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Bmx nonreceptor tyrosine kinase has an established role in endothelial and lymphocyte signaling; however, its role in the heart is unknown. To determine whether Bmx participates in cardiac growth, we subjected mice deficient in the molecule (Bmx knockout mice) to transverse aortic constriction (TAC). In comparison with wild-type mice, which progressively developed massive hypertrophy following TAC, Bmx knockout mice were resistant to TAC-induced cardiac growth at the organ and cell level. Loss of Bmx preserved cardiac ejection fraction and decreased mortality following TAC. These findings are the first to demonstrate a necessary role for the Tec family of tyrosine kinases in the heart and reveal a novel regulator (Bmx) of pressure overload–induced hypertrophic growth.

Bmx (also known as Etk) is a member of the Tec family of nonreceptor tyrosine kinases that has a critical role in B-cell development and proliferation.[1,2] The Tec family was originally discovered by the observation that mutations in the Btk family member induce X chromosome–linked agammaglobulinemia (XLA),[3,4] an immunologic disorder characterized by impaired antibody production and B-lymphocyte development. Although structurally similar to Btk, Bmx does not participate in XLA. Bmx has been shown to regulate wound healing in the epidermis[5] and, more recently, the response of skeletal muscle to prolonged ischemia.[6]

Very little is known about the functional significance of Bmx or other members of the Tec family in the heart. An early study documented expression of Bmx mRNA in the endocardium and vasculature of the adult myocardium,[7] but it was only recently that reports suggested that Bmx may be activated by nitric oxide[8] or ischemic preconditioning[9] in the heart. Whereas nonreceptor tyrosine kinases have been functionally implicated in cardiac phenotype, the role of the Tec family is unknown. In the present study, we demonstrate that absence of Bmx function prevents the hypertrophic response of the myocardium to pressure overload at the anatomic, functional, and cellular level. Our investigation uses genetic tools and physiological analyses to demonstrate a necessary role of this family of tyrosine kinase in cardiac function during stress.

Materials and Methods

The expanded Materials and Methods section in the online supplement, available at http://circres.ahajournals.org, provides a description of the mouse model of transverse aortic banding, the Bmx knockout (KO) mouse line, assessment of cardiac function by echocardiography, histology, gene expression analyses, and Western blotting.

Results

To determine whether Bmx nonreceptor tyrosine kinase is involved in cardiac signaling, we examined basal cardiac phenotype in homozygous Bmx KO mice.[5] As monitored by echocardiography, these mice manifest no abnormalities in cardiac function at baseline resting conditions.

Given that Bmx has been implicated in growth and proliferative processes in noncardiac cells, we sought to examine the role of this protein in cardiac growth following pressure overload. Transverse aortic constriction (TAC) surgery was performed on adult male Bmx KO or strain-matched Balb/c wild-type (WT) controls after which mice were allowed to recover for up to 8 weeks with weekly monitoring of cardiac parameters by echocardiography (efficacy of TAC operation was determined by evaluation of carotid pressure gradient; data not shown). In WT mice, TAC caused robust hypertrophy at 8 weeks following the surgery, as evidenced by increased heart weight to body weight (Figure 1A) or heart weight to tibia length (Figure I, A, in the online data supplement) ratios in TAC as compared to sham WT mice. Gross images also demonstrate changes in cardiac morphology, notably left atrial distention in TAC operated WT mice (Figure 1B). Remarkably, Bmx KO mice were resistant to pressure overload–induced hypertrophic growth even after 8 weeks of stress (Figure 1A and 1B and supplemental Figure I, A), demonstrating a previously unrecognized role for Bmx signaling in the heart and implicating the molecule as a necessary component of cardiac hypertrophy following stress.

To evaluate the role of Bmx in functional adaptation of the myocardium to pressure overload, echocardiography was used to evaluate cardiac parameters every 5 to 7 days following TAC or sham surgery. Figure 1D shows M-mode images taken 8 weeks following surgery; note the preservation of wall motion in the TAC operated Bmx KO mice as compared with their WT counterparts (see supplemental Table I for additional cardiac parameters). This sustained function translated into preserved ejection fraction and frac-

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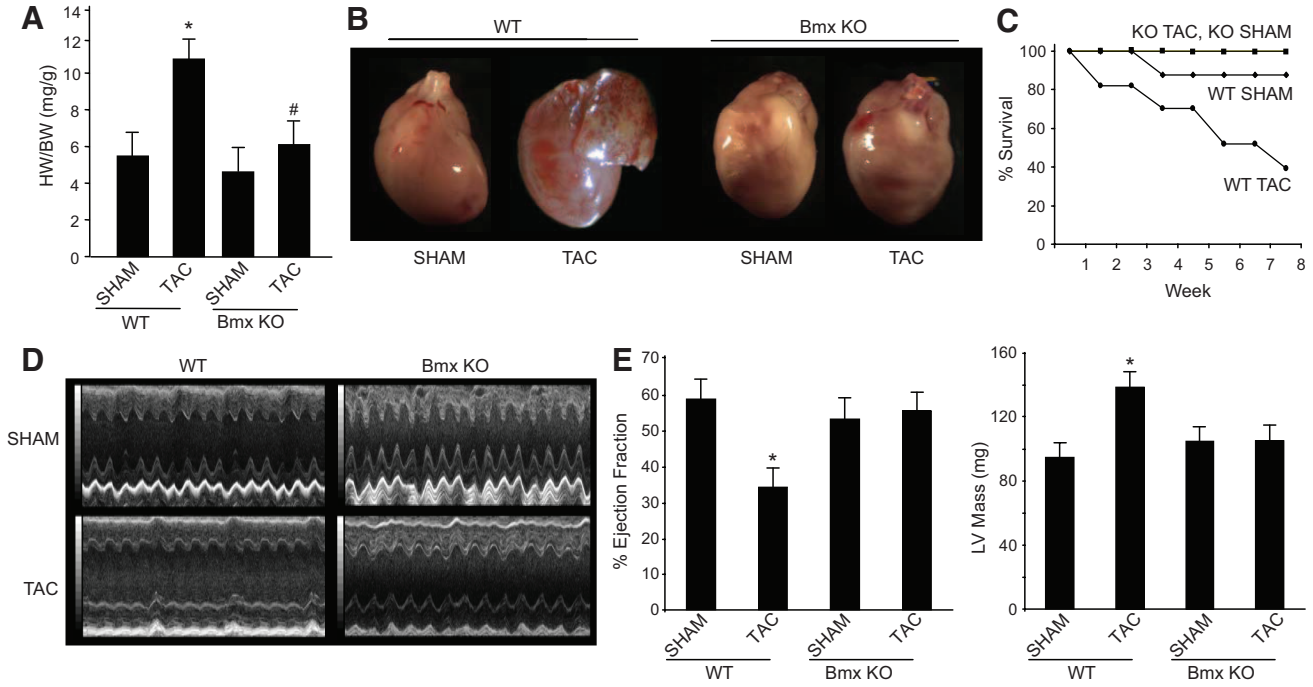

Figure 1. Loss of Bmx prevents pressure overload–induced hypertrophy: functional and anatomic indices. A, Mice were euthanized 8 weeks after TAC or sham surgery, and heart weight to body weight (HW/BW) ratio was determined. *P=0.004 vs WT sham for HW/BW, #P=NS vs Bmx KO sham. Bars are SEM. B, Images of hearts from mice euthanized 8 weeks after surgery. C, Kaplan–Meier survival curve (starting n values: WT TAC, 23; WT sham, 10; Bmx KO TAC, 9; Bmx KO sham, 7). D, M-mode echocardiographic images from WT and Bmx KO mice. E, Ejection fraction and left ventricular mass data obtained by echocardiography at 8 weeks after sham or TAC surgery. *P<0.01. Bars are SEM (n values for all groups: WT TAC, 6; WT sham, 9; Bmx KO TAC, 9; Bmx KO sham, 7).

tional shortening, as well as resistance to left ventricular hypertrophy in the Bmx KO myocardium (Figure 1E and supplemental Figure, B). The functional benefits conferred by loss of Bmx also manifest as better survival following TAC (Figure 1C). At this time, it is unknown whether these benefits extend beyond 8 weeks.

Cardiac hypertrophy induced by pressure overload or other stimuli can involve a conserved gene program that participates in reverting the myocyte to a more fetal-like state and precipitating pathological remodeling of the myocardium. We examined markers of this program in the Bmx KO myocardium and found that increases in ANF expression and aspects of the myosin heavy chain (MHC) switch following TAC remain intact in the absence of Bmx (Figure 2A). Bmx and other Tec family members have been implicated in regulating transcription in the heart[10] and various cultured cell types[11,12]; however, Bmx does not appear to be required for critical aspects of the fetal gene program. These features, along with the cardiac mass data, are reminiscent of the response of the NOS3-deficient mouse to pressure overload,[13] in which the loss of NOS3 protects against hypertrophic growth.

Having demonstrated that loss of Bmx renders mice resistant to the morphological changes associated with pressure overload and linking this to preserved function of the cardiac pump, we next sought to examine cellular level changes in the wake of TAC and the role of Bmx. Ventricular sections were analyzed by Masson's trichrome and wheat germ agglutinin (WGA) staining. WGA demarcates plasma membrane and was thus used for the dual purpose of evaluating changes in gross myocyte morphology (which were not observed; Figure 2B), as well as for calculating myocyte size. This latter index revealed that, akin to the organ and functional data, absence of Bmx function prevents hypertrophic growth at the myocyte level (Figure 2B and 2C). Histological analyses of cardiac sections by trichrome identified regions of fibrotic deposition, in particular around macrovasculature, following TAC that appeared unaltered by the loss of Bmx (Figure 2D). We also used terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling (TUNEL) staining to examine apoptosis in the hearts of WT and Bmx KO mice and observed no difference in the degree of TUNEL positivity between these groups (data not shown).

To investigate local signaling mechanisms through which Bmx is necessary for pressure overload hypertrophy, we examined known regulators of the protein also implicated in cardiac growth. In WT mice, we observed increased Bmx activation (as detected by phosphorylation on tyrosine 40; supplemental Figure I, C), as well as a trend toward increased total Bmx protein following TAC. Other investigators have implicated altered association of Bmx with membranous fractions,[14,15] as well as caspase-dependent degradation of the protein,[16] as means of regulation; the contribution of these and other processes to available active Bmx in the basal myocardium or following TAC are unknown at this time. Because Akt is a known regulator of cardiac growth[17] and has been implicated in Bmx signaling in noncardiac cells,[12,18] we examined Akt total protein and phosphorylation following TAC. Absence of Bmx function did not alter total Akt following TAC (supplemental Figure I, D). Likewise, proximal (protein kinase D) and distal (glycogen synthase kinase

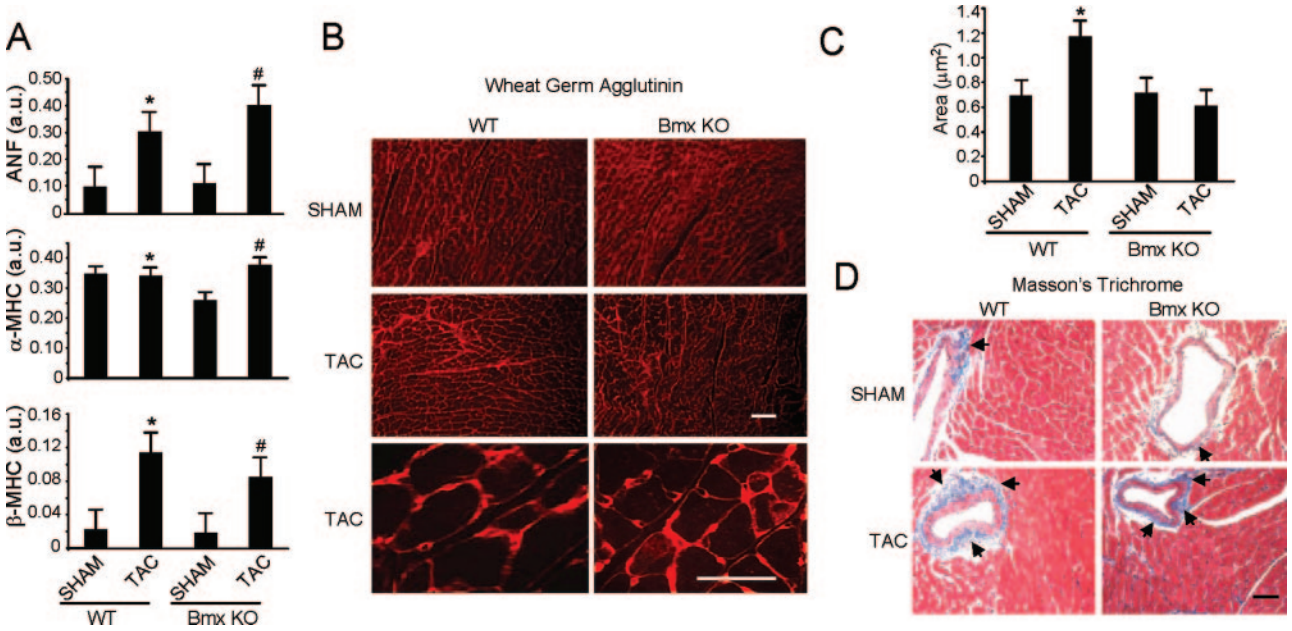

Figure 2. Altered cellular level responses in Bmx KO mice following pressure overload. A, RT-PCR analysis of cardiac hypertrophic marker genes was performed 8 weeks after surgery. *P=NS vs WT sham for ANF and α-MHC and P=0.01 for β-MHC, #P=0.05 vs Bmx KO sham for ANF and β-MHC and P=0.04 for α-MHC (n=3 per group; 2 technical replicates; all data normalized to GAPDH). B, WGA staining to evaluate tissue morphology and cell size. Magnification: ×20 magnification (top and middle images); ×60 (bottom images). Scale bars=50 μm. C, Quantitative data from WGA experiments (n=3 animals per group, 50 cells per animal). *P=0.002 vs WT sham. D, Trichrome staining to examine fibrotic deposition (×20 magnification). Scale bar=50 μm.

3β) signaling partners of Akt were ostensibly unaffected by the loss of Bmx. Furthermore, the behavior of Akt phosphorylation (at either T308 or S473), as detected by Western blotting, was not significantly changed between the normal and Bmx-deficient hearts (supplemental Figure I, D).

Discussion

This study provides the first definitive evidence for a role of the Tec family of tyrosine kinases in cardiac phenotype using genetic tools and physiological analyses. We demonstrate that Bmx is a necessary component of the morphological, functional, and cell level responses of the heart to pressure overload. Whether Bmx plays a role in other forms of cardiac hypertrophy, such as that induced by neural/hormonal stimuli, will be examined in future studies. A recent study showed that Bmx activity is necessary for acute and prolonged responses to ischemia in the rodent hindlimb.[6] The short-term recovery required Bmx signaling not in the skeletal muscle but rather in bone marrow–derived cells. Given the increasingly recognized role of noncardiac cells in injury responses in the myocardium, we recognize that some of the affects Bmx exerts in the WT animal may arise from cells other than cardiomyocytes.

As mentioned previously, the Bmx KO phenotype following TAC shares several features with the NOS3 KO, including resistance to hypertrophic growth, preserved cardiac function, and undisturbed fetal gene activation.[13] It is reasonable to hypothesize that Bmx modulates signaling via NOS3 during pressure overload; however, additional details regarding the relationship between these signaling systems will require further experimentation. The Bmx KO mice do not display altered cardiac development, indicating that although Bmx is indispensable in the response of the adult heart to pressure overload, it appears to not be required for normal embryonic cardiac development. These findings lend credence to task-specific roles for different signaling kinase family members and support the function of Bmx as a stress-activated isoform of the Tec family.

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Disclosures

None.

References

1. Tamagnone L, Lahtinen I, Mustonen T, Virtaneva K, Francis F, Muscatelli F, Alitalo R, Smith CI, Larsson C, Alitalo K. BMX, a novel nonreceptor tyrosine kinase gene of the BTK/ITK/TEC/TXK family located in chromosome Xp22.2. *Oncogene*. 1994;9:3683–3688.
2. Qiu Y, Robinson D, Pretlow TG, Kung HJ. Etk/Bmx, a tyrosine kinase with a pleckstrin-homology domain, is an effector of phosphatidylinositol 3′-kinase and is involved in interleukin 6-induced neuroendocrine differentiation of prostate cancer cells. *Proc Natl Acad Sci U S A*. 1998;95:3644–3649.
3. Tsukada S, Saffran DC, Rawlings DJ, Parolini O, Allen RC, Klisak I, Sparkes RS, Kubagawa H, Mohandas T, Quan S, Belmont JW, Cooper MD, Conley ME, Witte ON. Deficient expression of a B-cell cytoplasmic tyrosine kinase in human X-linked agammaglobulinemia. *Cell*. 1993;72:279–290.
4. Vetrie D, Vorechovsky I, Sideras P, Holland J, Davies A, Flinter F, Hammarstrom L, Kinnon C, Levinsky R, Bobrow M, Smith CIE, Bentley DR. The gene involved in X-linked agammaglobulinaemia is a member of the src family of protein-tyrosine kinases. *Nature*. 1993;361:226–233.
5. Rajantie I, Ekman N, Iljin K, Arighi E, Gunji Y, Kaukonen J, Palotie A, Dewerchin M, Carmeliet P, Alitalo K. Bmx tyrosine kinase has a

redundant function downstream of angiopoietin and vascular endothelial growth factor receptors in arterial endothelium. *Mol Cell Biol*. 2001;21:4647–4655.
6. He Y, Luo Y, Tang SB, Rajantie I, Salven P, Heil M, Zhang R, Luo DH, Li XH, Chi HB, Yu J, Carmeliet P, Schaper W, Sinusas AJ, Sessa WC, Alitalo K, Min W. Critical function of Bmx/Etk in ischemia-mediated arteriogenesis and angiogenesis. *J Clin Invest*. 2006;116:2344–2355.
7. Ekman N, Lymboussaki A, Vastrik I, Sarvas K, Kaipainen A, Alitalo K. Bmx tyrosine kinase is specifically expressed in the endocardium and the endothelium of large arteries. *Circulation*. 1997;96:1729–1732.
8. Zhang J, Ping P, Wang GW, Lu M, Pantaleon D, Tang XL, Bolli R, Vondriska TM. Bmx, a member of the Tec family of nonreceptor tyrosine kinases, is a novel participant in pharmacological cardioprotection. *Am J Physiol*. 2004;287:H2364–H2366.
9. Mathur P, Kaga S, Zhan LJ, Das DK, Maulik N. Potential candidates for ischemic preconditioning-associated vascular growth pathways revealed by antibody array. *Am J Physiol*. 2005;288:H3006–H3010.
10. Willey CD, Palanisamy AP, Johnston RK, Mani SK, Shiraishi H, Tuxworth WJ, Zile MR, Balasubramanian S, Kuppuswamy D. STAT3 activation in pressure-overloaded feline myocardium: role for integrins and the tyrosine kinase BMX. *Int J Biol Sci.* 2008;4:184–199.
11. Saharinen P, Ekman N, Sarvas K, Parker P, Alitalo K, Silvennoinen O. The Bmx tyrosine kinase induces activation of the Stat signaling pathway, which is specifically inhibited by protein kinase Cdelta. *Blood*. 1997;90:4341–4353.
12. Chau CH, Clavijo CA, Deng HT, Zhang QZ, Kim KJ, Qiu Y, Le AD, Ann DK. Etk/Bmx mediates expression of stress-induced adaptive genes VEGF, PAI-1, and iNOS via multiple signaling cascades in different cell systems. *Am J Physiol*. 2005;289:C444–C454.
13. Takimoto E, Champion HC, Li M, Ren S, Rodriguez ER, Tavazzi B, Lazzarino G, Paolocci N, Gabrielson KL, Wang Y, Kass DA. Oxidant stress from nitric oxide synthase-3 uncoupling stimulates cardiac pathologic remodeling from chronic pressure load. *J Clin Invest*. 2005;115:1221–1231.
14. Vargas L, Nore BF, Berglof A, Heinonen JE, Mattsson PT, Smith CI, Mohamed AJ. Functional interaction of caveolin-1 with Bruton's tyrosine kinase and Bmx. *J Biol Chem*. 2002;277:9351–9357.
15. Schwartzberg PL, Finkelstein LD, Readinger JA. TEC-family kinases: regulators of T-helper-cell differentiation. *Nat Rev Immunol*. 2005;5:284–295.
16. Wu YM, Huang CL, Kung HJ, Huang CY. Proteolytic activation of ETK/Bmx tyrosine kinase by caspases. *J Biol Chem*. 2001;276:17672–17678.
17. Shiojima I, Walsh K. Regulation of cardiac growth and coronary angiogenesis by the Akt/PKB signaling pathway. *Genes Dev*. 2006;20:3347–3365.
18. Zhang R, Xu Y, Ekman N, Wu Z, Wu J, Alitalo K, Min W. Etk/Bmx transactivates vascular endothelial growth factor 2 and recruits phosphatidylinositol 3-kinase to mediate the tumor necrosis factor-induced angiogenic pathway. *J Biol Chem*. 2003;278:51267–51276.

KEY WORDS: tyrosine kinase ■ cardiac hypertrophy ■ signal transduction

Supplement Material

Materials and Methods.

All experiments conform to the NIH Guide for the Care and Use of Laboratory Animals and were approved by the UCLA Animal Research Committee.

Antibodies, chemicals and reagents. Primary antibodies: GSK 3β #9315, phospho-GSK 3β (S9) #9336, AKT #9272, phospho-AKT (S473) #4058, phospho-AKT (T308) #9275, PDK1 #3062, phospho-PDK1 (S241) #3061, phospho-Bmx (Y-40) #3211 (from Cell Signaling Technologies); Bmx (BD Biosciences #610792), GAPDH (Abcam) ab9483. Secondary antibodies: Horse Radish Peroxidase (HRP) conjugate donkey anti-goat sc2304, goat anti-mouse sc2055 and chicken anti-rabbit sc 2936 (Santa Cruz Biotechnology). Staining and Immunohistochemistry: Texas red-X-conjugated wheat germ agglutinin was from Invitrogen and Masson's Trichrome Stain Kit was from Sigma.

Mice. All experiments were performed on adult (8-12 week old) male homozygous (-/-) Bmx knockout mice, generated by replacement of the first coding exon of the *Bmx* gene with the lac-Z reporter cassette,[1] or strain-matched balb/c wild type controls. Bmx knockout mice were back crossed to the balb/c background for at least eight generations prior to this study.

Transverse aortic constriction surgery. The murine model of transverse aortic banding-induced cardiac hypertrophy was performed as described previously.[2] Animals were anesthetized with 3.0% isoflurane, intubated, and ventilated with 2.0% isoflurane in 98% O_2 / 2% CO_2. After shaving hair from the animal, the chest was entered from the left side via the third intercostal space, the aorta identified at the T8 region and a venous vascular clamp (Fine Science Tools), outfitted with a band of silastic tubing at the distal edge of one of the clamps, placed around the vessel. The internal diameter of the resulting modified clamp was that of a 27 gauge needle, a common diameter for aortic banding in the mouse; the clamp is of a size that does not impede pulmonary function. Once the aorta was clamped, distal blood flow was measured and quantified using a flow probe (Transonic Instruments). The chest was then closed using 6-0 proline suture, during which negative pressure in the thorax reestablished by removing air with a PE-50 chest tube attached to a syringe. SHAM operated mice underwent the same procedure without placement of aortic clamp.

Echocardiography (ECHO). ECHO was used to determine cardiac parameters in live mice as described,[2,3] including the following indices: left ventricular size (end-diastolic and end-systolic dimension), wall thickness (intra-ventricular septum and posterior wall thickness), ventricular mass, ventricular function (ejection fraction), and blood flow. The mouse was sedated with isoflurane vaporized (2.5% for induction, 1.0% for maintenance) in oxygen, its chest shaved and positioned in the left lateral decubitus

position for ultrasonic imaging with Vevo 770 high-resolution ECHO system equipped with a 35 MHz transducer. The short axis view (M-mode) and the long axis view (Doppler) measurements were performed and data stored for off-line analysis. LV chamber dimensions, VST, PWT, and LV mass index (the ratio of LV mass to body weight) were obtained from M-mode images; LV systolic function is also assessed from these measurements by calculating EF. The peak velocity of early diastolic filling (E), the peak velocity of late filling associated with atrial contraction (A wave) and the E/A ratio were measured from the Doppler tracings to assess the LV diastolic function. All mice underwent ECHO analyses once before TAC or SHAM surgery, once a day after and then once every 5 days for the duration of the study. The ECHO data reported in the paper are only from mice that completed the study to eight weeks.

Western immunoblotting. Ventricular proteins were isolated in buffer containing: (in mmol/L) 150 NaCl, 20 Trs-HCl, 1 EDTA, 1 EGTA, 1 Na_3VO_4, 1 NaF, 1 PMSF phosphatase inhibitors, 1% Triton X-100 (Sigma) and protease inhibitor cocktail (Roche Diagnostics). After Bradford protein assay, proteins were separated by standard SDS-PAGE, transferred to nitrocellulose and blotted with corresponding antibodies. Protein loading was confirmed by Ponceau staining of membranes and GAPDH immunoblotting.[4]

Histological Assessment. Whole hearts were rapidly excised from animals, hung and perfused with 0.1M potassium (K^+) solution, subsequently fixed using 4%

paraformaldehyde and then embedded in paraffin. Hearts were then sectioned at 4μm and placed on slides. *Wheat Germ Agglutinin*: Heart sections were incubated with Texas red-X-conjugated wheat germ agglutinin (1:100) for 90 minutes and assessed for cell size. [5,6] *Hematoxylin and Eosin Staining (H/E), Trichrome*: H/E staining and Masson's Trichrome staining were performed according to the manufacturer's protocols. All images were visualized using Nikon eclipse TE2000-U microscope and imaging and cell size analysis was performed with the SPOT software (Diagnostic Instruments Inc.).

RNA Purification and Quantitative Real Time PCR Analysis. Total RNA was isolated from the left ventricles of the hearts using TRIzol (Invitrogen) according to the manufacturer's protocol. The total RNA was transcribed using SuperScript First-Strand Synthesis system for RT-PCR (Invitrogen) according to the manufacturer's protocol to produce cDNA. For SYBR green qRT-PCR, 25μl iQ SYBR Green Supermix (BioRad), 0.5 μl of the specific primers and 2 μl of cDNA was mixed and incubated using the iCycler (BioRad) thermocycler for 40 cycles of denaturing at 95°C, annealing at 60°C and amplification at 72°C for 45 seconds each. Three samples were analyzed for each mouse type and were duplicated to account for pipetting errors. To calculate the fold change of expression the mean of the cycle number for each group was calculated and was normalized against GAPDH by the formula: mean $_{sample}$ – mean $_{GAPDH.}$ The normalized means were then applied to the formula $[2^{(cycle\#)}]^{-1}$ to calculate the fold change.

Statistical analyses. All data are expressed ±SEM with $p \leq 0.05$ considered to be

statistically significant. Comparisons were made using student's t-test for unequal variances.

References

1. Rajantie I, Ekman N, Iljin K, Arighi E, Gunji Y, Kaukonen J, Palotie A, Dewerchin M, Carmeliet P, Alitalo K. Bmx tyrosine kinase has a redundant function downstream of angiopoietin and vascular endothelial growth factor receptors in arterial endothelium. *Molecular and Cellular Biology*. 2001;21:4647-4655.
2. Takimoto E, Champion HC, Li M, Ren S, Rodriguez ER, Tavazzi B, Lazzarino G, Paolocci N, Gabrielson KL, Wang Y, Kass DA. Oxidant stress from nitric oxide synthase-3 uncoupling stimulates cardiac pathologic remodeling from chronic pressure load. *J Clin Invest*. 2005;115:1221-31.
3. Eghbali M, Deva R, Alioua A, Minosyan TY, Ruan H, Wang Y, Toro L, Stefani E. Molecular and functional signature of heart hypertrophy during pregnancy. *Circ Res*. 2005;96:1208-16.
4. Vondriska TM, Zhang J, Song C, Tang XL, Cao X, Baines CP, Pass JM, Wang S, Bolli R, Ping P. Protein kinase C epsilon-Src modules direct signal transduction in nitric oxide-induced cardioprotection: complex formation as a means for cardioprotective signaling. *Circ Res*. 2001;88:1306-13.
5. Ha T, Hua F, Li Y, Ma J, Gao X, Kelley J, Zhao A, Haddad GE, Williams DL, Browder IW, Kao RL, Li C. Blockade of MyD88 attenuates cardiac hypertrophy and decreases cardiac myocyte apoptosis in pressure overload-induced cardiac hypertrophy in vivo. *Am J Physiol Heart Circ Physiol*. 2006;290:H985-94.
6. Satoh M, Ogita H, Takeshita K, Mukai Y, Kwiatkowski DJ, Liao JK. Requirement of Rac1 in the development of cardiac hypertrophy. *Proc Natl Acad Sci U S A*. 2006;103:7432-7.

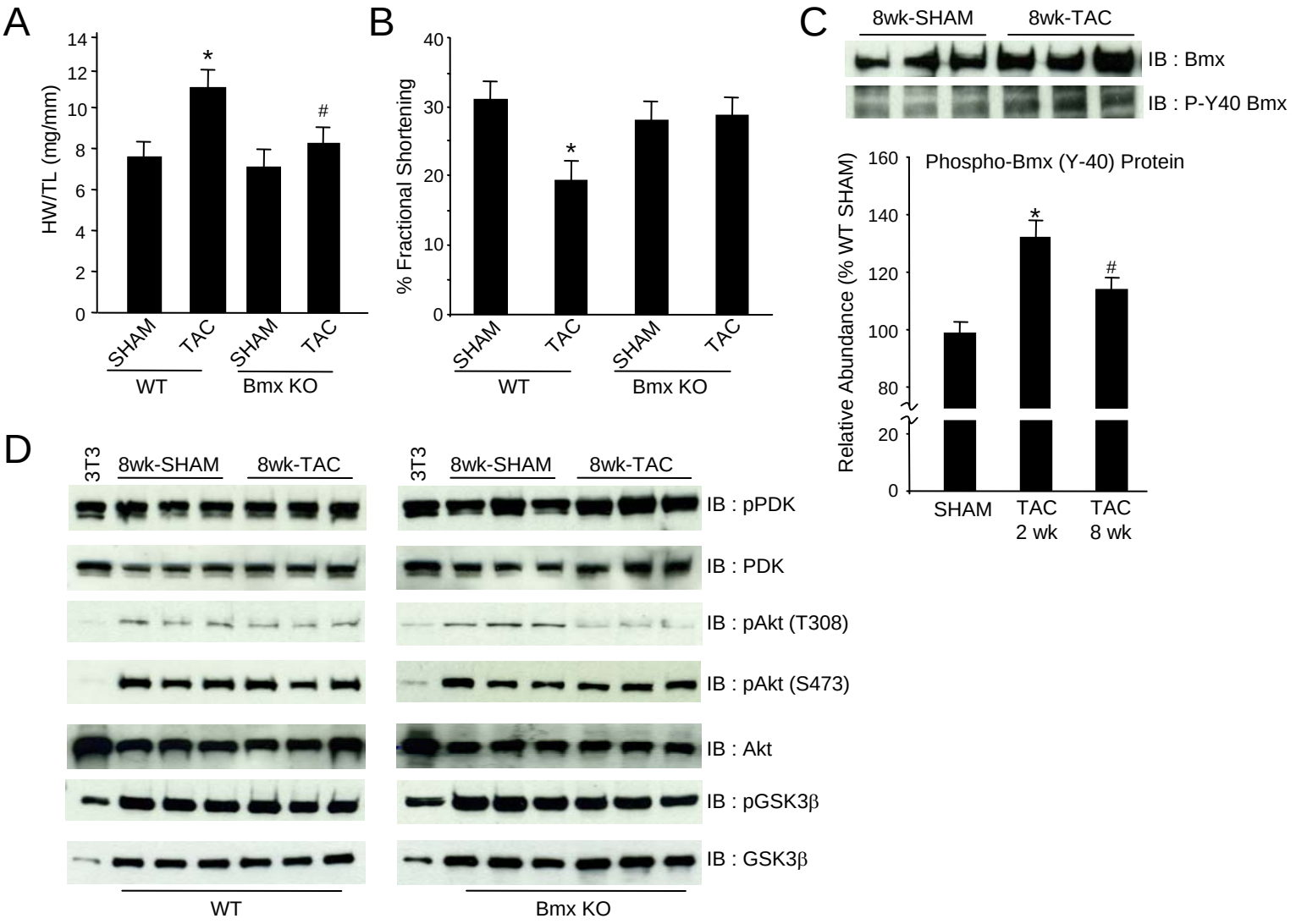
Online Table I: Cardiac Parameters Determined by Echocardiography

		SHAM	2week TAC	4week TAC	8week TAC
LVEDV	WT balb/c	70.31±4.94	50.40±5.88a	56.32±6.97b	71.35±6.16
	Bmx KO	57.21±3.63	46.65±4.36b	49.95±4.28	57.61±3.75
LVESV	WT balb/c	29.01±3.18	24.37±3.25	28.68±5.90	41.25±5.70
	Bmx KO	26.79±3.77	16.10±2.38c	19.03±2.22d	26.12±3.12
LV %EF	WT balb/c	58.97±2.76	51.73±1.69b	50.17±4.68b	43.28±4.29a
	Bmx KO	53.37±6.03	66.66±2.98c	62.23±3.27d	55.54±3.51
LV mass	WT balb/c	94.80±9.79	95.40±6.67	108.88±10.23	137.81±12.17a
	Bmx KO	104.15±12.24	91.58±9.4	97.58±6.25	104.78±11.99
LV %FS	WT balb/c	30.95±1.81	25.76±1.04b	25.03±2.82b	21.40±2.49b
	Bmx KO	27.95±4.32	36.38±2.39	33.13±2.31	28.66±2.18
HR; bpm	WT balb/c	409±14.32	402±18.7	431.4±14.44	407±20
	Bmx KO	424±15.47	413±19.1	408.44±27.6	401±24.94

a;p<0.01 compared to WT SHAM
b;p<0.05 compared to WT SHAM
c;p<0.01 compared to WT SHAM
d;p<0.05 compared to WT SHAM

	SHAM	2week TAC	4week TAC	8week TAC	
n values	9	19	16	6	WT balb/c
n values	7	9	9	9	Bmx KO

Online Figure I. ***A.*** Heart weight to tibia length (HW/TL) ratios were determined 8 weeks following TAC or SHAM surgery (* indicates p=0.007 vs. WT SHAM for HW/TL; # indicates p=NS vs. Bmx KO SHAM; bars are SEM). ***B.*** Fractional shortening indices as determined by ECHO 8 weeks following TAC or SHAM surgery (* indicates p<0.01, bars are SEM; n values for all groups: WT TAC, 6; WT SHAM, 9; Bmx KO TAC, 7; Bmx KO SHAM, 9). ***C.*** Expression and phosphorylation of Bmx in WT animals (* indicates p=0.002 and # indicates p=0.01 vs. SHAM). ***D.*** Local signaling responses involved in Bmx-dependent cell growth.



Online Figure I