

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
Wild Type (WT)	Jax labs	C57BL/6	Males and females	
C57BL/6-oxAKAP12 (Gravin)-Tg	In house lab*	C57BL/6	Males and Females	

* In process of facilitating mice availability at Jax labs

Genetically Modified Animals

	Species	Vendor or Source	Background Strain	Other Information	Persistent ID / URL
Parent - Male	Mus musculus	Texas A&M-IBT (in house lab)	FVB	Backcrossed (>5 times) into C57BL/6	
Parent - Female	Mus musculus	Texas A&M-IBT (in house lab)	FVB	Backcrossed (>5 times) into C57BL/6	

Antibodies

Target antigen	Vendor or Source	Catalog #	Working concentration	Lot # (preferred but not required)	Persistent ID / URL
AKAP12 – C-terminal <i>Used for western blots</i>	Abcam Inc. Cambridge, UK	ab204559	1:1000		https://www.abcam.com/products/primary-antibodies/akap12-antibody-c-terminal-ab204559.html
AKAP12 - Anti-Gravin Antibody, Mouse Monoclonal <i>Used for ICC</i>	Sigma-Aldrich	G3795	1:100	028M4766V & 0000123814	https://www.sigmaaldrich.com/id/id_en/g3795-200ul-id
PDE8A Antibody Polyclonal <i>Used for western blots</i>	Thermo Fisher Scientific™, Waltham, MA, USA	PA5-88405	1:1000		https://www.thermofisher.com/antibody/product/PDE8A-Antibody-Polyclonal/PA5-88405
PDE8A Polyclonal antibody <i>Used for ICC</i>	ProteinTech	13956-1-AP	1:100	00005031	https://www.ptglab.com/products/PDE8A-Antibody-13956-1-AP.htm
WGA (wheat germ agglutinin) Alexa Fluor 488 conjugate <i>Used for ICC</i>	Thermo Fisher Scientific™, Waltham, MA, USA	W11261	100µg/ml		https://www.thermofisher.com/order/catalog/product/W11261
troponin I Rabbit Ab <i>Used for western blots</i>	Cell Signaling, Danvers, MA, USA	4002S	1:1000	Ref:10/2015 Lot:2	https://www.cellsignal.com/products/primary-antibodies/troponin-i-antibody/4002
p-troponin I(cardiac)(S23/24) Rabbit Ab <i>Used for western blots</i>	Cell Signaling, Danvers, MA, USA	4004S	1:1000	Ref:05/2012 Lot:2	https://www.cellsignal.com/products/primary-antibodies/phospho-troponin-i-cardiac-ser23-24-antibody/4004

DOI [to be added]

Anti-rabbit IgG, HRP-linked Antibody <i>Used for western blots</i>	Cell Signaling, Danvers, MA, USA	7074	1:1000		https://www.cellsignal.com/products/secondary-antibodies/anti-rabbit-igg-hrp-linked-antibody/7074
Anti-mouse IgG, HRP-linked Antibody <i>Used for western blots</i>	Cell Signaling, Danvers, MA, USA	7076	1:1000		https://www.cellsignal.com/products/secondary-antibodies/anti-mouse-igg-hrp-linked-antibody/7076
Alexa Flour 488 goat anti-mouse <i>Used for ICC</i>	Invitrogen, life technologies	A11001	1:500	2714439	https://www.thermofisher.com/antibody/product/Goat-anti-Mouse-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-11001
Alexa Flour 568 donkey anti-rabbit <i>Used for ICC</i>	Invitrogen, life technologies	A10042	1:500	2540901	https://www.thermofisher.com/antibody/product/Donkey-anti-Rabbit-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A10042

DNA/cDNA Clones

Clone Name	Sequence	Source / Repository	Persistent ID / URL
Human AKAP12 (hAKAP12)	HG18215-UT	Sino Biologicals, Wayne, PA, USA	https://www.sinobiological.com/cdna-clone/human-akap12-hg18215-ut

Cultured Cells

Name	Vendor or Source	Sex (F, M, or unknown)	Persistent ID / URL
AC16 Human Cardiomyocyte Cell Line ¹	Millipore Sigma	Unknown	https://www.sigmaaldrich.com/US/en/product/mm/scc109?gclid=CjwKCAiAqNSsBhAvEiwAn_tmXy5Ra9IYHp0xTVteqErJ2Ou1pAzyrv0AV1aVeEJzUsWowUB9lpMaQBoCHNMQAvD_BwE

Data & Code Availability

Description	Source / Repository	Persistent ID / URL
RNAseq Data	data will be available using the online <i>figshare</i> data repository	
Raw data files and Prism analysis files organized by figure panels	data will be available using the online <i>figshare</i> data repository	

Other

Description	Source / Repository	Catalog Number
Reagents and Chemicals		
Dulbecco's modified Eagle's medium (DMEM) high glucose, pyruvate	Life Technologies, Grand Island, NY, USA	11-995-073

Fetal Bovine Serum (FBS)	Life Technologies, Grand Island, NY, USA	26-140-079
Antibiotic-Antimycotic (100X)	Life Technologies, Grand Island, NY, USA	15-240-062
DPBS, no calcium, no magnesium	Life Technologies, Grand Island, NY, USA	14-190-235
Opti-MEM™ I Reduced Serum Medium	Life Technologies, Grand Island, NY, USA	31-985-070
Bovine Serum Albumin	Sigma Aldrich, St. Louis, MO, USA	A7906-100G
10X RIPA Lysis Buffer	MilliporeSigma, Burlington, MA, USA	20-188
Paraformaldehyde Solution, 4% in PBS	Thermo Fisher Scientific™, Waltham, MA, USA	AAJ19943K2
Triton™ X-100	Sigma Aldrich, St. Louis, MO, USA	T8787
TWEEN® 20	Sigma Aldrich, St. Louis, MO, USA	P9416
ViaFect™ Transfection Reagent	Promega, Madison, WI, USA	E4982
Duolink® In Situ PLA® Probe Anti-Mouse MINUS	MilliporeSigma, Burlington, MA, USA	DUO92004
Duolink® In Situ PLA® Probe Anti-Rabbit PLUS	MilliporeSigma, Burlington, MA, USA	DUO92002
Duolink® In Situ Detection Reagents Red	MilliporeSigma, Burlington, MA, USA	DUO92008
PowerUp™ SYBR™ Green Master Mix	Thermo Fisher Scientific™, Waltham, MA, USA	A25776
Halt™ Protease and Phosphatase Inhibitor Single-Use Cocktail (100X)	Thermo Fisher Scientific™, Waltham, MA, USA	78442
SuperSignal West Pico PLUS Chemiluminescent Substrate	Thermo Fisher Scientific™, Waltham, MA, USA	34577

Kits		
Description	Source / Repository	Catalog Number
Trans-Blot Turbo RTA Mini 0.2 µm PVDF Transfer Kit	Bio Rad, Hercules, CA, USA	1704272
Stain Free Mini-PROTEAN® TGX™ Precast Gels	Bio Rad, Hercules, CA, USA	4568026

DOI [to be added]

Pierce™ BCA Protein Assay Kit	Thermo Fisher Scientific™, Waltham, MA, USA	23225
PureLink® RNA Mini Kit	Life Technologies, Grand Island, NY, USA	12-183-020
SuperScript IV VILO Kit	Thermo Fisher Scientific™, Waltham, MA, USA	11-756-050
ELISA cAMP assay	Abcam	ab290713

Drugs		
Description	Source / Repository	Catalog Number
(-) – Epinephrine [RRID: SCR_003281]	Sigma Aldrich, St. Louis, MO, USA	E4250-10G
Forskolin	Sigma Aldrich, St. Louis, MO, USA	F3917-10MG
PF-04597325	Thermo Fisher Scientific™, Waltham, MA, USA	50-202-9122-1MG
IBMX	Sigma Aldrich, St. Louis, MO, USA	I5879
Rolipram	Sigma Aldrich, St. Louis, MO, USA	R6520
PTX	Thermo Fisher Scientific™, Waltham, MA, USA	PHZ1174
ICI-118551	Sigma Aldrich, St. Louis, MO, USA	I127-25MG
CGP-20712A	Sigma Aldrich, St. Louis, MO, USA	C231-10MG

RTqPCR Primers – Human		
Description	Forward Primer	Reverse Primer
AKAP12	GCTGGACAGGAAACGGAGAA	CACTGCGGTTGACTCTGACT
PDE1A	AGGTCACCTCCAGCAAATTA	CCACATAGGAAGAAGTTTCG
PDE1C	AGATATTAGCCATCCAGCAA	CAACGGAGATGACAGAAT
PDE2A	CCTTCAACAAGCTAGAAGGA	CGGATCTCATAGCTCTCATC
PDE3A	CAACTGTGTGTGTGTGTG	CAAGTGGTGCATAGCAGTAA
PDE4A	GCTGAAGACCTCATCGTAAC	ATTCTGTTTGTCCAGGAATG
PDE4C	AGAGTGGTACCAGAGCAAGA	TGGGAGCCACCTATAACTAA
PDE4D	CACCAAATGACCTTACCTGT	AGCTCCACTGTTACCTTTCA
PDE5A	GAAAAGGACTTTGCTGCTTA	TGATTTTGTTCATCATGT
PDE7A	GCAATATGAATTTGGCTTTC	GGAAAGAGCTGCAGTCTAAA
PDE7B	TCTTCAATACCCATGGACTC	ATCCTGTGTCATTTCTTTG
PDE8A	ACCAATGTAATGGATTCTGC	TGAGTTACAAGCCCTGAGTT

PDE8B	AGAACAGGAGGAAAGAGTCC	CGTTTCCTGACAGTCTTCTC
GAPDH	GTCTCCTCTGACTTCAACAGCG	ACCACCCTGTTGCTGTAGCCAA
HPRT1	CTTGAGCACACAGAGGGCTACA	CATTATGCTGAGGATTTGGAAAG
RTqPCR Primers – Mouse		
Description	Forward Primer	Reverse Primer
AKAP12	GTCCAAGAGGAAAGCCAGGATG	CTGTGGAACTGGCTGCCATTTT
PDE1A	TGACGTCACTCAAACGTGCATT	CCATGGCTAAAATTTCCAGTTCA
PDE1C	GAAGACAGCCCTGCAGCAA	GTGTAACATGAGGGATAAGGCTTTC
PDE2A	ATGAGCTGCCACAGGAAGGA	ATTGCAACTCAGCCGCTTCT
PDE3A	TTCAGAATGGGACCACAAGAGA	TCACCCATCACAGCAATATCCA
PDE3B	GGGACTTGAAGCAGTGGTGAAA	AGCACTGAAAGATCAACTCCATTTT
PDE4D	CCTAACTAATTCATGTATCCCCAGGTT	GGCCCCACTTGTTCCACATCT
PDE5A	AATACCACCCCTGGAGCACC	TTCAAGGGCTCGCCAAAAGC
PDE8A	TCAGAGTGTGCAATGGCAAC	GTCCATCGAATGTTTCCTCC
AC5	CACCGCCAATGCCATAGAC	CTTCAGCGCCACCTTGGT
AC6	CTTCATCTGTTTTATCCAGCTCCTT	CGGCATAAATCCCGAGTATCA
AC8	GGATCTACATCCATCGCTATGAGA	CGTGGAGAGGTTGGTAAATCCT
HPRT1	GCTTGCTGGTGAAAAGGACCTCTCGAAG	CCCTGAAGTACTCATTATAGTCAAGGGCAT
RPL4	GCCGCTGGTGGTTGAAGATAA	CGTCGGTTTCTCATTTTGCCC
Genotyping Primers – Mouse		
Description	Forward Primer	Reverse Primer
AKAP12-OX	ACTTGCGTTTCTGATAGTTCTGA	CAGAGATGAGTTTCTGCTCGCTAG

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

Groups	Genotype	Sex	Age	Number (prior to experiment)	Number (after termination)	Littermates (Yes/No)	Other description
Group 1 (Sham; control for pump insertion and drug)	WT	M and F	8-12 Weeks	10	M: 10 F: 9	Yes	
Group 2 (Vehicle; control for drug only)	WT	M and F	8-12 Weeks	8	M: 6 F: 8	Yes	
Group 3 (ISO; experimental group)	WT	M and F	8-12 Weeks	10	M: 9 F: 8	Yes	
Group 4 (Sham; control for pump insertion and drug)	AKAP12 ^{OX}	M and F	8-12 Weeks	10	M: 7 F: 6	Yes	
Group 5 (Vehicle; control for drug only)	AKAP12 ^{OX}	M and F	8-12 Weeks	8	M: 4 F: 5	Yes	
Group 6 (ISO; experimental group)	AKAP12 ^{OX}	M and F	8-12 Weeks	10	M: 6 F: 9	Yes	

Sample Size: Please explain how the sample size was decided. Please provide details of any *prior* sample size calculation, if done.

For sample size calculations, we used power analysis to determine the minimum sample size required for statistical significance; providing high values for sample size (n=9) for physiological experiments.

Inclusion Criteria

The study included male and female mice of two genotypes (WT and AKAP12^{OX}), aged 8-12 weeks, with a healthy cardiac function before osmotic pump insertion.

Exclusion Criteria

DOI [to be added]

Mice that did not survive the 14-day study period or mice that survived but had a Heart Rate (HR) below 400 bpm or above 650 bpm were excluded from the analysis².

Randomization

Sample Randomization within Litter-Based Stratification; To achieve a balanced distribution of mice across experimental conditions, a sample randomization strategy was employed within the framework of litter-based stratification. Litters, defined as groups of mice born to the same mother during a single reproductive cycle served as the basis for stratification. The randomization process ensured that each experimental group included a representative yet random subset of mice from various litters. This approach was implemented to control potential genetic and environmental factors shared among littermates, contributing to the robustness and internal validity of the study design.

Blinding

Blinding procedures were implemented in the acquisition and analysis of echocardiography for mouse genotypes. Similarly, human samples remained blinded until both RT-qPCR and Western Blot analyses were finalized.

1. Davidson MM, Nesti C, Palenzuela L, Walker WF, Hernandez E, Protas L, et al. Novel cell lines derived from adult human ventricular cardiomyocytes. *J Mol Cell Cardiol.* 2005;39:133-147
2. Lindsey ML, Kassiri Z, Virag JAI, de Castro Bras LE, Scherrer-Crosbie M. Guidelines for measuring cardiac physiology in mice. *Am J Physiol Heart Circ Physiol.* 2018;314:H733-H752