



## Cardiac-specific overexpression of aldehyde dehydrogenase 2 exacerbates cardiac remodeling in response to pressure overload



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### ABSTRACT

Pathological cardiac remodeling during heart failure is associated with higher levels of lipid peroxidation products and lower abundance of several aldehyde detoxification enzymes, including aldehyde dehydrogenase 2 (ALDH2). An emerging idea that could explain these findings concerns the role of electrophilic species in redox signaling, which may be important for adaptive responses to stress or injury. The purpose of this study was to determine whether genetically increasing ALDH2 activity affects pressure overload-induced cardiac dysfunction. Mice subjected to transverse aortic constriction (TAC) for 12 weeks developed myocardial hypertrophy and cardiac dysfunction, which were associated with diminished ALDH2 expression and activity. Cardiac-specific expression of the human ALDH2 gene in mice augmented myocardial ALDH2 activity but did not improve cardiac function in response to pressure overload. After 12 weeks of TAC, ALDH2 transgenic mice had larger hearts than their wild-type littermates and lower capillary density. These findings show that overexpression of ALDH2 augments the hypertrophic response to pressure overload and imply that downregulation of ALDH2 may be an adaptive response to certain forms of cardiac pathology.

### 1. Introduction

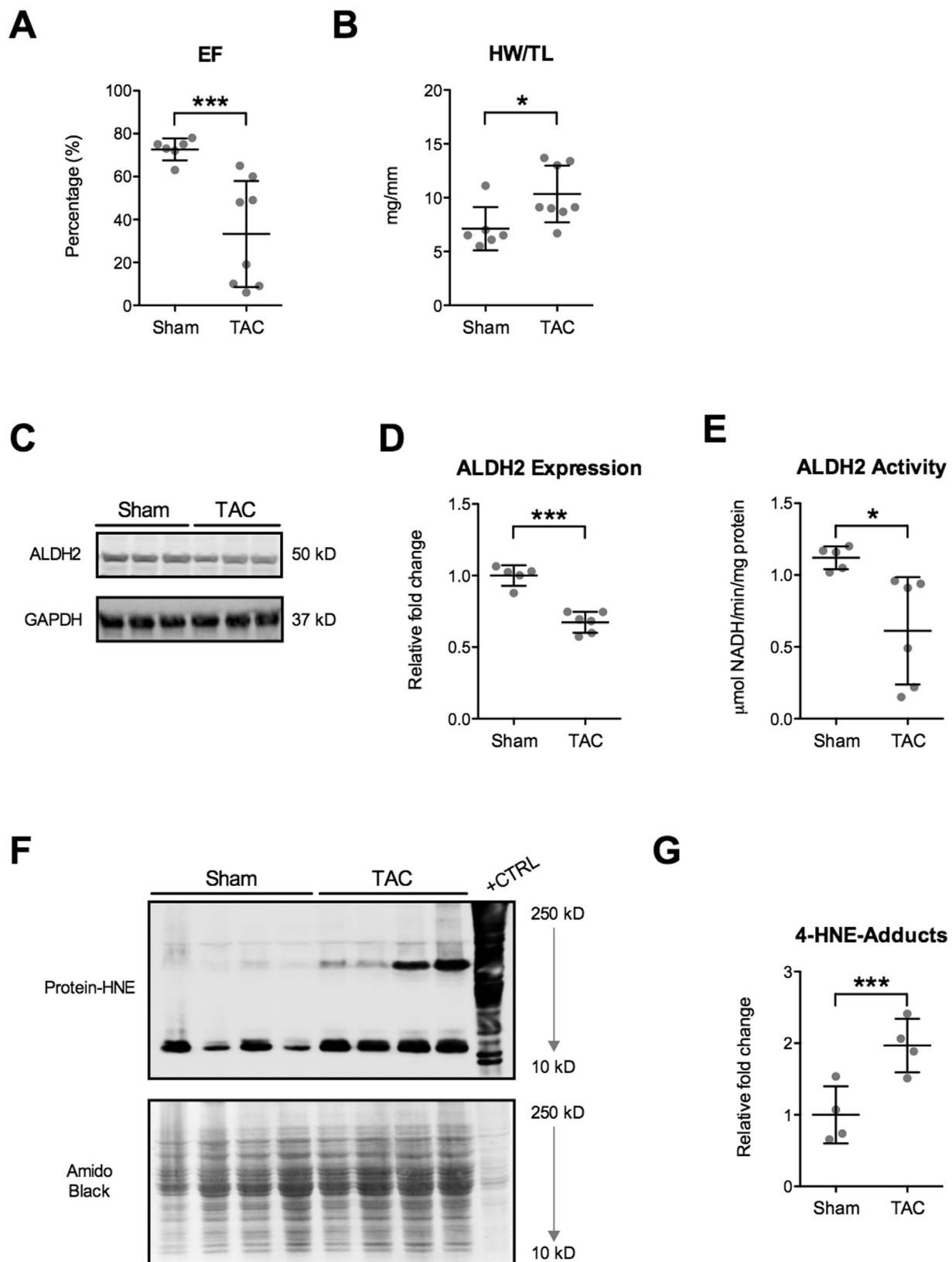
Heart failure is associated with lipid peroxidation and the accumulation of  $\alpha$ ,  $\beta$ -unsaturated aldehydes [1,2], which react avidly with cellular nucleophiles and can propagate or amplify tissue injury [3]. Although the heart possesses several aldehyde-detoxifying enzymes [4,5], its capacity to remove aldehydes is diminished in heart failure. For example, aldose reductase, which reduces reactive aldehydes such as 4-hydroxynonenal (HNE) to relatively inert alcohols, is down-regulated in the failing mouse heart [6], and glutathione-S-transferase activity, which conjugates aldehydes to glutathione, is lower in the pressure-overloaded rat heart [7]. Furthermore, the activity or abundance of aldehyde dehydrogenase 2 (ALDH2)—responsible for the majority of HNE detoxification in the heart [4,5]—is diminished in ischemia [4], after myocardial infarction [8,9], and in pressure overload-induced hypertrophy [10].

Although several studies indicate that ALDH2 protects the heart from injury and maladaptive remodeling (reviewed in [11–13]),

accumulating evidence indicates that deficiency of the enzyme could be protective as well. For example, mice expressing the defective *Aldh2*\*2 mutation show lower aldehyde detoxification capacity yet are protected against ischemia-reperfusion injury [14]. Moreover, overexpression of ALDH2 worsens aging-induced cardiac hypertrophy and dysfunction and shortens lifespan [15,16], and deficiency of ALDH2 prevents cardiac hypertrophy caused by pressure overload [17]. These studies suggest that diminishment of mitochondrial aldehyde dehydrogenase capacity may actually confer beneficial consequences, redolent of the hormetic effects of mitochondrial reactive species on survival and longevity [18–20] and the critical role of oxidants in tissue adaptation to injury [21]. Nevertheless, it remains unclear whether the decrease in ALDH2 activity occurring in heart failure is deleterious or adaptive.

In this study, we examined whether cardiac-specific overexpression of ALDH2 influences cardiac remodeling in the context of pressure overload. Our data indicate that ALDH2 expression and activity are lower in the pressure-overloaded heart and that overexpression of ALDH2 augments hypertrophic responses and limits capillary density

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**Fig. 1.** Pressure overload-induced heart failure is associated with decreases in cardiac ALDH2 activity. Cardiac function and ALDH2 expression and activity in wild-type mice subjected to sham surgery or transverse aortic constriction (TAC) for 12 weeks: Shown are (A) ejection fraction measured by echocardiography. Additional echocardiographic parameters can be found in [Table 1](#); (B) Gravimetric measurements of heart mass in Sham and TAC mice; (C) Representative Western blot of ALDH2; (D) Densitometric measurements from panel C; (E) ALDH2 activity measurements.  $n = 5-8$  per group; \* $p \leq 0.05$ , \*\*\* $p < 0.001$ , \*\*\*\* $p \leq 0.0001$ ; in panel E, Welch's correction was applied because equal variances cannot be assumed; (F) Representative Western blot of protein-4-hydroxynonenal (HNE) adducts from sham and TAC hearts. Heart homogenates incubated with reagent HNE served as a positive control; and (G) Densitometric measurements from panel F.  $n = 4$  per group, \* $p < 0.05$ .

**Table 1**

Phenotypic and echocardiographic characteristics of mice subjected to sham or transverse aortic constriction (TAC) surgery after 12 wk.

Group	n	Age (wk)	BW (g)	HR (bpm)	EDV ( $\mu$ L)	ESV ( $\mu$ L)	SV ( $\mu$ L)	LVIDd (mm)	LVIDs (mm)	LVAWd (mm)	LVPWd (mm)	LV mass (mg)
Sham	6	23.0 $\pm$ 0.1	29.0 $\pm$ 1.6	532 $\pm$ 19	41 $\pm$ 4	11 $\pm$ 3	30 $\pm$ 3	3.6 $\pm$ 0.2	2.0 $\pm$ 0.2	0.9 $\pm$ 0.1	0.8 $\pm$ 0.1	138 $\pm$ 43
TAC	8	23.0 $\pm$ 0.1	28.3 $\pm$ 1.7	551 $\pm$ 33	92 $\pm$ 49	71 $\pm$ 57	21 $\pm$ 9	4.8 $\pm$ 0.9	3.9 $\pm$ 1.4	1.0 $\pm$ 0.1	1.0 $\pm$ 0.1	191 $\pm$ 56

\*  $p < 0.05$  vs. Sham group (unpaired *t*-test).

without affecting fibrosis. These results provide support to the idea that downregulation of ALDH2 is an adaptive response to cardiac stress that influences hypertrophic programming.

## 2. Methods

### 2.1. Animal surgeries

All surgical animal procedures were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the University of Louisville Institutional Animal Care and Use Committee. Adult, 12–20-week-old, male wild-type (WT) mice on the C57BL/6J background were subjected to transverse aortic constriction (TAC) as previously described [22–24]. To examine the influence of ALDH2 expression, we used ALDH2 Tg mice and their nontransgenic (NTg) littermates. Briefly, mice were anesthetized with intra-peritoneal injections of ketamine (50 mg/kg) and pentobarbital (50 mg/kg). Mice were orally intubated and ventilated with 100% oxygen. The aorta was visualized following a thoracotomy and an intercostal incision. A 7-0 nylon suture was looped around the aorta between the brachiocephalic and left common carotid arteries. The suture was tied with a 27-gauge needle placed adjacent to the aorta to constrict the aorta to a reproducible diameter. The needle was removed and the chest was closed. Mice were extubated upon recovery of spontaneous breathing. Analgesia (Ketoprofen, 5 mg/kg) was provided prior to recovery and by 24 and 48 h post-surgery. Sham mice were subjected to the same procedure as the TAC cohort except the suture was not tied. The surgeon was blinded to mouse genotype.

### 2.2. Echocardiographic assessment of cardiac function

Transthoracic echocardiography of the left ventricle was performed as described [22,24–30]. The sonographer was blinded to mouse genotype.

### 2.3. Generation of cardiac-specific ALDH2 Tg mice

To generate cardiac-specific aldehyde dehydrogenase 2 transgenic (TG) mice, human ALDH2 cDNA (1.58 Kb) was ligated at the 5' end to the 5.5 kb murine cardiac-specific  $\alpha$ -myosin heavy chain ( $\alpha$ -MHC) promoter and a 0.6 kb human Growth Hormone (hGH) polyA was ligated at the 3' end of the ALDH2 cDNA. Nucleotide sequencing and comparisons with published sequences verified the construct as hALDH2 cDNA. The resultant 7.68 kb cDNA construct was treated with restriction endonuclease to release the transgene, and it was purified and microinjected into male pronuclei of fertilized ova to generate transgenic mice at the University of Cincinnati transgenic core. The transgenic mice were produced on the C57BL/6 mouse strain. PCR was used to amplify DNA isolated from ear tissue and identify mice carrying the transgene. Founder mice were identified by genomic PCR using the following primers, Forward: 5' AGT CCT GGT GGG AGA GCC ATA 3' and Reverse: 5' CAC TGC CTT GTC CAC ATC TTC 3' corresponding to the  $\alpha$ -MHC 5' UTR and the ALDH2 coding region, respectively.

### 2.4. ALDH2 activity assays

ALDH2 activity was measured in whole heart homogenates, as described [31]. Briefly, hearts were homogenized in phosphate-buffered saline, pH 7.4, containing protease inhibitor cocktail and 0.5% NP-40. The homogenates were centrifuged at 13,000g for 10 min, and protein in the supernatant was determined using the Lowry DC protein assay (Biorad). The activity assay was carried out at 37 °C in buffer containing 50 mM sodium pyrophosphate, pH 8.8, 0.1 mM 4-methylpyrazole, 1 mM EDTA, 2.5 mM NAD<sup>+</sup>, 1 mM DTT, and 100  $\mu$ g of homogenate protein. To initiate the reaction, 1 mM propionaldehyde was added to the reaction mix. Formation of NADH was determined spectrophotometrically ( $\epsilon_{340} = 6220 \text{ M}^{-1} \text{ cm}^{-1}$ ), and ALDH2 activity was expressed as  $\mu$ moles NADH/min/mg protein.

### 2.5. Protein abundance measurements

Tissues were homogenized in buffer containing 20 mM HEPES (pH 7.0), 110 mM KCl, 1 mM EDTA, 1% NP-40%, and 0.1% SDS; fresh phosphatase and protease inhibitors were added immediately prior to tissue homogenization. The homogenates were sonicated on ice and centrifuged at 13,000g for 20 min. Proteins in the supernatant were separated by SDS-PAGE, electroblotted to PVDF membranes, and probed with antibodies according to the manufacturers' protocols. With the exception of the GAPDH antibody, which was directly linked to horseradish peroxidase (HRP), HRP-linked secondary antibodies were used to detect immunoreactive proteins. Images were recorded using a Fujifilm LAS-300 imager. Where appropriate, relative protein abundance, measured via densitometric analysis using TotalLab or ImageJ software, was normalized to the loading control (GAPDH or Amido Black) prior to calculating fold change. A list of the antibodies is in the [Online Supplement](#).

### 2.6. Measurements of gene expression

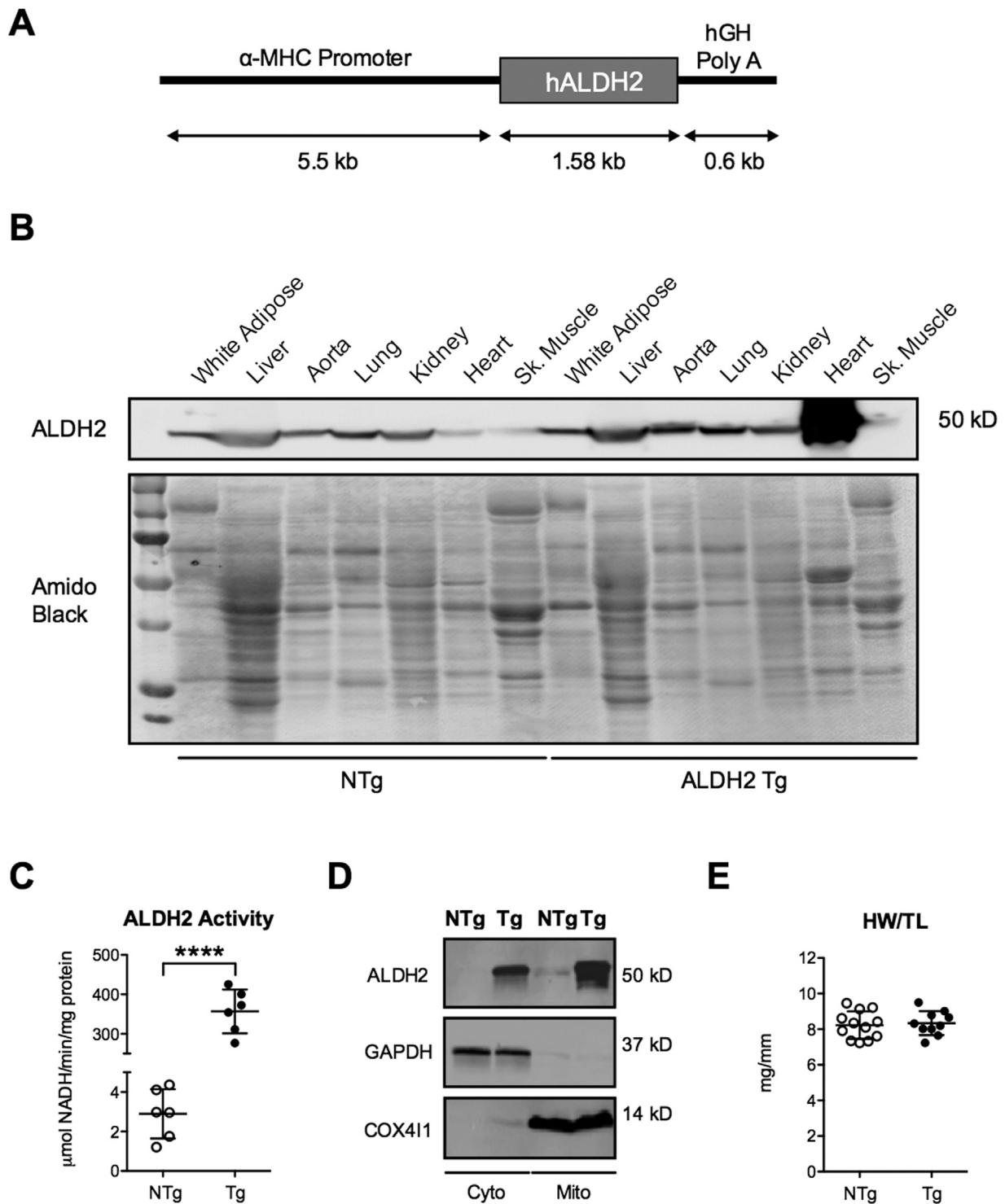
For gene expression analysis via RT-PCR, RNA was extracted from the heart using the RNeasy Plus Universal Kit (Qiagen, Germantown, MD), followed by cDNA synthesis. Real-time PCR amplification was performed with Power SYBR PCR Master Mix (ThermoFisher Scientific, Waltham, MA) using a 7900HT Fast Real-Time PCR System (Applied Biosystems, Foster, CA). Relative expression was determined by the  $2^{-\Delta\Delta Ct}$  method. A list of the primers is in the [Online Supplement](#).

### 2.7. Histopathology

Histology was performed following previously published methods [23,29,30]. Following final echocardiography, hearts were excised and arrested in diastole with KCl. Hearts were then sectioned into 1 mm cross-sectional slices. A mid-ventricular section was fixed with formalin, deparaffinized, and rehydrated.

### 2.8. Cardiomyocyte hypertrophy

Cardiac sections were stained with wheat germ agglutinin (WGA; AlexaFluor 555 conjugate, Invitrogen, Carlsbad, CA) to identify cell borders and DAPI to detect nuclei. WGA-stained cells were visualized

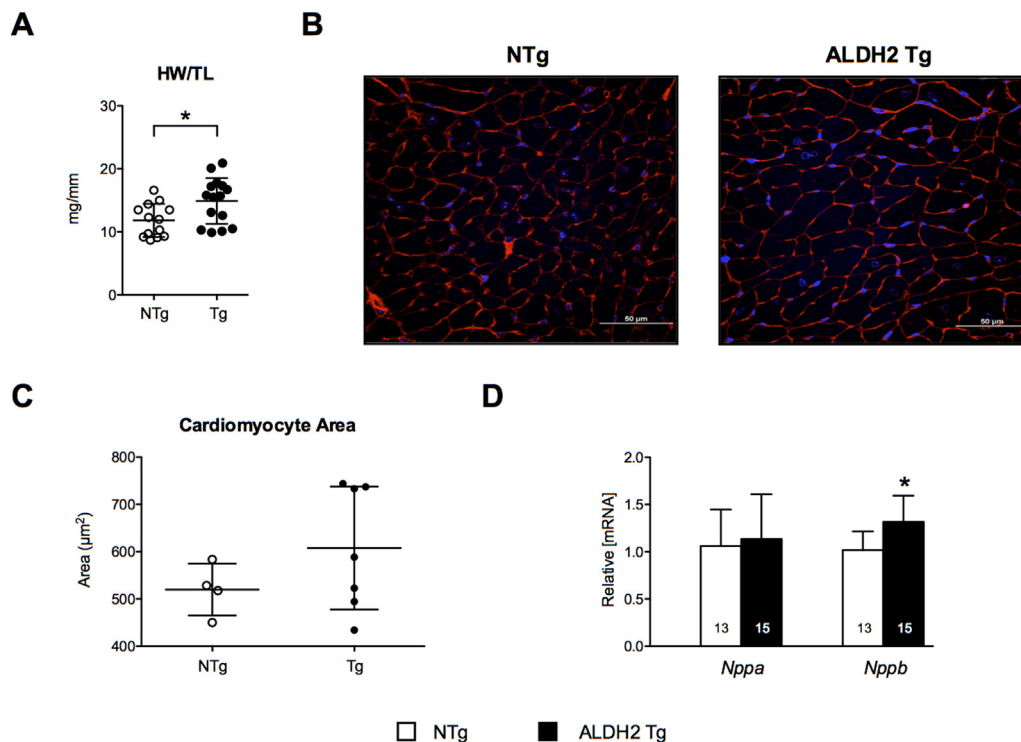


**Fig. 2. Generation of a cardiac-specific ALDH2 overexpressing mouse.** (A) Schematic of the  $\alpha$ -MHC-hALDH2 overexpression construct.  $\alpha$ -MHC,  $\alpha$ -myosin heavy chain; hGH poly A, human growth hormone poly-adenylation signal. (B) Western blot analysis of ALDH2 expression in tissues from NTg and ALDH2 Tg mice. Amido Black served as a loading control. (C) ALDH2 activity measurements.  $n = 6$  per group, \*\*\*\*  $p < 0.0001$ . (D) Localization of ALDH2 in the cytosolic and mitochondrial fractions of the heart. Cytosolic marker, GAPDH, glyceraldehyde-3-phosphate dehydrogenase; mitochondrial marker, COX4I1, cytochrome c oxidase subunit 4 isoform 1. (E) Gravimetric measurements of heart size in naive NTg and ALDH2 Tg mice.  $n = 10$ – $11$  per group, \* $p < 0.05$ .

using a Nikon TE-2000E microscope interfaced with a Nikon A1 confocal system. Cell areas were measured from cardiomyocytes with centrally located nuclei using Nikon Elements software [64-bit version 3.22.00(Build 710)].

### 2.9. Capillary density

Cardiac sections were stained with isolectin B4 (Fluorescein labeled Griffonia Simplicifolia Lectin I, Vector Labs, Burlingame, CA) following previously published protocols [30]. Capillary density was determined



**Fig. 3.** ALDH2 overexpression increases pressure overload-induced cardiac hypertrophy. Measurements of cardiac structure and gene expression in NTg and ALDH2 Tg mice subjected to 12 weeks of TAC: (A) Gravimetric measurements of heart size; (B) Representative images of WGA and DAPI-stained mid-ventricular histological sections from NTg and ALDH2 Tg TAC hearts; (C) Cardiomyocyte area; and (D) Expression of *Nppa* and *Nppb* measured by qRT-PCR.  $n = 12\text{--}15$  per group, \* $p < 0.05$ .

by dividing the total number of isolectin B4 positive vessels by the area of the image. Capillary density was expressed as the number of capillaries per mm<sup>2</sup>.

### 2.10. Cardiac fibrosis

Cardiac sections were stained with Fast Green (EMD, Gibbstown, NJ) and Sirius Red (Roboz, Washington, DC) using previously published protocols [29,32]. Fibrosis was expressed as a percentage of scar tissue divided by the total area of tissue.

### 2.11. Statistical considerations

Data are reported as mean  $\pm$  SD. Significance level was tested by unpaired *t*-test. A *p* value  $< 0.05$  was considered statistically significant.

## 3. Results

### 3.1. Heart failure is associated with a reduction in ALDH2 abundance and activity

As expected, TAC decreased ejection fraction (Fig. 1A) and increased heart weight (Fig. 1B). In addition, TAC hearts exhibited enlarged chamber dimensions (Table 1). Compared with Sham hearts, pressure-overloaded hearts had significantly lower ALDH2 protein abundance and activity (Figs. 1C–1E). Protein-4-hydroxynonenal (protein-HNE) adducts were increased 2-fold in TAC hearts compared with Sham hearts (Figs. 1F and 1G). These data indicate that decreases in ALDH2 expression and activity are associated with cardiac hypertrophy and are accompanied by elevated levels of oxidatively damaged proteins.

### 3.2. Generation of cardiac-specific ALDH2 overexpressing mice

To determine whether increasing ALDH2 in the heart affects cardiac remodeling and heart failure in response to pressure overload, we

generated a cardiac-specific, ALDH2 transgenic (ALDH2 Tg) mouse, with the human ALDH2 transgene driven by the  $\alpha$ MHC promoter (Fig. 2A). Mice carrying the ALDH2 transgene were crossed with WT mice to generate ALDH2 transgenic mice and respective NTg littermates. The progeny demonstrated a non-Mendelian distribution, with  $\sim 31\%$  (46/150) expressing the ALDH2 transgene.

To examine tissue specificity of the ALDH2 transgene, we examined ALDH2 abundance in multiple organs from ALDH2 Tg mice and NTg littermates. As anticipated, ALDH2 was markedly elevated in hearts from ALDH2 Tg mice but was not elevated above NTg levels in any other tissue (Fig. 2B). To determine whether the overexpressed ALDH2 protein was enzymatically active, we measured propionaldehyde oxidation activity in NTg and ALDH2 Tg hearts. Compared with NTg hearts, the ALDH2 Tg hearts demonstrated  $> 100$ -fold increase ALDH2 activity (Fig. 2C). To determine the subcellular localization of ALDH2, we examined ALDH2 abundance in cytosolic and mitochondrial fractions. As shown in Fig. 2D, ALDH2 in NTg hearts was observed only in the mitochondrial fraction. While the majority of the overexpressed ALDH2 protein was found in the mitochondrial fraction, the enzyme was also found in the cytosol. The size of naïve ALDH2 Tg hearts was similar to that from NTg littermates (Fig. 2E). These results show that ALDH2 Tg hearts have remarkably elevated cardiac ALDH2 activity without changes in cardiac size.

### 3.3. Overexpression of ALDH2 augments pressure-overload induced hypertrophy

To determine whether overexpression of ALDH2 affects cardiac structure and function following pressure overload, we subjected ALDH2 Tg mice and their NTg littermates to TAC for 12 weeks. After TAC, hearts from ALDH2 Tg mice were significantly larger than that of their NTg littermates (Fig. 3A); however, ALDH2 Tg hearts subjected to TAC showed chamber dimensions and indices of cardiac function similar to that of pressure-overloaded NTg hearts (Table 2). Although cardiomyocyte area was not statistically different between ALDH2 Tg and NTg mice (Fig. 3C), *Nppb* expression was modestly but significantly higher (Fig. 3D). The expression of genes that commonly change in

**Table 2**  
Phenotypic and echocardiographic characteristics of NTg and cardiac-specific ALDH2 Tg mice subjected to transverse aortic constriction (TAC) surgery after 12 wk.

Group	n	Age (wk)	BW (g)	HR (bpm)	SV (μL)	CO (mL/min)	EDV (μL)	ESV (μL)	EF (%)	SV (μL)	LVIDd (mm)	LVIDs (mm)	LVAWd (mm)	LVPWd (mm)	LV mass (mg)
NTg	13	26.0 ± 1.0	29.2 ± 2.8	522 ± 44	23 ± 8	11.9 ± 4.4	82 ± 45	59 ± 52	37 ± 21	23 ± 8	4.7 ± 0.8	3.9 ± 1.1	0.9 ± 0.2	1.1 ± 0.2	218 ± 47
Tg	15	27.0 ± 1.0	27.5 ± 3.8	536 ± 45	17 ± 9	8.9 ± 4.9	106 ± 56	90 ± 65	27 ± 24	17 ± 9	5.1 ± 1.1	4.4 ± 1.4	0.9 ± 0.2	1.1 ± 0.3	278 ± 70

\* p < 0.05 vs. Sham group (unpaired t-test).

heart failure (*Myh6*, *Myh7*, *Glut4*, *Pgc1a*, and *Ppara*) was similar between ALDH2 Tg and NTg TAC hearts (Supplemental Figure 1). These findings indicate that ALDH2 overexpression augmented left ventricular hypertrophy without improving cardiac function following TAC.

#### 3.4. ALDH2 overexpression modulates the cardiac redox landscape

To determine if the differences in cardiac remodeling in ALDH2 Tg hearts are associated with lower aldehyde burden, we measured the levels of protein-HNE adducts. ALDH2 Tg hearts subjected to TAC showed a trend toward lower levels of HNE-damaged proteins compared with corresponding NTg hearts ( $p = 0.059$ ; Fig. 4A). In these hearts, the expression of the antioxidant enzymes catalase and heme oxygenase 1 (HO-1) showed diametrically opposite changes (Fig. 4C–E), which suggests redox remodeling caused by ALDH2 overexpression. These findings indicate that ALDH2 Tg hearts show an aberrant redox phenotype, which might affect cardiac responses to pathological stress.

#### 3.5. Effects of ALDH2 on fibrosis and capillary density

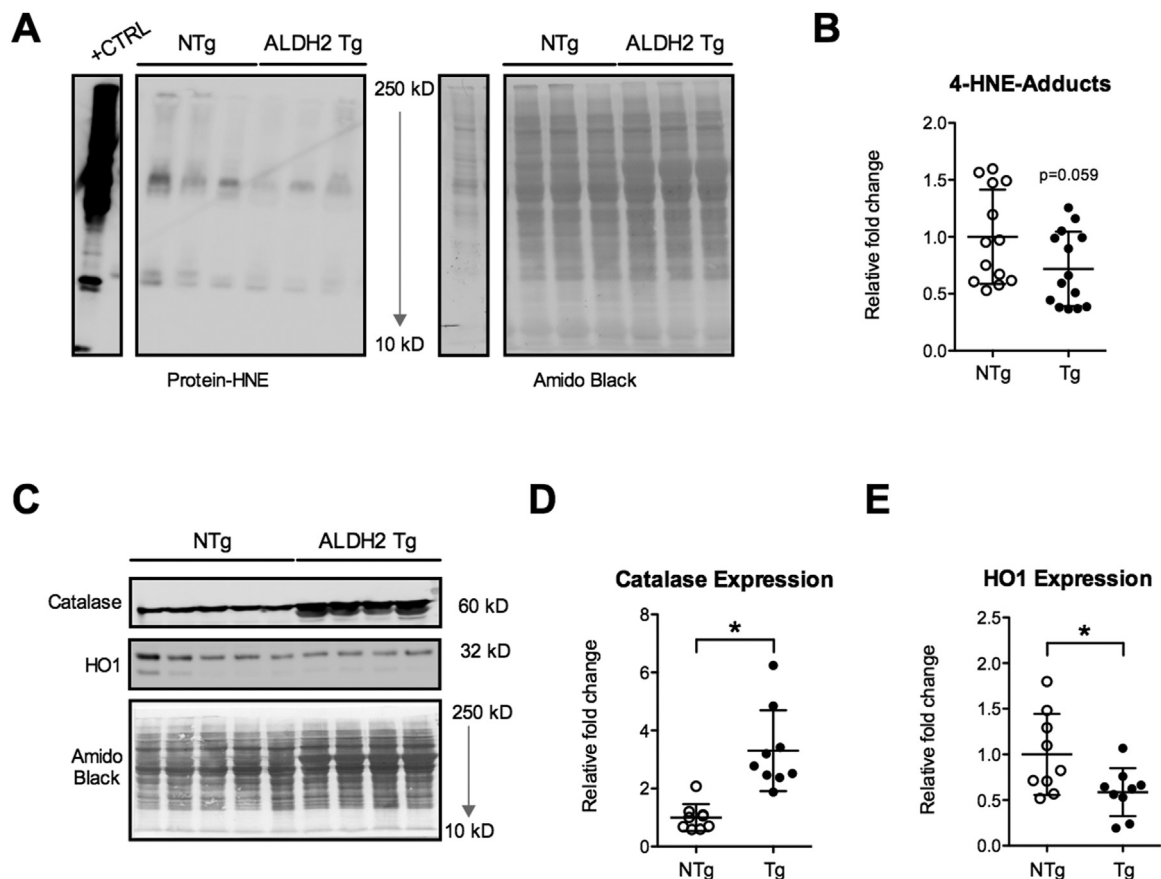
Because fibrosis is a hallmark of TAC-induced pathology, we first assessed fibrotic markers and the relative abundance of collagen. Although pressure-overloaded, ALDH2 Tg hearts showed lower expression of *Col1a1*, *Tgfb1*, and *Fgf1* than corresponding NTg hearts (Fig. 5A), fibrosis assessed via fast green/sirius red staining (Fig. 5B) showed no significant differences in collagen accumulation between the genotypes (Figs. 5C and 5D).

Because changes in angiogenesis are implicated in cardiac hypertrophy and heart failure [33], we also assessed molecular and gross indicators of capillary density. Although we found no differences in the expression of eNOS (*Nos3*) or VEGF receptor 2 (*Kdr*), expression of the iNOS (*Nos2*) and T-box 3 (*Tbx3*) genes, which can regulate angiogenesis [34,35], were lower in pressure-overloaded ALDH2 Tg hearts (Fig. 5A). Quantification of isolectin-stained sections showed significantly lower capillary density in ALDH2 Tg TAC hearts compared with NTg TAC hearts (Fig. 5E and F). Taken as a whole, these data suggest that ALDH2 overexpression exacerbates TAC-induced cardiac remodeling by exaggerating the hypertrophic response and by diminishing capillary density.

## 4. Discussion

The abundance of several aldehyde-detoxifying enzymes is lower in the failing heart. In particular, ALDH2 is downregulated in both the infarcted and pressure-overloaded heart [8–10]. Nevertheless, it remains unclear whether decreases in ALDH2 expression are beneficial or deleterious in the context of pathological stress. In this study, we tested the hypothesis that enforcing ALDH2 levels in the pressure-overloaded heart improves cardiac function and remodeling. Similar to previous findings, we found that ALDH2 abundance and activity were lower in hearts subjected to pressure overload; however, not only was cardiac-specific overexpression of ALDH2 insufficient to prevent cardiac dysfunction due to pressure overload, it augmented the hypertrophic response and diminished capillary density. These changes were associated with altered expression of the antioxidant enzymes catalase and HO-1. Our findings suggest that increasing ALDH2 expression or activity may not be beneficial in all contexts of heart failure and imply that the downregulation of ALDH2 may be an adaptive response that controls the redox landscape, permits angiogenesis, and limits hypertrophy under conditions of pressure overload.

Our findings are surprising given the relatively large number of studies that attest to a beneficial role of ALDH2 in cardiac pathology. Seminal studies showed that small molecule activation of ALDH2 diminishes ischemic damage to the heart [36–38]. Later studies



**Fig. 4. ALDH2 overexpression decreases protein-HNE adducts.** Western blots of indicators of aldehyde burden and antioxidant enzymes in NTg and ALDH2 Tg mice subjected to 12 weeks of TAC: (A) Representative Western blot of protein-4-hydroxynonenal (HNE) adducts. Heart homogenates incubated with reagent HNE served as a positive control; (B) Densitometric measurements from panel A.  $n = 13\text{--}14$  per group; (C) Representative Western blots of catalase and HO-1; (D) Densitometric measurement of catalase from panel C; (E) Densitometric measurement of HO-1 from panel C. E.  $n = 9$  per group,  $*p < 0.05$ .

demonstrated that whole-body ALDH2 deletion worsens injury due to ischemia-reperfusion, whereas whole-body ALDH2 overexpression diminishes injury [39]; other studies appear to confirm the protective effect of ALDH2 in the acutely injured heart (e.g., [40–42]). Nevertheless, mice expressing *Aldh2*\*2, which is a single nucleotide polymorphism that lowers ALDH2 activity, were found to be protected against cardiac ischemic injury [3,14], which suggests a potential Janus face of ALDH2.

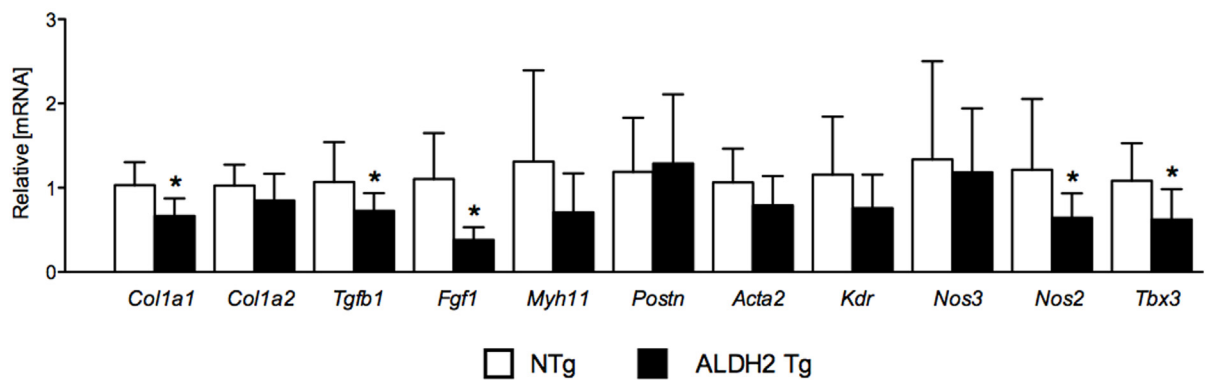
A beneficial role of ALDH2 is less clear in the context of cardiac hypertrophy. Whereas ALDH2 has been suggested to be protective in alcohol-induced cardiomyopathy [43], myocardial infarction-induced heart failure [9,44] and pressure overload-induced cardiac dysfunction [10], a nearly equivalent level of evidence exists for deleterious effects of ALDH2. General overexpression of ALDH2 was found not only to promote cardiac hypertrophy and contractile dysfunction in aged mice [15], but to shorten lifespan and promote cardiac aging as well [16]. Moreover, deletion of ALDH2 was shown to prevent cardiac hypertrophy caused by pressure overload [17]. Similarly, cardiac-specific overexpression of aldehyde reductase, which can reduce aldehydes such as HNE to their less reactive alcohols, causes cardiac hypertrophy and contractile dysfunction in aged mice [45]. These findings suggest that the abundance of ALDH2 and other aldehyde-detoxifying enzymes may modulate hypertrophic programs in the heart. Our results showing increased expression of a molecular marker of hypertrophy (*Nppb*) and an exaggerated hypertrophic response to TAC in ALDH2 Tg mice appear consistent with this idea.

While the mechanisms by which ALDH2 regulates cardiac responses to pathological stress remain unclear, the capillary rarefaction found in pressure-overloaded ALDH2 Tg hearts provides potential clues. Loss of

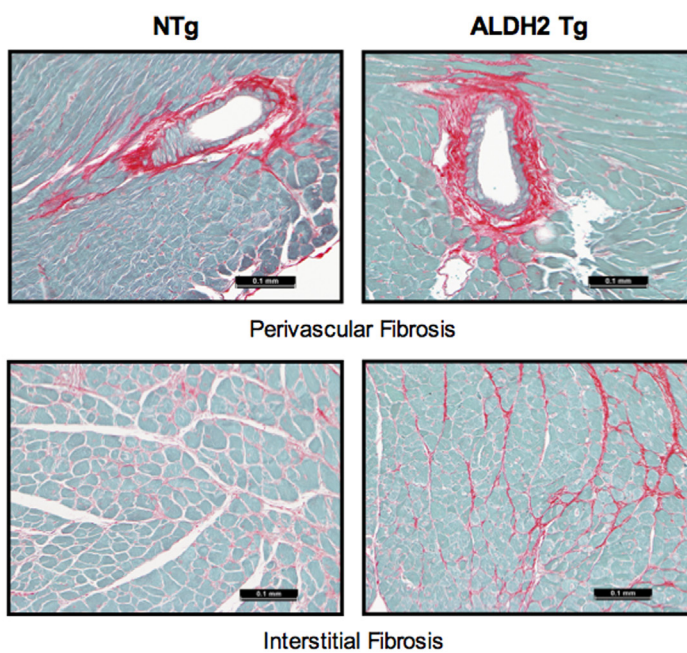
capillary density and diminished endothelial function occur in pathological hypertrophy [46–48] and have been suggested to play a causal role in idiopathic dilated cardiomyopathy [49] and heart failure with preserved ejection fraction [50,51]. Interestingly, reactive products normally detoxified by ALDH2, such as HNE, can promote angiogenesis and augment microvessel density in other tissues [52–54]. Thus, it makes sense that HNE may be important for regulating capillary density in the heart as well. Interestingly, compared with corresponding NTg mice, *Tbx3*—a strong inducer of angiogenesis in the context of cancer [35]—was lower in pressure-overloaded ALDH2 Tg hearts. Additional studies will be required to discern how ALDH2 and the products it detoxifies influence myocardial angiogenesis.

The fact that, in the context of pressure overload, ALDH2-overexpressing hearts showed higher catalase levels suggests a potential role of aldehyde dehydrogenase activity in modulating the detoxification of other classes of reactive species. Catalase, which catalyzes the reduction of hydrogen peroxide, is upregulated in end-stage human heart failure [43]. Generally, catalase is considered to be a protective enzyme in the context of cardiomyopathy [55–59]. It is possible that augmentation of ALDH2 and other classes of antioxidant enzymes such as catalase diminish the hormetic responses known to protect against tissue injury and dysfunction [14,19,60]. Whereas catalase was elevated in ALDH2 Tg hearts, HO-1 expression was lower. Because HO-1 is protective in the context of heart failure [61], the lower levels of HO-1 may also contribute to the worsened pathological hypertrophy observed in pressure-overloaded ALDH2 Tg hearts. Furthermore, it is possible that the imbalanced redox landscape caused by overexpression of ALDH2 leads to a form of reductive stress that could worsen cardiac pathology, as suggested by previous studies [62–64].

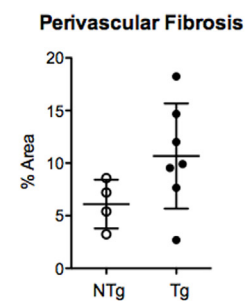
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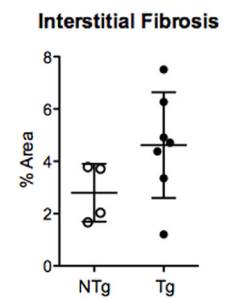
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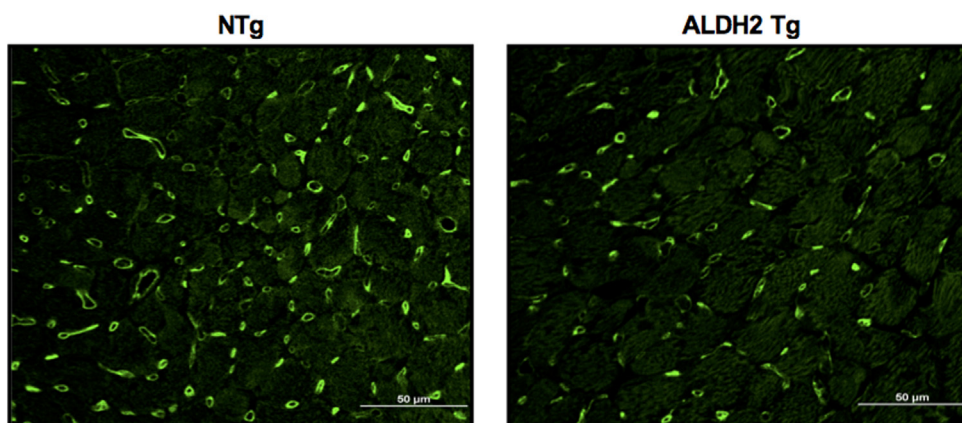
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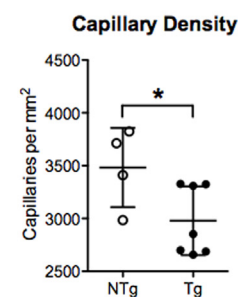
**D**



**E**



**F**



**Fig. 5. ALDH2 overexpression diminishes capillary density in the context of pressure overload.** Measurements of gene expression and cardiac structure in NTg and ALDH2 Tg mice subjected to 12 weeks of TAC: (A) Gene expression of fibrotic and vascular markers measured by qRT-PCR. n = 12–15 per group, \*p < 0.05; (B) Representative images of fast green/sirius red-stained NTg and ALDH2 Tg TAC hearts. Red staining indicates fibrotic regions. Green-blue staining depicts non-fibrotic regions; (C) Quantification of perivascular fibrosis from panel B; (D) Quantification of interstitial fibrosis from panel B; (E) Representative images of NTg and ALDH2 Tg TAC hearts stained with isolectin B4; (F) Quantification of capillary density. n = 4–6 per group, \*p < 0.05.



There are several limitations to our study. Foremost is the dramatically elevated levels of ALDH2 in transgenic hearts. The high levels of ALDH2 expression led to its higher abundance not only in mitochondria, but in the cytosol as well, which potentially obfuscates data interpretation. Although results of our activity assays suggest > 100-fold higher ALDH2 enzyme detoxification capacity in the heart, it is unlikely that the enzyme is as active in vivo as measured in vitro, where saturating substrate and cofactor conditions and optimal pH (8.8) for ALDH2 activity are maintained. Indeed, the levels of protein-HNE adducts, while trending toward diminished abundance, were not remarkably different in ALDH2 Tg hearts compared with NTg counterparts. Nevertheless, coherence of our finding with several other studies showing worsened hypertrophy in ALDH2-overexpressing models as well as diminished hypertrophy in ALDH2 knockout models [15–17] supports the idea that ALDH2 downregulation is not necessarily maladaptive under conditions of pressure overload. Another limitation is the lack of a clear mechanism for how ALDH2 affects pathological responses to pressure overload. It appears that ALDH2 overexpression not only augments cardiac hypertrophy, but it diminishes capillary density and remodels antioxidant enzyme expression as well. Further studies would be required to understand whether such changes relate causally to one another and to determine their relative contribution to pathological hypertrophy in this model.

In summary, we find that augmenting expression of cardiac ALDH2 does not improve the response to pressure overload. Rather, ALDH2 overexpression worsens cardiac hypertrophy and promotes capillary rarefaction. Integrating our findings with the literature, we posit that downregulation of ALDH2 may be an adaptive response to certain forms of pathological stress. Additional studies are required to determine how ALDH2 and other aldehyde-detoxifying enzymes influence cardiac remodeling.

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## Disclosures

The authors declare no competing or relevant financial interests.

## Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at <http://dx.doi.org/10.1016/j.redox.2018.05.016>.

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