Published in final edited form as:
Circ Res. 2006 September 15; 99(6): 636–645. doi:10.1161/01.RES.0000240498.44752.d6.

Myocyte-Restricted Focal Adhesion Kinase Deletion Attenuates Pressure Overload-Induced Hypertrophy

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Abstract

Focal adhesion kinase (FAK) is a ubiquitously expressed cytoplasmic tyrosine kinase strongly activated by integrins and neurohumoral factors. Previous studies have shown that cardiac FAK activity is enhanced by hypertrophic stimuli before the onset of overt hypertrophy. Herein, we report that conditional deletion of FAK from the myocardium of adult mice did not affect basal cardiac performance, myocyte viability, or myofibrillar architecture. However, deletion of FAK abolished the increase in left ventricular posterior wall thickness, myocyte cross-sectional area, and hypertrophy-associated atrial natriuretic factor induction following pressure overload. Myocyte-restricted deletion of FAK attenuated the initial wave of extracellular signal-regulated kinase activation and cFos expression induced by adrenergic agonists and biomechanical stress. In addition, we found that persistent challenge of mice with myocyte-restricted FAK inactivation leads to enhanced cardiac fibrosis and cardiac dysfunction in comparison to challenged genetic controls. These studies show that loss of FAK impairs normal compensatory hypertrophic remodeling without a concomitant increase in apoptosis in response to cardiac pressure overload and highlight the possibility that FAK activation may be a common requirement for the initiation of this compensatory response.

Keywords

FAK; integrins; heart; hypertrophy; heart failure; signaling

In the face of chronic pressure or volume overload, the adult heart undergoes pathological hypertrophic growth. This response is characterized by an increase in cardiomyocyte size and myofibrillar content as well as an altered pattern of cardiac gene expression, including induction of immediate early genes and reexpression of several fetal gene transcripts. Initially these changes are compensatory, but may eventually lead to decreases in cardiac performance and heart failure.[1] The precise molecular mechanisms that regulate anabolic or pathological myocardial hypertrophy are not completely known, but studies have implicated a variety of neuroendocrine and autocrine factors, many of which act through G protein-coupled receptors (in particular those coupled to $G\alpha_q$).[2]

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Disclosures
None.

Extensive evidence indicates that extracellular matrix (ECM)/integrin receptor signaling is also an important regulator of myocardial hypertrophy. Upregulation of collagen III, fibronectin, osteopontin, or their cognate integrin receptors, β_1, α_3, and α_5, correlates with the advancement of cardiac hypertrophy in animal models.[3] Also transgenic mice that express a myocyte-restricted activated α_5 integrin develop profound cardiac hypertrophy,[4] whereas myocyte-restricted deletion of the β_1 integrin in adult mouse hearts leads to a dilated cardiomyopathy and concomitant heart failure.[5,6] In addition, mutations in the structural focal adhesion proteins muscle LIM protein and metavinculin are associated with dilated cardiomyopathy in patients.[7,8] Collectively, these studies underscore the possibility that defects in integrin signaling may play a direct role in regulating cardiomyocyte sarcomere formation and function during cardiac development and disease.

One of the major proteins involved in the integrin intracellular signaling cascade is the nonreceptor protein tyrosine kinase, focal adhesion kinase (FAK), which is strongly and rapidly activated by various growth factors and by ligation of all β_1-, β_3-, or β_5-containing integrins.[9] Through multiple protein-protein interactions, activation of FAK results in the subsequent activation of the extracellular signal-regulated kinase (ERK) and c-Jun N-terminal kinase (JNK) growth promoting mitogen-activated protein (MAP) kinases and the cytoskeletal regulating small molecular weight GTPases Rac and Rho, some of the same molecules implicated in myocyte hypertrophy.[2,9]

Although a direct role for FAK in cardiac growth and development has yet to be examined, germline deletion of FAK results in general mesodermal defects and embryonic lethality between embryonic day 7.5 (E7.5) to E10 (similar to both fibronectin$^{-/-}$ and $\alpha_5^{-/-}$ mice) and *fak*$^{-/-}$ hearts lack separate mesocardial and endocardial layers, indicative of a defect in cardiomyocyte maturation.[10-12] Interestingly, recent work by our group and by others clearly indicates that FAK is activated in cultured cardiomyocytes by a variety of hypertrophic stimuli[13-16] and that increased cardiac FAK activity is induced following pressure overload in vivo.[17-20] The idea that FAK activation may play a direct role in the development of cardiomyocyte hypertrophy is evident from our initial findings that the activation of FAK is required for phenylephrine (PE)-stimulated hypertrophy of cultured cells and similar findings from others that FAK is required for maximal endothelin-1 and stretch-induced hypertrophy in vitro.[14,21,22]

To test the possibility that FAK inactivation could prevent cardiac hypertrophy in vivo, we generated a mouse model that produces a conditional myocyte-specific deletion of FAK in the adult heart. In support of recent findings from Peng et al, we show that inactivation of FAK in the adult myocardium does not affect basal cardiac function.[23] However, we show that hearts with myocyte-specific FAK depletion do not develop concentric hypertrophy on induction of biomechanical stress by transverse aortic constriction (TAC). These studies show that FAK plays a significant role in the development of compensatory hypertrophy in the intact myocardium and highlight the possibility that FAK serves to integrate growth signals from various hypertrophic stimuli.

Materials and Methods

Generation of MFKO Mice

Drs Louis Reichardt and Hilary Beggs (University of California, San Francisco) graciously provided the *fak*^{flox} mice, and Dr Kenneth Chien (Harvard Medical School) graciously provided the *mlc2v*^{Cre} knock-in mice.[24,25] All mice were backcrossed to the C57black6 background at least 8 generations before subsequent breeding. DNA isolated from tail snips or tissues was subjected to PCR analysis using primers specific for Cre and the presence of the targeted or recombined FAK allele as described previously.[24,25] Mice were housed in a

University Animal Care Facility accredited by the American Association for Accreditation of Laboratory Animal Care.

Statistics

Data are presented as mean±SEM. Means were compared by 2-tailed Student *t* test. $P<0.05$ was considered statistically significant.

See the online data supplement, available at http://circres.ahajournals.org, for complete details of the experimental procedures used herein.

Results

Myocyte-Restricted Deletion of FAK in Adult Mice

We used Cre/loxP technology to test the possibility that FAK inactivation could prevent cardiac hypertrophy in the intact myocardium. To inactivate the *fak* gene in a myocyte-restricted fashion in mice, we bred fak^{flox} mice to those that express Cre recombinase under the control of the ventricle specific *mlc2v* promoter ($mlc2v^{Cre/wt}$)[25] to obtain $fak^{flox/flox}/mlc2v^{Cre/wt}$ mice, hereafter, referred to as myocyte specific *fak* knockout mice (MFKO) (Figure 1A).

MFKO mice were born with the expected Mendelian frequency (Figure 1A), and we observed the selective appearance of the excised, recombined *fak* gene in the MFKO ventricles at 4 weeks postnatal (not shown), with maximal recombination occurring by 3 months (Figure 1B). Western analysis confirmed that FAK protein was dramatically reduced in MFKO ventricular lysate by 3 months postnatal, whereas FAK protein levels remained constant in other tissues including skeletal muscle, stomach, and brain (Figure 1C and 1D). The low level of FAK protein remaining in the ventricular lysates from 3 months onward was likely attributable to its continuing expression in resident nonmyocyte cells (ie, cardiac fibroblasts, smooth muscle, and endothelial cells) that do not express Mlc2v.

FAK Is Not Required for Basal Cardiac Function

Disruption of FAK expression in the adult heart did not lead to any overt abnormalities. Both sexes of MFKO mice lived a normal lifespan, were fertile, and females did not exhibit any complications during pregnancy, indicating that FAK is not required for the maintenance of normal heart function. In confirmation of this, we found no evidence of left ventricular (LV) dysfunction as assessed by fractional shortening (FS) and ejection fraction (EF) derived from M-mode echocardiographs of the left ventricle of young (4-month) or old (14-month) hearts from MFKO mice in comparison with aged matched genetic controls (Figure 2A and the Table). At 14 months of age, both lines of mice had a significant reduction in FS and MFKO mice had a significant reduction in EF when compared with the 4-month-old controls, but no significant differences in these parameters were observed between age-matched controls (Figure 2A and the Table). Importantly, no significant difference was observed in posterior (PW) or intraventricular septal (IVS) wall thickness or LV chamber size, between the 4-month-old MFKO and genetic controls ($fak^{flox/flox}$mlc2v^{wt} [Table] or $fak^{wt/wt}$mlc2v^{Cre} [data not shown]). Hemodynamic analysis by cardiac catheterization also revealed no significant differences in intrinsic contractility, diastolic function, and cardiac output between control and MFKO mice at 4 months of age, consistent with a previous report[23] (and data not shown). In addition, 4- to 5-month-old MFKO mice had similar LV weight (LVW):body weight (BW) ratios (Figure 3C), cardiomyocyte cross-sectional area, myofibrillar organization, and levels of fibrosis compared with littermate controls (Figures 2C and 2D and 3C), indicating that the young MFKO mice are phenotypically indistinguishable from genetic controls.

Myocyte-restricted FAK deletion did have a slight but significant effect on heart growth during aging. HW/BW was significantly decreased in 14-month MFKO mice relative to aged matched controls (Figure 2B), and myocyte cross-sectional area was reduced slightly (by 5%; P=0.04; data not shown). Consistent with these findings, serial echocardiographic measurements revealed a significant increase in thickness of LV posterior wall from 4 to 14 months in control mice but not in MFKO mice (Table). Histochemical analysis of hearts from aged MFKO mice (14 months) revealed an increase in interstitial fibrosis in comparison to age matched controls (Figure 2C and supplemental Table I), but no noticeable differences were found in sarcomeric integrity or myocyte alignment in comparison to hearts from age-matched control mice (Figure 2C and 2D). Thus, the absence of FAK in cardiac muscle is functionally tolerated, but FAK may regulate anabolic myocyte growth.

We next asked whether the depletion of FAK from myocytes was tolerated because of a compensatory increase in the FAK-related protein PYK2/CADTK, which is known to have increased expression in *fak*$^{-/-}$ embryos and cells.[26,27] However, we found comparable PYK2 protein levels (and activity) in control and MFKO hearts (Figure 2E; data not shown), indicating that PYK2 does not compensate for loss of FAK in this model.

Pressure Overload-Induced Structural Remodeling and Hypertrophic Gene Expression Is Attenuated in MFKO Mice

The absence of underlying developmental defects in the conditional MFKO mice at 4 to 5 months of age renders this model advantageous for determining whether FAK is important for the progression of pathological cardiac hypertrophy. Four-month-old control and MFKO mice were therefore subjected to a minimally invasive aortic banding procedure that provided an approximate 50% reduction in the lumen of the ascending aorta. Blinded echocardiographic measurements revealed several striking differences between the age-matched genetic controls and MFKO hearts following 4 weeks of TAC (Figure 3A through 3C and the Table). Notably, the control TAC mice developed significant thickening of LVPW and IVS (approximately 50% and 30% increases in systole respectively), a significant reduction in left ventricular end diameter (LVED) (approximate 40% decrease in end diastolic chamber dimension), and a 40% increase in FS compared with nonbanded control mice. In contrast, the MFKO mice did not exhibit significant increases in left ventricular posterior wall thickness systole (LVPWT) or FS comparison to the prebanded MFKO mice (Table). Indeed, the percent change of each parameter from pre- to post-TAC was significantly decreased in MFKO hearts in comparison to control hearts (Figure 3A). Measurements from serial cross-sections though banded control and MFKO hearts confirmed a significant reduction in relative LVPWT in 15 of 19 of the 4-week post-TAC MFKO hearts in comparison with the control hearts (Figure 3B; data not shown). Importantly, morphometric analysis revealed a significant decrease in myocyte cross-sectional area in the post-TAC MFKO hearts compared with post-TAC controls (Figure 3C). Although a significant increase in LVW/BW was observed following 4 weeks of TAC in MFKO hearts (possibly attributable to increased fibrosis; see below), the increase in MFKO hearts was reduced relative to control hearts (Figure 3C). Notably, no significant difference in the surgically induced intensity of pressure overload was observed between MFKO and the genetic controls as assessed by Doppler imaging (supplemental Figure I). A comparable concentric hypertrophic response was observed in our 2 groups of control mice (*fak*$^{flox/flox}$mlc2v^{wt} [shown] and *fak*$^{wt/wt}$mlc2v^{Cre} [data not shown]), indicating that the lack of concentric hypertrophy observed in the MFKO mice was not attributable to adverse effects of either the LoxP-targeted *fak* allele, expression of Cre, or haploinsufficiency of Mlc2v.

While this report was under review, a separate article was published in which the authors suggested that cardiac-restricted inactivation of FAK leads to cardiac dilation because of an eccentric hypertrophic response.[23] Therein, Peng et al examined mice at only 1 time point

following TAC (10 days), which was not sufficient to induce concentric hypertrophy in their control animals, but they observed a significant increase LV chamber dimension in their CFKO mice when compared with banded controls.[23] To determine whether the change in chamber size was a primary or secondary response in our model, we banded a second group of mice (n=7 control and MFKO) and examined their hypertrophic response by blinded echocardiography at 10 days and 4 weeks following TAC. As shown in Figure 3D, no significant differences were observed between either LVPW or LVED between MFKO and control hearts at 10 days following banding. However, similar to our previous large study of mice (the Table), this separate study revealed a significant decrease in LVPW in 4-week banded MFKO hearts in comparison with 4-week banded control hearts. This change in LVPWT was accompanied by an increase in LVED in MFKO hearts relative to banded genetic controls but, like in our previous study, MFKO LVED was not significantly increased from baseline MFKO hearts. Because the echocardiographic measurements for this new data set were taken while mice were under heavier sedation (see Materials and Methods for details), the data were analyzed separately and are presented in full in supplemental Table II. These studies confirm our contention that FAK inactivation inhibits the compensatory concentric hypertrophic response following TAC.

Enhanced perivascular fibrosis as assessed by Masson's Trichrome staining was observed following TAC in both control and MFKO TAC hearts with more interstitial fibrosis found in MFKO TAC hearts (blue staining, Figure 4A, top; supplemental Table I). However, there was no significant difference in sarcomeric structure or integrity between control and MFKO hearts following TAC as visualized by troponin T staining (Figure 4A and 4B). Also, we did not find any significant difference in the level of apoptosis in control or MFKO hearts at baseline or following banding (Figure 4B and supplemental Figure II). Although PYK2 expression did increase following TAC, we found no significant difference in PYK2 levels (or activity) in MFKO TAC hearts relative to control TAC hearts (Figure 4C; data not shown). Collectively, these data indicate that FAK is not required for the maintenance of proper myocyte architecture but is required for the promotion of biomechanical stress-induced myocyte hypertrophy.

Hearts from control and MFKO nonbanded and 4-week TAC-induced mice were also analyzed for hypertrophic marker gene expression by quantitative RT-PCR. We examined the expression of atrial natruretic factor (ANF) because increased levels of this gene correlate highly with the degree of hypertrophy observed in several animal models.[28] As shown in Figure 5, ANF expression was, as expected, dramatically increased in the control mice after TAC. This increase was virtually abolished in the MFKO hearts (Figure 5A). Interestingly, banded MFKO hearts displayed a significant increase in α-myosin heavy chain (α-MHC) compared with banded controls whereas no significant difference in β-MHC or skeletal α-actin was observed between the 2 groups postbanding (data not shown). Collectively our data indicate that loss of FAK alters the structural and a subset of the molecular changes induced by pressure overload.

Persistent Pressure Overload in MFKO Mice Leads to Cardiac Dysfunction

Because the MFKO TAC hearts exhibited a reduced compensatory response following 4 weeks of pressure overload, we asked whether the MFKO mice would progress to heart failure after a more persistent challenge. Although greater than 90% survival was observed in control and MFKO mice banded for 8 to 12 weeks, the wet lung weights from the MFKO 8-week TAC group were significantly higher than nonbanded mice, indicating substantial congestion and the likelihood that the MFKO hearts were failing (Figure 6A). Accordingly, significant depression of cardiac output, a measure of contractile performance, was apparent in MFKO mice 12 weeks after TAC (Figure 6B). The decreased contractility in the MFKO mice was associated with an increase in interstitial fibrosis relative to control-banded hearts at 8 and 12 weeks (supplemental Table I). However, sarcomeric integrity was maintained in these hearts

as assessed by troponin T staining (data not shown). These data indicate that the MFKO mice develop heart failure in response to chronic pressure overload and suggest the possibility that the blunted compensatory hypertrophic response in these mice was not sufficient to meet the increased hemodynamic demand.

Absence of FAK Impairs MAP Kinase Activation Following Hypertrophic Stimuli

It is possible that the requirement for functional signaling through FAK in pressure overload-induced hypertrophy may be attributable to the ability of FAK to coactivate an essential growth pathway. Because ERK activation by hypertrophic stimuli precedes the characteristic changes in gene expression and inhibition of this signaling pathway can lessen maladaptive hypertrophic growth,[29,30] we evaluated ERK activity in control and MFKO hearts subjected to aortic banding for 1 and 4 days. Immunoblot analysis revealed reduced levels of ERK phosphorylation in MFKO hearts in comparison with control hearts after acute aortic constriction, although no change was observed at baseline (Figure 7A). Because cardiac hypertrophy induced by pressure overload and neurohumoral signals share some common mechanisms, we also examined ERK activation induced by adrenergic agonists in control and MFKO hearts. We injected an adrenergic cocktail previously shown to induce a strong hypertrophic response in mice.[31] As shown in Figure 7B, ERK was strongly activated in control hearts following acute adrenergic agonist injection but was significantly lower in the MFKO hearts. We also examined activation of the immediate early gene cFos following acute banding. We found that cFos expression was significantly upregulated in control hearts 4 days after TAC but returned to baseline by the 7-day time point (Figure 7C). However, no significant increase in cFos expression was observed in MFKO hearts following banding, in accordance with data indicating that activation of cFos is dependent on ERK signaling (Figure 7C). These data indicate that ERK activation is uncoupled in the absence of FAK and highlight the possibility that FAK activity is required for the initial wave of signaling induced by both pressure overload and adrenergic stress.

Discussion

Previous work by our group and others indicate that FAK is important in the hypertrophic response. Herein, we have used a mouse model in which myocyte-restricted FAK deletion in early adulthood results in mice whose hearts are functionally and morphologically similar those of control mice, confirming recent work from Peng et al.[23] However, we found that myocyte-restricted deletion of FAK depressed heart growth during aging and markedly reduced the hypertrophic response to transverse aortic constriction as assessed by examination of several conserved features of pathological hypertrophy. Our data indicate that inhibition of FAK signaling alone is sufficient to suppress the diverse signaling inputs within the complex myocyte microenvironment in vivo that can modulate cardiac growth induced by stress and aging.

Our findings that FAK inactivation attenuates concentric hypertrophic remodeling differ with the conclusions drawn in the aforementioned manuscript by Peng et al, who observed chamber dilation (or eccentric growth) in FAK-null hearts following 10 days of TAC.[23] This discrepancy could be attributable to differences in the timing of FAK deletion, the extent of aortic constriction imposed in the 2 models, and/or the genetic background of the mice used in these studies. (See the online data supplement for further details.) The late onset of recombination in our model system (3 months postnatal) was somewhat surprising because the *mlc2v* promoter has been reported to drive Cre expression as early as E8.5; however, our results are consistent with several studies showing that maximal recombination of targeted genes is not observed until at least 6 weeks after birth (and some even later) using the same line.[5,32] A distinct advantage of using our MFKO line is that it enables examination of a direct role for

FAK in the progression of adult onset cardiac diseases in mice that do not have any underlying developmental defects.

It is interesting that FAK is dispensable for basal myocyte function, whereas depletion of integrin receptors or various cytoskeletal components including vinculin, muscle LIM protein, desmin, plakoglobin, or N-cadherin all lead to rapid defects in sarcomeric integrity accompanied by considerable interstitial fibrosis.[3] These data indicate that although FAK co-resides with these cytoskeletal proteins in Z-disks, it is not an integral component of the contractile apparatus in myocytes. However, we did find that following persistent challenge MFKO hearts exhibited systolic dysfunction relative to control-banded hearts. This was likely a secondary effect attributable to insufficient compensation in response to TAC, because this model induces a rapid and severe remodeling response in control animals. There is some evidence indicating that the switch from hypertrophy to cardiac failure may involve activation of apoptotic pathways. Although we did observe a greater extent of interstitial fibrosis in banded and aged MFKO hearts (likely because of increased myocyte dropout), we did not observe an increase in apoptosis or apoptotic markers at the time points examined. These data corroborate our previous studies showing that FAK inhibition in cultured cardiomyocytes did not induce programmed cell death and studies from others, indicating that targeted deletion of FAK in keratinocytes or neurons did not induce apoptosis (see the online data supplement for further discussion).[14,24,33] Taken together, these data indicate that the ensuing dysfunction in MFKO hearts is likely attributable to a passive necrotic process.

As a multifunctional adapter protein that associates with the integrin cytoplasmic tail, with actin, and with several adapter proteins that regulate various catalytic signaling molecules, FAK is an ideal candidate to sense and respond to hormonal imbalances and/or alterations in the force-generating actin cytoskeleton by inducing coordinate activation of downstream hypertrophic growth signaling pathways. Our study revealed that myocyte-specific depletion of FAK dramatically reduces biomechanical stress or hypertrophic agonist-stimulated ERK activation. Given that activation of the MEK1-ERK1/2 signaling pathway in vivo precedes the induction of hypertrophic gene expression and that inhibition of these signaling pathways can lessen hypertrophic growth, it is reasonable to assume that reduced ERK activation may be the primary mechanism responsible for the blunted hypertrophic response in MFKO hearts.[2,29,30]

In conclusion, our analysis of MFKO mice reveals that FAK is essential for the heart to sense and transduce a biomechanical insult into a compensatory hypertrophic response. This effect is likely attributable to the ability of FAK to modify the initial wave of ERK-dependent signaling induced by hypertrophic stimuli. Because cardiac hypertrophy induced by pressure overload and neurohumoral signals share common mechanisms, and because signaling from adrenergic receptors is depressed in MFKO hearts, it is possible that activation of FAK may be a common requirement for the initiation of the pathological hypertrophic response. Interestingly, recent studies have shown that whereas disruption of MEKK1 (like FAK) eventually leads to cardiac decompensation following pressure overload, inactivation of MEKK1 actually prevents systolic dysfunction induced by $G\alpha_q$.[34,35] Thus, further studies coupling various hypertrophic model systems with cardiac-targeted FAK disruption should lead to important new information with regard to the transition from compensatory to decompensated stages of cardiac hypertrophy and aid in determining under which circumstances FAK-dependent signaling pathways may be protective or detrimental. Whether targeted inhibition of FAK would be beneficial for the treatment of heart disease will likely depend on the contributing factors for disease.

Acknowledgments

We thank Dr Oliver Smithies (University of North Carolina) and Dr J. Thomas Parsons (University of Virginia) for a critical review of the manuscript.

Sources of Funding

This work was supported in part by grants from the NIH/National Heart, Lung, and Blood Institute (HL-081844 and HL-HL071054) and the American Heart Association (0355776U to J.M.T and NS19090 to L.F.R). L.A.D. was supported by an American Heart Predoctoral Fellowship (0415384U).

References

1. Frey N, Olson EN. Cardiac hypertrophy: the good, the bad, and the ugly. Annu Rev Physiol 2003;65:45–79. [PubMed: 12524460]
2. Dorn GW 2nd, Force T. Protein kinase cascades in the regulation of cardiac hypertrophy. J Clin Invest 2005;115:527–537. [PubMed: 15765134]
3. Brancaccio M, Hirsch E, Notte A, Selvetella G, Lembo G, Tarone G. Integrin signalling: the tug-of-war in heart hypertrophy. Cardiovasc Res 2006;70:422–433. [PubMed: 16466704]
4. Valencik ML, McDonald JA. Cardiac expression of a gain-of-function alpha(5)-integrin results in perinatal lethality. Am J Physiol Heart Circ Physiol 2001;280:H361–H367. [PubMed: 11123252]
5. Shai SY, Harpf AE, Babbitt CJ, Jordan MC, Fishbein MC, Chen J, Omura M, Leil TA, Becker KD, Jiang M, Smith DJ, Cherry SR, Loftus JC, Ross RS. Cardiac myocyte-specific excision of the beta1 integrin gene results in myocardial fibrosis and cardiac failure. Circ Res 2002;90:458–464. [PubMed: 11884376]
6. Valencik ML, Keller RS, Loftus JC, McDonald JA. A lethal perinatal cardiac phenotype resulting from altered integrin function in cardiomyocytes. J Card Fail 2002;8:262–272. [PubMed: 12397575]
7. Knoll R, Hoshijima M, Hoffman HM, Person V, Lorenzen-Schmidt I, Bang ML, Hayashi T, Shiga N, Yasukawa H, Schaper W, McKenna W, Yokoyama M, Schork NJ, Omens JH, McCulloch AD, Kimura A, Gregorio CC, Poller W, Schaper J, Schultheiss HP, Chien KR. The cardiac mechanical stretch sensor machinery involves a Z disc complex that is defective in a subset of human dilated cardiomyopathy. Cell 2002;111:943–955. [PubMed: 12507422]
8. Olson TM, Illenberger S, Kishimoto NY, Huttelmaier S, Keating MT, Jockusch BM. Metavinculin mutations alter actin interaction in dilated cardiomyopathy. Circulation 2002;105:431–437. [PubMed: 11815424]
9. Parsons JT. Focal adhesion kinase: the first ten years. J Cell Sci 2003;116:1409–1416. [PubMed: 12640026]
10. Furuta Y, Ilic D, Kanazawa S, Takeda N, Yamamoto T, Aizawa S. Mesodermal defect in late phase of gastrulation by a targeted mutation of focal adhesion kinase, FAK. Oncogene 1995;11:1989–1995. [PubMed: 7478517]
11. Ilic D, Furuta Y, Kanazawa S, Takeda N, Sobue K, Nakatsuji N, Nomura S, Fujimoto J, Okada M, Yamamoto T. Reduced cell motility and enhanced focal adhesion contact formation in cells from FAK-deficient mice. Nature 1995;377:539–544. [PubMed: 7566154]
12. Yang JT, Bader BL, Kreidberg JA, Ullman-Cullere M, Trevithick JE, Hynes RO. Overlapping and independent functions of fibronectin receptor integrins in early mesodermal development. Dev Biol 1999;215:264–277. [PubMed: 10545236]
13. Torsoni AS, Constancio SS, Nadruz W Jr, Hanks SK, Franchini KG. Focal adhesion kinase is activated and mediates the early hypertrophic response to stretch in cardiac myocytes. Circ Res 2003;93:140–147. [PubMed: 12805241]
14. Taylor JM, Rovin JD, Parsons JT. A role for focal adhesion kinase in phenylephrine-induced hypertrophy of rat ventricular cardiomyocytes. J Biol Chem 2000;275:19250–19257. [PubMed: 10749882]
15. Sadoshima J, Qiu Z, Morgan JP, Izumo S. Tyrosine kinase activation is an immediate and essential step in hypotonic cell swelling-induced ERK activation and c-fos gene expression in cardiac myocytes. EMBO J 1996;15:5535–5546. [PubMed: 8896447]

16. Kuppuswamy D, Kerr C, Narishige T, Kasi VS, Menick DR, Cooper G 4th. Association of tyrosine-phosphorylated c-Src with the cytoskeleton of hypertrophying myocardium. J Biol Chem 1997;272:4500–4508. [PubMed: 9020175]

17. Laser M, Willey CD, Jiang W, Cooper Gt, Menick DR, Zile MR, Kuppuswamy D. Integrin activation and focal complex formation in cardiac hypertrophy. J Biol Chem 2000;275:35624–35630. [PubMed: 10958798]

18. Franchini KG, Torsoni AS, Soares PH, Saad MJ. Early activation of the multicomponent signaling complex associated with focal adhesion kinase induced by pressure overload in the rat heart. Circ Res 2000;87:558–565. [PubMed: 11009560]

19. Melendez J, Welch S, Schaefer E, Moravec CS, Avraham S, Avraham H, Sussman MA. Activation of pyk2/related focal adhesion tyrosine kinase and focal adhesion kinase in cardiac remodeling. J Biol Chem 2002;277:45203–45210. [PubMed: 12228222]

20. Yi XP, Wang X, Gerdes AM, Li F. Subcellular redistribution of focal adhesion kinase and its related nonkinase in hypertrophic myocardium. Hypertension 2003;41:1317–1323. [PubMed: 12732587]

21. Pham CG, Harpf AE, Keller RS, Vu HT, Shai SY, Loftus JC, Ross RS. Striated muscle-specific beta (1D)-integrin and FAK are involved in cardiac myocyte hypertrophic response pathway. Am J Physiol Heart Circ Physiol 2000;279:H2916–H2926. [PubMed: 11087248]

22. Kovacic-Milivojevic B, Roediger F, Almeida EA, Damsky CH, Gardner DG, Ilic D. Focal adhesion kinase and p130Cas mediate both sarcomeric organization and activation of genes associated with cardiac myocyte hypertrophy. Mol Biol Cell 2001;12:2290–2307. [PubMed: 11514617]

23. Peng X, Kraus MS, Wei H, Shen TL, Pariaut R, Alcaraz A, Ji G, Cheng L, Yang Q, Kotlikoff MI, Chen J, Chien K, Gu H, Guan JL. Inactivation of focal adhesion kinase in cardiomyocytes promotes eccentric cardiac hypertrophy and fibrosis in mice. J Clin Invest 2006;116:217–227. [PubMed: 16374517]

24. Beggs HE, Schahin-Reed D, Zang K, Goebbels S, Nave KA, Gorski J, Jones KR, Sretavan D, Reichardt LF. FAK deficiency in cells contributing to the basal lamina results in cortical abnormalities resembling congenital muscular dystrophies. Neuron 2003;40:501–514. [PubMed: 14642275]

25. Chen J, Kubalak SW, Chien KR. Ventricular muscle-restricted targeting of the RXRalpha gene reveals a non-cell-autonomous requirement in cardiac chamber morphogenesis. Development 1998;125:1943–1949. [PubMed: 9550726]

26. Klingbeil CK, Hauck CR, Hsia DA, Jones KC, Reider SR, Schlaepfer DD. Targeting Pyk2 to beta 1-integrin-containing focal contacts rescues fibronectin-stimulated signaling and haptotactic motility defects of focal adhesion kinase-null cells. J Cell Biol 2001;152:97–110. [PubMed: 11149924]

27. Sieg DJ, Ilic D, Jones KC, Damsky CH, Hunter T, Schlaepfer DD. Pyk2 and Src-family protein-tyrosine kinases compensate for the loss of FAK in fibronectin-stimulated signaling events but Pyk2 does not fully function to enhance FAK-cell migration. EMBO J 1998;17:5933–5947. [PubMed: 9774338]

28. Caron KM, James LR, Kim HS, Knowles J, Uhlir R, Mao L, Hagaman JR, Cascio W, Rockman H, Smithies O. Cardiac hypertrophy and sudden death in mice with a genetically clamped renin transgene. Proc Natl Acad Sci U S A 2004;101:3106–3111. [PubMed: 14978280]

29. Wang Y, Huang S, Sah VP, Ross J Jr, Brown JH, Han J, Chien KR. Cardiac muscle cell hypertrophy and apoptosis induced by distinct members of the p38 mitogen-activated protein kinase family. J Biol Chem 1998;273:2161–2168. [PubMed: 9442057]

30. Bueno OF, De Windt LJ, Tymitz KM, Witt SA, Kimball TR, Klevitsky R, Hewett TE, Jones SP, Lefer DJ, Peng CF, Kitsis RN, Molkentin JD. The MEK1-ERK1/2 signaling pathway promotes compensated cardiac hypertrophy in transgenic mice. EMBO J 2000;19:6341–6350. [PubMed: 11101507]

31. Maass AH, Ikeda K, Oberdorf-Maass S, Maier SK, Leinwand LA. Hypertrophy, fibrosis, and sudden cardiac death in response to pathological stimuli in mice with mutations in cardiac troponin T. Circulation 2004;110:2102–2109. [PubMed: 15466629]

32. Chien KR. To Cre or not to Cre: the next generation of mouse models of human cardiac diseases. Circ Res 2001;88:546–549. [PubMed: 11282885]

33. Essayem S, Kovacic-Milivojevic B, Baumbusch C, McDonagh S, Dolganov G, Howerton K, Larocque N, Mauro T, Ramirez A, Ramos DM, Fisher SJ, Jorcano JL, Beggs HE, Reichardt LF, Ilic D. Hair cycle and wound healing in mice with a keratinocyte-restricted deletion of FAK. Oncogene 2006;25:1081–1089. [PubMed: 16247468]

34. Sadoshima J, Montagne O, Wang Q, Yang G, Warden J, Liu J, Takagi G, Karoor V, Hong C, Johnson GL, Vatner DE, Vatner SF. The MEKK1-JNK pathway plays a protective role in pressure overload but does not mediate cardiac hypertrophy. J Clin Invest 2002;110:271–279. [PubMed: 12122119]

35. Minamino T, Yujiri T, Terada N, Taffet GE, Michael LH, Johnson GL, Schneider MD. MEKK1 is essential for cardiac hypertrophy and dysfunction induced by Gq. Proc Natl Acad Sci U S A 2002;99:3866–3871. [PubMed: 11891332]

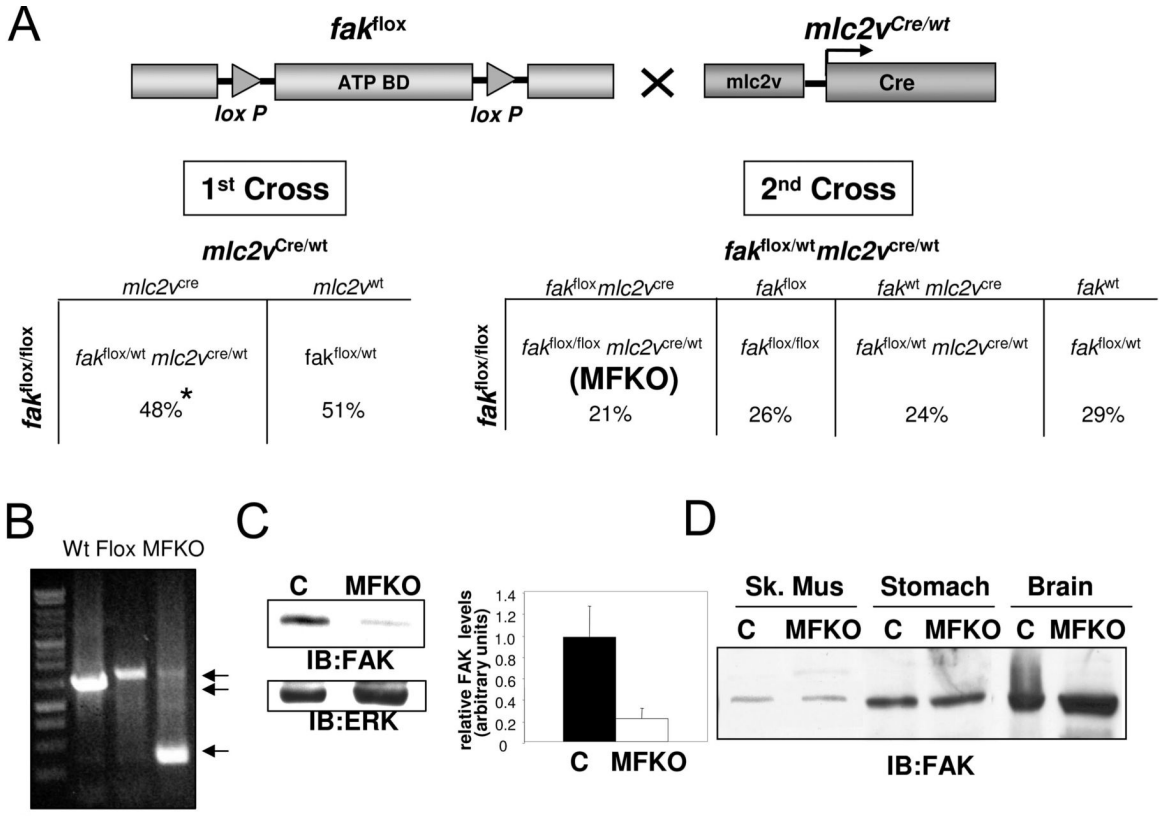

Figure 1.

Targeted myocyte specific disruption of mouse focal adhesion kinase. a, Diagrammatic representation of the *fak*$^{flox/flox}$ and *mlc2v*$^{Cre/wt}$ breeding strategy (n=725). b, PCR analyses of heart DNA from wild-type mice (wt) (1.4-kb band), Flox (1.6-kb band), and MFKO mice (recombined allele generates 327-bp band). c, Protein extracts from 3-month-old control (C) and MFKO ventricles were processed by SDS-PAGE and probed with anti-FAK or anti-ERK antibodies. Densitometry quantification of FAK expression compared with an ERK loading control (right, n=3). d, Protein extracts from skeletal muscle, stomach, and brain from 3-month-old control (C) and MFKO mice processed as described above using an anti-FAK antibody.

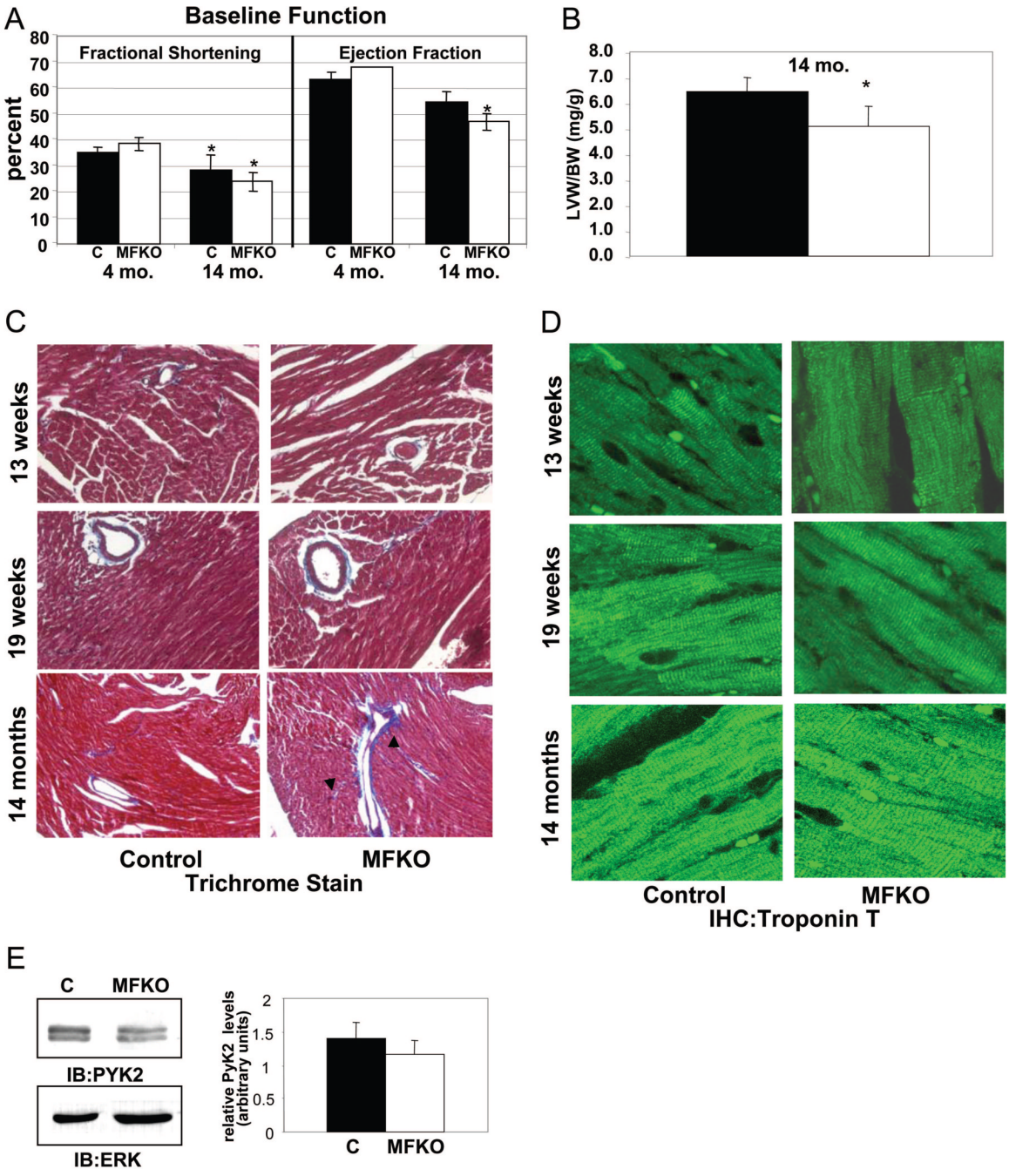

Figure 2.

FAK is required for myocyte growth but not for maintenance of myocyte cyto-architecture. a, Baseline function of 4-month and 14-month-old control (C) and MFKO hearts as determined by M-mode echocardiography (**P*<0.05 when compared with baseline genetic control; see the Table for measured values and number of mice/condition). b, LV weight to body weight ratio for 14-month-old control (n=10) and MFKO (n=6) mice. **P*<0.05. c, Left ventricles from indicated ages of control and MFKO mice were processed for histology as described in Materials and Methods and stained with Masson's Trichrome to reveal myocyte organization and level of fibrosis (blue). d, Sections of left ventricle from control and MFKO mice were stained with an anti-cardiac troponin T antibody to reveal myofibrillar organization. e,

Immunoblot of protein extracts from 4-month control or MFKO ventricles were probed with anti-PYK2 (top) or anti-ERK antibodies (bottom). Densitometry quantification of PYK2 expression compared with an ERK loading control (right, n=4).

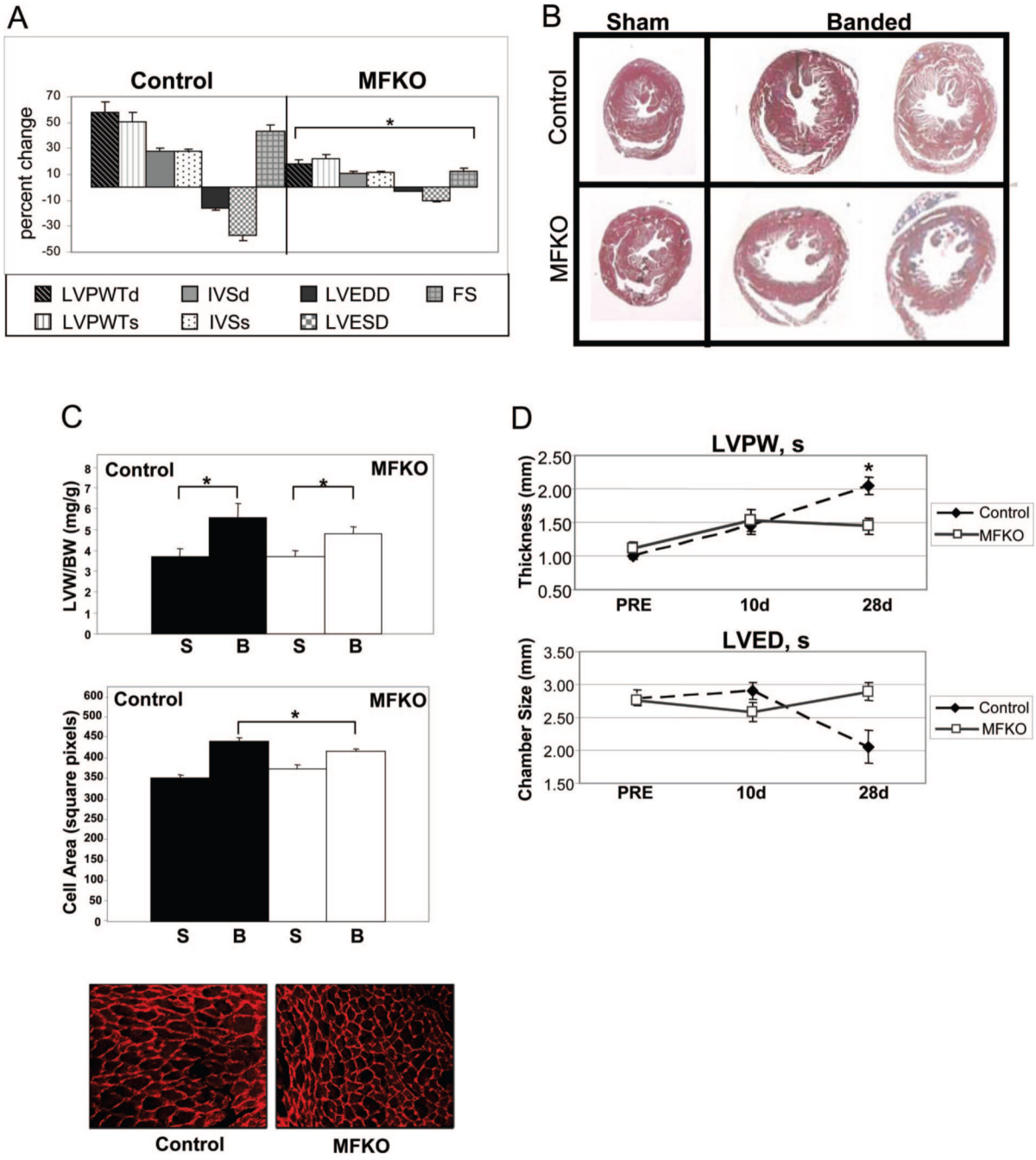

Figure 3.

FAK is essential for pressure overload-induced hypertrophy. a, M-mode echocardiograph measurements of the left ventricles from baseline and banded control and MFKO mice during diastole (d) and systole (s) were analyzed for percent change from pre- to post-TAC. **P*<0.05 when compared with percent change in control mice. b, Ventricles from sham and 4-week banded control and MFKO hearts were stained with Trichrome to reveal gross changes in wall thickness and chamber dimensions following 4-weeks of TAC. c, LVW to BW ratio (top). Cross-sectional myocyte area of sham and 4-week banded control and MFKO myocytes (middle, n=at least 175 per condition). **P*<0.05 for indicated comparison. Representative LV sections stained with lectin reveals reduced cross-sectional area in MFKO myocytes compared

with controls following 4-weeks of TAC (bottom). d, Comparison of LV posterior wall and chamber size in both control and MFKO mice. *P=0.01 vs control 4-week banded control.

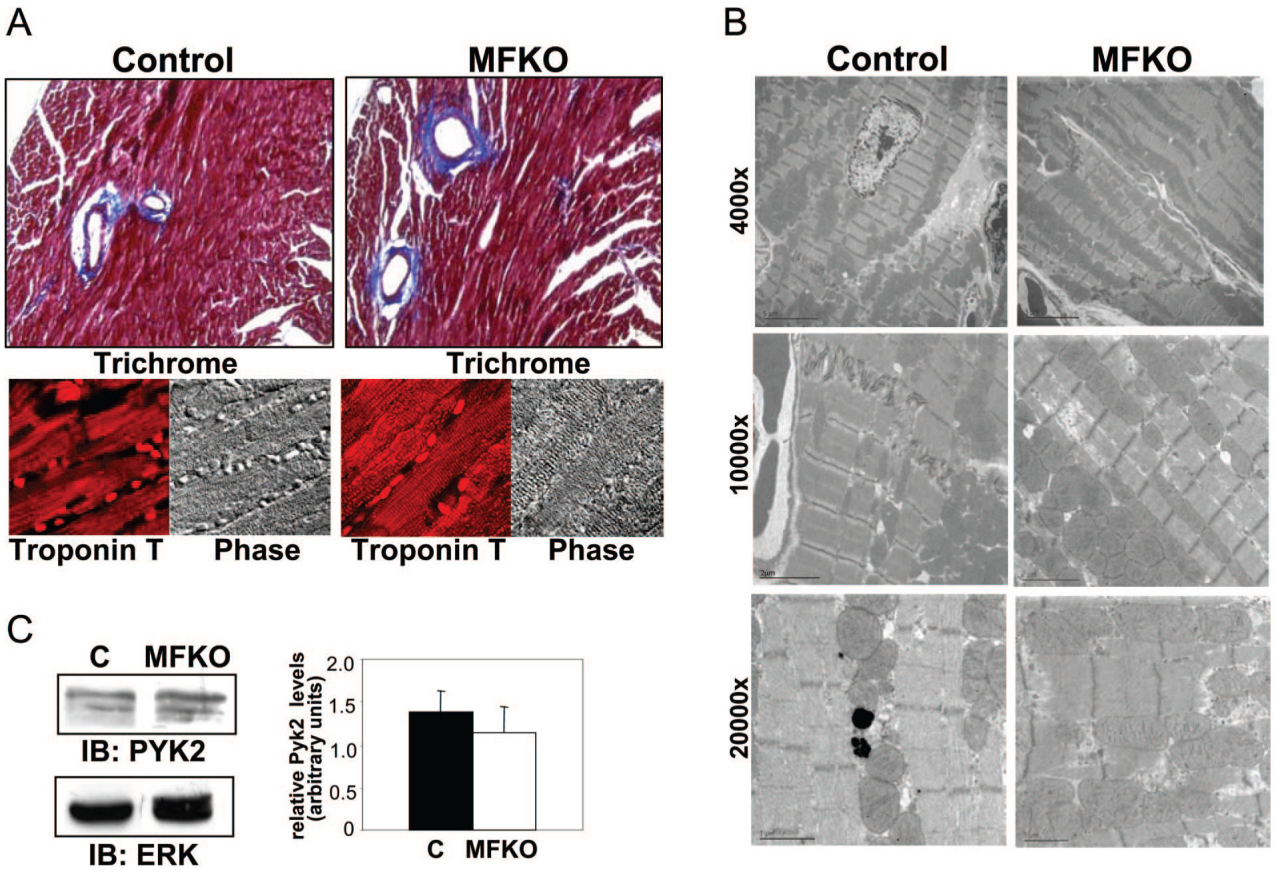

Figure 4.

Post-TAC MFKO hearts have increased fibrosis but MFKO myocytes display normal cytoarchitecture. a, Images of Trichrome-stained sections (×10) (top) or images of sections stained with cardiac troponin T antibody (bottom) (×40) reveal increased fibrosis but normal cell-cell interactions and myofibrillar organization in control and MFKO hearts. b, Transmission electron micrographs from the mid-wall region of the left ventricle of 4-week banded control and MFKO mice taken at ×4000, ×10 000, and ×20 000 magnification. c, Western blot analysis of PYK2 protein levels following 4-week TAC of control (C) and MFKO mice. Densitometry quantification of Pyk2 expression compared with an ERK loading control (right, n=5).

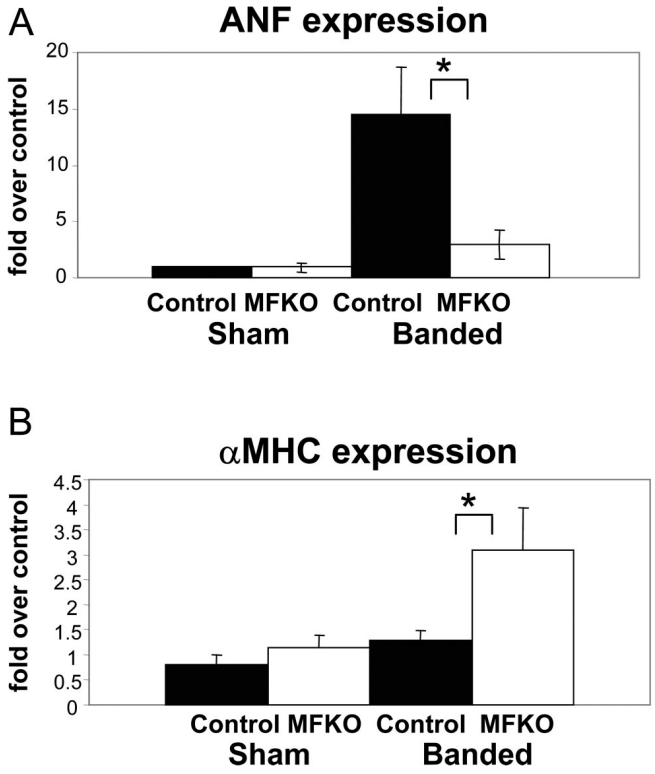

Figure 5.

FAK modulates ANF expression following TAC. Quantitative RT-PCR analysis for ANF (a) and α-MHC (b) RNA levels from sham and banded control and MFKO hearts. Data were normalized to β-actin levels and presented as fold over values from sham control mice. *P=0.03 vs banded control.

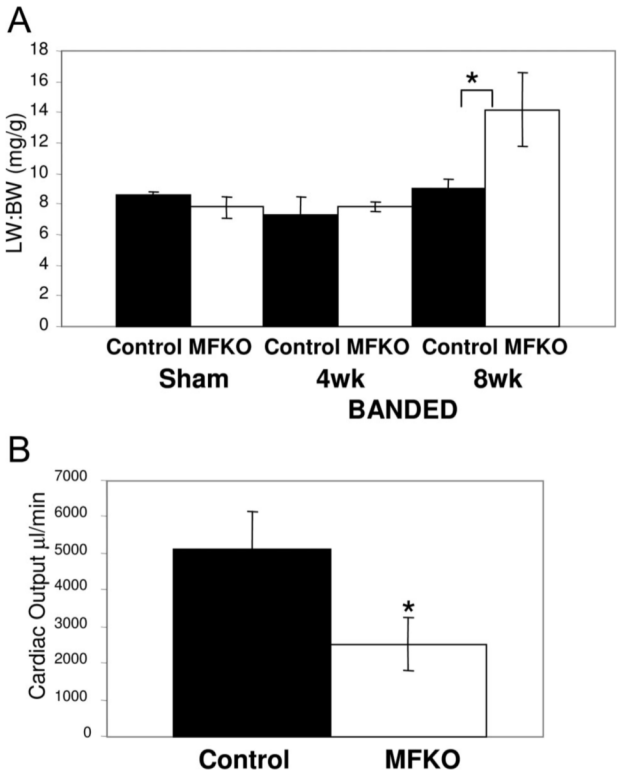

Figure 6.

Chronic banding leads to systolic dysfunction in MFKO mice. a, Wet lung and body weight measurements were taken from control and MFKO sham-treated mice or mice banded for 4 or 8-weeks. **P*=0.03 vs experimental control n=4 (or more) for each group. b, Sham or 12-week banded control and MFKO mice were subjected to cardiac catheterization as described in Materials and Methods to examine contractile function. **P*=0.03 vs 12-week banded control (n=at least 4 for each group).

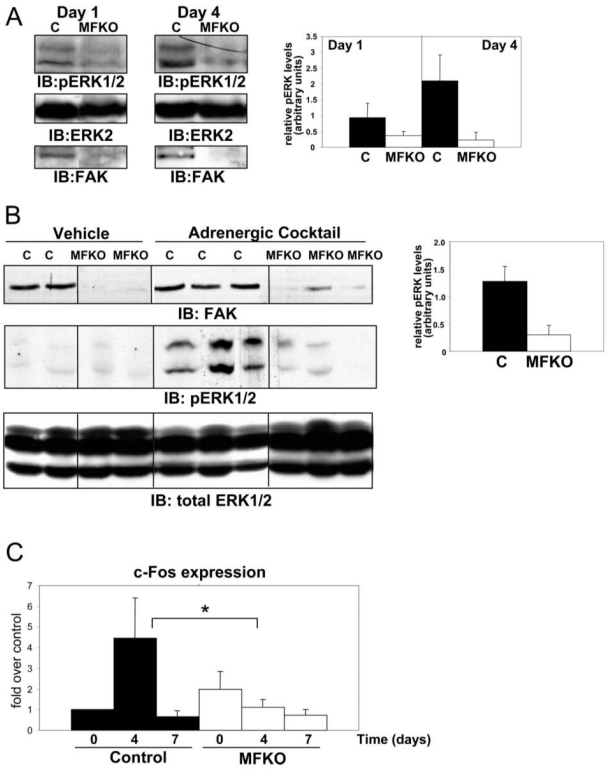

Figure 7.

FAK is required for maximal ERK activation induced by banding and adrenergic stress. a, Western blot analysis of phopspho-ERK1/2 following 1 or 4 days of aortic constriction in control and MFKO mice. Densitometry quantification of phospho-ERK1/2 compared with an ERK loading control (right, n=3). b, Control (C) and MFKO mice were injected with a adrenergic cocktail for 7 minutes containing PE and isoproterenol (30 mg/kg each) or PBS into the intraperitoneal cavity. Ventricular lysate was processed for Western blot analysis using anti-FAK, phospho-ERK1/2, and total ERK1/2 (loading control) antibodies. Densitometry quantification of phospho-ERK1/2 compared with an ERK loading control (right, n=3). c, RNA was extracted from control and MFKO hearts at days 0, 4, and 7 following banding and processed for quantitative RT-PCR using the c-*fos* probe and primers detailed in Materials and Methods. **P*=0.03 vs 4-day banded control mice.

Echocardiographic Analysis in Baseline, Banded, and Aged Mice

	Control			MFKO		
	4 Month (Baseline)	5 Month (Banded)	14 Month (Aged)	4 Month (Baseline)	5 Month (Banded)	14 Month (Aged)
LVEdD, mm	3.1±0.1	2.6±0.1†	3.9±0.3†	3.2±0.1	3.1±0.1§	4.2±0.2‡
LVEsD, mm	2.1±0.1	1.3±0.1†	2.8±0.4†	2.0± 0.1	1.8±0.2§	3.0±0.2‡
LVPWTd, mm	0.8±0.06	1.3±0.1†	1.3±0.2†	1.0±0.1	1.2±0.1	1.1±0.2
LVPWTs, mm	1.1±0.1	1.6±0.1†	1.6±0.2†	1.2±0.1	1.5±0.1¶	1.5±0.3
IVSd, mm	1.1±0.04	1.3±0.1†	1.3±0.2†	1.1±0.04	1.2±0.06¶	1.3±0.3¶
IVSs, mm	1.5±0.1	1.9±0.1†	1.7±0.2*	1.6±0.1	1.7±0.1¶	1.6±0.3
%FS	35.1±2.1	50.3±3.0†	28.5±5.6*	38.5±2.4	43.4±3.1	23.9±3.7¶
%EF	63.3±2.9	81.7±2.6†	54.5±4.0	68.2±0.03	72.7±0.04	47.0±3.3¶
HR	501±31	640±25	492±32	567±24	611±25	479±27

%FS, percent FS; %EF, percent EF; HR, heart rate; d, diastole; s, systole. Four-month (baseline) control, n=22; 14-month control, n=10; 4-month (baseline) MFKO, n=33; 14-month MFKO, n=6; 4 weeks banded control, n=15; 4 weeks banded MFKO, n=2.

LVEdD indicates left ventricular end diastolic diameter; LVEsD, left ventricular end systolic diameter; LVPWTd, left ventricular posterior wall thickness diastole; and LVPWTs, left ventricular posterior wall thickness systole.

† $P<0.001$

* $P<0.05$ vs baseline control;

‡ $P<0.01$

¶ $P<0.05$ vs baseline MFKO;

§ $P<0.01$ vs banded control, n=25.