Geniposide Protects against Cardiac Hypertrophy via GLP-1R/AMPKα Signalling Pathway

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Short title: Geniposide against Cardiac Hypertrophy

SUPPLEMENTAL MATERIAL

SUPPLEMENTAL METHOD

XBP-1 splicing assay

The XBP-1 splicing assay was preformed according to a previous study. The total RNA was extracted from hearts and cells, and then was reverse-transcribed into cDNA using the Transcriptor First Strand cDNA Synthesis Kit (Roche, 04896866001). Levels of spliced and unspliced XBP-1 by using primers flanking the intron excised by IRE1 exonuclease activity. The running conditions were: 50 ℃ (30 min); 95 ℃ (15 min); 30 cycles of 94° C (1 min), 55 ℃ (1 min), 72 ℃ (1min); 72 ℃ (10 min).

Table S1: Primer sequences used for RT-PCR

Gene	Species		Sequence (5'-3')
GAPDH	Mouse	Forward	ACTCCACTCACGGCAAATTC
		Reverse	TCTCCATGGTGGTGAAGACA
GAPDH	Rat	Forward	GACATGCCGCCTGGAGAAAC
		Reverse	AGCCCAGGATGCCCTTTAGT
ANP	Mouse	Forward	ACCTGCTAGACCACCTGGAG
		Reverse	CCTTGGCTGTTATCTTCGGTACCGG
ANP	Rat	Forward	AAAGCAAACTGAGGGCTCTGCTCG
		Reverse	TTCGGTACCGGAAGCTGTTGCA
		Reverse	GCCATTTCCTCCGACTTTTCTC
β-MHC	Mouse	Forward	CCGAGTCCCAGGTCAACAA
		Reverse	CTTCACGGGCACCCTTGGA
GRP78	Rat	Forward	TGGAGGTGGGCAAACCAAGACA
		Reverse	TTGGTTGCTTGTCGCTGGGCAT
XBP-1	Rat	Forward	AAGCGCTGCGGAGGAAACTGAA
		Reverse	TCGTCAGGATCCAGCGTGTCCATT
GLP-1R	Mouse	Forward	TGCAACCGGACCTTTGATGA
		Reverse	TTGTAGCACACTACTGGCCC
GLP-1R	Rat	Forward	GCTGCTGTTCGTTATCCCCT
		Reverse	AGGAAGTTGACCCCGATTGC
AMPKα1	Rat	Forward	ATCCGCAGAGAGATCCAGAA
		Reverse	CGTCGACTCTCCTTTTCGTC
AMPKα2	Rat	Forward	CGGAGGTCATCTCAGGAAGGCTG
		Reverse	ACGTGCTCATCGTCGAACGGG
XBP-1 (the splicing assay)	Mice	Forward	GAACCAGGAGTTAAGAACACG
		Reverse	AGGCAACAGTGTCAGAGTCC

Table S2: The information of the primary antibodies used in western

Antibody name	Company	Number	Dilution
GAPDH	Santa Cruz	sc-25778	1:200
PERK	Santa Cruz	sc-13073	1:200
phospho-PERK	Santa Cruz	sc-32577	1:200
GRP78	Santa Cruz	sc-13968	1:200
XBP-1	Santa Cruz	sc-7160	1:200
ANP	Santa Cruz	sc-20158	1:200
AMPKα	Cell Signaling Technology	2603P	1:1000
phospho-AMPKα	Cell Signaling Technology	2535	1:1000
ACC	Cell Signaling Technology	3676P	1:1000
phospho-ACC	Cell Signaling Technology	3661P	1:1000
mTOR	Cell Signaling Technology	2983	1:1000
phospho-mTOR	Cell Signaling Technology	2971	1:1000
ERK	Cell Signaling Technology	4695	1:1000
phospho-ERK	Cell Signaling Technology	4370P	1:1000
ATF6	Abcam	ab103673	1:1000

SUPPLEMENTAL FIGURES AND FIGURE LEGENDS:

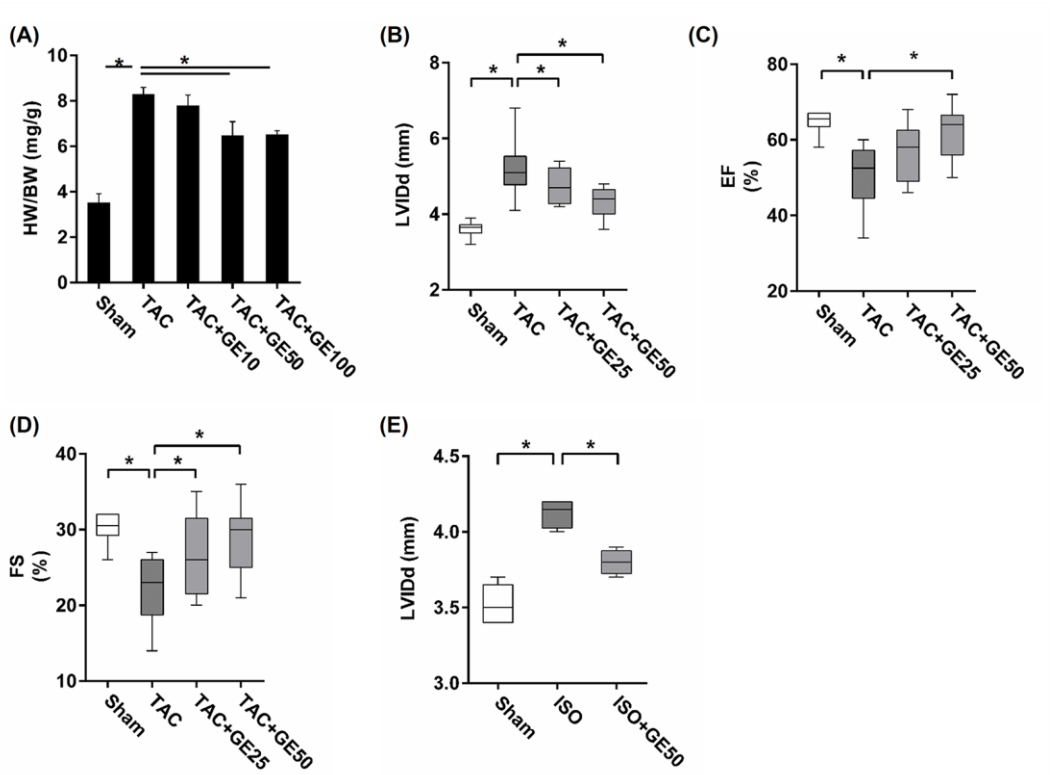

Figure S1. Effects of geniposide (GE) on cardiac hypertrophy induced by pressure overload. HW/BW: heart weight/body weight. A, The preliminary experiment indicated that GE (50mg/kg-100mg/kg) protected against cardiac hypertrophy (n=5). B-D, echocardiographic parameters in mice after 8 weeks of TAC (n=14). E, Left ventricular internal diastolic diameter (LVIDd) in mice subjected to isoprenaline (ISO) injection (n=8). $^{*}P<0.05$ versus matched control.

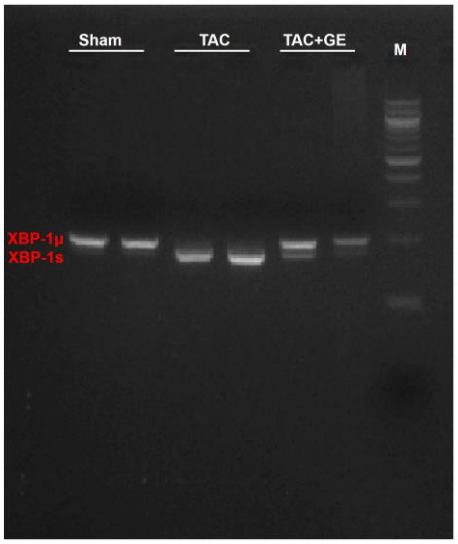

Figure S2. Effects of geniposide (GE) suppressed the production of transcription factor XBP1s. M: marker.

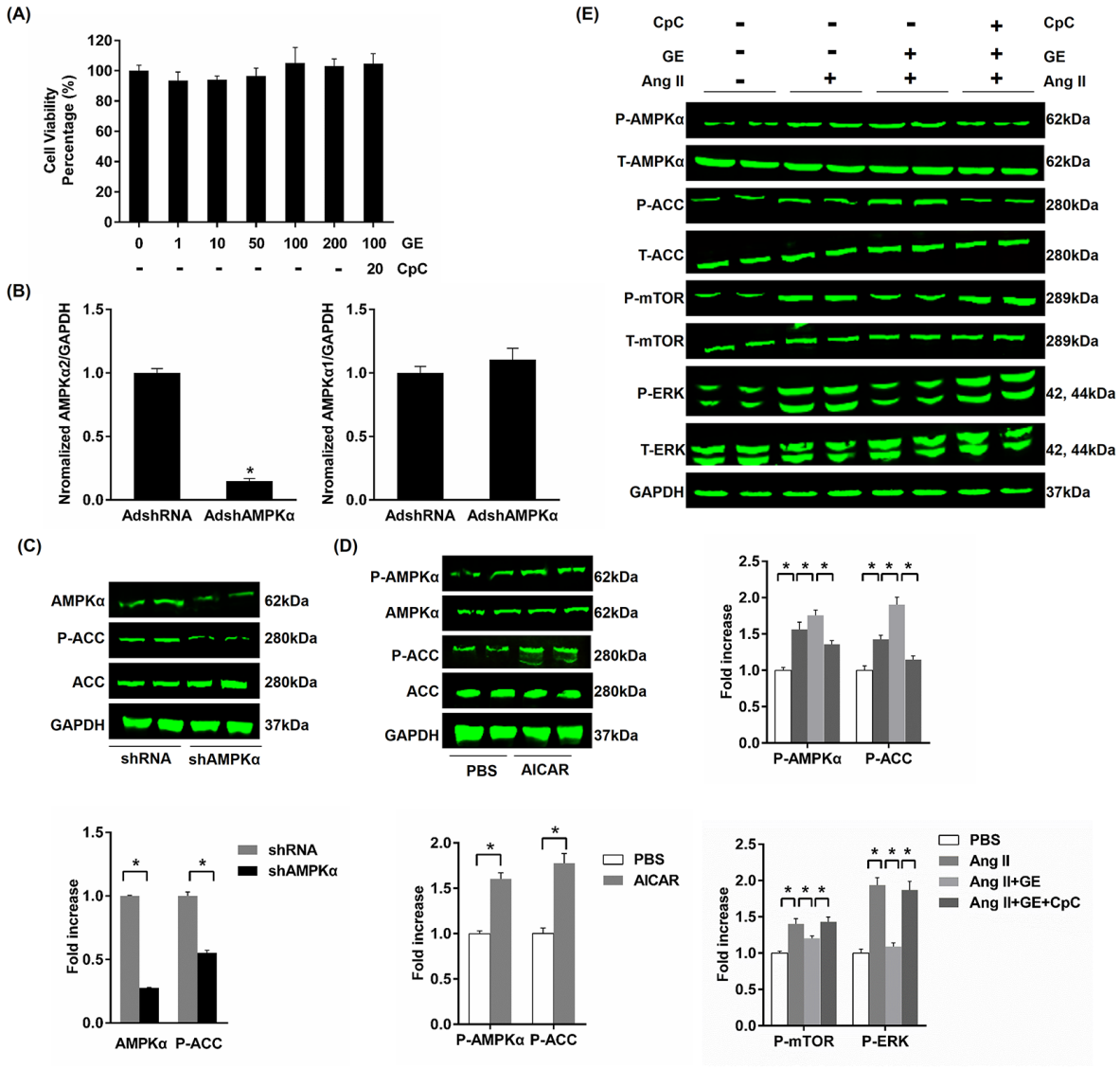

Figure S3. Geniposide (GE) suppressed hypertrophy of myocytes in vitro. A, Viability of H9c2 cells (n=3). B, mRNA of 5'-adenosine monophosphate-activated protein kinase-α (AMPKα)1 and AMPKα2 after adenovirus infection in neonatal rat cardiac myocytes (n=6). C, Changes of AMPKα and acetyl-CoA carboxylase (ACC) after adenovirus infection in neonatal rat cardiac myocytes (n=6). D, Changes of AMPKα and ACC after AICAR treatment in H9c2 (n=6). E, Phosphorylated AMPKα and downstream proteins after Compound (CpC) treatment in H9c2 cells (n=6). $^{*}P<0.05$ versus matched control.

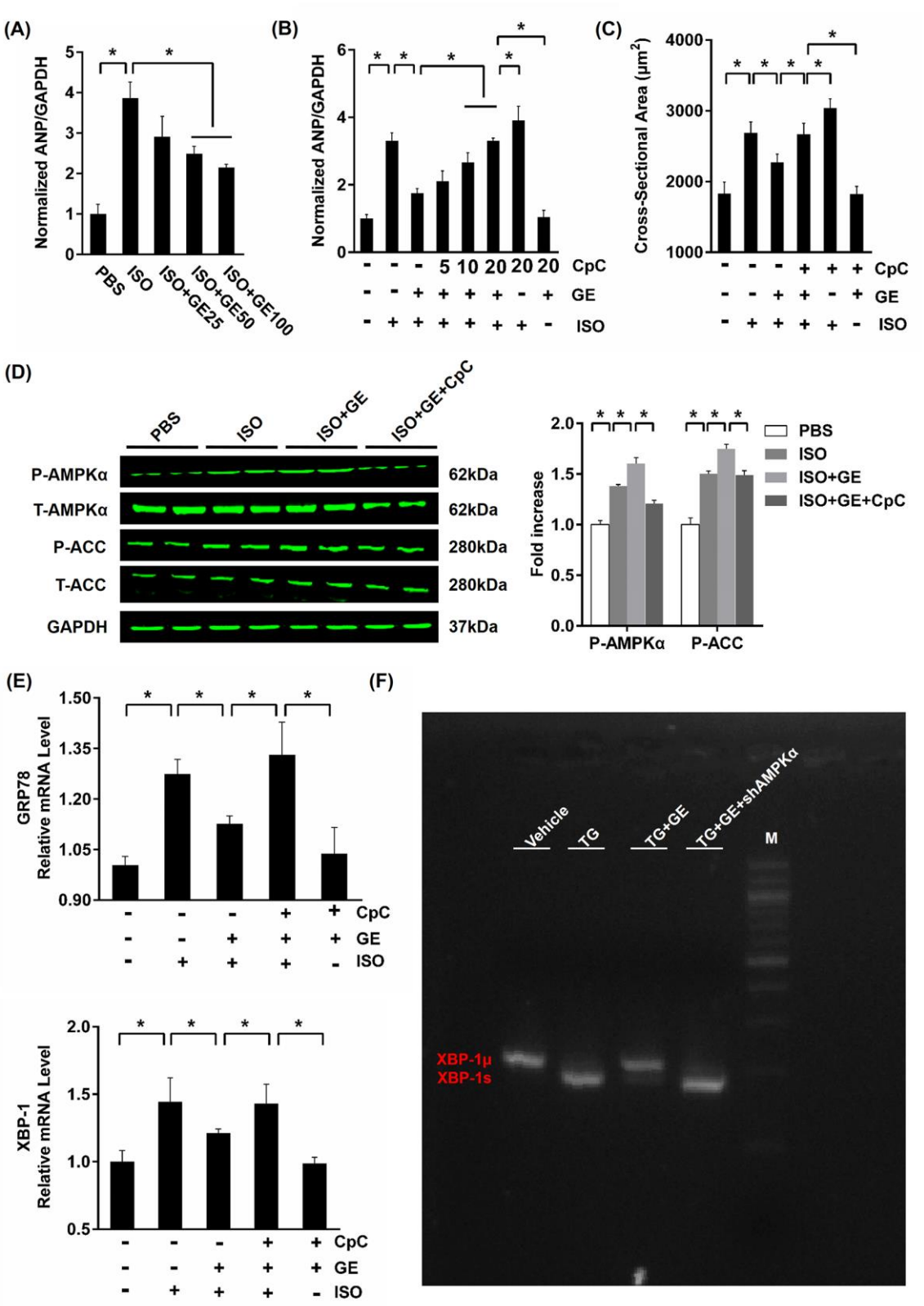

Figure S4. The effects of geniposide (GE) on hypertrophy induced by isoprenaline (ISO) (10mmol/L for 24h) in H9c2 cell. A, The levels of atrial natriuretic peptide (ANP) induced by ISO in indicated groups (n=5). B, Compound (CpC) (5-20 μM for 24h) reversed hypertrophic response induced by ISO (n=5). C, The cross-sectional area of H9c2 myocytes (n=5). D, The protein levels of phosphorylated 5'-adenosine

monophosphate-activated protein kinase-α (AMPKα) in indicated groups (n=6). E, GE suppressed ISO-induced ER stress (n=5). F, shAMPKα offset the effect of GE against the production of transcription factor XBP1s. M: marker. $^{*}P<0.05$.

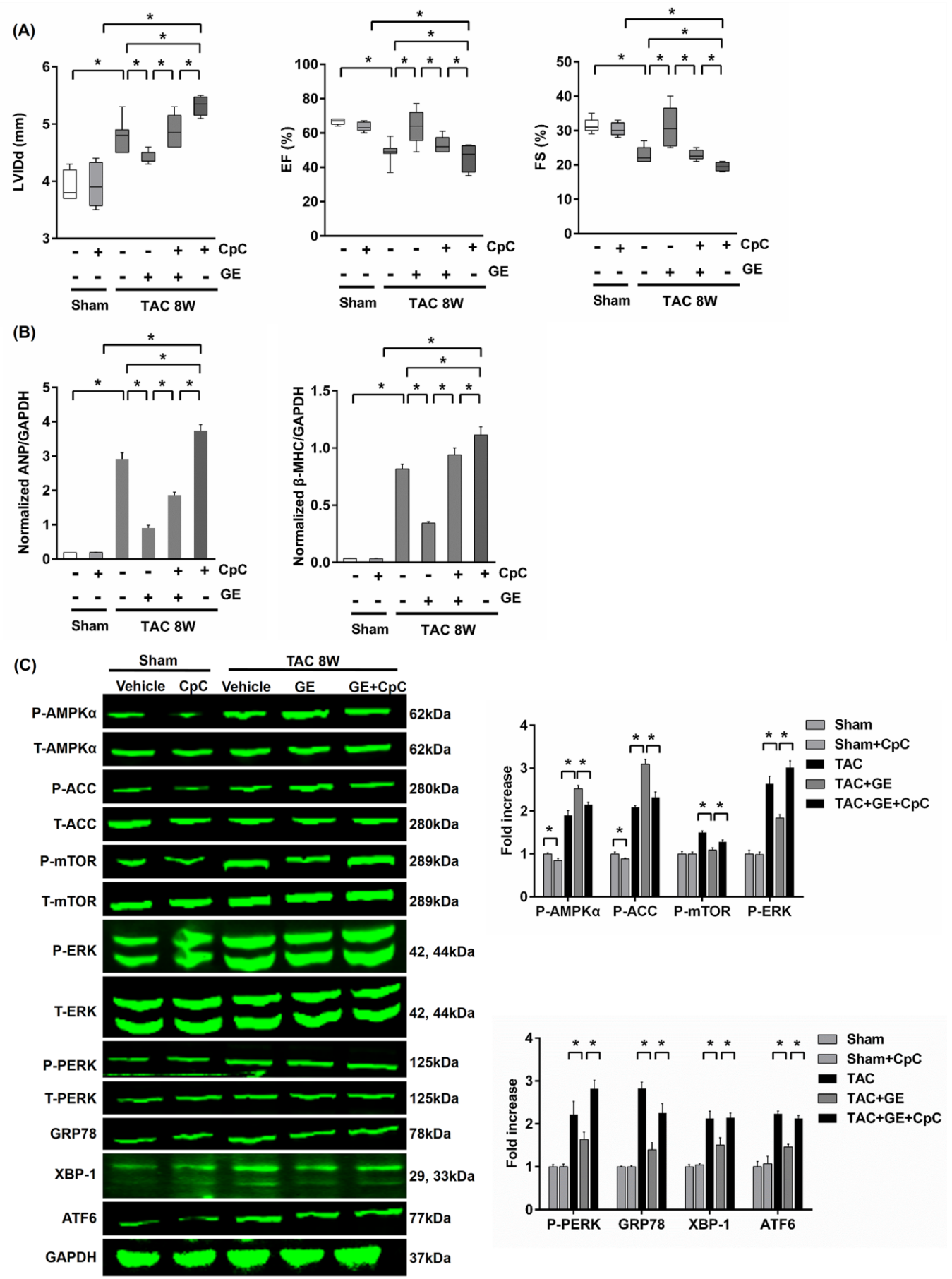
(A)
LVIDd (mm)
EF (%)
FS (%)
CpC
GE
Sham
TAC 8W
(B)
Normalized ANP/GAPDH
Normalized β-MHC/GAPDH
(C)
Sham
TAC 8W
Vehicle
CpC
Vehicle
GE
GE+CpC
P-AMPKα
T-AMPKα
P-ACC
T-ACC
P-mTOR
T-mTOR
P-ERK
T-ERK
P-PERK
T-PERK
GRP78
XBP-1
ATF6
GAPDH
62kDa
62kDa
280kDa
280kDa
289kDa
289kDa
42, 44kDa
42, 44kDa
125kDa
125kDa
78kDa
29, 33kDa
77kDa
37kDa
Fold increase
Sham
Sham+CpC
TAC
TAC+GE
TAC+GE+CpC
P-AMPKα
P-ACC
P-mTOR
P-ERK
P-PERK
GRP78
XBP-1
ATF6

Figure S5. Compound (CpC) offset effects of geniposide (GE) on hypertrophy in vivo. A, Echocardiographic parameters in mice subjected to CpC (n=7). B, The mRNA expressions of hypertrophic markers (n=6). C, CpC attenuates 5’-adenosine monophosphate-activated protein kinase-α (AMPKα) and related targets (n=6). $^{*}P<0.05$ versus matched control.

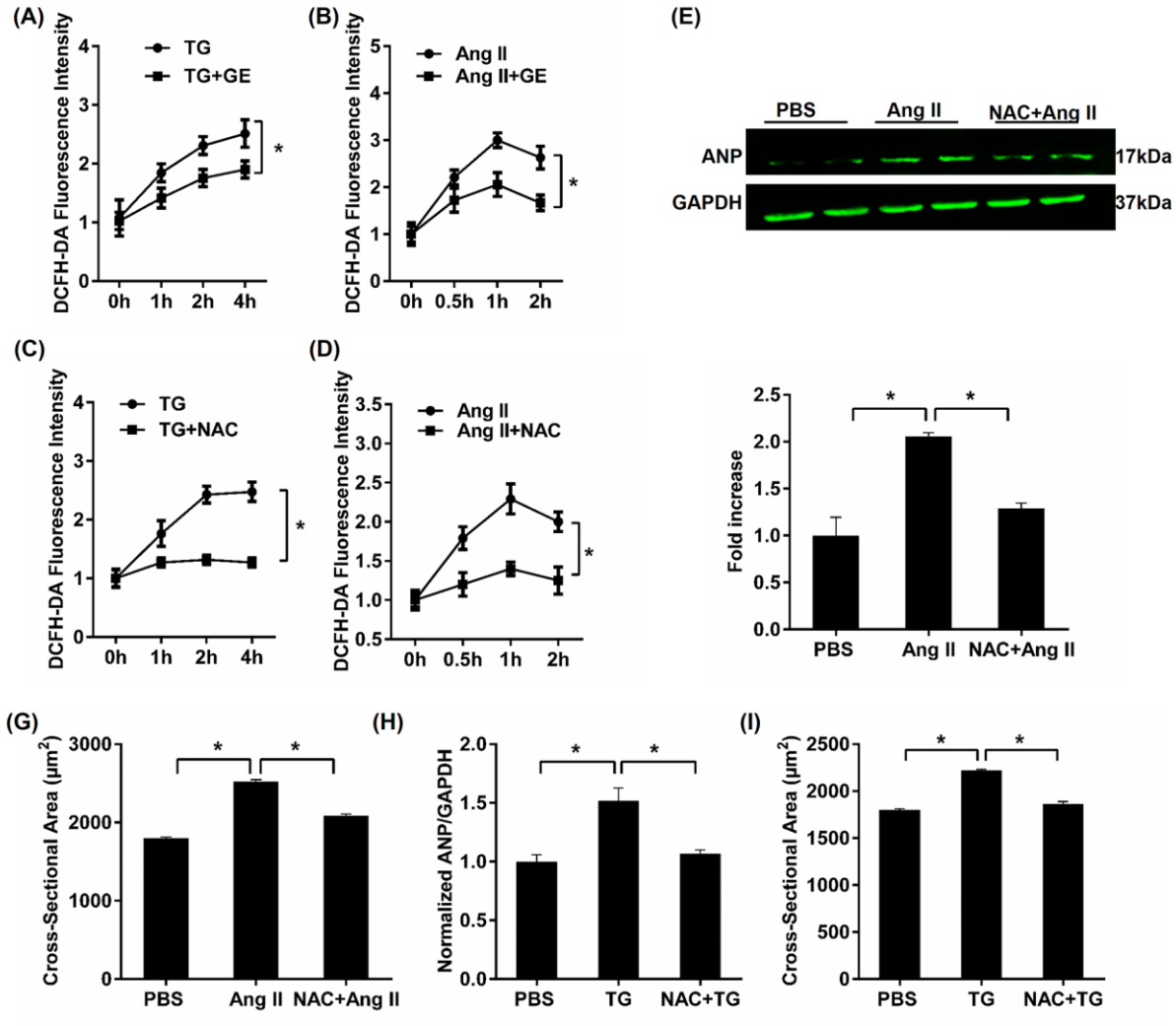

Figure S6. Endoplasmic stress induces accumulation of reactive oxygen species (ROS). A, Geniposide (GE) suppressed the accumulation of ROS induced by thapsigargin (TG). B, GE suppressed the accumulation of ROS induced by Angiotensin II (Ang II). C, N-acetylcysteine (NAC) blunted the accumulation of ROS induced by TG. D, NAC blunted the accumulation of ROS induced by Ang II. E-F, NAC almost completely abolished the increase of atrial natriuretic peptide (ANP) and cross-sectional area (n=6). G-H. NAC suppressed the increase of ANP and cross-sectional area induced by TG (0.5μM for 48h) (n=6). ROS were detected by DCFH-DA in 3 experiments independently. Statistical analysis of ROS was performed using a repeated measures ANOVA. $^{*}P<0.05$ versus matched control.

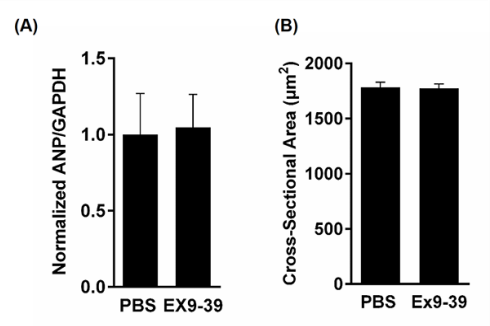

Figure S7. Atrial natriuretic peptide (ANP) and cross-sectional area of myocytes induced by Ex9-39 (n=6).

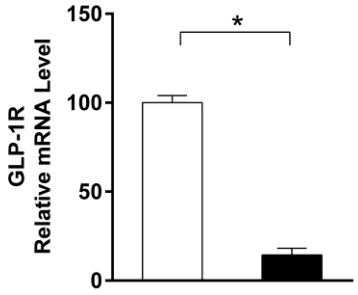

Figure S8. The mRNA levels of glucagon-like peptide 1 receptor (GLP-1R) in the hypertrophic heart after infection (n=8). $^{*}P<0.05$ versus matched control.

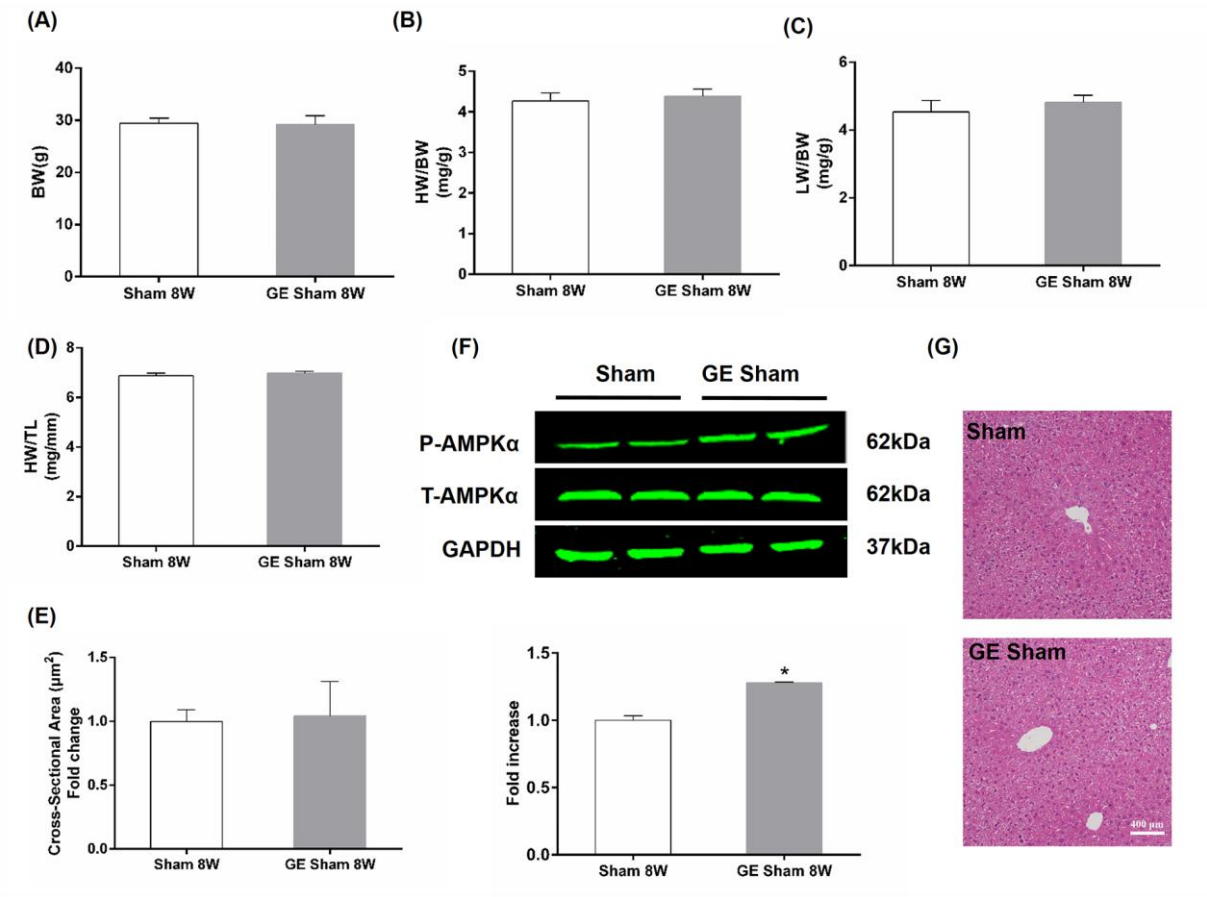

Figure S9. Effects of geniposide (GE) on the heart at baseline. A-D, Statistical results of body weight (BW), heart weight (HW)/BW, HW/tibial length (TL) of the indicated groups (n=6). E, The cross-sectional areas of myocytes (n=6). F, The protein levels of phosphorylated 5'-adenosine monophosphate-activated protein kinase-α (AMPKα) in mice from indicated groups (n=6). G, Hepatic morphology after GE treatment (n=5). $^{*}P<0.05$ versus sham.