

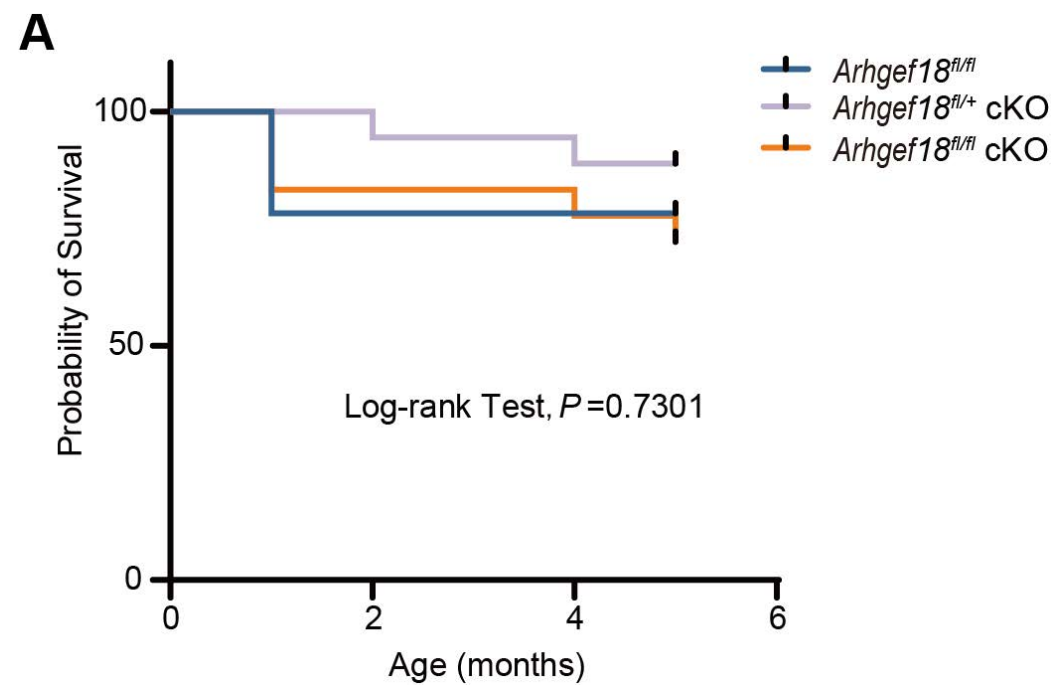


Fig. S1. Kaplan–Meier survival curves. (A) With no statistically significant difference in survival curves among $Arhgef18^{fl/fl}$ ($n=23$), $Arhgef18^{fl/+}$ cKO ($n=18$), and $Arhgef18^{fl/fl}$ cKO ($n=18$) mice according to the log-rank test.

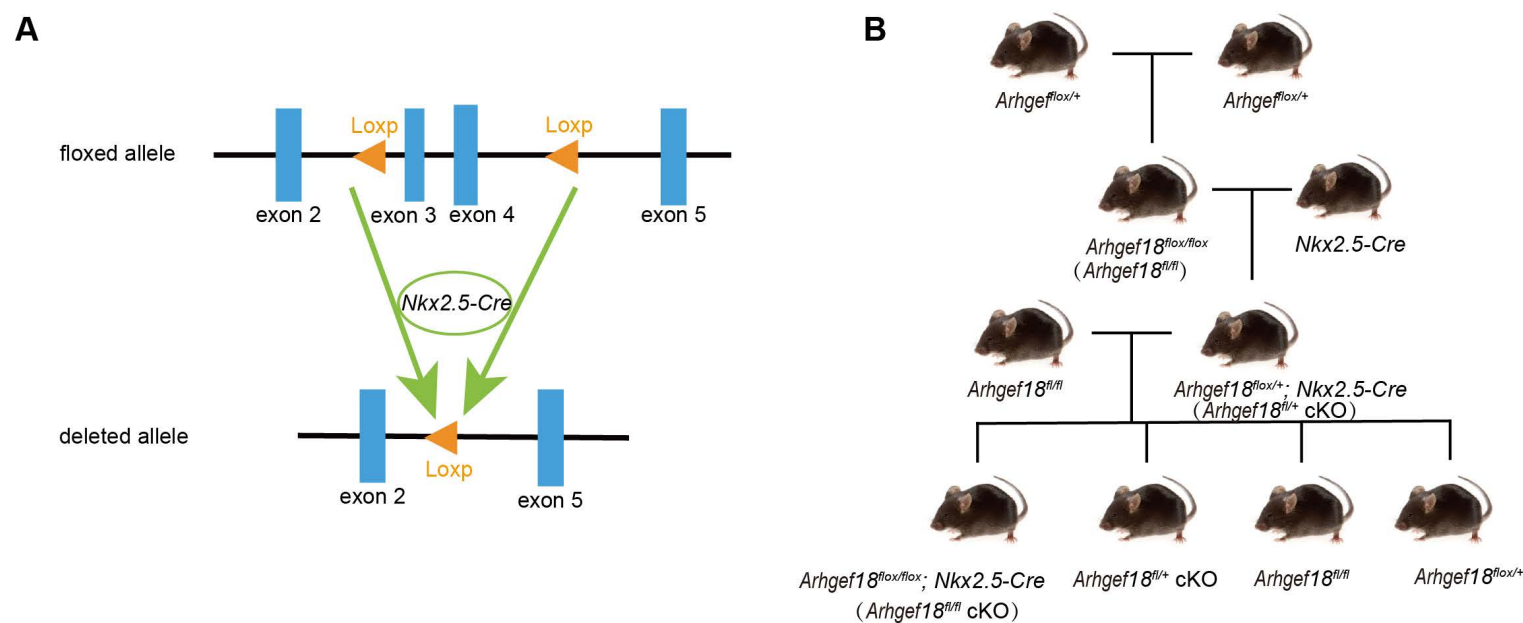


Fig. S2. Construction and breeding of *Arhgef18* cKO mice. Diagram of the construction strategy for *arhGEF18* cKO mice. (B) Flowchart for breeding *Arhgef18* cKO mice.

Table S1. Cardiac function measurements using echocardiography.

Parameters	2 W				4 W			12 W			24 W	
	<i>Arhgef18^{fl/fl}</i>	<i>Arhgef18^{fl/+}</i>	<i>Arhgef18^{fl/fl}</i>	<i>Arhgef18^{fl/fl}</i>	<i>Arhgef18^{fl/+}</i>	<i>Arhgef18^{fl/fl}</i>	<i>Arhgef18^{fl/fl}</i>	<i>Arhgef18^{fl/+}</i>	<i>Arhgef18^{fl/fl}</i>	<i>Arhgef18^{fl/fl}</i>	<i>Arhgef18^{fl/+}</i>	<i>Arhgef18^{fl/fl}</i>
		cKO	cKO		cKO	cKO		cKO	cKO		cKO	cKO
Heart rate (bpm)	493.7±15.19	504.1±8.99	501.4±9.24	498.2±16.96	515±12.26	498.6±13.93	507.2±5.30	515.5±5.21	508.8±3.38	508.1±13.48	497.1±14.64	504.8±11.76
LV end-diastolic												
LVAW (mm)	0.75±0.15	0.80±0.11	0.70±0.19	0.85±0.11	0.77±0.17	0.68±0.14	1.09±0.15	1.04±0.34	0.81±0.12	1.08±0.10	1.08±0.13	0.92±0.17
LVID (mm)	2.44±0.33	2.19±0.12	2.35±0.39	3.05±0.41	3.17±0.23	3.11±0.24	3.48±0.46	3.52±0.09	3.80±0.54	3.21±0.44	3.25±0.27	3.74±0.50
LVPW (mm)	0.67±0.11	0.67±0.16	0.60±0.11	0.68±0.14	0.69±0.05	0.66±0.04	0.78±0.11	0.76±0.20	0.77±0.04	0.94±0.15	0.95±0.28	0.86±0.20
LV volume (μl)	21.57±7.00	16.13±2.23	20.01±8.84	37.33±11.52	40.43±6.81	38.68±6.99	51.24±16.89	51.56±3.39	63.62±20.28	51.24±14.20	51.56±8.14	63.62±20.37
LV end-systolic												
LVAW (mm)	1.25±0.22	1.27±0.11	1.17±0.17	1.41±0.15	1.32±0.21	1.09±0.24*	1.80±0.25	1.71±0.30	1.38±0.10*	1.62±0.19	1.56±0.14	1.30±0.26*
LVID (mm)	1.11±0.26	1.09±0.11	1.18±0.36	1.65±0.23	1.80±0.14	1.97±0.15**	1.84±0.41	1.97±0.18	2.49±0.44*	2.02±0.33	2.16±0.33	2.94±0.61***†
LVPW (mm)	1.16±0.12	1.10±0.16	0.98±0.17	1.08±0.15	1.14±0.11	1.05±0.10	1.39±0.19	1.26±0.26	1.15±0.09	1.34±0.19	1.28±0.25	1.09±0.15
LV volume (μl)	3.02±1.69	2.64±0.67	3.84±3.56	8.00±2.69	9.84±1.91	12.42±2.32*	11.13±6.82	12.38±2.83	23.07±10.18*	13.62±5.24	16.04±5.84	35.27±19.46**†
LVEF (%)	86.86±4.77	83.73±3.00	83.3±6.63	78.65±2.22	75.73±1.48	67.54±4.91***††	79.52±5.46	76.06±4.81	64.31±6.94***†	67.57±9.41	63.33±8.74	44.62±11.08***†
LVFS (%)	54.97±5.89	50.45±3.88	50.58±6.54	45.95±2.25	43.26±1.28	36.51±4.01***††	47.53±5.19	44.1±4.62	34.67±4.95***†	37.02±7.58	33.74±6.50	21.87±6.04***†
LV mass (mg)	46.85±18.94	40.85±8.97	39.62±18.79	72.27±20.76	72.81±19.64	61.25±12.51	118.7±29.04	112.6±11.80	106.7±25.73	118.3±36.37	119.4±29.85	122.8±25.35
LV mass Corrected (mg)	37.48±15.15	32.68±7.18	31.69±15.03	57.82±16.61	58.25±15.71	49.00±10.01	94.95±13.23	90.11±9.44	85.36±20.59	94.67±29.10	95.55±23.88	98.26±20.28

Values are expressed as mean ± s.d. from at least 5-8 mice per group. Statistical significance was determined by ANOVA.

P*<0.05 *P*<0.01 ****P*<0.001 compared to *Arhgef18^{fl/fl}*, †*P*<0.05 ††*P*<0.01 †††*P*<0.001 compared to *Arhgef18^{fl/+}* cKO.

Table S2. Primer sequences of PCR.

Gene	Forward primer (5'-3')	Reverse primer (5'-3')
<i>Arhgef18-Loxp</i>	GCTTCAGACTCACATAGCACTGAG	TAGCTAGGTAGCACAAGCCTCA
<i>Nkx2.5-Cre</i>	GCCTCCGCCAACAGCAA	CCCAGATCAGATCCCATACAATG

Table S3. Primer sequences of q-PCR.

Gene	Forward primer (5'-3')	Reverse primer (5'-3')
<i>Arhgef18</i>	TCTTAGTCTCCAGGCGGTCA	CTCCTCACGCTGCTTCTCAA
<i>vinculin</i>	CATGGTGATGGATGGCCAAGG	GTTCAAGGTCTGGGTGGAGGA
<i>αβ-tubulin</i>	TGTTCGTAGACCTGGAACCC	CCTTGCCAATGGTGTAGTGGG
<i>α-actinin</i>	GGGTTACTCCTGTGGTGTC	CTCTCTGCCACATCAAAGGC
<i>cTNT</i>	CGACCACCTGAATGAAGACCA	TCCCAGGAGTTTTGGAGACTT
<i>Pard3</i>	TCTACTGGGCAAAGCCAACC	ATCCTGCGTTCTCGGTCATC
<i>Scrib</i>	GCGAGTGTGGAGGGAAAGAA	ATGACGCTGACGGAGGGATA
<i>18S</i>	CGCCGCTAGAGGTGAAATTCT	CGCCGCTAGAGGTGAAATTCT
<i>Nppa</i>	GCTTCCAGGCCATATTGGAG	GGGGGCATGACCTCATCTT
<i>Nppb</i>	GAGGTCACTCCTATCCTCTGG	GCCATTCCTCCGACTTTTCTC

Table S4. Antibodies used for western blots and immunofluorescence.

Protein	Product Number	Company	Country	WB	IF
Arhgef18	GTX102223	GenTex	USA	1:500	-
vinculin	v9131	Sigma	Germany	1:1000	1:800
αβ-tubulin	2148	Cell signaling	USA	1:1000	1:50
α-actinin	6487	Cell signaling	USA	1:1000	1:200
cTNT	ab209813	Abcam	USA	1:1000	1:1000
SCRIB	27083-1-AP	Proteintech	China	1:1000	-
CRB2	ab156286	Abcam	USA	1:1000	-
hSP90	TA500494	OriGene	China	1:2000	-
Goat anti-rabbit IgG (H+L)	SA00013-2	Proteintech	China	1:5000	-
Goat anti-mouse IgG (H+L)	SA00001-1	Proteintech	China	1:5000	-
555 HRP-goat anti-rabbit IgG	Y6088L	UELandy	China	-	1:200
488 HRP-goat anti-mouse IgG	Y6104L	UELandy	China	-	1:200