Supporting Information

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SI Materials and Methods

Generation and Identification of CFKO-2a Mice. Mouse embryos and pups were genotyped by PCR using forward (AGGGCTG-GTCTGCGCTGACAGG) and reverse (GCTGATGTC-CCAAGCTATTCC) primers. Cycling conditions were as follows: three cycles at 94°C for 3 min, 67°C for 2 min and 72°C for 2 min, 30 cycles at 94°C for 1 min, 67°C for 1 min and 72°C for 2 min, 1 cycle at 72°C for 10 min. The expected sizes of the wild-type allele and the floxed allele are 1.5 kb and 1.6 kb, respectively.

Mice were housed in a specific pathogen-free facility and handled in accordance with standard use protocols and animal welfare regulation. The genetic backgrounds of these mice are a mixture of 129 and B6. All study protocols were approved by the Institutional Animal Care and Use Committee of Cornell University and conform to the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH Publication No. 85-23, revised 1996).

X-Gal Whole-Mount Staining. The isolated E9.5, E10.5 embryos or E14.5 embryonic hearts were fixed in freshly prepared 4% paraformaldehyde for 1 h at 4°C, rinsed for 30 min with rinse buffer (100 mM sodium phosphate, pH 7.3/2 mM $MgCl_2$/0.01% deoxycholic acid/0.02% Igepal CA-630) three times at room temperature. After rinsing, the fixed embryos or embryonic hearts were stained by a staining solution (100 mM sodium phosphate, pH 7.3/2 mM $MgCl_2$/0.01% deoxycholic acid/0.02% Igepal CA-630/5 mM potassium ferricyanide/5 mM potassium ferrocyanide/1 mg/ml X-gal) overnight at room temperature and then postfixed in 10% formalin overnight at 4°C.

Southern, Northern, and Western Blots. Genomic DNA was extracted from the mouse left or right ventricle using phenol/chloroform and then digested with SacI, electrophoresed on 0.7% agarose gels and transferred to NYTRAN SuPerCharg-enylon membrane (Schleicher & Schuell) using alkaline method. The probe was prepared as described previously (1) and labeled with [α-^{32}P]dCTP by a random primer DNA labeling kit (Invitrogen). The blots were then hybridized at 65°C using QuikHyb hybridization solution (Stratagene) following manual instruction and exposed to Kodak film later.

Total RNA was isolated from cultured 3T3 cells and was used for Northern blotting as described previously (1). MEF2a probe was purchased from ResGen (Invitrogen) and labeled with [α-^{32}P]dCTP by a random primer DNA labeling kit (Invitrogen).

Isolated heart or cultured cells were homogenized with modified RIPA buffer and extracts were used for Western blotting as described previously (1). The rabbit antiserum specific for chicken FAK has been described previously (2). FAK, HA, and MEF2a antibodies were purchased from Santa Cruz Biotechnology. The vinculin and phosphorylated histone H3 (Ser-10) antibodies were purchased from Sigma and Upstate, respectively.

Transthoracic Echocardiography. The mice were anesthetized with an i.p. injection of 160 mg/kg sodium pentobarbital. Using a Vivid 7 ultrasound system (GE Medical Systems) equipped with a wide-band, multifrequency (5.8–14 mHz) linear array probe (i13L), measurements were obtained from the M-mode display using two-dimensional guidance.

Cardiomyocyte Isolation. Time-crossed pregnant female mice were euthanized and E14.5 or later embryonic hearts were harvested. Embryonic ventricles were dissected and washed twice with cold PBS. Cardiomyocytes were released by two 30 min rounds of Collagenase II digestion at 37°C. The isolated cells were pelleted by low speed centrifugation (800 rpm) and resuspended in DMEM containing 10% FBS. Cells were then plated on culture flasks for 45 min to remove attached non-cardiomyocytes, and the suspended cardiomyocytes were then harvested. Aliquots of the purified cardiomyocytes were plated on laminin-coated six-well plates for future studies.

Generation and Infection of Recombinant Adenovirus. The recombinant adenoviruses encoding wild-type FAK, FAK kinase-defective mutant, FAK Y397F, FAK D395A and FAK-RNAi were generated by using the Adeasy-1 system (Stratagene) according to the manufacturer's instruction. All viruses were amplified using Ad 293 cells, and the viral titers were determined by using Adeno-X rapid titer kit (BD Biosciences). An optimal viral titer (5–50 moi) was determined to give almost 100% infection efficiency (based on GFP expression) with no detectable cell toxicity. Cells were infected for 48–72 h before the experiments.

Microarray. Briefly, total RNAs were isolated from FAK or Mock cells 12 h after induction using RNAeasy kits (Qiagen) according to the manufacturer's protocol. Aliquots of the cell lysates were subjected to Western blotting to confirm the expression of exogenous FAK. Approximately 3 μg of mRNA from each sample were labeled with Cy3 or Cy5, respectively (Invitrogen) by reverse transcription to generate cDNA probes. The probes were then hybridized onto the cDNA microarray. The images were scanned and analyzed at Boyce Thompson Institute microarray facility of Cornell University. Data from two independent experiments for each mRNA sample were obtained and analyzed with Microsoft Excel software.

SI Results

Cardiac-Restricted Deletion of FAK in Embryonic Development. In our previous study, we have unraveled the critical role of FAK in eccentric ventricular hypertrophy in adult mice (1). However, it remains to be elucidated whether FAK exerts a role in cardiac development because of the insufficient FAK deletion efficiency by MLC2vKICre in embryonic heart. To determine the role of FAK in cardiac development, we crossed the floxed FAK mice with another cardiac-specific expressed Cre mouse line, MLC2a-Cre (3). To delineate the temporal and spatial pattern of Cre activity, we crossed MLC2a-Cre mice with R26RstoplacZ mice (4), in which expression of LacZ requires Cre-mediate excision of a stop cassette. MLC2vKICre mice were crossed with R26RstoplacZ at the same time as a control. Time-crossed pregnant female mice were euthanized at E9.5, E10.5, or E14.5, and yolk sacs were harvested to extract DNA for genotyping. Recombination in the heart was visible by E9.5 in both MLC2vKICre (Fig. S1*A*) and MLC2a-Cre (Fig. S1*B*) positive embryos. Thereafter, MLC2vKICre was exclusively expressed in heart by E10.5 (Fig. S1*C*). However, in addition to its expression in the heart, MLC2a-Cre also expressed in the embryonic tail part (Fig. S1*D*). At E14.5, the heart was isolated from the embryos and subsequently stained with X-Gal. Both left and right ventricles stained blue and no blue staining was evident in

the atria of the MLC2vKICre positive heart (Fig. S1*E*). However, the whole heart, including atria and ventricles was stained blue in MLC2a-Cre mouse (Fig. S1*F*). Furthermore, the blue staining in MLC2a-Cre embryonic heart was always stronger than that of MLC2vKICre, suggesting that the MLC2a-Cre recombination efficiency was higher than that of the MLC2vKICre in the embryonic heart.

We crossed the MLC2a-Cre transgenic mice with the floxed FAK mice to obtain double heterozygous ($FAK^{flox/+}$:Cre+) mice and further crossed these with the floxed FAK mice to obtain four types of mice: $FAK^{flox/+}$:Cre-; $FAK^{flox/+}$:Cre+ (designated as Cre); $FAK^{flox/flox}$:Cre- (designated as control) and $FAK^{flox/flox}$:Cre+ (designated as CFKO-2a for conditional FAK knockout mice from MLC2a-Cre). We screened 351 pups and found that CFKO-2a mice were born, but were underrepresented at weaning (6% of littermates, rather than 25% as expected), suggesting that the majority of CFKO-2a mice died in embryonic stage (Table S1). To examine the recombination efficiency of the floxed FAK allele in CFKO-2a embryonic heart, we performed immunofluorescence staining of FAK on E13.5 embryo. We found that FAK staining in CFKO-2a heart (Fig. S1*H*) was significantly decreased compared to the control heart (Fig. S1*G*), whereas the FAK staining in the liver was comparable between CFKO-2a (Fig. S1*J*) and control mice (Fig. S1*I*). To further confirm the recombination efficiency of the floxed FAK allele in CFKO-2a, we prepared protein extracts from hearts harvested from CFKO-2a or CFKO-2v (conditional FAK knockout mice from MLC2vKICre) (1) embryo at E13.5. Analyses of the lysates showed that FAK expression level is significantly decreased in the heart of CFKO-2a (0.46 ± 0.12), but not CFKO-2v (1.03 ± 0.28), when compared to the control embryonic hearts (whose relative level is defined as 1; Fig. 1*K*). Because a small fraction of the CFKO-2a mice survived to adult, we examined the FAK deletion efficiency in adult heart by Southern blotting analysis of genomic DNA extracted from the left or right ventricle of 2-month-old control, CFKO-2a and CFKO-2v mice. Fig. S1*L* shows higher recombination efficiency in the heart from CFKO-2a mice (i.e., the ratio of the deleted vs. the floxed alleles) than that from CFKO-2v mice. Western blotting analysis of extracts from the adult hearts of CFKO-2a and CFKO-2v mice confirmed a more significant reduction in the FAK protein level in CFKO-2a than that of CFKO-2v mice (Fig. S1*M Top*). Taken together, these results suggest an efficient inactivation of FAK gene by MLC2a-Cre in embryonic and adult heart. A previous paper reported the expression of MLC2a-Cre in the liver and kidney in adult mice by LacZ staining (3). Therefore, we also examined the FAK expression levels in CFKO-2a mice by Western blotting. Analyses of the lysates showed that FAK expression level is comparable in liver of CFKO-2a and CFKO-2v mice (Fig. S1*M Bottom*). Under the current condition, we cannot detect the FAK expression in kidney (Fig. S1*M Middle*). These results suggest that the cardiac phenotype in CFKO-2a is due to the FAK deletion in the heart.

1. Peng X, *et al*. (2006) Inactivation of focal adhesion kinase in cardiomyocytes promotes eccentric cardiac hypertrophy and fibrosis in mice. *J Clin Invest* 116:217–227.
2. Peng X, *et al*. (2004) Overexpression of focal adhesion kinase in vascular endothelial cells promotes angiogenesis in transgenic mice. *Cardiovasc Res* 64:421–430.
3. Wettschureck N, *et al*. (2001) Absence of pressure overload induced myocardial hypertrophy after conditional inactivation of Galphaq/Galpha11 in cardiomyocytes. *Nat Med* 7:1236–1240.
4. Mao X, Fujiwara Y, Orkin SH (1999) Improved reporter strain for monitoring Cre recombinase-mediated DNA excisions in mice. *Proc Natl Acad Sci USA* 96:5037–5042.

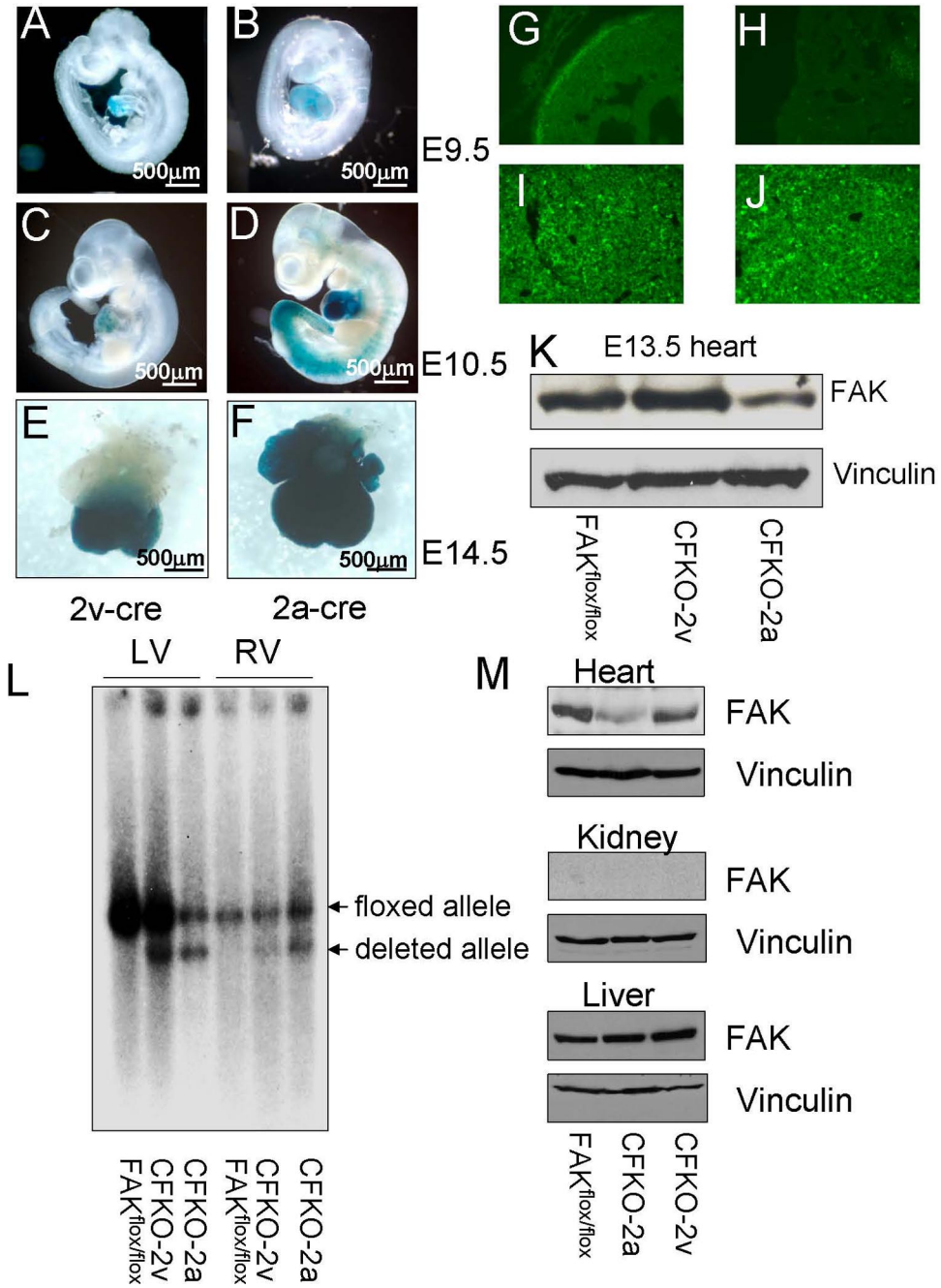

Fig. S1. Characterization of CFKO-2a mice. (*A–D*) Whole-mount LacZ reporter gene expression in the E9.5 or E10.5 heart when MLC2vKICre (*A* and *C*) or MLC2a-Cre (*B* and *D*) mice were crossed with R26R reporter mice. (*E* and *F*) LacZ staining of E14.5 embryonic heart harvested from MLC2vKICre (*E*) or MLC2a-Cre (*F*) crossing with R26R reporter mice. (*G–J*) Embryonic heart (*G* and *H*) and liver (*I* and *J*) sections from E13.5 control (*G* and *I*) or CFKO-2a (*H* and *J*) embryos were analyzed by immunofluorescence staining using anti-FAK antibody. (*K*) Lysates were prepared from E13.5 embryonic heart and then analyzed by α-FAK or α-vinculin as indicated. (*L*) Southern blotting analysis of genomic DNA extracted from the right or left ventricle from CFKO-2a, CFKO-2v, and control adult mice after SacI digestion. The positions of the floxed and deleted alleles are shown on the right. (*M*) Adult mice heart, kidney, and liver lysates were analyzed by Western blotting using α-FAK or α-vinculin as indicated.

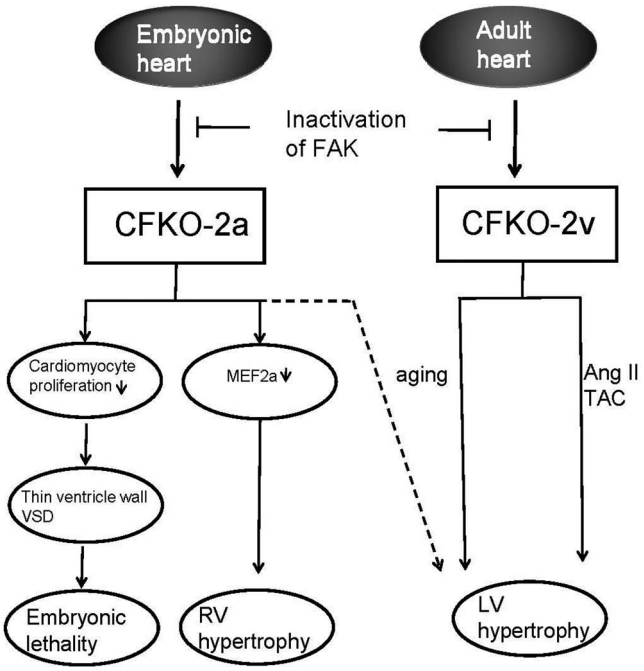

Fig. S2. Diagram of FAK inactivation in embryonic or postnatal heart affects heart development or hypertrophy. The deletion of FAK in the embryonic heart results in lethality in the majority of embryos due to the cardiomyocytes proliferation defects. A small fraction of CFKO-2a could survival to adulthood with right ventricle (RV) hypertrophy. The inactivation of FAK in postnatal heart accelerates left ventricle hypertrophy (LV) upon stimulation with angiotention II, TAC, or aging. The dotted line indicates that old CFKO-2a mice may also exhibit LV hypertrophy.

Table S1. Genotype of progeny from cross between $FAK^{flox/flox}$ and $FAK^{flox/+}$:MLC2a-Cre+ mice

Genotype	E11.5–E13.5	E14.5–E16.5	E17.5–E18.5	P21
$FAK^{flox/+}$	26	30	15	118
$FAK^{flox/+}$;Cre+	30	37 (2)	17	102
$FAK^{flox/flox}$	23 (1)	36	13 (1)	110
$FAK^{flox/flox}$;Cre+	31 (3)	29 (17)	7 (3)	21

Data in parentheses are the number of embryos that showed edema, hemorrhage, or degeneration. P21, postnatal day 21.

Table S2. Echocardiographic parameters in anesthetized mice

Mouse	No.	Heart rate, beats/min	RVIDd, cm	IVSd, cm	IVSs, cm	LVIDd, cm	LVIDs, cm	LVFWd, cm	LVFWs, cm	FS, %
Control	6	340 + 55.5	N/A	0.1 ± 0.02	0.14 ± 0.02	0.29 ± 0.05	019 ± 0.04	0.11 ± 0.01	0.12 ± 0.02	37.1 ± 12.7
CFKO	9	284 + 50.7	0.29 ± 0.05	0.09 ± 0.02	0.12 ± 0.02	0.34 ± 0.04	0.26 ± 0.03	0.11 ± 0.03	0.14 ± 0.02	30.9 ± 8.8

Values were quantified from three separate M-mode measurements. RVIDd, right ventricular internal diameter at end-diastole; IVSd, interventricular septal thickness at end-diastole; IVSs, interventricular septal thickness at end-systole; LVIDd, left ventricular internal diameter at end-diastole; LVFWd, left ventricular free wall thickness at end-diastole; LVIDs, left ventricular internal diameter at end-systole; LVFWs, left ventricular free wall thickness at end-systole. FS, fractional shortening.