

# **SUPPLEMENTAL MATERIAL**

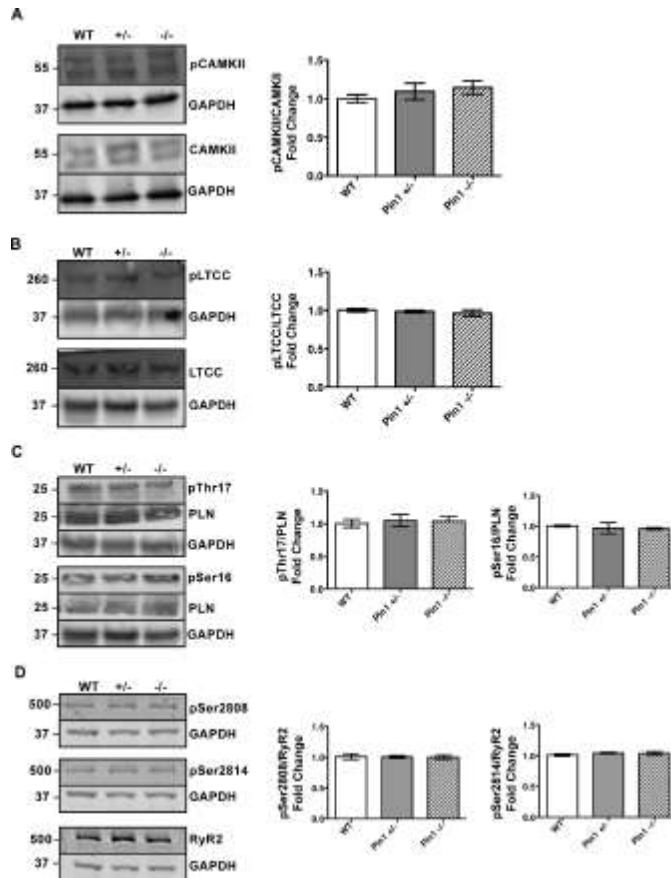
**Table S1. Antibody list.**

<b>Use: Western Blot</b>	<b>Company</b>	<b>Primary antibody dilution</b>	<b>Secondary antibody dilution</b>
Goat anti-SERCA2a	Santa Cruz Biotechnology (sc-8095)	1:1000	Donkey anti-goat 680 1:5000 (925-68074)
Rabbit anti NCX-1	Santa Cruz Biotechnology (sc-30304)	1:500	Donkey anti-rabbit 800 1:5000 (925-32213)
Rabbit anti-CamKII	ThermoFisher (PA5-39732)	1:500	Donkey anti-rabbit 800 1:5000 (925-68074)
Rabbit anti-pCamKII	Thermofisher (PA5-39731)	1:500	Donkey anti-rabbit 800 1:5000 (925-68074)
Rabbit anti-LTCC	Alomone Labs (ACC-003)	1:500	Donkey anti-rabbit 800 1:5000 (925-68074)
Rabbit anti-LTCC (pSer 1928)	Badrilla (A010-70)	1:250	Donkey anti-rabbit 800 1:5000 (925-68074)
Rabbit anti-RYR2	Millipore (AB9080)	1:500	Donkey anti-rabbit 800 1:5000 (925-68074)
Rabbit anti-RYR2 (pSer2814)	Badrilla (A010-31)	1:1000	Donkey anti-rabbit 800 1:5000 (925-68074)
Rabbit anti-RYR2 (pSer2808)	Badrilla (A010-30)	1:3000	Donkey anti-rabbit 800 1:5000 (925-68074)

Mouse anti-PLN	Thermofisher (MA3-922)	1:1000	Donkey anti-mouse 680 1:5000 (925-68072)
Rabbit anti-PLN (pSer16)	Badrilla (A010-12AP)	1:1000	Donkey anti-rabbit 800 1:5000 (925-68074)
Rabbit anti-PLN (pThr17)	Badrilla (A010-13)	1: 3000	Donkey anti-rabbit 800 1:5000 (925-68074)
Goat anti- GAPDH	SICGEN (AB0067- 200)	1:10000	Donkey anti-goat 680 1:5000 (925-68074)
Rabbit anti-GST	Cell Signaling Technology (#2622)	1:500	Donkey anti- rabbit 800 1:5000 (925-68074)
<b>Use: PLA on Paraffin Sections</b>	<b>Company</b>	<b>Primary antibody dilution</b>	<b>Secondary antibody dilution</b>
Goat anti-MYL2	Santa Cruz Biotechnology (sc-34490)	1:50	Donkey anti-goat 1:200 (A11055)
Mouse anti- SERCA2a	Thermofisher (MA3-919)	1:50	N/A
Mouse anti-NCX- 1	Thermofisher (MA3-926)	1:50	N/A
Rabbit anti-Pin1	Santa Cruz Biotechnology (sc- 15340)	1:20	N/A

Use: PLA on fixed cardiomyocyte	Company	Primary antibody dilution	Secondary antibody dilution
Goat anti-MYL2	Santa Cruz Biotechnology (sc-34490)	1:100	Donkey anti-goat 1:200 (A11055)
Mouse anti-SERCA2a	Thermofisher (MA3-919)	1:100	N/A
Mouse anti-NCX-1	Thermofisher (MA3-926)	1:100	N/A
Rabbit anti-Pin1	Santa Cruz Biotechnology (sc-15340)	1:50	N/A

**Figure S1. pCamKII/CamKII (A), pLTCC/LTCC(B), pPLN (Ser16 and Thr 17)/PLN (C) and pRyR2 (Ser2808 and Ser2814)/RyR2 (D) expression and phosphorylation levels are unaltered in Pin1 deficient hearts.** Phosphorylated Ca<sup>2+</sup>/Calmodulin-dependent kinase II (pCamKII)/CamKII (A), phosphorylated L-type Ca<sup>2+</sup> channel (LTCC)/LTCC(B), phosphorylated Phospholamban (PLN) (Ser16 and Thr 17)/PLN (C) and pRyR2 (Ser2808 and Ser2814)/RyR2 expression and phosphorylation levels are unaltered in Peptidyl Prolyl Isomerase (Pin1)<sup>+/-</sup> and Pin1<sup>-/-</sup> hearts compared to wild-type (WT) by immunoblot analysis. GAPDH used as loading control. Quantitation of protein expression shown below each representative blot as n-fold expression/WT. Data presented as mean±SEM, N=4.



**Figure S2. Putative consensus motifs for Pin1 binding on SERCA2a and NCX-1 protein sequences identified by in silico analysis. (A)** sarco(endo)plasmic reticulum calcium ATPase (SERCA2a) sequence accession number NP\_033852.1 (murine; NCBI protein database) or **(B)** Na<sup>2+</sup>/Ca<sup>2+</sup> exchanger 1 (NCX-1) sequence accession number NP\_035536.2 (murine; NCBI protein database) analyzed for serine next to a proline residue (black squares) or threonine next to a proline (red squares).

**A SERCA2a**

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1menahktkve evlghfgvne stqlsleqvK kkerwgsne lpaeeektll elviegfedl
61lvrillilaac isfvlawfee geetitafve pfvillilva naivgvwqer naenaiealk
121eyepengkvy rqdrksvqri kakdivpgdi veiavgdkvp adirltsiks ttlrvdqsil
181tgesvsvikh tdpvpdprav nqdkknmlfs gtniaagkam gvvvatgvnt eigkirdemv
241ateqertplq qkldefgeql skvislicia vwiinighfn dpvhggswir gaiyyfkiav
301alavaaapeg lpavittcla lgtrrmakkn aivrslpave tlgetsvics dktgtltnq
361msvcrnfile kvgedtcsln efsitgstya pigevqkddk pvkchgydgl velaticale
421ndsaldynea kvvyekvgea tetaltclve kmvfdtelk glskierana cnsvikqlmk
481keftleford rkamsvycrp nkpartsmek mfvkgapegv idrothirvg stkvpmrpgv
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601pprievassv klrcgagirv imitgdnkgt avaicrriqi fgqdedvtsk aftgrefdel
661pqsagrdacl narcfarvep shkskivefl qsfdeitam gdgvndapal kkseigiamg
721sgtavaktas envladdnfs tivaaveegr aiynnmkqfi rylissnvge vvcifltaal
781gfpealipvg llwnlvtdg lpatalgnfp pdldimakkpp rnpkeplisg wiffrylaig
841cyvgaatvga aawwflaadg gprvsfyqls hflgckednp dfdgvdcaif espypmtmal
901svlvtiemcn ainslsengs llrmpwewi wlvgsiclsz slhflilyve plplifqlrp
961lnltqwlmlv kislpvilmd etlklfvarny leqpalle

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**B NCX-1**

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1mlrlslppnv smgfrlyalv allfshvdhi tadteaetgg nettectgsy yckkqvilpi
61wepqdpstfgd kiaratvyfv anvymflgvs iia drfnasi evitsaqekei tikkngett
121kttvriwnet vsnltlmalg ssapeillsv ievcghnfta gdigpativg saafnmfiil
181alcvyvvpdg etrkikhrlv fvtvaawsif aytwlyiils vsppgvvevw eglltffffg
241icvvfawvad rrllyfykyv kryragkqrg mliehegdrp asktelendg kvvnshvdfn
301ldgalvlevd erdqddeear remarilkel kqkhpekeie glielanyqv lsqqkkralf
361yriqatrlmt gagnilkrha adqarkavsm hevnmeneaen dpvskiffeg gtyqclencg
421tvaltimrrg gdlettvfvd frtedgtana gsdyeftegt vifkpggetqk eirvgliidd
481ifeedenflv hlsnvrssd vsedgileen hassiaclgs pstatitifd ddhagiftfe
541epvthvsesi gimevkvirt sgargnviip ykctiegtarg ggedfedtgc elefqndeiv
601ktisvkvidd eeyeknktff ieigeprlve msekkalln eiggftltgk emyqqpifrk
661vhardhpips tvitiseeyd dkqpltskee eerriaemgr pilgehtkle viieesyefk
721stvdkliktk nialvvgtns wreqlieait vsageddddd ecgeeklpac fdymhfltv
781fwkvlfafvp pteywnqvac fivsilmlgl ltafigdlas hfgctiglkd svtavvfvai
841gtsvpdtfas kvaatdqya gasignvtgs navnvlqig vwsiaaiyh aangeqfkvs
901pgtlafsvtl ftifafinvg vlllyrrrpei ggelggprta klltsslfvl lwllyiffss
961leavchikqf

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**Figure S3 SERCA2a and NCX-1 physically interact with GST-Pin1**

**(A)** Immunoblot for Glutathione S-Transferase (GST) (left) and GST-Pin1 (right) showing GST and GST-Pin1 protein expression at 25 and 45 kDa respectively. **(B)** Na<sup>2+</sup>/Ca<sup>2+</sup> exchanger 1 (NCX-1) and sarco(endo)plasmic reticulum calcium ATPase (SERCA2a) interacted with GST-Pin1 but not with GST upon pull-down as shown by immunoblot. β-catenin and AKT were used as positive control for the pull-down since are known targets of Pin1. N=3

