

Supplemental Information

**The lncRNA Plscr4 Controls Cardiac
Hypertrophy by Regulating miR-214**

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α -actinin/DAPI

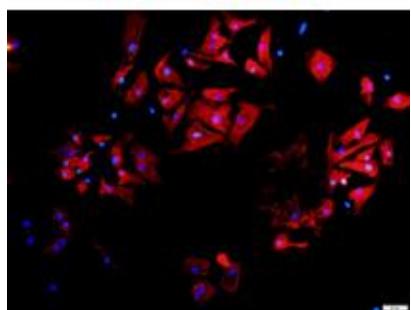


Figure S1 Identification of cultured neonatal mice cardiomyocytes.

Cardiomyocytes were stained with antibody against α -actinin for detecting of CMs and with DAPI for the nuclear of cells. scale bars, 20 μ m.

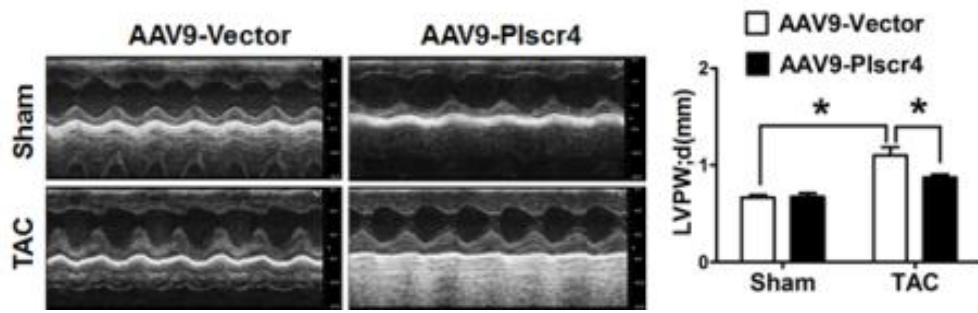


Figure S2 Overexpression of Plscr4 attenuates the pressure overload–induced cardiac left ventricular wall thickness. Quantitative analysis of the diastolic left ventricular posterior wall diameter (LVPW:d) by echocardiography in the AAV9-Vector or AAV9-Plscr4 overexpressing mice subjected to the Sham or TAC operation for 4 weeks. (n = 6, *P < 0.05).

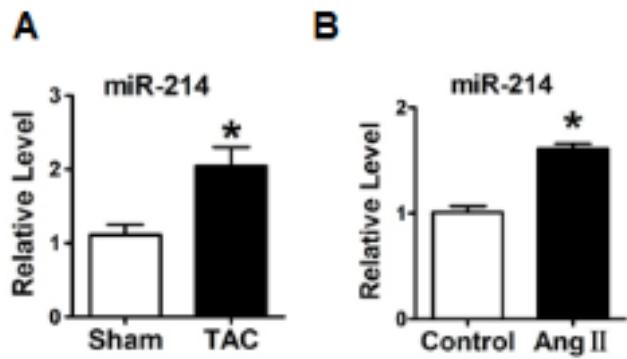


Figure S3 miR-214 is upregulated in response to hypertrophic stress. A. The relative level of miR-214 in mice 4 weeks after the sham or TAC treatment ($n = 6$, $*P < 0.05$ v.s. Sham). **B.** The relative level of miR-214 in the cardiomyocytes after a 48 h treatment of PBS or Ang II. ($n = 6$, $*P < 0.05$ v.s. Control).

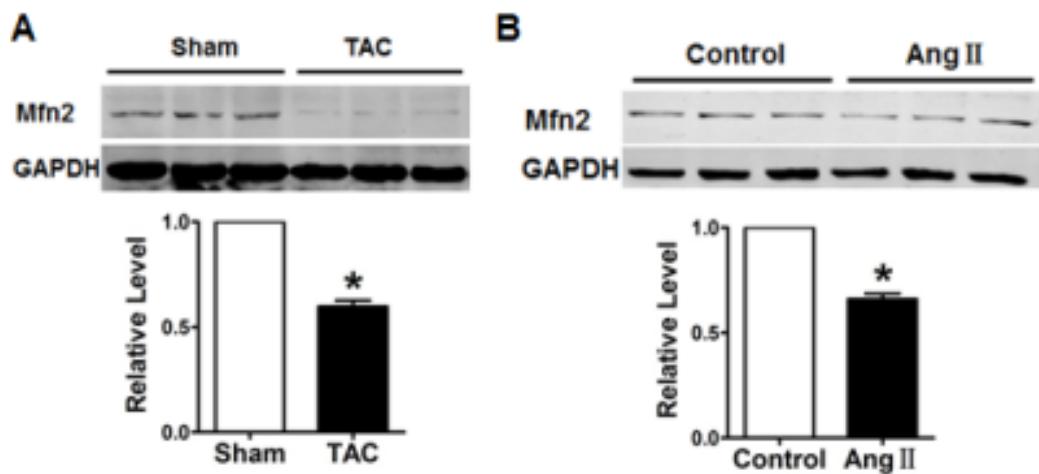


Figure S4 Protein level of Mfn2 is downregulated in response to hypertrophic stress. A. Representative western blot bands of Mfn2 in the Sham and TAC group. The protein expression was quantified and normalized to GAPDH ($n = 6$ per group; $*P < 0.05$ v.s. Sham). **B.** Representative western blot bands of Mfn2 in the CMs after a 48 h treatment with PBS or Ang II. The protein expression was quantified and normalized to GAPDH ($n = 6$; $*P < 0.05$ v.s. Control).

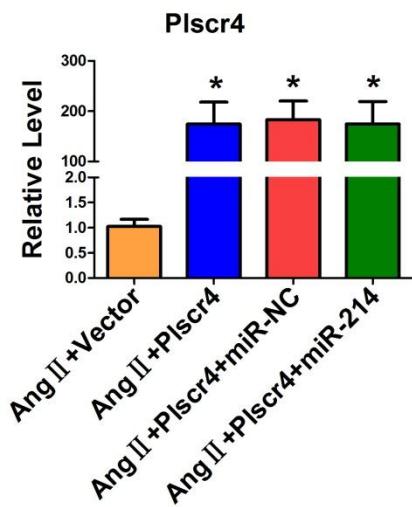


Figure S5 The successful transfection of Plscr4. The relative mRNA levels of Plscr4 in the CMs transfected with Plscr4 or the Vector alone or in combination with miR-214 or miR-NC followed by 48 h of Ang II treatment ($n = 6$, $*P < 0.05$ v.s. Ang II+Vector).

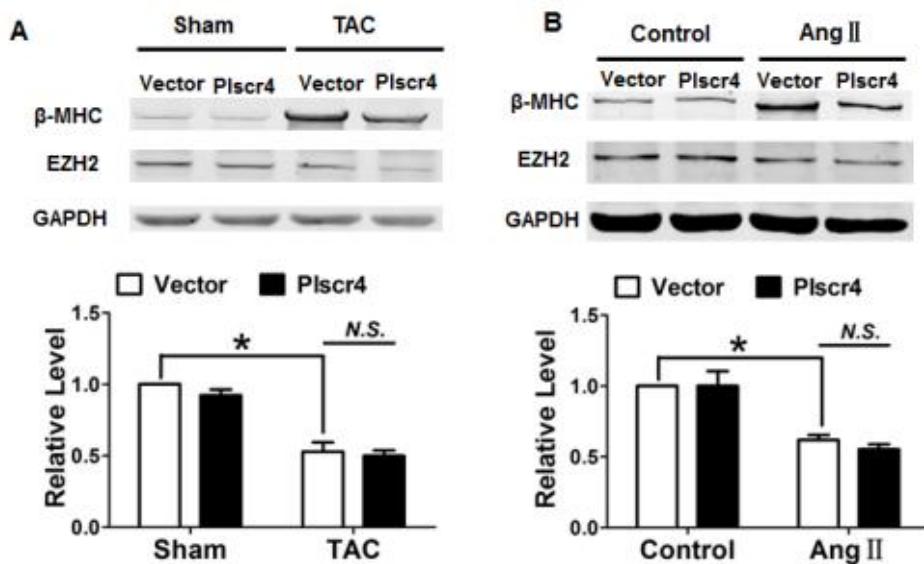


Figure S6 Protein level of EZH2 are downregulated in response to hypertrophic stress which is not influenced by Plscr4. **A.** Representative western blot bands of EZH2 in the AAV9-Vector or AAV9-Plscr4 overexpression mice after 4 weeks of the TAC or sham operation. (n = 3, *P < 0.05). **B.** Representative western blot bands of EZH2 in the CMs transfected with lncRNA-plscr4 or the Vector control that were subsequently treated with phosphate-buffered saline (PBS) or Ang II (1 μ mol/L) for 48 h (n = 4, *P < 0.05).

Table S1 Conservation analysis of Plscr4 between mice and human.

This figure displays a sequence alignment between Mouse and Human DNA. The alignment highlights conserved regions in yellow and mutated regions in blue. The x-axis represents the sequence position, and the y-axis lists the species: Mouse and Human.

Conservation: Yellow boxes indicate positions where the sequence is identical between Mouse and Human. These are predominantly found in the upper and lower sections of the alignment.

Mutations: Blue boxes indicate positions where the sequence differs between Mouse and Human. These are scattered throughout the alignment, often appearing as single nucleotide changes or small insertion/deletion events.

Key Features:

- Top Section:** Shows a highly conserved region followed by a series of mutations (blue) in both species.
- Second Section:** A large block of yellow (conservation) is followed by a series of mutations (blue).
- Third Section:** A large block of blue (mutations) is followed by a series of mutations (blue).
- Fourth Section:** A large block of yellow (conservation) is followed by a series of mutations (blue).
- Bottom Section:** A large block of blue (mutations) is followed by a series of mutations (blue).

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Mouse  gctgcttggcagtagttactttctgtatgaatttaggtacttataggaaaggacagtaatcatatgtataacaatggtatcaacatattccacagatgaca  
Human  atagtgttagtcaataatta=====  
          * * * * * *  
Mouse  aatgggaaggactttgattgcaggagccaatcacagcctacagtttct  
Human  =====
```

Table S2 Primers used for the qRT-PCR analysis.

| RNA name | Primers from 5' to 3' |
|---------------|--|
| Plscr4—F | GAGGCTGCTTGGCAGTAGT |
| Plscr4—R | GCTCCTGCAATCAAAAGTCC |
| ANP—F | ACCTGCTAGACCACCTGGAG |
| ANP—R | CCTTGGCTGTTATCTTCGGTACCGG |
| BNP—F | GAGGTCACTCCTATCCTCTGG |
| BNP—R | GCCATTCCCTCCGACTTTCTC |
| β-MHC-F | CCGAGTCCCAGGTCAACAA |
| β-MHC-R | CTTCACGGGCACCCTTGGA |
| GAPDH-F | TCTACATGTTCCAGTATGACTC |
| GAPDH-R | ACTCCACGACATACTCAGCACC |
| U6-RT | CGCTTCACGAATTGCGTGTCA |
| U6-F | GCTTCGGCAGCACATATACTAAAAT |
| U6-R | CGCTTCACGAATTGCGTGTCA |
| miR-214-3p-RT | GTCGTATCCAGTGCAGGGTCCGAGGTATTG GCACTGGATACGACACTGCC |
| miR-214-3p-F | GCGGACAGCAGGCACAGACA |
| miR-214-3p-R | ATCCAGTGCAGGGTCCGAGG |