Supplementary Data

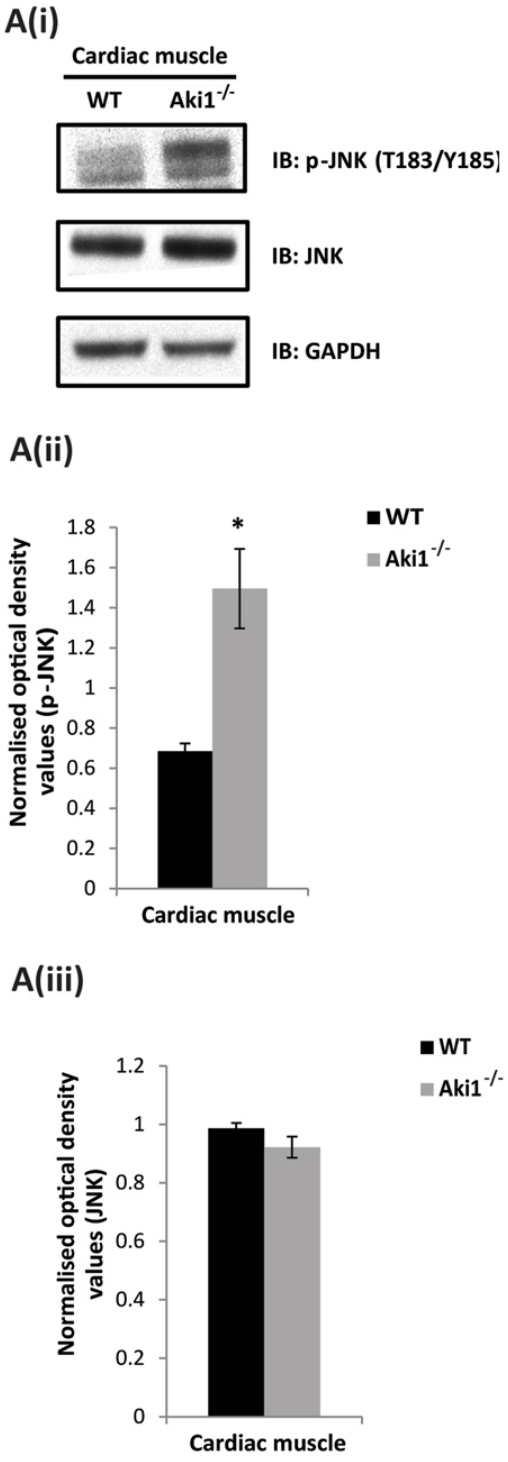
Citation: Rao VV et al (2018) Akirin1, a nuclear factor, induces cardiac hypertrophy in mice via SRF and *miR-1*, Research Reports 2:e1-e20 doi:10.9777/rr.2018.10327

S1. Lack of Akirin1 leads to activation of JNK signaling pathway in the cardiac muscle

Haq et al. showed that the three mitogen activated protein kinase (MAPK) pathways are activated during cardiac hypertrophy i.e., c-Jun N-terminal kinase (JNK), p38 and Extracellular signal-regulated kinase (ERK1/2) pathways (Haq et al., 2001). The protein levels of both phospho-JNK (T183/Y185) and total JNK in Akirin1 knock-out and wild type cardiac muscles using anti-phospho-JNK (T183/Y185) and anti-JNK antibodies respectively were analyzed. Western blotting results showed that Akirin1 knock-out cardiac muscle showed increased levels of phospho-JNK (T183/Y185) compared to the wild type cardiac muscle while the total JNK levels were unchanged between Akirin1 knock-out and wild type cardiac muscle [Figure 5.9A(i), A(ii) and A(iii)]. The ratio of phospho-JNK (T183/Y185) to total JNK was also significantly increased in Akirin1 knock- out cardiac muscle compared to the wild type cardiac muscle [Figure 5.9A(iv)].

S2. Lack of Akirin1 leads to activation of p38 signaling pathway in the cardiac muscle

Another important member of the MAPK pathways is p38 MAPK pathway. We wanted to investigate if p38 pathway was up-regulated in Akirin1 knock- out cardiac muscle as it was shown by Haq et al., 2001 that p38 pathway is up- regulated during cardiac hypertrophy. The protein lysates from Akirin1 knock- out and wild type cardiac muscle were subjected to western blotting with anti- phospho-p38 MAPK (T180/Y182) and anti-p38 MAPK antibodies. Protein expression analysis showed that Akirin1 knock-out cardiac muscle showed increased levels of both phospho-p38 MAPK (T180/Y182) and p38 MAPK protein levels compared to the wild-type [Figure 5.10A(i)]. Densitometry analysis also showed that Akirin1 knock-out cardiac muscle showed increased levels of both phospho-p38 MAPK (T180/Y182) and p38 MAPK protein levels compared to the wild type cardiac muscle [Figure 5.10A(ii) and A(iii)]. The ratio of phospho-p38 MAPK (T180/Y182) to p38 MAPK was also significantly up-regulated in Akirin1 knock-out cardiac muscle compared to the wild type cardiac muscle [Figure 5.10A(iv)].



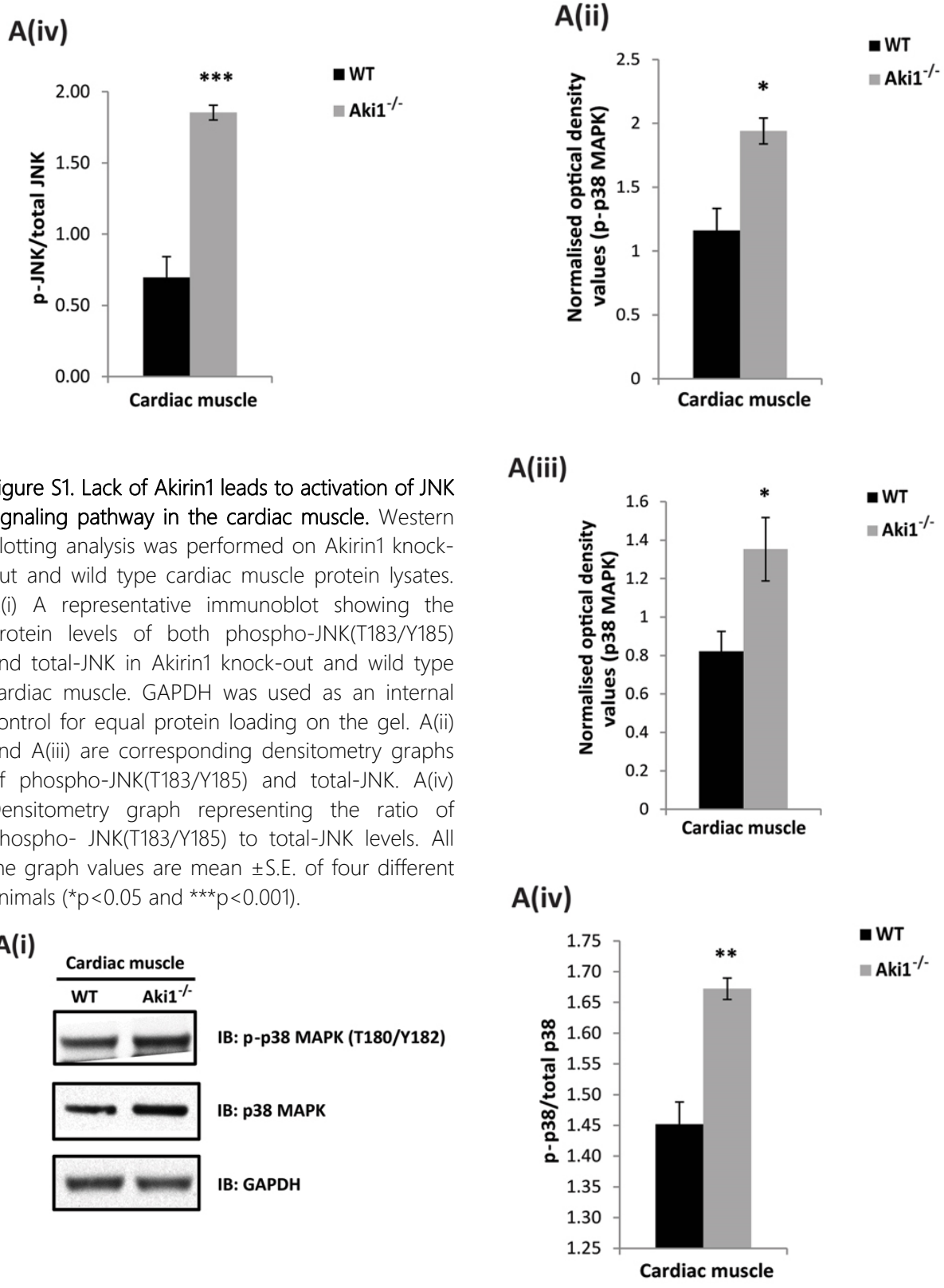
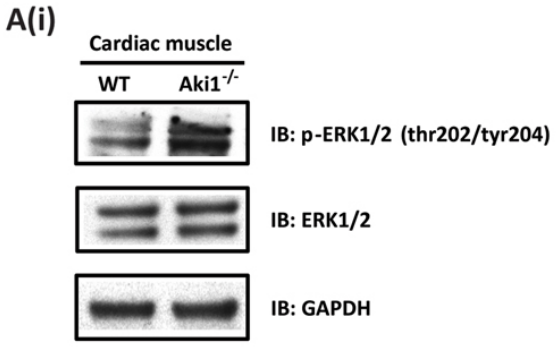
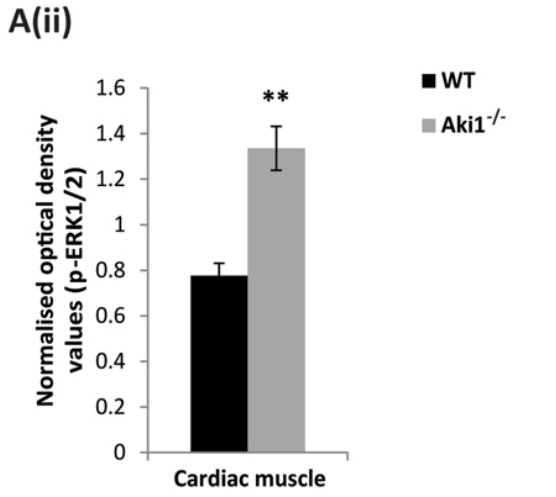


Figure S1. Lack of Akirin1 leads to activation of JNK signaling pathway in the cardiac muscle. Western blotting analysis was performed on Akirin1 knock-out and wild type cardiac muscle protein lysates. A(i) A representative immunoblot showing the protein levels of both phospho-JNK(T183/Y185) and total-JNK in Akirin1 knock-out and wild type cardiac muscle. GAPDH was used as an internal control for equal protein loading on the gel. A(ii) and A(iii) are corresponding densitometry graphs of phospho-JNK(T183/Y185) and total-JNK. A(iv) Densitometry graph representing the ratio of phospho- JNK(T183/Y185) to total-JNK levels. All the graph values are mean ±S.E. of four different animals (*p<0.05 and ***p<0.001).





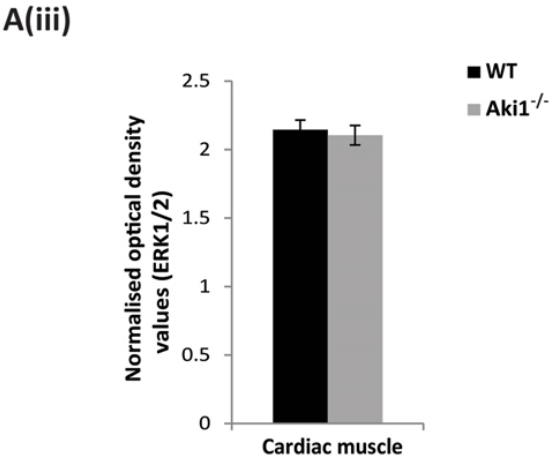


Figure S2.Lack of Akirin1 leads to activation of p38 MAPK signaling pathway in the cardiac muscle. Western blotting analysis was performed on Akirin1 knock-out and wild type cardiac muscle protein lysates. A(i) A representative immunoblot showing the protein levels of both phospho-p38MAPK (T180/Y182) and total-p38 MAPK in Akirin1 knock-out and wild type cardiac muscle. GAPDH was used as an internal control for equal protein loading on the gel. A(ii) and A(iii) Corresponding densitometry graphs of phospho-p38 MAPK (T180/Y182) and total-p38 MAPK. A(iv) Densitometry graph representing the ratio of phospho-p38 MAPK (T180/Y182) to total-p38MAPK levels. All the graph values are mean ±S.E. of four different animals (*p<0.05 and **p<0.01).

S3. Lack of Akirin1 leads to activation of ERK1/2 signaling pathway in the cardiac muscle

We investigated the levels of another member of MAPK pathway like ERK1/2, as it was shown by Haq et al., 2001 that ERK pathway is up- regulated during cardiac hypertrophy. Akirin1 knock-out and wild type cardiac muscle protein lysates were probed with anti-phospho-ERK1/2 (thr202/tyr204) and ERK1/2 antibodies. Western blotting results showed that Akirin1 knock-out cardiac muscle showed increased levels of phospho- ERK1/2 (thr202/tyr204) compared to the wild type cardiac muscle while the total ERK1/2 levels were unchanged between Akirin1 knock-out and wild type cardiac muscle [Figure 5.11A(i), A(ii) and A(iii)]. The ratio of phospho- ERK1/2 (thr202/tyr204) to total ERK1/2 was also significantly increased in Akirin1 knock-out cardiac muscle compared to the wild type cardiac muscle [Figure 5.11A(iv)]. These results together suggest that all the three main MAPK signaling pathways are activated in the absence of Akirin1 in cardiac muscle indicating cardiac hypertrophy.

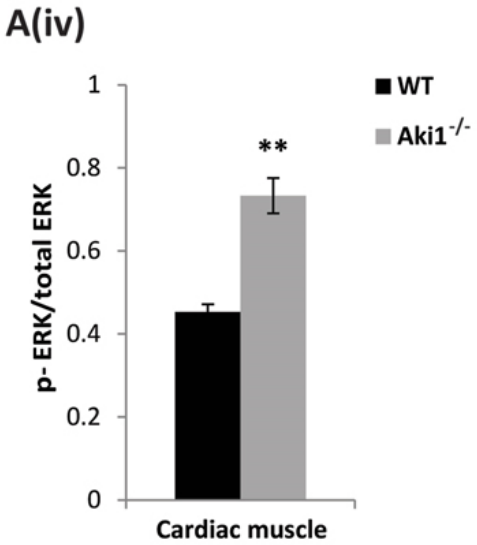


Figure S3. Lack of Akirin1 leads to activation of ERK1/2 signaling pathway in the cardiac muscle. Western blotting analysis was performed on Akirin1 knock-out and wild type cardiac muscle protein lysates. A(i) A representative immunoblot showing the protein levels of both phospho-ERK1/2 (thr202/tyr204) and total-ERK1/2 in Akirin1 knock-out and wild type cardiac muscle. GAPDH was used as an internal control for equal protein loading on the gel. A(ii) and A(iii) Corresponding densitometry graphs of phospho-ERK1/2 (thr202/tyr204) and total-ERK1/2. A(iv) Densitometry graph representing the ratio of phospho-ERK1/2 (thr202/tyr204) to total- ERK1/2 levels. All the graph values are mean ±S.E. of four different animals (**p<0.01).

Table 1. *RT-qPCR primers.*

Gene	Forward primer 5′-3′	Reverse primer 5′-3′
U6	CTCGCTTCGGCAGCACA	AACGCTTCACGAATTTGCGT
Pri- miR-1	GGTGGGCTGCTTCATGTT	TTTCCTTTAGCTTCTTCTTGGC
Cyclophilin	TTGCCATTCCTGGACCCAAA	ATGGCACTGGTGGCAAGTCC
Akirin1	ATAACCATTCCCACAGTTCCACAGC	TAAAAGAGCACTGGTGGGCTCTCA
β-MyHC/Myh7	TGCAAAGGCTCCAGGTCTGAGGGC	GCCAACACCACCCTGTCCAAGTTC
ANP	CCAGGCCATATTGGAGCAAA	GAAGCTGTTGCAGCCTAGTC
mature miR-1	TGGAATGTAAAGAAGTATGTAT	TTTTTTTTTTTTTTTTT
SRF	TACCAGGTGTCGGAGTCTGACA	GGCAGGTTGGTGACTGTGAA

Table 2. *Western antibodies and dilutions.*

Antibody	Catalogue No.	Source	Dilution used
4eBP1	sc-9977	Santa Cruz	1:500
Akt	sc-8312	Santa Cruz	1:2000
ANP	sc-80686	Santa Cruz	1:500
Desmin	D76	DSHB	1:500
GAPDH	sc-166545	Santa Cruz	1:10000
GSK3β	612312	BD Pharmingen	1:500
IGF-1	sc-9013	Santa Cruz	1:300
mTOR	2983	Cell signaling	1:300
p-4eBP1 (ser65/thr70)	sc-12884	Santa Cruz	1:500

p-Akt (ser473-R)	sc-7985-R	Santa Cruz	1:500
p-GSK3β (ser9)	9336	Cell signaling	1:500
p-mTOR (ser2448)	2971	Cell signaling	1:300
PI3-Kp85	4292	Cell signaling	1:500
pPI3-Kp85(tyr458)/p55(tyr199)	4228	Cell signaling	1:200
SRF	5147	Cell signaling	1:1000
α-actin	sc-58670	Santa Cruz	1:1000
α-Myh (cardiac)	Ab50967	Abcam	1:100
β-Myh (cardiac MYH7)	sc- 71575	Santa Cruz	1:200

Table 3. *Secondary antibodies.*

Antibody	Catalogue no.	Source	Dilution used	Usage
Goat anti-mouse IgG HRP conjugate	170-6516	Bio-Rad	1:5000	Western blot
Goat anti-rabbit IgG HRP conjugate	170-6515	Bio-Rad	1:5000	Western blot
Goat anti-rat IgG HRP conjugate	sc-2006	Santa Cruz	1:2000	Western blot