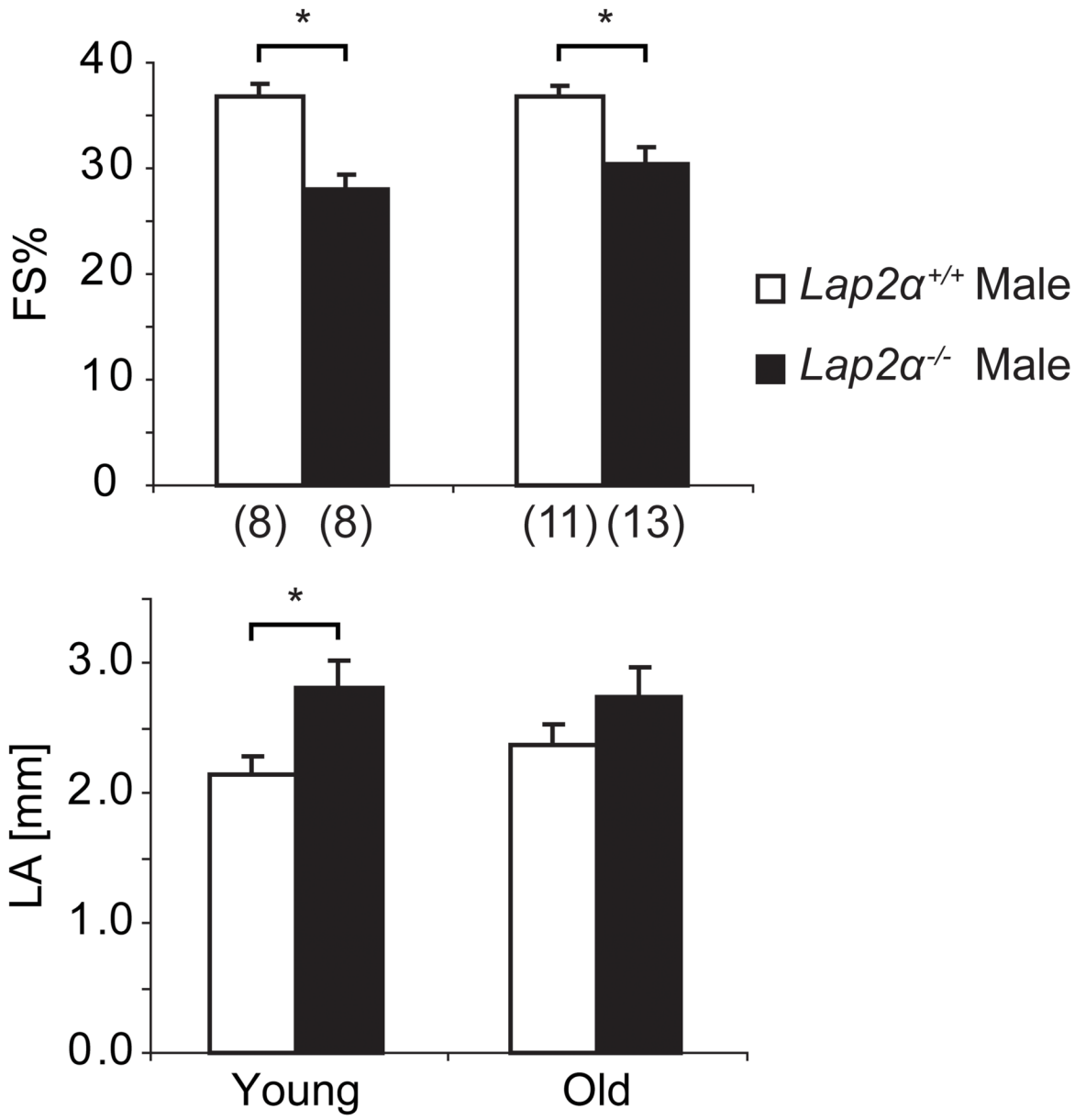

Figure 2. Loss of LAP2α impairs heart function in mice

Echocardiography data show left ventricular systolic dysfunction in young (10 weeks) and old (10–12 months) male *Lap2α*$^{-/-}$ mice. (FS% – fractional shortening; LA – left atrium diameter; (n) - number of mice; *p<0.05 ANOVA, values are means ± SE).

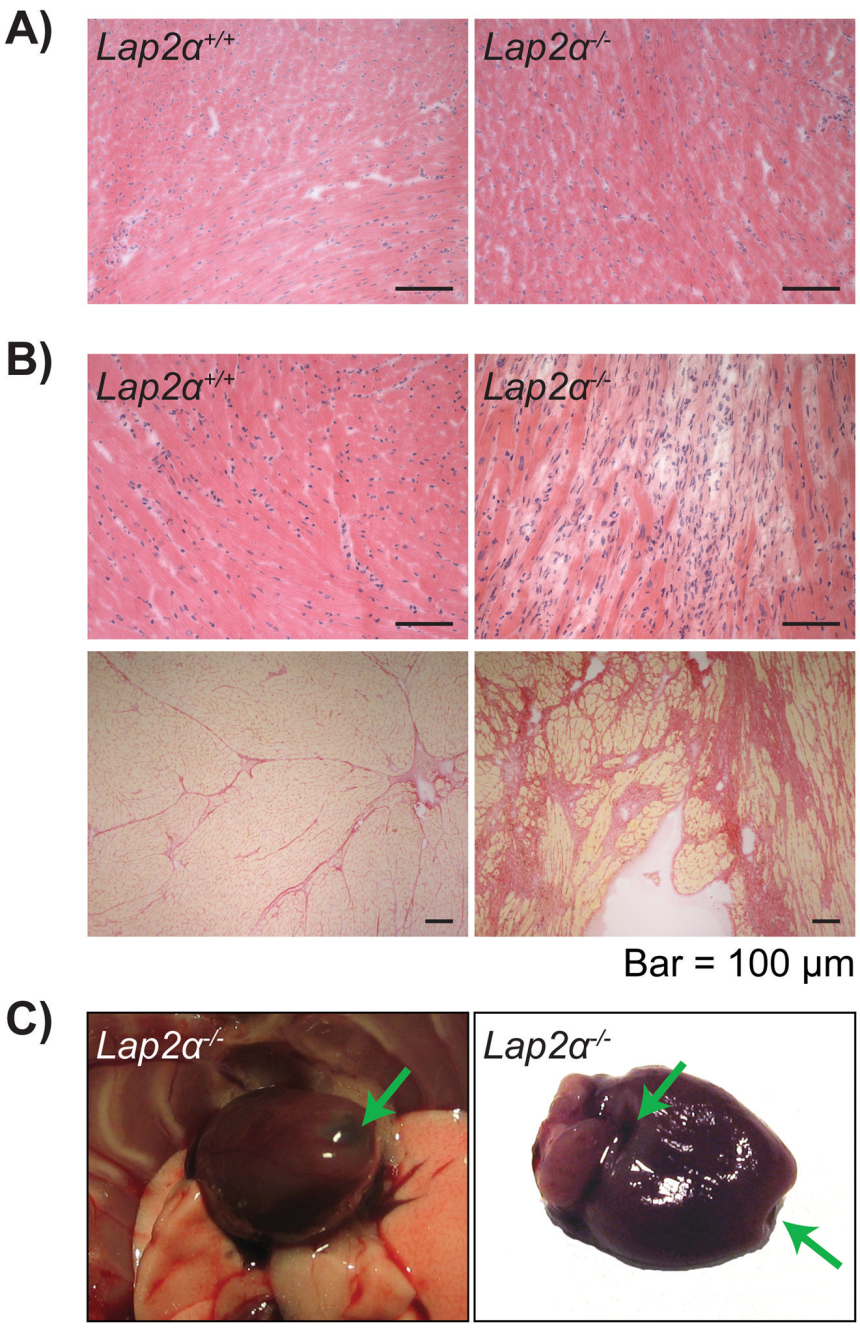

Figure 3. Old *Lap2α*$^{-/-}$ mice show sporadic cases of fibrosis

A) Young male *Lap2α*$^{-/-}$ myocardium is histologically indistinguishable from the WT. **B)** 18% of the old male *Lap2α*$^{-/-}$ hearts show disperse fibrotic foci (upper panels haematoxylin & eosin-stained, lower panels Picrosirius Red-stained heart sections). **C)** Thinning of the old *Lap2α*$^{-/-}$ myocardium – arrows mark the transparent fibrotic regions of the heart.

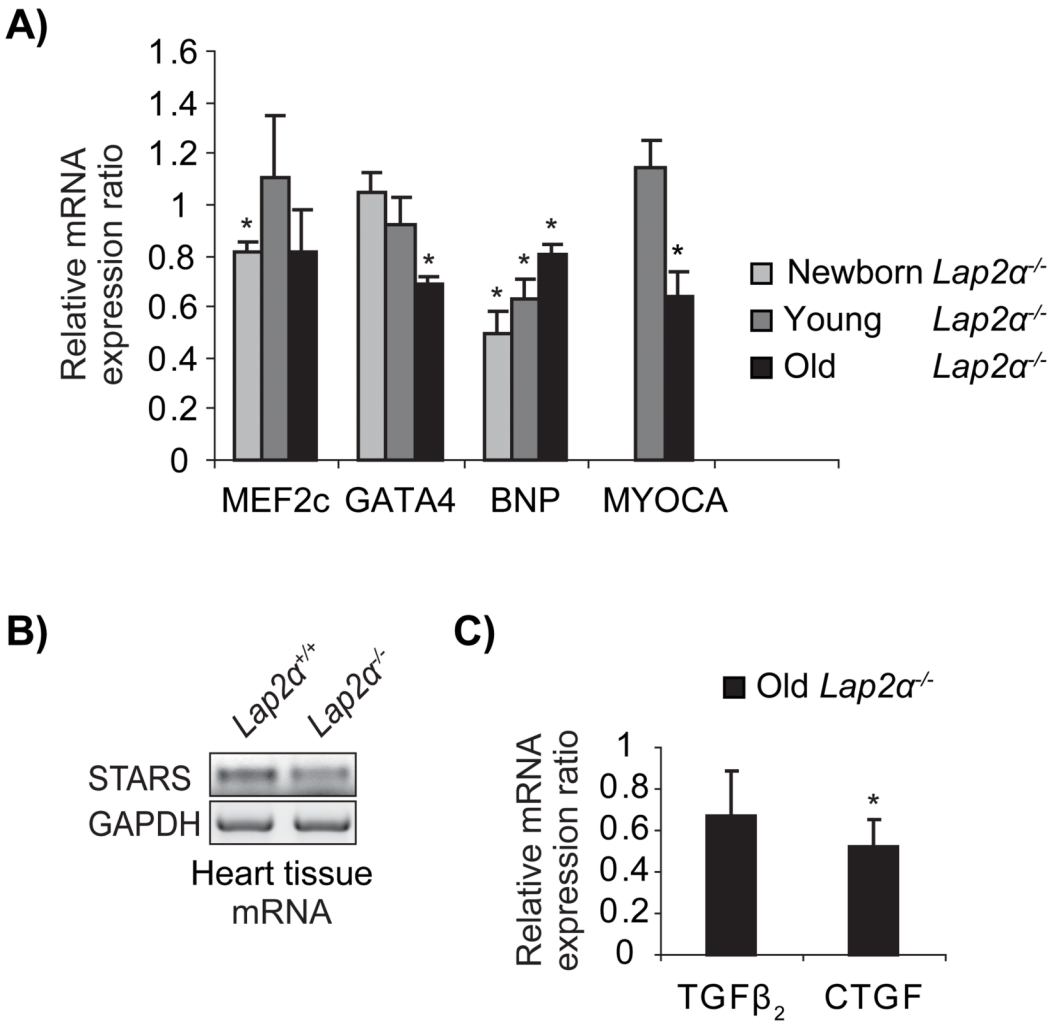

Figure 4. Deregulated expression of major cardiac transcription factors in LAP2α-deficient mice

A) MEF2c, GATA4 and their downstream targets, BNP and Myocardin A (MYOCA), are downregulated on mRNA level at specific stages in *Lap2α*$^{-/-}$ mice. **B)** STARS mRNA levels are lower only in old LAP2α-deficient hearts. **C)** Old *Lap2α*$^{-/-}$ mice exhibit decreased CTGF expression and normal TGFβ2 mRNA content. (Quantitative PCR (qPCR) and semiqPCR analyses of heart tissue. n = 4 – 5 male littermate pairs of each age. One outlier pair was excluded according to Grubb's test. Samples were normalized for endogenous GAPDH levels in semiqPCR and HPRT (Hypoxanthine-Guanine Phosphoribosyltransferase) levels in qPCR and analyzed according to Pfaffl method[44]. Each knockout (KO) sample was compared to its respective WT littermate sample. Values are mean KO/WT expression ratios ± SE. *p<0.05, ANOVA).

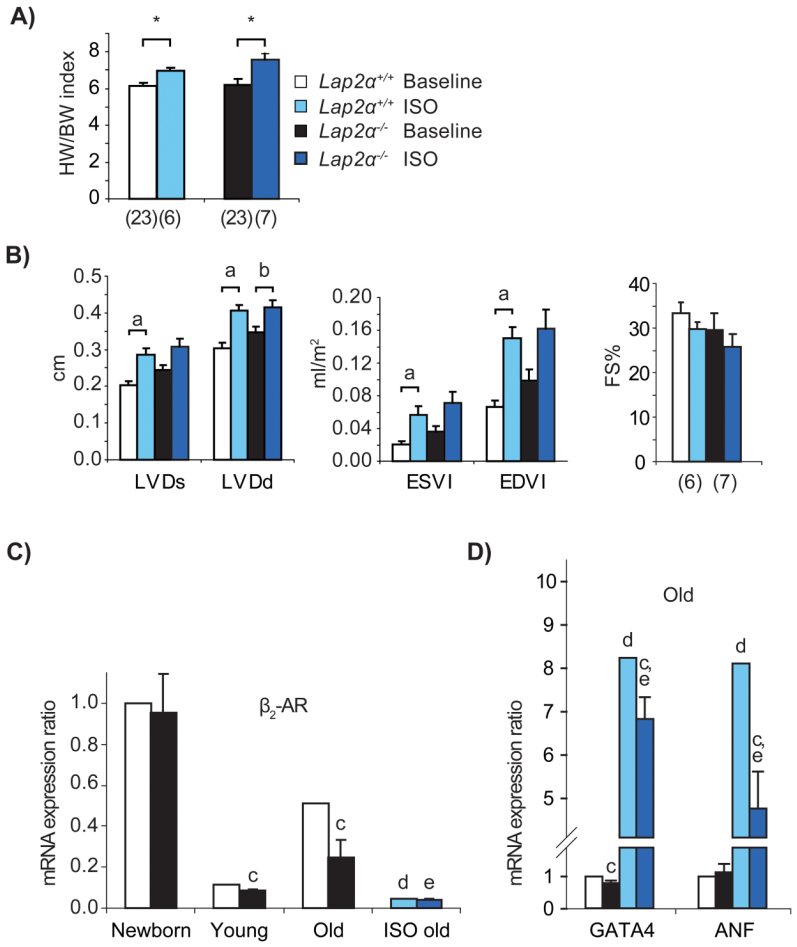

Figure 5. *Lap2α*$^{-/-}$ mice show a blunted response to chronic isoproterenol infusion

A) *Lap2α*$^{+/+}$ and *Lap2α*$^{-/-}$ male mice exhibit a similar increase in heart weight/body weight (HW/BW) indexes after 7 days of ISO treatment. (*p<0.05 ANOVA). **B)** Echocardiography reveals the absence of significant left ventricular chamber dilation in *Lap2α*$^{-/-}$ mice and preserved basal systolic function in both genotypes. [LVDd/s – left ventricular diameter in diastole/systole; ESVl – end-systolic left ventricular volume index; EDVl – end-diastolic left ventricular volume index; (n) - sample size. Data were analyzed using Student's paired t-test (comparisons within one genotype before and after the treatment) and one way-ANOVA (comparisons between the two genotypes). (a) p<0.05, (b) p = 0.05]. **C)** Relative baseline mRNA levels of β_2-AR are lower in *Lap2α*$^{-/-}$ hearts compared to WT, whereas ISO treatment causes its downregulation in both genotypes. **D)** ISO-induced increase in expression of fetal and pro-fibrotic genes is diminished in the absence of LAP2α. (qPCR analyses of heart tissue, n = 5 ISO-treated + 4 – 5 untreated male littermate pairs of each age; ANOVA, p<0.05 for (c) WT vs. KO of the respective age, (d) WT baseline vs. WT ISO-treated and (e) KO baseline vs. KO ISO-treated animals, mean ± SE).

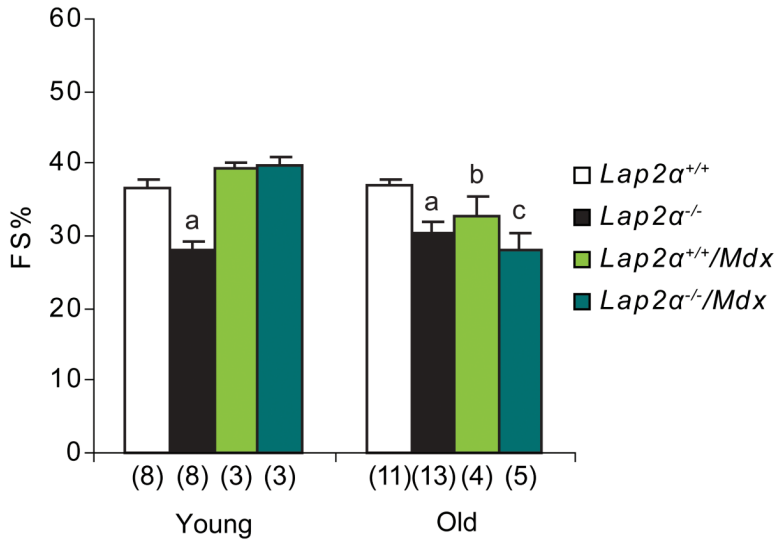

Figure 6. Absence of dystrophin delays the onset of ventricular dysfunction in *Lap2α*$^{-/-}$*/Mdx* mice

FS% values in young male *Lap2α*$^{-/-}$*/Mdx* mice are higher compared to *Lap2α*$^{-/-}$ animals, whereas old mice of both genotypes show similar systolic dysfunction (ANOVA; $p<0.05$ for (a) WT vs. KO, (b) WT vs. *Mdx* and (c) WT vs. *Lap2α*$^{-/-}$*/Mdx* mice; (n) - sample size; mean ± SE).

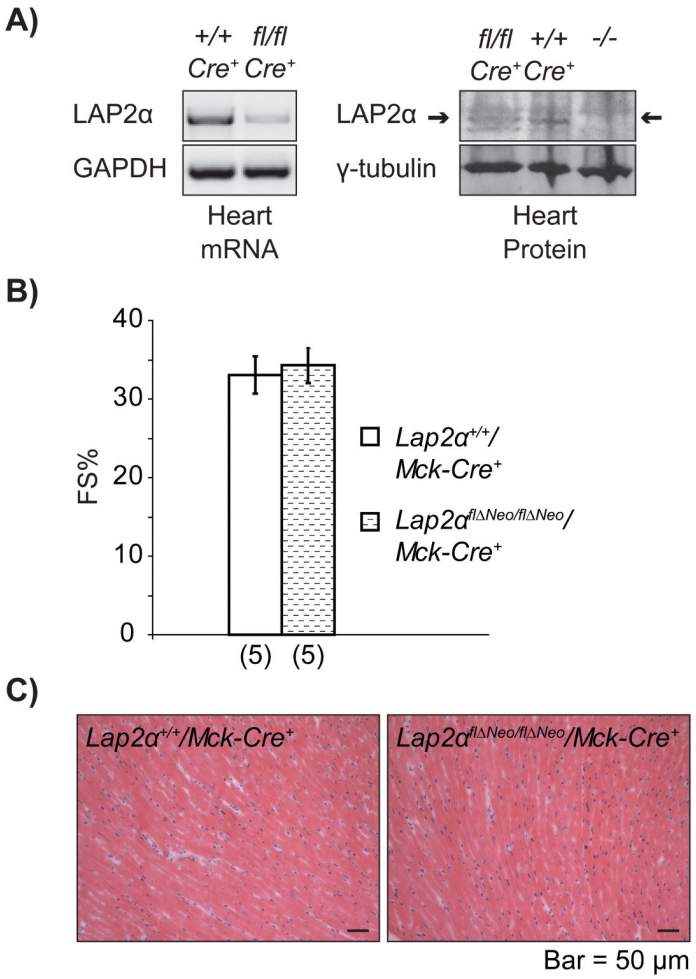

Figure 7. LAP2α is dispensable in late-embryonic and adult cardiomyocytes

A) Reduced LAP2α mRNA and protein levels in *Lap2α*$^{flΔNeo/flΔNeo}$*/Mck-Cre*$^{+}$ [fl/fl] vs. *Lap2α*$^{+/+}$*/Mck-Cre*$^{+}$ heart tissue [+/+]. (Samples were normalized to GAPDH in semiqPCR and to γ-tubulin in western blot analyses). **B)** Normal FS% values in young male *Lap2α*$^{flΔNeo/flΔNeo}$*/Mck-Cre*$^{+}$ mice ($p>0.05$, ANOVA; (n) - sample size, mean ± SE). **C)** Normal heart structure in conditional striated muscle-specific LAP2α knockout mice.