

SUPPLEMENTAL METHODS

Cell cultures

Mouse neonatal hearts were dissected and incubated with F-10 nutrient medium, containing 140 µg/ml of collagenase type-2 (Sigma) and 440 µg/ml pancreatin (Sigma) until cells were dissociated. The cells were then washed, resuspended in 1:1 mix of Dulbecco's minimal Eagle's medium (DMEM) and F10 nutrient mix with 5% each of equine and bovine calf serum. These cells were pre-plated in tissue culture dishes for 1 h to allow for attachment of nonmyocytes. The resulting non-adherent myocytes were plated on fibronectin-coated (Sigma) tissue culture dishes. Cells from one heart were plated in each 35 mm² petridish and cultured for 24 h, after which the medium was replaced with 1:1 mix of DMEM and F10 nutrient medium containing 1 µg/ml thyroxin, 5 µg/ml of transferrin, 1 µg/ml of insulin, 10 pM each of LiCl and selenite. A 0.1 mM bromodeoxyuridine concentration was used to check fibroblast growth.

CaMKIIN mice

The HA eptiope and membrane association domain (MEM), encoding a palmitoylation sequence, were added to the 5' of βCaMKIIN sequence by PCR using the 5' primer: gccattaagcttatgctctgctgtatgagaagaaccaaacaggttgaaaagaatgatgaggacaaaagatcatgtccg-agatcctaccctacggc and the 3' primer ccgtatggatccctacactccggacggaggcttctc (Integrated DNA Technologies) with *HindIII* and *BamHI* sites at the extreme ends. The DNA template for PCR was pSB3-His-CaMKIIN. CaMKIIN cDNA construct was a kind gift from Dr. Tom Soderling (Vollum Institute). The product (~360 base pairs) was purified from agarose gel (Quiagen gel extraction kit) and was digested with restriction enzymes, *HindIII* and *BamHI*. The resulting DNA product was ligated into pCMV5-HA1 previously digested with *HindIII* and *BamHI*. The HA-tagged MEM-CaMKIIN sequence was subcloned into pα-MHC promoter vector by PCR using the following primers: 5'-ccatgagtcgacgccgccaccatgtacccatagcagctcccagactatgctgg taccacgcgtatcgataaactt and 3'-gccattaagcttctacactccggacggaggcttctc that incorporated *SalI* and

kozak sites in the 5' region and a *HindIII* site in the 3' region. PCR products digested with *SaI* and *HindIII* were ligated into the p α MHC-script-Hgh vector (9.2 kb). Mouse embryonic stem cells were injected with the linearized DNA (digested with *NotI*) in the Vanderbilt Transgenic Mouse Core Facility and implanted into B6D2 pseudo-pregnant females. Insertion of the transgene into the mouse genome was confirmed by PCR analysis (not shown) using the above primers.

Quantitative RT-PCR

For quantitative RT-PCR, 1 μ l of reverse transcription reaction was mixed with 10 pmoles each specific primer and 12.5 μ l SYBR PCR Master Mix (BioRad). The reaction was incubated in an iQ5 model thermocycler (BioRad) for 40 cycles consisting of denaturation at 95°C for 10 s and annealing/extension at 59.9°C for 1 min. The quality of the PCR product was routinely checked by thermal denaturation curve following the qPCR reactions. The threshold cycle (C_T) was determined by the iCycler software, and quantification of relative mRNA levels was performed by $\Delta\Delta C_T$ method. We thank Dr. Mollie Meffert (Johns Hopkins) for I κ B-DN lentivirus expression construct. mCfbFor 5'-GAAACCCTGTCACTGTCATTC-3' and mCfbRev 5'-CCCCAAACACATACACA TCC-3'.

SUPPLEMENTAL FIGURE LEGENDS

Suppl. Figure 1:

Complement factor B expression in normal hearts. (A) Quantitative RT-PCR analyses of RNA from hearts (n=3) shows increased *Cfb* mRNA levels in AC3-I compared to WT. (B) Immunoblotting of heart extracts (n=3) shows similar amounts of CFB in both WT and AC3-I. (C) Immunoblot of WT and AC3-I heart extracts was probed with anti-CFB antibody (upper panel) as described in the Methods. Following the signal detection, the blot was stained with coomassie blue (lower panel).

Suppl. Figure 2:

Cfb expression in cardiomyocytes. (A) RT-PCR analysis of *Cfb* and *Hprt* performed on cultured neonatal (Neo) and isolated adult cardiomyocytes. The RT-PCR lanes representing reverse transcriptase reaction with and without reverse transcriptase enzyme are designated as '+' and '-', respectively. The far left and far right columns show molecular size markers marked in base pairs (bp). (B) Immunoblotting for CFB protein expression in neonatal and adult cardiomyocytes, and heart tissue. Equal amount of total protein from each sample was fractionated using NuPAGE gels. Antibody to CFB was used to detect the CFB protein band. Immunoblots of actin were used as a loading control.

Suppl. Figure 3:

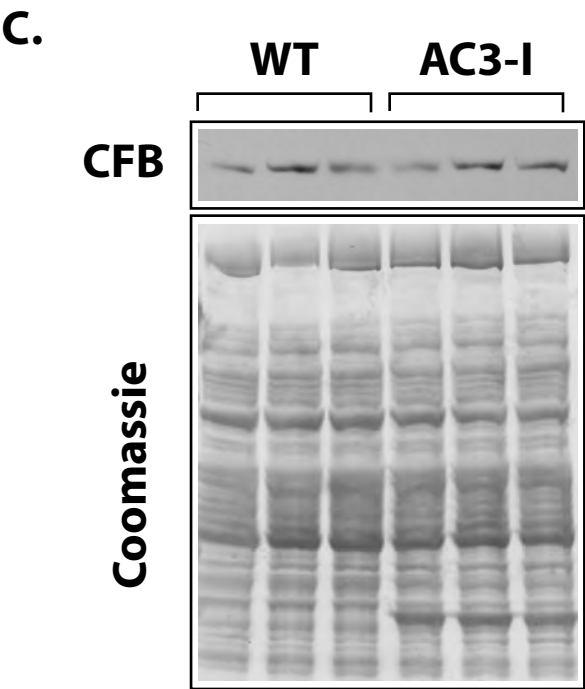
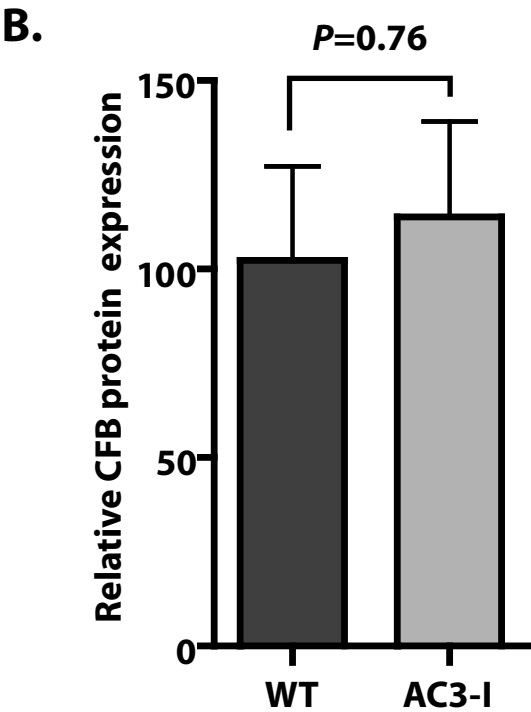
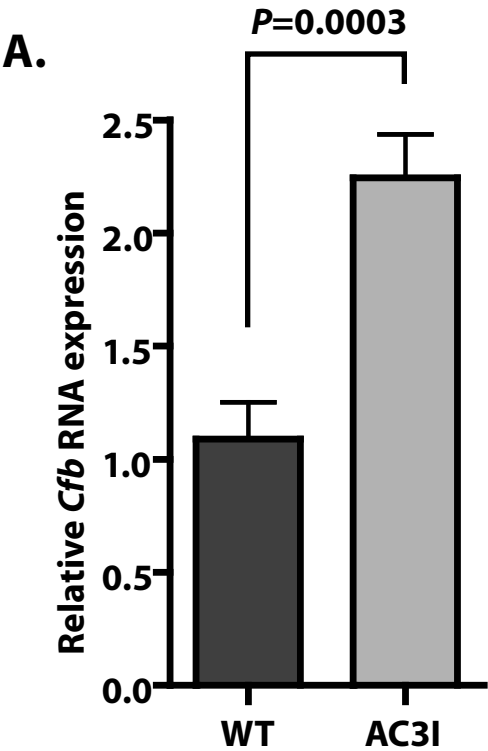
Death and apoptosis markers in neonatal cardiomyocytes treated with LPS. (A) Fraction of dead cardiomyocytes determined by flow cytometry using propidium iodide (PI) fluorescent vital dye (Molecular Probes). A total of 18,344 and 14,647 cells were sorted for control (serum alone) and treatment (serum and LPS), respectively. The significance of the results of PI-positive cells in each treatment was determined by Fisher's Exact test. (B) TUNEL staining data from mouse serum and LPS treated cultured cardiomyocytes. All data are from n>3 separate experiments.

Suppl. Figure 4:

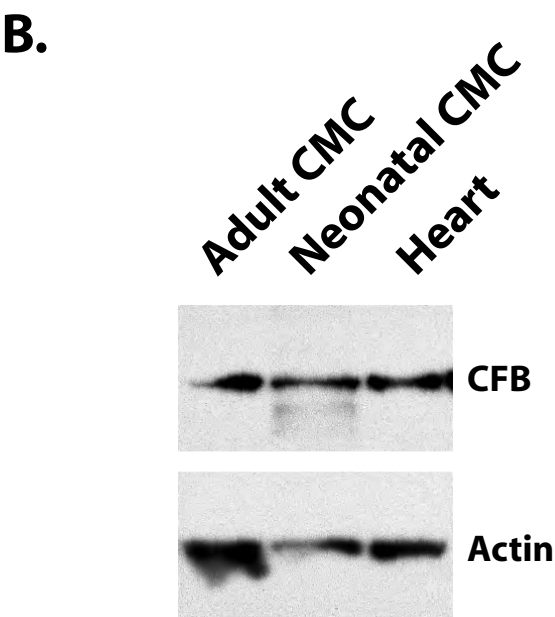
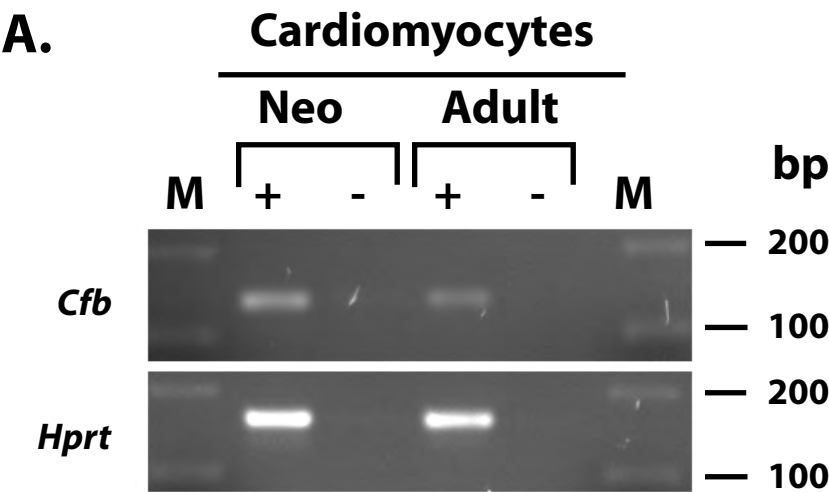
(A) Expression of β CaMKIIN in transgenic mouse heart and inhibition of CaMKII mediated phosphorylation of T17 residue of phospholamban (PLN). Heart extracts from wild type (WT) and β CaMKIIN transgenic mice (CaMKIIN) were fractionated on a NuPage gel (Invitrogen) and transferred onto a PVDF membrane (BioRad). Blots were probed with either a monoclonal

antibody to detect HA-epitope tagged cardiomyocyte-delimited expression of transgenic β CaMKIIN or antibodies to phospholamban (PLN). Specific antibodies to either total or phospho-specific epitope were used. For PLN, the same blot was stripped and reprobed in the following order: PLN-T17, PLN-S16, and PLN-total. **(B)** Membrane localization of β CaMKIIN. DNA binding fluorescent stain DAPI was used to visualize the nuclei. Images were obtained by BioRad multiphoton confocal microscopy and rendered pseudocolors (Red= anti-HA, Blue= DAPI). Scale bars = 20 μ m.

Suppl Figure-1

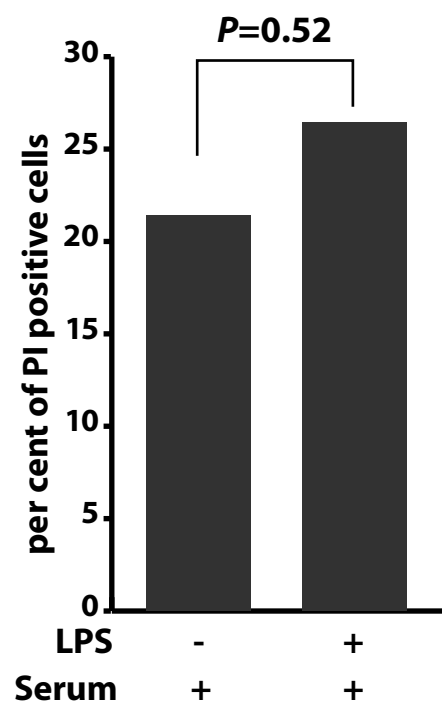


Suppl Figure-2

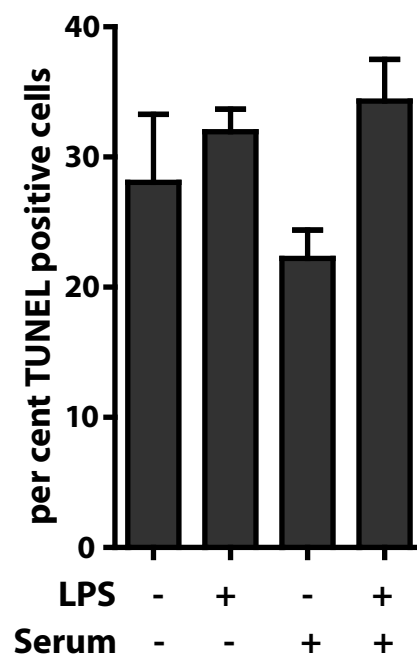


Suppl Figure-3.

A.

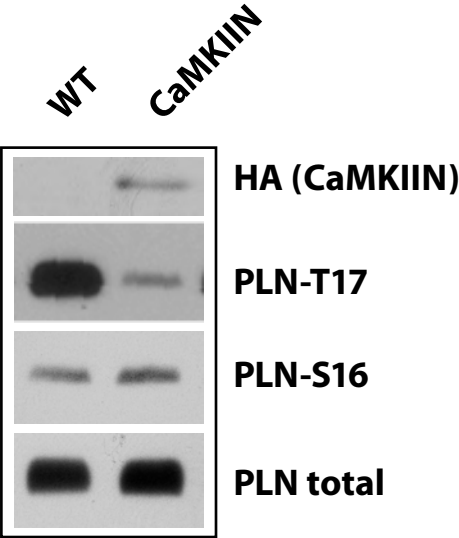


B.



Suppl Figure-4.

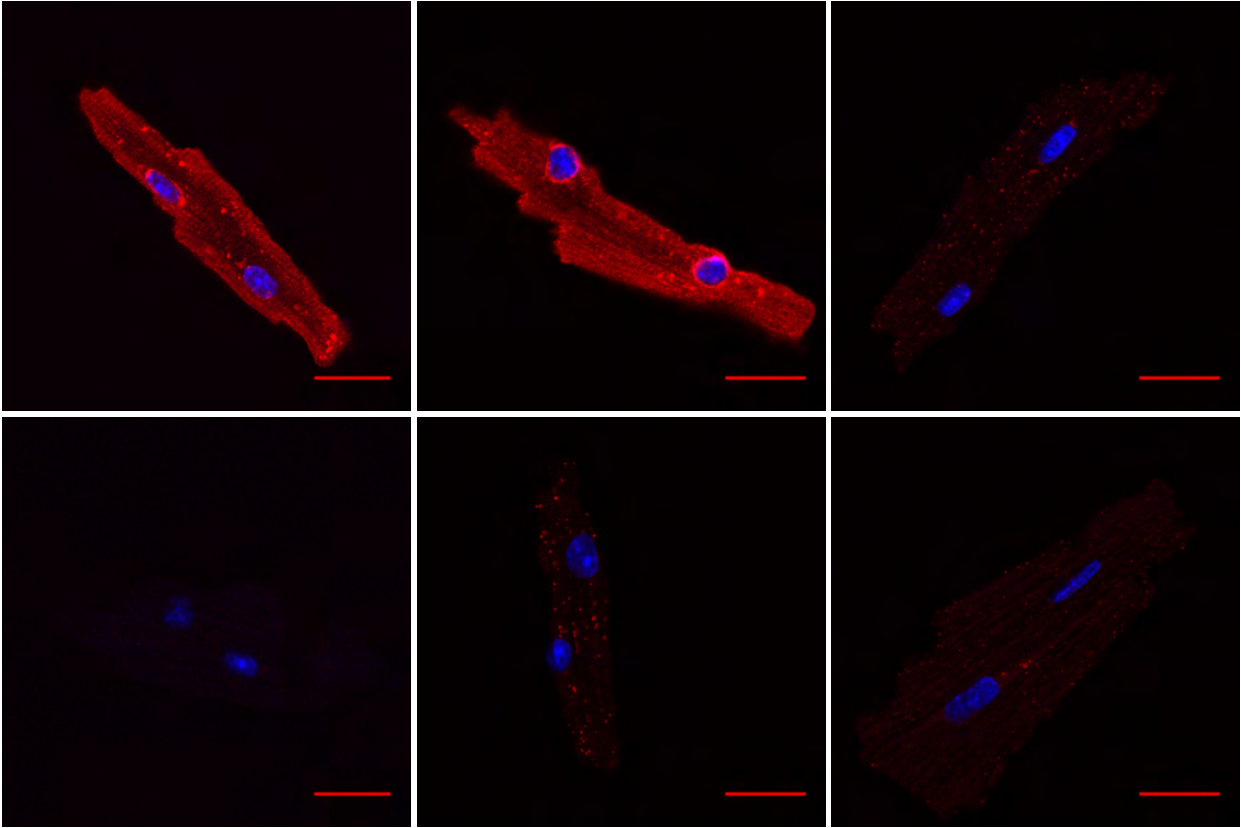
A.



B.

**Anti-HA
(CaMKIIN)**

**2° antibody
only**



CaMKIIN

HLL x CaMKIIN

HLL

Supplemental Table 1.
cutoff)

List of genes upregulated in post-MI AC3-C hearts (1.8-fold increase

1110002B05Rik
AC124413.2
AC138640.7
Adamts15
Adn
AK024941.1
AL732556.7
Ankrd1
Arntl
Atp13a1
ATXN1
AY172581.1
Bf
Btln2
BX883045.1
C1s
C2
C4a
Ca3
Cacna2d1
Cald1
Cap1
Capzb
Cd2
Cd37
Cd74
Cdo1
Centg1
Cetn3
Cfp
Cish
Clk4
Cma1
Col3a1
Col5a2
Cpne3
Cpxm1
Ctgf
Ctss
Cyp2e1
Cyp2f2
Cyp4f5

Dym
Efemp1
Eif3s10
Eln
Enpp2
Eprs
Fasn
Fn1
Fos
Gpc3
Gpnmb
Hbg1
Hmgn3
Hspa1a
Ifi30

IgG-2a
Igh-6

Il18
Itgam
Itih4
Klf9
Kras
L22655
LOC64305
Loxl1
M21780.1
Man2a1
Map2k6
Matr3
Mbnl2
Mgl1
Mgp

Mmp9

Msln
Myo1g
Ncl
Neb
Nfia
Nif3l1
Nppa
Nppb
Nr3c1
Plcb1
Plcb4
Plk2
Pnkd

Postn
Ppp4r2

Prei3
Prkcb1
Ptk2b
Rasa1
Rcan1
RGD1564450
Rock2
Rpl5
RT1-Aw2
Rtn4

S100a9
Saa
Sacm1l
Scd1
Sfrs12
Sorl1

Stat1
Stch
Stk25
Sv2b
Tbc1d5
Tbca
Tf

Tgfb2
Timp1
Tpp2
Ttc3
Ugt1a1
Uqcrrs1
Usp15
Vcp
Vps54
X53054
X68312
XM_234581.2
XM_341386.1
Zfp265
Zfp692

Supplementary Table 2. Genes downregulated in post-MI AC3-C hearts (1.8 fold decrease cutoff).

Angptl4
AY172581.1
BX883045.1
Camk2b
Cd164
Cd36
Cd63
Cish
Dbp
Dhrs7c
Dtx2
Eno3
Idh1
Irx3
Kbtbd10
Pam
Pdk4
Pfkfb1
Pim3
Ptgds
Ptp4a3
Pttg1
Rbp7
Slc9a3r1
Tcap
Uqcrbl
XM_342364.1
XM_347242.1