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Supplemental information

Neutrophil-like cell membrane-coated siRNA of lncRNA *AABR07017145.1* therapy for cardiac hypertrophy via inhibiting ferroptosis of CMECs

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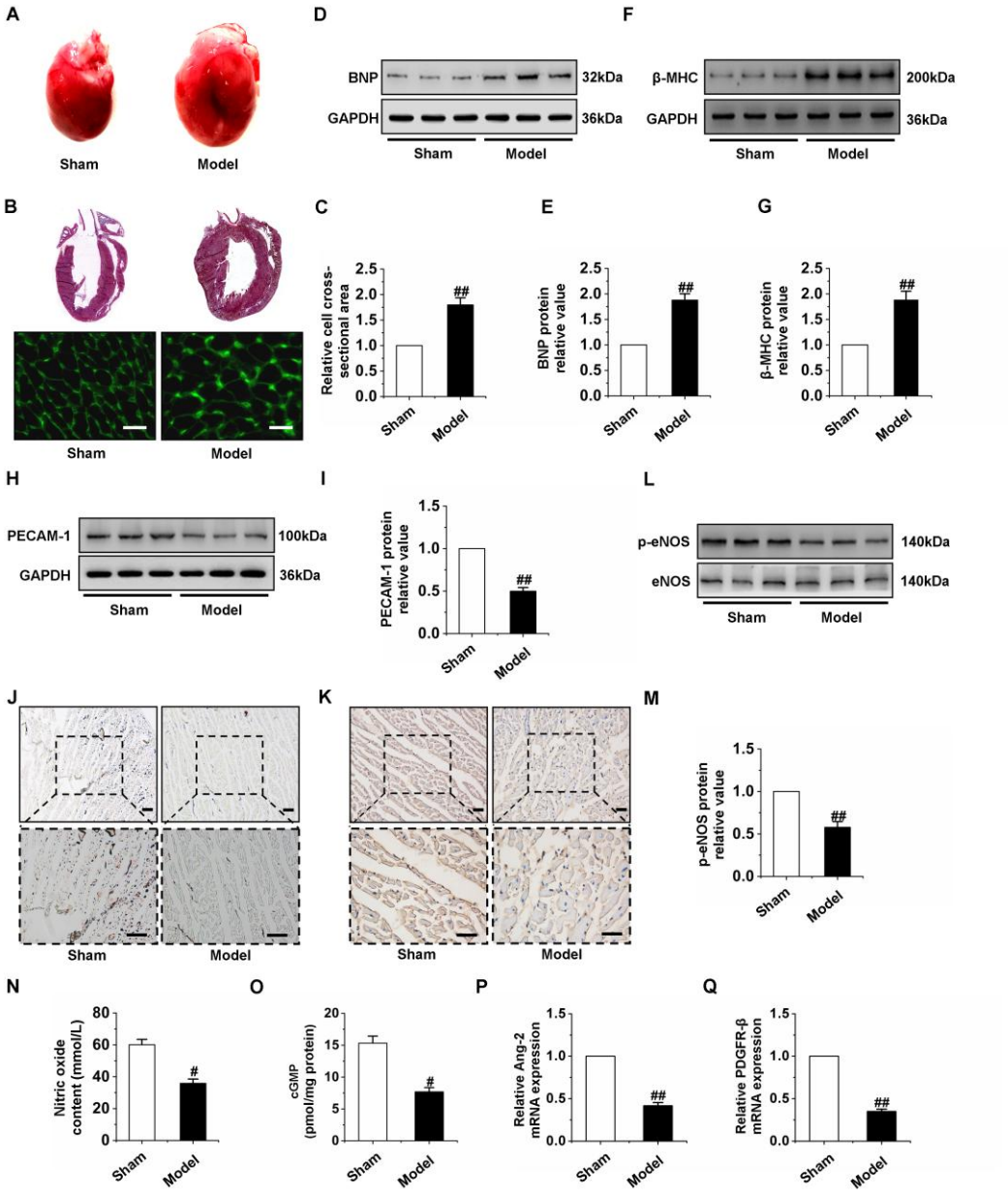
Supplementary figure.1

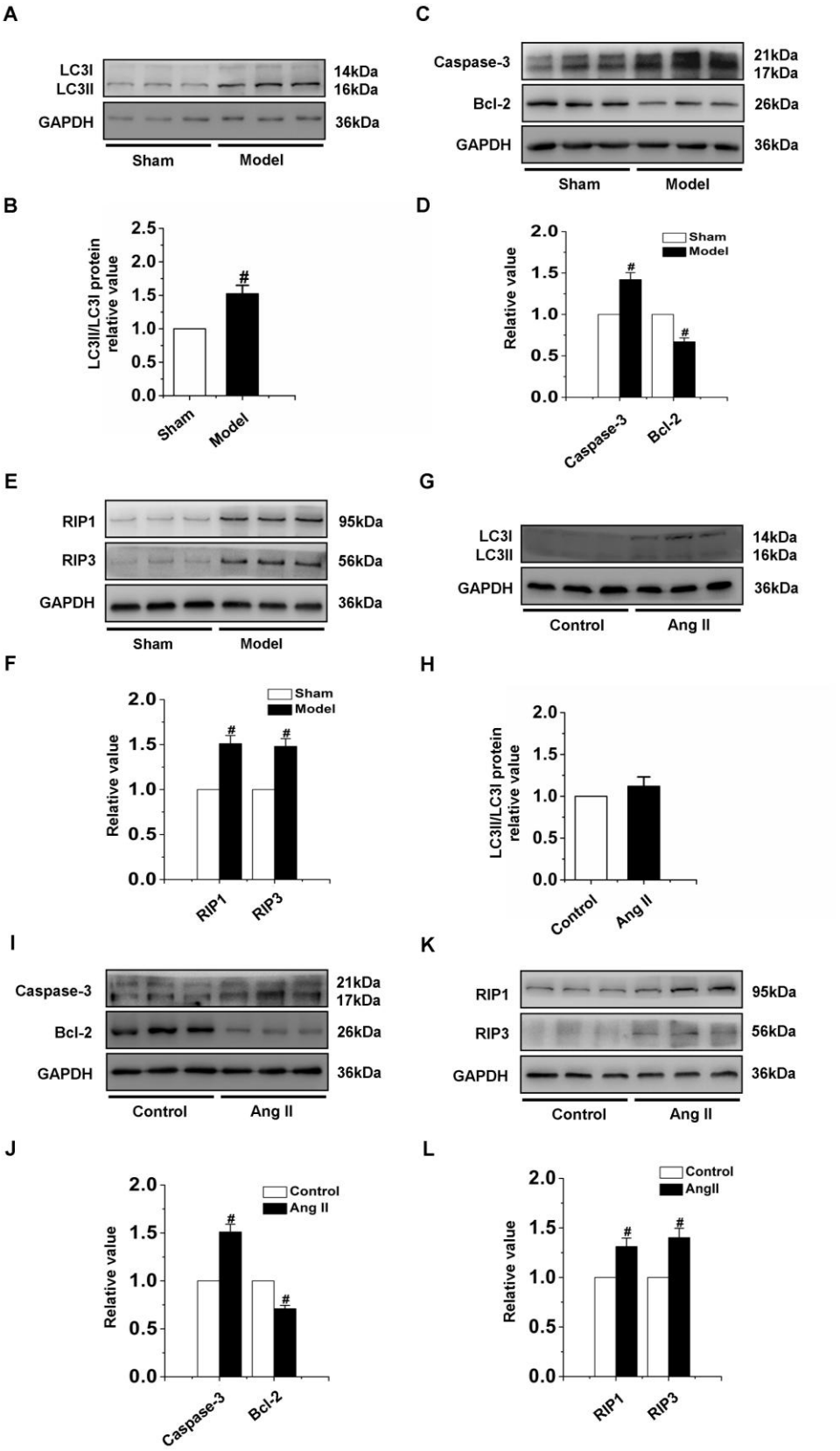


Supplementary figure S1. Cardiac microvascular damage is induced in hypertrophic hearts

(A) Cardiac volume representative images of rats induced by AAC for 4 weeks. (B-C) Representative sections of heart stained for HE and WGA staining in rats induced by AAC for 4 weeks. (D-E) Detection of *BNP* protein expression by western blot analysis in cardiac tissue. (F-G) Western blot analysis of *β-MHC* expression in cardiac tissue. (H-I) Detection of *PECAM-1* protein expression by western blot analysis in

cardiac tissue. (J) The effect of treatment with AAC for 4 weeks on microvascular perfusion. (K) Representative image of immunohistochemistry of *PECAM-1* in the AAC-induced rat hearts. (L-M) Western blot results of *p-eNOS* protein in cardiac tissue. (N) Statistical analysis of NO content in cardiac tissue of rats. (O) Statistical analysis of cGMP content in cardiac tissue of rats. (P-Q) Quantification of the mRNA expressions of *Ang-2* and *PDGFR-β* was validated by qRT-PCR in cardiac tissue. NO: nitric oxide. AAC: abdominal aorta constriction. WGA: wheat germ agglutinin. Model group: Rats were treated with AAC for 4 weeks. Data were represented by means ± SEM (n=6). $^{\#}P<0.05$ and $^{\#\#}P<0.01$ vs. sham group. Scale bars, Figure S1B, 50 μm; Figure S1J, 100 μm (×100); 100 μm (×200); Figure S1K, 50 μm (×200); 50 μm (×400).

Supplementary figure.2

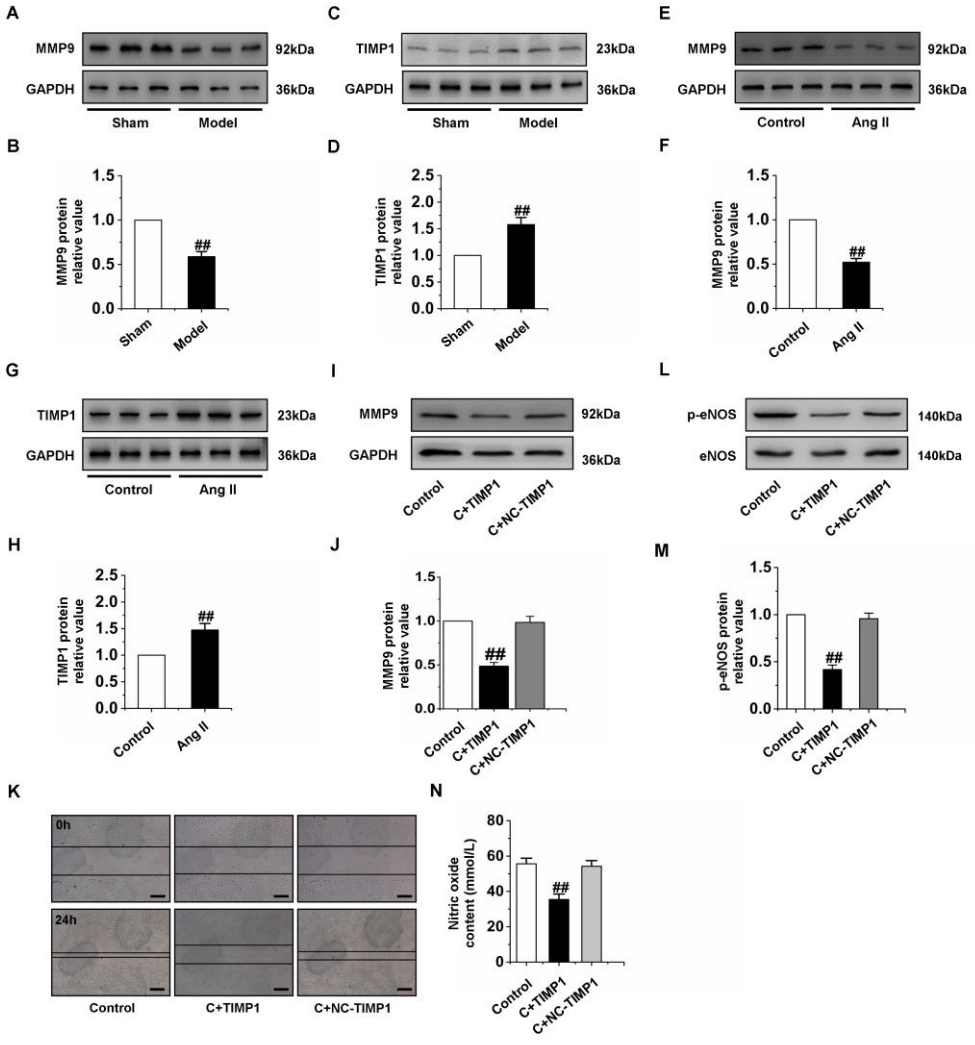


Supplementary figure S2. Autophagy, apoptosis and necroptosis occur in

myocardial hypertrophy rat hearts, while apoptosis and necroptosis in CMECs

(A-B) Western blots were performed to analyze the *LC3II* expression in cardiac tissue isolated from rats exposed to AAC for 4 weeks. (C-D) Western blot results of *caspase-3* and *Bcl-2* protein expression in cardiac tissue. (E-F) Western blot results of *RIP1* and *RIP3* protein expression levels in cardiac tissue isolated from rats. Model group: Rats were treated with AAC for 4 weeks. Data were represented by means ± SEM (n=6). $^{\#}P<0.05$ vs. sham group. (G-H) Western blot results of *LC3II* protein expression in CMECs induced by Ang II for 24 h. (I-J) Western blot analysis of *caspase-3* and *Bcl-2* protein expression in CMECs. (K-L) Protein levels of *RIP1* and *RIP3* in CMECs. Ang II group: CMECs were exposed to Ang II for 24 h. Data were represented by means ± SEM (n=6). $^{\#}P<0.05$ vs. control group.

Supplementary figure.3

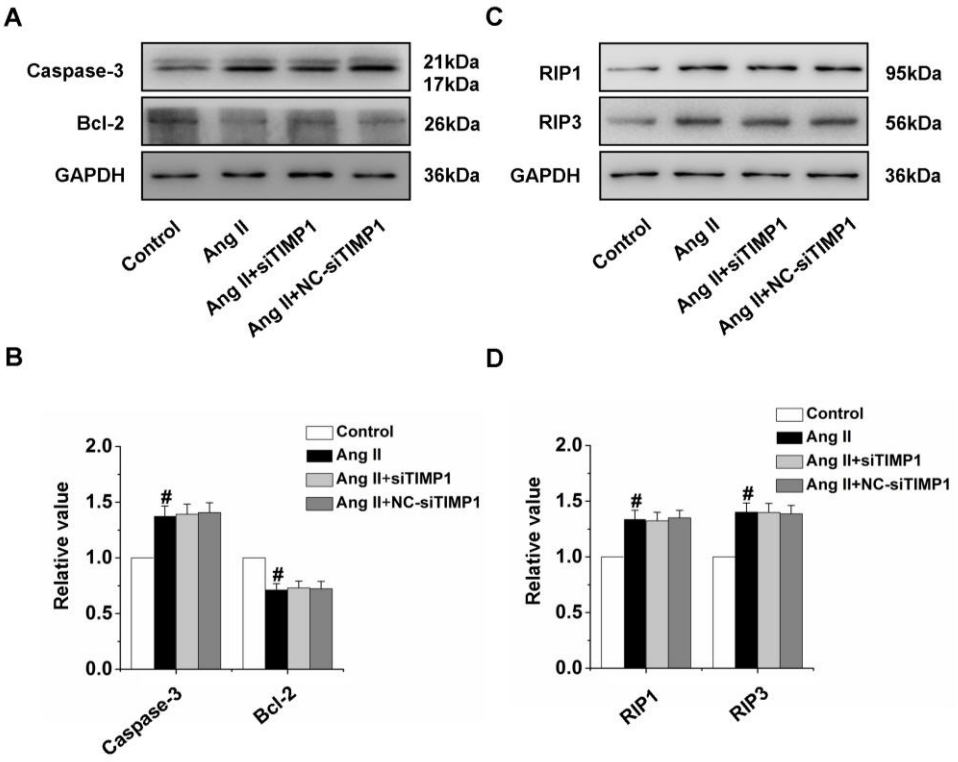


Supplementary figure S3. Overexpression of *TIMP1* leads to dysfunction of CMECs

(A-D) Detection of *MMP9* and *TIMP1* protein expression of cardiac tissue after AAC treatment for 4 weeks by western blot analysis. Data were represented by means ± SEM (n=6). $^{\#\#}P<0.01$ vs. sham group. (E-H) Detection of *MMP9* and *TIMP1* protein expression in CMECs treated with 100 nM Ang II for 24 h. (I-J) The protein expression of *MMP9* in CMECs were detected by western blot after *TIMP1* or control vectors (NC-*TIMP1*) and x-treme GENE were added to the CMECs and incubated at 37℃ for 24 h. (K) The scratch test was used to detect the effect of overexpression of *TIMP1* on migration ability of CMECs. (L-M) Western blot analysis of *p-eNOS* in CMECs. (N) Statistical analysis chart of NO content in CMECs. C+*TIMP1*: *TIMP1*

and x-treme GENE were added to the CMECs and incubated at 37℃ for 24 h. C+NC-*TIMP1*: pcDNA3.1 empty vector was transfected into CMECs for 24 h. Data were represented by means ± SEM (n=6). $^{\#\#}P<0.01$ vs. control group. Scale bars: 10 μm.

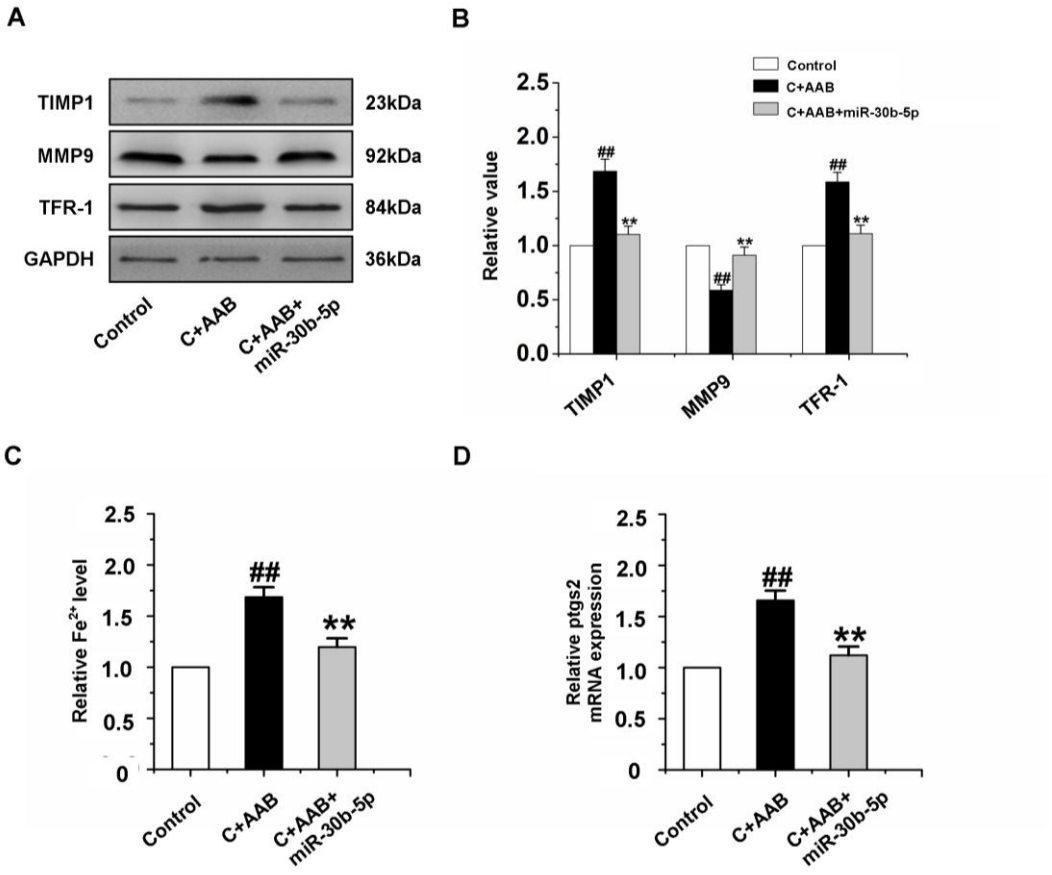
Supplementary figure.4



Supplementary figure S4. Silencing *TIMP1* does not inhibit cell apoptosis and necroptosis

(A-B) Western blot results of *caspase-3* and *Bcl-2* protein expression in CMECs treated with *siTIMP1* for 24 h and then exposed to Ang II for 24 h. (C-D) Western blot results of *RIP1* and *RIP3* protein expression levels in CMECs. *siTIMP1*: *TIMP1* was silenced. Ang II+*siTIMP1*: CMECs were treated with *siTIMP1* for 24 h and then exposed to Ang II for 24 h. Ang II+NC-*siTIMP1*: CMECs were treated with negative control of *siTIMP1* for 24 h and then exposed to Ang II for 24 h. Data were represented by means ± SEM (n=6). $^{\#}P<0.05$ vs. control group.

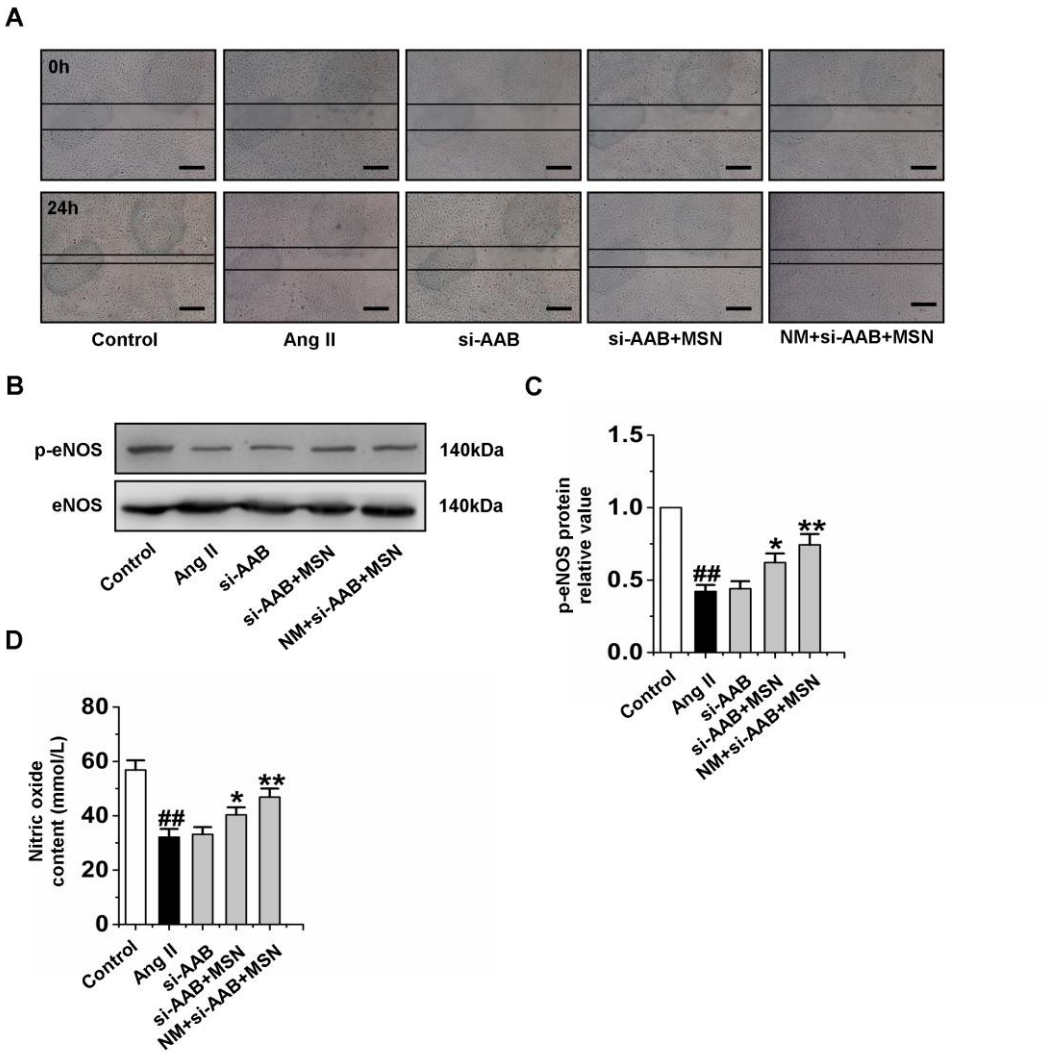
Supplementary figure.5



Supplementary figure S5. LncRNA *AAB* regulates ferroptosis via *miR-30b-5p* in CMEC

(A-B) Western blot analysis of *TIMP1*, *MMP9* and *TFR-1* in CMECs co-transfected with lncRNA *AAB* and *miR-30b-5p* for 24 h. (C) Statistical analysis chart of Fe^{2+} content in CMECs. (D) qRT-PCR was used to detect the *ptgs2* mRNA expression in CMECs. *AAB*: lncRNA *AAB*. C+*AAB*: CMECs were transfected with lncRNA *AAB* for 24 h. C+*AAB*+*miR-30b-5p*: CMECs were co-transfected with lncRNA *AAB* and *miR-30b-5p* for 24 h. Data were represented by means ± SEM (n=6). $^{\#\#}P<0.01$ vs. control group. $^{**}P<0.01$ vs. C+*AAB* group.

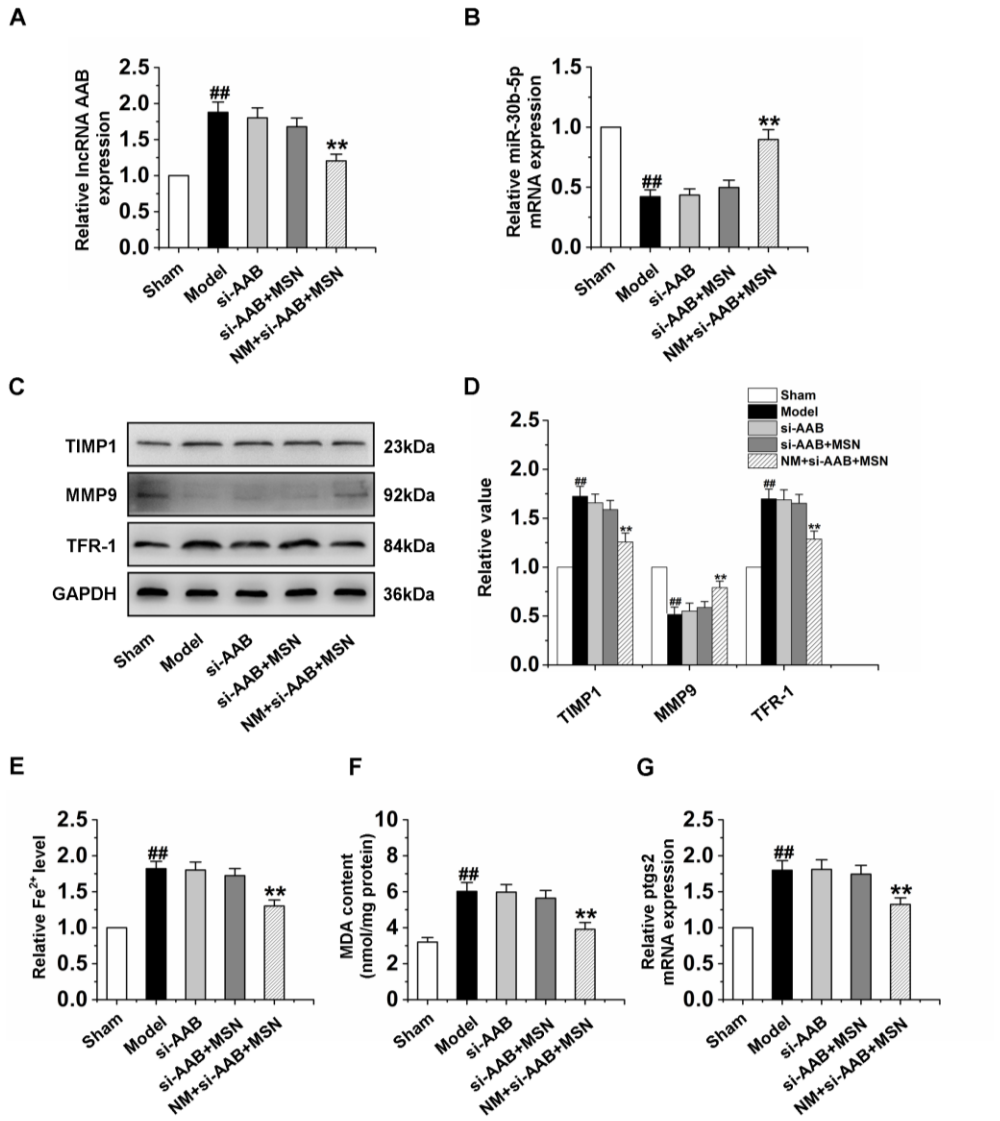
Supplementary figure.6



Supplementary figure S6. NM+si-*AAB*+MSN improves the function of CMECs induced by Ang II

(A) The scratch test was used to detect the effect of si-*AAB*, si-*AAB*+MSN and NM+si-*AAB*+MSN on migration ability of CMECs exposed to Ang II for 24 h. (B-C) Western blot analysis of *p-eNOS*. (D) Statistical analysis chart of NO content. Ang II group: The CMECs were given 100 nM Ang II for 24 h. si-*AAB*, si-*AAB*+MSN and NM+si-*AAB*+MSN groups: The CMECs were given 100 nM Ang II for 24 h following by treatment with si-*AAB*, si-*AAB*+MSN and NM+si-*AAB*+MSN for 48 h, respectively. Data were represented by means ± SEM (n=6). $^{\#\#}P<0.01$ vs. control group; $^{*}P<0.05$ and $^{**}P<0.01$ vs. Ang II group. Scale bars: 10 μm.

Supplementary figure.7

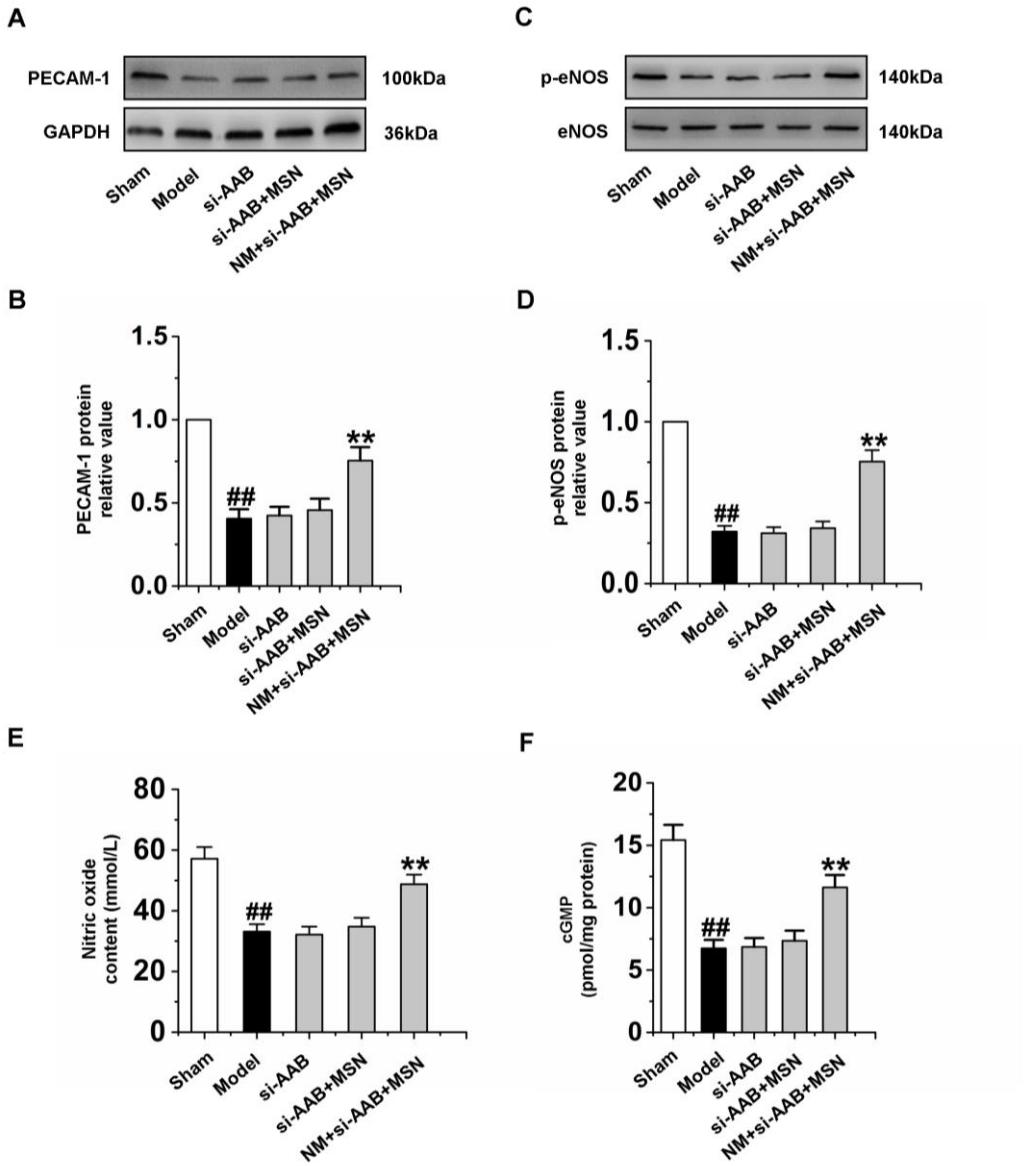


Supplementary figure S7. NM+si-*AAB*+MSN inhibits the ferroptosis of hypertrophic heart

(A) qRT-PCR analysis of lncRNA *AAB* in the hearts of cardiac hypertrophy rats induced by AAC for 4 weeks. (B) The *miR-30b-5p* mRNA level was detected by qRT-PCR in different groups. (C-D) Western blot analysis of *TIMP1*, *MMP9* and *TFR-1* in cardiac tissue of rats. (E) Statistical analysis chart of iron ions content in hearts of rats. (F) Statistical analysis chart of MDA content in cardiac tissue. (G) mRNA expression of *ptgs2* in cardiac tissue was measured by qRT-PCR. Model group: Rats were subjected to AAC and received vehicle (PBS, caudal vein injection) 24 h later once every 2 days for 4 weeks. si-*AAB* group: Rats were subjected to AAC and

treated with si-*AAB* via tail vein injection 24 h later every 2 days for 4 weeks. si-*AAB*+MSN group: Rats were subjected to AAC and treated with si-*AAB*+MSN via tail vein injection 24 h later every 2 days for 4 weeks. NM+*si-AAB*+MSN group: Rats were subjected to AAC and treated with NM+si-*AAB*+MSN via tail vein injection 24 h later every 2 days for 4 weeks. Data were represented by means ± SEM (n=6). $^{\#\#}P<0.01$ vs. sham group; $^{**}P<0.01$ vs. model group.

Supplementary figure.8

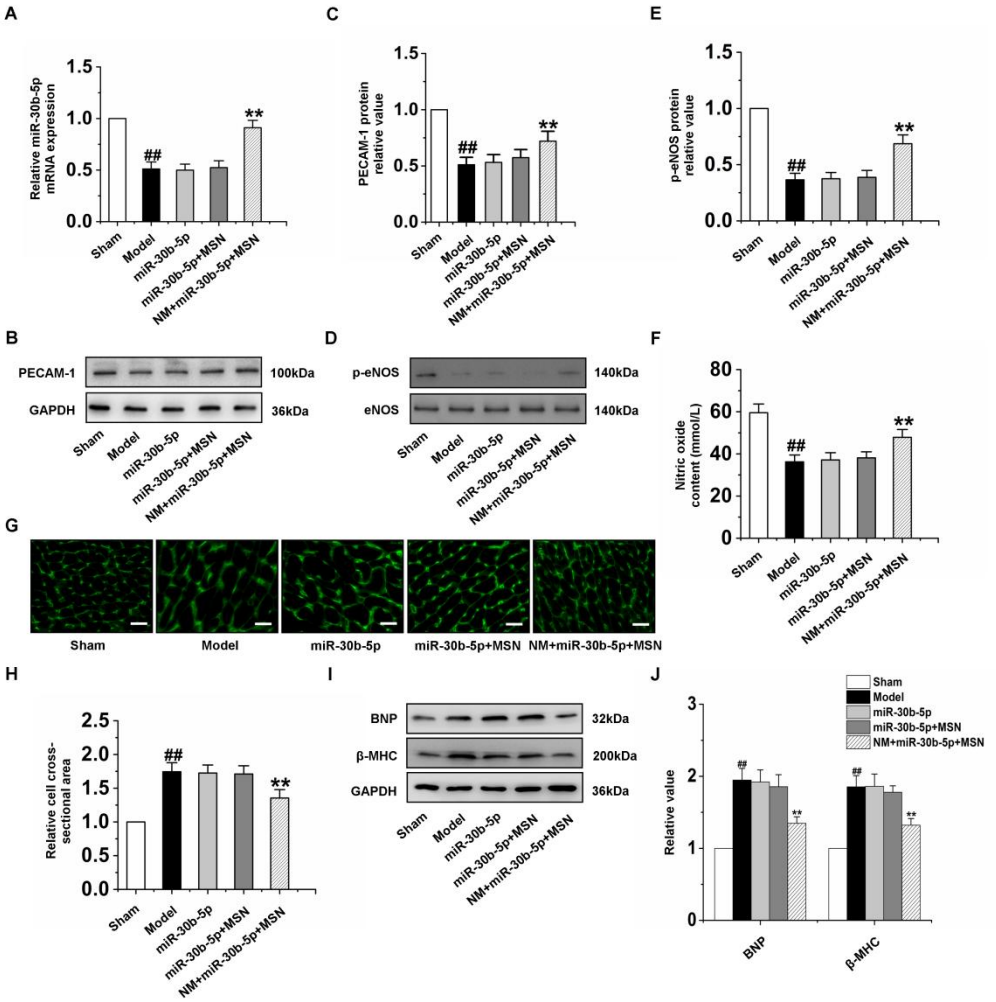


Supplementary figure S8. NM+si-*AAB*+MSN plays a protective role in the cardiac microvessels

(A-B) Protein levels of *PECAM-1* in the cardiac hypertrophy rat hearts were assessed by western blot analysis. (C-D) Detection of *p-eNOS* protein expression by western blot analysis. (E) Statistical analysis chart of NO content. (F) Statistical analysis chart of cGMP content. Model group: Rats were subjected to AAC and received vehicle (PBS, caudal vein injection) 24 h later once every 2 days for 4 weeks. si-*AAB* group: Rats were subjected to AAC and treated with si-*AAB* via tail vein injection 24 h later every 2 days for 4 weeks. si-*AAB*+MSN group: Rats were subjected to AAC and

treated with si-*AAB*+MSN via tail vein injection 24 h later every 2 days for 4 weeks. NM+*si-AAB*+MSN group: Rats were subjected to AAC and treated with NM+si-*AAB*+MSN via tail vein injection 24 h later every 2 days for 4 weeks. Data were represented by means ± SEM (n=6). $^{\#\#}P<0.01$ vs. sham group; $^{**}P<0.01$ vs. model group.

Supplementary figure.9

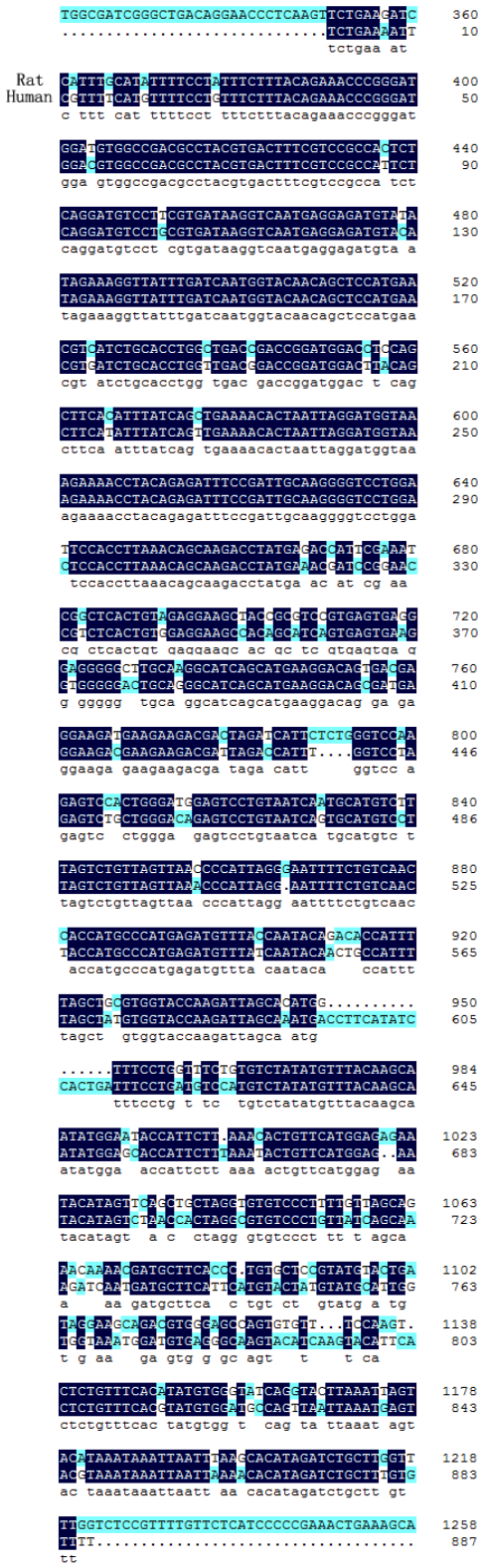


Supplementary figure S9. NM+*miR-30b-5p*+MSN inhibits cardiac hypertrophy by improving cardiac microvascular function

(A) qRT-PCR was used to detect the *miR-30b-5p* expression in cardiac tissue of rats. (B-C) Protein levels of *PECAM-1* in the cardiac hypertrophy rat hearts were assessed by western blot analysis. (D-E) Detection of *p-eNOS* protein expression by western blot analysis. (F) Statistical analysis chart of NO content. (G-H) Representative sections of hearts stained for WGA staining. (I-J) Detection of *BNP* and *β-MHC* protein expression by western blot analysis in cardiac tissue of rats. WGA: wheat germ agglutinin. Model group: Rats were subjected to AAC and received vehicle (PBS, caudal vein injection) 24 h later once every 2 days for 4 weeks. *miR-30b-5p* group: Rats were subjected to AAC and treated with *miR-30b-5p* via tail vein injection 24 h later every 2 days for 4 weeks. *miR-30b-5p*+MSN group: Rats were

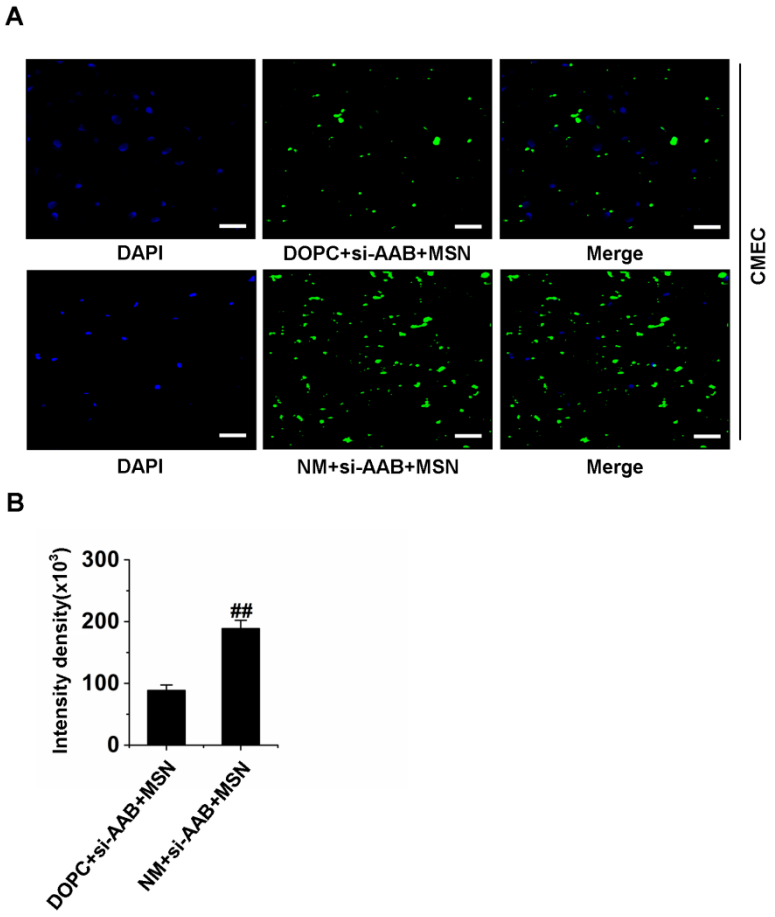
subjected to AAC and treated with *miR-30b-5p*+MSN via tail vein injection 24 h later every 2 days for 4 weeks. NM+*miR-30b-5p*+MSN group: Rats were subjected to AAC and treated with NM+*miR-30b-5p*+MSN via tail vein injection 24 h later every 2 days for 4 weeks. Data were represented by means ± SEM (n=6). $^{\#\#}P<0.01$ vs. sham group; $^{**}P<0.01$ vs. model group. Scale bars, 50 μm.

Supplementary figure 10



Supplementary figure S10. The conserved sites of lncRNA *AAB* between rat and human

Supplementary figure.11



Supplementary figure S11. The cell uptake is evaluated by immunofluorescence

(A-B) Cellular uptake of NM+si-*AAB*+MSN and DOPC+si-*AAB*+MSN by CMECs exposed to Ang II for 24 h, was observed by fluorescence microscopy. DOPC: dioleoylphosphatidylcholine. Data were represented by means ± SEM (n=6). $^{\#\#}P<0.01$ vs. DOPC+si-*AAB*+MSN group. Scale bars, 50 μm.

Table S1. Changes of cardiac structural and functional indicators in cardiac hypertrophy rat

Index	Sham	Model
HW/BW (mg/g)	2.69±0.22	3.67±0.29##
LVW/BW (mg/g)	2.03±0.18	3.19±0.21##
HW/TL (mg/mm)	28.45±1.89	39.62±2.25##
LVPWd (mm)	1.21±0.11	1.52±0.12#
LVIDd (mm)	3.82±0.29	5.10±0.28#
LVIDs (mm)	2.11±0.13	3.91±0.24##
LVFS (%)	41.2±2.31	24.87±1.46##
LVEF (%)	78.15±4.52	57.86±3.21##

Data were represented by means ± SEM (n=6). #*P*<0.05 and ##*P*<0.01 vs. sham group.

Table S2. Changes of hemodynamic index in cardiac hypertrophy rat

Hemodynamic index	Sham	Model
LVSP (mmHg)	125.36±6.74	81.32±4.30##
LVEDP (mmHg)	5.12±0.24	13.27±1.04##
$+dp/dt_{max}$ (mmHg/ms)	4.82±0.27	2.81±0.21##
$-dp/dt_{max}$ (mmHg/ms)	-4.58±0.26	-2.78±0.18##

Data were represented by means ± SEM (n=6). ##*P*<0.01 vs. sham group.

Table S3. Changes of oxidative stress indexes in cardiac hypertrophy rat

Oxidative stress index	Sham	Model
SOD（U/mg protein）	130.55±7.63	70.9±4.44##
Mn-SOD (U/mg protein)	19.87±1.34	9.86±0.85##
Cu/Zn-SOD (U/mg protein)	14.80±1.03	7.62±0.56##
CAT（U/mg protein）	42.36±3.17	20.4±2.33##
MDA（nmol/mg protein）	3.2±0.34	5.99±0.45##
GSH（nmol/mg protein）	40.35±2.87	18.0±1.92##
GSH-PX (U/mg protein)	60.21±3.57	35.45±2.31##

Data were represented by means ± SEM (n=6). ##*P*<0.01 vs. sham group.

Table S4. Changes of oxidative stress indexes in CMECs induced by Ang II

Oxidative stress index	Control	Model
SOD（U/mg protein）	121.36±6.85	88.52±4.22#
Mn-SOD (U/mg protein)	18.22±1.41	9.52±0.92##
Cu/Zn-SOD (U/mg protein)	15.03±1.12	6.99±0.60##
CAT（U/mg protein）	38.96±3.25	22.35±2.38##
MDA（nmol/mg protein）	3.31±0.30	5.55±0.42##
GSH（nmol/mg protein）	38.65±2.62	20.30±1.65##
GSH-PX (U/mg protein)	58.65±3.68	40.35±2.25#

Data were represented by means ± SEM (n=6). #*P*<0.05 and ##*P*<0.01 vs. control group.

Table S5. Eleven lncRNAs shared a highly conserved binding site with miR-30b-5p in rat

>rno-miR-30b-5p	ENSRNOT00000084731	164	-14.9	2 17	28 49	15	86.67%	86.67%	149.1
>rno-miR-30b-5p	ENSRNOT00000090427	167	-18.42	2 21	947 969	20	75.00%	85.00%	148.58
>rno-miR-30b-5p	ENSRNOT00000086616	162	-14.58	2 21	960 980	19	78.95%	78.95%	147.42
>rno-miR-30b-5p	ENSRNOT00000077548	168	-21.46	2 21	52 71	19	84.21%	89.47%	146.54
>rno-miR-30b-5p	ENSRNOT00000081630	162	-16.5	2 19	102 123	17	76.47%	82.35%	145.5
>rno-miR-30b-5p	ENSRNOT00000079518	162	-17.11	2 16	281 303	15	86.67%	93.33%	144.89
>rno-miR-30b-5p	ENSRNOT00000075897	164	-19.33	2 20	3269 3288	18	83.33%	83.33%	144.67
>rno-miR-30b-5p	ENSRNOT00000084787	163	-19.08	2 20	196 217	18	77.78%	77.78%	143.92
>rno-miR-30b-5p	ENSRNOT00000080464	162	-18.56	2 20	730 752	19	73.68%	84.21%	143.44
>rno-miR-30b-5p	ENSRNOT00000043280	165	-22.17	2 19	714 736	18	83.33%	83.33%	142.83
>rno-miR-30b-5p	ENSRNOT00000087948	160	-18.78	2 21	923 941	19	78.95%	78.95%	141.22

Table S6. Changes of cardiac structural and functional indicators in cardiac hypertrophy rat

Indicator	Sham	Model	si-*AAB*	si-*AAB*+MSN	NM+si-*AAB*+MSN
HW/BW (mg/g)	2.64±0.25	3.88±0.28$^{\#\#}$	3.73±0.27	3.71±0.25	3.06±0.19**
LVW/BW (mg/g)	2.21±0.19	3.22±0.23$^{\#\#}$	3.26±0.26	3.08±0.18	2.68±0.21**
HW/TL (mg/mm)	28.34±1.91	40.03±2.33$^{\#\#}$	39.83±2.08	38.88±2.12	32.18±2.35**
LVPWd (mm)	1.22±0.13	1.71±0.14$^{\#}$	1.66±0.12	1.65±0.14	1.34±0.12**
LVIDd (mm)	3.90±0.25	5.48±0.23$^{\#\#}$	5.19±0.20	5.10±0.22	4.21±0.25**
LVIDs (mm)	2.08±0.12	4.05±0.22$^{\#\#}$	3.91±0.23	3.82±0.23	2.65±0.20**

Data were represented by means ± SEM (n=6). $^{\#}P<0.05$ and $^{\#\#}P<0.01$ vs. sham group; $^{**}P<0.01$ vs. model group.

Table S7. Primer sequences

Name	Forward (5'-3')	Reverse (5'-3')
ptgs2	CAACCAGCAGTTCCAGTATCAG	GAGCAAGTCCGTGTTCAAGG
Ang-2	AGCCAGTCTCCCTTCCAG	AGGCAAGCCATTCTCACA
PDGFR-β	GCACCGAAACAAACACACCTT	ATGTAACCACCGTCGCTCTC
miR-30b-5p	CCAGCAACTGTAAACATCCTACAC	TATGGTTTTGACGACTGTGTGAT
lncRNA *AAB*	TTCGTCCGCCACTCTCAGGATG	TCGGTCAGCCAGGTGCAGATG
TIMP1 siRNA	GGAACGGAAAUUUGCACAUTT	AUGUGCAAAUUUCCGUUCCTT
negative control of *TIMP1* siRNA	UUCUCCGAACGUGUCACGUTT	ACGUGACACGUUCGGAGAATT
miR-30b-5p mimics	UGUAAACAUCCUACACUCAGCU	CUGAGUGUAGGAUGUUUACAUU
negative control of *miR-30b-5p* mimics	UUGAGCCACAGCUGCAUACTT	ACCCGGAAGGCCGCCAAGCTT
lncRNA *AAB* siRNA	GUCCUGGAUUCCACCUUAATT	UUAAGGUGGAAUCCAGGACTT