SUPPLEMENTARY   MATERIAL

The cross-talk between PARylation and SUMOylation in C/EBPβ at K134 site participates in pathological cardiac hypertrophy

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Supplementary Figures and Figure Legends
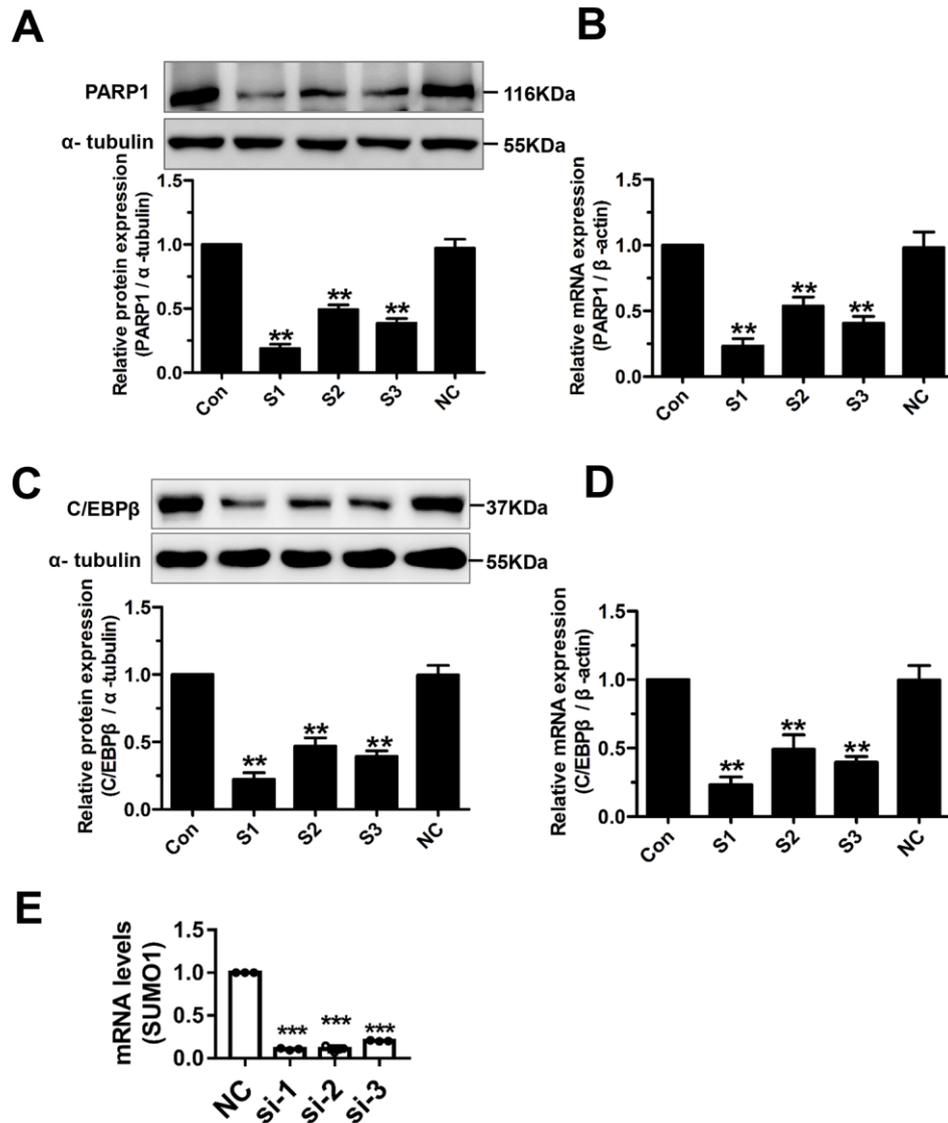
Figure S1. qPCR and Western blot were conducted to confirm the silencing efficiency of siRNAs. (**A** and **B**) The silencing efficiency of three different siRNA against PARP1 (S1, S2 and S3) was tested. (**C** and **D**) The silencing efficiency of three different siRNA against C/EBPβ (S1, S2 and S3) was tested. (**E**) The silencing efficiency of three different siRNA against SUMO1 (si-1, si-2 and si-3) was tested. **$P < 0.01$ *vs.* the NC group, ***$P < 0.001$ *vs.* the NC group. *n*=5.
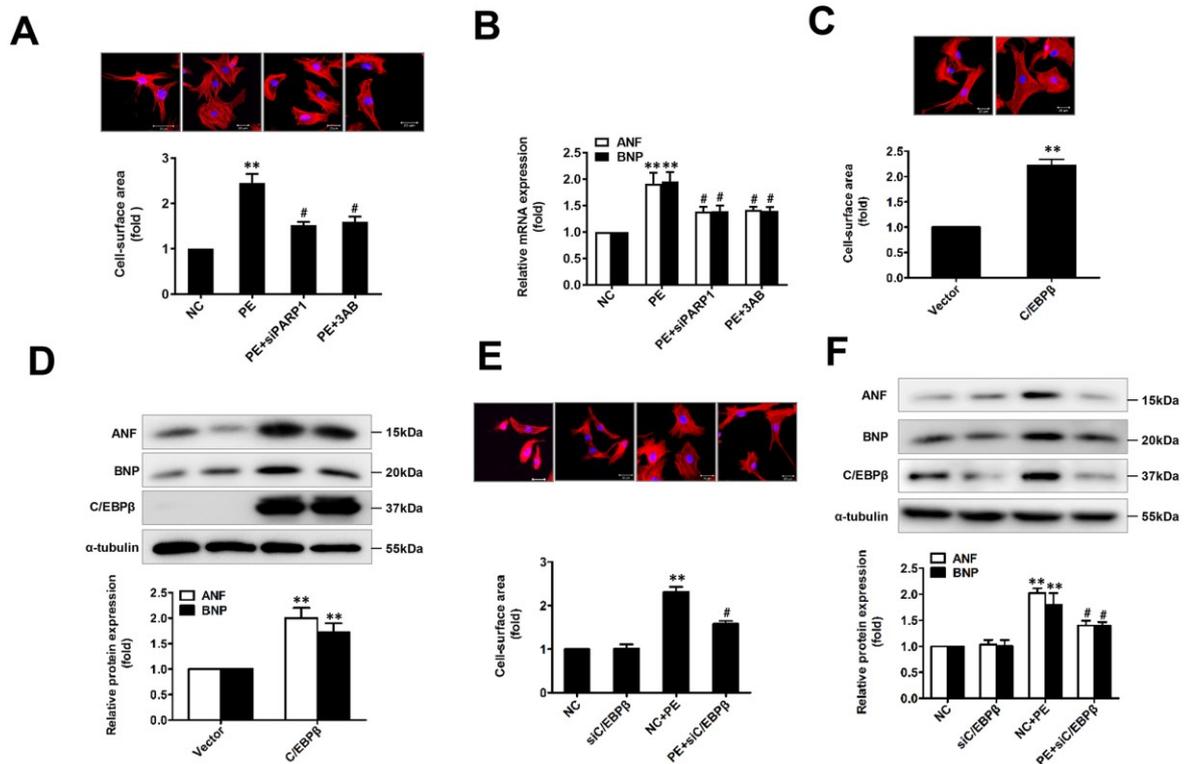
Figure S2. PARP1 and C/EBPβ participate in cardiac hypertrophy. NRCMs were transfected with siPARP1 or incubated with 3-aminobenzamide (3AB) (20 μmol/L) followed by incubation with phenylephrine (PE) (100 μmol/L for 24 h). (**A**) the cell surface area was observed using rhodamine-phalloidin staining, and the mRNA levels of atrial natriuretic factor (ANF) and brain natriuretic polypeptide (BNP) (**B**) were determined by qRT-PCR. NRCMs were transfected with HA-C/EBPβ plasmid and the hypertrophic responses were determined by measuring cell surface area (**C**) and the protein levels of ANF and BNP (**D**). NRCMs were transfected with siC/EBPβ followed by incubation with PE (100 μmol/L for 24 h), the reversed hypertrophic responses were demonstrated by cell surface area (**E**) and the protein levels of ANF and BNP (**F**). $*P < 0.05$ vs. the Control group, $**P < 0.01$ vs. the Control group, $^{\#}P < 0.05$ vs. the PE treatment group. $n=3$.
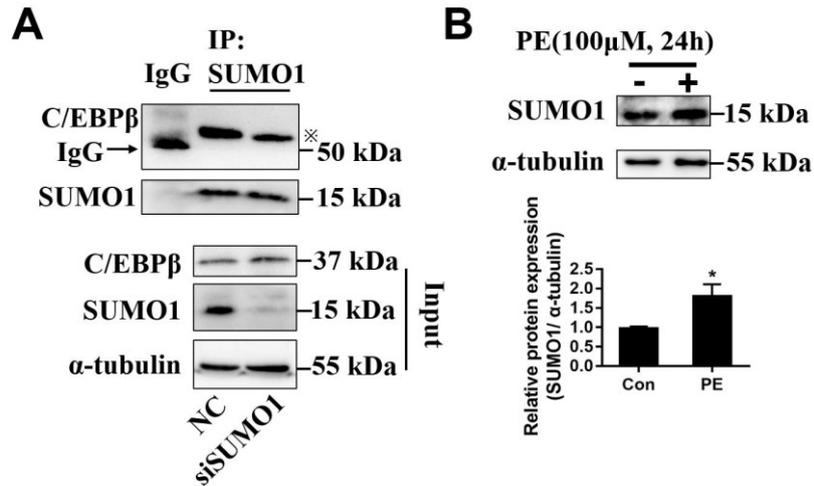
Figure S3. A, NRCMs were transfected with SUMO1 siRNA, immunoprecipitated with anti-SUMO1 antibody and subsequently subjected to immunoblotting analysis. ※ indicates C/EBPβ-SUMO. **B**, Neonatal rat cardiomyocytes (NRCMs) were treated with PE (100 µmol/L for 24 h), the protein level of SUMO1 was determined by western blotting analysis. *n*=3.

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Figure S4. The protein sequences of PARP1 and its mutant (E988K) were aligned using Basic Local Alignment Search Tool (BLAST). We produced a mutant form of PARP1, E988K, which lacks catalytic activity. Glutamic acid (E) in the 988 site of PARP1 was replaced by lysine residue (K), and its protein sequences were aligned to wild type PARP1.

Sequence ID: Query_186913　Length: 297　Number of Matches: 1

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Figure S5. The protein sequences of C/EBPβ and its mutant (K134A) were aligned using Basic Local Alignment Search Tool (BLAST). We produced a mutant form of C/EBPβ, Lysine acid (K) at the 134 site of C/EBPβ was replaced by Arginine residue (A), and its protein sequences were aligned to wild type C/EBPβ.

Sequence ID: Query_223603　Length: 292　Number of Matches: 1

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Figure S6. The protein sequences of C/EBPβ and its truncated mutant (ΔSUMO) were aligned using Basic Local Alignment Search Tool (BLAST). The conserved motif (LKAEP) was truncated, which lacks the sites for poly(ADP-ribosyl)ation, and its protein sequences were aligned to wild type C/EBPβ.

Supplementary Tables

Table S1. Rat-specific primer sequences for quantitative reverse transcription polymerase chain reaction (qRT-PCR)

Target gene	Sequences
ANF	Forward: 5'-GGAAGTCAACCCGTCTCA-3'
	Reverse: 5'-AGCCCTCAGTTTGCTTTT-3'
BNP	Forward: 5'-TTTGGGCAGAAGATAGACCG-3'
	Reverse: 5'-AGAAGAGCCGCAGGCAGAG-3'
C/EBPβ	Forward:5'-AAGATGCGCAACCTGGAGAC-3'
	Reverse:5'-CCTTCTTCTGCAGCCGCTC-3'
SUMO1	Forward:5'-TCCAAAAGTGGCTTCACTGGA -3'
	Reverse:5'-AGCAGTGTCTGTTGCGTACA-3'
β-actin	Forward: 5'-TCGTGCGTGACATTAAAGAG-3'
	Reverse: 5'-ATTGCCGATAGTGATGACCT-3'

Table S2. Sequences of siRNAs for C/EBPβ, PARP1 and SUMO1

siRNA	Sequences
C/EBPβ-rat-01	Sense: 5'-ACCUCUUCGCCGACGACUATT-3'
	Antisense:5'-UAGUCGUCGGCGAAGAGGUTT-3'
C/EBPβ-rat-02	Sense:5'-AGCUGAGCGACGAGUACAATT-3'
	Antisense: 5'-UUGUACUCGUCGCUCAGCUTT-3'
C/EBPβ-rat-03	Sense:5'-CCAUGGAAGUGGCCAACUUTT-3'
	Antisense:5'-AAGUUGGCCACUUCCAUGGTT-3'
PARP1-rat-01	Sense: 5'-GGGACUAACUCCUAUUACATT-3'
	Antisense:5'-UGUAAUAGGAGUUAGUCCCTT-3'
PARP1-rat-02	Sense: 5'-CCAUGUUCGAUGGAAAAGUTT -3'
	Antisense:5'-ACUUUUCCAUCGAACAUGGTT -3'
PARP1-rat-03	Sense: 5'-CCUACCUCAAGAAAUUAAATT -3'
	Antisense:5'-UUUAAUUUCUUGAGGUAGGTT-3'

SUMO1-rat-01	Sense: 5'-GCAGUGAGAUCCAUUUCAATT-3'	
	Antisense:5'-UUGAAAUGGAUCUCACUGCTT-3'	
SUMO1-rat-02	Sense: 5'-GGAAGAAGACGUGAUUGAATT-3'	
	Antisense:5'-UUCAAUCACGUCUUCUUCCTT-3'	
SUMO1-rat-03	Sense: 5'- GACAGGGAGUUCCAAUGAATT-3'	
	Antisense:5'-UUCAUUGGAACUCCCUGUCTT-3'	
NC	Sense:5'- UUCUCCGAACGUGUCACGUTT-3'	
	Antisense:5'- ACGUGACACGUUCGGAGAATT-3'	

Table S3. Echocardiographic parameters of rats submitted to intramyocardial injection of adenovirus encoding PARP1 and ALA intraperitoneal injection

Group	Ad-GFP	Ad-PARP1	Ad-PARP1+ALA
LVAWd (mm)	1.62±0.04	2.32±0.09*	2.08±0.07
LVAWs(mm)	2.05±0.19	3.12±0.12*	2.89±0.04
LVIDd(mm)	7.62±0.29	7.36±0.47*	7.78±0.08[#]
LVIDs(mm)	4.90±0.13	4.32±0.13*	4.55±0.11
LVPWd(mm)	2.16±0.08	2.32±0.16*	2.21±0.04[#]
LVPWs(mm)	3.08±0.09	3.49±0.26*	3.28±0.02[#]
EF%	62.38±4.9	70.32±2.91*	64.92±2.25[#]
FS%	34.95±2.8	40.59±1.65*	38.46±1.02[#]
SV(μL)	219.25±12.8	274.36±14.2*	252.27±13.2[#]
LVEDV(μL)	358.05±18.3	390.25±17.8*	382.36±16.2
LVSV(μL)	165.35±10.8	175.26±9.6	168.26±10.3

Data were presented as mean ± SD. *$P<0.05$ *vs*. Ad-GFP group, [#]$P < 0.05$ *vs*. Ad-PARP1 group, *n*=8.

Table S4. Echocardiographic parameters of rats submitted to abdominal aortic constriction (AAC) surgery and 3AB intraperitoneal injection

Group	Sham	AAC	AAC+3AB
IVS-d (mm)	1.64±0.06	2.25±0.06*	1.82±0.04[#]
IVS-s (mm)	2.26±0.10	3.16±0.08*	2.69±0.07[#]
LVID-d (mm)	8.01±0.09	7.32±0.07*	7.67±0.09[#]
LVID-s (mm)	5.34±0.21	4.21±0.14*	4.97±0.11[#]
LVPW-d (mm)	1.82±0.08	2.43±0.09*	2.09±0.04[#]
LVPW-s (mm)	2.64±0.06	3.34±0.09*	2.98±0.04[#]
EF%	61.80±1.60	71.63±1.84*	64.92±2.29[#]
FS%	36.00±1.50	45.48±1.10*	40.46±1.04[#]
SV(μL)	221.25±11.6	278.36±14.1*	248.22±11.2[#]
LVSV(μL)	348.05±15.7	387.24±18.2*	378.36±14.3
LVEDV(μL)	159.35±11.4	165.26±9.6	164.26±10.3

Data are presented as mean ± SD. *$P<0.05$ *vs*. the sham group, [#]$P < 0.05$ *vs*. the AAC group, *n* = 8.