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
Mouse	The Jackson Laboratory (Jax)	C57BL/6J	M/F	https://www.jax.org/strain/000664
Rat	Charles River Laboratories	Sprague Dawley	M/F	https://www.criver.com/products-services/find-model/sas-sprague-dawley-rat?region=3611

Genetically Modified Animals

	Species	Vendor or Source	Background Strain	Other Information	Persistent ID / URL
Parent - Male	Mouse	Jax	C57BL/6J	*Myh6* Cre	Strain #:005657
Parent - Male	Mouse	Jax	C57BL/6J		
Parent - Female	Mouse	University of Michigan Transgenic Core	C57BL/6J	*Robo1$^{fl/fl}$*	N/A
Parent - Male	Mouse	University of Michigan Transgenic Core	C57BL/6J	*Robo1$^{fl/fl}$*	N/A
Parent - Female	Mouse	Dr. Sean McLean, University of North Carolina	CD1	*Slit3$^{-/-}$*	N/A
Parent - Male	Mouse	Dr. Sean McLean, University of North Carolina	CD1	*Slit3$^{-/-}$*	N/A
Parent - Male	Mouse	Dr. Michelle Tallquist, University of Hawaii	C57BL/6J	*Tcf21*-MerCreMer	N/A
Parent - Male	Mouse	Dr. Sylvia Evans, UCSD	C57BL/6J	*Tbx18*-CreERT2	N/A
Parent – Male	Mouse	Jax	C57BL/6J	*Rosa26*-MerCreMer	
Parent – Male	Mouse	Dr. Sun-Kyeong Lee, University of Connecticut	C57BL/6J	*Slit3$^{fl/fl}$*	N/A
Parent - Male	mouse	The Jackson Laboratory	C56 BL/6J	Td Tomato	Stock No: 007909
Parent - Female	mouse	The Jackson Laboratory	C56 BL/6J	Td Tomato	Stock No: 007909
Parent - Male	mouse	The Jackson Laboratory	C56 BL/6J	R26-CreERT2	Stock No: 008463

DOI [to be added]

Parent - Female	mouse	The Jackson Laboratory	C56 BL/6J	R26-CreERT2	Stock No: 008463
Neonates-Female and male	mouse	Dr. Alexander Jaworski, Brown University	CD1	*Robo1^{-/-}*	N/A

Antibodies

Target antigen	Vendor or Source	Catalog #	Working concentration	Lot # (preferred but not required)	Persistent ID / URL
SLIT3	Thermo Fisher Scientific	PA5-104142	1:1000		PA5-104142
a-actinin	abcam	Ab68194	1:100		Ab68194
ROBO1	abcam	Ab256791	1:100		Ab256791
cTNT	abcam	Ab209813	1:100		Ab209813
PDGFRa	Cell Signaling Technology	3174	1:100		3174
Myh11	Thermofisher	MA5-11971	1:100		MA5-11971
NG2	Thermofisher	PA5-100235	1:100		PA5-100235
Goat anti-Mouse IgG2b Cross-Adsorbed Secondary Antibody, Alexa Fluor 488	ThermoFisher	A-21141	1:100		A-21141
Goat anti-Mouse IgG2b Cross-Adsorbed Secondary Antibody, Alexa Fluor 488	ThermoFisher	A-21141	1:100		A-21141
Goat anti-rabbit IgG (H+L), F(ab')2 Fragment (Alexa Fluor® 594 Conjugate)	cell signaling technology	8889	1:100		8889
goat anti-rabbit IgG (H+L), F(ab')2 Fragment (Alexa Fluor® 488 Conjugate)	cell signaling technology	4412	1:100		4412
Alexa Fluor® 488 WGA	ThermoFisher	W11261	1:100		W11261
SLIT3	Sigma Aldrich	SAB2109223	1:100		SAB2109223
tdTomato	abcam	EPR18992	1:100		EPR18992

DNA/cDNA Clones

Cultured Cells

Name	Vendor or Source	Sex (F, M, or unknown)	Persistent ID / URL
iCell iPS-CMs	FUJIFILM Cellular Dynamics, Inc.		R1105
Mouse cardiac fibroblasts	Isolated from C57BL/6J mice	F and M	
Primary human aortic vascular smooth muscle cells	Promocell GmbH		C-12533
Neonatal rat cardiomyocytes	Isolated from Sprague Dawley neonatal rats	F and M	

Other

Description	Source / Repository	Persistent ID / URL
Ad-GFP-h-SLIT3	Vector Biolabs	Cat. No: ADV-223657
Ad-Cre-GFP	Vector Biolabs	Cat. No: 1700
Pierce™ Primary Cell Isolation Kits	Invitrogen	Cat. No: 88280
siRNA human *SLIT3*	Origene Technologies, Inc.	
siRNA mouse *Robo1*	Origene Technologies, Inc.	
Ad-GFP	Vector Biolabs	Cat. No: 1060

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	Sex	Age	Number (prior to experiment)	Number (after first postop echo)	Littermates (Yes/No)	Other description
TAC Exp1- $Slit3^{fl/fl}$	M/F	8-10 weeks	N=13	N=8	Yes	N=4 surgical death N=1 inadequate TAC gradient
TAC Exp1-$Rosa26$-CreERT2;$Slit3^{fl/fl}$	M/F	8-10 weeks	N=12	N=8	Yes	N=3 surgical deaths N=1 inadequate gradient
TAC Exp2- $Slit3^{fl/fl}$	M/F	8-10 weeks	N=12	N=8	Yes	N=1 inadequate gradient N=3 surgical deaths
TAC Exp2-$Tcf21$-MerCreMer;$Slit3^{fl/fl}$	M/F	8-10 weeks	N=12	N=8	Yes	N=3 surgical deaths N=1 inadequate gradient
TAC Exp3- $Slit3^{fl/fl}$	M/F	8-10 weeks	N=11	N=8	Yes	N=1 surgical death N=2 inadequate TAC gradient
TAC Exp3-$Tbx18$-CreERT2;$Slit3^{fl/fl}$	M/F	8-10 weeks	N=10	N=8	Yes	N=1 inadequate gradient N=1 surgical death
Exp4- $Slit3^{+/+}$	M/F	7 days	N=3	N=3	Yes	Planned euthanasia
Exp4- $Slit3^{-/-}$	M/F	7 days	N=3	N=3	Yes	Planned euthanasia
Exp5- $Robo1^{+/+}$	M/F	7 days	N=3	N=3	Yes	Planned euthanasia
Exp5- $Robo1^{-/-}$	M/F	7 days	N=3	N=3	Yes	Planned euthanasia
TAC Exp6-$Robo1^{fl/fl}$	M/F	8-10 weeks	N=10	N=8	Yes	N=1 inadequate TAC gradient N=1 surgical death
TAC Exp6-$Myh6$-MerCreMer;$Robo1^{fl/fl}$	M/F	8-10 weeks	N=9	N=8	Yes	N=1 surgical death
Sham Exp1- $Slit3^{fl/fl}$	M/F	8-10 weeks	N=8	N=8	Yes	
Sham Exp1-$Rosa26$-CreERT2;$Slit3^{fl/fl}$	M/F	8-10 weeks	N=8	N=8	Yes	
Sham Exp2- $Slit3^{fl/fl}$	M/F	8-10 weeks	N=8	N=8	Yes	
Sham Exp2-$Tcf21$-MerCreMer;$Slit3^{fl/fl}$	M/F	8-10 weeks	N=8	N=8	Yes	
Sham Exp3- $Slit3^{fl/fl}$	M/F	8-10 weeks	N=8	N=8	Yes	

DOI [to be added]

Sham Exp3-*Tbx18-*CreERT2;*Slit3*$^{fl/fl}$	M/F	8-10 weeks	N=8	N=8	Yes	
Sham Exp6-*Robo1*$^{fl/fl}$	M/F	8-10 weeks	N=8	N=8	Yes	
Sham Exp6-*Myh6-*MerCreMer;*Robo1*$^{fl/fl}$	M/F	8-10 weeks	N=8	N=8	Yes	

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

We performed a power analysis to determine the appropriate number of animals in each TAC group. Given our historical measurements (mean±SD) of these outcomes on control animals, our intention to detect a 20% difference between control and experimental groups, a probability of Type I error (◌) of 0.05, and the risk of Type II error (◌) of 0.2, we have determined the minimum number animals needed for each experiment: n=8 mice/group for HW/BW ratio measurement. Initial group size was at least 11 animals to account for attrition or dropout rate from surgical mortality. Approximately 20% of animals were excluded from the study because of TAC procedure mortality or inadequate TAC gradients.

Inclusion Criteria – healthy animals to be considered for surgery. No signs of preoperative distress. Post surgery – no signs of distress, adequate TAC gradient.

Exclusion Criteria – TAC gradient <25 mmHg. Signs of distress. Death from surgery.

Randomization – Animals were randomized to TAC or Sham procedures by alternating to each group.

Blinding – Echocardiograms were performed by a technician who was blinded to the genotype. Cell size and cardiomyocyte cross-sectional area was measured from coded digital images that lacked genotype or group assignment identifiers.