Supplementary Fig. 1: DKO mice accumulate excess bile acids and demonstrate liver damage, but exhibit no fibrosis or overt injury in the heart.

(A) Serum bile acid levels were dramatically elevated in DKO compared to the individual *FXRKO* and *SHPKO* mice. **(B)** Serum ALT and AST levels were also increased in DKO mice than the WT controls. This data is in line with our previous findings. (n=5-6 mice/group; * $p<0.05$ compared to WT mice; Mean ± SEM). Heart tissues from WT and DKO mice were processed, sectioned and stained with Hematoxylin & Eosin **(C)**, and Masson's trichrome **(D)** in order to analyze for changes in the cardiac architecture. (20X Magnification)

Supplementary Fig. 2: DKO hearts exhibit hypertrophy, bradycardia, distress and poor response to pressure overload.

(A) DKO hearts show increased heart-to-body weight ratio, which is corroborated by induced expression of cytoskeleton gene *β-MyH7* **(B).** DKO hearts exhibit reduced heart rate **(C)**, along with activation of stress as well as pro-apoptotic pathways **(D).** Upon TAC, DKO mice displayed an increase in heart weight and decrease in ejection fraction (EF) with modest decrease in shortening fraction (FS) in comparison to WT mice **(E-F)** (n=5-6/group; *****$p<0.05$ when compared to WT animals; Mean ± SEM)**.**

Supplementary Fig. 3: Pgc1α downstream signaling is repressed in DKO hearts.

qRT-PCR analysis was performed on the RNA isolated from 5 months old WT and DKO hearts for Pgc1α downstream target genes (**A-D**). The expression levels were normalized to *36b4*. (n=4-6 mice/ group; *****p<0.05; **p<0.001 when compared to WT mice; Mean± SEM).

Supplementary Fig. 4: Deletion of FXR does not alter electrophysiological parameters of the heart but induces FAO and inhibits glucose oxidation.

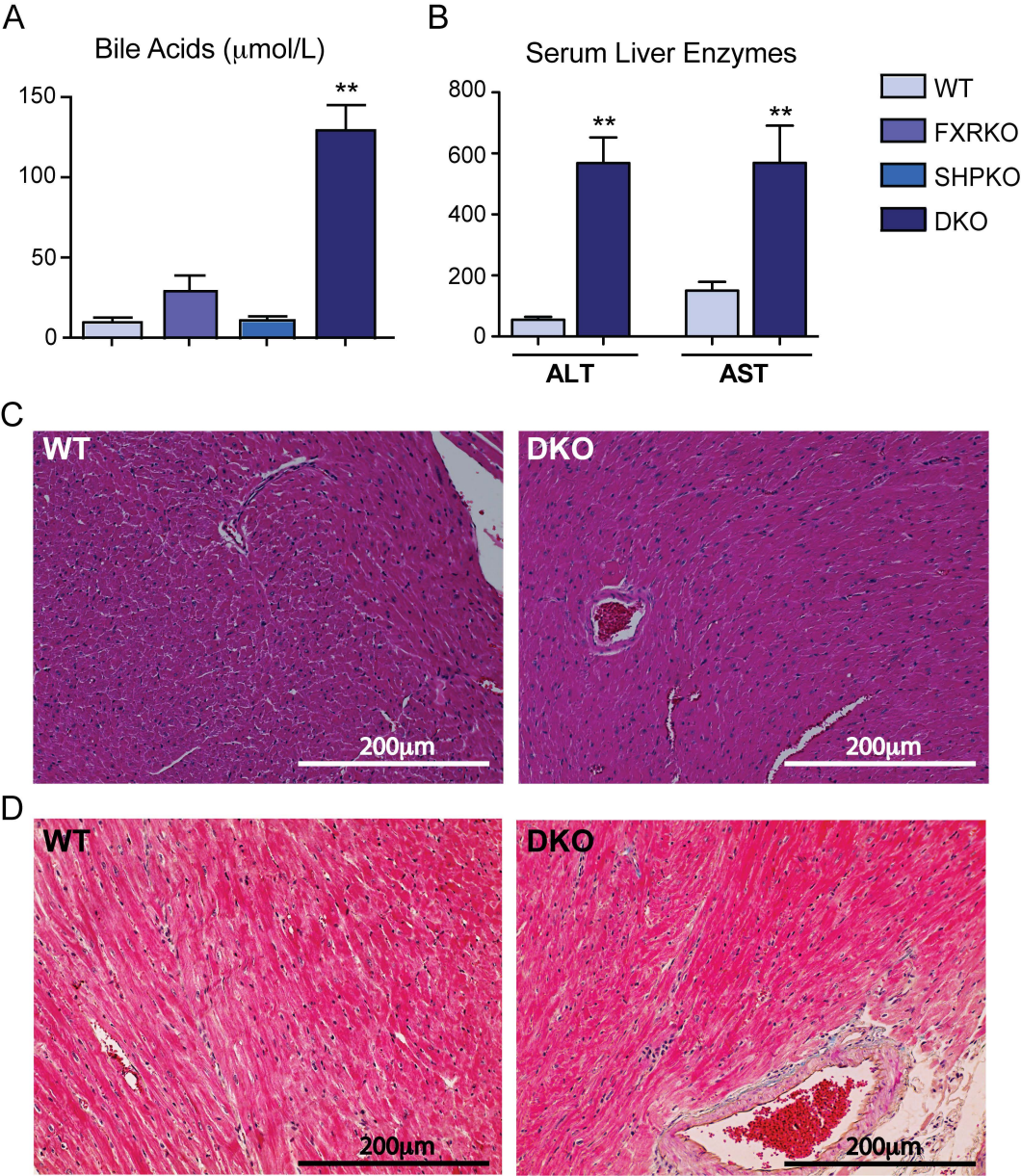
qRT-PCR analysis was performed on the RNA isolated from 5 months old WT, and *FXRKO* hearts for the genes regulating fatty acid oxidation and glucose utilization (**A-E**). The expression levels were normalized to *36b4*. *FXRKO* mice showed normal cardiovascular parameters as evaluated by echocardiography **(F)** compared to WT controls. (n=3-6 mice/ group; *****p<0.05; **p<0.001 when compared to WT mice; Mean± SEM).

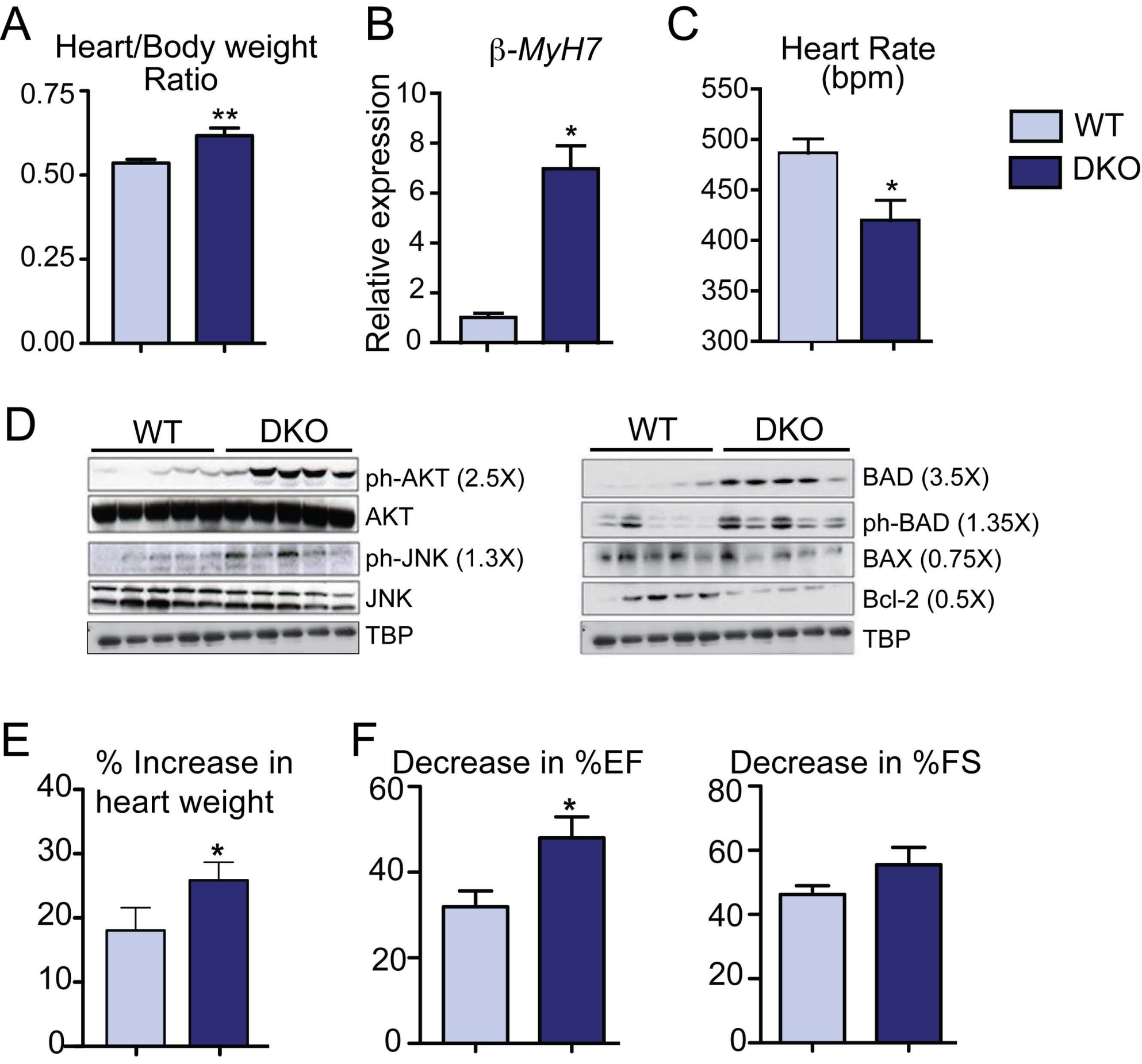
Supplementary Fig. 5: Deletion of SHP reduces heart rate, *mCpt1* but increases glucose oxidation.

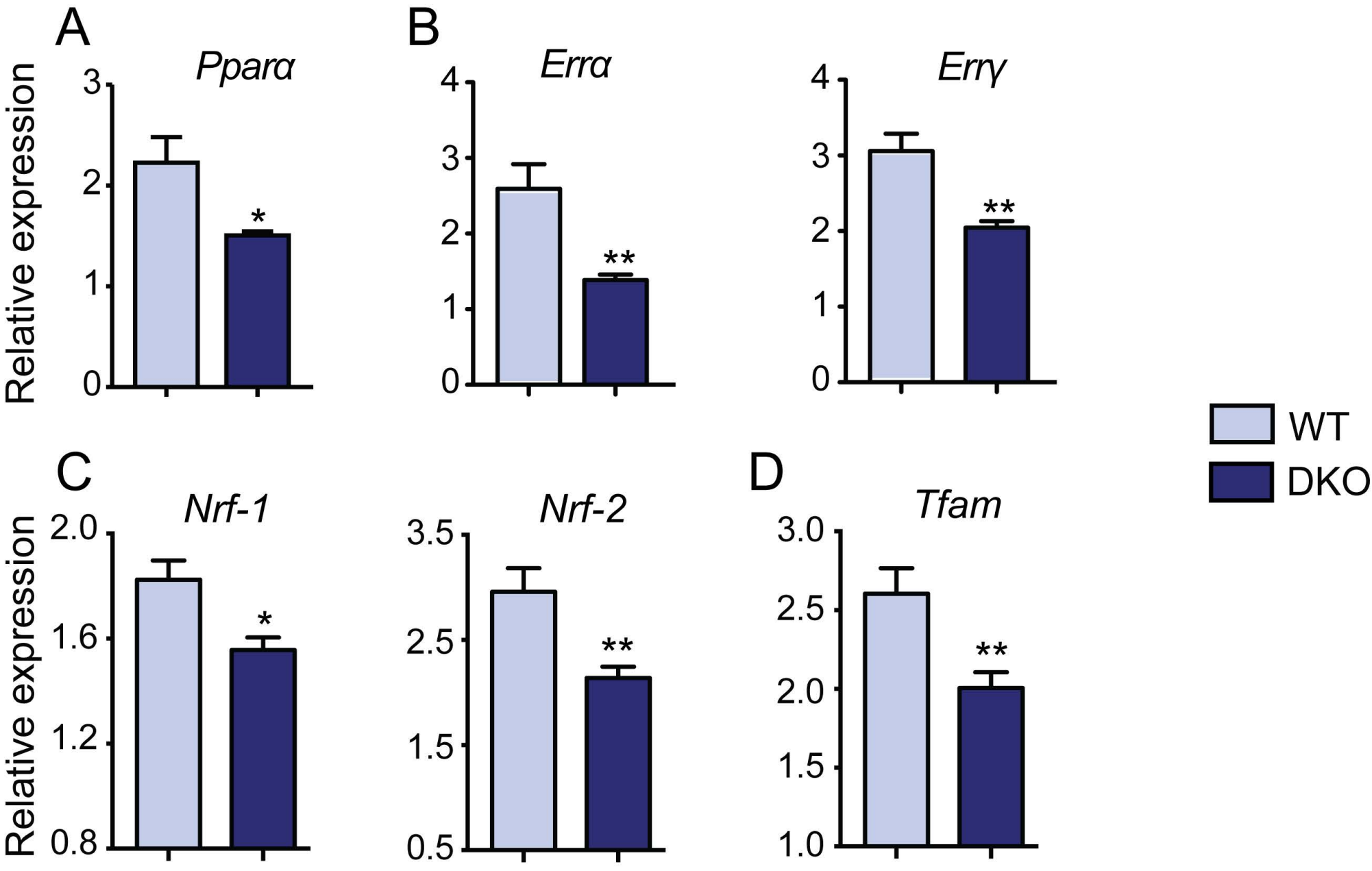
qRT-PCR analysis was performed on the RNA isolated from 5 months old WT, and *SHPKO* hearts for the genes regulating fatty acid oxidation and glucose utilization **(A-E)**. The expression levels are normalized to *36b4*. *SHPKO* mice showed normal cardiovascular parameters, except for a decrease in heart rate **(F)** compared to WT controls. (n=3-6 mice/ group; *****p<0.05; **p<0.001 when compared to WT mice; Mean± SEM).

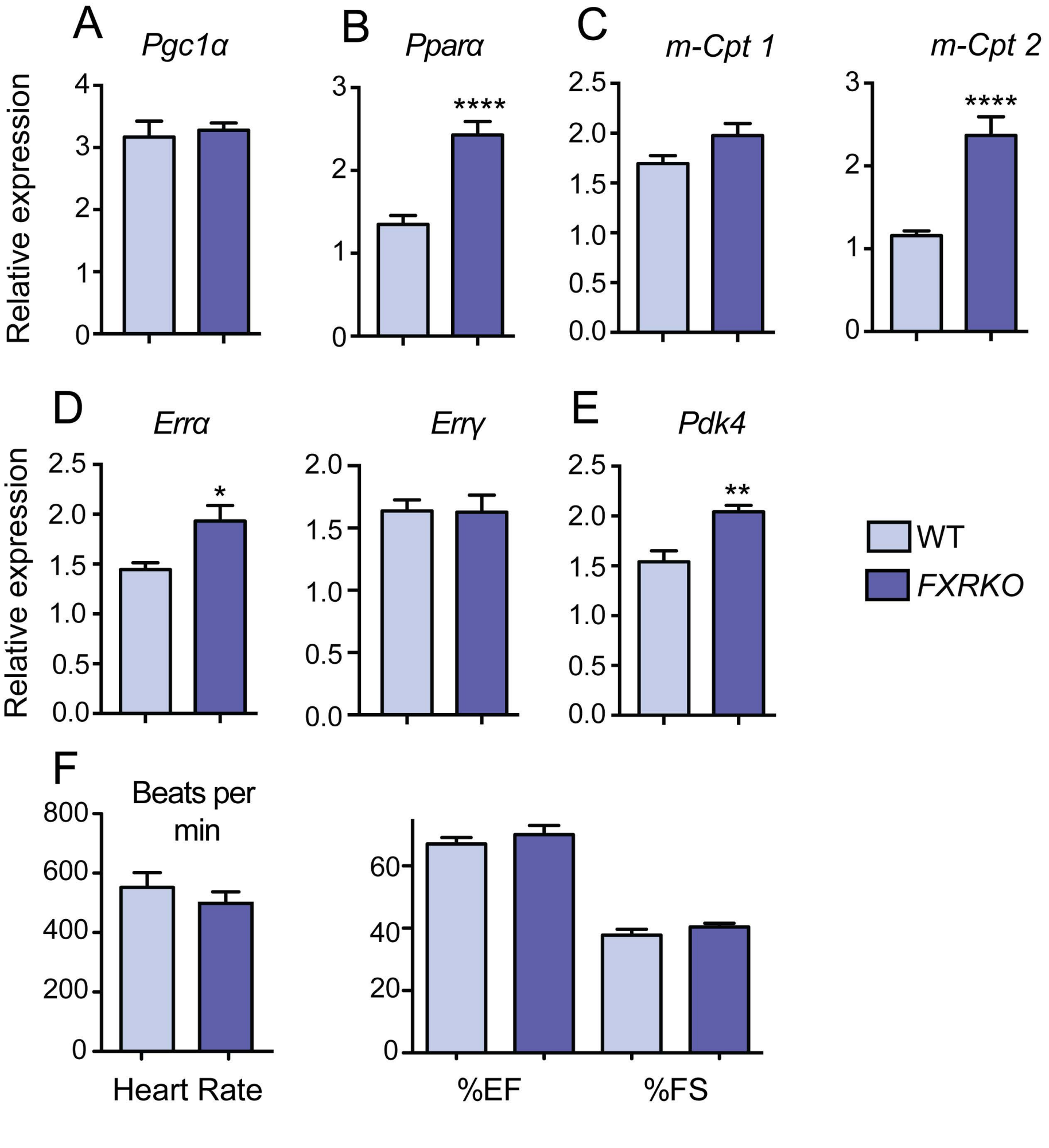
Supplementary Fig. 6: Exogenous administration of bile acids induces bradycardia and metabolic reprogramming in the hearts.

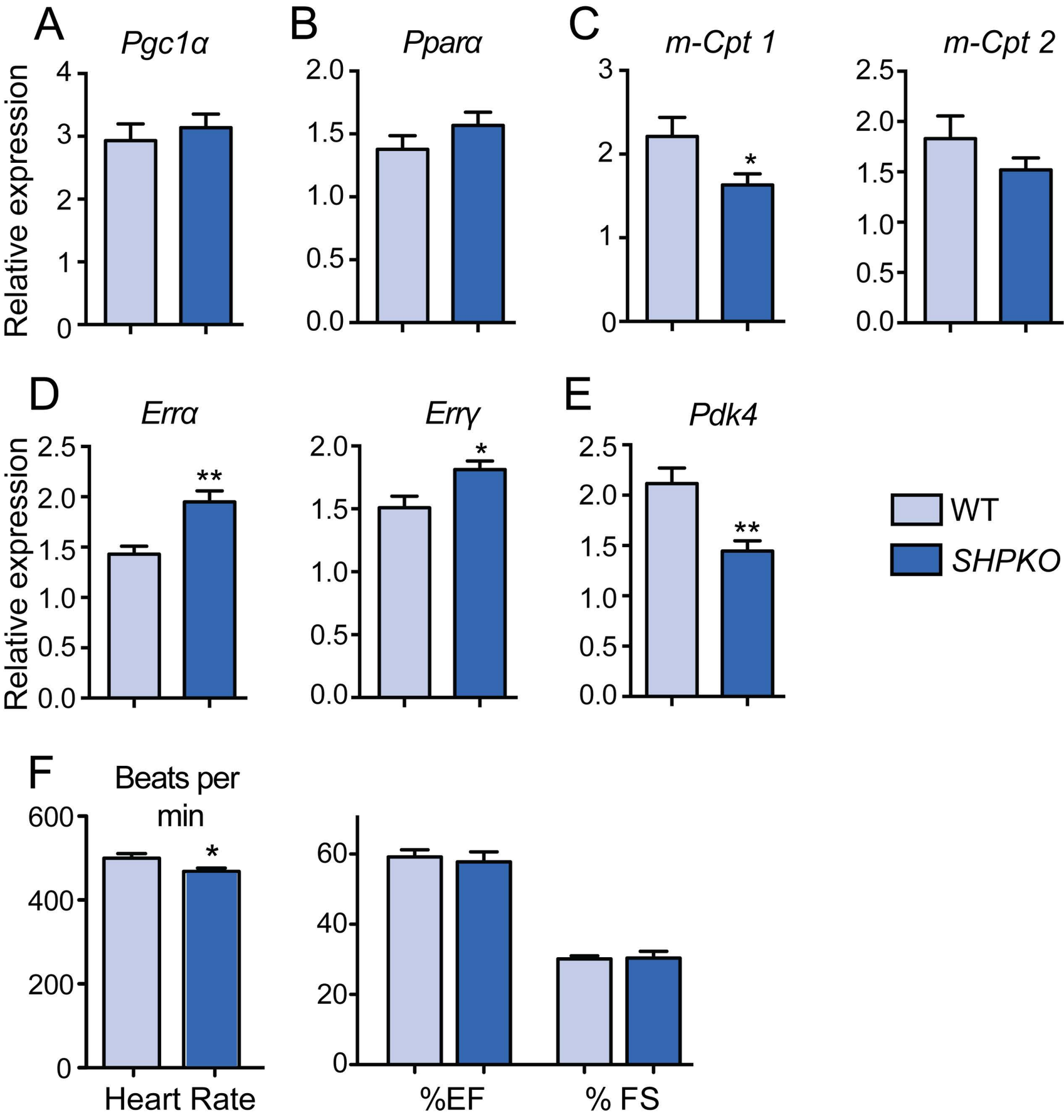
WT mice were injected with 100 mg/kg Lithocholic Acid (LCA) or corn oil vehicle for 4 days (1 dose/day). **(A-B)** Bar graph show significant reduction in heart rate and left ventricular inner diameter. **(C)** However the acute exposure to bile acids *in vivo* was not sufficient to alter ejection fraction or shortening fraction. **(D-F)** WT mice injected with 100mg/kg Taurocholic Acid (TCA) demonstrated bradycardia, decreased cycle amplitude and oleate oxidation, compared to the mice injected with corn oil. (n=3-4 mice/group; *$p<0.05$; **$p<0.001$ when compared to vehicle treated WT; Mean± SEM).

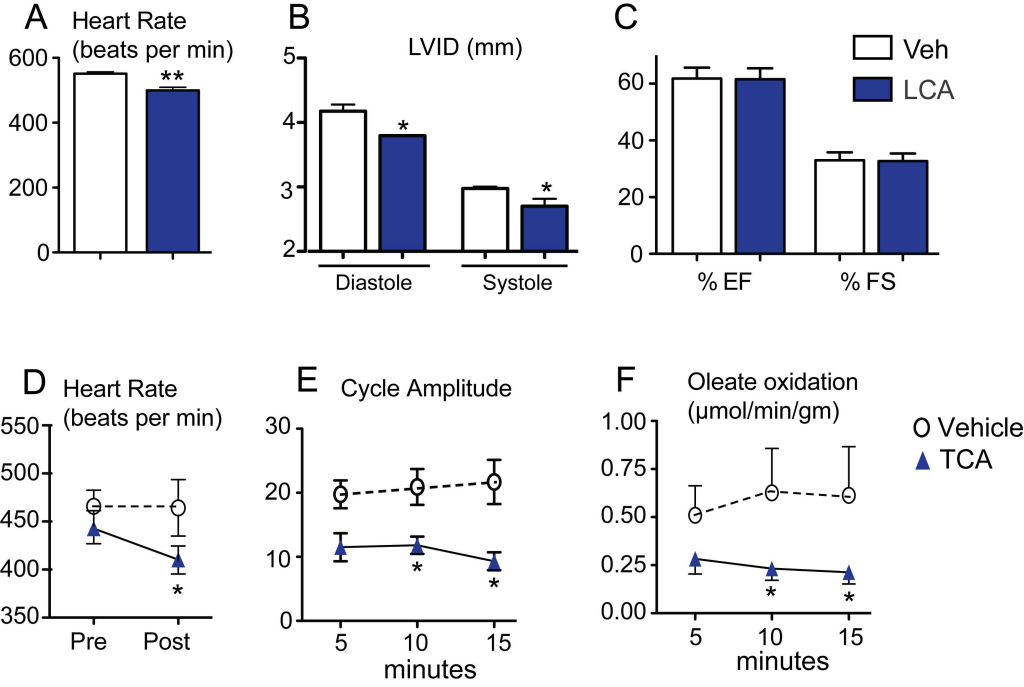

A
Bile Acids (μmol/L)
150
100
50
0
**
B
Serum Liver Enzymes
800
600
400
200
0
**
**
ALT
AST
WT
FXRKO
SHPKO
DKO
C
WT
200μm
DKO
200μm
D
WT
200μm
DKO
200μm


A
Heart/Body weight Ratio
0.75
0.50
0.25
0.00
**
B
β-MyH7
Relative expression
10
8
6
4
2
0
*
C
Heart Rate (bpm)
550
500
450
400
350
300
*
WT
DKO
D
WT
DKO
ph-AKT (2.5X)
AKT
ph-JNK (1.3X)
JNK
TBP
WT
DKO
BAD (3.5X)
ph-BAD (1.35X)
BAX (0.75X)
Bcl-2 (0.5X)
TBP
E
% Increase in heart weight
40
30
20
10
0
*
F
Decrease in %EF
60
40
20
0
*
Decrease in %FS
80
60
40
20
0


A
Pparα
B
Errα
Errγ
C
Nrf-1
Nrf-2
D
Tfam
Relative expression
Relative expression
WT
DKO
*
**
**
*
**
**


A
Pgc1α
B
Pparα
C
m-Cpt 1
m-Cpt 2
Relative expression
****
****
D
Errα
Errγ
E
Pdk4
Relative expression
*
**
WT
FXRKO
F
Beats per min
Heart Rate
%EF
%FS


A
Pgc1α
B
Pparα
C
m-Cpt 1
m-Cpt 2
Relative expression
D
Errα
Errγ
E
Pdk4
WT
SHPKO
F
Beats per min
Heart Rate
%EF
% FS


A
Heart Rate
(beats per min)
600
400
200
0
**
B
LVID (mm)
5
4
3
2
*
*
Diastole
Systole
C
Veh
LCA
60
40
20
0
% EF
% FS
D
Heart Rate
(beats per min)
550
500
450
400
350
*
Pre
Post
E
Cycle Amplitude
30
20
10
0
*
*
5
10
15
minutes
F
Oleate oxidation
(µmol/min/gm)
1.00
0.75
0.50
0.25
0.00
*
*
5
10
15
minutes
O Vehicle
▲ TCA

Supplementary Table 1: Overlapping similarities in the cardiovascular pathophysiology observed in DKO mouse model compared to human cirrhotic cardiomyopathy.

PARAMETERS	DKO mouse model	Human CCM
Bradycardia	DKO mice demonstrate lower heart rate (Fig.1D). Bradycardia was also observed in DDC induced mouse model of biliary fibrosis as well as cholic acid fed Abcb11$^{-/-}$ mice, which also exhibit pathologic cholanemia and jaundice (1, 2).	Cirrhotic patients often exhibit tachycardia secondary to a chronic inflammation and low systemic vascular resistance due to vasodilation (3). However, patients with obstructive jaundice exhibit bradycardia –described as *"icteric bradycardia"* (4-6).
Electrocardiographic abnormality	DKO mice demonstrate prolonged QTc interval and prolonged PR interval (Fig.1E). Similarly, DDC fed mouse model of biliary fibrosis also demonstrated prolonged QT interval (1).	Prolongation of the QT interval is one of the key features of human CCM and is associated with increased risk for mortality in both adults and children (7-10). Bile acids are sufficient to induce rhythm disturbance in humans (9, 11,12).
Increased EF and FS	DKO mice show increased ejection and shortening fraction (Fig.1E), which indicates hyperdynamic contractility of the LV.	Hyperdynamic LV is seen in both adults and children with end stage liver disease (13,14).
Diastolic dysfunction	Diastolic dysfunction is difficult to evaluate using mouse echocardiograms. LVID - the internal diameter of the LV in diastole can be used as a surrogate marker to estimate LV filling. DKO mice have smaller LVID than the WT mice (Fig. 1B) indicating less LV filling.	Diastolic dysfunction is a consistent finding of human CCM (14,15).
Catecholamine resistance	DKO mice took a longer time to achieve peak heart rate and showed an attenuated rise in the shortening fraction (contractility) in response to isoprenaline, indicating resistance to catecholamine stimulation (Fig	Inadequate response to catecholamine has been shown in human CCM (14, 20).

	2B). This data is consistent with published findings in other mouse models of biliary fibrosis and bile acid overload (2,16-19).	
Exercise intolerance	DKO mice show exercise intolerance (Fig. 2A).	Exercise fatigue is seen in patients with cirrhosis and has been attributed to CCM (21).
Biochemical evidence of myocardial injury	Elevated cardiac troponin levels are detected in DKO mice (Fig.1G) suggesting injury to myocardium (22,23).	Elevations of cardiac troponin levels in serum have been detected in human CCM (24).
Possible metabolic etiology	In this study we suggest that bile acid mediated alteration in substrate utilization as one of the possible mechanism responsible for the cardiomyopathy observed in this model.	Alteration in cardiac metabolism as a cause of cardiomyopathy in cirrhotic patients has been previously speculated (25).

References

1. Desai MS, Shabier Z, Taylor M, Lam F, Thevananther S, Kosters A, Karpen SJ. Hypertrophic cardiomyopathy and dysregulation of cardiac energetics in a mouse model of biliary fibrosis. Hepatology 2010 Jun;51(6):2097-107

2. Desai MS, Eblimit Z, Thevananther S, Kosters A, Moore DD, Penny DJ, Karpen SJ. Cardiomyopathy reverses with recovery of liver injury, cholestasis and cholanemia in mouse model of biliary fibrosis. Liver Int. 2013 Dec 14;

3. Moller S, Henriksen JH. Cardiovascular complications of cirrhosis. Postgrad.Med.J. 2009 Jan;85(999):44-54

4. Bashour TT, Antonini C, Sr., Fisher J. Severe sinus node dysfunction in obstructive jaundice. Ann.Intern.Med. 1985 Sep;103(3):384-5

5. John Wickham Legg. On the bile, jaundice and biliary diseases. H.K.Lewis, 136 Gower Street ,London.; 1879.

6. Song E, Segal I, Hodkinson J, Kew MC. Sinus bradycardia in obstructive jaundice--correlation with total serum bile acid concentrations. S.Afr.Med.J. 1983 Sep 28;64(14):548-51

7. Arikan C, Kilic M, Tumgor G, Levent E, Yuksekkaya HA, Yagci RV, Aydogdu S. Impact of liver transplantation on rate-corrected QT interval and myocardial function in children with chronic liver disease*. Pediatr.Transplant. 2009 May;13(3):300-6

8. Bernardi M, Calandra S, Colantoni A, Trevisani F, Raimondo ML, Sica G, Schepis F, Mandini M, Simoni P, Contin M, et al. Q-T interval prolongation in cirrhosis: prevalence, relationship with severity, and etiology of the disease and possible pathogenetic factors. Hepatology 1998 Jan;27(1):28-34

9. Finucci G, Lunardi F, Sacerdoti D, Volpin R, Bortoluzzi A, Bombonato G, Angeli P, Gatta A. Q-T interval prolongation in liver cirrhosis. Reversibility after orthotopic liver transplantation. Jpn.Heart J. 1998 May;39(3):321-9

10. Zambruni A, Trevisani F, Caraceni P, Bernardi M. Cardiac electrophysiological abnormalities in patients with cirrhosis. J.Hepatol. 2006 May;44(5):994-1002

11. Desai MS, Penny DJ. Bile acids induce arrhythmias: old metabolite, new tricks. Heart 2013 Aug 22;

12. Rainer PP, Primessnig U, Harenkamp S, Doleschal B, Wallner M, Fauler G, Stojakovic T, Wachter R, Yates A, Groschner K, et al. Bile acids induce arrhythmias in human atrial myocardium--implications for altered serum bile acid composition in patients with atrial fibrillation. Heart 2013 Jul 26;

13. Desai MS, Zainuer S, Kennedy C, Kearney D, Goss J, Karpen SJ. Cardiac structural and functional alterations in infants and children with biliary atresia, listed for liver transplantation. Gastroenterology 2011 Oct;141(4):1264-72

14. Wiese S, Hove JD, Bendtsen F, Moller S. Cirrhotic cardiomyopathy: pathogenesis and clinical relevance. Nat.Rev.Gastroenterol.Hepatol. 2014 Mar;11(3):177-86

15. Wong F. Cirrhotic cardiomyopathy. Hepatol.Int. 2009 Mar;3(1):294-304

16. Binah O, Bomzon A, Blendis LM, Mordohovich D, Better OS. Obstructive jaundice blunts myocardial contractile response to isoprenaline in the dog: a clue to the susceptibility of jaundiced patients to shock? Clin.Sci.(Lond) 1985 Dec;69(6):647-53

17. Ceolotto G, Papparella I, Sticca A, Bova S, Cavalli M, Cargnelli G, Semplicini A, Gatta A, Angeli P. An abnormal gene expression of the beta-adrenergic system contributes to the pathogenesis of cardiomyopathy in cirrhotic rats. Hepatology 2008 Dec;48(6):1913-23

18. Joubert P. An in vivo investigation of the negative chronotropic effect of cholic acid in the rat. Clin.Exp.Pharmacol.Physiol 1978 Jan;5(1):1-8

19. Zavecz JH, Battarbee HD. The role of lipophilic bile acids in the development of cirrhotic cardiomyopathy. Cardiovasc.Toxicol. 2010 Jun;10(2):117-29

20. Torregrosa M, Aguade S, Dos L, Segura R, Gonzalez A, Evangelista A, Castell J, Margarit C, Esteban R, Guardia J, et al. Cardiac alterations in cirrhosis: reversibility after liver transplantation. J.Hepatol. 2005 Jan;42(1):68-74

21. Wong F, Girgrah N, Graba J, Allidina Y, Liu P, Blendis L. The cardiac response to exercise in cirrhosis. Gut 2001 Aug;49(2):268-75

22. Mair J, Dienstl F, Puschendorf B. Cardiac troponin T in the diagnosis of myocardial injury. Crit Rev.Clin.Lab Sci. 1992;29(1):31-57

23. Mair P, Mair J, Seibt I, Wieser C, Furtwaengler W, Waldenberger F, Puschendorf B, Balogh D. Cardiac troponin T: a new marker of myocardial tissue damage in bypass surgery. J.Cardiothorac.Vasc.Anesth. 1993 Dec;7(6):674-8

24. Wiese S, Mortensen C, Gotze JP, Christensen E, Andersen O, Bendtsen F, Moller S. Cardiac and proinflammatory markers predict prognosis in cirrhosis. Liver Int. 2014 Jul;34(6):e19-e30

25. Fukazawa K, Gologorsky E, Manmohansingh V, Nishida S, Vigoda MM, Pretto EA, Jr. Is the immediate reversal of diastolic dysfunction of cirrhotic cardiomyopathy after liver transplantation a sign of the metabolic etiology? Liver Transpl. 2009 Nov;15(11):1417-9

Supplementary Table 2: qRT-PCR primer sequences used in this study

Primer Sequence	Forward Primer	Reverse Primer
β-Myh7	5'- TTCATCCGAATCCATTTTGGGG -3	5'- GCATAATCGTAGGGGTTGTTGG -3'
Troponin, cardiac	5'- TCTGCCAACTACCGAGCCTAT-3'	5'- CTCTTCTGCCTCTCGTTCCAT -3'
Pgc1a	5'- CCCACAGAAAACAGGAACAG -3'	5'- CTGGGGTCAGAGGAAGAGAT -3'
Ppara	5'- ACAAGGCCTCAGGGTACCA -3'	5'- GCCGAAAGAAGCCCTTACAG -3'
m-Cpt 1	5'- GCGACAGGCATTTTCTTC -3'	5'- AGGAGACGGACACAGATAGC -3'
m-Cpt 2	5'- GCTGCCTATCCCTAAACTTG -3'	5'- CTTCCCAATGCCGTTCTC -3'
Erra	5'- GTACGTCCTGCTGAAAGCTC -3'	5'- CCAGCTTCATACTCCAGCAG -3'
Errg	5'- GTTGTGTACCGATCGCTTTC -3'	5'- CCAGCTGCAGGATAGCATTA -3'
Pdk4	5'- GGATTACTGACCGCCTCTTTAGT -3'	5'- AGATGATAGCGTCTGTCCCATAA -3'
Nrf-1	5'- GCACCTTTGGAGAATGTGGT -3'	5'- CTGAGCCTGGGTCATTTTGT -3'
Nrf-2	5'- CTCTCTGAACTCCTGGACGG -3'	5'- GGGTCTCCGTAAATGGAAG -3'
Tfam	5'- CAAGTCAGCTGATGGGTATGG -3'	5'- TTTCCCTGAGCCGAATCATCC -3'

Supplementary Table 3: Coefficient of Variation for the different Cardiovascular Parameters obtained from Echocardiography.

	MEAN	**SD**	**SEM**	**CV**	**Mann-Whitney**
WT (HR) bpm	490	37	13	6.85%	P= 0.0148
DKO (HR) bpm	386	59	21	12.75%	
WT (PR) msec	22.5	1.4	0.54	6%	P=0.015
DKO (PR) msec	26.6	2.2	0.85	8%	
WT (% EF)	57	10	2	16%	P=0.018
DKO (% EF)	66	9.5	1.8	14%	
WT (% FS)	30	5.9	1.3	19%	P=0.005
DKO (%FS)	37	7.9	1.5	21%	