**In vivo cardiac role of migfilin during experimental pressure overload**

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**Aims**

Increased myocardial wall strain triggers the cardiac hypertrophic response by increasing cardiomyocyte size, reprogramming gene expression, and enhancing contractile protein synthesis. The LIM protein, migfilin, is a cytoskeleton-associated protein that was found to translocate in vitro into the nucleus in a Ca²⁺-dependent manner, where it co-activates the pivotal cardiac transcription factor Csx/Nkx2.5. However, the in vivo role of migfilin in cardiac function and stress response is unclear.

**Methods and results**

To define the role of migfilin in cardiac hypertrophy, we induced hypertension by transverse aortic constriction (TAC) and compared cardiac morphology and function of migfilin knockout (KO) with wild-type (WT) hearts. Heart size and myocardial contractility were comparable in untreated migfilin KO and WT hearts, but migfilin-null hearts presented a reduced extent of hypertrophic remodelling in response to chronic hypertensile stress. Migfilin KO mice maintained their cardiac function for a longer time period compared with WT mice, which presented extensive fibrosis and death due to heart failure. Migfilin translocated into the nucleus of TAC-treated cardiomyocytes, and migfilin KO hearts showed reduced Akt activation during the early response to pressure overload.

**Conclusions**

Our findings indicate an important role of migfilin in the regulation of cardiac hypertrophy upon experimental TAC.

**Keywords**

Migfilin • Cardiac hypertrophy • Cardiac function • Pressure overload • Myocardial remodelling

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1. Introduction

Cardiovascular disease is the number one cause of mortality in the western world, with heart failure representing the fastest growing subclass.¹ Heart failure can be caused by a variety of disease stimuli, of which chronic hypertension and aortic stenosis are prominent.² These pathologies induce a hypertrophic myocardial response which eventually results in myocardial remodelling and deterioration of heart function, exemplified by reduced left ventricular (LV) contractility (e.g. fractional shortening, FS) and an increase in LV volume over time.³ At the molecular level, chronic hypertension triggers intracellular stress-signalling pathways, such as Akt and MAPK pathways, that converge on a few critical transcription factors. These transcription factors orchestrate a genetic response, leading to increased synthesis of sarcomeric proteins in cardiomyocytes and overall cell hypertrophy.⁴ One pivotal cardiac transcription factor is Csx/Nkx2.5, which is essential for heart development and post-natal cardiac function,⁵⁻⁷ but also essential for the hypertrophic stress response.⁸⁻⁹ For instance, the promoter activities of the important cardiac genes atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) are under the control of Csx/Nkx2.5.⁹ To study chronic pressure overload in vivo, it can be experimentally induced in animal models such as mice by transverse aortic constriction (TAC).¹⁰

A recently identified co-activator of Csx/Nkx2.5 is migfilin. Migfilin is a cell adhesion protein that in vitro was shown to translocate into the nucleus in a Ca²⁺-dependent manner and co-activate Csx/Nkx2.5.⁵ It is one of the seven mammalian zyxin family proteins and comprises an N-terminal domain followed by a proline-rich region containing a nuclear export signal, and three C-terminal LIM domains.¹¹⁻¹³ All
zyxin family proteins have been found to shuttle into the nucleus and function as co-activators to specific transcription factors, hinting at an important function in development, homeostasis, and disease. However, single genetic ablation of migfilin or other zyxin family members in mice did not cause a basal phenotype. This apparent lack of phenotypes is likely due to compensation by the remaining zyxin family members. Accordingly, in flies, the targeted deletion of one of just two zyxin family members is lethal. Detailed analysis revealed an important role of several zyxin family proteins in response to specific diseases and/or stressors. Migfilin is highly expressed in the heart, and specifically localizes to intercalated discs in cardiomyocytes. We previously did not find a basal phenotype in migfilin-deficient hearts, but suspected that migfilin could have a role in cardiac hypertrophy due to its function as a co-activator of Csx/Nkx2.5. We therefore studied the changes of cardiac morphology and function in migfilin-deficient mice caused by experimental pressure overload. Here, we show that migfilin plays an important regulatory role in the cardiac response to experimental pressure overload in vivo.

2. Methods

2.1 Mice
Migfilin knockout (KO) mice have been described previously. Migfilin-null mice were backcrossed onto a C57BL6N background. Migfilin wild-type (WT) and KO littermates were generated from migfilin heterozygous parents. Only male animals were used for the experiments. Animals were handled in accordance with the institutional guidelines and the investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health. Mice were maintained in controlled environmental conditions at a constant room temperature of 22 and 24 °C on a 12-h light/dark cycle and provided with standard food and water ad libitum.

2.2 Cardiac pressure overload (transverse aortic constriction)
Nine- to 12-week-old (see Supplementary material online, Figure S2A) control WT and migfilin KO mice were subjected to TAC as described previously (n = 12 per genotype). Animals were anaesthetized with a mixture of medetomidin (0.5 mg/kg), midazolam (5 mg/kg), and fentanyl (0.05 mg/kg) administered via an intraperitoneal injection. In brief, following sedation, intubation, ventilation, and thoracotomy, aortic constriction was performed by a 7-0 monofilament non-absorbable polypropylene suture located around the aorta between the innominate and left carotid artery. The ligature narrows the aortic diameter to 0.4–0.5 mm, which correlates with a TAC of 65–70% (see Supplementary material online, Figure S1). The overall periprocedural mortality was below 10%. Mice were euthanized, hearts quickly removed, and the left ventricle was immediately snap-frozen in liquid nitrogen. Proteins were extracted using Tissue Protein Extraction Reagent (T-PER), containing Halt Protease and Phosphatase Inhibitor Cocktail (PIERCE, Rockford, USA). Western blot and RT-PCR analyses after defined time points (40 min, 3 h, 6 h, 12 h, 24 h, 1 week, and 3 weeks; n = 4 per group).

2.3 Echocardiography
Echocardiographic measurements were performed as described previously. Myocardial contractility was determined by transthoracic echocardiography, using a sector scanner (AcuNav 10 MHz, Acuson Sequioa C256, Acuson Corporation, Mountain View, CA, USA). Short-axis 2D-targeted M-mode images of the left ventricle were obtained. LV end-diastolic diameter (LVEDD) and LV end-systolic diameter (LVESD) were measured, and FS was calculated using the formula: FS = (LVEDD – LVESD)/LVEDD × 100. Cardiac function in TAC-treated migfilin KO and WT control animals was assessed at baseline and weekly after the TAC surgery until the eighth week after TAC.

2.4 Histological assessment, fibrosis determination, and cardiomyocyte cell size
Hearts were fixed in 4% paraformaldehyde at 4 °C overnight and embedded in paraffin. Longitudinal sections of 2.5 μm thickness were cut from defined levels of the left ventricle, stained with Masson trichrome with aniline blue, scanned with a 3DHistech slide scanner, and analysed using the Panoramic viewer software (3DHistech, Budapest, Hungary). Fibrosis was analysed on Trichrome-stained heart sections by quantifying the area of fibrosis (blue) in relation to the area of cardiomyocytes (red) using Photoshop CS5 (Adobe, San Jose, CA, USA). Cross-sectional areas of 20 cardiomyocytes of each left ventricle were measured and statistically analysed.

2.5 IF and Tunel staining
The generation of the migfilin antisera and IF stainings of heart cryosections were reported earlier. Briefly, hearts were quickly harvested, washed in ice-cold PBS, and embedded in OCT. Sections of 10 μm thickness were stained with antibodies against migfilin11 and vinculin (Sigma, St Louis, MO, USA) or troponin T (Thermo Scientific, Waltham, MA, USA). Moreover, biotinylated isocitrate B4 (Szabo Scandic, Vienna, Austria) was used to stain blood vessels, and a CD45 antibody (BD Biosciences, San Jose, CA, USA) was used to mark leucocytes. Blood vessels were quantified in silico by using Photoshop CS5 (Adobe, Thereby, the blood vessel-dependent area was put in relation to the remaining myocardium. Leucocytes were counted manually in five standardized fields of views (× 20 magnification) throughout the left ventricle (basal free wall, middle free wall, apex, middle septum, and basal septum). Goat anti-mouse Alexsa 555, goat anti-rabbit Alexsa 488, goat anti-rat Alexsa 488, and streptavidin-conjugated Alexsa 647 (Life Technologies, Carlsbad, CA, USA) were used as secondary antibodies at room temperature for 1 h. Cell death was visualized, using a DeadEnd fluorometric Tunel kit (Promega, Madison, WI, USA). Samples were counterstained with DAPI for 15 min and mounted in DAKO fluorescent mounting medium (DAKO, Denmark). Immunofluorescent images were taken with an LSM 780 confocal microscope with a spectral GaAsp detector (Zeiss, Jena, Germany) or were scanned using a 3DHistech scanner (3DHistech).

2.6 Protein extraction and western blotting
Mice were euthanized, hearts quickly removed, and the left ventricle was immediately snap-frozen in liquid nitrogen. Proteins were extracted using Tissue Protein Extraction Reagent (T-PER), containing Halt Protease and Phosphatase Inhibitor Cocktail (PIERCE, Rockford, USA). Western blotting was performed as described previously with a self-made antibody against migfilin. Protein lysates from LV tissue were used. Moreover, antibodies against phospho-Akt (Ser473), phospho-ERK (T202/Y204), phospho-JNK (T183/Y185), and phospho-p38 (T180/Y182) were used in combination with total Akt, ERK, JNK, and p38 antibodies as controls. An anti-GAPDH
2.7 Quantitative real-time PCR

Total RNA was extracted from LV tissue (RNaseasy Fibrous Tissue Mini Kit, Qiagen GmbH, Germany) and reverse-transcribed, using MMLV reverse transcriptase (Promega) or iScript (Bio-Rad Laboratories, Hercules, CA, USA). RNA quantification was performed by real-time PCR in a CFX96 real-time PCR detection System (Bio-Rad Laboratories) with oligonucleotides for ANP (Nppa), B-type natriuretic peptide (Nppb), (Adora1), (Ankrd), calreticulin (Calr), connexin 43 (Gaj5), Gata 4 (Gata4), (Myocd), (Slc8a1), alpha actinin (Actn1), alpha myosin heavy chain (Myh6), and beta-myosin heavy chain (Myh7). Transcript levels were normalized to the levels of Pum1 and further normalized to WT baseline levels. RT-PCR for migfilin was performed as described earlier. All reactions were performed in triplicate. Relative mRNA levels were calculated by the ‘delta delta Ct’ method.

2.8 Statistical analysis of data

All data are presented as mean values ± SD. Unpaired t-test or ANOVA with Bonferroni multiple comparison analysis was used for normally distributed data. Two-way ANOVA with Sidak’s multiple comparison test was used for the time course echocardiographic data in the first 3 weeks. After the third week, statistical power deteriorated as half of the WT mice were dead and the data from surviving animals converged. Three WT mice died before the third week and were excluded from this analysis. Probability values (P < 0.05) were considered significant. GraphPad Prism version 6 (GraphPad Software, La Jolla, CA, USA) was used for statistical analysis.

3. Results

3.1 Migfilin-null hearts are comparable with control hearts under baseline conditions

Previously, we found that migfilin KO mice have no overt phenotype during development or ageing. A more detailed analysis of adult hearts confirmed that control (WT) and migfilin-null (KO) mice had a similar body weight (BW), tibia length (TL), and heart weight (HW) at the age of 8 weeks (see Supplementary material online, Figure S2). Accordingly, HW ratios to BW and TL were also similar. There was also no difference in cardiac morphology, cell composition, and collagen deposition (Figure 1A). Migfilin KO hearts demonstrated a minor increase in LV diameter during diastole (LVEDD) compared with WT controls (Figure 1B). However, this significant difference was lost using two-way ANOVA analysis of the same study cohort where three WT mice that did not survive the first 3 weeks of TAC were excluded (see Supplementary material online, Figure S3B). In contrast, LVESD and cardiac function as measured by FS were not affected (Figure 1C and D). These results confirm that loss of migfilin expression does not affect cardiac morphology and function under basal conditions.

3.2 Migfilin expression is regulated by cardiac stress

Although KO hearts were normal under baseline conditions, we suspected that migfilin has a role in the cardiac stress response. To
test this, we randomized WT mice into sham surgery (SHAM) and TAC-treated cohorts. We harvested LV cardiac tissue immediately (SHAM) or after defined time periods of TAC (40 min to 3 weeks) and determined migfilin transcript levels by quantitative RT-PCR and migfilin protein levels by immunoblotting. Migfilin transcript levels were reduced in the acute TAC response within 24 h, but consequently increased to 2.5-fold compared with SHAM treatment after 1 week of TAC (Figure 2A). Migfilin protein levels were at first largely unchanged (40 min to 24 h), but were significantly increased at 1 week of TAC and fell below initial levels in SHAM-treated hearts after 3 weeks of pressure overload (Figure 2B and C). These results show that migfilin expression is regulated as part of the cardiac pressure overload response, indicating a function for migfilin in compensatory cardiac hypertrophy.

### 3.3 Migfilin-null hearts show decreased hypertrophy and fibrosis under pressure overload and less cardiac failure

To test the function of migfilin as a regulator of the myocardial stress response, we subjected 12 migfilin KO mice and 12 migfilin WT littermate controls to TAC. The randomized animals underwent experimental TAC surgery between the 9th and 12th week of age (see Supplementary material online, Figure S3A). Whereas three migfilin WT mice died within 3 weeks after the induction of pressure overload, there was no lethality during this time in the migfilin-null group. Overall, all but one WT mouse died during 8-week follow-up, whereas only two KO mice died. Loss of migfilin therefore provided a drastic survival benefit following cardiac pressure overload (Figure 3A).

Myocardial function in TAC-treated migfilin KO and WT control animals was assessed by weekly serial echocardiographic measurements at baseline and until eighth week after TAC. Both LVEDD and LVESD constantly increased during the study in WT and KO hearts compared with baseline, with a stronger dilatation observed in WT hearts (Figure 3C and D). Cardiac function defined by FS gradually decreased in WT and KO mice in this period and was lower in WT control mice relative to migfilin KO mice (Figure 3B). The heart parameters of the sole surviving WT mouse at eighth week TAC were comparable with KO cardiac parameters. Taken together, this shows that the survival benefit in the migfilin-null cohort is caused by slower deterioration of heart function under cardiac pressure overload. Hearts were harvested for further analysis at the time of death or at the end of follow-up 8 weeks after TAC initiation. Morphological analysis confirmed the echocardiography data and survival advantage: BW and TL were significantly higher in KO than in WT mice at the time of death or eighth week of TAC, reflecting the increased lifespan and reduced cardiac cachexia of KO animals (see Supplementary material online, Figure S3B and C). A TAC-induced hypertrophic increase of HW was lower in the mutant cohort (Figure 4A and see Supplementary material online, Figure S3D). Accordingly, the ratios of HW to BW, and to TL, were significantly lower in the migfilin-null cohort compared with WT controls (Figure 4B and C).

**Figure 2** Migfilin transcript and protein expression during TAC. Migfilin expression levels determined from protein lysate or reverse-transcribed mRNA isolated from ventricular tissue after sham operation or the indicated times of TAC. (A) Transcript levels were determined by quantitative RT-PCR and normalized to transcript of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and further normalized to sham transcript levels. (B) Migfilin protein levels were determined by densitometry of immunoblots for migfilin, relative to GAPDH and normalized to sham protein levels. Error bars indicate SD. N = 4 per group. **p < 0.01.
As migfilin KO hearts had a lower hypertrophic increase in HW upon TAC, we next determined the hypertrophic response of cardiomyocytes after TAC. The cross-sectional area of cardiomyocytes in heart trichrome sections was similar at baseline level (Figure 4D). TAC induced hypertrophic growth of cardiomyocytes in both control and migfilin-null mice, but the increase was less pronounced in KO hearts (Figure 4E). This confirms that loss of migfilin attenuates cardiomyocyte hypertrophy after TAC-induced pressure overload. Moreover, WT hearts presented massive subendocardial fibrosis evidenced by collagen deposition at the study endpoint. In contrast, this was absent in corresponding migfilin KO hearts (Figure 4A and F).

To test if the decrease in fibrosis during pressure overload is caused by secondary effects like differences in vascularization or inflammation, we immunofluorescently labelled blood vessels with isolectin B4 and infiltrating leucocytes with anti-CD45. We did not detect any obvious differences in blood vessel number or infiltrating leucocytes between WT and KO at baseline or during cardiac pressure overload (Figure 5A–C). In addition, we examined cell death after TAC as defined by TUNEL (TdT-mediated dUTP-biotin nick end labelling)-positive staining. We rarely detected TUNEL-positive nuclei in the left ventricle, and there was no noticeable difference between the two genotypes (see Supplementary material online, Figure S4). We therefore focused on cardiomyocyte signalling as an explanation for the observed differences.

### 3.4 Migfilin translocates to the nucleus of cardiomyocytes upon pressure overload

Migfilin can translocate into the nucleus and transactivate Csx/Nkx 2.5 in vitro. To test if this also occurs in vivo in TAC-treated heart, we determined the cardiac subcellular localization of migfilin at different time points. Migfilin co-localized with vinculin at intercalated discs of cardiomyocytes as previously shown, and there was no convincing nuclear signal for migfilin in baseline cardiomyocytes (ref.11 and Figure 6A). However, following experimental pressure overload, we observed a marked induction of nuclear migfilin in all cardiomyocyte nuclei analysed (Figure 6B and C, and see Supplementary material online, Figure S5, N = 3/3 sections per heart and time point). We also carefully examined other cardiac cell populations such as fibroblasts and endothelial cells, which are troponin T-negative and can be differentiated from cardiomyocytes by location, size, and shape. However, these cells did not have migfilin-positive nuclei. This shows that migfilin accumulates specifically in cardiomyocyte nuclei in a strain-dependent manner.

To test if the nuclear accumulation of migfilin affects Csx/Nkox2.5 activity, we determined the mRNA expression levels of cardiac natriuretic peptides ANP (Nppa) and brain natriuretic peptide (BNP, Nppb) as a downstream readout for Csx/Nkox2.5 activity in baseline...
and TAC-treated hearts. Indeed, Nppb transcript levels were reduced in migfilin-deficient mice at baseline level (Figure 6D and E), whereas Nppa transcript levels were unaffected by loss of migfilin (Figure 6F). However, both transcripts were up-regulated to a similar extent in TAC-treated WT and KO hearts. Analysis of further known Csx/Nkx 2.5 response and heart failure genes revealed no differences in mRNA level between Migfilin KO and WT mice (see Supplementary material online, Figure S6). Thus, we could not confirm a reduced function of Csx/Nkx 2.5 in response to pressure overload implied by previous in vitro studies.

3.5 Migfilin influences Akt and Erk signalling in the heart

Akt and MAPK pathways have been shown to orchestrate cardiac hypertrophy at the molecular level. To examine the impact of migfilin loss on crucial hypertrophic- and stress-signaling pathways in the heart, we harvested additional migfilin KO and littermate control hearts under baseline conditions (Figure 7A) and after 24 h (Figure 7B), 1 week (Figure 7C), and 3 weeks of TAC (Figure 7D, N = 3 per genotype and time point). We subjected the protein lysates to immunoblotting, using antibodies against the phosphorylated and total forms of Akt, ERK, JNK, and p38 (Figure 7). Whereas a significant reduction in Akt phosphorylation in migfilin-null hearts, compared with WT hearts, was found under baseline conditions, ERK signalling was opposite in the same samples (Figure 7A). Interestingly, the decreased phospho-Akt signal persisted in the 24 h TAC migfilin KO hearts compared with WT controls (Figure 7B). There was no difference in phosphorylated or total p38 and JNK1/2 between migfilin-null and control hearts at any time point. To further emphasize the time course-dependent character of Akt and ERK phosphorylation, we compared one sample of each time point and genotype on an additional membrane (Figure 7E). The difference in Akt phosphorylation after 1 week of TAC found in the time course immunoblot (Figure 7E) could not be confirmed in the side-by-side comparison of three biological replicates (Figure 7C).

Taken together, whereas both Akt and ERK phosphorylation were significantly changed between migfilin KO and control hearts, only Akt signalling showed a similar pattern during baseline and early TAC conditions.

4. Discussion

Accumulating evidence points to crucial roles for migfilin in several fundamental cellular processes, including shape modulation, motility, and differentiation. Based on in vitro data pointing to a co-activating function of migfilin for the cardiac transcription factor Csx/Nkx2.5, we hypothesized that migfilin has a specific role in response to cardiac stress or diseases. We could indeed find that loss of migfilin in mice has a striking pro-survival effect in a chronic hypertensile stress model: during prolonged pressure overload, migfilin KO mice maintained their cardiac function for a longer time period with strongly
reduced hypertrophic remodelling of cardiomyocytes and fibrosis compared with WT mice.

Adaptive cardiac hypertrophy is initially beneficial for survival, ensuring heart function under conditions of increased mechanical workload by increasing cardiomyocyte force generation. However, chronic hypertrophic stimulation results in cardiomyopathy and eventually heart failure due to escalating fibrosis at the expense of cardiomyocyte function. As a corollary, reduced hypertrophy was shown to be beneficial for survival under chronic hypertension, similar to what we observed in TAC-treated migfilin KO mice. Importantly, another well-studied LIM domain-containing protein, FHL1 (four-and-a-half LIM domain 1), proved similar characteristics following TAC compared with migfilin: (i) FHL1 is also up-regulated following experimental pressure overload and (ii) FHL1 KO mice showed attenuated hypertrophic response after TAC.

Moreover, we did not detect differences in leucocyte infiltration, cell death, and vessel density between migfilin KO and control hearts. Since heart failure progression is strongly driven by destructive inflammatory processes, the observed similarity in inflammatory infiltration and cell death points to a direct role of migfilin in the hypertrophic remodelling of cardiomyocytes. This also reflects the high expression levels of migfilin in cardiomyocytes compared with endothelial or hematopoietic cells.

Cardiomyocyte hypertrophy and remodelling due to mechanical stress are accompanied by increased intracellular Ca\(^{2+}\) levels, a known trigger for nuclear accumulation of migfilin. The nuclear accumulation of migfilin upon prolonged hypertensile stress is a further indicator for a role for migfilin in this process. This behaviour of stress-dependent nuclear translocation was also found earlier in other LIM domain-containing proteins, for example, muscle-specific LIM-only protein (MLP) and lipoma-preferred partner (LPP). However, although migfilin was previously shown to modulate the transcriptional activity of Csx/Nkx2.5 in vitro, most prominent Csx/Nkx2.5 target genes were largely unaffected by loss of migfilin in basal and stressed hearts. Only the level of the Csx/Nkx2.5 downstream target BNP was strongly decreased in basal migfilin KO hearts, but quickly reached levels comparable with WT hearts upon TAC. Taken together, these results hint at only a modest function of migfilin in the

Figure 5 Analysis of blood vessels and leucocyte infiltration in migfilin KO and control hearts during baseline conditions and after TAC. (A) Representative images of control (WT) and migfilin KO hearts under baseline conditions (left panel) and after 3 weeks of TAC (right panel) that were immunofluorescently labelled with an antibody against CD45 (leucocytes, upper row) and isolectinB4 (blood vessels, second row). Colour combined images are shown in the third row. Blue: DAPI (nuclei); red: blood vessels (isolectinB4); green: leucocytes (CD45). Yellow arrowheads indicate leucocytes. (B and C) Quantitative analysis of leucocytes (B) and blood vessels (C) in control (black) and migfilin KO (orange) hearts under baseline conditions, after 1 week, and after 3 weeks of TAC. Values are shown as mean ± SD. N = 3 per group.
Csx/Nkx2.5 activity is secured through a highly redundant regulatory network, since it is essential for cardiac function in development, homeostasis, and disease. Therefore, compensating activation of Csx/Nkx2.5 during myocardial development and strain could mask the effects of migfilin loss in our mouse heart model, since other regulators of Csx/Nkx2.5 transcriptional activity were not targeted by deletion of migfilin.

Akt and MAPK signalling pathways have been shown to play critical roles in the response of cardiomyocytes to pressure overload. Especially, Akt is a very well-studied signalling molecule that has been shown to promote cardiac hypertrophy. Moreover, integrins in combination with several associated cytoskeletal proteins have been proved to influence the above-mentioned key pathways. We found a clear reduction of Akt activation in migfilin KO hearts under baseline conditions and in the early phase of pressure overload. Conversely, ERK signalling showed an opposite pattern compared with AKT signalling at baseline in migfilin KO mice that could indicate a compensating mechanism of the two pro-hypertrophic kinases. In addition, reduced ERK signalling at later TAC time points might reflect the reduced remodeling phenotype of the migfilin KO hearts. Shiojima et al. reported that prolonged augmented Akt activation leads to the transition from compensatory hypertrophy to heart failure due to a dysbalance in cardiomyocyte hypertrophy to angiogenesis. In contrast, we could not detect a difference in Akt signalling at later TAC time points nor did we find a difference in vessel density at time points when the mutant cohort phenotypically diverges from the control mice. Additional tested potential mechanisms, for example, cell death and infiltrating leukocytes, did not reveal any significant difference in migfilin KO to WT mice. However, the activation of the key hypertrophic protein kinase B (Akt) is reduced in migfilin-null hearts at baseline and 24 h of TAC. This offers a tempting starting point for further mechanistic studies. It will be crucial to ask the question if the observed differences in signalling are primarily due to the loss of migfilin or secondary to the reduced remodelling phenotype of the mutant hearts.

While all seven members of the zyxin family are expressed in heart, the only other family member where a role in cardiac stress was implicated is the LPP: LPP is up-regulated similar to migfilin in response to cardiac banding in rats. However, functional consequences of LPP loss in response to cardiac stress have not been explored. Murine KO are available for most of the zyxin family genes, but there are no reported developmental or post-natal basal cardiac phenotypes. It will be interesting in the future to generate compound KO mouse models for several zyxin family members to gain insights into their combined function in cardiac development and disease.

We present here the first report of a role for a zyxin family protein in response to cardiac overload. Loss of migfilin results in prolonged survival and partially conserved cardiac function combined with reduced interstitial fibrosis. Reduced Akt signalling and the fact that migfilin...
translocates into the nucleus in a cardiac strain-dependent manner point to a potential mechanistic explanation for the mutant phenotype. Nonetheless, these mechanistic data are hypothesis-generating and in depth analyses are warranted.

**Supplementary material**

Supplementary material is available at Cardiovascular Research online.

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Migfilin in the heart


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