

Supplementary information

**The mechanosensitive Piezo1 channel mediates heart
mechano-chemo transduction**

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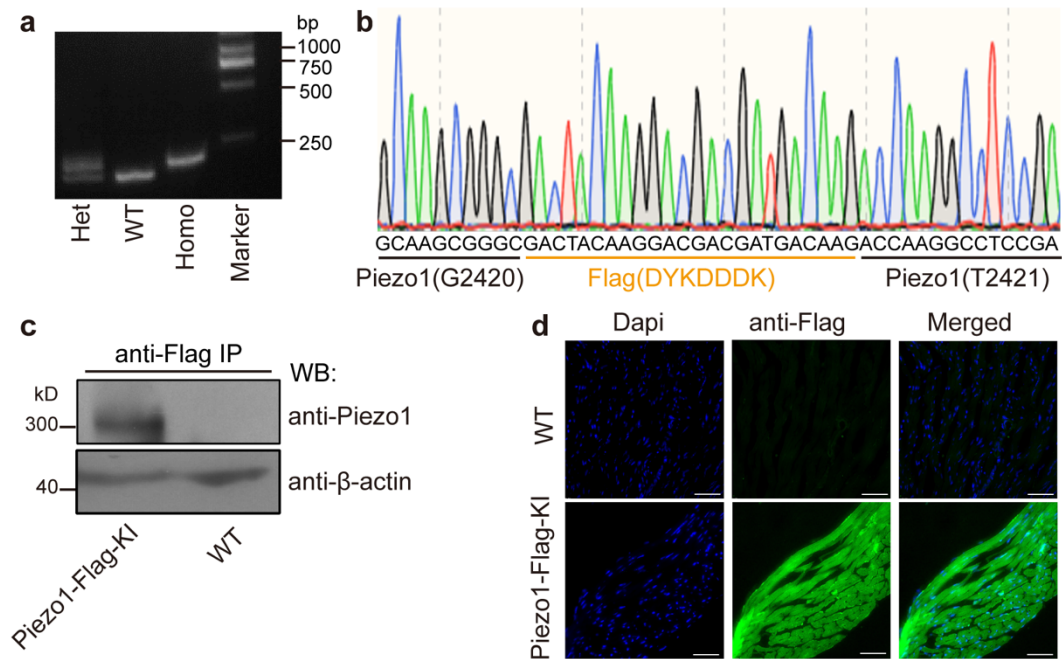
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Supplementary Figure 1 Generation and characterization of the Piezo1-Flag-KI mice

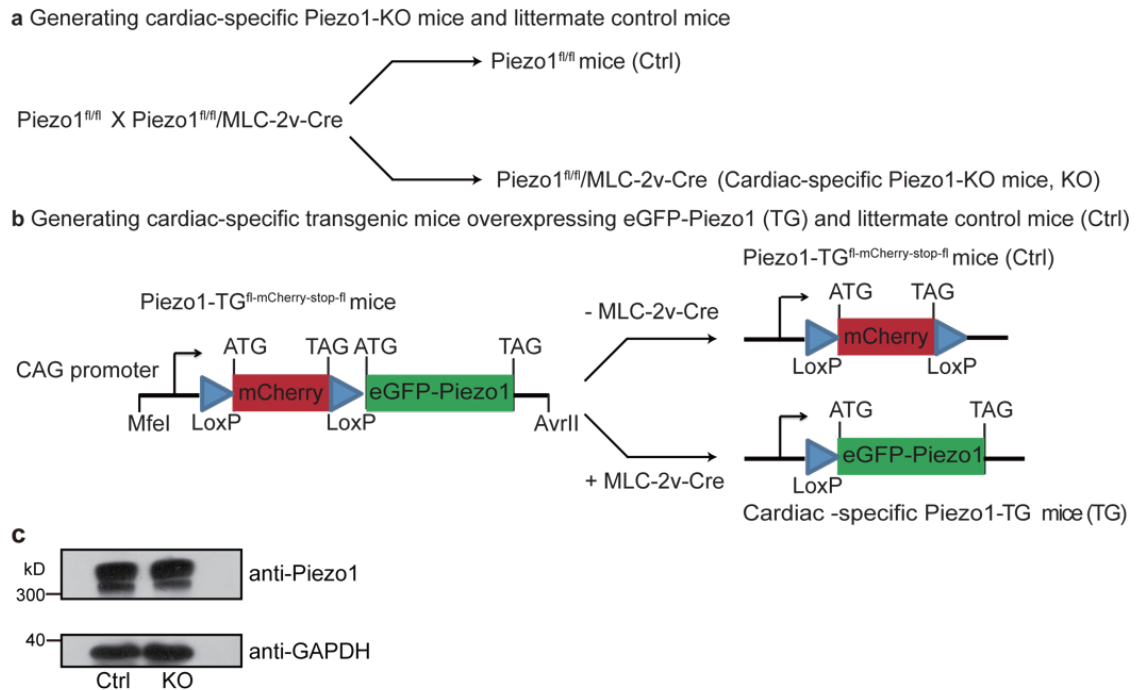
a, Genotyping results of the heterozygous (Het) or homozygous (Homo) Piezo1-Flag-KI mice and their wild-type (WT) littermate control mice.

b, DNA sequencing verification of the insertion of the Flag-coding sequence after the genetic sequence coding the residue G2420 of mouse Piezo1.

c, Representative western blotting result of anti-Flag-immunoprecipitated Piezo1-Flag proteins from the Piezo1-Flag-KI and WT littermate control heart homogenates using the anti-Piezo1 antibody. The β -actin level was used for loading control.

d, Representative images showing Dapi staining or immunostaining of heart tissues derived from WT and Piezo1-Flag-KI mice using the anti-Flag antibody. Scale bar, 100 μ m.

Each experiment was repeated independently three times with similar results.

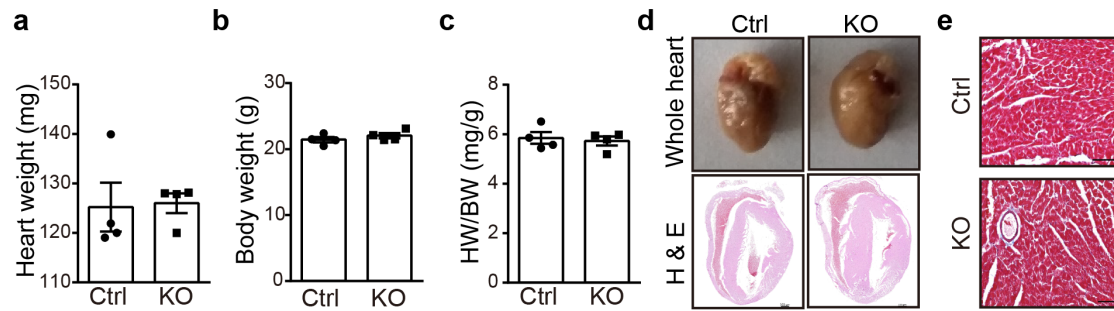


Supplementary Figure 2 Generation of cardiac-specific Piezo1-KO mice and Piezo1-transgenic (Piezo1-TG) mice overexpressing the eGFP-Piezo1 fusion protein

a, Breeding strategy for generating the Piezo1^{fl/fl} littermate control mice and the cardiac-specific Piezo1-KO mice using the MLC-2v-Cre mice.

b, Breeding strategy for generating the Piezo1-TG^{fl-mCherry-stop-fl} littermate control mice (Ctrl) and the cardiac-specific Piezo1-TG mice using the MLC-2v-Cre mice. The Piezo1-TG^{fl-mCherry-stop-fl} mice allow tissue-specific Cre-dependent expression of eGFP-Piezo1. In the absence of Cre recombinase, the upstream floxed mCherry coding sequence with a TAG stop codon blocks the translation of the downstream eGFP-Piezo1 fusion protein, resulting in the Piezo1-TG^{fl-mCherry-stop-fl} littermate control mice that express mCherry. When TG^{flox-mCherry} mice are crossed with the cardiac-specific MLC-2v-Cre line, the mCherry sequence is cleaved, leading to generation of the cardiac-specific Piezo1-TG mice that express eGFP-Piezo1 in cardiomyocytes.

c, Representative western blotting result of red blood cells from the KO and Ctrl littermate using the anti-Piezo1 antibody. The GAPDH level was used for loading control. Similar results were obtained from 3 independent experiments.

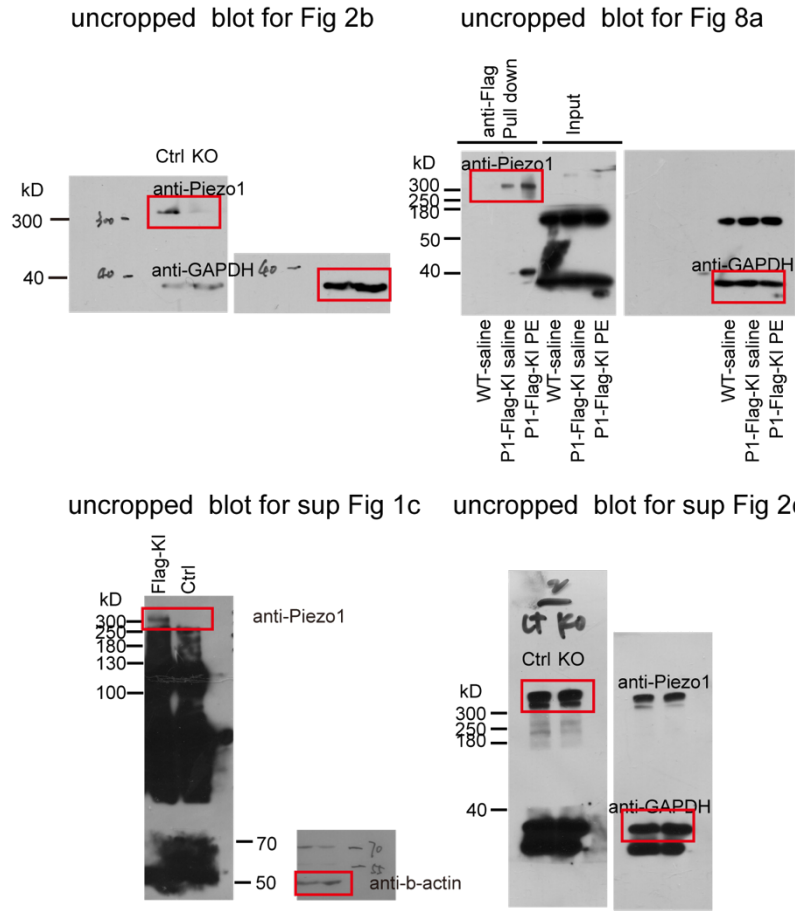


Supplementary Figure 3 8-week-old Piezo1-KO mice show normal heart structure and function

a-c, Scatter plot of heart weight (**a**), body weight (**b**), and HW/BW ratio (**c**) of 8-week old (n=4 mice for each group) littermate Ctrl and Piezo1-KO male mice. Unpaired student's t-test. Values are mean \pm SEM.

d, Histologic analysis of whole hearts or H & E stained longitudinal heart sections derived from 8-week old littermate Ctrl and Piezo1-KO male mice. Each experiment was repeated independently three times with similar results.

e, Histologic analysis of the left ventricles of 8-week old littermate Ctrl and Piezo1-KO hearts sectioned longitudinally and subjected to Masson's trichrome staining. Each experiment was repeated independently three times with similar results.



Supplementary Figure 4 Uncropped western blots

Red boxes in the uncropped blots indicate the cropped regions shown in the corresponding figures.

a, Echocardiographic analysis of 8-week old Piezo1-KO and littermate control mice (Ctrl)

	A'(mm/s)	E'(mm/s)	A (mm/s)	E (mm/s)	E'/A'	MVE/A	LVAW;d	LVAW;s	LVID;d	LVID;s	LVPW;d	LVPW;s	EF	FS	LV Mass AW	LV Vol;d	LV Vol;s
Ctrl (n=3)	-18.70 ±2.24	-21.96 ±1.71	432.86 ±50.97	731.45 ±33.98	1.19 ±0.06	1.72 ±0.14	0.63 ±0.05	0.73 ±0.05	3.84 ±0.16	3.06 ±0.19	0.82 ±0.09	0.99 ±0.11	48.60 ±3.02	26.90 ±1.38	96.19 ±8.18	63.66 ±8.09	37.28 ±5.46
KO (n=3)	-20.23 ±3.31	-17.61 ±4.27	478.18 ±95.48	694.98 ±119.49	0.86 ±0.07	1.46 ±0.04	0.71 ±0.01	0.84 ±0.02	3.72 ±0.17	2.68 ±0.08	0.82 ±0.09	1.04 ±0.10	54.83 ±1.63	27.88 ±1.16	97.49 ±0.19	58.98 ±6.45	26.54 ±1.95

Unpaired student's t-test,two-sided. Values are mean ±SEM.

b, Echocardiographic analysis of 18-week old cardio-specific Piezo1-KO and Ctrl mice

	A'(mm/s)	E'(mm/s)	A (mm/s)	E (mm/s)	E'/A'	MVE/A	LVAW;d	LVAW;s	LVID;d	LVID;s	LVPW;d	LVPW;s	EF	FS	LV Mass AW	LV Vol;d	LV Vol;s
Ctrl (n=12)	-17.29 ±2.11	-24.14 ±1.95	520.26 ±26.81	697.80 ±39.11	1.81 ±0.37	1.34 ±0.04	0.89 ±0.03	1.23 ±0.08	3.79 ±0.09	2.48 ±0.12	0.80 ±0.04	1.13 ±0.06	64.27 ±2.56	34.85 ±1.93	116.56 ±5.72	62.14 ±3.39	22.87 ±2.55
KO (n=8)	-23.02 ±1.73	-31.79 ±1.60*	515.25 ±40.79	789.66 ±34.94	1.42 ±0.09*	1.59 ±0.14	0.82 ±0.08	1.17 ±0.10	4.10 ±0.24*	3.13 ±0.20**	0.76 ±0.02	1.05 ±0.06	50.94 ±4.58*	26.21 ±2.88*	133.42 ±8.95	81.80 ±8.70*	40.62 ±6.27**

Unpaired student's t-test,two-sided. *P < 0.05, **P<0.01. Values are mean ±SEM.

c, Echocardiographic analysis of 4-week old cardio-specific Piezo1-TG and Ctrl mice

	A'(mm/s)	E'(mm/s)	A (mm/s)	E (mm/s)	E'/A'	MVE/A	LVAW;d	LVAW;s	LVID;d	LVID;s	LVPW;d	LVPW;s	EF	FS	LV Mass AW	LV Vol;d	LV Vol;s
Ctrl (n=4)	-20.46 ±5.29	-24.13 ±4.16	238.30 ±36.80	650.99 ±57.48	1.25 ±0.01	2.81 ±0.26	0.83 ±0.04	1.16 ±0.07	3.66 ±0.16	2.43 ±0.22	0.69 ±0.08	0.95 ±0.15	63.17 ±4.45	33.74 ±3.21	93.92 ±5.99	56.90 ±5.92	21.48 ±4.45
TG (n=4)	-20.55 ±2.68	-20.22 ±3.84	395.07 ±91.95	556.70 ±34.36	0.96 ±0.08	1.59 ±0.28*	0.77 ±0.03	0.98 ±0.05	3.76 ±0.03	3.08 ±0.05*	0.70 ±0.06	0.75 ±0.04	37.83 ±3.49*	17.96 ±1.85*	94.34 ±2.68	60.46 ±1.03	37.49 ±1.50*

Unpaired student's t-test,two-sided. *P < 0.05. Values are mean ±SEM.

d, Echocardiographic analysis of 8-week old cardio-specific Piezo1-TG and Ctrl mice

	A'(mm/s)	E'(mm/s)	A (mm/s)	E (mm/s)	E'/A'	MVE/A	LVAW;d	LVAW;s	LVID;d	LVID;s	LVPW;d	LVPW;s	EF	FS	LV Mass AW	LV Vol;d	LV Vol;s
Ctrl (n=4)	-18.41 ±5.84	-22.82 ±5.29	576.50 ±15.31	758.38 ±138.98	1.28 ±0.30	1.10 ±0.16	0.72 ±0.03	1.18 ±0.08	4.11 ±0.21	2.92 ±0.26	0.65 ±0.04	0.91 ±0.07	56.12 ±5.51	29.34 ±3.72	101.00 ±10.37	75.53 ±8.99	34.01 ±6.83
TG (n=4)	-18.95 ±6.21*	-16.91 ±3.53	268.47 ±41.50	665.36 ±109.92	1.05 ±0.18	2.48 ±0.84	0.78 ±0.05	1.09 ±0.10	4.44 ±0.16	3.70 ±0.15*	0.88 ±0.03	0.81 ±0.04	34.52 ±6.80*	16.64 ±3.51*	123.38 ±2.84*	90.27 ±7.57	58.48 ±5.72*

Unpaired student's t-test,two-sided. *P < 0.05. Values are mean ±SEM.

e, Echocardiographic analysis of 18-week old cardio-specific Piezo1-TG and Ctrl mice

	A'(mm/s)	E'(mm/s)	A (mm/s)	E (mm/s)	E'/A'	MVE/A	LVAW;d	LVAW;s	LVID;d	LVID;s	LVPW;d	LVPW;s	EF	FS	LV Mass AW	LV Vol;d	LV Vol;s
Ctrl (n=7)	-20.86 ±2.24	-26.29 ±2.37	473.55 ±47.88	751.63 ±40.72	1.25 ±0.12	1.47 ±0.15	0.83 ±0.05	1.32 ±0.04	3.83 ±0.12	2.54 ±0.10	0.75 ±0.04	1.07 ±0.03	63.22 ±2.08	33.82 ±1.48	107.30 ±5.35	63.83 ±4.44	23.56 ±2.20
TG (n=5)	-16.78 ±1.96	-13.07 ±1.33*	457.75 ±35.40	514.51 ±37.24*	0.63 ±0.04	1.15 ±0.14	0.97 ±0.06	1.29 ±0.05	4.06 ±0.10	3.40 ±0.18**	0.85 ±0.05	0.95 ±0.02*	34.74 ±5.45***	16.57 ±2.81***	144.68 ±13.72*	72.92 ±4.07	48.25 ±6.51**

Unpaired student's t-test,two-sided. *P < 0.05, **P<0.01, ***P<0.001. Values are mean ±SEM.

f, Echocardiographic analysis of Dox-induced mouse model

	MVE/A	LVAW;d	LVAW;s	LVID;d	LVID;s	LVPW;d	LVPW;s	EF	FS	LV Mass AW	LV Vol;d	LV Vol;s
Ctrl-Saline (9)	1.42 ±0.05	0.83 ±0.03	1.03 ±0.09	3.80 ±0.11	2.72 ±0.17	0.78 ±0.03	1.02 ±0.03	60.28 ±2.94	31.36 ±2.43	116.67 ±8.05	68.60 ±5.99	29.09 ±4.01
Ctrl-Dox (10)	1.56 ±0.15	0.84 ±0.04	1.07 ±0.08	4.16 ±0.11*	3.16 ±0.13	0.76 ±0.01	0.95 ±0.03	48.38 ±2.70**	24.30 ±1.65*	127.03 ±9.86	77.54 ±4.92	40.63 ±4.17*

Unpaired student's t-test,two-sided. *P < 0.05, **P<0.01. Values are mean ±SEM.

Supplementary Table 1 Echocardiographic analysis of the indicated mice

mANP-qPCR-F	GCCATATTGGAGCAAATCCT
mANP-qPCR-R	GCAGGTTCTTGAAATCCATCA
m β -MHC-qPCR-F	AAGCAGCAGTTGGATGAGCG
m β -MHC-qPCR-R	CCTCGATGCGTGCCTGAAGC
mBNP-qPCR-F	CATGGATCTCCTGAAGGTGC
mBNP-qPCR-R	CCTTCAAGAGCTGTCTCTGG
m α -MHC-F	GGAAGAGTGAGCGGCCATCAAGG
m α -MHC-R	CTGCTGGAGAGGTTATTCTCG
mSERCA2a-F	GAGAACGCTCACACAAAGACC
mSERCA2a-R	CAATTCGTTGGAGCCCCAT
mGAPDH-F	GCACCACCAACTGCTTAG
mGAPDH-R	GGATGCAGGGATGATGTTC
sgRNA-T7-F	taggGTGGGGAGCAAGCGGGCACCA
sgRNA-T7-R	aaacTGGTGCCCGCTTGCTCCCCAC
mP1-Flag-KI-HA-oligo	GTGCGCATCCAGCTGCGGAGGGAGCAAGTGG GCACAGGGGCCTCTGGGGAGCAAGCGGGCGA CTACAAGGACGACGATGACAAGACCAAGGCC TCCGACTTCCTCGAGTGGTGGGTCATCGAGCT GCAGGACTGCAAGGCTGAC
mouse-sequence-F	CCGACTCTAACTATCCCACTCAAC
mouse-sequence-R	CTGACCTTGTCACTGAAGATGACC
Flag-F	GACTACAAGGACGACGATGACAAG
Flag-R	AGGCAGCTCCTTCATTCCCG

Supplementary Table 2 Sequences of primers and sgRNAs