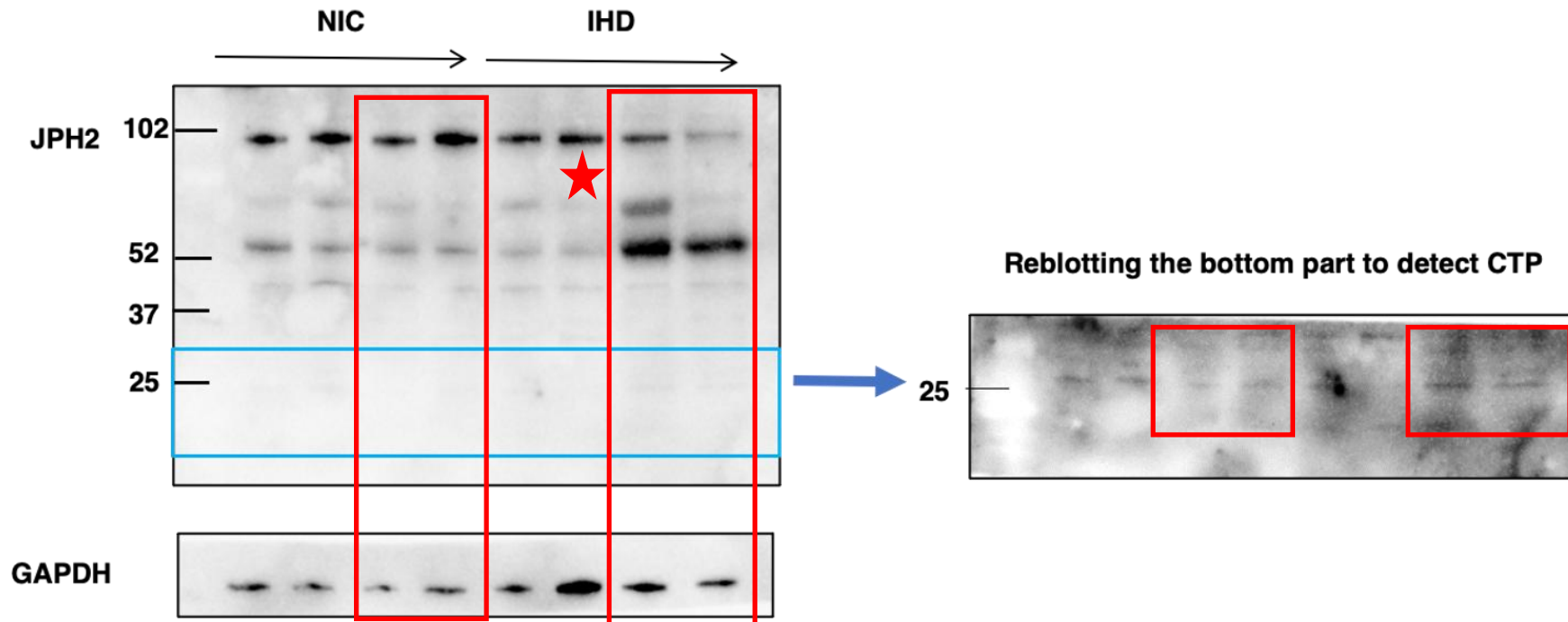


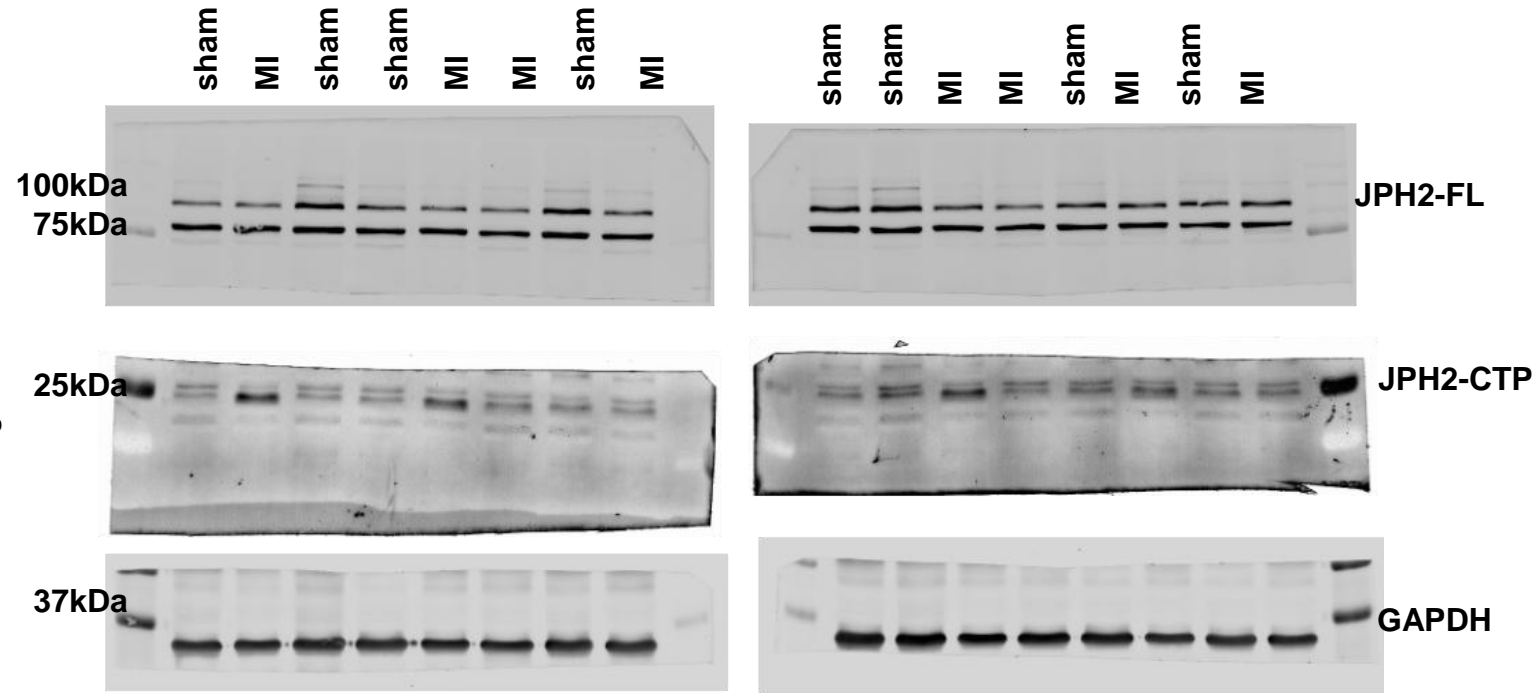
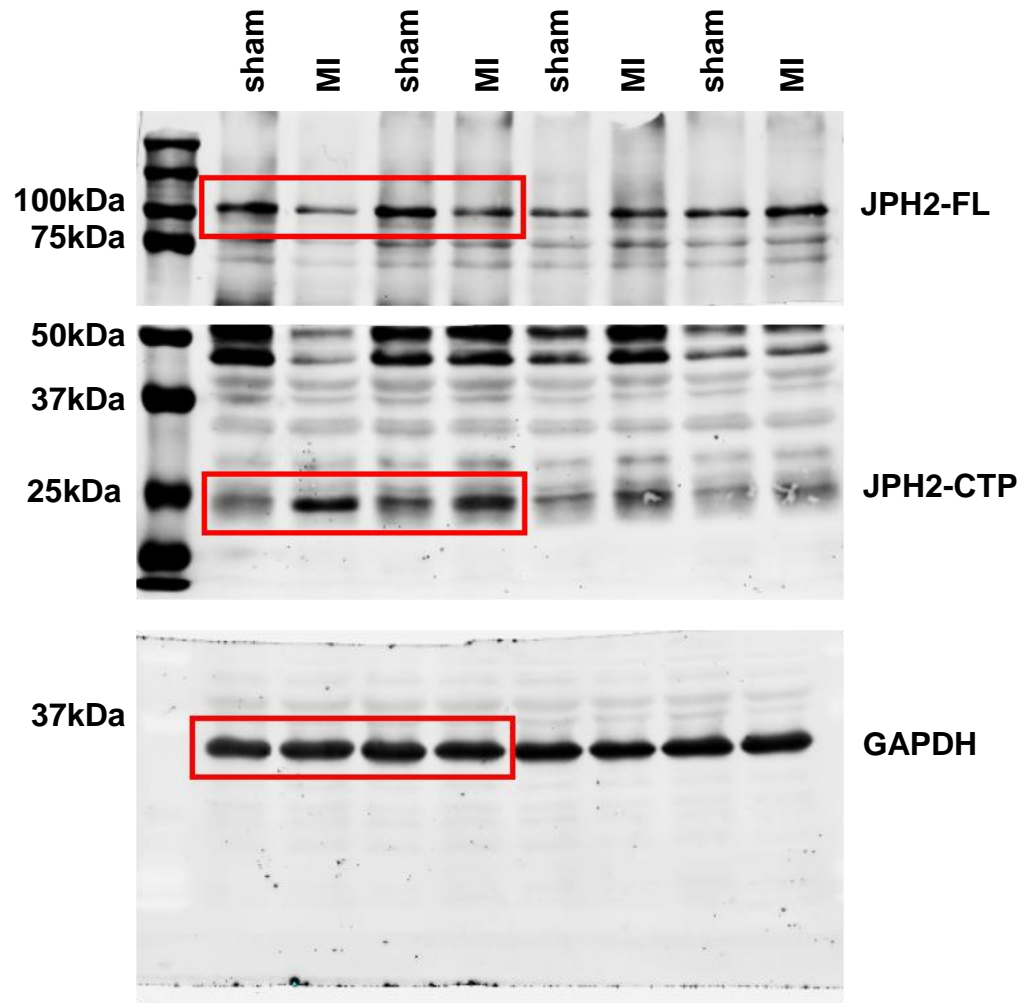
Fig 1A-C



★ Excluded in the analysis as CTP was outlier in Graphpad Prism outlier test.

□ Representative blot for final figure

Fig 1D-F



 Representative blot for final figure

Fig 2F-G

 Representative blot for final figure

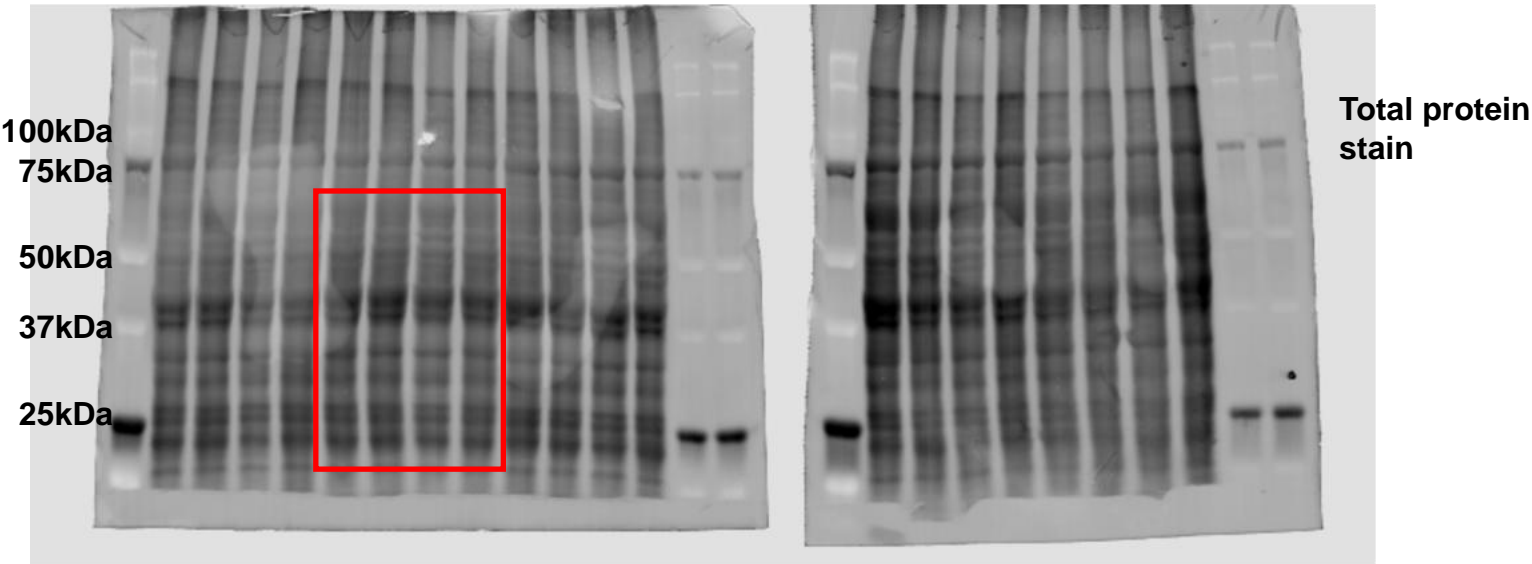
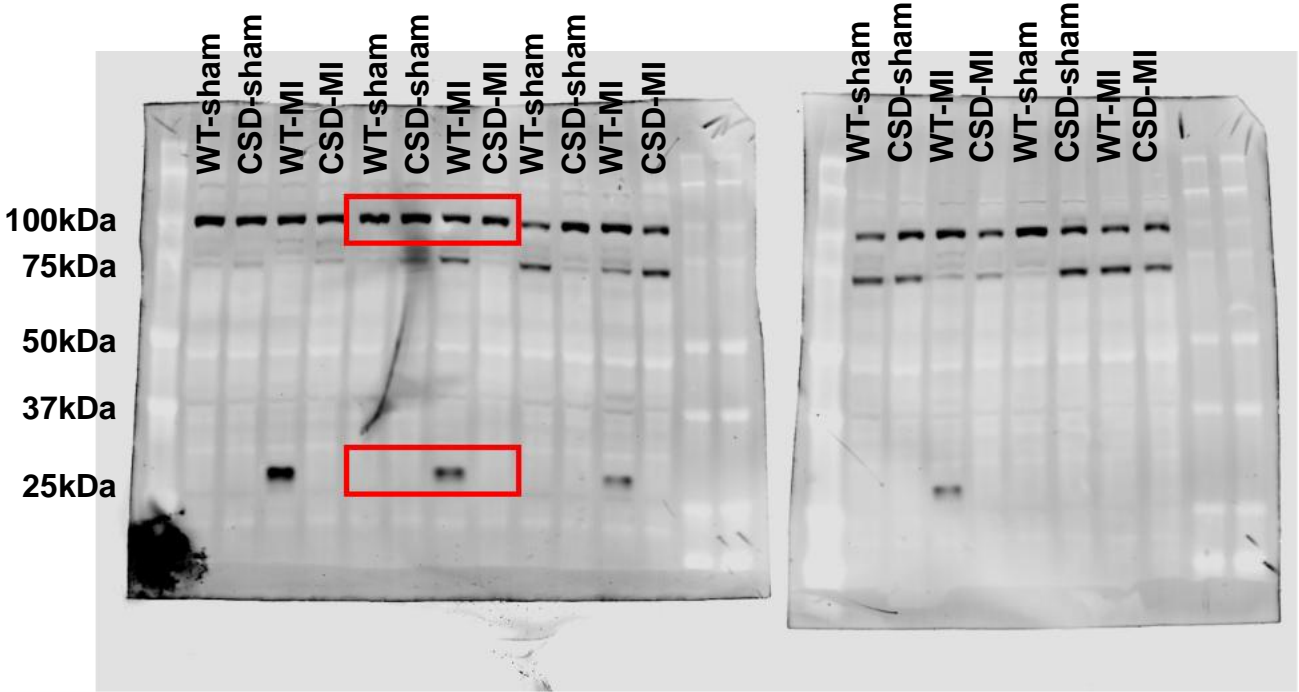
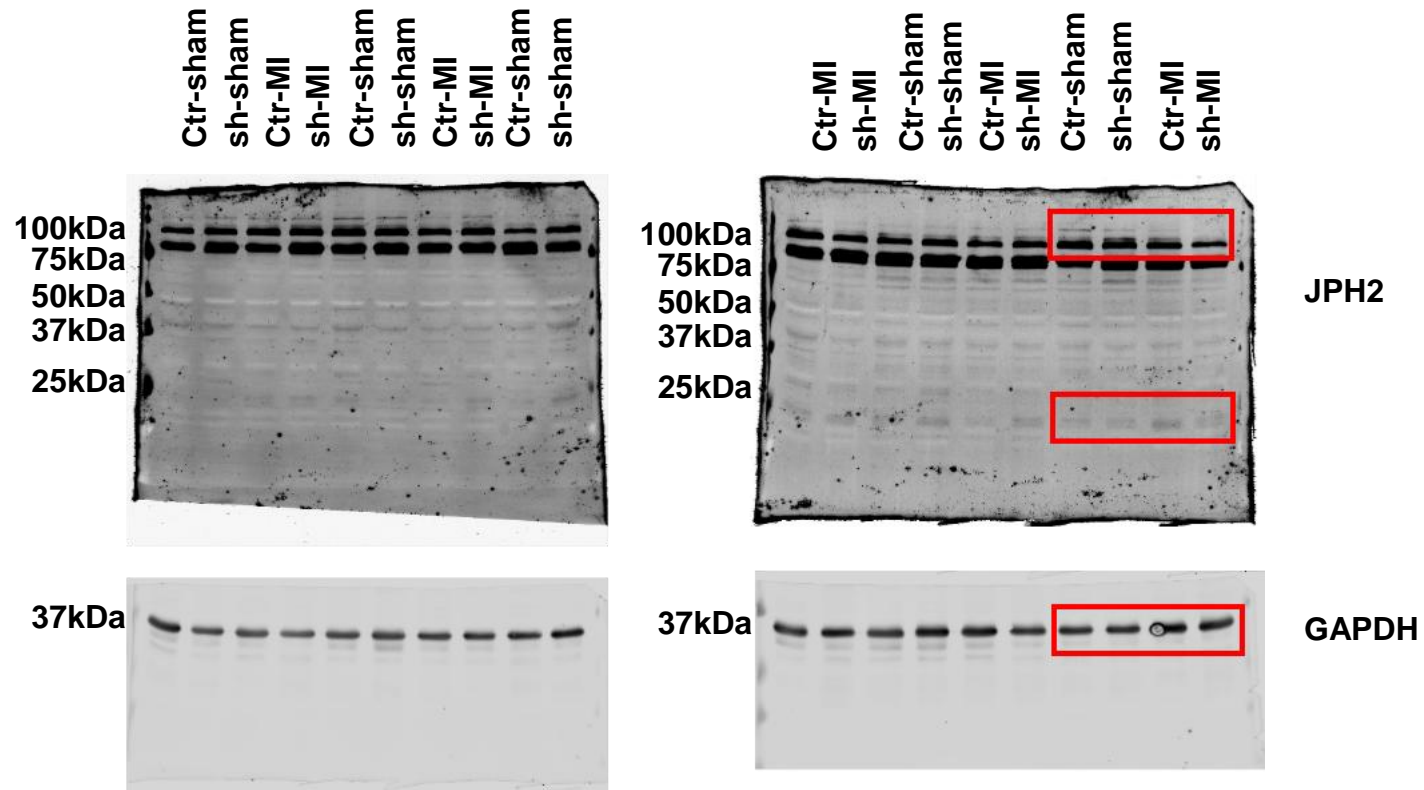


Fig 6E-F



 Representative blot for final figure



Representative blot for final figure

Fig 6G-H

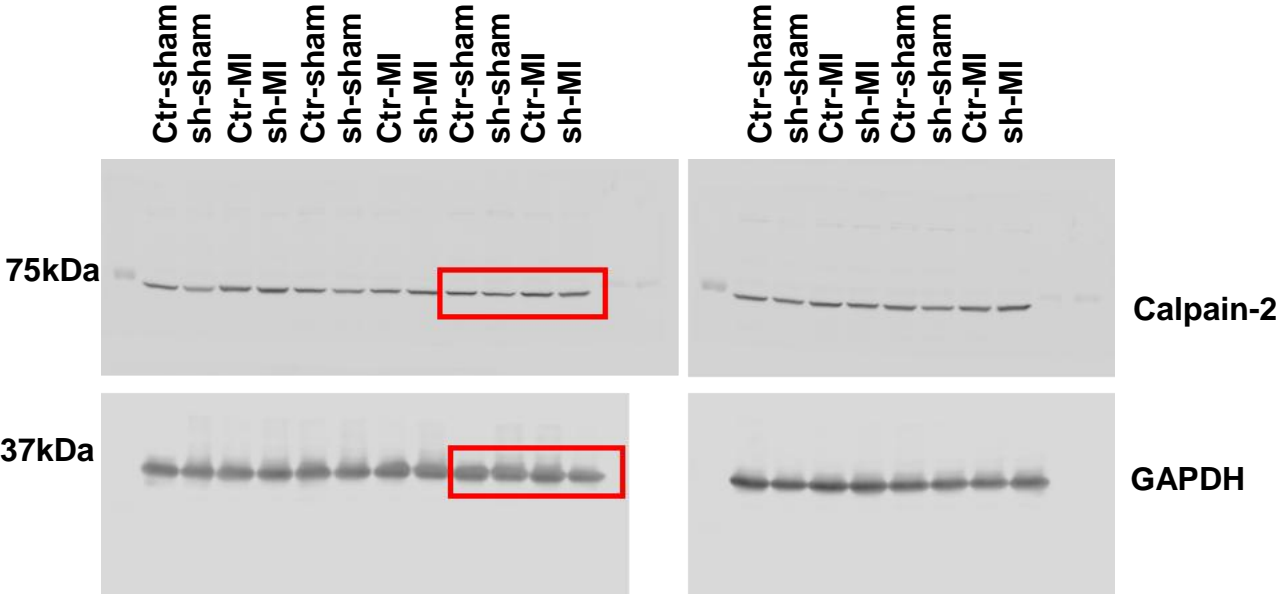


Fig 6I-J

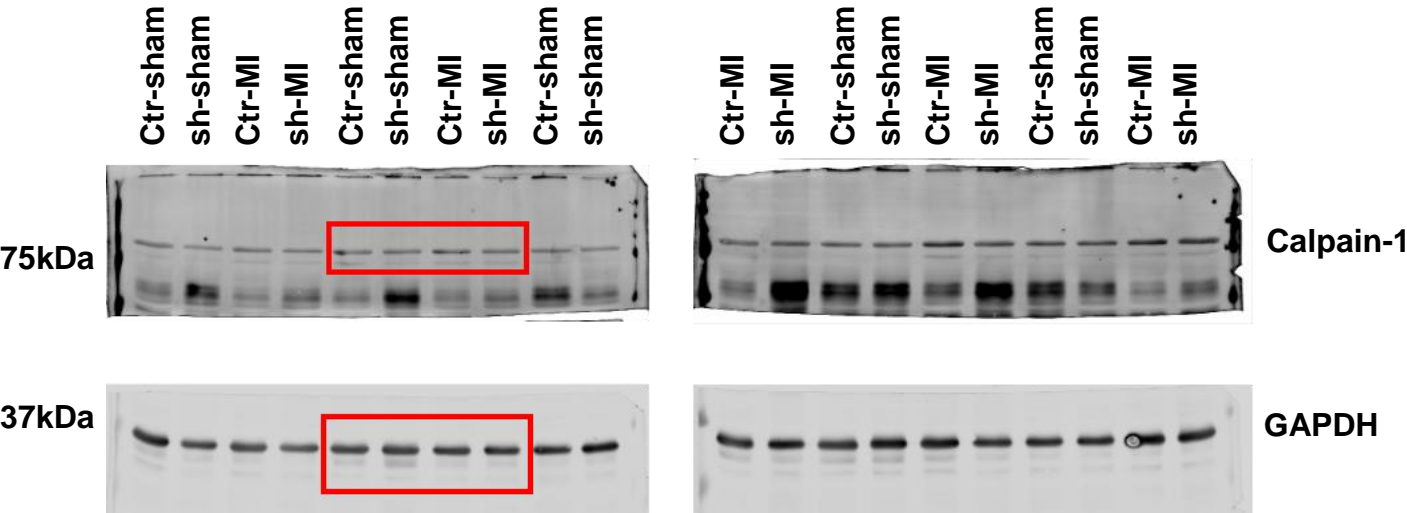


Fig 8A

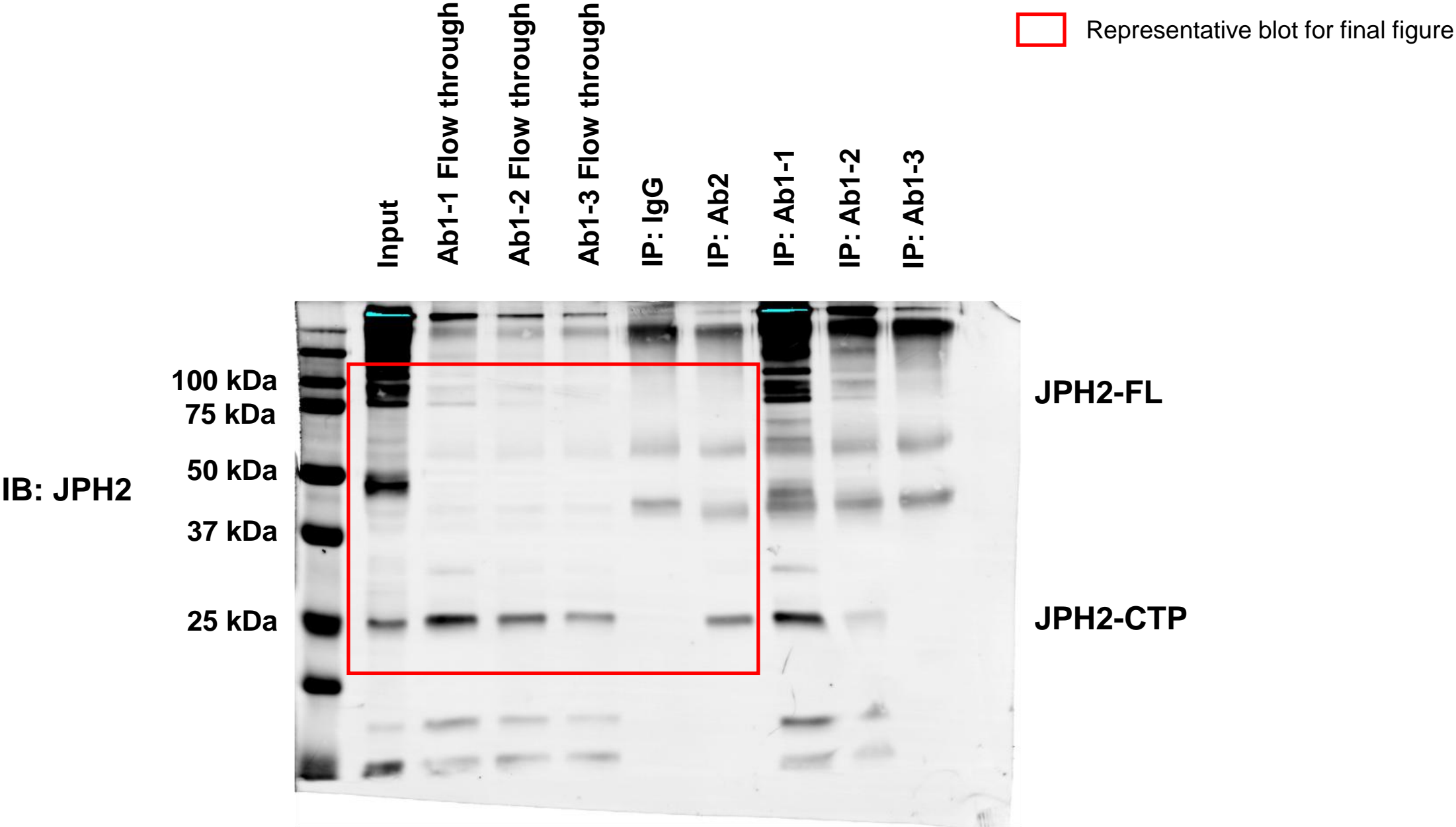
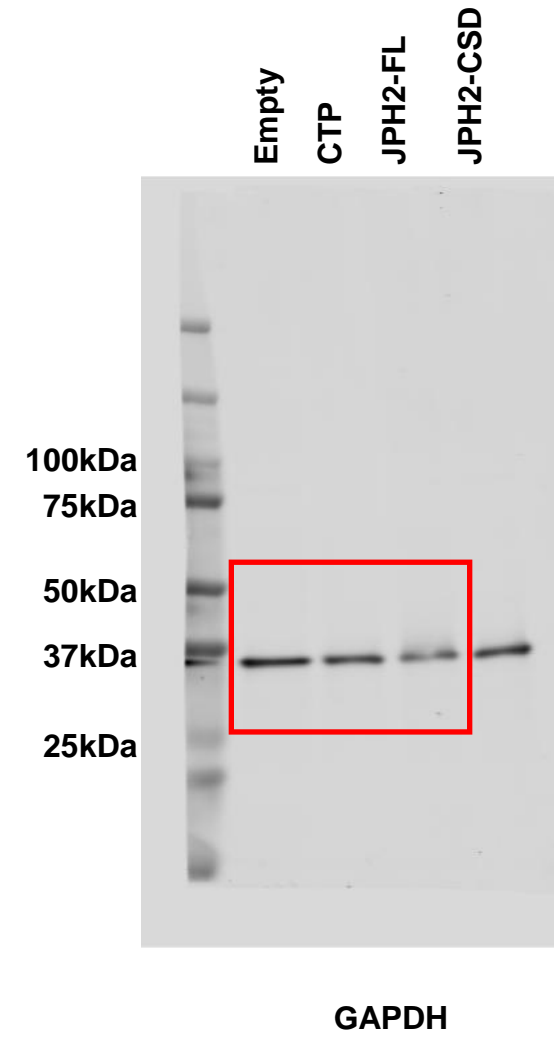
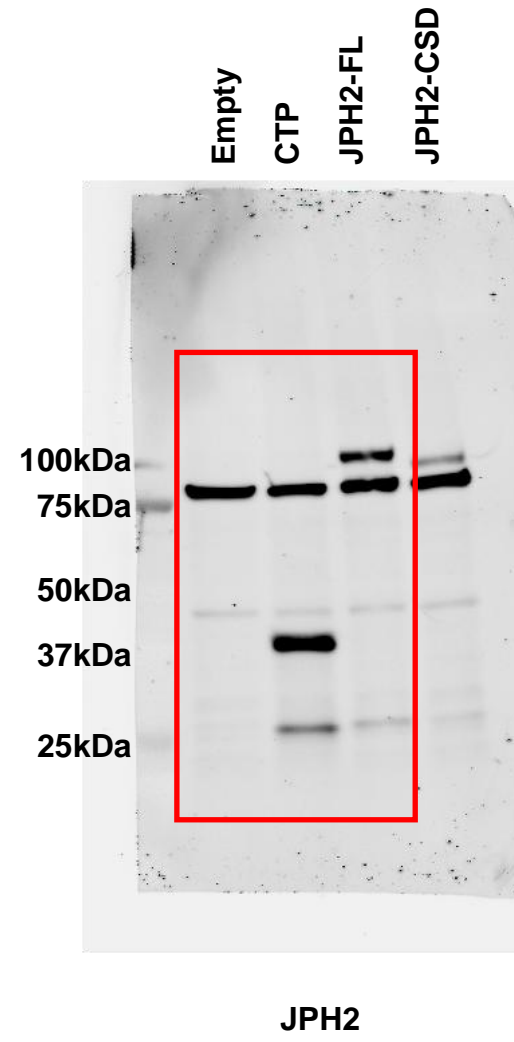


Fig 8C

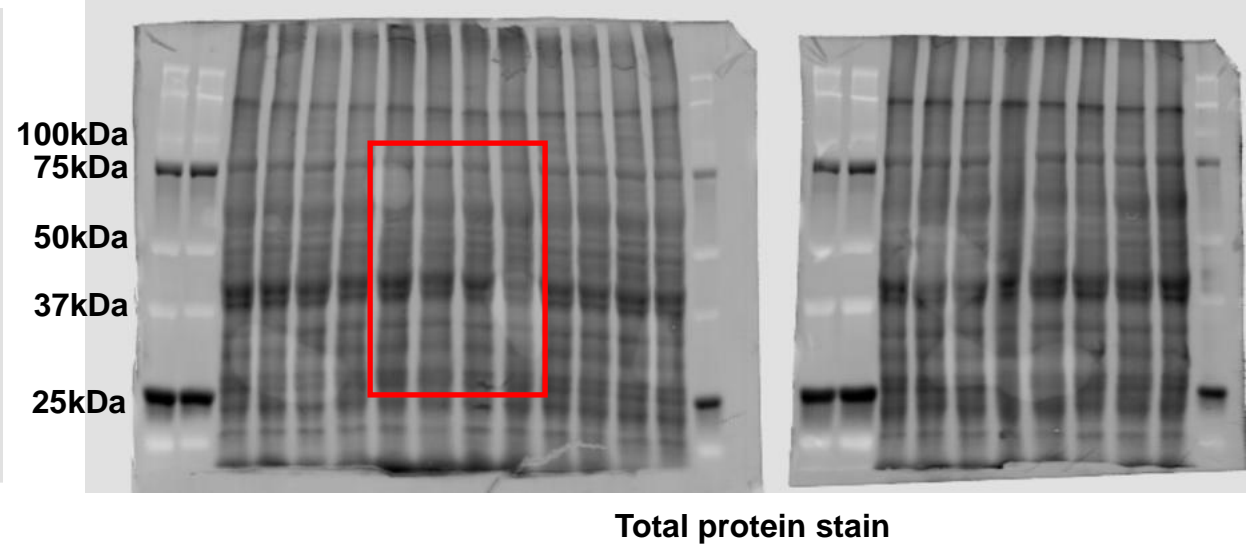
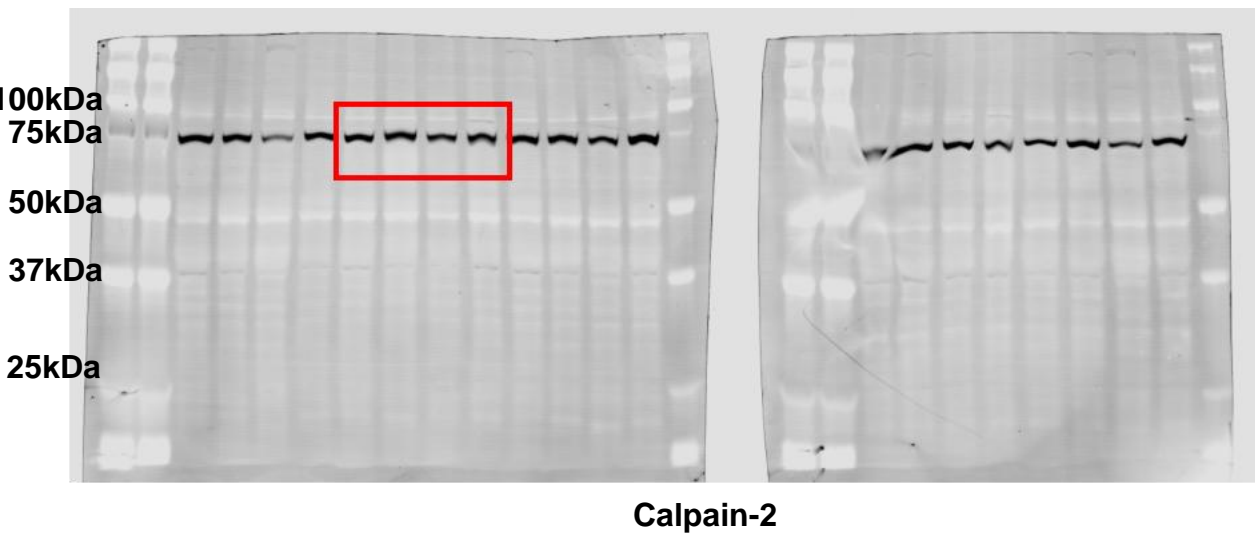
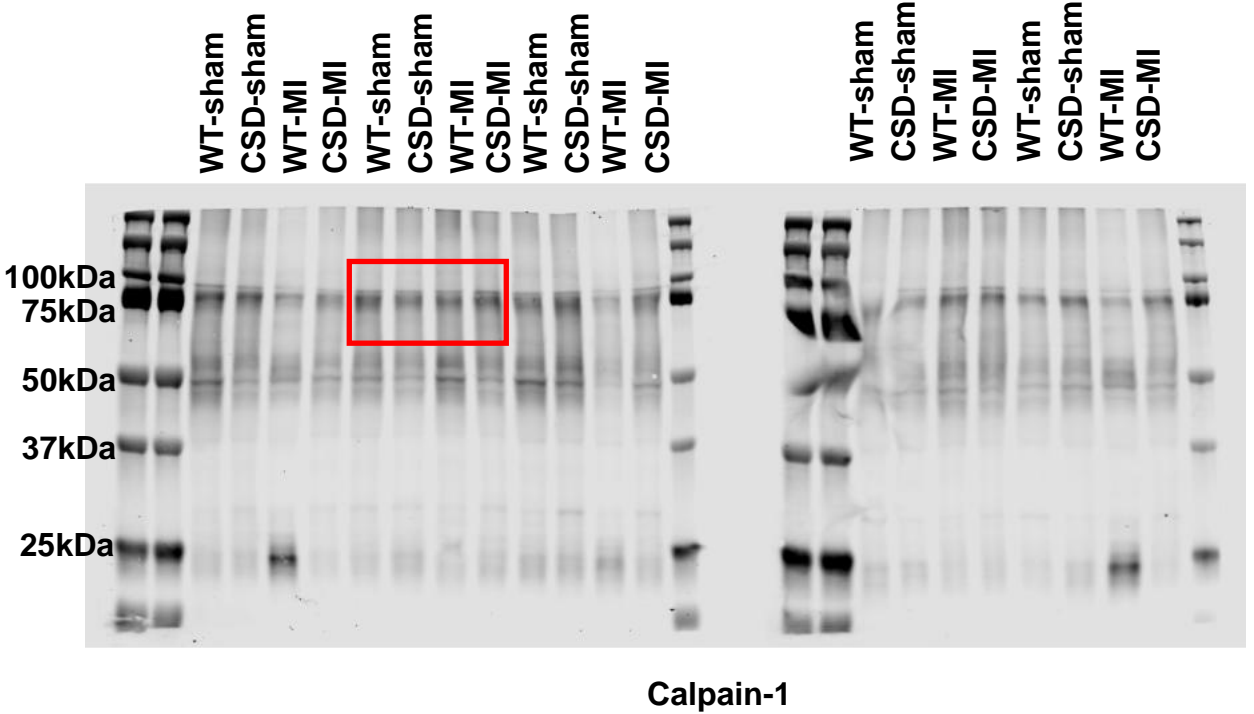
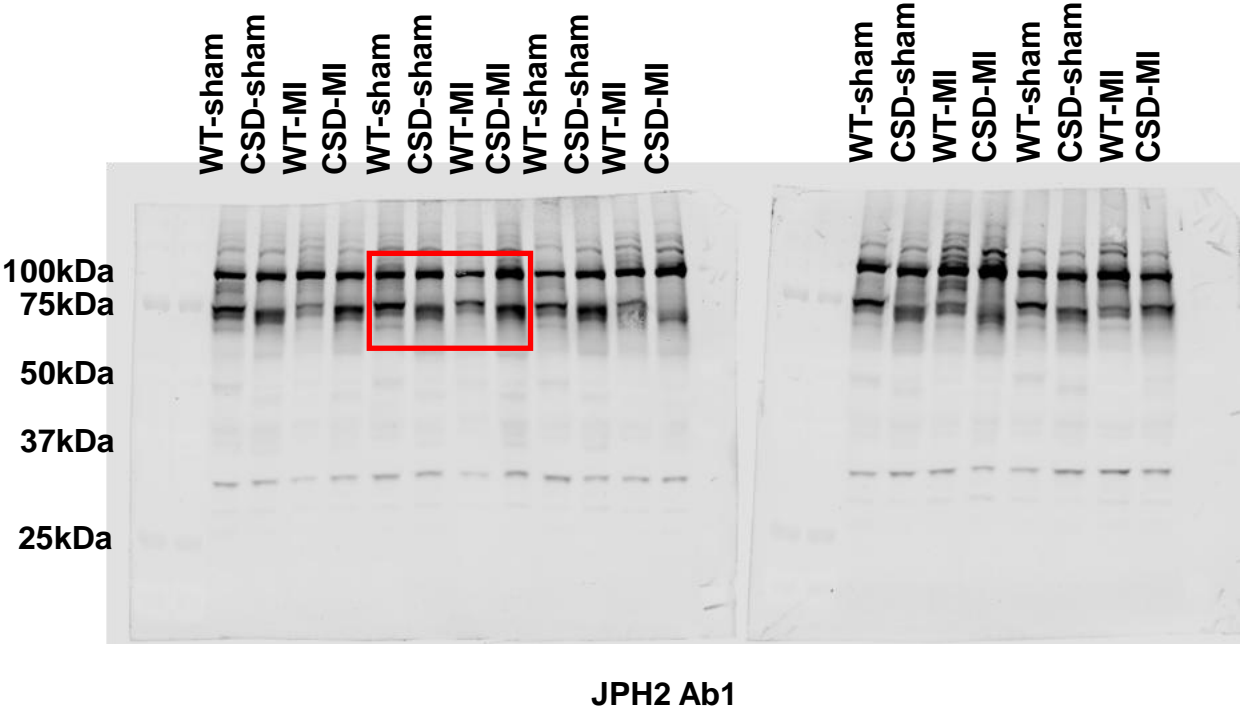


 Representative blot for final figure



Representative blot for final figure

Supplemental Fig 2A-D



Supplement Materials

Targeting Calpain-2 Mediated Junctophilin-2 Cleavage Delays Heart Failure Progression following Myocardial Infarction

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Supplemental Methods

Generation of JPH2-CSD knock-in mice. JPH2-CSD knock-in mice were generated using CRISPR-Cas9 with cytosolic Cas9 protein embryo microinjections. A JPH2 S479-Q486-del sgRNA (CTTGGGTTGCGGGGGCGTCCCGG) was designed by the BCM Genetically Engineered Rodent Model (GERM) core targeting the JPH2 cleavage motif (S479-Q486) encoded by exon 4 of the *Jph2* gene. Around 200 embryos were injected with a mix of Cas9 mRNA, sgRNA and ssOligo and implanted in a female with a C57BL/6J background supplied by Jackson Laboratories (stock number: 000664). Pups from this initial injection were genotyped to identify JPH2-CSD mosaic founders. A pair of JPH2 genotyping primers (forward primer – GAGTATCAGAAGCGTCGGCT, and reverse primer – CCTCGGGCTCCATCGTTAC) were used to amplify a 390-bp region around the deletion site within the WT allele, while the JPH2-CSD band was observed at 366-bp (24bp fewer, corresponding to the deleted 8 amino acids). The mosaic CSD mice were crossed with C57BL/6J mice to obtain heterozygous founder mice. These mice were backcrossed for at least three generations prior to being used in experiments. All studies were performed in both male and female mice with WT littermates as controls.

Myocardial infarction surgery. Mice at 16-weeks-of-age were prepared for surgery by shaving the chest and application of depilatory cream. Prior to surgery, 1mg/kg buprenorphine-SR (s.c.) and 2mg/kg meloxicam-SR (s.c.) were administered subcutaneously. Following induction of anesthesia in a chamber filled with 3% isoflurane in 100% oxygen, mice were intubated endotracheally, ventilated at a tidal volume of 150 μ L and at a respiratory rate of 175 breaths/minute, and anesthesia was maintained with 2.5% isoflurane for the duration of the procedure. Body temperature was maintained at $37.0^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ using a heated platform guided by a rectal thermometer. Surface ECG was monitored throughout the surgery. A left thoracotomy was performed through the 3rd intercostal space, and the left anterior descending (LAD) artery was ligated with a 7-0 silk suture in mice randomized to the MI group. Proper ligation of the LAD was confirmed visually by the pale discoloration of the left ventricle distal to the ligation and by ST segment depression followed by ST segment elevation on the ECG monitor. Mice in the sham group

underwent the same procedure but no suture was tied. The ribs and skin were closed with 6-0 prolene sutures. All the mice were randomized for either sham or MI surgeries. These mice were monitored every day for 6 days to assess any clinical signs of discomfort and pain. In addition, 1mg/kg buprenorphine-SR (s.c.) was injected every 72-h and 2mg/kg meloxicam-SR (s.c.) was injected every 48-h during the 6 days post-operative period.

Echocardiography. Nair cream was used to depilate the mice's chest to remove all hair. Mice were anesthetized with isoflurane (1.5-2.0% in 100% oxygen). Mice were placed on a heated platform to maintain body temperature between 36.5°C to 37.5°C. Echocardiography was performed using Vevo 2100 (Fujifilm VisualSonics, Toronto, ON) with a 30 MHz frequency probe. Short axis images of both B- and M- mode were recorded, and the M-mode images were analyzed using Vevo2100 software.

Western blotting. Heart samples were crushed in liquid nitrogen and resuspended in RIPA buffer containing 1% CHAPS, Phos-STOP (Sigma-Aldrich; St. Louis, MO; catalog # 4906837001) and complete mini protease inhibitor cocktail (Sigma-Aldrich; St. Louis, MO; catalog # 4693124001), 20mM sodium fluoride (NaF), 1mM Na₃VO₄. Lysates were further homogenized with steel beads using a homogenizer (Tissue Lyser LT; Qiagen, Germantown, MD) at 50-Hz for 8 minutes. These samples were sonicated 3 times for 2 seconds each and centrifuged at 14,000 rpm for 20 mins at 4°C. Supernatants were collected as lysates, and protein concentration was measured using a NanoDrop spectrophotometer (Thermo Fisher Scientific, Waltham, MA). Human heart, mouse heart, or cell lysates (75 mg) were denatured in 2x Laemmli sample buffer (Biorad; Hercules, CA; catalog # 1610737) with 5% 2-mercaptoethanol (Sigma-Aldrich; St. Louis, MO; catalog # M3148) for 10 mins at 70°C. SDS gel electrophoresis was done on 10-12% acrylamide gel at 100 Volts. Proteins were further transferred onto 0.45-micron polyvinylidene fluoride (PVDF) membranes for 1.5-h at 100 Volts. Membranes were blocked for 1-h at room temperature and incubated overnight at 4°C with primary antibodies diluted in blocking buffer. Primary antibodies used were JPH2-Ab1 (1:2,000; Rabbit polyclonal, custom made); JPH2-Ab2 (1:2,000; rabbit polyclonal, Thermo Fisher Scientific, Waltham, MA; catalog # 40-5300); calpain-1 (1:2,000; mouse monoclonal,

Thermo Fisher Scientific, Waltham, MA; catalog # MA3-940), calpain-2 (1:2,000; Cell Signaling, Danvers, MA; catalog # 2539), and GAPDH (1:10,000; mouse monoclonal, EMD Milipore, Burlington, MA; catalog # MAB374). Membranes were washed 3 times with TBST (0.1% tween-20) for 10 minutes each and incubated with secondary antibody (1:10,000) for 1-h at room temperature. Secondary antibodies used were anti-mouse-Alexa-Fluor-680 (goat polyclonal, Thermo Fisher Scientific, Waltham, MA; catalog # A-21057) and anti-rabbit-IR800 (goat polyclonal, Rockland Immunochemicals, Pottstown, PA; catalog # 611-145-122). After washing 3x10 minutes in TBST, membranes were developed using LICOR Odyssey infrared imager (LICOR, Lincoln, NE). The Revert™ 520 stain was used to quantify total protein levels (LICOR, Lincoln, NE; catalog #926-10011). Bands were quantified using the ImageJ software and normalized to GAPDH levels.

Ca²⁺ imaging studies. Mouse hearts were quickly excised, cannulated, and perfused retrogradely using a heated Langendorff system via the aorta, as described (1). The perfusion buffer consisted of Ca²⁺-free tyrode solution containing (mM): 140 NaCl, 5.4 KCl, 1 MgCl₂, 5 HEPES, and 10 Glucose, pH 7.4, supplemented with liberase TH (22µg/mL; Sigma-Aldrich, St. Louis, MO; catalog # 5401119001). After digesting, hearts were perfused with 5mL Kraft-Brühe (KB) solution containing (mM): 90 KCl, 30 K₂HPO₄, 5 MgSO₄, 5 pyruvic acid, 5 B-hydroxybutyric acid, 5 creatine, 20 taurine, 0.5 EGTA, 5 HEPES, 10 glucose, pH 7.2. Only left ventricular tissue distal of the suture was teased apart and strained through 250µm nylon mesh to isolate single cells. In hearts from sham mice, a corresponding area was selected. Ventricular cardiomyocytes were re-adapted to 1.8mM Ca²⁺ and loaded with 4µM Cal520AM (Santa Cruz Biotechnology, Dallas, TX, USA; catalog # sc-477280) for Ca²⁺ imaging. Cells were plated onto laminin-coated coverslips and imaged using an LSM880 confocal microscope (Carl Zeiss, Thornwood, NY, USA) in line scan mode with 1024 pixels per line 982Hz using 40X objective. Cardiomyocytes were paced at 1-Hz for 15s, unstimulated for 60s, and then perfused with 10mM caffeine to assess SR load. The Ca²⁺ spark frequency (CaSpF) was analyzed using the SparkMaster ImageJ plugin (2).

Mass spectrometric analyses. Mass spectrometric analyses were performed at the Core Facility Proteomics of the University Medical Center Göttingen. Samples were reconstituted in 1× NuPAGE LDS Sample Buffer (Invitrogen) and run for 1.5 cm into a 4-12 % NuPAGE Novex Bis-Tris Minigels (Invitrogen). Gels were stained with Coomassie Blue for visualization purposes, and each lane sliced into 3 equidistant parts regardless of staining. After washing, gel slices were reduced with dithiothreitol (DTT), alkylated with 2-iodoacetamide and digested with Endopeptidase Trypsin (sequencing grade, Promega) overnight. The resulting peptide mixtures were then extracted, dried in a SpeedVac, reconstituted in 2% acetonitrile/0.1% formic acid/ (v:v) and prepared for nanoLC-MS/MS as described previously.⁽³⁾ For mass spectrometric analysis samples were enriched on a self-packed reversed phase-C18 precolumn (0.15 mm ID x 20 mm, Reprosil-Pur120 C18-AQ 5 µm, Dr. Maisch, Ammerbuch-Entringen, Germany) and separated on an analytical reversed phase-C18 column (0.075 mm ID x 200 mm, Reprosil-Pur 120 C18-AQ, 3 µm, Dr. Maisch) using a 73 min linear gradient of 5-42 % acetonitrile/0.1% formic acid (v:v) at 300 nl min⁻¹). The eluent was analyzed on a Q Exactive HF hybrid quadrupole/orbitrap mass spectrometer (ThermoFisher Scientific, Dreieich, Germany) equipped with a Flexlon nanoSpray source and operated under Excalibur 2.4 software using a data-dependent acquisition method. Raw data were processed using MaxQuant Software version 1.6.5.0 (Max Planck Institute for Biochemistry, Martinsried, Germany). Proteins were identified against the UniProtKB mouse reference proteome (v2019.02) along with a set of common lab contaminants. The search was performed with trypsin excluding proline-proximal cleavage as enzyme, and iodoacetamide as cysteine blocking agent. Up to two missed tryptic cleavages were allowed for, and methionine oxidation and protein N-terminal acetylation variable modifications. Instrument type 'Orbitrap' was selected to adjust for MS acquisition specifics. Protein and peptide results lists were thresholded at False Discovery Rates (FDR) of 0.01, respectively, using a forward-and-reverse decoy database approach. Perseus Software version 1.6.15.0 (Max Planck Institute for Biochemistry, Martinsried, Germany) was used to obtain relative protein quantitation values from the MaxQuant Software results using the LFQ algorithm at default settings, and to perform statistical evaluation.

Supplemental references

1. Quick AP, Wang Q, Philippen LE, Barreto-Torres G, Chiang DY, Beavers D, et al. SPEG (Striated Muscle Preferentially Expressed Protein Kinase) Is Essential for Cardiac Function by Regulating Junctional Membrane Complex Activity. *Circ Res*. 2017;120(1):110-9.
2. Picht E, Zima AV, Blatter LA, Bers DM. SparkMaster: automated calcium spark analysis with ImageJ. *Am J Physiol Cell Physiol*. 2007;293(3):C1073-81.
3. Atanasov I, Urlaub H. Increased proteome coverage by combining PAGE and peptide isoelectric focusing: comparative study of gel-based separation approaches. *Proteomics*. 2013;13(20):2947-55.

Supplemental Table 1. Patient demographics.

Groups of comparison	Non-ischemic controls (NIC)	Ischemic heart disease (IHD)	P-value
Total number patients, n	4	3	
Mean age, years (mean \pm SD)	68.0 \pm 10.3	69.0 \pm 6.4	0.800
Men, n (%)	4 (100)	2 (66.6)	0.429
Surgical procedure, n (%)			
CABG	0 (0)	1 (33.3)	0.429
CABG + AVR / MVR	1 (25)	2 (66.6)	0.486
AVR / MVR	3 (75)	0 (0)	0.143
Medical history, n (%)			
Smoker/ex-smoker	1 (25)	1 (33.3)	>0.999
Hypertension	4 (100)	2 (66.7)	0.429
Diabetes mellitus	2 (50)	0 (0)	0.429
Heart Failure	0 (0)	0 (0)	>0.999
Previous MI	0(0)	2 (66.7)	0.143
COPD/asthma	1 (25)	0 (0)	>0.999
Medications, n (%)			
Anticoagulants	0 (0)	0 (0)	>0.999
Antiplatelets	1 (25)	1 (33.3)	>0.999
β -Blockers	2 (50)	2 (66.7)	>0.999
Statins	1 (25)	2 (66.7)	0.486
Calcium channel blockers	0 (0)	1 (33.3)	0.429
ACEIs and ARBs	0 (0)	1 (33.3)	0.429
Diuretics	1 (25)	1 (33.3)	>0.999
Echocardiography, (mean \pm SD)			
EF	59.0 \pm 4.3	60.0 \pm 5.0	>0.999
LVEDD	4.48 \pm 0.49	4.73 \pm 0.60	0.629

ACEI, angiotensin-converting enzyme (ACE) inhibitors; ARBs, angiotensin II receptor blockers; AVR, aortic valve replacement; CABG, coronary artery bypass grafting; MI, myocardial infarction; MVR, mitral valve replacement; COPD, chronic obstructive pulmonary disease; EF, ejection fraction; LVEDD, left ventricular end-diastolic diameter; SD, standard deviation. P value determine using Fisher's exact test, with the exception of age (t-test), except for Mann-Whitney test used for age, EF and LVEDD comparisons.

Supplemental Table 2. Echocardiography parameters from WT and CSD mice after myocardial infarction or sham procedure

Baseline	WT-Sham (n=5) (3M, 2F)	CSD-Sham (n=5) (3M, 2F)	WT-MI (n=11) (5M, 6F)	CSD-MI (n=10) (4M, 6F)	P value (WT- MI vs WT- sham)	P value (CSD-MI vs WT-MI)
Heart rate (bpm)	494.88 ± 14.14	526.74 ± 18.99	472.12 ± 18.02	457.04 ± 16.13	0.756	0.923
ESD (mm)	2.6 ± 0.17	2.47 ± 0.12	2.4 ± 0.06	2.22 ± 0.15	0.714	0.677
EDD (mm)	3.96 ± 0.19	3.7 ± 0.17	3.8 ± 0.08	3.68 ± 0.08	0.845	0.736
ESV (uL)	25.45 ± 3.9	21.98 ± 2.68	20.51 ± 1.31	18.3 ± 2.04	0.652	0.801
EDV (uL)	69.44 ± 7.56	58.86 ± 6.34	62.27 ± 3.21	57.68 ± 3	0.819	0.726
SV (uL)	43.99 ± 4.78	36.88 ± 4.2	41.77 ± 2.07	38.35 ± 1.63	0.972	0.574
EF (%)	63.78 ± 3.42	62.65 ± 2.14	67.19 ± 0.86	67.2 ± 1.72	0.925	>0.999
FS (%)	34.42 ± 2.49	33.27 ± 1.57	36.69 ± 0.66	36.72 ± 1.3	0.998	>0.999
CO (mL/min)	21.72 ± 2.37	19.25 ± 1.9	19.92 ± 1.49	17.35 ± 0.47	0.915	0.393
LVAW;s (mm)	1.17 ± 0.06	1.1 ± 0.08	1.19 ± 0.04	1.11 ± 0.05	0.976	0.466
LVAW;d (mm)	0.68 ± 0.04	0.72 ± 0.06	0.78 ± 0.04	0.69 ± 0.04	0.388	0.528
LVPW;s (mm)	1.16 ± 0.04	1.07 ± 0.04	1.13 ± 0.05	0.99 ± 0.06	0.981	0.271
LVPW;d (mm)	0.75 ± 0.06	0.68 ± 0.01	0.78 ± 0.08	0.61 ± 0.03	0.983	0.305

2-weeks post- surgery	WT-Sham (n=5) (3M, 2F)	CSD-Sham (n=5) (3M, 2F)	WT-MI (n=11) (5M, 6F)	CSD-MI (n=10) (4M, 6F)	P value (WT- MI vs WT- sham)	P value (CSD-MI vs WT-MI)
Heart rate (bpm)	481.29 ± 22.42	518.17 ± 13.43	497.4 ± 13.6	484.9 ± 15.34	0.924	0.927
ESD (mm)	2.49 ± 0.2	2.39 ± 0.08	4.84 ± 0.23	3.56 ± 0.24	<0.001	0.012
EDD (mm)	3.68 ± 0.17	3.69 ± 0.06	5.7 ± 0.23	4.62 ± 0.21	<0.001	0.023
ESV (uL)	23.21 ± 4.59	20.26 ± 1.67	111.92 ± 11.8	55.41 ± 8.46	<0.001	0.012
EDV (uL)	58.2 ± 6.47	58.12 ± 2.21	162.21 ± 14.67	100.1 ± 10.1	<0.001	0.024
SV (uL)	34.98 ± 2.13	37.87 ± 1.13	50.29 ± 3.82	44.69 ± 2.61	0.028	0.634
EF (%)	61.7 ± 3.29	65.34 ± 2.01	31.49 ± 1.65	46.39 ± 3.54	<0.001	0.026
FS (%)	32.71 ± 2.17	35.23 ± 1.56	15.1 ± 0.84	23.34 ± 2.05	<0.001	0.032
CO (mL/min)	16.82 ± 1.26	19.66 ± 0.96	25.04 ± 2.21	21.73 ± 1.61	0.042	0.634
LVAW;s (mm)	1.15 ± 0.09	1.27 ± 0.09	0.98 ± 0.04	0.91 ± 0.08	0.390	0.869
LVAW;d (mm)	0.82 ± 0.07	0.88 ± 0.06	0.69 ± 0.03	0.60 ± 0.05	0.429	0.041
LVPW;s (mm)	1.2 ± 0.11	1.26 ± 0.12	0.66 ± 0.06	0.95 ± 0.06	0.014	0.025
LVPW;d (mm)	0.81 ± 0.06	0.82 ± 0.08	0.49 ± 0.04	0.62 ± 0.01	0.008	0.079

4-weeks post-surgery	WT-Sham (n=5) (3M, 2F)	CSD-Sham (n=5) (3M, 2F)	WT-MI (n=11) (5M, 6F)	CSD-MI (n=10) (4M, 6F)	P value (WT-MI vs WT-sham)	P value (CSD-MI vs WT-MI)
Heart rate (bpm)	505.03 ± 25.02	483.17 ± 8.14	502.83 ± 5.89	496.81 ± 10.84	0.999	0.960
ESD (mm)	2.77 ± 0.13	2.71 ± 0.15	5.73 ± 0.27	4.4 ± 0.22	<0.001	0.007
EDD (mm)	4.16 ± 0.14	4.03 ± 0.17	6.2 ± 0.25	5.18 ± 0.21	<0.001	0.029
ESV (uL)	29.17 ± 3.09	27.77 ± 4.08	167.61 ± 16.64	90.89 ± 9.72	<0.001	0.005
EDV (uL)	77.21 ± 5.82	71.97 ± 7.13	198.55 ± 17.38	131.37 ± 11.74	<0.001	0.024
SV (uL)	48.04 ± 3.27	44.2 ± 3.19	30.94 ± 3.17	40.48 ± 3.24	0.014	0.187
EF (%)	62.55 ± 1.99	62.03 ± 1.71	16.89 ± 2.5	32.1 ± 2.5	<0.001	0.002
FS (%)	33.5 ± 1.37	32.99 ± 1.1	7.84 ± 1.24	15.38 ± 1.31	<0.001	0.003
CO (mL/min)	24.37 ± 2.38	21.34 ± 1.49	15.57 ± 1.58	20.09 ± 1.64	0.062	0.228
LVAW;s (mm)	1.16 ± 0.11	1.15 ± 0.1	0.59 ± 0.05	0.62 ± 0.05	0.012	0.982
LVAW;d (mm)	0.67 ± 0.04	0.72 ± 0.04	0.47 ± 0.03	0.42 ± 0.03	0.019	0.712
LVPW;s (mm)	0.99 ± 0.02	1.07 ± 0.05	0.66 ± 0.04	0.89 ± 0.04	<0.001	0.076
LVPW;d (mm)	0.59 ± 0.03	0.74 ± 0.1	0.52 ± 0.04	0.63 ± 0.04	0.634	0.206

Bpm, beats per minute; ESD, end-systolic diameter; EDD, end-diastolic diameter; ESV, end-systolic volume; EDV, end-diastolic volume; SV, stroke volume; EF, ejection fraction; FS, fractional shortening; CO, cardiac output; LVAW;s, left ventricular anterior wall thickness in systole; LVAW;d, left ventricular anterior wall thickness in diastole; LVPW;s, left ventricular posterior wall thickness in systole; LVPWd, left ventricular posterior wall thickness in diastole; Two-way ANOVA and Tukey's multiple comparison test were performed between two selected groups. Bolded P values are statistically significant (P<0.05).

Supplemental Table 3. P-value table of WT and CSD mice post MI and Sham (Two-way ANOVA with Tukey's multiple comparison test)

Baseline	P value (WT-Sham vs CSD-Sham)	P value (WT-Sham vs WT-MI)	P value (WT-Sham vs CSD-MI)	P value (CSD-Sham vs WT-MI)	P value (CSD-Sham vs CSD-MI)	P value (CSD-MI vs WT-MI)
Heart rate (bpm)	0.565	0.756	0.335	0.217	0.078	0.923
ESD (mm)	0.916	0.714	0.385	0.968	0.599	0.677
EDD (mm)	0.733	0.845	0.542	0.954	>0.999	0.736
ESV (uL)	0.881	0.652	0.431	0.958	0.704	0.801
EDV (uL)	0.715	0.819	0.524	0.961	0.998	0.726
SV (uL)	0.690	0.972	0.696	0.732	0.987	0.574
EF (%)	0.736	0.925	0.953	0.304	0.394	>0.999
FS (%)	0.960	0.998	0.999	0.870	0.907	>0.999
CO (mL/min)	0.847	0.915	0.380	0.992	0.774	0.393
LVAW;s (mm)	0.020	0.053	0.015	0.201	0.461	0.593
LVAW;d (mm)	0.491	0.003	<0.001	0.005	<0.001	0.528
LVPW;s (mm)	0.509	0.981	0.160	0.734	0.767	0.271
LVPW;d (mm)	0.674	0.983	0.398	0.534	0.586	0.305

2-weeks post-surgery	P value (WT-Sham vs CSD-Sham)	P value (WT-Sham vs WT-MI)	P value (WT-Sham vs CSD-MI)	P value (CSD-Sham vs WT-MI)	P value (CSD-Sham vs CSD-MI)	P value (CSD-MI vs WT-MI)
Heart rate (bpm)	0.533	0.924	>0.999	0.705	0.409	0.927
ESD (mm)	0.967	<0.001	0.031	<0.001	0.014	0.013
EDD (mm)	>0.999	<0.001	0.028	<0.001	0.021	0.023
ESV (uL)	0.926	<0.001	0.044	<0.001	0.030	0.012
EDV (uL)	>0.999	<0.001	0.032	0.001	0.030	0.024
SV (uL)	0.650	0.028	0.072	0.064	0.168	0.634
EF (%)	0.957	<0.001	0.047	<0.001	0.017	0.026
FS (%)	0.993	<0.001	0.006	<0.001	0.006	0.032
CO (mL/min)	0.345	0.042	0.147	0.194	0.699	0.634
LVAW;s (mm)	0.773	0.394	0.264	0.124	0.078	0.831
LVAW;d (mm)	0.914	0.429	0.159	0.124	0.041	0.571
LVPW;s (mm)	0.982	0.014	0.261	0.015	0.191	0.025
LVPW;d (mm)	>0.999	0.008	0.088	0.026	0.160	0.080

4-weeks post-surgery	P value (WT-Sham vs CSD-Sham)	P value (WT-Sham vs WT-MI)	P value (WT-Sham vs CSD-MI)	P value (CSD-Sham vs WT-MI)	P value (CSD-Sham vs CSD-MI)	P value (CSD-MI vs WT-MI)
Heart rate (bpm)	0.838	>0.999	0.989	0.276	0.748	0.961
ESD (mm)	0.989	<0.001	<0.001	<0.001	<0.001	0.007
EDD (mm)	0.933	<0.001	0.006	<0.001	0.004	0.029
ESV (uL)	0.992	<0.001	<0.001	<0.001	<0.001	0.005
EDV (uL)	0.938	<0.001	0.006	<0.001	0.004	0.024
SV (uL)	0.834	0.014	0.397	0.054	0.845	0.187
EF (%)	0.997	<0.001	<0.001	<0.001	<0.001	0.002
FS (%)	0.991	<0.001	<0.001	<0.001	<0.001	0.003
CO (mL/min)	0.713	0.062	0.491	0.086	0.941	0.228
LVAW;s (mm)	>0.999	0.012	0.016	0.007	0.010	0.983

LVAW;d (mm)	0.829	0.019	0.006	0.006	0.002	0.712
LVPW;s (mm)	0.491	<0.001	0.132	<0.001	0.045	0.076
LVPW;d (mm)	0.553	0.635	0.620	0.310	0.941	0.207

Supplemental Table 4. Echo parameters of Control and shCapn2 injected mice post MI and Sham

Baseline	Saline-sham (n=4) (4M)	shCapn2-sham (n=5) (3M, 2F)	Saline-MI (n=6) (4M, 2F)	shCapn2-MI (n=5) (2M, 3F)	P value (Saline-MI vs Saline-sham)	P value (shCapn2-MI vs Saline-MI)
Heart rate (bpm)	495.4 ± 23.9	489.7 ± 23.5	461.5 ± 8.95	538.0 ± 14.3	0.399	0.006
ESD (mm)	2.70 ± 0.12	2.64 ± 0.09	2.62 ± 0.06	2.54 ± 0.14	0.270	0.710
EDD (mm)	4.19 ± 0.16	3.96 ± 0.11	3.97 ± 0.11	3.85 ± 0.19	0.488	0.743
ESV (uL)	27.3 ± 2.78	25.7 ± 2.15	25.3 ± 1.46	23.8 ± 3.14	0.297	0.256
EDV (uL)	78.9 ± 6.85	68.6 ± 4.38	69.4 ± 4.42	64.9 ± 7.81	0.530	0.748
SV (uL)	51.6 ± 4.77	42.9 ± 2.46	44.1 ± 3.04	41.2 ± 4.92	0.675	0.770
EF (%)	65.4 ± 2.08	62.7 ± 1.29	63.4 ± 0.65	63.6 ± 1.65	0.157	0.916
FS (%)	35.6 ± 1.54	33.4 ± 0.90	34.0 ± 0.51	34.0 ± 1.16	0.172	0.936
CO (mL/min)	25.9 ± 3.52	21.1 ± 1.96	20.3 ± 1.29	22.2 ± 2.60	0.961	>0.999
LVAW;s (mm)	0.97 ± 0.14	0.89 ± 0.04	1.06 ± 0.06	0.99 ± 0.08	0.509	0.769
LVAW;d (mm)	0.53 ± 0.05	0.50 ± 0.05	0.56 ± 0.03	0.61 ± 0.04	>0.999	0.998
LVPW;s (mm)	1.11 ± 0.05	1.09 ± 0.11	0.99 ± 0.07	1.01 ± 0.08	0.318	0.928
LVPW;d (mm)	0.66 ± 0.02	0.74 ± 0.05	0.60 ± 0.05	0.60 ± 0.05	0.175	0.898

2-weeks post- surgery	Saline-sham (n=4) (M-4)	shCapn2-sham (n=5) (3M, 2F)	Saline-MI (n=6) (4M, 2F)	shCapn2-MI (n=5) (2M, 3F)	P value (Saline-MI vs Saline-sham)	P value (shCapn2-MI vs Saline-MI)
Heart rate (bpm)	490.49 ± 18.88	506.01 ± 12.77	477.17 ± 14.36	520.81 ± 22.06	0.871	0.157
ESD (mm)	2.76 ± 0.13	2.68 ± 0.11	4.39 ± 0.35	3.81 ± 0.28	0.016	0.551
EDD (mm)	4.30 ± 0.15	4.14 ± 0.15	5.16 ± 0.29	5.13 ± 0.22	0.089	0.889
ESV (uL)	28.97 ± 3.5	26.81 ± 2.85	90.41 ± 15.24	63.85 ± 10.99	0.050	0.520
EDV (uL)	83.65 ± 6.93	76.54 ± 6.62	129.49 ± 16	126.29 ± 12.6	0.128	0.838
SV (uL)	54.68 ± 3.76	49.74 ± 4.27	39.08 ± 3.06	62.45 ± 2.12	0.594	0.029
EF (%)	65.58 ± 1.66	64.97 ± 1.67	32.04 ± 4.4	50.49 ± 3.8	<0.001	0.013
FS (%)	35.81 ± 1.23	35.28 ± 1.28	15.36 ± 2.29	25.97 ± 2.34	<0.001	0.009
CO (mL/min)	26.63 ± 1.09	25.07 ± 1.97	18.69 ± 1.63	32.39 ± 0.59	0.315	0.007
LVAW;s (mm)	1.22 ± 0.03	1.09 ± 0.06	1.15 ± 0.23	1.21 ± 0.08	>0.999	0.892
LVAW;d (mm)	0.78 ± 0.07	0.72 ± 0.06	0.84 ± 0.17	0.67 ± 0.05	0.866	0.795
LVPW;s (mm)	1 ± 0.1	1.12 ± 0.06	0.85 ± 0.07	0.95 ± 0.11	0.818	0.946
LVPW;d (mm)	0.69 ± 0.04	0.73 ± 0.02	0.69 ± 0.1	0.76 ± 0.05	0.971	0.995

4-weeks post-surgery	Saline-sham (n=4) (4M)	shCapn2-sham (n=5) (3M, 2F)	Saline-MI (n=6) (4M, 2F)	shCapn2-MI (n=5) (2M, 3F)	P value (Saline-MI vs Saline-sham)	P value (shCapn2-MI vs Saline-MI)
Heart rate (bpm)	489.6 ± 15.0	502.2 ± 17.8	521.5 ± 18.1	527.4 ± 16.5	0.510	0.995
ESD (mm)	3.07 ± 0.11	2.69 ± 0.07	5.01 ± 0.29	4.53 ± 0.22	0.002	0.562
EDD (mm)	4.40 ± 0.15	4.01 ± 0.06	5.49 ± 0.27	5.43 ± 0.26	0.059	0.829
ESV (uL)	37.2 ± 3.24	27.0 ± 1.81	121.9 ± 15.9	95.3 ± 10.4	0.007	0.528
EDV (uL)	88.3 ± 7.00	70.7 ± 2.52	149.6 ± 16.4	145.4 ± 16.1	0.036	0.538
SV (uL)	51.1 ± 4.13	43.7 ± 2.16	27.71 ± 2.94	50.1 ± 6.74	0.098	0.088
EF (%)	57.9 ± 1.41	61.8 ± 2.04	19.5 ± 2.42	34.5 ± 2.36	<0.001	0.006
FS (%)	30.4 ± 0.96	32.9 ± 1.44	9.00 ± 1.17	16.7 ± 1.26	<0.001	0.006
CO (mL/min)	25.1 ± 2.45	21.9 ± 1.21	14.5 ± 1.68	26.6 ± 4.26	0.199	0.143
LVAW;s (mm)	0.95 ± 0.05	0.85 ± 0.06	0.60 ± 0.06	0.81 ± 0.06	0.008	0.148
LVAW;d (mm)	0.55 ± 0.03	0.53 ± 0.03	0.44 ± 0.05	0.46 ± 0.08	0.296	0.998
LVPW;s (mm)	1.02 ± 0.02	0.94 ± 0.03	0.55 ± 0.05	0.68 ± 0.10	<0.001	0.649
LVPW;d (mm)	0.69 ± 0.02	0.59 ± 0.03	0.50 ± 0.05	0.54 ± 0.07	0.032	0.954

Bpm, beats per minute; ESD, end-systolic diameter; EDD, end-diastolic diameter; ESV, end-systolic volume; EDV, end-diastolic volume; SV, stroke volume; EF, ejection fraction; FS, fractional shortening; CO, cardiac output; LVAW;s, left ventricular anterior wall thickness in systole; LVAW;d, left ventricular anterior wall thickness in diastole; LVPW;s, left ventricular posterior wall thickness in systole; LVPWw, left ventricular posterior wall thickness in diastole; Two-way ANOVA and Tukey's multiple comparison test were performed between different groups. Bolded P values are statistically significant (P<0.05).

Supplemental Table 5. P-value table of Control and shCapn2 injected mice post MI and Sham (Two-way ANOVA with Tukey's multiple comparison test)

Baseline	P value (Saline-sham vs shCapn2- sham)	P value (Saline-sham vs Saline-MI)	P value (Saline-sham vs shCapn2- MI)	P value (shCapn2- sham vs Saline-MI)	P value (shCapn2- sham vs Saline-sham)	P value (shCapn2-MI vs Saline-MI)
Heart rate (bpm)	>0.999	0.400	0.730	0.242	0.598	0.006
ESD (mm)	0.593	0.270	0.833	0.764	0.991	0.710
EDD (mm)	0.765	0.488	0.956	0.867	0.976	0.743
ESV (uL)	0.628	0.297	0.997	0.738	0.546	0.256
EDV (uL)	0.790	0.503	0.947	0.838	0.988	0.748
SV (uL)	0.914	0.675	0.995	0.897	0.976	0.770
EF (%)	0.534	0.157	0.580	0.866	>0.999	0.916
FS (%)	0.552	0.172	0.575	0.903	>0.999	0.937
CO (mL/min)	0.952	0.961	0.934	>0.999	>0.999	>0.999
LVAW;s (mm)	0.964	0.509	0.885	0.777	0.998	0.769
LVAW;d (mm)	>0.999	>0.999	0.993	>0.999	>0.999	0.998
LVPW;s (mm)	0.925	0.318	0.733	0.884	0.997	0.928
LVPW;d (mm)	0.522	0.175	0.458	0.026	0.077	0.898

2-weeks post- surgery	P value (Saline-sham vs shCapn2- sham)	P value (Saline-sham vs Saline-MI)	P value (Saline-sham vs shCapn2- MI)	P value (shCapn2- sham vs Saline-MI)	P value (shCapn2- sham vs Saline-sham)	P value (shCapn2-MI vs Saline-MI)
Heart rate (bpm)	0.986	0.871	0.782	0.399	0.817	0.157
ESD (mm)	0.992	0.016	0.011	0.016	0.008	0.552
EDD (mm)	0.991	0.089	0.039	0.066	0.009	0.890
ESV (uL)	0.991	0.050	0.027	0.047	0.025	0.520
EDV (uL)	0.990	0.128	0.034	0.107	0.014	0.838
SV (uL)	0.992	0.594	0.206	0.686	0.113	0.029
EF (%)	>0.999	<0.001	0.004	<0.001	0.003	0.013
FS (%)	>0.999	<0.001	0.003	<0.001	0.004	0.009
CO (mL/min)	>0.999	0.315	0.043	0.447	0.048	0.008
LVAW;s (mm)	0.995	>0.999	0.591	0.993	0.264	0.892
LVAW;d (mm)	0.989	0.866	0.993	0.928	0.922	0.795
LVPW;s (mm)	0.054	0.818	>0.999	0.028	0.178	0.946
LVPW;d (mm)	0.373	0.971	0.839	0.855	0.904	0.995

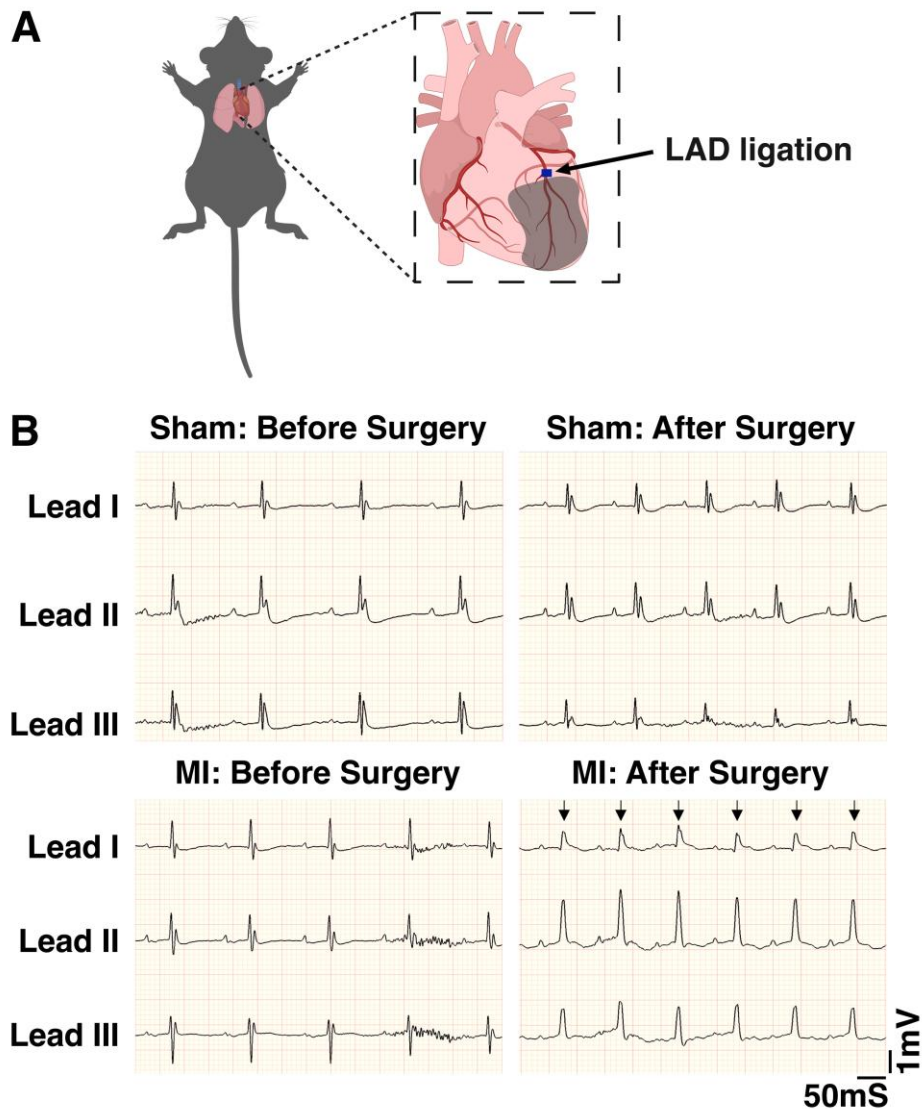
4-weeks post-surgery	P value (Saline-sham vs shCapn2-sham)	P value (Saline-sham vs Saline-MI)	P value (Saline-sham vs shCapn2-MI)	P value (shCapn2-sham vs Saline-MI)	P value (shCapn2-sham vs Saline-sham)	P value (shCapn2-MI vs Saline-MI)
Heart rate (bpm)	0.828	0.510	0.387	0.863	0.704	0.995
ESD (mm)	0.131	0.002	0.006	<0.001	0.002	0.562
EDD (mm)	0.398	0.060	0.126	0.006	0.014	0.830
ESV (uL)	0.156	0.007	0.015	0.004	0.009	0.528
EDV (uL)	0.406	0.037	0.107	0.012	0.024	0.538
SV (uL)	0.717	0.098	0.998	0.003	0.868	0.088
EF (%)	0.093	<0.001	0.002	<0.001	<0.001	0.006
FS (%)	0.130	<0.001	<0.001	<0.001	<0.001	0.006
CO (mL/min)	0.897	0.199	0.990	0.012	0.789	0.144
LVAW;s (mm)	0.268	0.008	0.214	0.031	0.956	0.148
LVAW;d (mm)	0.881	0.296	0.640	0.449	0.821	0.998
LVPW;s (mm)	0.308	<0.001	0.077	<0.001	0.158	0.649
LVPW;d (mm)	0.055	0.032	0.201	0.372	0.830	0.954

Supplemental Table 6. Top 15 binding partners of JPH2-CTP

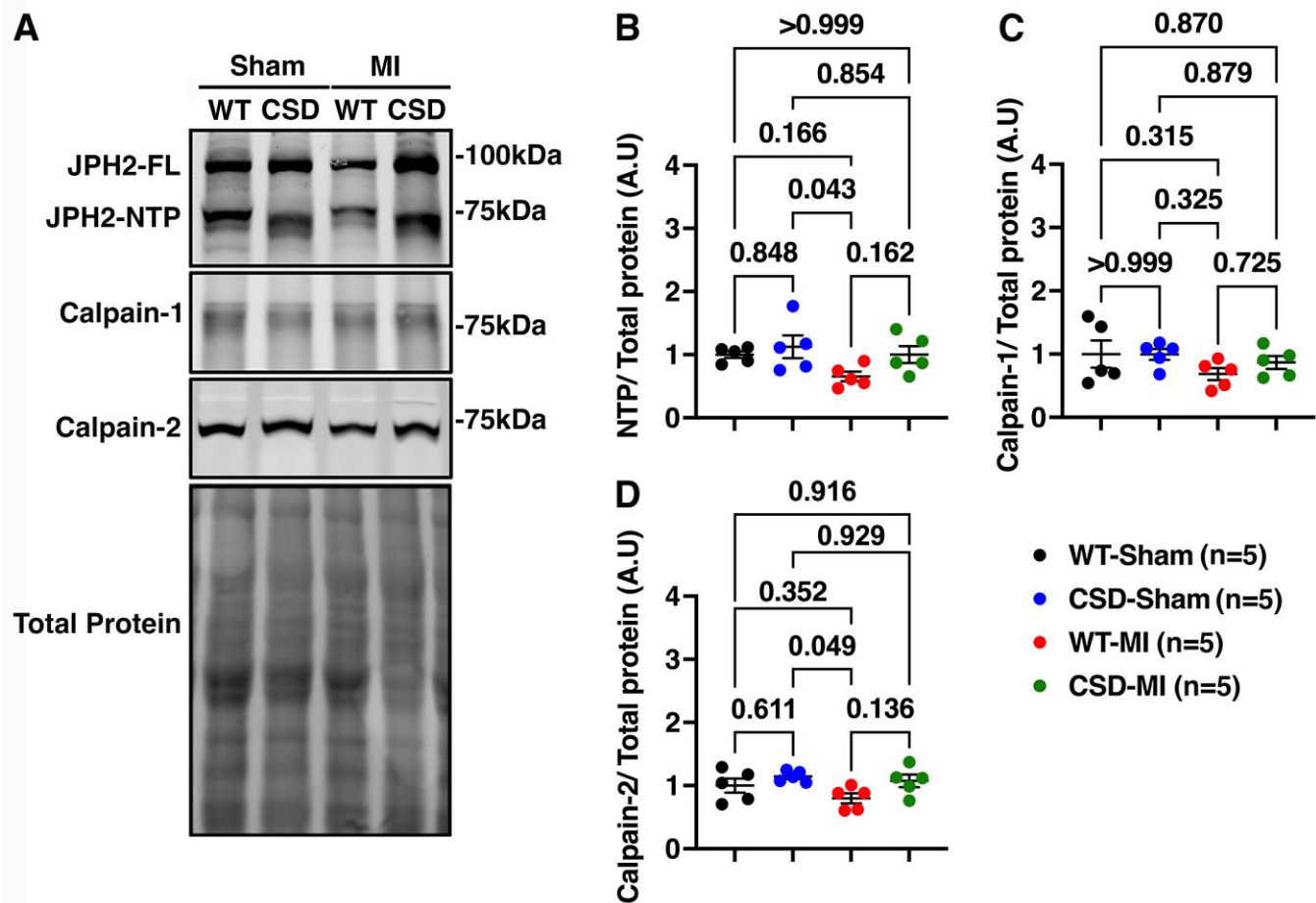
Protein ID	Protein name	Fold Change (Ab2/IgG)	P value
Nes	Nestin	92.83	0.002
C4b	Complement C4-B	82.03	0.066
Ndufs7	NADH dehydrogenase [ubiquinone] iron-sulfur protein 7	50.67	0.103
Anxa5	Annexin A5	35.31	0.083
Bag3	BAG family molecular chaperone regulator 3	31.24	0.083
C1qbp	Complement C1q binding partner	4.50	0.044
C4bpa	C4b-binding protein	4.24	0.146
Cox5b	Cytochrome c oxidase subunit 5B	3.73	0.147
Ak2	Aspartokinase 2	3.55	0.162
Arf1;Arf3;Arf5;Arf2;Arf4	ADP-ribolysation factor 1	3.44	0.035
Lyz2	Lysozyme C-2	3.43	0.196
Opa1	Dynamin-like 120 kDa protein	3.25	0.161
Vcp	Valosin-containing protein	3.11	0.024
Tnnc1	Troponin C	3.01	0.125

JPH2-CTP binding factors from mass spec proteomics after clearing full length JPH2. Student T-test was done between IgG and JPH2-Ab2 pull down to get P value.

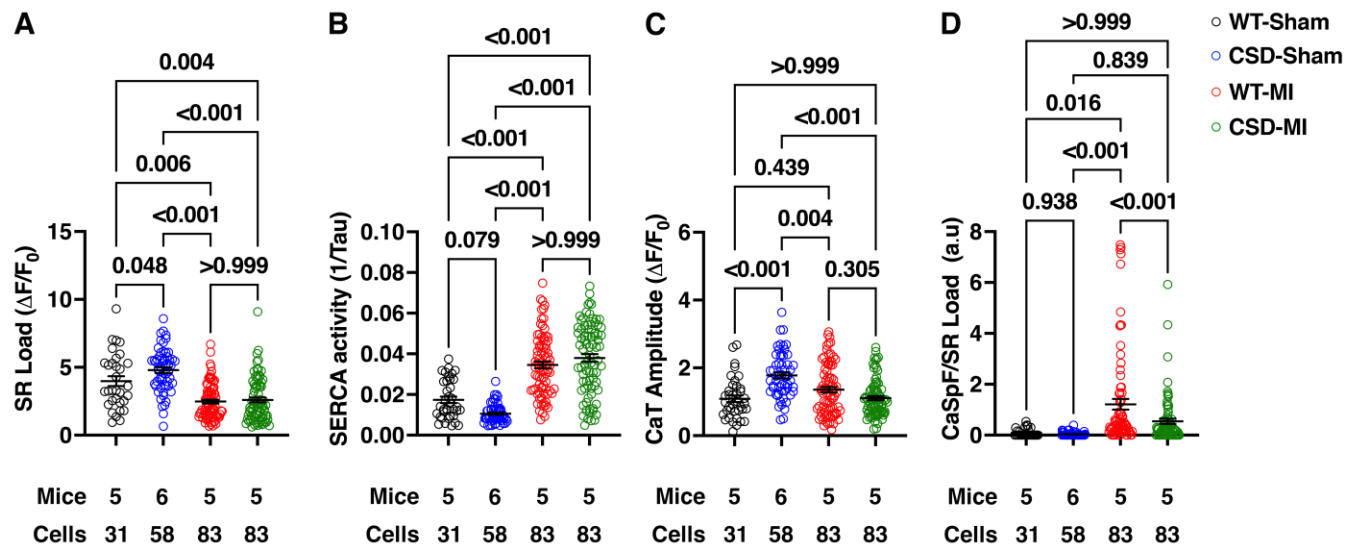
Supplemental Fig. 1. Surgical model of myocardial infarction. (A) Schematic diagram showing permanent ligation of the left anterior descending (LAD) coronary artery to induce ischemia in the left ventricle (marked brown as the area). **(B)** Sample ECG traces from C57BL/6J mice right before and after myocardial infarction (MI) or sham surgeries. Small arrows mark S-T elevation indicating successful ligation of the LAD in mice.



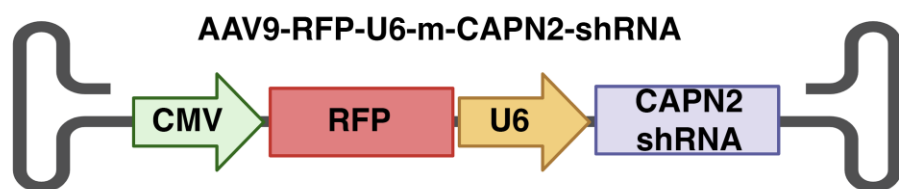
Supplemental Fig. 2. Unaltered levels of JPH2 N-terminal peptide following MI. (A) Representative western blots of full length JPH2 (FL) and N-terminal peptide (NTP), calpain-1, calpain-2, and total protein as loading control. (B) Quantification of JPH2 N-terminal peptide, (C) calpain-1, and (D) calpain-2, normalized to total protein levels. N, number of mice per group. Two-way ANOVA with Tukey's multiple comparison test was performed to assess statistical significance between 4 groups at different timepoints.



Supplemental Fig 3. Ca^{2+} dynamics in CSD post MI. (A) Quantification of sarcoplasmic reticulum load after 10mM caffeine perfusion in isolated cardiomyocytes from WT or CSD mice that underwent sham or MI surgery. **(B)** Quantification of Ca^{2+} transient decay dynamics representing SERCA activity ($1/\text{Tau}$), **(C)** Ca^{2+} transient amplitude during 1Hz pacing and **(D)** Quantification of Ca^{2+} spark frequency (CaSpF) to SR load ratio in isolated cardiomyocytes from WT or CSD mice that underwent sham or MI surgery. P-value from two-way ANOVA with Tukey's multiple comparison test.



Supplemental Fig. 4. *In vivo* calpain-2 knockdown using AAV9-shCapn2. Schematic diagram of the plasmid map of the AAV9-CMV-RFP-U6-shCapn2 virus.



Supplemental Fig. 5. Unaltered mitochondrial membrane potential following CTP overexpression.

Quantification of the mitochondrial membrane potential in mouse embryonic fibroblasts (MEFs) transfected with empty vector controls, full-length (FL) JPH2, or JPH2-CTP plasmid cloned in a pcDNA3.1 vector backbone. MEFs overexpressing these constructs were stained with mitochondrial membrane potential dye TMRE (1 nM), which was analyzed via flow cytometry. n = 4 biological replicates per group, where each data point represents the membrane potential of one well of MEFs (10,000 cells analyzed per well) overexpressing a specific construct. The Shapiro-Wilk test was performed to assess normality in the data. Statistical testing was performed using the one-way ANOVA multiple comparison test.

