

## Major Resources Table

In order to allow validation and replication of experiments, all essential research materials listed in the Methods should be included in the Major Resources Table below. Authors are encouraged to use public repositories for protocols, data, code, and other materials and provide persistent identifiers and/or links to repositories when available. Authors may add or delete rows as needed.

### Animals (in vivo studies)

Species	Vendor or Source	Background Strain	Sex	Persistent ID / URL

### Genetically Modified Animals

	Species	Vendor or Source	Background Strain	Other Information	Persistent ID / URL
Parent - Male	Mouse	TJU-WUSTL (GW Dorn II)	Mixed C57Bl/6J, FVB/N	<sup>Tg/Tg</sup> (Myh6/-tetO-linker) x <sup>Tg/Tg</sup> (Myh6/tTA) x Mfn2 <sup>fl/fl</sup>	
Parent - Female				See text.	

### Antibodies

Target antigen	Vendor or Source	Catalog #	Working concentration	Lot # (preferred but not required)	Persistent ID / URL/RRID
Anti-MCU	Cell signaling	D2Z3B	1:500 WB, 1:100 IF		RRID:AB_2721812
Anti-RFP	Life Technologies	R10367	1:100 IG	1425235	RRID:AB_10563941
Anti-RyR2	Invitrogen	MA3-916	1:500 WB, 1:50 IF		RRID:AB_2183054
Alexa fluor 488 chicken anti rabbit	Invitrogen	A21441	1:200		RRID:AB_2535859
Alexa fluor 647 donkey anti mouse	Invitrogen	A31571	1:200		RRID:AB_162542
Calreticulin	Cell Signaling Technologies	2891	WB 1:1000		RRID:AB_2275208
Calsequestrin	Abcam	ab3516	WB 1:1000		RRID:AB_303865
Fluoronanogold Alexa Fluor 488	Nanoprobes	7202	1:200		
LiCor IRDye 680RD anti-mouse	Li-Cor	926-68022	1:10000		RRID:AB_10715072
LiCor IRDye 800CW anti-rabbit	Li-Cor	926-32213	1:5000		RRID:AB_621848
PGC1 $\alpha$	Abcam	ab72230	WB 1:1000		RRID:AB_1640773
SERCA	Thermo Fisher Scientific	MA3-919	WB 1:1000		RRID:AB_325502

**Chemicals, Peptides, and Recombinant proteins**

<b>Name</b>	<b>Vendor or Source</b>	<b>Catalog #</b>	<b>Lot #</b>	<b>Persistent ID/URL/RRID</b>
Acetone	Electron microscopy Sciences	10000		<a href="https://pubchem.ncbi.nlm.nih.gov/compound/180">https://pubchem.ncbi.nlm.nih.gov/compound/180</a>
2, 3- butanedione monoxime	Sigma-Aldrich	B0753-25g		<a href="https://pubchem.ncbi.nlm.nih.gov/substance/329827967">https://pubchem.ncbi.nlm.nih.gov/substance/329827967</a>
Antimycin A	Sigma-Aldrich	A8674		<a href="https://pubchem.ncbi.nlm.nih.gov/substance/24891355">https://pubchem.ncbi.nlm.nih.gov/substance/24891355</a>
Bovine Serum Albumin Fraction V, heat shock	Sigma-Aldrich	03116964001		
Calcium chloride solution	Sigma-Aldrich	21115		<a href="https://pubchem.ncbi.nlm.nih.gov/substance/57647902">https://pubchem.ncbi.nlm.nih.gov/substance/57647902</a>
Caffeine	Sigma-Aldrich	C0750–500 g		<a href="https://pubchem.ncbi.nlm.nih.gov/substance/24277682">https://pubchem.ncbi.nlm.nih.gov/substance/24277682</a>
Calf serum	Cytiva	SH30073		
Collagenase type 2	Worthington-Biochem	LS004176		
Creatine	Sigma-Aldrich	C0780-50g		<a href="https://pubchem.ncbi.nlm.nih.gov/substance/24892273">https://pubchem.ncbi.nlm.nih.gov/substance/24892273</a>
Cyclosporin A	Santa Cruz Biotechnologies	sc-3503		
Dulbecco's modified eagle's medium	Sigma-Aldrich	D5030-10L		
Durcupan, ACM, Epoxy resin kit	Electron microscopy Sciences	RT14040		
EPON, Embed 812 Embedding kit	Electron microscopy Sciences	14900		
Ethanol, 200 proof	Decon laboratories, Inc	2701		
FCCP				
Formvar Solution in Ethylene Dichloride	Electron Microscopy Sciences	15810		
Fura-IoAff K+ salt	Teflabs	0137		
GoldEnhance EM Plus	nanoprobes	2114		
Glucose (dextrose)	Sigma-Aldrich	D9434-250g		<a href="https://pubchem.ncbi.nlm.nih.gov/substance/24894295">https://pubchem.ncbi.nlm.nih.gov/substance/24894295</a>
Glutaraldehyde, 25% solution	Electron Microscopy Sciences	16210		
HALT Protease & Phosphatase Inhibitor	ThermoFisher Scientific	78440		
HEPES, powder	Fisher Bioreagents	BP310-1		

DOI [to be added]

HEPES, buffer solution	Gibco	15630-080		
Heparin, 1000 usp units per ml	Sagent Pharmaceuticals	NDC 25021-400-01		
Isoproterenol hydrochloride	Sigma-Aldrich	I6504-1g		<a href="https://pubchem.ncbi.nlm.nih.gov/substance/24278495">https://pubchem.ncbi.nlm.nih.gov/substance/24278495</a>
ITS (insulin-transferrin-selenium)	Sigma-Aldrich	I-1884		
Krebs-Henseleit buffer modified	Sigma-Aldrich	K3753-10X1L		
Laminin	Corning	354232		
L-glutamine	Gibco	25030-081		
Lead nitrate	Sigma Aldrich	467790-50G		<a href="https://pubchem.ncbi.nlm.nih.gov/substance/24870438">https://pubchem.ncbi.nlm.nih.gov/substance/24870438</a>
Li-Cor Blocking Buffer	Li-Cor	927-50000		
Luna® Universal One-Step Reaction buffer	New England Biolabs	E3005		
Magnesium chloride	Sigma-Aldrich	M8266-100g		<a href="https://pubchem.ncbi.nlm.nih.gov/substance/24897235">https://pubchem.ncbi.nlm.nih.gov/substance/24897235</a>
Magnesium sulfate heptahydrate	Sigma-Aldrich	M2773-500g		<a href="https://pubchem.ncbi.nlm.nih.gov/substance/24896765">https://pubchem.ncbi.nlm.nih.gov/substance/24896765</a>
Mannitol	Thermo Fisher Scientific	M120-3		
Methyl Alcohol	Electron Microscopy Sciences	18510		
MEM + glutamax	Gibco	41090-036		
MitoTracker Red CMXros	Invitrogen	M7512		
Monarch Total RNA miniprep kit	New England Biolabs	T2010		
Oligomycin from streptomyces diastatochromogenes	Sigma-Aldrich	O4876-25mg		<a href="https://pubchem.ncbi.nlm.nih.gov/substance/24898006">https://pubchem.ncbi.nlm.nih.gov/substance/24898006</a>
Osmium tetroxide, 4%	Electron Microscopy Sciences	RT19150		
Paraformaldehyde, 32%	Electron Microscopy Sciences	15714-S		
PBS, pH 7.4 (1X)	Gibco	10010-023		
Penicilin/streptomycin	Gibco	15140-122		
Percoll	Sigma-Aldrich	P1644-100ml		
Pluronic® F127	Sigma-Aldrich	P-2443		

Potassium chloride	Sigma-Aldrich	P9541-500g		<a href="https://pubchem.ncbi.nlm.nih.gov/substance/24899042">https://pubchem.ncbi.nlm.nih.gov/substance/24899042</a>
Potassium hexacyanoferrate(II) -13C6 trihydrate	Sigma-Aldrich	736716		<a href="https://pubchem.ncbi.nlm.nih.gov/substance/329765135">https://pubchem.ncbi.nlm.nih.gov/substance/329765135</a>
Potassium phosphate monobasic	Sigma-Aldrich	P9791-100g		<a href="https://pubchem.ncbi.nlm.nih.gov/substance/24898921">https://pubchem.ncbi.nlm.nih.gov/substance/24898921</a>
Proteinase K	Thermo Fisher Scientific	25530049		
Resolving buffer	Biorad	161-0798		
RNA-Later solution	Thermo Fisher Scientific	AM7921		
Rotenone				
Seahorse XFe24 FluxPak	Agilent Technologies	102340-100		
SlowFade Gold Antifade Mountant	Thermo Fisher Scientific	S36937		
Sodium aspartate				
Sodium bicarbonate	Sigma-Aldrich	S5761-500g		<a href="https://pubchem.ncbi.nlm.nih.gov/substance/24899673">https://pubchem.ncbi.nlm.nih.gov/substance/24899673</a>
Sodium chloride	Sigma-Aldrich	S5886-500g		<a href="https://pubchem.ncbi.nlm.nih.gov/substance/24899675">https://pubchem.ncbi.nlm.nih.gov/substance/24899675</a>
Sodium cyanide	Sigma-Aldrich	S3296		Product has been discontinued.
Sodium Cacodylate Buffer	Electron Microscopy Sciences	11652		
Sodium phosphate dibasic	Sigma-Aldrich	S5136-100g		<a href="https://pubchem.ncbi.nlm.nih.gov/substance/24899638">https://pubchem.ncbi.nlm.nih.gov/substance/24899638</a>
Sodium pyruvate	Sigma-Aldrich	P8574-25g		<a href="https://pubchem.ncbi.nlm.nih.gov/substance/24898951">https://pubchem.ncbi.nlm.nih.gov/substance/24898951</a>
Spurr's resin	Electron Microscopy Sciences	RT14300		
Stacking buffer	Biorad	161-0799		
Tail Lysis Buffer1	BioWorld	10450042		
Tannic Acid	Electron Microscopy Sciences	21700		
Taurine	Sigma-Aldrich	T0625-100g		<a href="https://pubchem.ncbi.nlm.nih.gov/substance/24278721">https://pubchem.ncbi.nlm.nih.gov/substance/24278721</a>
Thiocarbohydrazide	Electron Microscopy Sciences	RT21900		
Triphenyl tetrazolium chloride	Hach Company	2406042		
Triton X-100	Calbiochem	648462		
Uranyl Acetate	Electron Microscopy Sciences	22400		
		E3005		

WarmStart RT Enzyme mix	New England Biolabs			
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**DNA/cDNA Clones**

Clone Name	Sequence	Source / Repository	Persistent ID / URL

**Cultured Cells**

Name	Vendor or Source	Sex (F, M, or unknown)	Persistent ID / URL

**Data & Code Availability**

Description	Source / Repository	Persistent ID / URL

**Other**

Description	Source / Repository	Persistent ID / URL
Doxy-diet Modified 5001 diet w/200ppm doxycycline	Animal Specialties and Provisions, Quakertown, PA	

## ARRIVE GUIDELINES

The ARRIVE guidelines (<https://arriveguidelines.org/>) are a checklist of recommendations to improve the reporting of research involving animals. Key elements of the study design should be included below to better enable readers to scrutinize the research adequately, evaluate its methodological rigor, and reproduce the methods or findings.

**Study Design –for In vivo cardiac function (Echocardiography) experiments shown in Fig.2,S2 for basal recordings Basal includes the the animals assigned for Isoproterenol (iso) challenge as well, for their basal values before Iso injection. For other, smaller-scale survival and non-survival experiments, see Table S2.**

Groups	Sex	Age	Number (prior to experiment)	Number (after termination)	Littermates (Yes/No)	Other description
Group 1 (Control)	F	12-18 wk	23 (basal-Fig3) 13 (iso-Figs7,S5)	23 (basal-Fig3) 10 (iso-Fg7,S5)	No/partial	3 Iso-injected females died before termination
Group 2 (Control)	M	12-18 wk	22 (basal-Fig3) 10 (iso-Figs7,S5)	22 (basal-Fig3) 10 (iso-Fg7,S5)	No/partial	
Group 3 (Linker)	F	12-18 wk	21 (basal-Fig3) 11 (iso-Figs7,S5)	21 (basal-Fig3) 10 (iso-Fg7,S5)	No/partial	1 Iso-injected female died before termination
Group 4 (Linker)	M	12-18 wk	32 (basal-Fig3) 15 (iso-Figs7,S5)	32 (basal-Fig3) 13 (iso-Fg7,S5)	No/partial	2 Iso-injected males died before termination
Group 5 (Doxy-Linker)	F	12-18 wk	6 (basal-Fig3) 6 (iso-Figs7,S5)	6 (basal-Fig3) 4 (iso-Fg7,S5)	No/partial	2 Iso- injected females died before termination
Group 6 (Doxy-Linker)	M	12-18 wk	5 (basal-Fig3) 5 (iso-Figs7,S5)	5 (basal-Fig3) 5 (iso-Fg7,S5)	No/partial	

**Sample Size:** Please explain how the sample size was decided Please provide details of any a *prior* sample size calculation, if done. Since both genders were used, for each group minimum 5-6 animals from each sex were the original target for the injurious stress (isoproterenol injection) experiments, with the notion that the experiments are exploratory and significant findings will require further studies. For the first experiment, only Control and Linker groups were compared. This was followed by a second round of study/experiments, where Control, Linker and Doxy-linker groups were compared. This effectively doubled N for the Control and Linker groups. Basal Echo recordings were also obtained from other smaller cohorts that were then assigned to other in vivo survival assays (e.g. blood pressure measurement, treadmill exercise performance); that is why N for basal are higher.

### Inclusion Criteria

Besides the genotype, mouse inclusion was primarily based on age range. The assigned animals should have stayed visibly healthy and non-distressed until the start of the experimental use.

### Exclusion Criteria

Animals displaying distress or appearing sick before the experimental use were excluded. When echocardiography was performed with heart rate recording, animals with <400/min basal heart rate were excluded from the analysis (concern of relative isoflurane overdose).

### Randomization

Whenever more than one pair of Ctr vs Lnk (vs Doxy-Lnk) animals were used for in vivo experiments (e.g. echocardiography), attention was paid to perform the procedure on the animals by alternating between groups to avoid potential biased variations in the individual waiting time in the cages at the performance site. When males and females were both used, the alternation between groups was done in male-male, female-female pairs.

### Blinding

DOI [to be added]

The person, who did the imaging, performed the primary Echocardiography analysis. He was provided with the animal (ear tag) ID number without the group information and the IDs were 'decoded'/un-blinded during the secondary comparative analyses.