Supplementary information

The mechanosensitive Piezo1 channel mediates heart mechano-chemo transduction

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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, 100μm.

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\(^{floxed-mCherry}\) 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-e, 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 ±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.
### Table 1

#### Supplementary Table 1

| K (mm) | EF (%) | FS (%) | LV Mass (g) | LV Vol (ml) | LV Vt (ml) |
|--------|--------|--------|-------------|-------------|------------|
| 1.37    | 37.02   | 13.57  | 0.92         | 0.80        | 0.75       |
| 1.57    | 33.97   | 11.26  | 1.03         | 0.85        | 0.78       |
| 1.77    | 29.85   | 9.94   | 1.14         | 0.98        | 0.82       |

Unpaired student's t-test, two-sided. *P < 0.05, **P < 0.01. Values are mean ± SEM.
**Supplementary Table 2 Sequences of primers and sgRNAs**

| Primer Name | Sequence |
|-------------|----------|
| mANP-qPCR-F | GCCATATTGGAGCAATATCTCT |
| mANP-qPCR-R | GCAGGTTCTTGAATCCATCA |
| mβ-MHC-qPCR-F | AAGCAGCGAGTTGGATGAGCG |
| mβ-MHC-qPCR-R | CCTCGATGCGTGCTGACAGG |
| mBNP-qPCR-F | CATTGATCTCCTGAAAGTGC |
| mBNP-qPCR-R | CCTTCAAAGAGCTGTCTCTGG |
| mα-MHC-F | GGAAGAGTGGAGCCGCCATCAAGG |
| mα-MHC-R | CTGCTGGAGAGTTATTCCTG |
| mSERCA2a-F | GAGAACGCTCACACAAAGACC |
| mSERCA2a-R | CAAATTCGTTGGAGCCCAT |
| mGAPDH-F | GCACCACCACACTGCCTAG |
| mGAPDH-R | GGAAGGAGGATGATGTTC |
| sgRNA-T7-F | taggGTGGGGGAGCAAGCGGGGCAAC |
| sgRNA-T7-R | aaacTGGTCGCCGGCATCGTCTCCCAC |
| mP1-Flag-KI-HA-oligo | GTGCGCATCCAGCTGCGGAGGGAGCGCAAAGTGG |
|                | GCACAGGGGCTCTGGGAGGAGCAAGCGGGGCA |
|                | CTCAAGGACGACAGTGACAAAGACCAAGGCC |
|                | TCCGACTTCTCGAGTGGGTGGTGCAGCTG |
|                | GCAGGACTGCAAGGCTGAC |
| mouse-sequence-F | CCGACTCTTAACTATCCCACTCAAC |
| mouse-sequence-R | CTGACCTTGTCACTGAGATGACC |
| Flag-F | GACTCAAGAGCGAGCATGACAAG |
| Flag-R | AGGCAGCTCTTCAATTCCCG |