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Supplemental Information

The lncRNA Plscr4 Controls Cardiac

Hypertrophy by Regulating miR-214

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α -actinin/DAPI

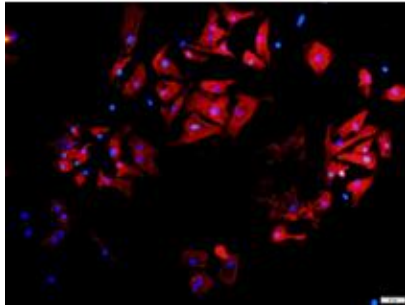


Figure S1 Identification of cultured neonatal mice cardiomyocytes.

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.

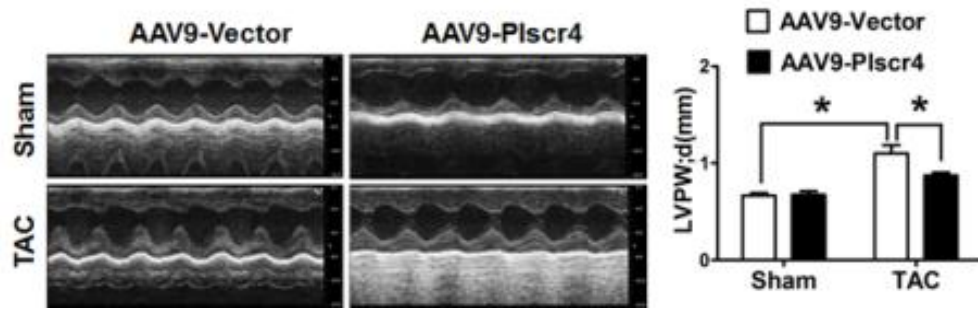


Figure S2 Overexpression of Plscr4 attenuates the pressure overload–induced cardiac left ventricular wall thickness. Quantitative analysis of the diastolic left ventricular posterior wall diameter (LVPW:d) by echocardiography in the AAV9-Vector or AAV9-Plscr4 overexpressing mice subjected to the Sham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

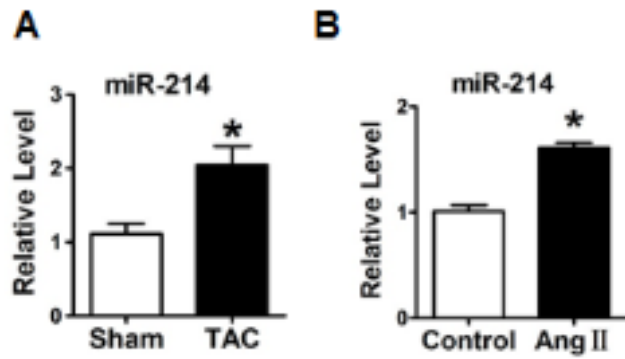


Figure S3 miR-214 is upregulated in response to hypertrophic stress. A. The relative level of miR-214 in mice 4 weeks after the sham or TAC treatment (n = 6, * $P < 0.05$ v.s. Sham). **B.** The relative level of miR-214 in the cardiomyocytes after a 48 h treatment of PBS or Ang II. (n = 6, * $P < 0.05$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

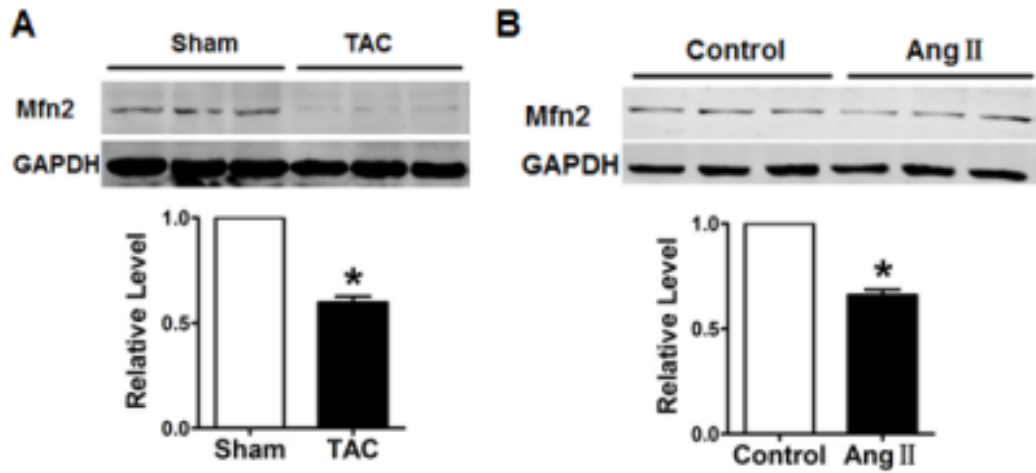


Figure S4 Protein level of Mfn2 is downregulated in response to hypertrophic stress. **A.** Representative western blot bands of Mfn2 in the Sham and TAC group. The protein expression was quantified and normalized to GAPDH (n = 6 per group; * $P < 0.05$ v.s. Sham). **B.** Representative western blot 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).

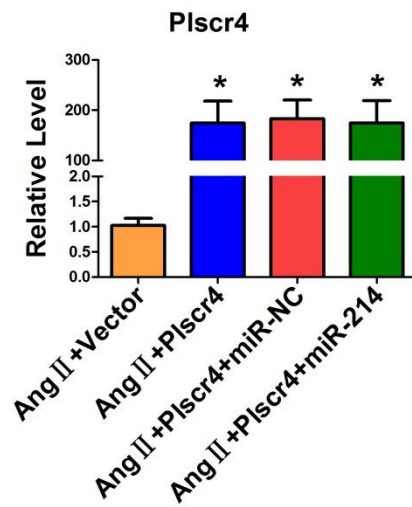


Figure S5 The successful transfection of Plscr4. The relative mRNA levels of Plscr4 in the CMs transfected with Plscr4 or the Vector alone or in combination with miR-214 or miR-NC followed by 48 h of Ang II treatment (n = 6, * $P < 0.05$ v.s. Ang II+Vector).

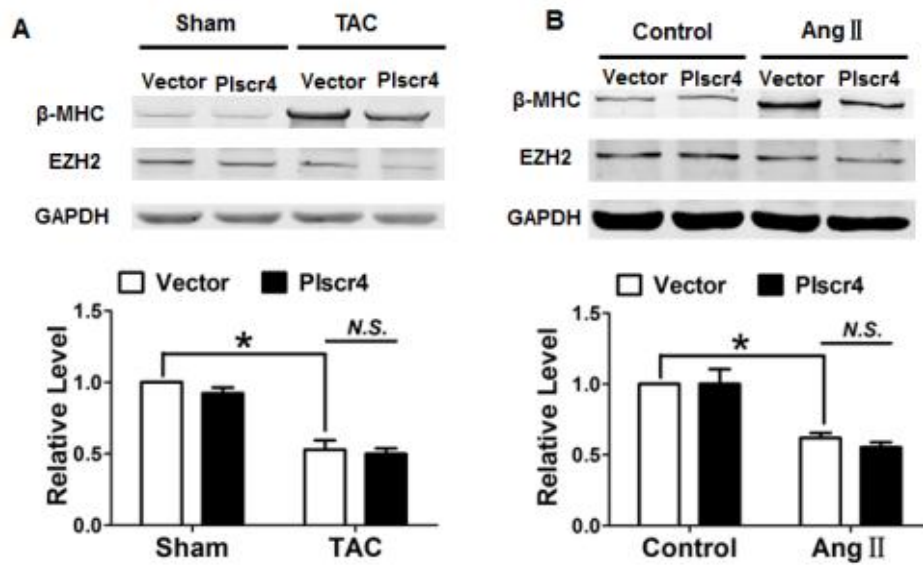


Figure S6 Protein level of EZH2 are downregulated in response to hypertrophic stress which is not influenced by Plscr4. **A.** Representative western blot bands of EZH2 in the AAV9-Vector or AAV9-Plscr4 overexpression mice after 4 weeks of the TAC or sham operation. (n = 3, * $P < 0.05$). **B.** Representative 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 $\mu\text{mol/L}$) for 48 h (n = 4, * $P < 0.05$).

Mouse gctgctttggcagtagttactttctgatgaattaggtacttataggaaggacagtaatacatatgttatacaatggtatcaacatattccacagatgaca

Human atagtgtagtcaataatta=====

* * * * *

Mouse aatggggaaggacttttgattgcaggagccaatcacagcctacagttttct

Human =====

Table S2 Primers used for the qRT-PCR analysis.

RNA name	Primers from 5' to 3'
Plscr4—F	GAGGCTGCTTTGGCAGTAGT
Plscr4—R	GCTCCTGCAATCAAAAGTCC
ANP—F	ACCTGCTAGACCACCTGGAG
ANP—R	CCTTGGCTGTTATCTTCGGTACCGG
BNP—F	GAGGTCACTCCTATCCTCTGG
BNP—R	GCCATTTCTCCGACTTTTCTC
β -MHC-F	CCGAGTCCCAGGTCAACAA
β -MHC-R	CTTCACGGGCACCCTTGGA
GAPDH-F	TCTACATGTTCCAGTATGACTC
GAPDH-R	ACTCCACGACATACTCAGCACC
U6-RT	CGCTTCACGAATTTGCGTGTCAT
U6-F	GCTTCGGCAGCACATATACTAAAAT
U6-R	CGCTTCACGAATTTGCGTGTCAT
miR-214-3p-RT	GTCGTATCCAGTGCAGGGTCCGAGGTATTC GCACTGGATACGACACTGCC
miR-214-3p-F	GCGGACAGCAGGCACAGACA
miR-214-3p-R	ATCCAGTGCAGGGTCCGAGG