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Title Page

mPGES-1 deletion attenuates isoproterenol-induced myocardial fibrosis in mice

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Running Title Page

a) Running Title: mPGES-1 and myocardial fibrosis.

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d) Abbreviations

ANF	atrial natriuretic factor
ANOVA	analysis of variance
BNP	brain natriuretic peptide
COX	cyclooxygenase
CTGF	connective tissue growth factor
DMEM	dulbecco's modified eagle's medium
EF	ejection fraction
FBS	fetal bovine serum
FS	fractional shortening
GFP	green fluorescence protein
H&E	haematoxylin and eosin staining
IL	interleukin
ISO	isoproterenol
LV	left ventricular
LVPW	left ventricular posterior wall thickness

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MCP	monocyte chemoattractant protein
mPGES1	microsomal prostaglandin E synthase-1
PCR	polymerase chain reaction
PGE2	prostaglandin E2
siRNA	small interfering RNA
TNF-α	tumor necrosis factor α
VEGF	vascular endothelial growth factor
β-AR	β-adrenergic receptor

e) Section: Cardiovascular

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Abstract

Deletion of microsomal PGE2 synthase-1 (mPGES-1) inhibits inflammation and protects against atherosclerotic vascular diseases, but displayed variable influence on pathological cardiac remodeling. Overactivation of β-adrenergic receptors (β-ARs) causes heart dysfunction and cardiac remodeling, while the role of mPGES-1 in β-ARs induced cardiac remodeling is unknown. Here we addressed this question using mPGES-1 knockout mice, and subjecting them to isoproterenol, a synthetic nonselective agonist for β-ARs, at 5mg/kg/day or 15mg/kg/day to induce different degrees of cardiac remodeling in vivo. Cardiac structure and function were assessed by echocardiography 24 hours after the last of seven consecutive daily injections of isoproterenol, and cardiac fibrosis was examined by Masson trichrome stain in morphology and by real-time PCR for the expression of fibrosis related genes. The results showed that deletion of mPGES-1 had no significant effect on isoproterenol-induced cardiac dysfunction or hypertrophy. However, the cardiac fibrosis was dramatically attenuated in the mPGES-1 knockout mice after either low dose or high dose isoproterenol exposure. Furthermore, in vitro study revealed that overexpression of mPGES-1 in cultured cardiac fibroblasts increased isoproterenol induced fibrosis, while knocking-down mPGES-1 in cardiac myocytes decreased the fibrogenesis of fibroblasts. In conclusion, mPGES-1 deletion protects against isoproterenol-induced cardiac fibrosis in mice, and targeting mPGES-1 may represent a novel strategy to attenuate pathological cardiac fibrosis, induced by β-ARs agonists.

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Significance Statement

Inhibitors of mPGES-1 are being developed as alternative analgesics which are less likely to elicit cardiovascular hazards than COX-2 selective NSAIDs. We have demonstrated that deletion of mPGES-1 protects inflammatory vascular diseases and promotes post-MI survival. The role of mPGES-1 in β-adrenergic receptors induced cardiomyopathy is unknown. Here we illustrated that deletion of mPGES-1 alleviated isoproterenol-induced cardiac fibrosis without deteriorating cardiac dysfunction. These results illustrated that targeting mPGES-1 may represent an efficacious approach to the treatment of inflammatory cardiovascular diseases.

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Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are one of the most widely used drugs in the world. Based on the inhibition of the production of inflammatory PGE2, traditional NSAIDs and COX-2 selective inhibitors have become the best choice of antipyretic and analgesic drugs (Chen, Yang, & Grosser, 2013a). However, increasing studies have shown that long-term use of such drugs is accompanied by significant cardiovascular side effects, such as hypertension, stroke, myocardial infarction and so on (Grosser, Ricciotti, & FitzGerald, 2017). There has been intense interest in developing new NSAIDs that might preserve the anti-inflammatory efficacy while limiting the cardiovascular risks.

Microsomal prostaglandin E synthase-1 (mPGES-1), the inducible PGE2 terminal synthase, which is usually coupled with COX-2 to mediate the production of PGE2 in inflammatory states (Deng et al., 2019; Thoren & Jakobsson, 2000; Uematsu, Matsumoto, Takeda, & Akira, 2002), has gained considerable attention as a preferable target for new generation of antipyretic and analgesic drugs (Bergqvist, Morgenstern, & Jakobsson, 2019; Yang & Chen, 2016). It has been reported that, unlike COX-2 selective inhibitors, deletion of mPGES-1 in mice is protective to inflammatory vascular diseases, for example, retards atherogenesis (Wang et al., 2006), suppresses abdominal aortic aneurysm formation (Wang et al., 2008a), limits post-injury neointima hyperplasia (Wang et al., 2011). However, the role of mPGES-1 in pathological cardiac remodeling and heart dysfunction is still in debate. Although studies had reported impaired post-ischemic heart function and cardiac remodeling in mPGES-1 deficient mice (Degousee et al., 2008; Zhu et al., 2019), we and others failed to observe any adverse influence on cardiac remodeling after mPGES-1 was deleted globally or selectively in myeloid cells (Chen et al., 2019; Wu et al., 2009b). Moreover, in a model of Ang II mediated cardiac remodeling, although lack of mPGES-1 did not affect cardiac hypertrophy and fibrosis, nevertheless poor cardiac function was observed (Harding, Yang, He, &

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Lapointe, 2011). These paradoxes suggest a complex role of mPGES-1 in cardiac repair and more work is required to refine the role of mPGES-1 in mediating pathological myocardial remodeling and heart dysfunction.

In the present study, by using an isoproterenol (ISO)-induced cardiac remodeling model to simulate the hyperactivation of β-ARs under acute stress conditions, we found that, although deletion of mPGES-1 fails to improve ISO-induced cardiac hypertrophy and heart dysfunction, lack of mPGES-1 is protective to ISO-induced cardiac fibrosis. Although clinical application of β-blockers can eliminate the cardiac injury and its adverse consequences caused by sympathetic nervous system overactivation to a certain extent, since β-ARs is essential to many physiological cardiac function and metabolism as well, application of β-blockers is also associated with some adverse effects (Everly, Heaton, & Cluxton, 2004). Therefore, it is necessary to further explore the molecular mechanism of β-ARs overactivation mediated pathological cardiac remodeling, so as to provide novel therapeutic approaches. Our results demonstrated that targeting mPGES-1 might be a novel approach to prevent deleterious cardiac fibrosis, induced by β-ARs agonists.

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Materials and Methods

Mice and Isoproterenol treatment

mPGES-1 knockout mice in the C57BL/6 background were created as previously described (Wang et al., 2006). Male mPGES-1 KO mice and their wild-type (WT) littermates at the age of 3 to 4 month were maintained under 12:12 hours light/dark cycle and were subjected to isoproterenol (sigma) or saline subcutaneously injection at 5mg/kg/day or 15mg/kg/day for 7 consecutive days. The mice were euthanized 24h after the last injection of isoproterenol and the hearts were removed and prepared for further analyses. All procedures were in accordance with the guidelines approved by the Dalian Medical University Animal Care and Use Committee. Female mice had not been used in the current study which represents a limitation.

Echocardiography measurements

Echocardiography was performed to evaluate cardiac systolic and diastolic function of WT and mPGES-1 KO mice before and 24h after the last ISO injection. Mice were anesthetized with 1% isoflurane. The heart rate was stabilized at 400 to 500 b.p.m. The Vevo 3100 high-resolution imaging system (Fujifilm VisualSonics Inc.) was used to measure the cardiac function on a short axis view and 3 frames were analyzed for each animal. The percentage of ejection fraction (EF%), fractional shortening (FS%), left ventricular wall thickness, left ventricular mass, left ventricular internal dimension and interventricular septum were identified and calculated as described in previous studies (Collins, Korcarz, & Lang, 2003; Gao, Ho, Vatner, & Vatner, 2011; Xiao et al., 2018a).

Histology and Morphometry

Mice were euthanized and heart tissues were harvested and fixed in 4% paraformaldehyde. Then the tissues were embedded in paraffin and sectioned at 5-μm intervals. Masson trichrome staining was performed according to standard

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procedures. For immunohistochemistry, heart sections were stained with antibodies against macrophage marker Mac-2 (Santa cruz), T lymphocyte marker CD3, (GeneTex). Tissue morphometric features were evaluated by PerkinElmer Mantra tissue imaging analysis system (PerkinElmer Inc.).

Enzyme-Linked Immunosorbent Assay

Plasma were harvested for measurement of the levels of PGE2, PGI2 and PGF2α by validated enzyme-linked immunosorbent assay according to manufacturer instructions (Elabscience).

Real-time PCR

Total RNA was extracted from cardiac cells or heart tissues using Trizol reagent and reverse transcribed according to the manufacturer's protocol. Quantitation PCR was performed using SYBR Green to detect PCR products in real time with LightCyler96 Sequence Detection System (Roche). All mRNA measurements were normalized to 18S or actin levels. The primers were shown in table 1 (Wang et al., 2018).

Cell culture

We used neonatal SD rats, both genders, for the in vitro cell culture experiments. Cardiac fibroblasts and myocytes were isolated and cultured as described previously (Nuamnaichati, Sato, Moongkarndi, Parichatikanond, & Mangmool, 2018). In brief, 1 or 2 day old neonatal SD rats were euthanized and the hearts were digested with collagenase I. Cells were pelleted and plated on 10-cm dishes and cultured in DMEM supplemented with 10% FBS plus 1% penicillin and streptomycin. Unattached cardiac myocytes were removed to a new dish 3 to 4h later, and cardiac fibroblasts were attached to the bottoms of the dish. Cells at passage one were used for the experiment and analysis. Both cardiac myocytes and fibroblasts were changed to serum-free DMEM for 12h before isoproterenol stimulation or other treatment.

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For the conditioned medium collection, primary cultured neonatal rat cardiac myocytes were treated with or without 20μM ISO for 12h and the supernatant were collected as conditioned medium. Basically, 2ml of conditioned medium was collected from 1 $\times 10^6$ cardiac myocytes.

RNA interference

Primary cultured neonatal rat cardiac myocytes were transfected with Opti-MEM and Lipofectamine 3000 (Invitrogen), along with double stranded siRNA (80-150nM) according to the manufacturer's protocol to validate the knockdown effect. The siRNA sequence targeting rat mPGES-1 is sense (5'-CACUGCUGGUCAAGAUTT-3') and antisense (5'-AUCUUGAUGACCAGCAGUGTT-3'). For the conditioned medium experiment, cells were pretreated with 20μM ISO and 100nM siRNA, and the conditioned medium was collected and applied to the fibroblasts for 24 hours.

Adenovirus overexpression

Adenoviruses carrying full-length rat mPGES-1 were purchased from Vigene biosciences (Shandong, China), and rat GFP adenoviruses were used as a negative control. After 24-48h, the infection efficiency was determined by real-time PCR and western blot. Sham infection had not been used in the current study which might represent a limitation.

Statistical analysis

In all cases, quantitative results were expressed as mean ± SD. Student's t-test were used to analyze the statistical differences between two groups, one-way analysis of variance (ANOVA) plus a post hoc analysis using a Tukey's or a Bonferroni test were used to analyze the statistical differences among multiple groups. All data were analyzed using GraphPad Prism (version 8.0). $P<0.05$ was considered statistically significant.

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Results

mPGES-1 deletion does not affect ISO-induced cardiac hypertrophy

To investigate the effect of mPGES-1 deletion on isoproterenol (ISO)-induced cardiac remodeling, mPGES-1 KO and the WT mice were initially subjected to isoproterenol at a relatively low dose, 5mg/kg/day, and the cardiac structure and function were assessed by echocardiography before and after 7 consecutive days of ISO injection. As shown, although the cardiac function (reflected by ejection fraction and fractional shortness) was not altered by low dose ISO exposure (Figure 1A), obvious cardiac hypertrophy (reflected by increased end-diastolic left ventricular posterior wall thickness and LV mass) was clearly observed in both WT and KO groups (Figure 1B). However, no obvious difference was observed between the two genotypes (Figure 1B). Similarly, lack of mPGES-1 did not alter the expression of cardiac hypertrophic marker ANF, BNP (Figure 1C) and the mouse heart weight (Figure 1D).

mPGES-1 deletion protects ISO-induced cardiac fibrosis

Fibrosis is the most important feature of ISO-induced cardiac remodeling (Wang et al., 2019). Although most studies have demonstrated obvious morphological cardiac fibrosis with ISO treatment at 5 mg/kg/day for 7 days, we only observe marginal interstitial fibrosis by Masson's trichrome staining in either WT or mPGES-1 KO mice (Figure 2A), which might reflects the resistance of C57/BL6 mice to ISO-induced cardiac fibrosis (Park et al., 2018). In any event, the expression of mRNA for types I and III collagen, the main components of extracellular matrix for cardiac fibrosis, as well as other fibrosis related genes, such as fibronectin, were all significantly increased in WT mice (Figure 2B), suggesting the occurrence of fibrosis in these mice. Importantly, the KO mice had significantly reduced expression of collagen I, collagen III and fibronectin when compared with the WT (Figure 2B), implying a protective effect of mPGES-1 deficiency on ISO-induced fibrosis.

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Given the lack of morphological fibrosis in the low dose ISO models in our mice, we further treated the mice with ISO at a relatively high dose (15mg/kg/day) which was reported to induce heart failure and worse cardiac fibrosis (Oudit et al., 2003). As expected, unlike low dose ISO models, the Masson's trichrome staining did display markedly accumulated collagen deposition in heart sections from WT mice, and most importantly, dramatically attenuated cardiac collagen deposition was observed in the KO sections (Figure 3A). Similarly, the expression of collagen I, collagen III and fibronectin were significantly reduced in the KOs as well (Figure 3B).

Previous studies have proved that the cardio-protective properties of mPGES-1 deletion on atherogenesis and vascular injury may reflect both the suppressed PGE2 production and the augmented biosynthesis of PGI2 (Wang et al., 2011; Wang et al., 2006). Similarly, here we detected a significant reduction of serum PGE2 concentration in the mPGES-1 KO mice, and this suppression was also concomitant with increased PGI2 production, while PGF2a was unaltered (Figure 3C). Thus, both the depression of PGE2 and the increase of PGI2 may synergistically contribute to the beneficial effect of mPGES-1 deficiency on ISO-induced cardiac fibrosis.

mPGES-1 deletion does not affect ISO-induced heart dysfunction

In addition to severe cardiac fibrosis, high dose ISO evoked clearly impaired cardiac function, reflected by significantly decreased ejection fraction and fractional shortening. However, despite the profound protective effect on cardiac fibrosis, mPGES-1 deficiency failed to improve ISO induced cardiac dysfunction (Figure 4A). Cardiac hypertrophy reflected by end-diastolic left ventricular posterior wall thickness, LV mass (Table 3), and the HW/BW were not affected by mPGES-1 deletion as well (Figure 4B).

Inflammation was not affected by mPGES-1 deletion

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It has been established that activation of β-AR by ISO may lead to cardiac inflammation which is known to contribute to cardiac fibrosis and lead to progressive impairment of cardiac function. To determine whether mPGES-1 deletion might influence ISO induced inflammatory responses, we compared the cardiac expression of several proinflammatory cytokines. While variable effects were observed for IL-1β, IL-6, MCP-1 and TNF-α gene expression (Figure 4C), the overall inflammatory response was not differed between the two groups. IHC staining of the macrophage marker Mac-2 and lymphocyte marker CD3 displayed unaltered inflammatory cell infiltration in the KO heart sections (Figure 4D).

Knockdown of mPGES-1 decreased fibrosis

Cardiac fibroblasts play a critical role in ISO-induced cardiac fibrosis. To identify the mechanism by which mPGES-1 deletion protects ISO-induced cardiac fibrosis, we firstly examined the effects of mPGES-1 knockdown on primary cultured neonatal rat cardiac fibroblasts upon ISO stimulation. Surprisingly, ISO failed to directly increase fibrotic gene expression in fibroblasts (Figure 5A). However, when we applied the conditioned medium from ISO-pretreated neonatal rat cardiac myocytes to the fibroblasts, the expression level of fibrosis-related genes, including collagen I, collagen III and fibronectin, were all remarkably increased (Figure 5B). This finding is consistent with previous reports that cardiomyocytes directly response to ISO and secrete paracrine factors such as CTGF and VEGF and then activate fibroblasts to augment cardiac fibrosis (Nuamnaichati et al., 2018). Indeed, here we also observed increased expression of CTGF, VEGF and mPGES-1 in ISO-pretreated neonatal rat cardiac myocytes (Supplemental Figure S1). Moreover, knockdown of mPGES-1 in neonatal rat cardiac myocytes with siRNA transfection reversed the conditioned medium induced upregulation in cardiac fibroblasts (Figure 5C). The expression of mPGES-1 was used to validate the efficiency of siRNA (Figure 5D).

Overexpression of mPGES-1 increased fibrosis

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Furthermore, we examined the effects of mPGES-1 overexpression on primary cultured neonatal rat cardiac fibroblasts. As expected, adenovirus overexpression of mPGES-1 dramatically promotes the synthesis of PGE2 (Figure 6A) and the expression of fibrosis related genes in a dose dependent manner (Figure 6B). The efficiency of adenovirus infection was evaluated as well (Figure 6C).

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Discussion

Growing evidence has illustrated that, functionally coupled to COX-2, mPGES-1 is induced in various models of inflammation and is the dominant source of PGE2 production involved in inflammation and pain hypersensitivity, thus has been suggested to be an anti-inflammatory drug target alternative to NSAIDs (Kamei et al., 2004; Koeberle & Werz, 2015; Wang, Song, Cheng, & Fitzgerald, 2008b). Using genetic manipulated mouse models, we and others have proved that deletion of mPGES-1, especially in myeloid cells, restrains atherogenesis (Wang et al., 2006) (Lihong Chen & Jonathan H. DeLonge, 2014), attenuates vascular injury responses (Wang et al., 2011) (Chen et al., 2013b), and suppresses aortic aneurysm formation (Wang et al., 2008a), without significantly affecting blood pressure or thrombogenesis (Cheng et al., 2006).

While these results favor targeted delivery of mPGES-1 inhibitors for inflammatory vascular diseases, the role of mPGES-1 inhibition in pathological remodeling in heart remains unclear. Wu et al found that, unlike COX-2 inhibition, loss of mPGES-1 avoided the post-infarction death and did not increase ischemic myocardial injury after coronary occlusion in mice (Wu et al., 2009a, b). In contrast, Harding et al demonstrated a deleterious effect of mPGES-1 deletion on cardiac function after stress with Ang II, including reduced ejection fraction and dilated left ventricle chamber, while unaltered cardiac hypertrophy and fibrosis were observed (Harding et al., 2011). Moreover, Degousee et al showed that either global or myeloid cell mPGES-1 deletion adversely interfered with cardiac remodeling and cut down survival after experimental myocardial infarction in mice (Degousee et al., 2008; Degousee et al., 2012). However, in a sharp contrast, we recently demonstrated that inhibition of mPGES-1, especially in macrophages, might be beneficial to survival without worsen post-MI cardiac dysfunction (Chen et al., 2019). Although there are many possible explanations for the above

discrepancies, the complexity of the role of mPGES-1 in myocardial remodeling requires further investigation.

In the current study, we further addressed this question using an isoproterenol cardiac remodeling model. Isoproterenol is a synthetic nonselective agonist for β-adrenergic receptors (β-ARs). Overstimulation of β-ARs has been reported to cause cardiac remodeling including cardiac hypertrophy and fibrosis and lead to the deterioration of cardiac function (Dunser & Hasibeder, 2009; Lohse, Engelhardt, & Eschenhagen, 2003). Indeed, it has been documented that the pathophysiological alterations induced by isoproterenol in the heart tissues of experimental animals highly mimics those observed in infarcted myocardial tissues of humans (El-Armouche & Eschenhagen, 2009).

Unlike the insignificant or adverse role of mPGES-1 deficiency in post-MI cardiac remodeling, here we found that, although the cardiac function not significantly improved, the abnormal collagen deposition and fibrotic genes expression were clearly suppressed in mPGES-1 KO mice. These data clearly indicated that mPGES-1 is a crucial enzyme involved in isoproterenol induced myocardial fibrosis. Moreover, substrate rediversion resulting from mPGES-1 deletion was seen for PGI2 after ISO challenge. Given that PGI2 has been well characterized as a cardio-protective lipid mediator (Wang et al., 2008b), it is reasonable for us to expect that both the reduction of PGE2 and increased formation of PGI2 may have jointly contributed to the protective effect of mPGES-1 inhibition on ISO-induced cardiac fibrosis. Notably, here we only observed a small (16.4%) decrease in serum PGE2 levels of the mPGES-1 KO mice, while nearly 80% decrease was reported in other studies (Cheng et al., 2006). Differences in tissue sources (plasma versus urine) or detection techniques (ELISA versus Mass spectrometry) may account for this discrepancy.

Mechanistically, cardiac fibroblasts are critical in the accumulation of extracellular matrix, secrete collagen and induce fibrosis following ischemic or chemical injury (Porter & Turner, 2009). Herein we demonstrated that

overexpression of mPGES-1, which accompanied with increased PGE2 production, significantly promoted the isoproterenol induced fibrosis-related gene expression in primary cultured cardiac fibroblasts. Therefore, the observed attenuated fibrosis in the mPGES-1 KO mice might be largely attributable to the suppressed fibrogenesis in fibroblasts. However, the contribution of mPGES-1 in cardiomyocytes to ISO-induced cardiac fibrosis cannot be excluded. Indeed, here we found that, the fibroblasts failed to response to ISO stimuli directly in vitro. On the contrary, when we applied the conditioned medium from ISO-pretreated cardiac myocytes to the fibroblasts, the expression of fibrosis-related genes increased. This supports an idea that the cardiomyocytes directly response to ISO and secrete paracrine factors such as CTGF and VEGF and then activate fibroblasts to augment cardiac fibrosis (Nuamnaichati et al., 2018). Our results demonstrated that inhibition of mPGES-1 in cardiac myocytes would reverse the conditioned medium induced expression of fibrosis-related genes in fibroblasts. Thus it is possible for us to expect that, upon ISO stimulation, PGE2 may also act as a paracrine factor secreted from cardiac myocytes by mPGES-1 to stimulate the fibrogenesis in the adjacent fibroblasts. Nevertheless, more evidence is needed to illustrate the contribution of mPGES-1 in cardiomyocytes to ISO-induced cardiac remodeling. Moreover, it was previously reported that inflammatory cells are the likely major source of PGE2 biosynthesis in the heart after myocardial infarction (Degousee et al., 2008). Overactivation of β-ARs also includes activation of inflammation (Murray, Prabhu, & Chandrasekar, 2000) and blocking inflammation has displayed effectively attenuation on cardiac fibrosis. According to the most recent publication (Xiao et al., 2018b), the infiltration of macrophages in the heart was mostly detected 24h after ISO injection, which reached a peak at 72h and then gradually disappeared 7 days after ISO treatment. Thus, in the current study, although we did not see a clear effect of mPGES-1 deletion on ISO-induced cardiac inflammatory cell infiltration and pro-inflammatory cytokine expression 7 days post ISO treatment, we cannot completely exclude the contribution of

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inflammation to the protective effect of mPGES-1 deletion on ISO-induced cardiac fibrosis. Evidence from experiments at an earlier time point or using the myeloid specific mPGES-1 deficient mice would shed light on this important issue. Identification of the cellular source of PGE2 in the heart after β-AR activation warrants further investigation.

In conclusion, our results suggested that mPGES-1 deficiency alleviated β-adrenergic stress-induced cardiac fibrosis by decreasing the expression of fibrotic genes in fibroblasts. Cardiac fibrosis is a common feature and characteristic of many heart diseases, these findings further strengthen the evidence and pave the way for targeting mPGES-1 as a safe pharmacological approach for the cardio-protective NSAIDs.

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Authorship Contributions

Participated in research design: Chen, Guan, Yang.

Conducted experiments: Ji, Guo, Wang, Qian, Liu, Xu, Zhang.

Performed data analysis: Ji, Guo, Yang, Chen.

Wrote or contributed to the writing of the manuscript: Ji, Chen.

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References

Bergqvist, F., Morgenstern, R., & Jakobsson, P. J. 2019. A review on mPGES-1 inhibitors: From preclinical studies to clinical applications. ***Prostaglandins Other Lipid Mediat***, 147: 106383.

Chen, L., Yang, G., & Grosser, T. 2013a. Prostanoids and inflammatory pain. ***Prostaglandins Other Lipid Mediat***, 104-105: 58-66.

Chen, L., Yang, G., Jiang, T., Tang, S. Y., Wang, T., Wan, Q., Wang, M., & FitzGerald, G. A. 2019. Myeloid Cell mPges-1 Deletion Attenuates Mortality Without Affecting Remodeling After Acute Myocardial Infarction in Mice. ***J Pharmacol Exp Ther***, 370(1): 18-24.

Chen, L., Yang, G., Xu, X., Grant, G., Lawson, J. A., Bohlooly, Y. M., & FitzGerald, G. A. 2013b. Cell selective cardiovascular biology of microsomal prostaglandin E synthase-1. ***Circulation***, 127(2): 233-243.

Cheng, Y., Wang, M., Yu, Y., Lawson, J., Funk, C. D., & Fitzgerald, G. A. 2006. Cyclooxygenases, microsomal prostaglandin E synthase-1, and cardiovascular function. ***J Clin Invest***, 116(5): 1391-1399.

Collins, K. A., Korcarz, C. E., & Lang, R. M. 2003. Use of echocardiography for the phenotypic assessment of genetically altered mice. ***Physiol Genomics***, 13(3): 227-239.

Degousee, N., Fazel, S., Angoulvant, D., Stefanski, E., Pawelzik, S. C., Korotkova, M., Arab, S., Liu, P., Lindsay, T. F., Zhuo, S., Butany, J., Li, R. K., Audoly, L., Schmidt, R., Angioni, C., Geisslinger, G., Jakobsson, P. J., & Rubin, B. B. 2008. Microsomal prostaglandin E2 synthase-1 deletion leads to adverse left ventricular remodeling after myocardial infarction. ***Circulation***, 117(13): 1701-1710.

Degousee, N., Simpson, J., Fazel, S., Scholich, K., Angoulvant, D., Angioni, C., Schmidt, H., Korotkova, M., Stefanski, E., Wang, X. H., Lindsay, T. F., Ofek, E., Pierre, S., Butany, J., Jakobsson, P. J., Keating, A., Li, R. K., Nahrendorf, M., Geisslinger, G., Backx, P. H., & Rubin, B. B. 2012. Lack of microsomal prostaglandin e2 synthase-1 in bone marrow-derived myeloid cells impairs left ventricular function and increases mortality after acute myocardial infarction. ***Circulation***, 125(23): 2904-2913.

Deng, Y., Liu, B., Mao, W., Shen, Y., Fu, C., Gao, L., Zhang, S., Wu, J., Li, Q., Li, T., Liu, K., & Cao, J. 2019. Regulatory roles of PGE2 in LPS-induced tissue damage in bovine endometrial explants. ***Eur J Pharmacol***, 852: 207-217.

Dunser, M. W., & Hasibeder, W. R. 2009. Sympathetic overstimulation during critical illness: adverse effects of adrenergic stress. ***J Intensive Care Med***, 24(5): 293-316.

El-Armouche, A., & Eschenhagen, T. 2009. Beta-adrenergic stimulation and myocardial function in the failing heart. ***Heart Fail Rev***, 14(4): 225-241.

Everly, M. J., Heaton, P. C., & Cluxton, R. J., Jr. 2004. Beta-blocker underuse in secondary prevention of myocardial infarction. ***Ann Pharmacother***, 38(2): 286-293.

Gao, S., Ho, D., Vatner, D. E., & Vatner, S. F. 2011. Echocardiography in Mice. ***Curr Protoc Mouse Biol***, 1: 71-83.

Grosser, T., Ricciotti, E., & FitzGerald, G. A. 2017. The Cardiovascular Pharmacology of Nonsteroidal Anti-Inflammatory Drugs. ***Trends Pharmacol Sci***, 38(8): 733-748.

Harding, P., Yang, X. P., He, Q., & Lapointe, M. C. 2011. Lack of microsomal prostaglandin E synthase-1 reduces cardiac function following angiotensin II infusion. ***Am J Physiol Heart Circ Physiol***, 300(3): H1053-1061.

Kamei, D., Yamakawa, K., Takegoshi, Y., Mikami-Nakanishi, M., Nakatani, Y., Oh-Ishi, S., Yasui, H., Azuma, Y., Hirasawa, N., Ohuchi, K., Kawaguchi, H., Ishikawa, Y., Ishii, T., Uematsu, S., Akira, S., Murakami, M., & Kudo, I. 2004. Reduced pain hypersensitivity and inflammation in mice lacking microsomal prostaglandin e synthase-1. ***J Biol Chem***, 279(32): 33684-33695.

Koeberle, A., & Werz, O. 2015. Perspective of microsomal prostaglandin E2 synthase-1 as drug target in

inflammation-related disorders. ***Biochem Pharmacol***, 98(1): 1-15.

Lihong Chen, G. Y., James Monslowb, Leslie Toddb, David P. Cormodec, Jun Tangd, Gregory R. Granta,, & Jonathan H. DeLonge, S. Y. T., John A. Lawsona, Ellen Pureb, and Garret A. FitzGeralda,1. 2014. Myeloid cell microsomal prostaglandin E synthase-1 fosters atherogenesis in mice.pdf. ***PNAS***.

Lohse, M. J., Engelhardt, S., & Eschenhagen, T. 2003. What is the role of beta-adrenergic signaling in heart failure? ***Circ Res***, 93(10): 896-906.

Murray, D. R., Prabhu, S. D., & Chandrasekar, B. 2000. Chronic beta-adrenergic stimulation induces myocardial proinflammatory cytokine expression. ***Circulation***, 101(20): 2338-2341.

Nuamnaichati, N., Sato, V. H., Moongkarndi, P., Parichatikanond, W., & Mangmool, S. 2018. Sustained beta-AR stimulation induces synthesis and secretion of growth factors in cardiac myocytes that affect on cardiac fibroblast activation. ***Life Sci***, 193: 257-269.

Oudit, G. Y., Crackower, M. A., Eriksson, U., Sarao, R., Kozieradzki, I., Sasaki, T., Irie-Sasaki, J., Gidrewicz, D., Rybin, V. O., Wada, T., Steinberg, S. F., Backx, P. H., & Penninger, J. M. 2003. Phosphoinositide 3-kinase gamma-deficient mice are protected from isoproterenol-induced heart failure. ***Circulation***, 108(17): 2147-2152.

Park, S., Ranjbarvaziri, S., Lay, F. D., Zhao, P., Miller, M. J., Dhaliwal, J. S., Huertas-Vazquez, A., Wu, X., Qiao, R., Soffer, J. M., Rau, C., Wang, Y., Mikkola, H. K. A., Lusis, A. J., & Ardehali, R. 2018. Genetic Regulation of Fibroblast Activation and Proliferation in Cardiac Fibrosis. ***Circulation***, 138(12): 1224-1235.

Porter, K. E., & Turner, N. A. 2009. Cardiac fibroblasts: at the heart of myocardial remodeling. ***Pharmacol Ther***, 123(2): 255-278.

Thoren, S., & Jakobsson, P. J. 2000. Coordinate up- and down-regulation of glutathione-dependent prostaglandin E synthase and cyclooxygenase-2 in A549 cells. Inhibition by NS-398 and leukotriene C4. ***Eur J Biochem***, 267(21): 6428-6434.

Uematsu, S., Matsumoto, M., Takeda, K., & Akira, S. 2002. Lipopolysaccharide-dependent prostaglandin E(2) production is regulated by the glutathione-dependent prostaglandin E(2) synthase gene induced by the Toll-like receptor 4/MyD88/NF-IL6 pathway. ***J Immunol***, 168(11): 5811-5816.

Wang, L., Zhang, Y. L., Lin, Q. Y., Liu, Y., Guan, X. M., Ma, X. L., Cao, H. J., Liu, Y., Bai, J., Xia, Y. L., Du, J., & Li, H. H. 2018. CXCL1-CXCR2 axis mediates angiotensin II-induced cardiac hypertrophy and remodelling through regulation of monocyte infiltration. ***Eur Heart J***, 39(20): 1818-1831.

Wang, M., Ihida-Stansbury, K., Kothapalli, D., Tamby, M. C., Yu, Z., Chen, L., Grant, G., Cheng, Y., Lawson, J. A., Assoian, R. K., Jones, P. L., & Fitzgerald, G. A. 2011. Microsomal prostaglandin e2 synthase-1 modulates the response to vascular injury. ***Circulation***, 123(6): 631-639.

Wang, M., Lee, E., Song, W., Ricciotti, E., Rader, D. J., Lawson, J. A., Pure, E., & FitzGerald, G. A. 2008a. Microsomal prostaglandin E synthase-1 deletion suppresses oxidative stress and angiotensin II-induced abdominal aortic aneurysm formation. ***Circulation***, 117(10): 1302-1309.

Wang, M., Qian, L., Li, J., Ming, H., Fang, L., Li, Y., Zhang, M., Xu, Y., Ban, Y., Zhang, W., Zhang, Y., Liu, Y., & Wang, N. 2019. GHSR Deficiency Exacerbates Cardiac Fibrosis: Role in Macrophage Inflammasome Activation and Myofibroblast Differentiation. ***Cardiovasc Res***.

Wang, M., Song, W. L., Cheng, Y., & Fitzgerald, G. A. 2008b. Microsomal prostaglandin E synthase-1 inhibition in cardiovascular inflammatory disease. ***J Intern Med***, 263(5): 500-505.

Wang, M., Zukas, A. M., Hui, Y., Ricciotti, E., Puré, E., & FitzGerald, G. A. 2006. Deletion of microsomal prostaglandin E synthase-1 augments prostacyclin and retards atherogenesis. ***Proc Natl Acad Sci U S A***,

103(39): 14507-14512.

Wu, D., Mennerich, D., Arndt, K., Sugiyama, K., Ozaki, N., Schwarz, K., Wei, J., Wu, H., Bishopric, N. H., & Doods, H. 2009a. Comparison of microsomal prostaglandin E synthase-1 deletion and COX-2 inhibition in acute cardiac ischemia in mice. ***Prostaglandins Other Lipid Mediat***, 90(1-2): 21-25.

Wu, D., Mennerich, D., Arndt, K., Sugiyama, K., Ozaki, N., Schwarz, K., Wei, J., Wu, H., Bishopric, N. H., & Doods, H. 2009b. The effects of microsomal prostaglandin E synthase-1 deletion in acute cardiac ischemia in mice. ***Prostaglandins Leukot Essent Fatty Acids***, 81(1): 31-33.

Xiao, H., Li, H., Wang, J. J., Zhang, J. S., Shen, J., An, X. B., Zhang, C. C., Wu, J. M., Song, Y., Wang, X. Y., Yu, H. Y., Deng, X. N., Li, Z. J., Xu, M., Lu, Z. Z., Du, J., Gao, W., Zhang, A. H., Feng, Y., & Zhang, Y. Y. 2018a. IL-18 cleavage triggers cardiac inflammation and fibrosis upon beta-adrenergic insult. ***Eur Heart J***, 39(1): 60-69.

Xiao, H., Li, H., Wang, J. J., Zhang, J. S., Shen, J., An, X. B., Zhang, C. C., Wu, J. M., Song, Y., Wang, X. Y., Yu, H. Y., Deng, X. N., Li, Z. J., Xu, M., Lu, Z. Z., Du, J., Gao, W., Zhang, A. H., Feng, Y., & Zhang, Y. Y. 2018b. IL-18 cleavage triggers cardiac inflammation and fibrosis upon β-adrenergic insult. ***Eur Heart J***, 39(1): 60-69.

Yang, G., & Chen, L. 2016. An Update of Microsomal Prostaglandin E Synthase-1 and PGE2 Receptors in Cardiovascular Health and Diseases. ***Oxid Med Cell Longev***, 2016: 5249086.

Zhu, L., Xu, C., Huo, X., Hao, H., Wan, Q., Chen, H., Zhang, X., Breyer, R. M., Huang, Y., Cao, X., Liu, D. P., FitzGerald, G. A., & Wang, M. 2019. The cyclooxygenase-1/mPGES-1/endothelial prostaglandin EP4 receptor pathway constrains myocardial ischemia-reperfusion injury. ***Nat Commun***, 10(1): 1888.

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Footnotes

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Legends for Figures:

Figure 1. Effect of mPGES-1 deletion on isoproterenol induced cardiac dysfunction and hypertrophy. (A) Echocardiography of ejection fraction (EF%), fractional shortening (FS%) and heart rate (HR) of mice post 7 consecutive days injection of ISO at 5 mg/kg/day (Scale bar, 1 mm; Vehicle n=7-9; ISO n=13-14). (B) Echocardiography of end-diastolic left ventricular posterior wall thickness (LVPW;d) and left ventricular mass (Vehicle n=7-9; ISO n=13-14). (C) Quantitative real-time PCR analysis of atrial natriuretic factor (ANF) and brain natriuretic peptide (BNP) mRNA levels in the hearts (Vehicle n=5; ISO n=9). (D) Heart weight to body weight (HW/BW) ratio (Vehicle n=7-9; ISO n=13-14). * $P<0.05$; ** $P<0.01$; *** $P<0.001$. Data are represented as mean ± SD. The P values were obtained by one-way ANOVA plus a post hoc analysis using a Bonferroni test.

Figure 2. Effect of mPGES-1 deletion on low dose isoproterenol induced cardiac fibrosis. (A) Masson's trichrome staining of myocardial fibrosis from mice post 7 consecutive days of ISO injection at 5 mg/kg/day (Vehicle n=7-9; ISO n=13-14). (B) Real-time PCR analysis of the mRNA expression levels of collagen I, collagen III and fibronectin in the heart tissues (Vehicle n=3-5; ISO n=6-7). * $P<0.05$; ** $P<0.01$; *** $P<0.001$. Data are represented as mean ± SD. The P values were obtained by one-way ANOVA plus a post hoc analysis using a Bonferroni test.

Figure 3. Effect of mPGES-1 deletion on high dose isoproterenol induced cardiac fibrosis. (A) Masson's trichrome staining of myocardial fibrosis from mice post 7 consecutive days of ISO injection at 15 mg/kg/day (left). Quantification of fibrosis area (right, WT n=8; KO n=8). (B) Real-time PCR

analysis of the mRNA expression of fibrotic genes in the hearts (WT n=9; KO n=6). (C) Plasma concentrations of PGE2, PGI2 and PGF2α in mPGES-1 WT and KO mice 7 d post ISO treatment (WT n=9; KO n=6). * P<0.05; ** P<0.01; *** P<0.001. Data are represented as mean ± SD. The P values were obtained by two-tailed Student's t test.

Figure 4. Effect of mPGES-1 deletion on isoproterenol induced heart dysfunction and inflammation. (A) Echocardiography of EF% , FS% and heart rate (HR) of mice post 7 consecutive days of injection of ISO at 15 mg/kg/day (Scale bar, 1 mm; WT n=9; KO n=6). (B) Heart weight to body weight (HW/BW) ratio (WT n=9; KO n=6). (C) Real-time PCR analysis of the mRNA expression of inflammatory genes in the hearts (WT n=9; KO n=6). (D) Immunostainings of Mac-2 (macrophage marker) and CD3 (lymphocyte marker) in the heart sections (WT n=9; KO n=6). *P<0.05; ** P<0.01; *** P<0.001. Data are represented as mean ± SD. The P values were obtained by one-way ANOVA plus a post hoc analysis using a Bonferroni test for A and by two-tailed Student's t test for B, C and D.

Figure 5. Effect of mPGES-1 knockdown on fibrogenesis in primary cultured neonatal rat cardiac fibroblasts and myocytes. (A) Real-time PCR analysis of the mRNA expression levels of collagen I, collagen III and fibronectin in primary cultured cardiac fibroblasts treated by ISO (20μM) (n=3). (B) Primary cultured neonatal rat cardiac myocytes were treated with or without ISO (20μM) for 12h. After ISO stimulation, the supernatant were collected and added to the cardiac fibroblasts and further incubated for 24h. Real-time PCR analysis of the mRNA expression levels of fibrotic genes in cardiac fibroblasts (n=3). (C) The neonatal rat cardiac myocytes were treated with ISO (20μM) for 12h and transfected with mPGES-1 specific siRNA or negative control siRNA

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(100 nM). After treatment, the supernatant were collected and added to cardiac fibroblasts and further incubated for 24h. Quantitative real-time PCR analysis of the mRNA levels of fibrotic genes in the treated cardiac fibroblasts (n=3). (D) Western blot analysis of mPGES-1 in cardiomyocytes transfected with scrambled or mPGES-1 siRNA (80, 100, 150nM). * $P<0.05$; ** $P<0.01$; *** $P<0.001$. Data are represented as mean ± SD. The P values were obtained by two-tailed Student's t test.

Figure 6. Effect of mPGES-1 overexpression on primary cultured neonatal rat cardiac fibroblasts in vitro. Cardiac fibroblasts were infected with mPGES-1 adenovirus virus for different doses (from 5 to 50 MOI), GFP (10 MOI) adenovirus virus was used as control. (A) ELISA analysis of PGE2 level in cell culture supernatant (n=3). (B) Real-time PCR analysis of the expression of collagen I, collagen III and fibronectin (n=3). (C) Real-time PCR and western blot analysis of the expression of mPGES-1. * $P<0.05$; ** $P<0.01$; *** $P<0.001$. Data are represented as mean ± SD. The P values were obtained by one-way ANOVA plus a post hoc analysis using a Tukey's test.

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Tables

Table 1. Primers for quantitative real-time PCR.

PCR production	Forward primer	Reverse primer
Mouse ANF	CACAGATCTGATGGATTTCAAGA	CCTCATCTTCTACCGGCATC
Mouse BNP	GAAGGTGCTGTCCCAGATGA	CCAGCAGCTGCATCTTGAAT
Mouse Collagen I	GAGCGGAGAGTACTGGATCG	GTTCGGGCTGATGTACCAGT
Mouse Collagen III	TCCCCTGGAATCTGTGAATC	TGAGTCGAATTGGGGAGAAT
Mouse Fibronectin	AAGGTTCGGGAAGAGGTTGT	GAGCTTAAAGCCAGCGTCAG
Mouse IL-1β	CTTCCCCAGGGCATGTTAAG	ACCCTGAGCGACCTGTCTTG
Mouse IL-6	GCTACCAAACTGGATATAATCAGGA	CCAGGTAGCTATGGTACTCCAGAA
Mouse MCP-1	CCTGGATCGGAACCAAATGA	ACCTTAGGGCAGATGCAGTTTTA
Mouse TNF-α	ATGGCCTCCCTCTCATCAGT	CTTGGTGGTTTGCTACGACG
Mouse 18S	GAAACGGCTACCACATCCAAGG	GCCCTCCAATGGATCCTCGTTA
Mouse β-actin	GATCTGGCACCACACCTTCT	GGGGTGTTGAAGGTCTCAAA
Rat Collagen I	GAGACAGGCGAACAAGGTGA	GGGAGACCGTTGAGTCCATC
Rat Collagen III	AGAGGCTTTGATGGACGCAA	GGTCCAACCTCACCCTTAGC
Rat Fibronectin	CGTGGAGTATGTGGTTAGTGTCT	CTCAGGGCTTGAGTAGGTCA
Rat mPGES-1	TGTGAGGACCACGAGGAAATG	CGCAACGACATGGAGACGAT
Rat β-actin	TCCTAGCACCATGAAGATC	AAACGCAGCTCAGTAACAG
Rat CTGF	GGCAGGGCCAACCACTGTGC	CAGTGCACTTGCCTGGATGG
Rat VEGF	TGCCAAGTGGTCCCAG	CGCACACCGCATTAGG

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Table 2. Echocardiographic analysis of cardiac function in mice with low dose isoproterenol (5mg/kg/day) treatment for 7 consecutive days.

Treatment	Vehicle		ISO	
Genotype	WT (n=9)	KO (n=7)	WT (n=13)	KO (n=14)
LVID;d(mm)	4.18±0.17	4.14±0.26	4.31±0.31	4.30±0.30
LVID;s(mm)	2.86±0.22	2.77±0.39	3.17±0.40*	3.05±0.40
LVPW;s(mm)	1.34±0.17	1.24±0.09	1.23±0.18	1.41±0.19#
IVS;d(mm)	0.92±0.13	0.95±0.08	0.99±0.16	0.98±0.11
IVS;s(mm)	1.43±0.20	1.50±0.13	1.43±0.21	1.44±0.19

Data are mean ± SD. *P <0.05, compared with vehicle group. #P <0.05, compared with WT group. (One-way ANOVA plus a post hoc analysis using a Bonferroni test).

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Table 3. Echocardiographic analysis of cardiac function in high dose isoproterenol (15mg/kg/day) treated mice.

Days after ISO	0d		7d	
Genotype	WT (n=9)	KO (n=6)	WT (n=9)	KO (n=6)
LV mass(mg)	140.0±21.13	144.8±32.11	151.8±31.59	148.7±14.44
LVID;d(mm)	3.93±0.31	4.09±0.35	3.95±0.16	4.19±0.28#
LVID;s(mm)	2.62±0.26	2.74±0.32	2.86±0.34	3.07±0.25*
LVPW;d(mm)	0.98±0.15	0.91±0.07	1.01±0.22	0.92±0.06
LVPW;s(mm)	1.43±0.15	1.25±0.10	1.41±0.20	1.26±0.09
IVS;d(mm)	0.87±0.08	0.89±0.13	0.94±0.11	0.87±0.12
IVS;s(mm)	1.36±0.15	1.52±0.26	1.42±0.19	1.37±0.13

Data are mean ± SD. *P <0.05, compared with 0 d KO group. #P <0.05, compared with 7 d WT group. (One-way ANOVA plus a post hoc analysis using a Bonferroni test).

Figure 1

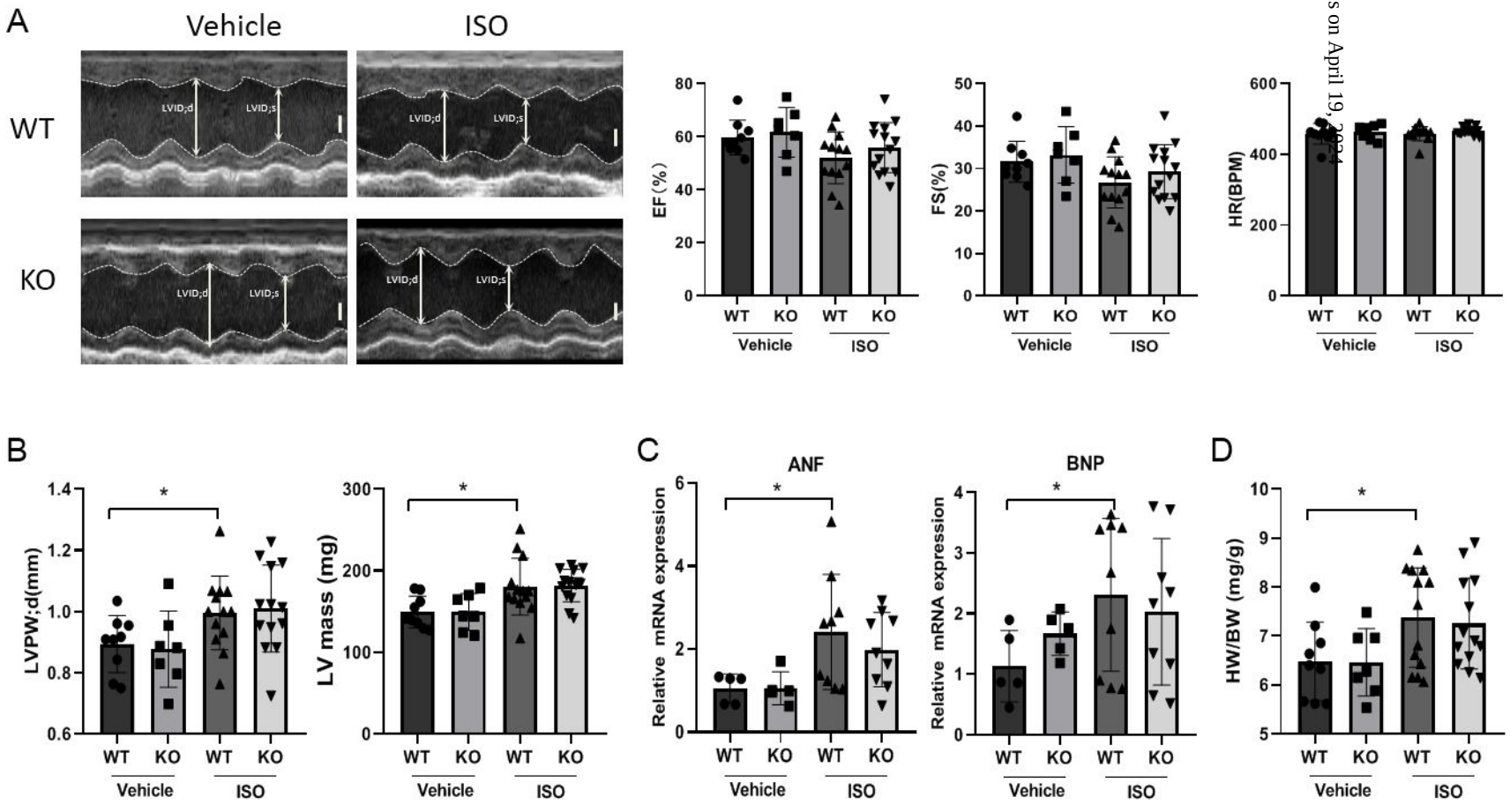

Figure 2

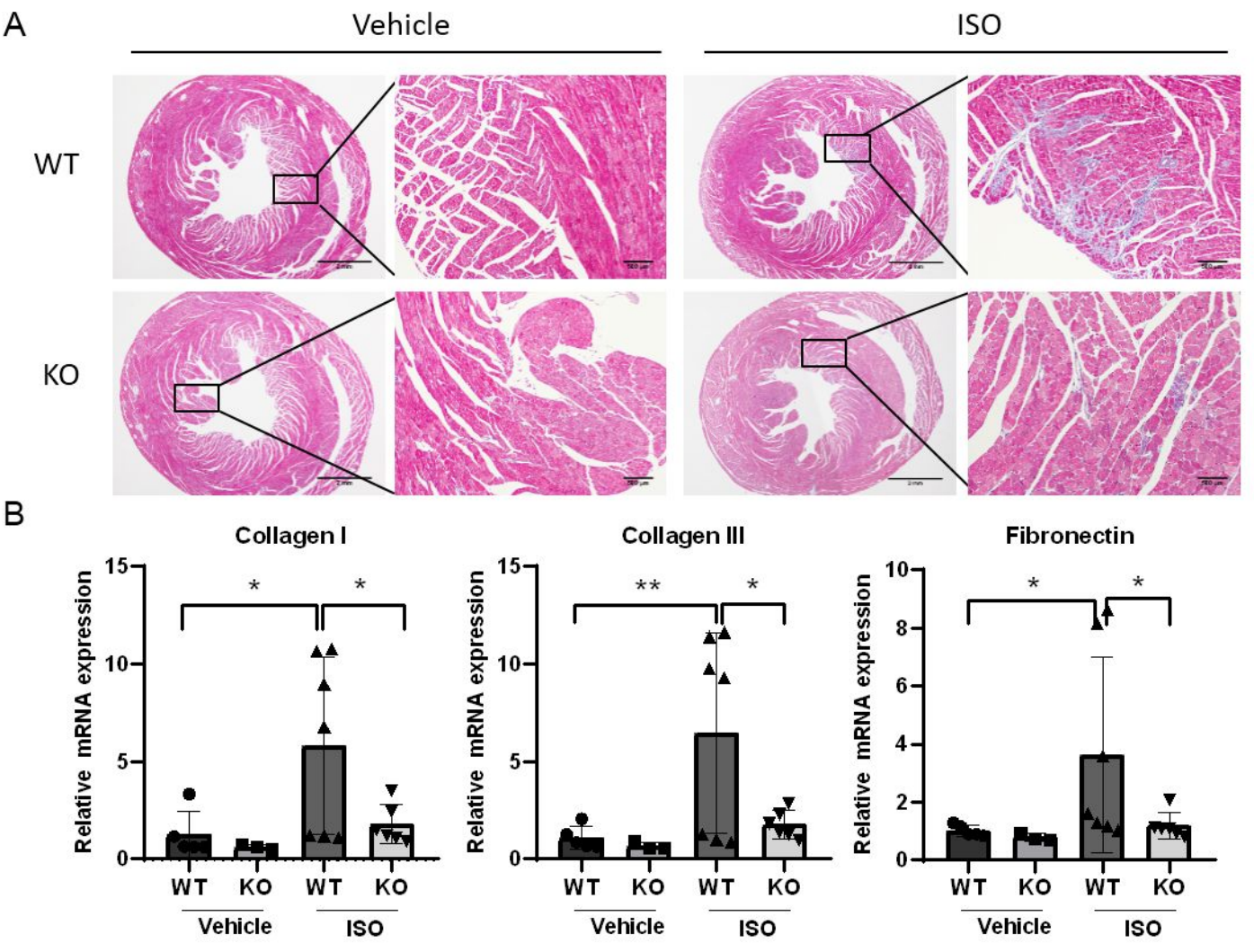
A
Vehicle
ISO
WT
KO
B
Collagen I
Collagen III
Fibronectin
Relative mRNA expression
15
10
5
0
8
6
4
2
*
**
WT
KO
Vehicle
ISO

Figure 3

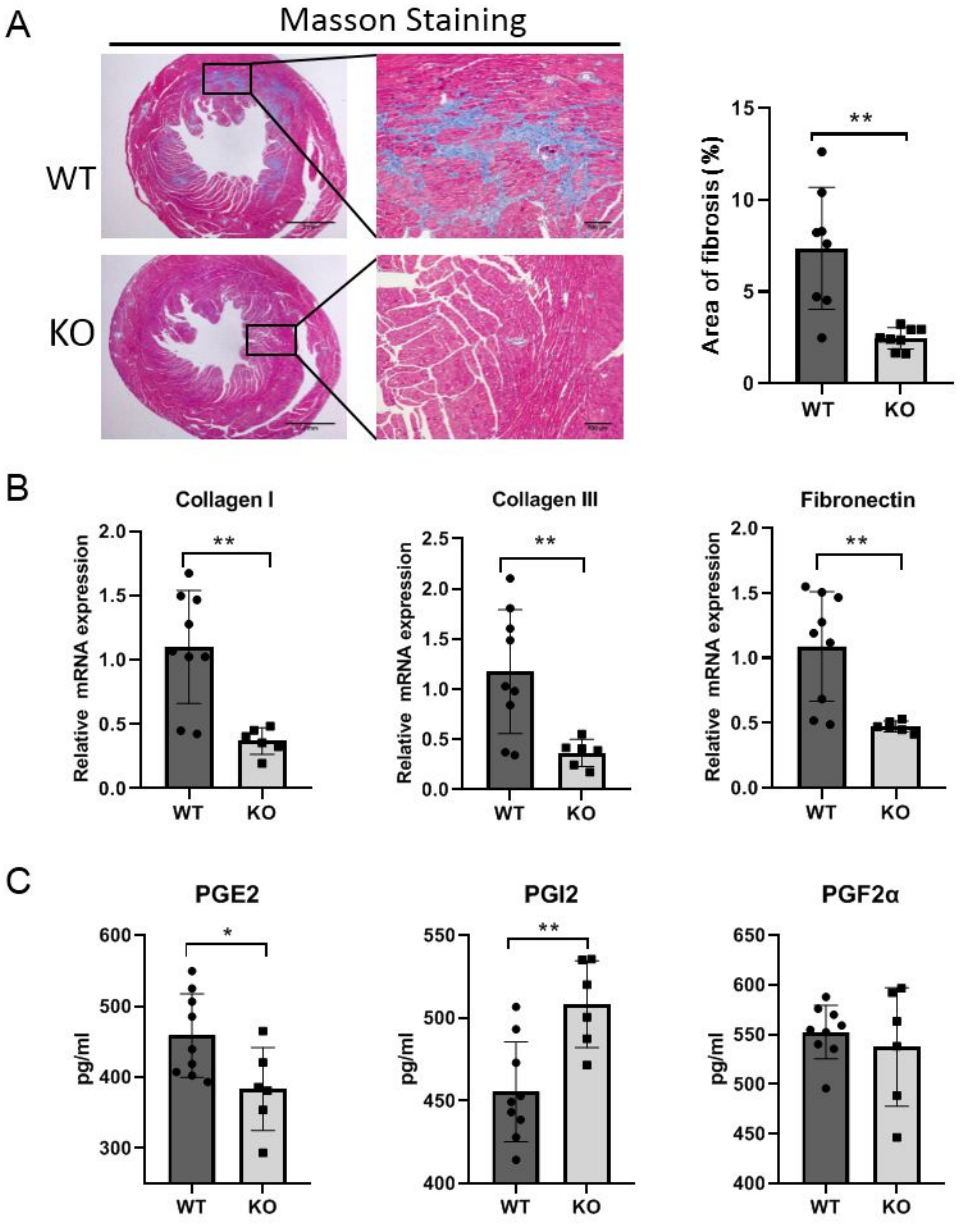
A
Masson Staining
WT
KO
Area of fibrosis (%)
15
10
5
0
**
WT
KO
B
Collagen I
Relative mRNA expression
2.0
1.5
1.0
0.5
0.0
**
WT
KO
Collagen III
Relative mRNA expression
2.5
2.0
1.5
1.0
0.5
0.0
**
WT
KO
Fibronectin
Relative mRNA expression
2.0
1.5
1.0
0.5
0.0
**
WT
KO
C
PGE2
pg/ml
600
500
400
300
*
WT
KO
PGI2
pg/ml
550
500
450
400
**
WT
KO
PGF2α
pg/ml
650
600
550
500
450
400
WT
KO

Figure 4

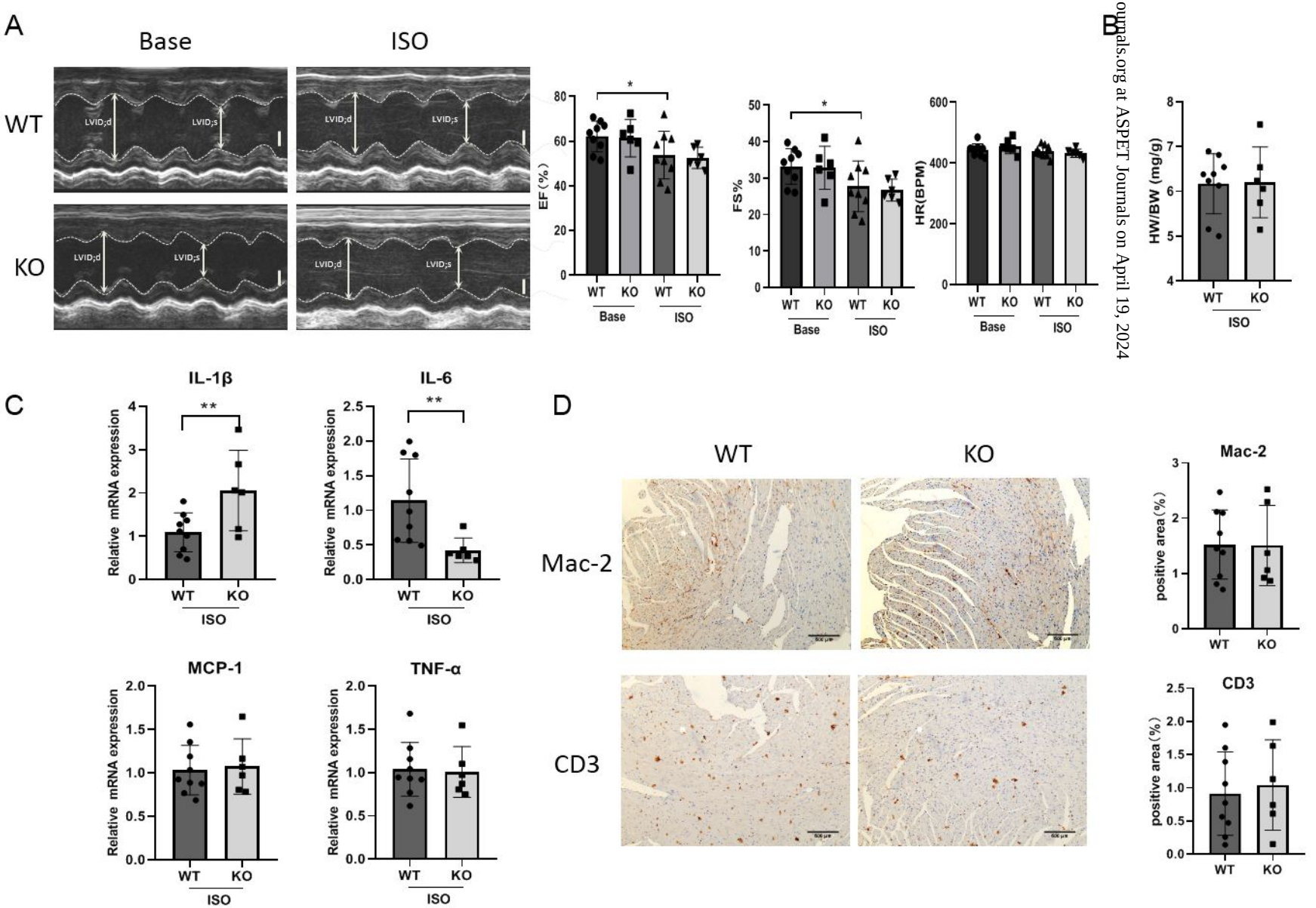

Figure 5

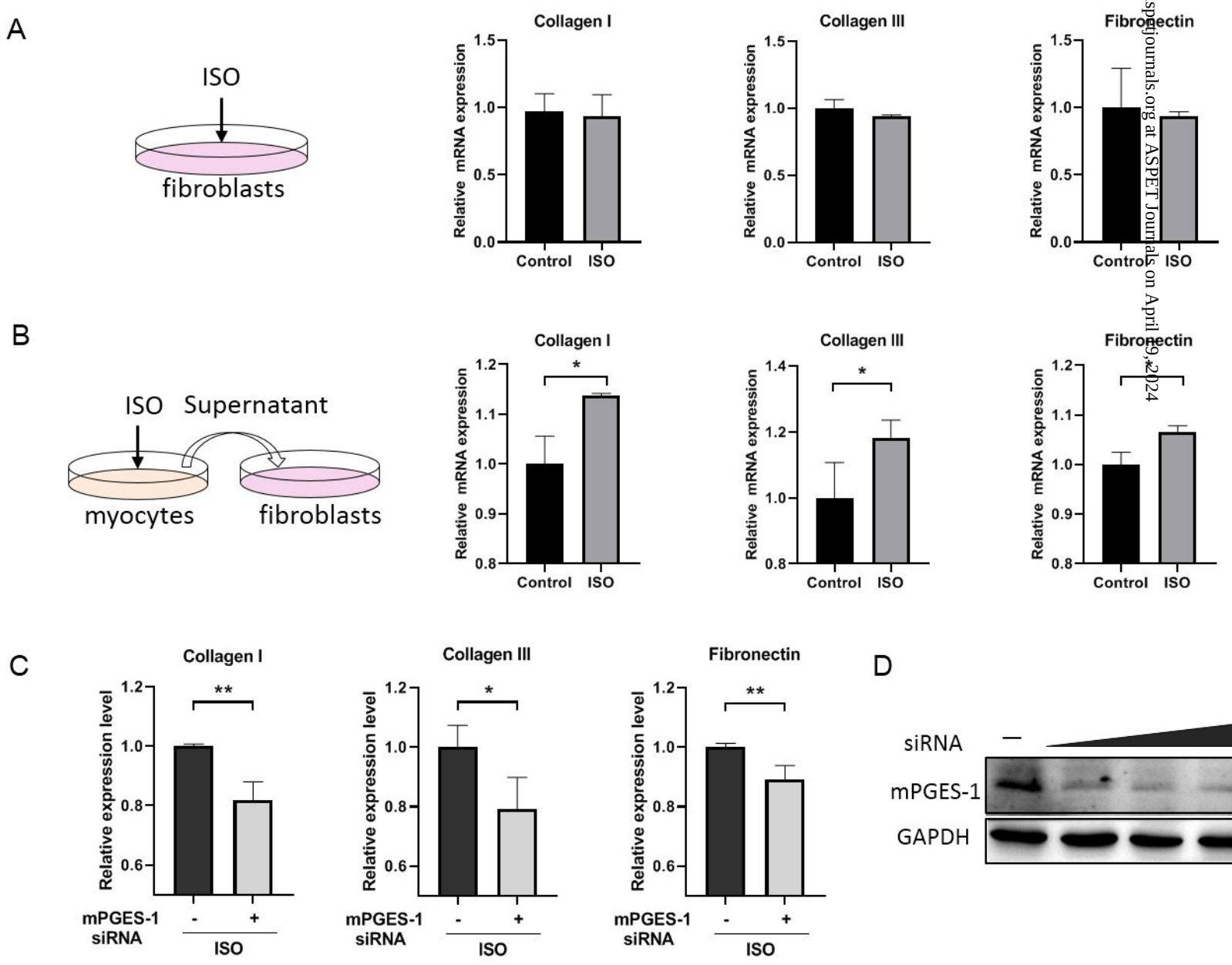

Figure 6