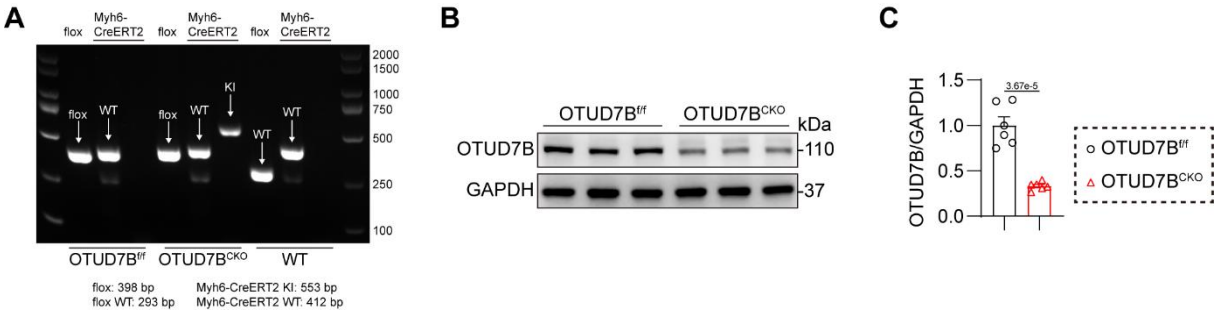
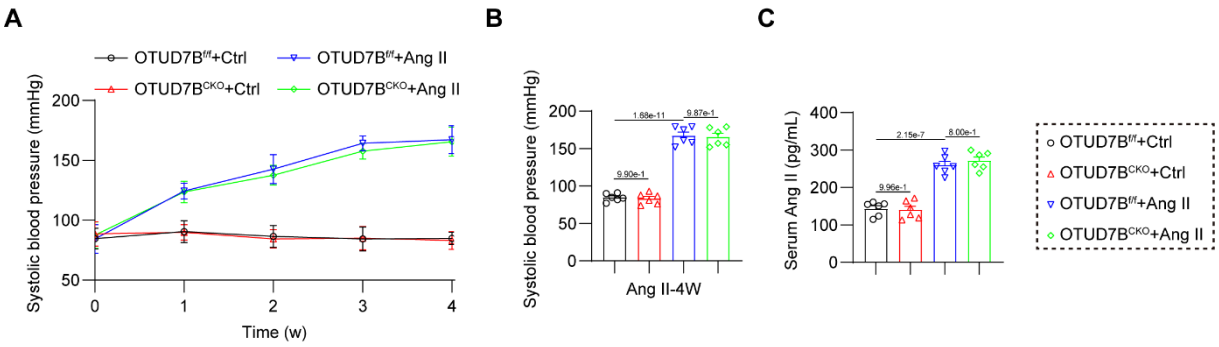
Supplemental Material

Cardiomyocyte-derived OTUD7B promotes cardiac hypertrophy by deubiquitinating SERCA2a

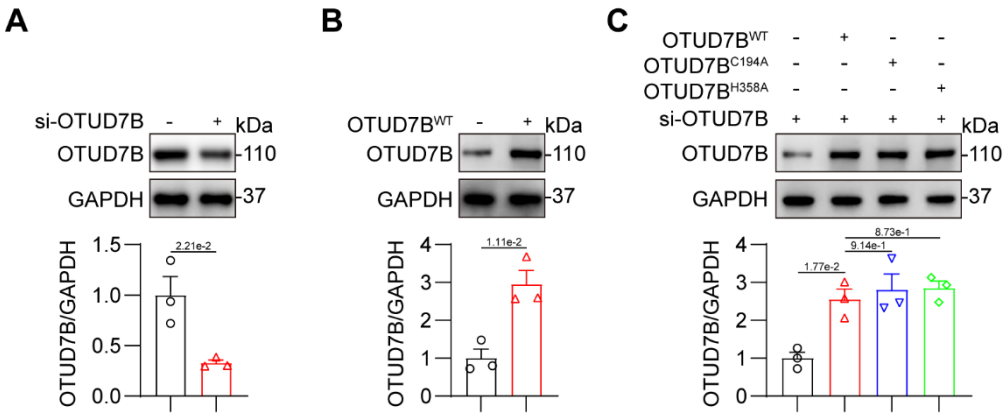
The Supplemental Material includes 10 figures and 6 tables.



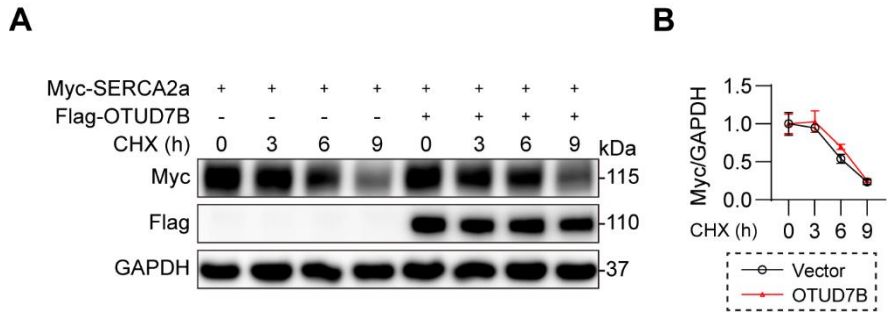
Supplementary Figure S1. Generation of cardiomyocyte-specific OTUD7B knockout mice (OTUD7B^{CKO}). (A) To identify the genotypes of OTUD7B$^{f/f}$ and OTUD7B^{CKO} mice, cDNA was extracted from mouse tail and the primers for identification of OTUD7B$^{f/f}$ and OTUD7B^{CKO} mice were used for PCR assay. (B-C) Western blotting detection (B) and quantitative analysis (C) of OTUD7B in heart tissues of OTUD7B$^{f/f}$ mice and OTUD7B^{CKO} mice. GAPDH served as the internal loading reference (n = 6). OTUD7B$^{f/f}$ mice and OTUD7B^{CKO} mice were intraperitoneally injected with tamoxifen for 1 week. Another week later, mice were euthanized and samples were harvested.



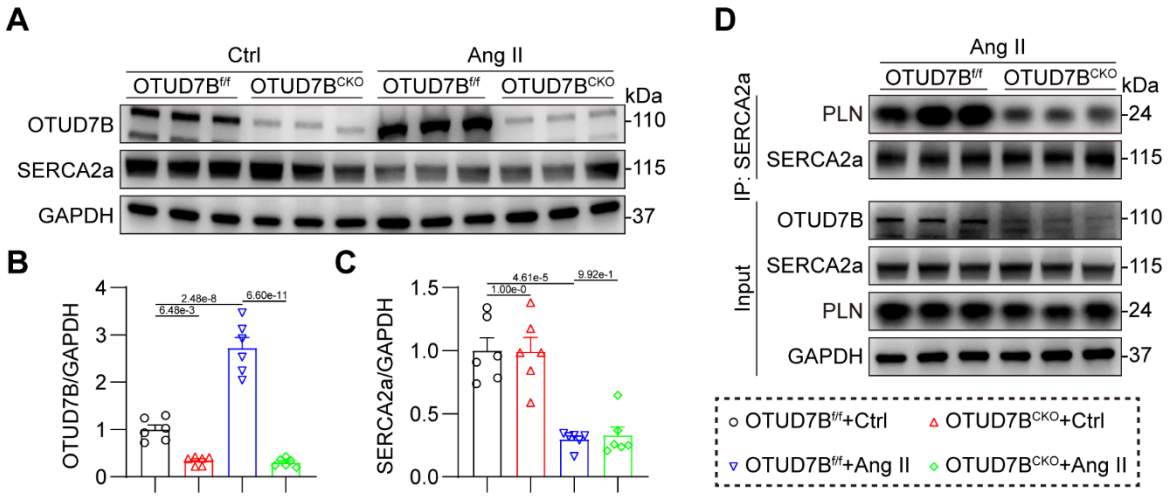
Supplementary Figure S2. Cardiomyocyte-specific OTUD7B deletion did not affect systolic blood pressure and Ang II level in Ang II-induced mice. (A) Systolic blood pressure was recorded during the Ang II-challenged mouse experiments (n = 6). (B) Systolic blood pressure of mice in the fourth week of Ang II treatment (n = 6). (C) Serum Ang II level was analyzed using ELISA (n = 6).



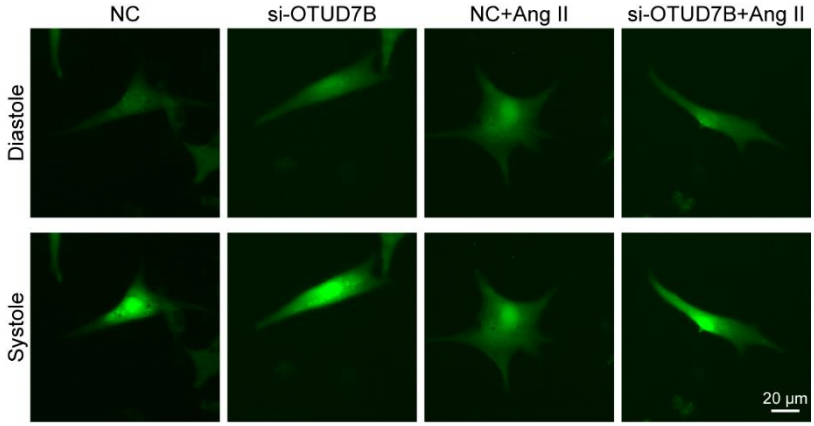
Supplementary Figure S3. Effects of OTUD7B knockdown or overexpression in NRCMs. (A) NRCMs were exposed to OTUD7B-targeting siRNA (si-OTUD7B) for 24 hours, while control cells received a non-targeting siRNA (NC). OTUD7B protein expression was assessed by Western blotting (n = 3). (B) NRCMs were exposed to $OTUD7B^{WT}$ or empty vector (EV) for 24h. OTUD7B protein expression was assessed by Western blotting (n = 3). (C) NRCMs were exposed to si-OTUD7B, $OTUD7B^{WT}$, $OTUD7B^{C194A}$ or $OTUD7B^{H358A}$ for 24h. OTUD7B protein expression was assessed by Western blotting (n = 3).



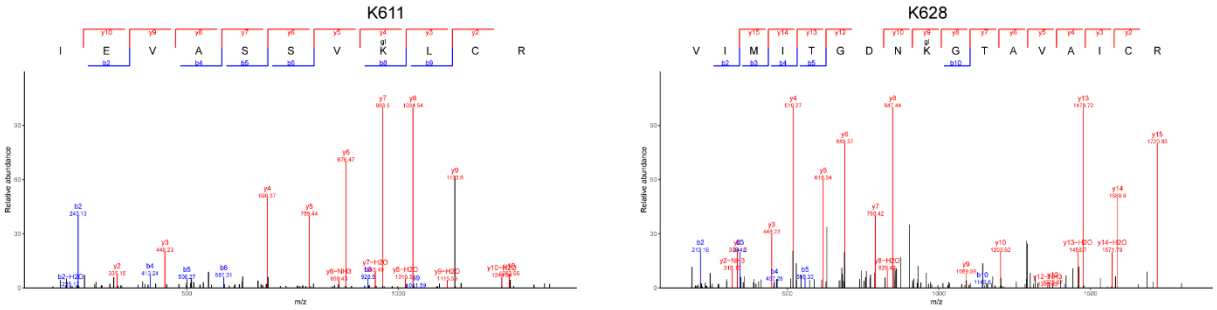
Supplementary Figure S4. OTUD7B does not affect the protein stability of SERCA2a. (A-B) Western blotting detection (A) and quantitative analysis (B) of Myc. 293T cells transfected with Flag-OTUD7B and Myc-SERCA2a were treated with cycloheximide (CHX, 50ug/ml) at the indicated time points. GAPDH was used as the loading control (n = 3).



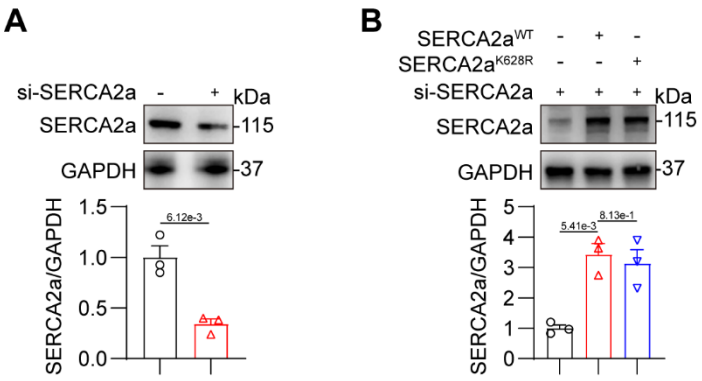
Supplementary Figure S5. Cardiomyocyte-specific OTUD7B deletion inhibits the binding of SERCA2a and PLN in Ang II-induced cardiac hypertrophy. (A-C) Western blotting detection (A) and quantitative analysis (B-C) of OTUD7B and SERCA2a in heart tissues (n = 6). (D) Co-immunoprecipitation of SERCA2a and PLN in heart tissues. SERCA2a was immunoprecipitated by anti-SERCA2a antibody.



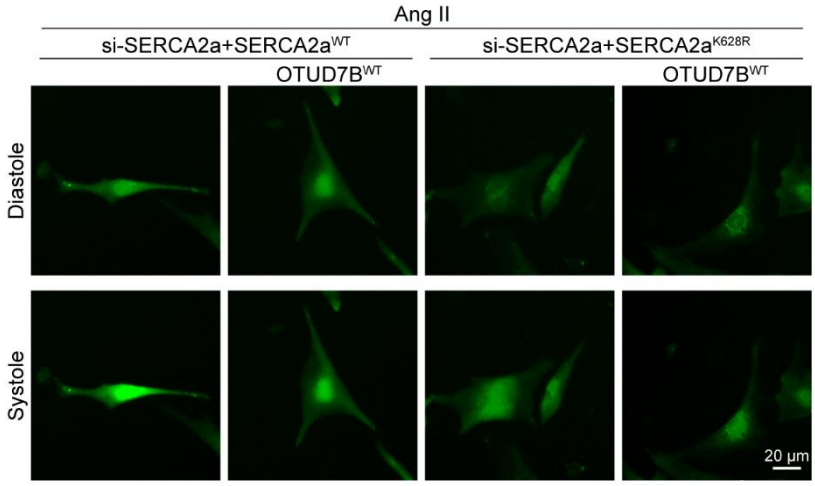
Supplementary Figure S6. OTUD7B regulates sarcoplasmic reticulum (SR) calcium content in cardiomyocytes. Representative confocal images of cytosolic Ca^{2+} determined by the Fluo-4 AM fluorescence signal in NRCMs transfected with si-OTUD7B or NC and then challenged with Ang II for 24h.



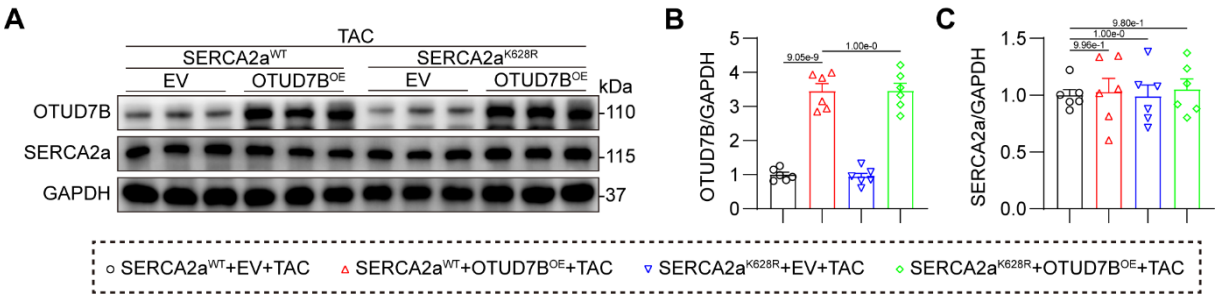
Supplementary Figure S7. Second-order spectrums of the peptide of SERCA2a at K611 and K628.



Supplementary Figure S8. Effects of SERCA2a knockdown or overexpression in NRCMs. (A) NRCMs were exposed to SERCA2a siRNA (si-SERCA2a) for 24h, while control cells received NC siRNA. SERCA2a protein expression was assessed by Western blotting (n = 3). (B) NRCMs were exposed to si-SERCA2a, $SERCA2a^{WT}$ or Myc-$SERCA2a^{K628R}$ for 24h. SERCA2a protein expression was assessed by Western blotting (n = 3).



Supplementary Figure S9. OTUD7B regulates sarcoplasmic reticulum (SR) calcium content in cardiomyocytes by regulating SERCA2a deubiquitination at residue K628. Representative confocal images of cytosolic Ca^{2+} determined by the Fluo-4 AM fluorescence signal in NRCMs transfected with si-SERCA2a, OTUD7B^{WT}, and SERCA2a^{WT} or SERCA2a^{K628R} and then challenged with Ang II for 24 h.



Supplementary Figure S10. Effects of OTUD7B and SERCA2a overexpression in vivo. (A-C) Western blotting detection (A) and quantitative analysis (B-C) of OTUD7B and SERCA2a in heart tissues (n = 6).

Supplementary Table S1. Clinical measurements for the human specimens

Type	Age (years)	Sex	IVS,mm	EF%
NCH-1	66	Male	11	68
NCH-2	48	Male	10	71
NCH-3	53	Female	9	64
NCH-4	81	Male	9	60
NCH-5	52	Male	10	67
NCH-6	76	Male	10	64
CH-1	46	Male	15	50
CH-2	77	Female	16	40
CH-3	73	Female	15	42
CH-4	80	Male	18	32
CH-5	57	Male	17	42
CH-6	55	Male	16	49

NCH, non-cardiac hypertrophy; CH, cardiac hypertrophy; IVS, interventricular septal thickness; EF, ejection fraction.

Supplementary Table S2. Primer sequences for PCR genotyping analysis

Gene	Species	Sequence
Otud7b-flox	Mouse	GTAGCTCACAGTTTCAGCCTGGATATT
		CCCAGCCTGTATGACTCTTTATATCTTC
Myh6-CreERT2 WT	Mouse	CAGCAAAACCTGGCTGTGGATC
		ATGAGCCACCATGTGGGTGTC
Myh6-CreERT2 KI	Mouse	GGCACATGAGTAACAAAGGCATG
		AGCCAACCTTTGTTCATGGCAG

Supplementary Table S3. Primer sequences for real-time qPCR assay

Gene	Species	Sequence
Myh7	Mouse	ACTGTCAACACTAAGAGGGTCA
		TTGGATGATTTGATCTTCCAGGG
Nppa	Mouse	GCTTCCAGGCCATATTGGAG
		GGGGGCATGACCTCATCTT
Nppb	Mouse	GAGGTCACTCCTATCCTCTGG
		GCCATTTCCTCCGACTTTTCTC
Actb	Mouse	GGCTGTATTCCCCTCCATCG
		CCAGTTGGTAACAATGCCATGT
Otud7b	Mouse	TGCTGTCCTGTCGGATTTTGT
		TGGACTTGACGCAACTGTTCA
Myh7	Rat	CTGAGGAGACACAGCGTTCT
		GGGTCAGCTGAGAGATAAGA
Nppa	Rat	GAGCGAGCAGACCGATGAAGC
		TCCATCTCTCTGAGACGGGTTGAC
Nppb	Rat	AGTCTCCAGAACAATCCACGATGC
		GCCTTGGTCCTTTGAGAGCTGTC
Actb	Rat	ACTATCGGCAATGAGCGGTTCC
		TGGCATAGAGGTCTTTACGGATGTC
Myh7	Human	GGAGTTCACACGCCTCAAAGAG
		TCCTCAGCATCTGCCAGGTTGT
Nppa	Human	CACCGTGAGCTTCCTCCTTT
		CCAAATGGTCCAGCAAATTCTTG
Actb	Human	CATGTACGTTGCTATCCAGGC
		CTCCTTAATGTCACGCACGAT
Otud7b	Human	GTCAGATTTTGTCCGTTCCACA
		CATGGACTTGACGTAGCTGTT

Supplementary Table S4. Echocardiographic data in all groups

	Ctrl		Ang II	
	$OTUD7B^{f/f}$	$OTUD7B^{CKO}$	$OTUD7B^{f/f}$	$OTUD7B^{CKO}$
EF (%)	72.96±0.66	$71.28±1.39^{ns}$	$58.6±1.27^{*}$	$65.83±1.5^{\#}$
FS (%)	39.34±0.68	$37.81±1.38^{ns}$	$27.64±0.84^{*}$	$33.12±1.31^{\#}$
IVSd (mm)	0.81±0.04	$0.83±0.04^{ns}$	$1.08±0.06^{*}$	$0.88±0.03^{\#}$
LVIDd (mm)	3.49±0.06	$3.49±0.1^{ns}$	$4.03±0.2^{ns}$	$3.64±0.2^{NS}$
LVPWd (mm)	0.93±0.06	$1.0±0.06^{ns}$	$1.0±0.16^{ns}$	$1.03±0.07^{NS}$
E/A	1.82±0.06	$1.74±0.06^{ns}$	$1.22±0.04^{*}$	$1.5±0.06^{\#}$
HR (bpm)	482±7.24	$479.5±6.2^{ns}$	$473.5±5.82^{ns}$	$484.3±3.02^{NS}$
HW/BW (mg/g)	4.49±0.08	$4.51±0.06^{ns}$	$6.26±0.39^{*}$	$5.15±0.2^{\#}$
HW/TL (mg/mm)	7.04±0.14	$6.74±0.21^{ns}$	$8.67±0.38^{*}$	$7.36±0.25^{\#}$

EF, ejection fraction; FS, fractional shortening; IVSd, diastole interventricular septum; LVIDd, diastole left ventricular internal diameter; LVPWd, diastole left ventricular posterior wall; E/A, E peak/A peak; HR, heart rate; HW, heart weight; BW, body weight; TL, tibial length. Data were shown as mean ± SEM. ns, $p>0.05$ vs $OTUD7B^{f/f}$ + Ctrl; *, $p<0.05$ vs $OTUD7B^{f/f}$ + Ctrl. NS, $p>0.05$ vs $OTUD7B^{f/f}$ + Ang II; #, $p<0.05$ vs $OTUD7B^{f/f}$ + Ang II.

Supplementary Table S5. Echocardiographic data in all groups

	Sham		TAC	
	$OTUD7B^{f/f}$	$OTUD7B^{CKO}$	$OTUD7B^{f/f}$	$OTUD7B^{CKO}$
EF (%)	76.4±1.94	$75.01±2.21^{ns}$	$56.32±2.36^{*}$	$67.62±2.66^{\#}$
FS (%)	39.59±1.7	$38.44±1.85^{ns}$	$25.22±1.39^{*}$	$32.69±1.94^{\#}$
IVSd (mm)	0.77±0.02	$0.76±0.04^{ns}$	$1.02±0.04^{*}$	$0.78±0.04^{\#}$
LVIDd (mm)	3.93±0.06	$4.01±0.09^{ns}$	$4.25±0.1^{ns}$	$4.1±0.14^{NS}$
LVPWd (mm)	0.85±0.06	$0.9±0.08^{ns}$	$1.0±0.11^{ns}$	$0.96±0.07^{NS}$
E/A	1.65±0.05	$1.7±0.08^{ns}$	$1.03±0.06^{*}$	$1.41±0.06^{\#}$
HR (bpm)	468.5±6.01	$477±4.31^{ns}$	$485.8±10.41^{ns}$	$479.5±5.7^{NS}$
HW/BW (mg/g)	4.43±0.05	$4.3±0.04^{ns}$	$6.3±0.25^{*}$	$5.15±0.26^{\#}$
HW/TL (mg/mm)	6.5±0.2	$6.61±0.19^{ns}$	$8.75±0.21^{*}$	$7.34±0.29^{\#}$

EF, ejection fraction; FS, fractional shortening; IVSd, diastole interventricular septum; LVIDd, diastole left ventricular internal diameter; LVPWd, diastole left ventricular posterior wall; E/A, E peak/A peak; HR, heart rate; HW, heart weight; BW, body weight; TL, tibial length; TAC, transverse aortic constriction. Data were shown as mean ± SEM. ns, $p>0.05$ vs $OTUD7B^{f/f}$ + Sham; *, $p<0.05$ vs $OTUD7B^{f/f}$ + Sham. NS, $p>0.05$ vs $OTUD7B^{f/f}$ + TAC; #, $p<0.05$ vs $OTUD7B^{f/f}$ + TAC.

Supplementary Table S6. Echocardiographic data in all groups

	TAC			
	$SERCA2a^{WT}$+ EV	$SERCA2a^{WT}$+ $OTUD7B^{OE}$	$SERCA2a^{K628R}$+ EV	$SERCA2a^{K628R}$+ $OTUD7B^{OE}$
EF (%)	75.84±2.16	64.82±2.45*	65.38±1.82	66.37±2.36NS
FS (%)	43.07±2.64	32.45±1.85*	32.8±1.6	33.98±1.57NS
IVSd (mm)	0.93±0.04	1.17±0.06*	1.11±0.03	1.08±0.03NS
LVIDd (mm)	3.82±0.1	4.06±0.13ns	4.02±0.04	3.99±0.08NS
LVPWd (mm)	1.08±0.07	1.25±0.07ns	1.27±0.06	1.36±0.09NS
HR (bpm)	462.8±8.24	482.5±4.25ns	470.2±18.8	457.8±13.14NS
HW/BW (mg/g)	5.62±0.14	6.78±0.33*	6.73±0.14	6.98±0.29NS
HW/TL (mg/mm)	7.71±0.16	9.33±0.4*	9.09±0.31	8.99±0.33NS

EF, ejection fraction; FS, fractional shortening; IVSd, diastole interventricular septum; LVIDd, diastole left ventricular internal diameter; LVPWd, diastole left ventricular posterior wall; HR, heart rate; HW, heart weight; BW, body weight; TL, tibial length; EV, empty vector; TAC, transverse aortic constriction. Data were shown as mean ± SEM. ns, $p>0.05$ vs $SERCA2a^{WT}$+EV+TAC; *, $p<0.05$ vs $SERCA2a^{WT}$+EV+TAC. NS, $p>0.05$ vs $SERCA2a^{K628R}$+EV+TAC.