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Research Paper

Hypertrophic preconditioning cardioprotection after myocardial ischaemia/reperfusion injury involves ALDH2-dependent metabolism modulation

Lei-Lei Ma [a,b,c,1], Zhi-Wen Ding [a,b,c,1], Pei-Pei Yin [a,b,c,1], Jian Wu [a,b,c,1], Kai Hu [a,b,c], Ai-Jun Sun [a,b,c,***], Yun-Zeng Zou [a,b,c,**], Jun-Bo Ge [a,b,c,*]

[a] Department of Cardiology, Shanghai Institute of Cardiovascular Diseases, Zhongshan Hospital, Fudan University, Shanghai, China
[b] NHC Key Laboratory of Viral Heart Diseases, Shanghai, China
[c] Key Laboratory of Viral Heart Diseases, Chinese Academy of Medical Sciences, Shanghai, China

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ABSTRACT

Brief episodes of ischaemia and reperfusion render the heart resistant to subsequent prolonged ischaemic insult, termed ischaemic preconditioning. Here, we hypothesized that transient non-ischaemic stress by hypertrophic stimulation would induce endogenous cardioprotective signalling and enhance cardiac resistance to subsequent ischaemic damage. Transient transverse aortic constriction (TAC) or Ang-II treatment was performed for 3–7 days in male mice and then withdrawn for several days by either aortic debanding or discontinuing Ang-II treatment, followed by subsequent exposure to regional myocardial ischaemia by *in situ* coronary artery ligation. Following ischaemia/reperfusion (I/R) injury, myocardial infarct size and apoptosis were markedly reduced and contractile function was significantly improved in the TAC preconditioning group compared with that in the control group. Similar results were observed in mice receiving Ang-II infusion. Mechanistically, TAC preconditioning enhanced ALDH2 activity, promoted AMPK activation and improved mitochondrial energy metabolism by increasing myocardial OXPHOS complex expression, elevating the mitochondrial ATP content and improving viable myocardium glucose uptake. Moreover, TAC preconditioning significantly mitigated I/R-induced myocardial iNOS/gp91^{phox} activation, inhibited endoplasmic reticulum stress and ameliorated mitochondrial impairment. Using a pharmacological approach to inhibit AMPK signalling in the presence or absence of preconditioning, we demonstrated AMPK-dependent protective mechanisms of TAC preconditioning against I/R injury. Furthermore, treatment with adenovirus-encoded ALDH2 partially emulated the actions of hypertrophic preconditioning, as evidenced by improved mitochondrial metabolism, inhibited oxidative stress-induced mitochondrial damage and attenuated cell death through an AMPK-dependent mechanism, whereas genetic ablation of ALDH2 abrogated the aforementioned actions of TAC preconditioning. The present study demonstrates that preconditioning with hypertrophic stress protects the heart from I/R injury *via* mechanisms that improve mitochondrial metabolism, reduce oxidative/nitrative stress and inhibit apoptosis. ALDH2 is obligatorily required for the development of cardiac hypertrophic preconditioning and acts as the mediator of this process.

* Corresponding author. Department of Cardiology, Shanghai Institute of Cardiovascular Diseases, Zhongshan Hospital, Yi Xue Yuan Rd, Xu Hui District, Shanghai, 200032, China.
** Corresponding author. Department of Cardiology, Shanghai Institute of Cardiovascular Diseases, Zhongshan Hospital, Yi Xue Yuan Rd, Xu Hui District, Shanghai, 200032, China.
*** Corresponding author. Department of Cardiology, Shanghai Institute of Cardiovascular Diseases, Zhongshan Hospital, Yi Xue Yuan Rd, Xu Hui District, Shanghai, 200032, China.
E-mail addresses: sun.aijun@zs-hospital.sh.cn (A.-J. Sun), zou.yunzeng@zs-hospital.sh.cn (Y.-Z. Zou), jbge@zs-hospital.sh.cn (J.-B. Ge).
[1] These author contributed equally to this work.

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1. Introduction

Acute myocardial infarct (AMI) commonly caused by thrombotic occlusion of a coronary artery remains a leading cause of mortality worldwide [1]. Timely reperfusion is the most effective treatment to limit myocardial infarct and rescue cardiomyocytes from death. However, despite early reperfusion therapy, morbidity and mortality are still substantial in patients with AMI. It has been reported that approximately 7% of patients with AMI die and 22% of survivors develop chronic heart failure at 1 year after AMI [2]. One reasonable explanation for the substantial morbidity and mortality of subjects with AMI is that the restoration of blood flow to the occluded coronary artery can produce additional myocardial injury, a process termed myocardial ischaemia/reperfusion (I/R) injury [3]. Therefore, it is urgent to develop novel therapeutic strategies to further reduce infarct size, preserve cardiac function, ameliorate adverse cardiac remodelling, and improve the outcomes of patients with AMI.

More than 3 decades ago, myocardial ischaemic preconditioning (IPC), involving brief cycles of coronary occlusion and reperfusion, was reported to protect the heart from I/R injury [4]. Regarding its potential application in clinical practice, numerous investigations have been performed to detect the potential mechanisms underlying the cardioprotection of preconditioning. In contrast, non-ischaemic preconditioning has received relatively little consideration and remains largely underdeveloped in cardiovascular physiology. Preconditioning with mechanical stretch, heat challenge and pharmacological agents can partially emulate the actions of IPC [3,5–7]. It was noted that intermittent pressure overload improves myocardial performance and induces a mild hypertrophic response [8–10]. Moreover, pretreatment with angiotensin II or norepinephrine activates protein kinase C and mitigates myocardial infarction in isolated perfused rabbit hearts [11,12], and brief left ventricular pressure overload reduces myocardial infarct size and mitigates the apoptotic response in rats subjected to I/R [13]. Recently, mechanically reducing left ventricular preload through percutaneous left atrial-to-femoral artery bypass before coronary reperfusion was shown to reduce myocardial infarct size by activating the reperfusion injury salvage kinase pathway [14]. More recently, Wei et al. reported that the removal of short-term pressure overload makes the heart more resistant to the development of pathological hypertrophy, called myocardial hypertrophic preconditioning [15]. These studies indicate that transient hypertrophic stimulation may induce endogenous cardioprotective gene expression against subsequent prolonged pressure overload and ischaemic insult [16,17]. However, it is completely unknown whether the removal of short-term or long-term pressure overload renders the heart resistant to subsequent prolonged ischaemic damage. Therefore, our hypothesis is that short-term hypertrophic stimulation can render the heart resistant to subsequent ischaemic insult and slow the progression to heart failure. In this study, we demonstrated that hypertrophic preconditioning exerted cardioprotection against reperfusion injury and involved improvements in mitochondrial energy production and the activation of ALDH2.

2. Methods

2.1. Animals

Male C57BL/6J mice were supplied by the Shanghai Laboratory Animal Center. ALDH2 knockout (ALDH2 KO) mice were produced by a method described previously [18]. All the protocols used conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (the 8th Edition, NRC 2011), and they were approved by the Institutional Review Board of Zhongshan Hospital at Fudan University.

2.2. Drug-induced hypertrophic preconditioning

The animals were anaesthetized by 1% pentobarbital sodium (50 mg/kg, *i.p.*). Then, the osmotic minipump (Alzet) filled with Ang-II (1.1 mg kg^{-1} d^{-1}) or vehicle (0.9% saline) was implanted subcutaneously in the back. Changes from Ang-II to vehicle were performed by replacing the implanted pump (filled with Ang-II) with a new pump (filled with saline).

(1) Effects of Ang-II-induced hypertrophic preconditioning on myocardial I/R (Fig. 1A). Three groups were used: 1) sham group: saline infusion for 4 days; 2) vehicle group: saline infusion for 4 days followed by 40 min of ischaemia and 24 h of reperfusion; and 3) Ang-II group: Ang-II infusion for 1 day and then stopping for 1 day, followed by Ang-II infusion for 2 days and stopping for 2 days, followed by 40 min of ischaemia and 24 h of reperfusion.
(2) Effects of ALDH2 deficiency on cardioprotection by Ang-II-induced hypertrophic preconditioning. Six groups were used: 1) sham group: saline infusion for 4 days; 2) vehicle group: saline infusion for 4 days followed by 40 min of ischaemia and 24 h of reperfusion; 3) Ang-II group: Ang-II infusion for 1 day and then stopping for 1 day, followed by Ang-II infusion for 2 days and stopping for 2 days, followed by 40 min of ischaemia and 24 h of reperfusion; 4) ALDH2 KO group: saline infusion for 4 days in ALDH2 KO mice; 5) ALDH2 KO + vehicle group: saline infusion for 4 days followed by 40 min of ischaemia and 24 h of reperfusion in ALDH2 KO mice; and 6) ALDH2 KO + Ang-II group: Ang-II infusion for 1 day and then stopping for 1 day, followed by Ang-II infusion for 2 days and stopping for 2 days, followed by 40 min of ischaemia and 24 h of reperfusion in ALDH2 KO mice.

2.3. Transverse aortic constriction-induced hypertrophy preconditioning

Male C57BL6/J mice at 8 weeks of age were randomly assigned to the following experimental groups:

(1) Effects of hypertrophic preconditioning by 3–7 days of TAC followed by several days of debanding on myocardial I/R injury. Four groups were used: 1) sham group: wild-type (WT) mice given a sham operation; 2) IR group: ligation of the left coronary artery for 40 min followed by reperfusion for 24 h; 3) T3D4+IR group: debanding of the aorta after 3 days of TAC and ligation of the left coronary artery for 40 min 4 days later followed by reperfusion for 24 h; and 4) T7D7+IR group: debanding of the aorta after 7 days of TAC and ligation of the left coronary artery for 40 min 7 days later followed by reperfusion for 24 h.
(2) To obtain more direct evidence that ALDH2 protects the heart from I/R, four groups were used: 1) sham + Adv-EGFP group: WT mice given control virus (Adv-EGFP, intramyocardial injection) without IR; 2) sham + Adv-ALDH2 group: WT mice given ALDH2 adenoviruses (Adv-ALDH2, intramyocardial injection) without IR; 3) IR + Adv-EGFP group: WT mice given control virus (Adv-EGFP, intramyocardial injection) following 40 min of ischaemia and 24 h of reperfusion; and 4) IR + Adv-ALDH2 group: WT mice given ALDH2 adenoviruses (Adv-ALDH2, intramyocardial injection) following 40 min of ischaemia and 24 h of reperfusion.
(3) To examine the effects of ALDH2 deficiency on cardioprotection by hypertrophic preconditioning, six groups were used: 1) sham group: WT mice given a sham operation; 2) IR group: ligation of the left coronary artery for 40 min followed by reperfusion for 24 h; 3) T3D4+IR group: debanding of the aorta after 3 days of TAC and ligation of the left coronary artery for 40 min 4 days later followed by reperfusion for 24 h. 4) ALDH2 KO group: ALDH2 KO mice given a sham operation; 5) ALDH2 KO + IR group: ligation of the left coronary artery for 40 min followed by reperfusion for 24 h in ALDH2 KO mice; and 6) ALDH2 KO + T3D4+IR group:

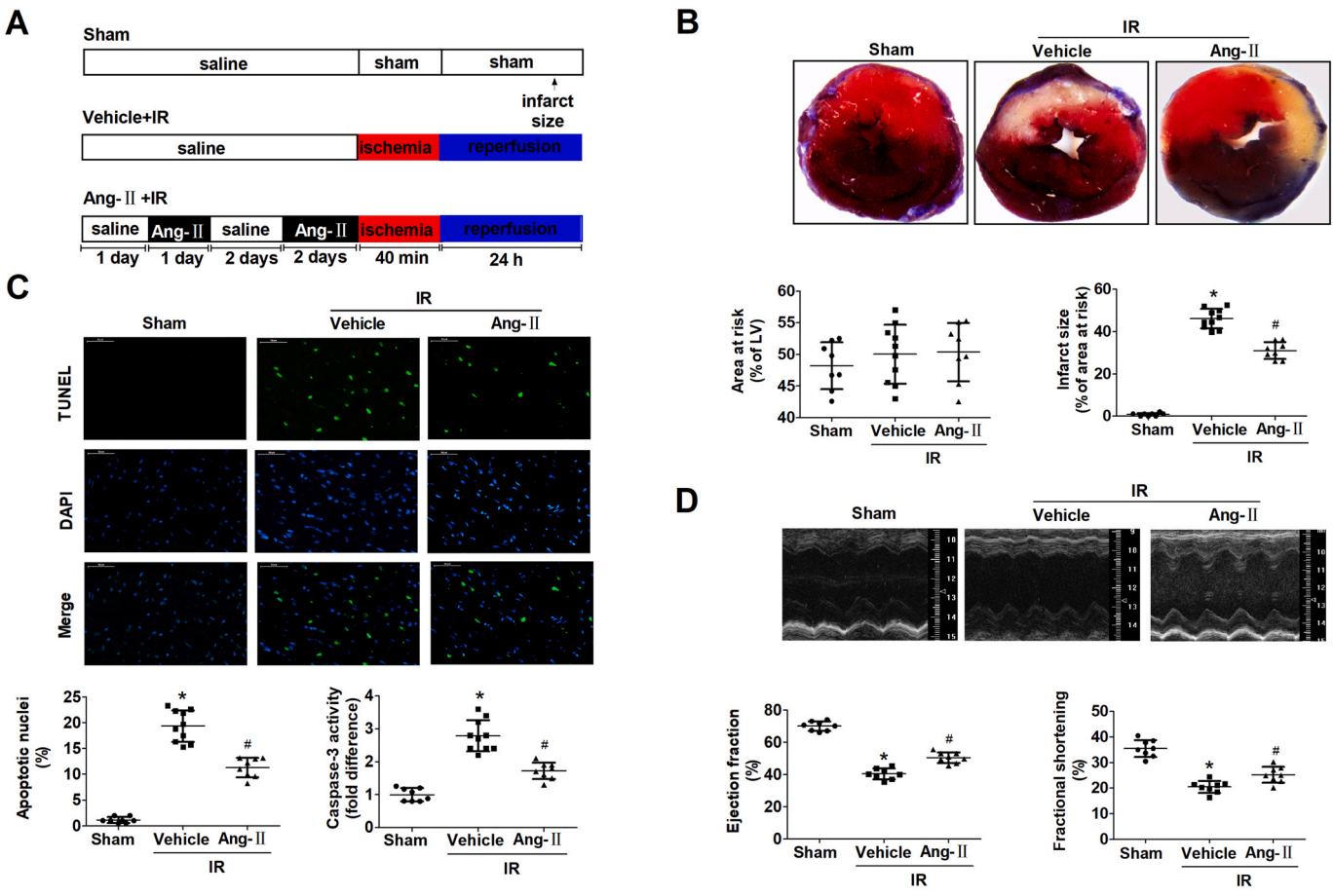

Fig. 1. Drug-induced hypertrophic preconditioning inhibited I/R-induced myocardial apoptosis, decreased infarct size and improved cardiac function following I/R. (A) Experimental protocols for the detection of myocardial hypertrophic preconditioning in mice subjected to myocardial I/R.(B) Myocardial infarct size determined by Evans blue and TTC staining at 24 h after I/R.(C) Cardiomyocyte apoptosis assessed by TUNEL staining (400 ×) and caspase-3 activity measurement following *in situ* I/R, scale bar = 50 μm D) Cardiac function (left ventricular ejection fraction and fractional shortening) measured using Doppler echocardiography at 24 h after I/R. $^{*}P < 0.05$ *vs.* sham and $^{\#}P < 0.05$ *vs.* vehicle; n = 8–10 per group. . (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

debanding of the aorta after 3 days of TAC and ligation of the left coronary artery for 40 min 4 days later followed by reperfusion for 24 h in ALDH2 KO mice.

(4) To determine the essential role of ALDH2 in the cardioprotection by hypertrophic preconditioning, six groups were used: 1) Adv-EGFP + ALDH2 KO group: ALDH2 KO mice given control virus (Adv-EGFP, intramyocardial injection) without IR; 2) Adv-EGFP + ALDH2 KO + IR group: ALDH2 KO mice given Adv-EGFP following 40 min of ischaemia and 24 h of reperfusion; 3) Adv-EGFP + ALDH2 KO + T3D4+IR group: debanding of the aorta after 3 days of TAC in ALDH2 KO mice given Adv-EGFP and ligation of the left coronary artery for 40 min 4 days later followed by reperfusion for 24 h; 4) Adv-ALDH2+ALDH2 KO group: ALDH2 KO mice given ALDH2 adenoviruses (Adv-ALDH2, intramyocardial injection) without IR; 5) Adv-ALDH2+ALDH2 KO + IR group: ALDH2 KO mice given Adv-ALDH2 following 40 min of ischaemia and 24 h of reperfusion; and 6) Adv-ALDH2+ALDH2 KO + T3D4+IR group: debanding of the aorta after 3 days of TAC in ALDH2 KO mice given Adv-ALDH2 and ligation of the left coronary artery for 40 min 4 days later followed by reperfusion for 24 h.

(5) To investigate the role of the AMPK pathway in ALDH2-induced cardioprotection, four groups were used: 1) IR + Adv-EGFP group; 2) IR + Adv-ALDH2 group: 3) IR + Adv-EGFP + CC group; and 4) IR + Adv-ALDH2+CC group. Compound C (CC, an inhibitor of the AMPK pathway, 0.25 mg/kg) was administered intraperitoneally (*i.p.*) 1 h before coronary occlusion.

(6) To investigate the role of the AMPK pathway in hypertrophic preconditioning-induced cardioprotection, six groups were used: 1) sham 2) IR group; 3) T3D4+IR group; 4) sham + CC (Compound C); 5) IR + CC group; and 6) T3D4+IR + CC group. Compound C (CC, an inhibitor of the AMPK pathway, 0.25 mg/kg) was administered intraperitoneally (*i.p.*) 1 h before coronary occlusion.

2.4. Transverse aortic constriction protocol

Male C57BL/6 mice at 8 weeks of age were given minimally invasive transverse aortic constriction (TAC) or debanding or sham operation as described elsewhere [19]. Briefly, the animals were anaesthetized by 1% pentobarbital sodium (50 mg/kg, *i.p.*). A longitudinal skin incision was performed to locate the trachea along the suprasternal notch, and then a horizontal sternum cut was made to locate the thymus and aorta. The aorta between the origin of the right innominate and left common carotid arteries was constricted with a 6-0 silk suture by tying the aorta with a bent 27-gauge needle, which was removed after ligation. Sham-operated animals underwent the same procedure except that the artery was not ligated. After the surgery, the mice were housed in standard animal housing conditions. At the indicated time, a debanding operation was performed by carefully removing the ligature.

2.5. Myocardial infarction protocol

Myocardial infarction surgery was performed as described elsewhere [20]. Briefly, the mouse heart was manually exposed *via* a left thoracotomy at the fifth intercostal space during inhalational 2% isoflurane, and a 6-0 silk suture was passed through the left anterior descending coronary artery. Then, a slipknot was made. After 40 min of ischaemia, the slipknot was removed, and the myocardium was reperfused for the indicated time. Mice that fully recovered from the surgical procedure were returned to the standard animal housing conditions.

2.6. Doppler echocardiography

The mouse was anaesthetized with 1% isoflurane after reperfusion for the indicated time. M-mode images of the left ventricle were obtained at the level of the papillary muscle tips using a Vevo 770 imaging system (VisualSonics, Toronto, Canada). The left ventricular internal diastolic diameter (LVIDd) and left ventricular internal systolic diameter (LVIDs) were recorded. Left ventricular fractional shortening (LVFS) was calculated according to the following formula: LVFS = [(LVIDd - LVIDs)/LVIDd] × 100. Left ventricular ejection fraction (LVEF) was calculated by using the spherical formula.

2.7. ^{18}F-FDG micro-PET/CT scanning and analysis

Micro-PET/CT scanning was used to detect viable myocardium. Mice were given a single injection of ^{18}F-FDG *via* the tail vein (100 μl of ^{18}F-FDG with an activity of 10 MBq) and placed in the anaesthetic chamber filled with 2% isoflurane for 1 h. Then, the animals were placed on the scanner bed and received continuous isoflurane anaesthesia during scanning by the Inveon Acquisition Workplace (IAW). After static PET scanning for 10 min, the photographs were reconstructed by maximum-a-posteriori (MAP). Cardiac ^{18}F-FDG uptake was assessed by Inveon Research Workplace software 3.0. The standard uptake value (SUV) was calculated following the formula by dividing the region of interest (ROI) concentration by the ratio of the injected dose to the body weight [21,22].

2.8. Determination of infarct size

The myocardial infarct size was assessed by 2,3,5-triphenyltetrazolium chloride (TTC, Sigma-Aldrich, St. Louis, MO, USA) staining after reperfusion for 24 h [20]. Briefly, the coronary artery was re-ligated, and 0.2 ml of 2% Evans blue dye was injected into the right ventricular cavity to identify the unstained area as the area at risk. The hearts were harvested and frozen, sectioned into 2-mm slices, and stained in 1% TTC solution at 37°C for 10 min. The areas of infarct (pale) and risk (red) were measured by planimetry using ImageJ 1.37 (National Institutes of Health, Bethesda, MD, USA). The myocardial infarct size was expressed as a percentage of infarct area over ischaemic area (area at risk).

2.9. In vivo adenovirus-mediated cardiac-specific gene overexpression

The mouse cDNA of ALDH2 was cloned into the adenoviral shuttle vector (GV135-CMV-EGFP vector) to generate a vector carrying the ALDH2 sequence (GV135-CMV-ALDH2-EGFP vector). ALDH2 adenoviruses were produced according to the instructions of the AdMax™ Adenoviral Vector Creation System. The mouse hearts were manually exposed *via* a left thoracotomy at the fifth intercostal space during inhalation of 2% isoflurane. A total of 30 μl of ALDH2 adenovirus (Adv-ALDH2, 4×10^9 IFU/ml) or the control virus (Adv-EGFP, 4×10^9 IFU/ml) was injected into the left ventricular free wall using a 32.5-gauge needle (three sites, 10 μl/site). These operations were conducted in a sterilizing room. The expression of ALDH2 in the myocardium was determined at 4 days post-injection [23,24].

2.10. Detection of myocardial apoptosis

Myocardial apoptosis was assessed by terminal deoxynucleotidyl transferase dUTP nick-end labelling (TUNEL) staining using a fluorescein *in situ* cell death detection kit (Roche, Indianapolis, IN, USA) as we described elsewhere [25]. Green fluorescein staining indicates apoptotic nuclei. TUNEL-positive nuclei (green nuclei) were expressed as the percentage of the total cell population.

2.11. Detection of caspase activities in heart tissue

Myocardial caspase-3, caspase-8, caspase-9 and caspase-12 activity was assessed using Caspase Fluorometric Assay Kits (BioVision, Mountain View, CA, USA) according to the manufacturer's instructions. The activities of caspase-3, caspase-8, caspase-9 and caspase-12 were expressed as the fold change over the corresponding control.

2.12. Mitochondrial ALDH2 activity assessment

The myocardial mitochondria were isolated after reperfusion. ALDH2 activity was determined by utilizing the Mitochondrial Aldehyde Dehydrogenase (ALDH2) Activity Assay Kit (Abcam, Cambridge, USA) according to the manufacturer's protocol.

2.13. Mitochondrial ATP content assessment

Myocardial ATP levels were determined using a commercial ATP assay kit (Beyotime Institute of Biotechnology, Shanghai, China) following the manufacturer's protocol. After reperfusion for 3 h, the supernatant was harvested after homogenization and centrifugation (12,000 g, 5 min) and mixed with the working dilution in a 96-well plate. Relative light units were assessed by utilizing a microplate reader.

2.14. Measurement of ROS generation

Dihydroethidium (DHE) and Mito-SOX staining were used to assess *in situ* ROS levels [26]. Five-micrometre-thick frozen slices without fixation were stained with DHE or MitoSOX Red Mitochondrial Superoxide Indicator at 37°C for 30 min. The photographs were acquired using a fluorescence microscope. Fluorescence intensity was assessed by using ImageJ 1.37. Myocardial superoxide production was measured by lucigenin-enhanced chemiluminescence [27]. The relative light units (RLUs) emitted were recorded and integrated over 30-s intervals for 5 min. Superoxide production was normalized with the heart weight.

2.15. Determination of nitrotyrosine content in cardiac tissue

The hearts were harvested after 3 h of reperfusion and cut into sections 5 μm thick after 4% paraformaldehyde fixation. The slices were embedded in paraffin and stained with anti-nitrotyrosine antibody (1:100; Millipore, Billerica, MA, USA). The immunostaining was conducted by utilizing the Vectastain ABC kit (1:200, Vector Laboratories, Burlingame, CA, USA), and the images were acquired under light microscopy. The cardiac nitrotyrosine content was quantified by utilizing the Nitrotyrosine ELISA Kit (Abnova, Taiwan, China). The nitrotyrosine content was expressed as micrograms/milligram of protein.

2.16. Measurement of myocardial NO content

Myocardial NO content was assessed by determining nitrite using the Griess method [28]. The samples from the ischaemic area were harvested after 3 h of reperfusion. The NO content was detected by utilizing the Total Nitric Oxide Assay Kit (Beyotime Institute of Biotechnology, Shanghai, China) following the manufacturer's instructions.

2.17. Transmission electron microscopy

The cardiac tissue from the ischaemic zone was fixed with 2% glutaraldehyde for 2 h, fixed in 1% OsO4 for 2 h, and embedded in resin. The ultrathin sections were stained with uranyl acetate and lead citrate and observed under a transmission electron microscope (Hitachi Model H-7650, Tokyo, Japan). Random horizons were acquired by an electron microscope technicist.

2.18. Immunoblotting

The samples were taken from the ischaemic zone. The expression levels of myocardial ALDH2 (1:1000, Abcam, Cambridge, USA), CHOP [Cell Signaling Technology (CST); 1:1000], caspase-12 (CST, 1:1000), caspase-9 (CST, 1:1000), caspase-3 (CST, 1:1000), Bax (CST, 1:1000), Bcl-2 (CST, 1:1000), gp^{91phox} (Abcam; 1:1000), iNOS (Abcam; 1:1000), p-AMPKα (CST, 1:1000), AMPKα (CST, 1:1000), mTOR (CST, 1:1000), p-mTOR (CST, 1:1000), p70s6k (CST, 1:1000), p-p70s6k (CST, 1:1000), mitochondrial OXPHOS complexes, and GAPDH (CST, 1:1000) were determined by immunoblotting [25]. The quantitative protein band density was assayed by ImageJ 1.37.

2.19. Statistical analysis

Data are shown as the mean ± SD. Statistical analysis was performed with unpaired Student's *t*-test for two group comparisons. For multi-group comparisons, one-way ANOVA followed by the Bonferroni *post hoc* test was used. A value of $P < 0.05$ was considered to be statistically significant. All statistical analyses were performed using GraphPad Prism Version 7.0 (GraphPad Inc, San Diego, CA, USA).

3. Results

3.1. Ang-II-induced hypertrophic preconditioning reduced I/R injury and improved cardiac function

To determine whether the removal of short-term pro-hypertrophic stimulation would render the heart resistant to subsequent ischaemic stress, Ang-II infusion was performed for 3 days in male mice and then withdrawn for several days by the removal of a minipump, followed by subsequent exposure to regional myocardial ischaemia by *in situ* coronary artery ligation (Fig. 1A). Transient administration of Ang-II for 3 days markedly decreased myocardial infarct size in the Ang-II group (31.04 ± 3.55% in the Ang-II group *vs.* 46.18% ± 4.32% in the vehicle group, $p < 0.05$, Fig. 1B), while the AAR did not significantly differ among all groups. Moreover, short-term Ang-II infusion markedly decreased I/R-induced cardiomyocyte apoptosis, as demonstrated by a reduced number of TUNEL-positive cell nuclei, and inhibited caspase-3 activation ($p < 0.05$, Fig. 1C). To assess LV performance, echocardiography was performed to measure cardiac function after 24 h of reperfusion. As shown in Fig. 1D, I/R markedly reduced left ventricular ejection fraction (LVEF) and left ventricular fractional shortening (LVFS). Compared with vehicle, short-term Ang-II infusion improved cardiac function, as evidenced by enhanced LVEF and LVFS (50.46% ± 2.38% and 25.25% ± 2.20% in the Ang-II group *vs.* 40.44% ± 2.23% and 20.10% ± 1.07% in the IR group, $p < 0.05$, Fig. 1C). Taken together, these findings indicate that hypertrophic preconditioning by short-term Ang-II infusion rescues cardiomyocyte death and improves cardiac function against I/R injury.

3.2. Aortic debanding-induced hypertrophic preconditioning inhibited I/R injury and improved cardiac function

To further determine whether the removal of short-term pro-hypertrophic stimulation would render the heart resistant to subsequent ischaemic stress, transient transverse aortic constriction (TAC) for 3–7 days was performed in male mice and then withdrawn for several days by aortic debanding, followed by subsequent exposure to regional myocardial ischaemia by *in situ* coronary artery ligation (Fig. 2A). Transient aortic constriction for 3–7 days ameliorated I/R-induced myocardial necrosis as assessed by infarct size (24.37 ± 6.28% in the T3D4+IR group, 25.12 ± 6.57% in the T7D7+IR group, *vs.* 45.36% ± 7.07% in the IR group, $p < 0.05$, Fig. 2B), while the AAR did not significantly differ among all groups. Moreover, short-term aortic constriction markedly decreased I/R-induced cardiomyocyte apoptosis, as demonstrated by a reduced number of TUNEL-positive cell nuclei, and inhibited caspase-3 activation ($p < 0.05$, Fig. 2C). To assess LV performance, echocardiography was performed to measure cardiac function after 24 h of reperfusion. As shown in Fig. 2D, I/R markedly reduced left ventricular ejection fraction (LVEF) and left ventricular fractional shortening (LVFS). Compared with the sham group, the retraction of aortic banding promoted the recovery of cardiac function, as evidenced by increased LVEF and LVFS (55.46% ± 6.60% and 27.25% ± 4.12% in the T3D4+IR group, 55.53% ± 5.33% and 26.76% ± 2.99% in the T7D7+IR group, *vs.* 40.44% ± 2.23% and 20.49% ± 1.16% in the IR group, $p < 0.05$, Fig. 2D). Taken together, these findings indicate that hypertrophic preconditioning induced by aortic debanding rescues cardiomyocyte death and improves cardiac function against I/R injury.

3.3. Hypertrophic preconditioning promoted ALDH2 activation in mouse hearts in vivo

Accumulating evidence suggests a vital role of aldehyde dehydrogenase-2 (ALDH2) in cardioprotection against I/R [29–33]. Moreover, approximately 540 million people or 8% of the population worldwide carry a loss-of-function allele of ALDH2 [34,35]. It is intriguing to know whether ALDH2 functions as a host factor regulating the therapeutic potential of hypertrophic preconditioning. Therefore, we detected myocardial ALDH2 expression and activity after the removal of stimulation. Interestingly, we found that short-term pressure overload for 3–7 days decreased both ALDH2 protein expression and activity, whereas the removal of short-term pressure overload promoted ALDH2 activation without altering ALDH2 protein expression (Fig. 3A and B).

3.4. ALDH2 overexpression alleviated I/R injury by modulating mitochondrial metabolism through an AMPK-dependent mechanism in the intact heart

To investigate whether cardiac ALDH2 overexpression can emulate the cardioprotective effects of TAC preconditioning in intact hearts, cardiac-specific gene overexpression of ALDH2 by *in vivo* intramyocardial adenovirus-encoded ALDH2 (Adv-ALDH2) transfection was performed 4 days before I/R, and I/R damage determinants were assessed. We found that the levels of ALDH2 were significantly upregulated in the Adv-ALDH2 group compared with those in the Adv-EGFP group (Supplemental Fig. 1). Compared with the adenovirus control (Adv-EGFP), administration of Adv-ALDH2 significantly inhibited I/R-induced myocardial apoptosis, as evidenced by increased TUNEL-positive nuclei as well as caspase-3 activity (Fig. 4A). Most importantly, Adv-ALDH2 markedly decreased myocardial infarct size (26.02% ± 6.44% in the Adv-ALDH2 group *vs.* 46.10% ± 6.47% in the Adv-EGFP group, $p < 0.05$, Fig. 4B), whereas the AAR did not significantly differ among all groups. To assess left ventricular systolic function, echocardiography was performed after 24 h of reperfusion. As shown in Fig. 4C, Adv-ALDH2 treatment markedly hindered I/R-induced cardiac dysfunction, as evidenced by the increase in LVEF and LVFS (52.20% ± 2.24% and 24.96% ± 2.01% in the Adv-ALDH2 group *vs.* 39.16% ± 3.67% and 20.05% ± 2.44% in the Adv-EGFP group, $p < 0.05$, Fig. 4C). These results imply that ALDH2 plays an important role in promoting cardiac survival and functional recovery during I/R.

We next sought to determine the potential mechanisms underlying

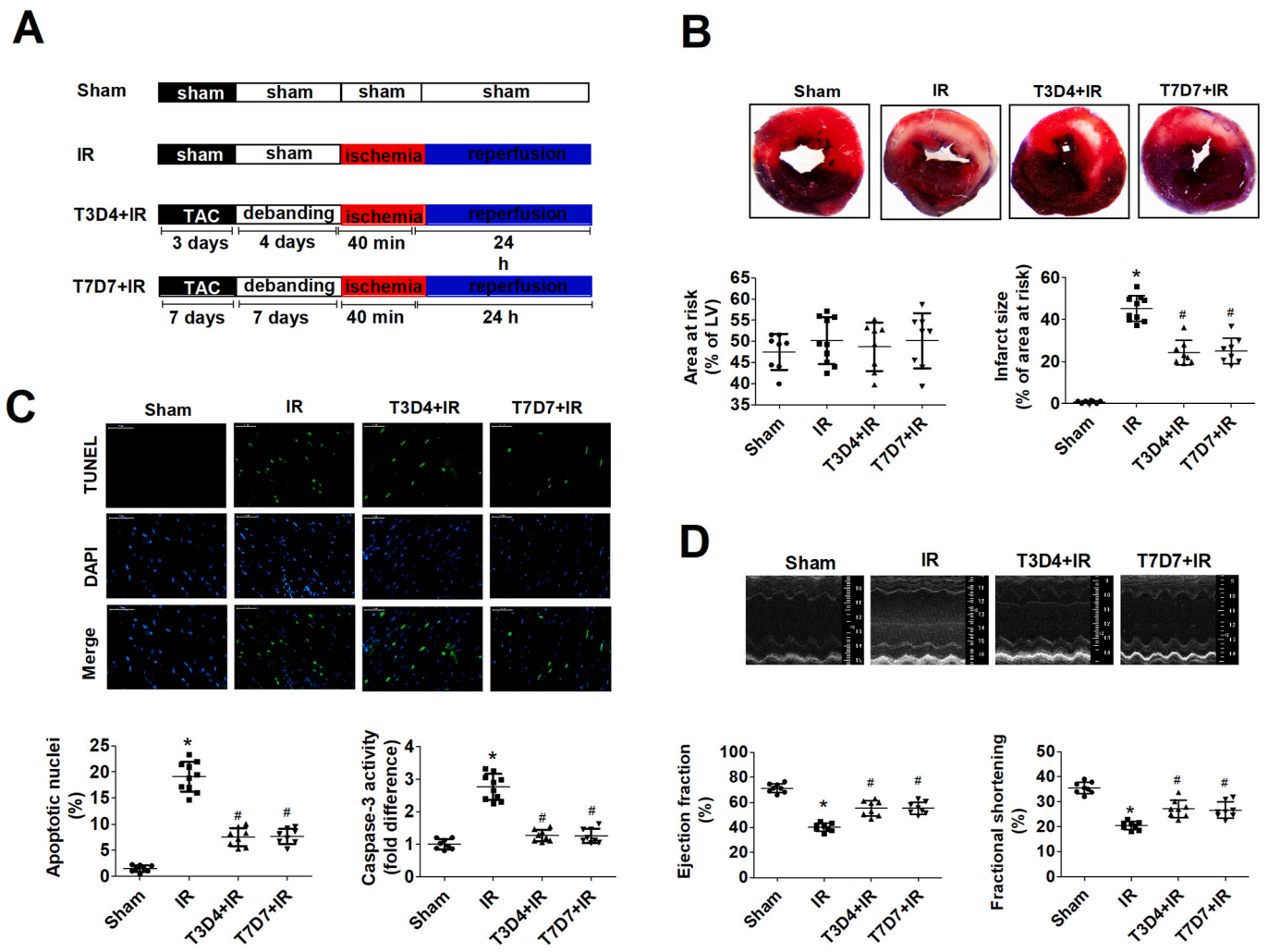

Fig. 2. Aortic debanding-induced hypertrophic preconditioning inhibited I/R-induced myocardial apoptosis, decreased infarct size and improved cardiac function following I/R.(A) Experimental protocols for the detection of myocardial hypertrophic preconditioning in mice subjected to myocardial I/R.(B) Myocardial infarct size determined by Evans blue and TTC staining at 24 h after I/R.(C) Cardiomyocyte apoptosis assessed by TUNEL staining (400 ×) and caspase-3 activity measurement following *in situ* I/R, scale bar = 50 μm. (D) Cardiac function (left ventricular ejection fraction and fractional shortening) measured using Doppler echocardiography at 24 h after I/R. $^{*}P < 0.05$ *vs.* sham and $^{\#}P < 0.05$ *vs.* IR; n = 8–10 per group. . (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

the cardioprotection of ALDH2 against I/R. We found that cardiac-specific ALDH2 overexpression further promoted I/R-elicited AMPK phosphorylation (Fig. 4D), and the inhibition of the AMPK pathway by Compound C completely eliminated the cardioprotective effects of ALDH2 overexpression, as evidenced by augmented myocardial infarct size, worsening cardiac function and increased cardiomyocyte apoptosis (Supplemental Fig. 2). Next, we analysed the expression profile of key complexes of the mitochondrial respiratory chain (OXPHOS complexes). Strikingly, under conditions of ALDH2 overexpression, we found increased expression of mitochondrial-encoded genes in complexes I, II, III, IV, and V in cardiac tissue following I/R (Fig. 4E and F), suggesting improved respiratory efficiency by ALDH2. Importantly, we observed markedly increased mitochondrial ATP production and improved mean myocardial standardized uptake values (SUVs) of ^{18}F-FDG in the ALDH2-transfected mice following I/R (Fig. 4G and H), suggesting improved viable myocardium metabolism by ALDH2. Last, we demonstrated that direct ALDH2 overexpression inhibited the mitochondrial-dependent apoptotic pathway and alleviated oxidative/nitrative stress-induced myocardial injury, as shown by preserved intact mitochondria (Fig. 4I), reduced caspase-9 and caspase-12 expression (Fig. 4J), hindered ROS production (Fig. 4K) and decreased myocardial nitrotyrosine content (Fig. 4L). Taken together, these results suggest that ALDH2 exerts its cardioprotective effects by improving mitochondrial metabolism, hindering cardiomyocyte apoptosis and reducing oxidative/nitrative stress through an AMPK-dependent mechanism.

3.5. *ALDH2 deficiency attenuated hypertrophic preconditioning-induced cardioprotective effects*

To explore whether and how host ALDH2 regulates the therapeutic efficacy of hypertrophic preconditioning, we examined myocardial morphology and function in response to I/R injury in the presence or absence of hypertrophic preconditioning in wild-type (WT) or gene-targeted ALDH2 knockout (KO) mice (Fig. 5A). Western blotting confirmed the lack of ALDH2 protein in the myocardium from ALDH2-deficient mice (Supplemental Fig. 3). Compared to the wild-type mice, I/R did not produce additional myocardial injury in the ALDH2-deficient mice. Neither the TUNEL-positive nuclei, the myocardial infarct size nor the left ventricular systolic function were significantly different between wild-type and ALDH2 null mice following I/R. However, ALDH2 deletion abrogated the aforementioned cardioprotective effects of TAC preconditioning, as shown by augmented infarct size (Fig. 5B), increased cardiomyocyte apoptosis (Fig. 5C), and worsening cardiac function (Fig. 5D). Similarly, ALDH2 deletion abrogated the cardioprotective

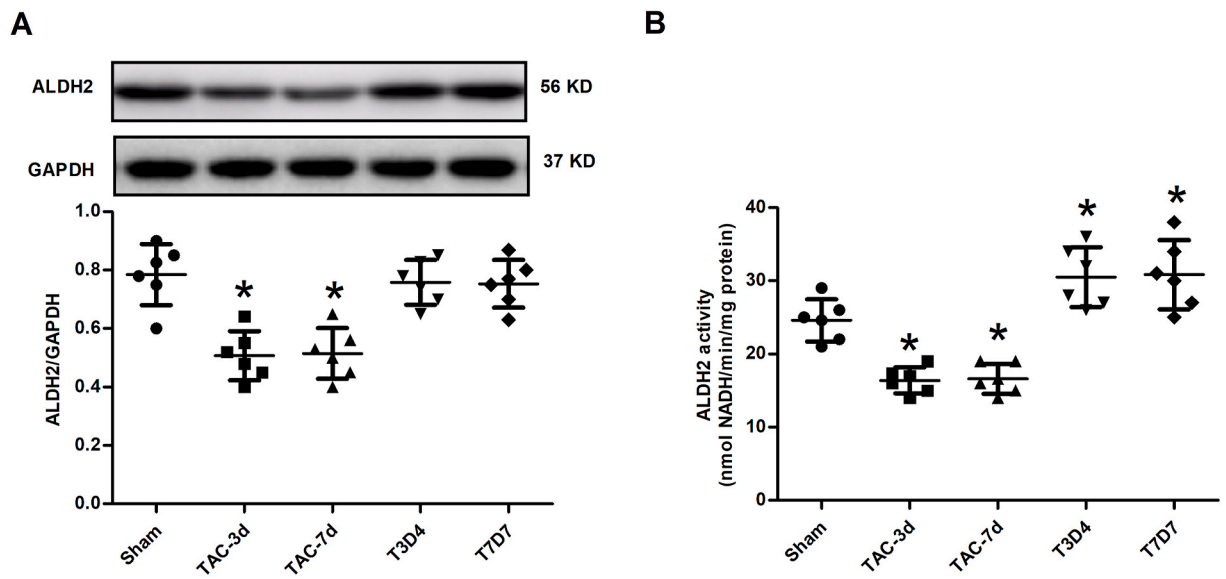

Fig. 3. The removal of short-term pressure overload promoted myocardial ALDH2 activation in mice *in vivo*.(A) Representative immunoblots and quantitative analysis of ALDH2 in wild-type mice with or without aortic debanding.(B) ALDH2 activity. *$P < 0.05$ *vs.* sham; n = 6 per group.

effects of Ang-II pretreatment, as shown by augmented infarct size (Supplemental Fig. 4B), increased cardiomyocyte apoptosis (Supplemental Fig. 4C), and worsening cardiac function (Supplemental Fig. 4D). Furthermore, we demonstrated that delivery of ALDH2 into ALDH2-deficient mouse hearts by intramyocardial Adv-ALDH2 transfection restored the cardioprotective effects of TAC preconditioning, as evidenced by reduced infarct size (Supplemental Fig. 5B), inhibited cardiomyocyte apoptosis (Supplemental Fig. 5C), and improved cardiac function (Supplemental Fig. 5D). Taken together, these findings indicate that ALDH2 plays an obligatory role in hypertrophic preconditioning-induced cardioprotection against I/R injury.

3.6. *ALDH2 deficiency attenuated hypertrophic preconditioning regulation of mitochondrial energy metabolism by inactivating AMPK*

We next sought to determine the potential mechanism underlying myocardial hypertrophic preconditioning-induced cardioprotective effects. We hypothesized that ALDH2 deficiency may result in the loss of improved mitochondrial energy metabolism by preconditioning. Indeed, we found that the removal of short-term aortic banding markedly promoted AMPK-mTOR-$p70^{S6K}$ pathway activation in normal mice subjected to I/R, whereas myocardial ALDH2 ablation completely eliminated such alterations in preconditioned mice with I/R (Fig. 6A–D). To test whether inactivated AMPK signalling was associated with decreased mitochondrial energy metabolism, we analysed the expression profile of OXPHOS complexes. Strikingly, under conditions of hypertrophic preconditioning, we found decreased expression of mitochondrial-encoded genes in complexes I, II, III, IV, and V in cardiac tissue from ALDH2-deficient mice following I/R (Fig. 6E and F), suggesting hindered respiratory efficiency by ALDH2 ablation after myocardial hypertrophic preconditioning. Importantly, we observed markedly decreased mitochondrial ATP content as well as a mean myocardial SUV of ^{18}F-FDG in the preconditioned ALDH2-deficient mice following I/R (Fig. 6G and H), suggesting reduced viable myocardium metabolism by ALDH2 deficiency after preconditioning. Last, we demonstrated that AMPK inhibition by Compound C completely eliminated the cardioprotective effects of hypertrophic preconditioning, as evidenced by augmented myocardial infarct size, worsening cardiac function and increased cardiomyocyte apoptosis (Supplemental Fig. 6). These findings suggest that ALDH2 deficiency attenuates hypertrophic preconditioning regulation of mitochondrial energy metabolism by inactivating AMPK signalling.

3.7. *ALDH2 deficiency attenuated hypertrophic preconditioning regulation of the endoplasmic reticulum stress- and mitochondrial-mediated apoptosis pathway*

To further provide mechanistic insights into hypertrophic preconditioning-induced myocardial protective effects against apoptotic cell death, endoplasmic reticulum stress and the mitochondria-mediated apoptotic pathway were assessed. In the WT mouse heart, I/R exacerbated mitochondrial damage and promoted the activation of caspase-12 (a mediator of endoplasmic reticulum stress), caspase-9 (a participator in mitochondrial-mediated apoptosis) and caspase-8 (a mediator of death receptor signalling), as evidenced by increased caspase-12 activity and higher levels of cleaved caspase-12, increased activated caspase-9 expression, and enhanced caspase-8 activity (Fig. 7A–E). In contrast, hypertrophic preconditioning inhibited I/R-elicited mitochondrial damage and caspase-12 and caspase-9 activation but did not alter caspase-8 activity (Fig. 7A–E). Moreover, I/R promoted the activation of CCAAT/enhancer-binding protein homologous protein (CHOP) (Fig. 7F), enhanced Bax activation (Fig. 7H), inhibited Bcl 2 expression (Fig. 7I) and promoted cleaved caspase-3 expression (Fig. 7J), all of which were markedly ameliorated by hypertrophic preconditioning in normal mice. In contrast, ALDH2 deficiency completely eliminated the cardiac effects by hypertrophic preconditioning, as evidenced by aggravated mitochondrial impairment (Fig. 7A), enhanced caspase-9 and caspase-12 activity (Fig. 7B–E), increased CHOP expression (Fig. 7F) and activation of the mitochondria-mediated apoptotic pathway (Fig. 7G–J). Taken together, these findings demonstrated that hypertrophic preconditioning ameliorated I/R-elicited endoplasmic reticulum stress and mitochondrial impairment *via* the ALDH2 pathway.

3.8. *ALDH2 deficiency attenuated hypertrophic preconditioning regulation of oxidative/nitrative stress in ischaemic/reperfused myocardium*

Oxidative/nitrative stress has been proven to be an important upstream mediator of apoptosis [36]; therefore, we investigated the roles of hypertrophic preconditioning in regulating oxidative/nitrative stress to provide mechanistic insights into the cardioprotection of hypertrophic preconditioning against apoptosis. We found that hypertrophic

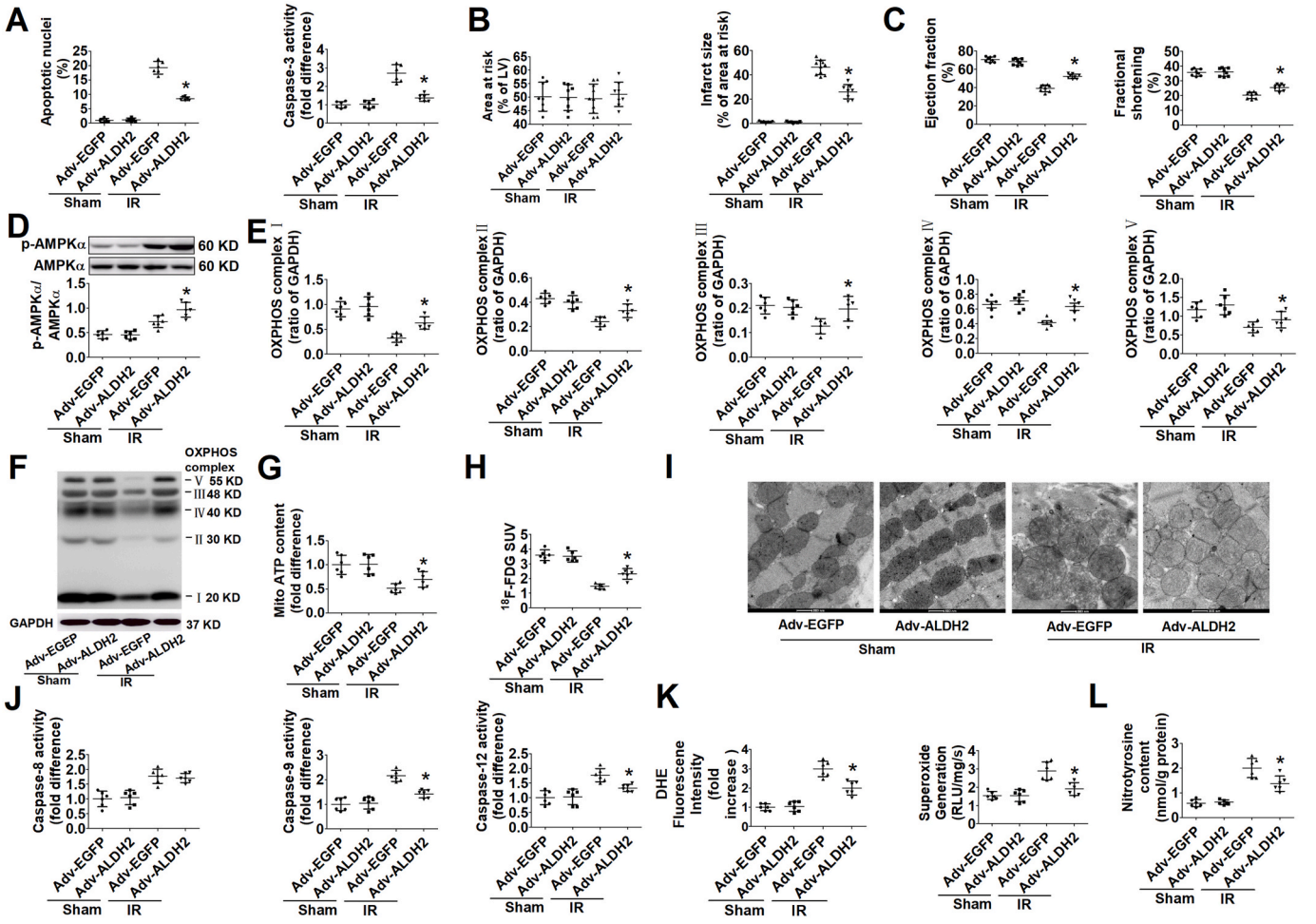

Fig. 4. Adenovirus-encoded ALDH2 attenuated I/R injury and partially emulated the actions of hypertrophic preconditioning.(A) Cardiomyocyte apoptosis assessed by TUNEL staining and caspase-3 activity measurement following *in situ* I/R, scale bar = 50 μm(B) Myocardial infarct size detected by Evans blue and TTC staining at 24 h after I/R. (C) Cardiac function (left ventricular ejection fraction and fractional shortening) measured using Doppler echocardiography at 24 h after I/R (D) Representative immunoblots and quantitative analysis of phosphorylated AMPK in mice transfected with Adv-EGFP or Adv-ALDH2 during I/R.(E) Expression of several OXPHOS complexes was measured by western blot on heart lysates.(F) Representative immunoblots of several OXPHOS complexes.(G) Mitochondrial ATP content.=(H) Myocardial ^{18}F-FDG SUV evaluated by ^{18}F-FDG uptake utilizing micro-PET/CT. (I) Representative mitochondrial photographs detected by transmission electron microscopy, scale bar = 2 μm(J) Myocardial caspase activity following *in situ* I/R.(K) Quantitative analyses of DHE fluorescence intensity, superoxide production and nitrotyrosine content in ischaemic-reperfused cardiac tissue.(L) Quantitative analyses of the nitrotyrosine content in ischaemic-reperfused cardiac tissue. *$P < 0.05$ *vs.* sham; n = 6–10 per group.

preconditioning markedly ameliorated I/R-elicited superoxide production (Fig. 8A–C) and gp91^{phox} overexpression (Fig. 8D). In addition, the myocardial nitrotyrosine content (Fig. 8E and F), inducible nitric oxide synthase (iNOS) expression and NO content were also markedly reduced by hypertrophic preconditioning (Fig. 8G and H). In contrast, ALDH2 deficiency significantly attenuated the aforementioned myocardial effects of hypertrophic preconditioning, as evidenced by aggravated superoxide production (Fig. 8A–C), elevated myocardial gp91^{phox} levels (Fig. 8D), increased myocardial nitrotyrosine content (Fig. 8E and F), enhanced iNOS expression, and promoted total NO production (Fig. 8G and H). Altogether, these findings imply that hypertrophic preconditioning induces cardioprotection by ameliorating oxidative/nitrative stress through ALDH2 signalling.

4. Discussion

Although several lines of studies have reported that the removal of short-term pressure overload makes the heart more resistant to the development of pathological hypertrophy, termed hypertrophic preconditioning [10,15], whether such cardioprotection can be extended beyond pathological hypertrophy to myocyte viability remains unexplored. The current study was performed to address these issues.

The novel contributions of the present work can be summarized as follows. First, hypertrophic preconditioning is a novel endogenous self-defensive and cardioprotective strategy that protects the heart from I/R injury. Second, ALDH2 is obligatorily required for the development of cardioprotective effects by hypertrophic preconditioning. Third, hypertrophic preconditioning improves mitochondrial energy metabolism, inhibits cardiomyocyte apoptosis and reduces oxidative/nitrative stress by activating ALDH2-AMPK signalling, whereas direct ALDH2 overexpression can partially emulate the cardiac effects of preconditioning.

Preconditioning with TAC for 3 or 7 days induces mild myocardial hypertrophy and makes the heart more resistant to the development of pathological hypertrophy [15]. A similar concept of 3 days in advance of preconditioning was also reported by another study. Zhu et al. reported that preconditioning with three episodes of 5 min of occlusion of the left femoral artery followed by 5 min of reperfusion for 3 consecutive days protects diabetic rats from myocardial IR injury [37]. Therefore, transient aortic banding for 3 or 7 days was adopted to induce a myocardial hypertrophic response in the animals.

The potential mechanisms underlying myocardial hypertrophic preconditioning-elicited cardioprotection remain elusive. In the present

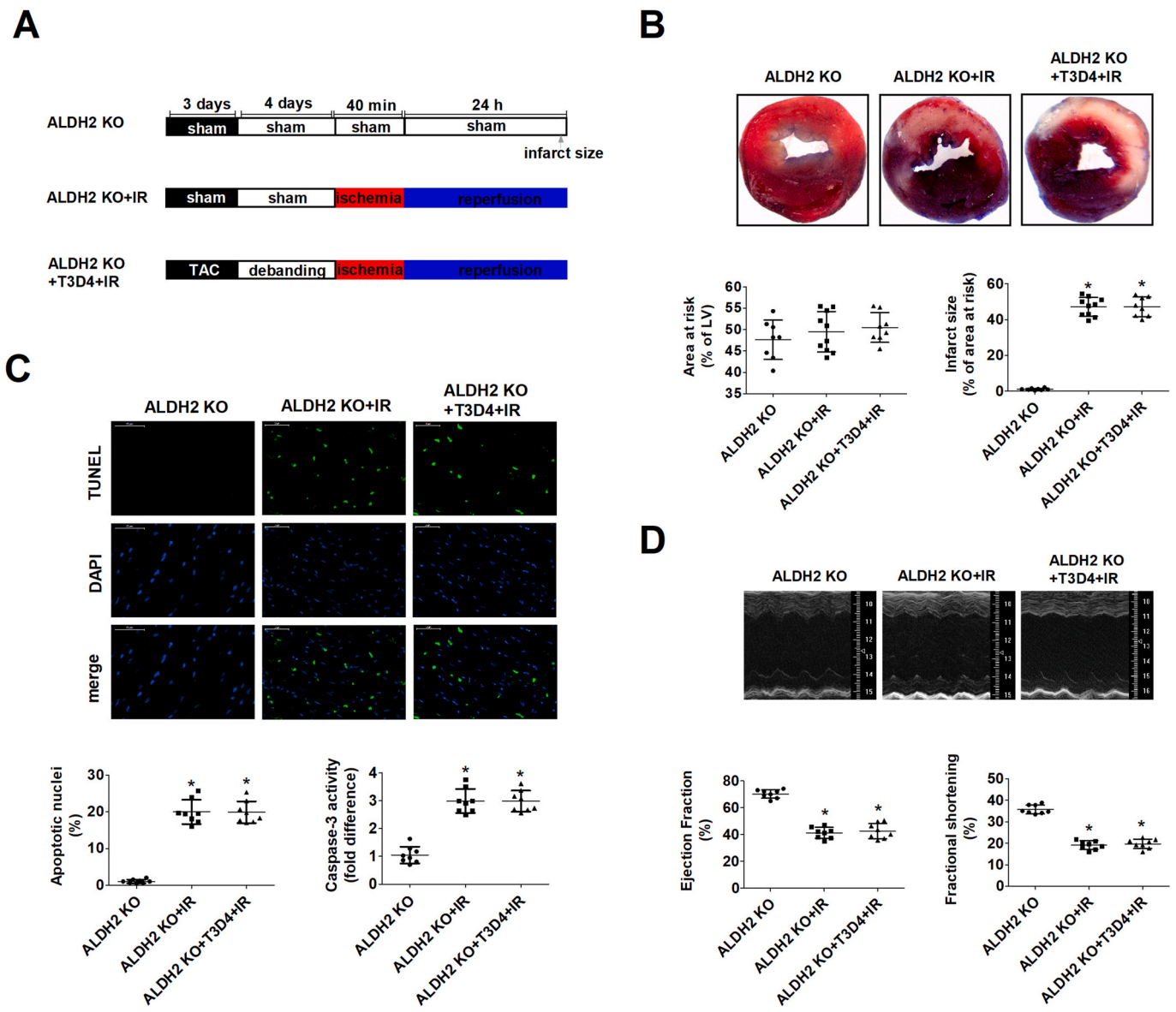

Fig. 5. ALDH2 deficiency eliminated the cardioprotection of hypertrophic preconditioning by aggravating cell death and worsening cardiac function.(A) Experimental protocols for the detection of myocardial hypertrophic preconditioning in ALDH2-deficient mice subjected to myocardial I/R.(B) Myocardial infarct size detected by Evans blue and TTC staining at 24 h after I/R. (C) Cardiomyocyte apoptosis assessed by TUNEL staining (400 ×) and caspase-3 activity measurement following *in situ* I/R, scale bar = 50 μm. (D) Cardiac function at 24 h after I/R. *$P < 0.05$ *vs.* ALDH2 KO group; n = 8–10 per group. . (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

study, we found that both ALDH2 protein expression and activity were decreased in response to chronic pressure overload, whereas the removal of short-term pressure overload enhanced ALDH2 protein activity without altering ALDH2 protein expression. Mitochondrial ALDH2 is a direct SIRT3 substrate, and its deacetylation increases acetaminophen toxic-metabolite binding and enzyme inactivation [38]. In addition, ethanol was also found to enhance myocardial ALDH2 activation by inducing the phosphorylation of ALDH2 expression in Langendorff perfused mouse hearts [30]. Therefore, we speculated that post-translational modification may play an essential role in regulating ALDH2 enzyme activity, and future studies are warranted to reveal the underlying regulatory mechanisms of hypertrophic preconditioning. Moreover, hypertrophic preconditioning was shown to increase AMPK phosphorylation, enhance myocardial OXPHOS complex levels, elevate mitochondrial ATP content and improve viable myocardium glucose metabolism. The aforementioned results suggest the involvement of ALDH2 in hypertrophic preconditioning-elicited cardioprotection. We then used gain- and loss-of-function approaches to further address this issue. We demonstrated that ALDH2 deficiency abrogated hypertrophic preconditioning-induced cardioprotection by aggravating cardiomyocyte death, deteriorating cardiac function and reducing mitochondrial energy metabolism in ischaemic-reperfused myocardium through an AMPK-dependent mechanism. Furthermore, using an adenovirus encoding ALDH2, we demonstrated that ALDH2 signalling played a crucial role in maintaining cardiac function and ameliorating cardiomyocyte death by improving mitochondrial metabolism through AMPK signalling. Especially important, we showed that the inhibition of AMPK activation by Compound C completely eliminated the cardiac effects of both ALDH2 overexpression and hypertrophic preconditioning, as shown by augmented myocardial infarct size, worsening cardiac function and increased cardiomyocyte apoptosis. Collectively, these findings indicate that hypertrophic preconditioning plays a crucial role in rescuing cardiomyocyte fate and maintaining cardiac function by regulating mitochondrial energy metabolism *via* an

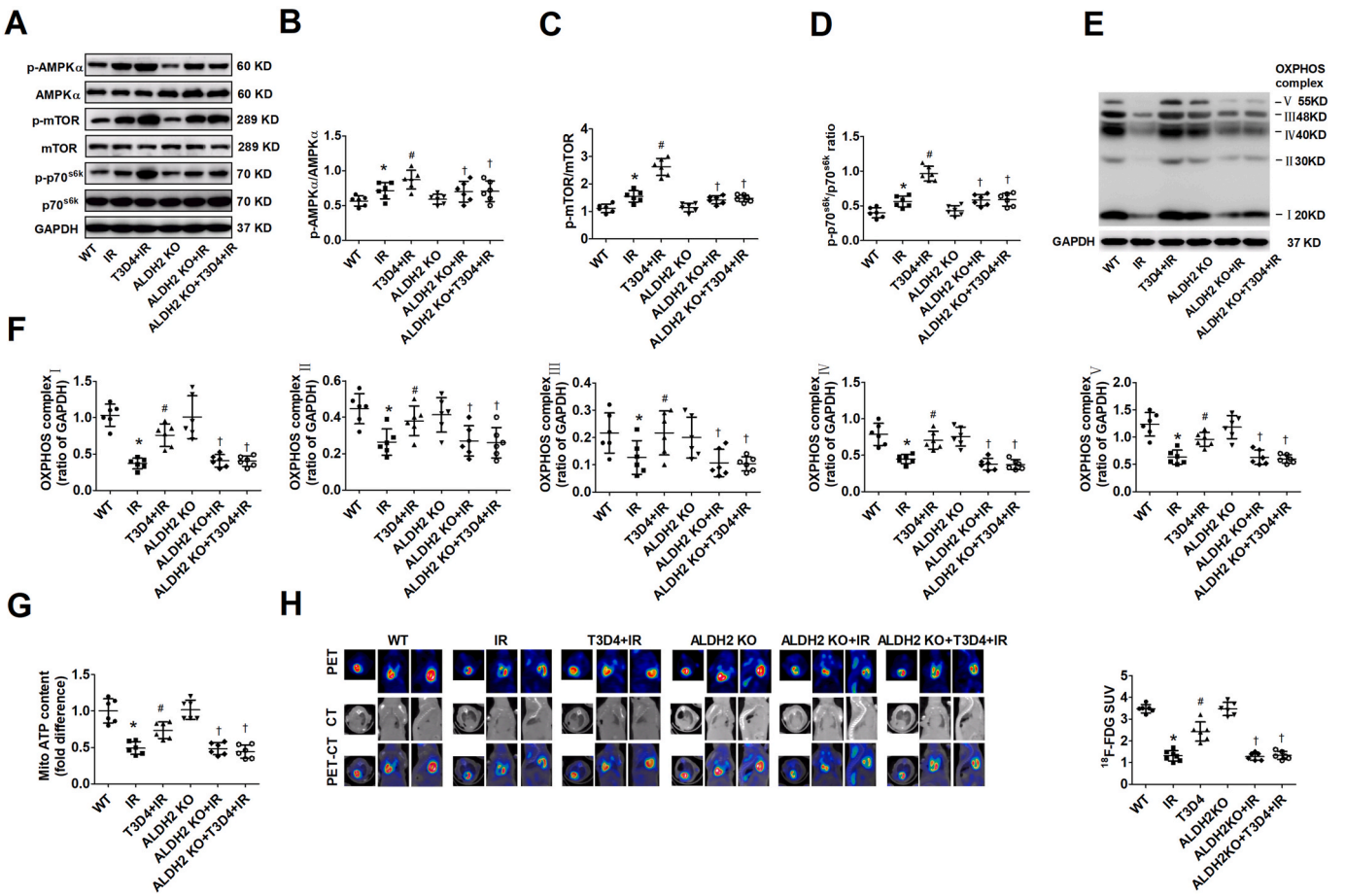

Fig. 6. ALDH2 deficiency attenuated hypertrophic preconditioning regulation of mitochondrial energy metabolism by inactivating AMPK.(A) Representative immunoblots and quantitative analysis of phosphorylated AMPK, mTOR and $p70^{S6K}$ in mice following *in situ* I/R. (B–D) Quantitative analysis of phosphorylated AMPK, mTOR and $p70^{S6K}$.(E) Representative immunoblots of several OXPHOS complexes.(F) Expression of several OXPHOS complexes was measured by western blot on heart lysates.(G) Mitochondrial ATP content.(H) Myocardial ^{18}F-FDG SUV, evaluated by ^{18}F-FDG uptake utilizing micro-PET/CT. $^{*}P < 0.05$ *vs.* WT, $^{\#}P < 0.05$ *vs.* IR and $^{\dagger}P < 0.05$ *vs.* T3D4+IR; n = 6–7 per group.

ALDH2-AMPK-dependent mechanism.

We further investigated the effects of hypertrophic preconditioning on endoplasmic reticulum stress and the mitochondria-mediated apoptotic pathway [6]. Our study showed that during I/R, three apoptotic pathways were activated, as evidenced by the activation of different caspase markers (caspase-8, caspase-9, and caspase-12). Hypertrophic preconditioning ameliorated I/R-induced endoplasmic reticulum and mitochondrial stress but did not alter caspase-8 activity, as evidenced by inhibited CHOP elevation, reduced caspase-12 activation, and decreased cleaved caspase-9 levels. In contrast, ALDH2 deficiency completely eliminated the cardiac effects by hypertrophic preconditioning, as evidenced by aggravated mitochondrial impairment, enhanced caspase-9 and caspase-12 activity, increased CHOP expression and activation of the mitochondria-mediated apoptotic pathway. Furthermore, we demonstrated that ALDH2 overexpression inhibited the mitochondrial-dependent apoptotic pathway and alleviated oxidative/nitrative stress-induced myocardial injury, as shown by preserved intact mitochondria and reduced caspase-9 and caspase-12 expression. These findings suggest that hypertrophic preconditioning exerts its anti-apoptotic effect by ameliorating mitochondrial impairment through an ALDH2-dependent mechanism.

I/R-induced DNA damage and superoxide production are the primary contributors to cardiomyocyte death and are causally related to endoplasmic reticulum stress and mitochondrial injury [39–41]. Therefore, we investigated the role of hypertrophic preconditioning in regulating oxidative stress during I/R. We provided the first direct evidence that hypertrophic preconditioning markedly reduced gp^{91phox} activation and superoxide production, thus ameliorating oxidative stress-induced myocardial impairment. Notably, NO itself does not lead to additional myocardial impairment under physiological conditions; however, NO interacts with superoxide and subsequently induces oxidative/nitrative injury to mitochondria, proteins and lipids under pathological conditions [42]. In addition, our previous studies have proven the harmful effects of nitrative stress, and scavenging peroxynitrite ameliorates reperfusion injury [40,41]. Our present study demonstrated that hypertrophic preconditioning inhibited iNOS activation, hindered NO production and reduced myocardial nitrotyrosine accumulation. ALDH2 deficiency significantly attenuated the aforementioned cardiac effects of hypertrophic preconditioning, as evidenced by aggravated superoxide production, elevated myocardial $gp91^{phox}$ levels, increased myocardial nitrotyrosine content, enhanced iNOS expression and increased total NO production. Furthermore, we demonstrated that ALDH2 overexpression by adenovirus-encoded ALDH2 infection alleviated myocardial oxidative/nitrative stress, as shown by hindered ROS production and decreased myocardial nitrotyrosine content. These results suggest that hypertrophic preconditioning inhibits oxidative/nitrative-induced cardiac injury during I/R and that ALDH2 is necessary for this process. Collectively, our study provided the first direct evidence that ALDH2 plays fundamentally diverse roles in preconditioned and non-preconditioned mice during I/R. We demonstrated that basic expression of ALDH2 did not affect myocardial infarction in the non-preconditioned state but is essential for the

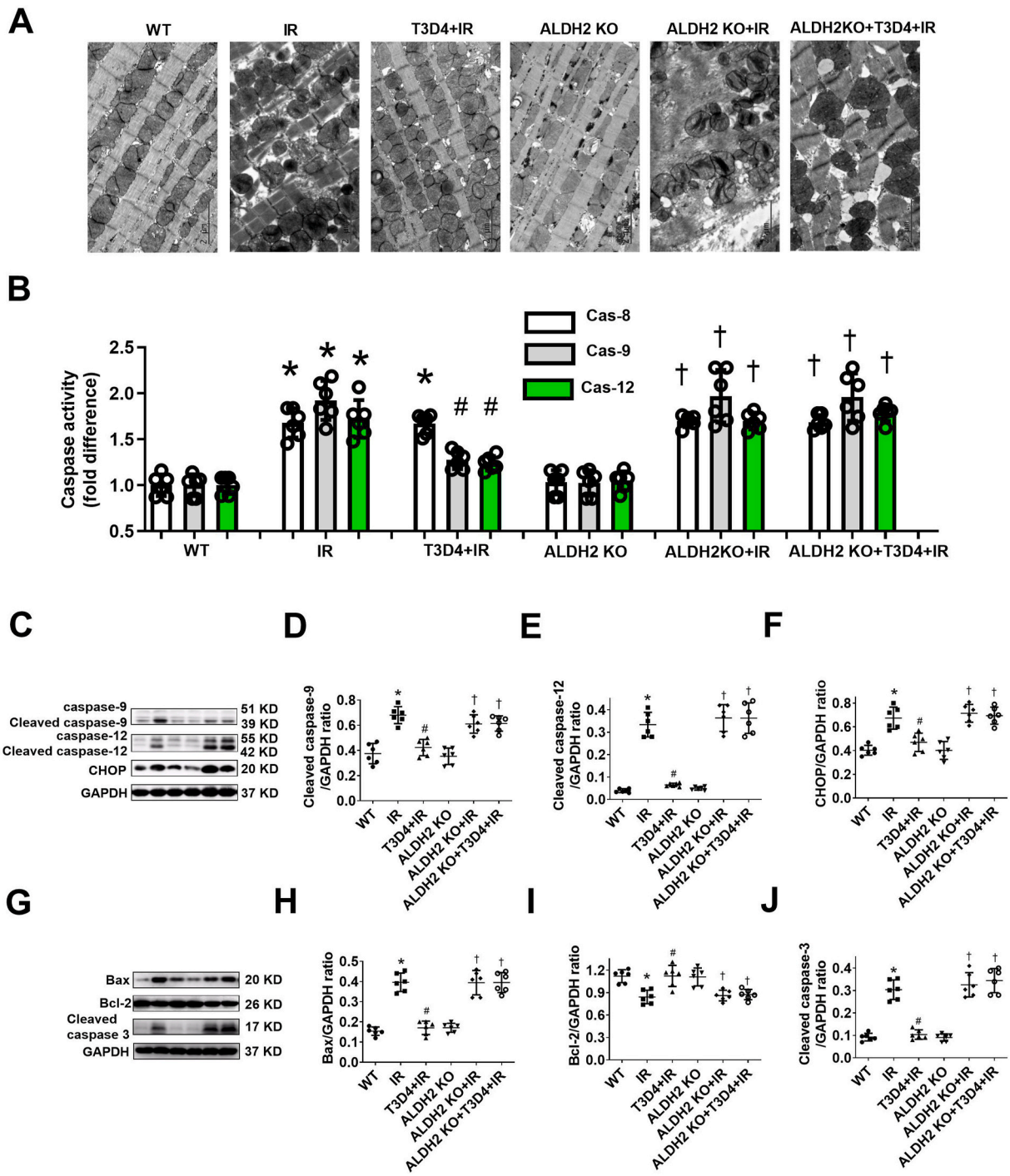

Fig. 7. ALDH2 deficiency attenuated hypertrophic preconditioning regulation of endoplasmic reticulum stress- and mitochondrial-mediated apoptosis pathways. (A) Representative mitochondrial photographs detected by transmission electron microscopy, scale bar = 2 μm(B) Myocardial caspase activity following *in situ* I/R.(C–H) Representative immunoblots and quantitative analysis of caspase-9 (C), caspase-12 (D), CHOP (E), caspase-3 (F), Bax (G), and Bcl-2 (H). *$P < 0.05$ *vs.* WT, $^{\#}P < 0.05$ *vs.* IR and †$P < 0.05$ *vs.* T3D4+IR; n = 6 per group.

development of myocardial protection due to preconditioning and acted by improving mitochondrial metabolism, hindering oxidative/nitrative stress and attenuating mitochondrial-mediated cell death.

The findings provided here are important in that they demonstrated that hypertrophic preconditioning is a novel endogenous self-defensive and cardioprotective strategy against I/R injury. However, there are several limitations in our current work. First, non-invasive remote ischaemic preconditioning has been shown to protect the human heart against myocardial ischaemia-reperfusion injury during open heart surgery in a recent study [43]. In comparison with non-invasive ischaemic preconditioning, hypertrophic preconditioning by the removal of aortic banding is an invasive and challenging operation. Second, although preconditioning with hypertrophic stress renders the heart resistant to subsequent IR injury, the clinical setting of AMI needs a post-treatment strategy, and further study should demonstrate whether postconditioning with hypertrophic stress protects against myocardial IR injury.

In summary, hypertrophic preconditioning is a novel endogenous

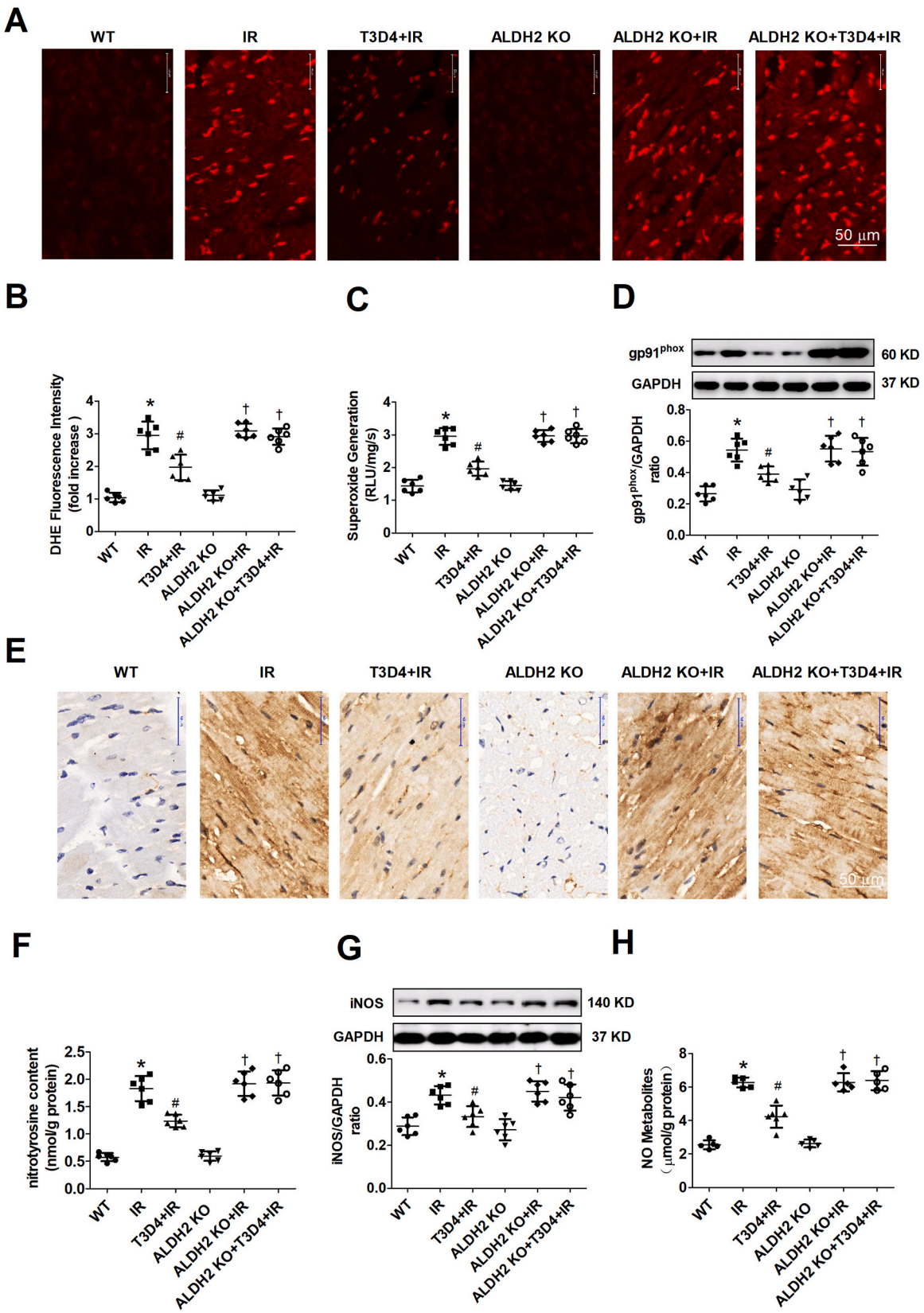

Fig. 8. ALDH2 deficiency attenuated hypertrophic preconditioning regulation of oxidative/nitrative stress in ischaemic/reperfused myocardium. (A) Myocardial superoxide at steady-state level measured by *in situ* dihydroethidium (DHE) staining, scale bar = 50 μm(B) Quantitative analyses of DHE fluorescence intensity (C) Superoxide production in ischaemic-reperfused cardiac tissue evaluated by lucigenin-enhanced chemiluminescence.(D) The expression of gp^{91phox} analysed by immunoblotting.(E) Representative images of nitrotyrosine immunostaining, scale bar = 50 μm(F) Nitrotyrosine content.(G) iNOS.(H) NO content in ischaemic-reperfused myocardial tissue measured by using the Griess methods. $^{*}P < 0.05$ *vs.* WT, $^{\#}P < 0.05$ *vs.* IR and †$P < 0.05$ *vs.* T3D4+IR; n = 6 per group.

self-defensive and cardioprotective strategy against I/R injury by improving mitochondrial metabolism, reducing oxidative/nitrative stress and inhibiting cardiomyocyte apoptosis; therefore, it represents a potentially attractive therapeutic strategy for the treatment of ischaemic heart diseases. Future studies are warranted to reveal the underlying cardioprotective mechanisms of hypertrophic preconditioning and may help us to find promising therapeutic strategies against I/R.

Author contributions

LLM, ZWD, PPY, JW and AJS performed the experiments and analysed the data. LLM performed the cardiac I/R injury surgery. ZWD and KH revised the manuscript. AJS, YZZ, and JBG designed and supervised the study and wrote the paper. All authors read and approved the final manuscript.

Declaration of competing interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.redox.2021.101960.

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