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# Title Page

# Myeloid cell mPges-1 deletion attenuates mortality without affecting remodeling after acute myocardial infarction in mice

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**Running Title Page**

a) Running Title: Myeloid mPges-1 and myocardial infarction.

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d) Abbreviations

ANP, atria natriuretic peptide; BNP, brain natriuretic peptide; COX, cyclooxygenase; KO, knockout; CTGF, connective tissue growth factor; NSAID, nonsteroidal anti-inflammatory drug; Mac, macrophage; MHC, major histocompatibility complex; MI, myocardial infarction; mPges-1, microsomal

$PGE_2$ synthase-1; PCR, polymerase chain reaction; $PGE_2$, prostaglandin $E_2$; $PGH_2$, prostaglandin $H_2$; $PGI_2$; prostacyclin; SEM, standard error of mean; TGF, transforming growth factor; WT, wild type.

**e)** Section: Cardiovascular

## Abstract

Selective deletion of microsomal $PGE_2$ synthase (mPges) -1 in myeloid cells retards atherogenesis and suppresses the vascular proliferative response to injury, while it does not predispose to thrombogenesis or hypertension. However, studies using bone marrow transplants from irradiated mice suggest that myeloid cell mPGES-1 facilitates cardiac remodeling and prolongs survival after experimental myocardial infarction (MI). Here we addressed this question using mice lacking mPges-1 in myeloid cells, particularly macrophages (Mac-mPges-1 KO), generated by crossing mPges-1 floxed mice with LysMCre mice, and subjecting them to coronary artery ligation. Cardiac structure and function were assessed by morphometric analysis, echocardiography, and invasive hemodynamics 3, 7 and 28 days after MI. Despite a similar infarct size, in contrast to the prior report, the post-MI survival rate was markedly improved in the Mac-mPges-1 KO mice compared to WT controls. Left ventricular systolic (reflected by ejection fraction, fractional shortness end systolic volume and +dP/dt) and diastolic function (reflected by end diastolic volume, -dP/dt and Tau), cardiac hypertrophy (reflected by LV dimensions) and staining for fibrosis did not differ between the groups. In conclusion, we find that Cre-loxP mediated deletion of mPges-1 in myeloid cells has favorable effects on post-MI survival, with no detectable adverse influence on post-MI remodeling. These results add to evidence that targeting macrophage mPGES-1 may represent a safe and efficacious approach to the treatment and prevention of cardiovascular inflammatory disease.

## Introduction

Cycloxygenases (COX-1 and -2) convert arachidonic acid to prostanoids. The inducible COX-2, and one of its products, $PGE_2$, dominates prostanoid production in inflammation and pain. COX-2 selective nonsteroidal anti-inflammatory drugs (NSAIDs) have been used in the management of pyrexia, alleviation of pain and inflammation (Grosser et al., 2017b). However, despite their efficacy, it has become evident that COX-2 inhibitors increase the incidence of cardiovascular adverse events, including myocardial infarction, stroke, hypertension, heart failure and sudden cardiac death(Grosser et al., 2017a). This results from suppression of COX-2 derived cardioprotective prostanoids, particularly prostacyclin ($PGI_2$), that attenuate atherogenesis and the response to thrombogenic and hypertensive stimuli (Yu et al., 2012). Given these observations and the opioid crisis, there is considerable interest in the development of novel, non-addictive analgesic and anti-inflammatory drugs.

Interest in the prostanoid pathway has focused on mPGES-1, which is functionally coupled with COX-2 and dominates inflammatory $PGE_2$ production (Friesen and Mancini, 2008; Wang and FitzGerald, 2010). We recently reported that deletion of mPges-1 in myeloid cells recapitulates effects of global enzyme deletion in that it attenuates the vascular proliferative response to injury and mitigates high-fat diet induced atherogenesis. By contrast, deletion of mPges-1 in vascular cells promotes the proliferative response to injury and does not affect atherogenesis (Chen et al., 2013; Chen et al., 2014). This prompts consideration of targeted delivery of mPGES-1

inhibitors to inflammatory macrophages as a novel therapeutic strategy.

Here, to address concerns that macrophage mPGES-1 might contribute to healing after myocardial infarction (MI), we have used genetic approaches to reveal that although cardiac remodeling is not profoundly altered after experimental myocardial infarction, myeloid deletion of mPges-1 improves post infarct survival.

## Materials and methods

### Mice and the MI model

The mPges-1-flox mice, kindly provided by Mohammad Bohlooly of Astra Zeneca, were crossed with LysMCre mice (Jackson laboratory, ME) to yield mice with mPges-1 deletion in myeloid cells, termed Mac-mPges-1-KO. Floxed mice without cre in same litters were used as controls, termed Mac-mPges-1-WT. Global mPges-1 KO (gKO) and their littermate controls (gWT) were obtained from (Jackson laboratory, ME). All mice were maintained under 12-hour/12-hour light/dark condition with free access to food and water before and throughout experiments. Only male mice at 3 to 4 months of age were used in this study.

Mice were subjected to coronary artery ligation to induce MI as previously reported (Gao et al., 2010). In brief, after anesthetization, a skin incision was made over the left chest and the pectoral muscles were retracted to expose the fourth intercostal space. A small hole was then made at the fourth intercostal space with a mosquito clamp to open the pleural membrane and pericardium. The heart was then carefully exteriorized. The left main descending coronary artery was located, sutured, and ligated approximately 3mm from its origin using a silk suture. After ligation, the heart was immediately placed back into the intrathoracic space followed by manual evacuation of air and closure of muscle and the skin. The mice were then allowed to breathe room air and monitored during the recovery period. All procedures were in accordance with the guidelines

approved by the University of Pennsylvania Institutional Animal Care and Use Committee and the Animal Center at Dalian Medical University, Dalian, China.

**Echocardiography and invasive hemodynamics**

Mice were anesthetized by inhalation of 1–2% isofluorane via nose cone and placed on a heating pad to keep the body temperature. Echocardiography was performed using a Vevo 770 (VisualSonics, Toronto, ON, Canada) with a linear 30-MHz probe (RMV 707B) as previously (Yuan et al., 2010). For invasive hemodynamics, a 1.4 French catheter (Millar Instruments, Houston, TX) was inserted through the right carotid artery, and then advanced into the left ventricle to record pressure and left ventricular function as previously reported (Xu et al., 2009).

**Histopathology**

The hearts from mice were fixed with 4% paraformaldehyde and then routinely dehydrated and embedded in blocks of paraffin wax and cross-sectioned at a thickness of 6μm. Morphometric analysis of collagen accumulation in heart tissues was assayed by Masson Trichrome and Picrosirius red staining as previously (Xu et al., 2009).

**Real-time RT-PCR**

Total RNA from infarcted and non-infarcted heart was extracted using RNeasy kit following the manufacturer's instructions and the concentration of RNA was measured by nanodrop, 0.5μg of each sample was applied reverse transcription. Taqman universal PCR Master Mix and probes were used for real-time PCR.

## Statistical analysis

All data were expressed as means ± SEM. Statistical differences were determined 2-way analysis of variance or 2-tailed Student's t-test as appropriate. A P value less than 0.05 was considered a significant difference.

## Results

### Myeloid mPges-1 deletion improved post-MI survival with little effect on cardiac function

To study the effects of myeloid mPges-1 deletion on cardiac response to myocardial infarction, Mac-mPges-1-WT or -KO male mice were subjected to coronary artery ligation, and the cardiac function was assessed by echocardiography before and 7, 28 days after the surgery. Invasive hemodynamics was performed at the end of the experiment. Although the cardiac function was clearly impaired upon the ligation at 7- and 28- days post-surgery, no significant difference was observed between the WT and KO groups, either for the left ventricular systolic function (reflected by ejection fraction, fractional shortness, end systolic volume and +dP/dt) or diastolic function (reflected by end diastolic volume, -dP/dt and Tau) (Table 1, Fig.1A & B). However, the survival rate was significantly improved in the KO groups, with 9 of 26 death in the WT mice, while only 2 of 24 in the KOs (Fig.1C).

### Post-MI cardiac hypertrophy and fibrosis were not affected by myeloid mPges-1 deletion

Masson Trichrome and Picrosirius red staining showed no obvious difference between the WT and KO groups for the extracellular matrix deposition, which is a feature of post-MI ventricular remodeling (Fig.2A & B). We also compared the mRNA level for several cardiac hypertrophy and fibrosis markers in both non-infarcted and infarcted heart zones. Irrespective of genotype, the infarcted heart showed markedly increased

expression of both fibrosis (collagen synthases, TGFβ, CTGF) and hypertrophy related genes (ANP, BNP and β-MHC) (Fig.2C & D).

**Inflammation and apoptosis were not affected by myeloid mPges-1 deletion**

Inflammation and myocyte apoptosis soon after experimental MI may cause fibrosis and lead to progressive impairment of cardiac function. To determine whether mPges-1 deletion in myeloid cells might influence these processes, Mac-mPges-1-WT and -KO mice were subjected to acute coronary artery ligation and heart function was assessed 3 days after. Similar to 7- or 28- days post infarction, left ventricular function showed no significant difference between the two groups 3 days after the occlusion (Fig.3). Gene expression relevant to both inflammation and apoptosis was also uninfluenced by genotype (Fig.4).

**Global mPges-1 deletion did not worsen post-MI cardiac dysfunction**

In addition to conditional myeloid cell deletion, we also studied the contribution of global mPges-1 deletion to post-MI remodeling. As shown in Fig.5 and Table.2, left ventricular function, hypertrophy and fibrosis were all unaltered in the global mPges-1 knockouts compared to controls.

## Discussion

Both the cardiovascular adverse effects of nonsteroidal anti-inflammatory drugs and the opioid crisis have focused interest on mPGES-1 the dominant source of $PGE_2$ biosynthesis downstream of COX-2, as an analgesic and anti-inflammatory drug target (Kamei et al., 2004; Wang et al., 2008b; Koeberle and Werz, 2015). Compared to disruption of the COX-2/$PGI_2$ signaling pathway, deletion of mPges-1 is less likely to predispose to thrombogenesis, hypertension (Cheng et al., 2006), atherogenesis (Wang et al., 2006), a proliferative response to vascular injury (Wang et al., 2011), or aortic aneurysm formation (Wang et al., 2008a) in mice. This altered profile reflects, at least in part, the differential impact of the two strategies on biosynthesis of $PGI_2$; this is enhanced by mPges-1 deletion or blockade in mice and humans due to increased utilization of the mPGES-1 substrate, $PGH_2$, by $PGI_2$ synthase (Tang et al., 2016). Specific inhibitors of mPGES-1 have been under development (Koeberle et al., 2016; Di Micco et al., 2018; Ding et al., 2018) and the clinical pharmacology of several mPGES-1 inhibitors has been described (Jin et al., 2015). However, progress in bringing these drugs to the market is slow. Factors that have complicated the development of mPGES-1 inhibitors include off target hepatotoxicity of some compounds (Jin et al., 2015) and the theoretical possibility that while augmented $PGI_2$ formation may attenuate cardiovascular risk but also restrain analgesic efficacy in some settings(Pulichino et al., 2006). More recently, the opioid crisis has highlighted the

limited number of analgesic options and has reinvigorated interest in inhibitors of mPGES-1.

Interestingly, deletion of mPges-1 in myeloid cells, within which the enzyme is most abundantly expressed in macrophages, attenuates the vascular proliferative response to injury and mitigates high-fat diet induced atherogenesis while conserving the efficacy of NSAIDs in models of analgesia; by contrast deletion of mPges-1 in vascular cells enhances the response to vascular injury (Chen, 2013; Chen et al., 2013; Chen et al., 2014). These observations have prompted us to pursue the possibility that targeting mPGES-1 selectively in activated macrophages might further improve the therapeutic index of mPGES-1 inhibitors.

However, despite the relatively benign preclinical cardiovascular profile of targeting mPGES-1, doubts have been raised about the safety of this strategy in the setting of acute myocardial infarction. Unlike COX-2 inhibition, Wu et al reported that deletion of mPges-1 did not increase ischemic myocardial injury or death after coronary occlusion in mice (Wu et al., 2009a; Wu et al., 2009b). However, in a model of angiotensin II mediated stress, Harding et al found that mPges-1 deletion resulted in myocardial dysfunction, albeit without evidence of cardiomyocyte hypertrophy or fibrosis (Harding et al., 2011). Finally, Degousee and colleagues reported a reduction in survival and impaired cardiac remodeling after experimental MI in mice subject to either global or myeloid cell deletion of mPges-1, the latter by bone marrow transfer in irradiated mice (Degousee et al., 2008; Degousee et al., 2012). Unexpectedly, they

observed enhanced inflammation and higher $PGE_2$ in the infarct area in the myeloid cell mPges-1 deficient mice (Degousee et al., 2012).

Here, we generated mice lacking mPges-1 in myeloid cells by crossing mPges-1 floxed mice with LysMCre mice. As we previously reported, the dominant consequence of the enzyme deletion is to render macrophages deficient in mPges-1 (Chen et al., 2013). Here, by contrast, we found that survival post-MI was improved, and we did not observe any alteration in LV function or post-infarction remodeling by genotype. Similarly, we failed to observe any detectable adverse influence on post-MI remodeling after mPges-1 was depleted globally. Several differences may explain this discrepancy from the reports of Degousee et al (Degousee et al., 2008; Degousee et al., 2012) including the cells deficient in mPges-1 (myeloid cells versus bone marrow–derived leukocytes), the genetic background of the mice used in the two studies (C57BL/6 versus DBA/1lacJ), the surgical approaches to coronary artery ligation (non-thoracotomy versus ventilated thoracotomy), and possibly the sex of the mice studied (males versus female recipients for the bone marrow transplantation).

So far, many strategies for targeting disease-relevant macrophages have been developed. These include liposome-encapsulated drug delivery(Ahsan et al., 2002; Ergen et al., 2017); glucan particle-based delivery of siRNA, protein, or small molecules to phagocytic cells(Aouadi et al., 2009; Tesz et al., 2011); nanotherapeutic delivery of high-density lipoprotein nanoparticle to atherosclerotic plaque macrophages(Duivenvoorden et al., 2014); and folate receptor-mediated therapeutic

intervention(Xia et al., 2009; Ayala-Lopez et al., 2010). Thus, many options can be explored to target specifically inflammatory macrophages with inhibitors of mPGES-1.

Taken together, our findings predict a beneficial cardiovascular impact of mPGES-1 inhibition, even in the setting of myocardial infarction. However, given the conflicting results from distinct approaches to addressing this question in mice, it must be modelled further with macrophage directed inhibitors before proceeding with their clinical development.

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## Authorship Contributions

*Participated in research design:* Chen, Yang, Wang M, FitzGerald.

*Conducted experiments:* Chen, Yang, Jiang, Tang, Wang T, Wan.

*Performed data analysis:* Chen, Yang, Jiang.

*Wrote or contributed to the writing of the manuscript:* Chen, Yang, FitzGerald.

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**Legends for Figures:**

**Figure 1.** Effect of myeloid mPges-1 deletion on post-MI survival and heart function. Mac-mPges-1-WT or -KO mice were subjected to coronary artery ligation, echocardiography and hemodynamics was performed to evaluate the heart function before and 7&28d after the ligation. A, Ejection fraction (EF), fractional shortness (FS), left ventricular end-systolic volume (ESV) and end-diastolic volume (EDV); B, The maximum ascending rate of left ventricular pressure (+dP/dt max); the maximum descending rate of left ventricular pressure (-dP/dt max); left ventricular relaxation time constant (Tau); n=10-15 mice/group. * P<0.05; ** P<0.01; *** P<0.001; 0 vs 7 or 28 days after MI; C, Survival of Mac-mPges-1-WT and -KO mice after MI. n=24-26, P=0.0317, log-rank test.

**Figure 2.** Effect of myeloid mPges-1 deletion on post-MI fibrosis and hypertrophy. A, Representative images of Masson Trichrome staining and the quantitative analysis. B, Representative images of Picrosirius red staining and the quantitative analysis. C, Real-time PCR analysis of the expression of fibrosis related genes in the heart from mice 28d post-MI. D, Real-time PCR analysis of the expression of hypertrophy related genes in the heart from mice 28d post-MI. n=6-8 for immunostaining; n=4-5 for real-time PCR. * P<0.05; ** P<0.01; *** P<0.001 non-infarcted vs infarcted heart.

**Figure 3.** Echocardiographic analysis of cardiac function in myeloid mPges-1 KO mice 3 days post-MI. BW: body weight; HR: heart rate; LV mass: left ventricular mass; LV mass/BW: ratio of left ventricular mass to body weight; CO: cardiac output; SV: stroke volume; EF: Ejection fraction; FS: Fractional shortness; ESV: LV end-systolic volume;

EDV: LV end-diastolic volume; LVIDs: LV systolic internal dimensions; LVIDd: LV diastolic internal dimensions. n=3 mice/group.

**Figure 4.** Effect of myeloid mPges-1 deletion on post-MI inflammation and apoptosis. A, Real-time PCR analysis of the expression of inflammation related genes in the heart from mice 3d post-MI. D, Real-time PCR analysis of the expression of apoptosis related genes in the heart from mice 3d post-MI. n=4-5. * P<0.05; ** P<0.01; *** P<0.001 non-infarcted vs infarcted heart.

**Figure 5.** Effect of global mPges-1 deletion on post-MI heart remodeling. A, mPges-1 global KO and the control mice were subjected to coronary artery ligation and heart function was assessed by echocardiography before and 7&28d after the ligation. EF: Ejection fraction; FS: Fractional shortness; ESV: LV end-systolic volume; EDV: LV end-diastolic volume. n=4-14 mice/group. B, Real-time PCR analysis of the expression of fibrosis related genes in the heart from mice 28d post-MI. n=6-8. C, Real-time PCR analysis of the expression of cardiac hypertrophy related genes in the heart from mice 28d post-MI. n=6-8.

## Tables

Table 1: Echocardiographic and hemodynamic analysis of cardiac function in myeloid mPges-1 KO mice.

| | Days after coronary ligation | 0 ( WT n=13 ; KO n=15) | | 7 ( WT n=11 ; KO n=13) | | 28 ( WT n=10 ; KO n=13) | |
|---|---|---|---|---|---|---|---|
| | Genotype | Mac-mPges-1-WT | Mac-mPges-1-KO | Mac-mPges-1-WT | Mac-mPges-1-KO | Mac-mPges-1-WT | Mac-mPges-1-KO |
| Echocardiography | Body weight (g) | 27.69±0.58 | 27.57±0.65 | 28.88±0.84 | 27.75±0.86 | 30.68±0.84* | 28.53±0.85 |
| | LV mass (mg) | 85.40±2.61 | 82.02±3020 | 110.25±6.00** | 96.97±5.10 | 109.19±4.96** | 103.88±6.69** |
| | LV mass/BW (mg/g) | 3.09±0.08 | 2.97±0.09 | 3.86±0.28** | 3.47±0.13 | 3.56±0.14 | 3.61±0.19** |
| | Heart rate (bpm) | 519.15±14.64 | 480.60±16.52 | 526.18±10.57 | 537.46±14.25 | 537.80±10.05** | 543.62±11.01** |
| | Cardiac output (ml/min) | 19.53±0.87 | 17.73±1.10 | 14.51±0.75** | 16.65±1.21 | 17.00±1.51 | 20.16±1.00 |
| | Stroke volume (ul) | 37.50±0.94 | 36.63±1.69 | 27.75±1.70** | 31.38±2.68 | 32.04±3.21 | 37.00±1.69 |
| | Ejection fraction (%) | 54.14±1.84 | 52.85±1.05 | 26.59±2.84*** | 35.84±4.67*** | 28.81±3.37*** | 37.14±3.83** |
| | Fractional shortness (%) | 27.50±1.54 | 30.72±1.10 | 15.37±2.15*** | 20.18±2.58*** | 15.83±2.45*** | 20.15±2.05*** |
| | LV volume, end systole (ul) | 32.56±2.41 | 32.80±1.79 | 86.62±12.48** | 71.04±13.61* | 85.23±9.74** | 80.02±15.82** |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | LV volume, end diastole (ul) | 70.06±2.62 | 69.43±3.11 | 114.37±11.98** | 102.41±12.0* | 117.27±8.71** | 117.02±15.87** |
| | LV internal diameter, end systole (mm) | 2.74±0.12 | 2.52±0.09 | 4.03±0.26*** | 3.43±0.28* | 4.16±0.3*** | 3.65±0.29** |
| | LV internal diameter, end diastole (mm) | 3.77±0.10 | 3.63±0.09 | 4.72±0.19** | 4.23±0.21* | 4.90±0.22*** | 4.50±0.25** |
| Hemodynamics | LV end systolic pressure (mmHg) | ND | ND | ND | ND | 91.90±2.95 | 87.36±1.97 |
| | LV end diastolic pressure (mmHg) | ND | ND | ND | ND | 7.48±0.97 | 5.10±0.75 |
| | LV +dP/dt max (mmHg/s) | ND | ND | ND | ND | 7071.68±563.29 | 6516.17±308.48 |
| | LV -dP/dt max (mmHg/s) | ND | ND | ND | ND | 6634.24±403.96 | 6665.88±241.52 |
| | Tau (ms) | ND | ND | ND | ND | 11.31±0.63 | 10.84±0.40 |

LV: left ventricular; BW: Body weight; ND: not determined. * P<0.05; ** P<0.01; *** P<0.001; 0 vs 7 or 28 days post-MI

Table 2: Echocardiographic analysis of cardiac function in global mPges-1 KO mice.

| Days after coronary ligation | 0 | | 7 | | 28 | |
|---|---|---|---|---|---|---|
| Genotype | gWT (n=4) | gKO (n=4) | gWT (n=8) | gKO (n=7) | gWT (n=14) | gKO (n=12) |
| Body weight (g) | 28.73±1.37 | 26.38±1.25 | 24.28±3.21 | 25.97±3.58 | 25.13±.08 | 26.90±2.29 |
| LV mass (mg) | 92.78±9.89 | 82.71±1058 | 90.88±15.79 | 91.19±16.42 | 86.35±16.32 | 84.05±19.79 |
| LV mass/BW (mg/g) | 3.24±0.40 | 3.13±0.30 | 3.76±0.53 | 3.51±0.45 | 3.49±0.82 | 3.11±0.63 |
| Heart rate (bpm) | 454.00±49.77 | 401.75±21.28 | 471.63±62.80 | 481.43±58.20 | 456.36±64.58 | 460.17±50.38 |
| Cardiac output (ml/min) | 23.91±4.61 | 16.84±0.82 | 12.70±5.43 | 15.87±4.98 | 15.34±3.46 | 15.54±2.33 |
| Stroke volume (ul) | 53.69±14.46 | 41.97±2.56 | 28.06±13.25 | 33.38±10.70 | 34.01±7.78 | 34.26±6.76 |
| Ejection fraction (%) | 59.91±5.83 | 57.02±2.49 | 33.14±19.57 | 37.48±23.40 | 40.07±16.68 | 49.96±19.42 |
| Fractional shortness (%) | 28.40±2.48 | 28.60±3.16 | 19.07±10.14 | 20.18±12.49 | 22.63±10.52 | 28.27±12.79 |
| LV internal diameter, end systole (mm) | 2.92±0.43 | 2.83±1.89 | 3.82±1.29 | 4.06±1.82 | 3.45±1.51 | 3.08±1.72 |
| LV internal diameter, end diastole (mm) | 4.07±0.50 | 3.97±0.11 | 4.63±1.04 | 4.89±1.52 | 4.32±1.22 | 4.08±1.40 |

**Figure 1**

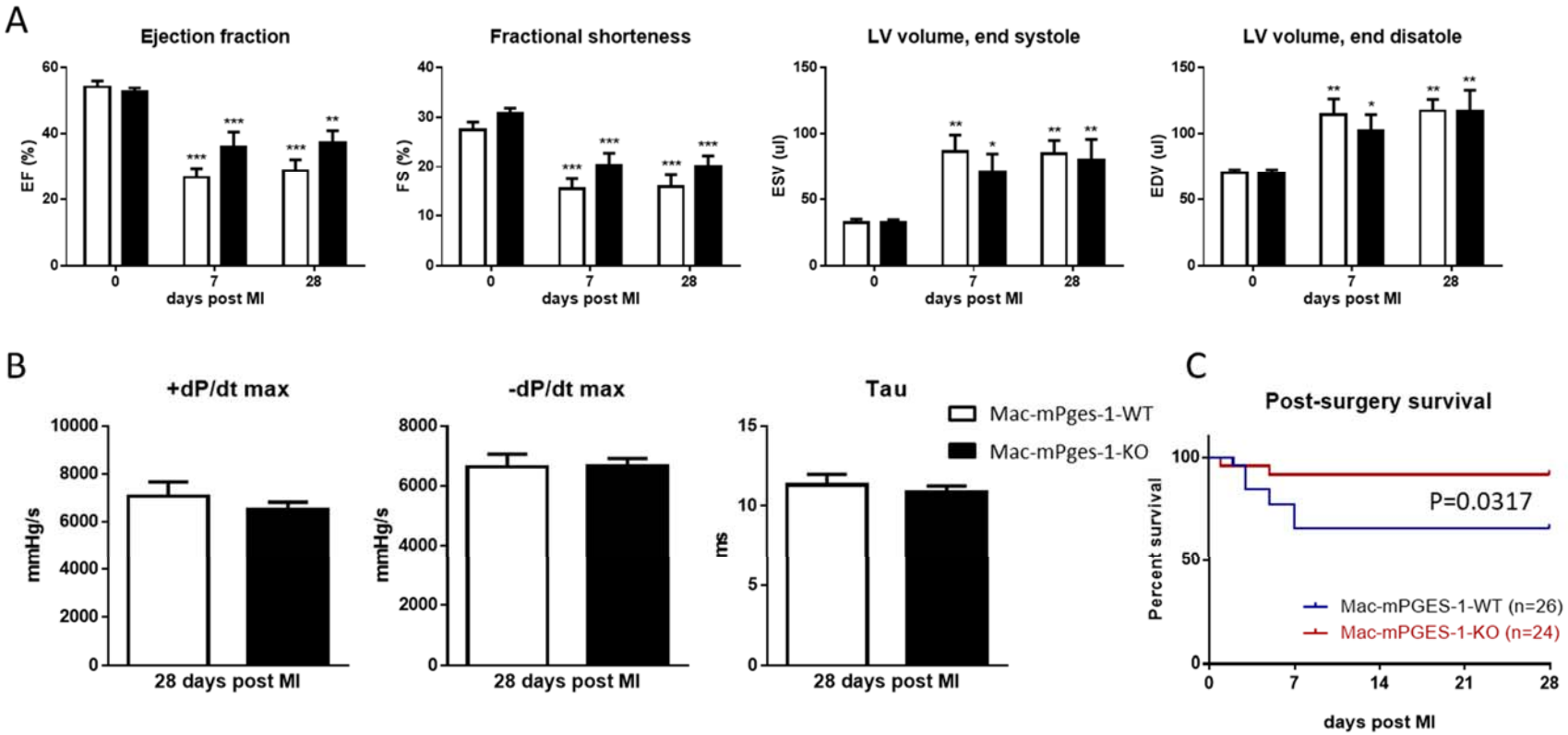

**Figure 2**

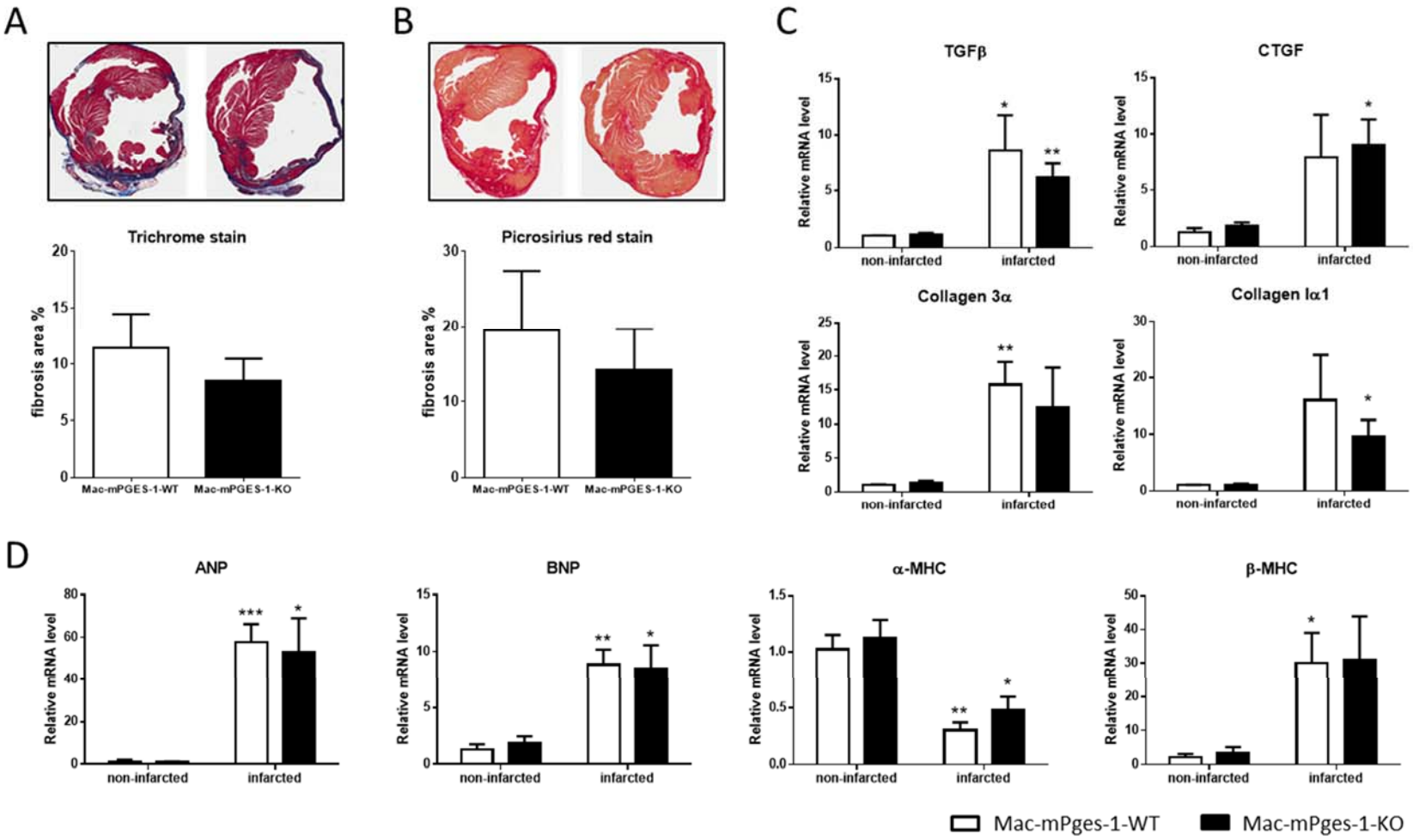

**Figure 3**

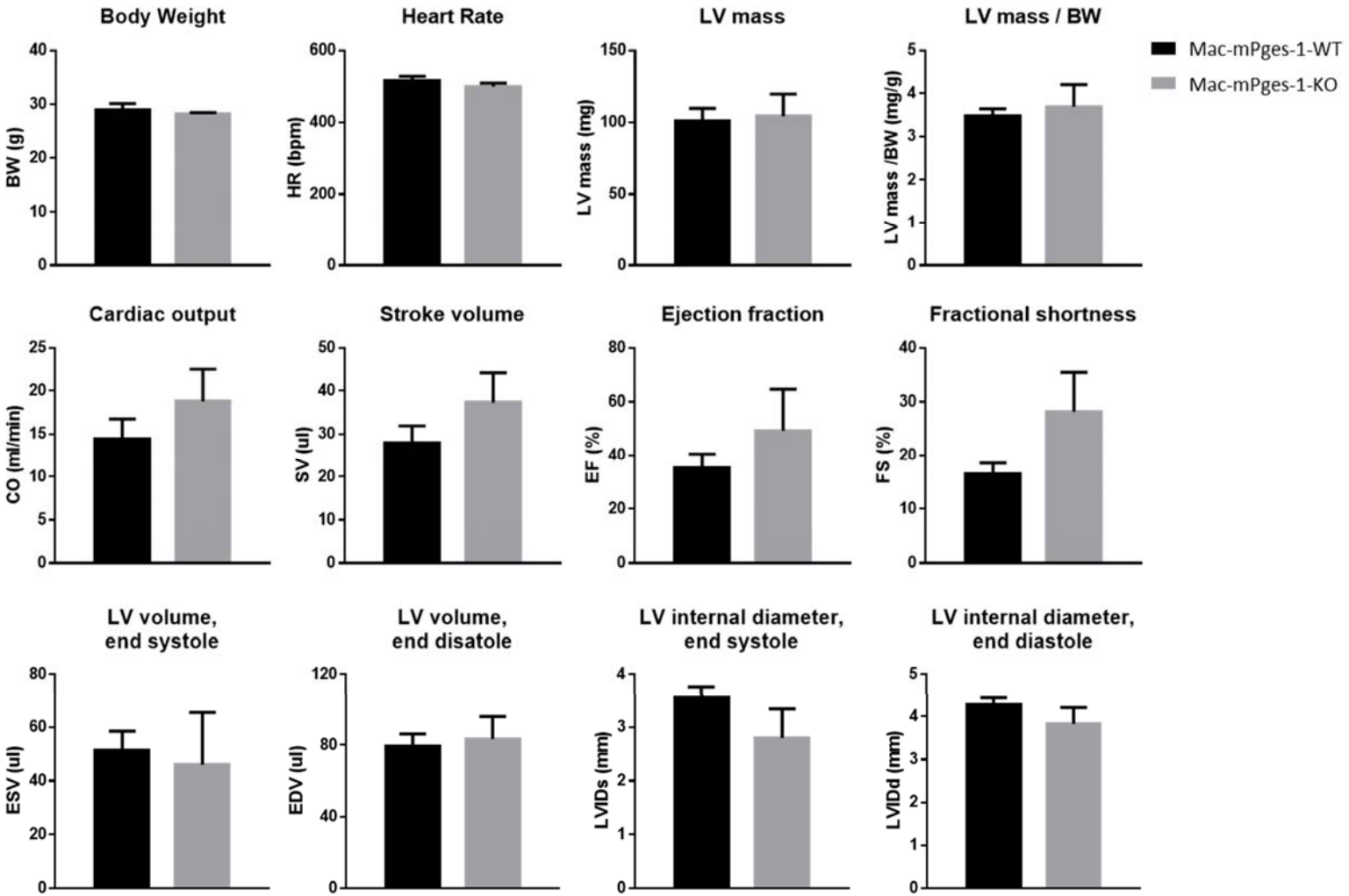

**Figure 4**

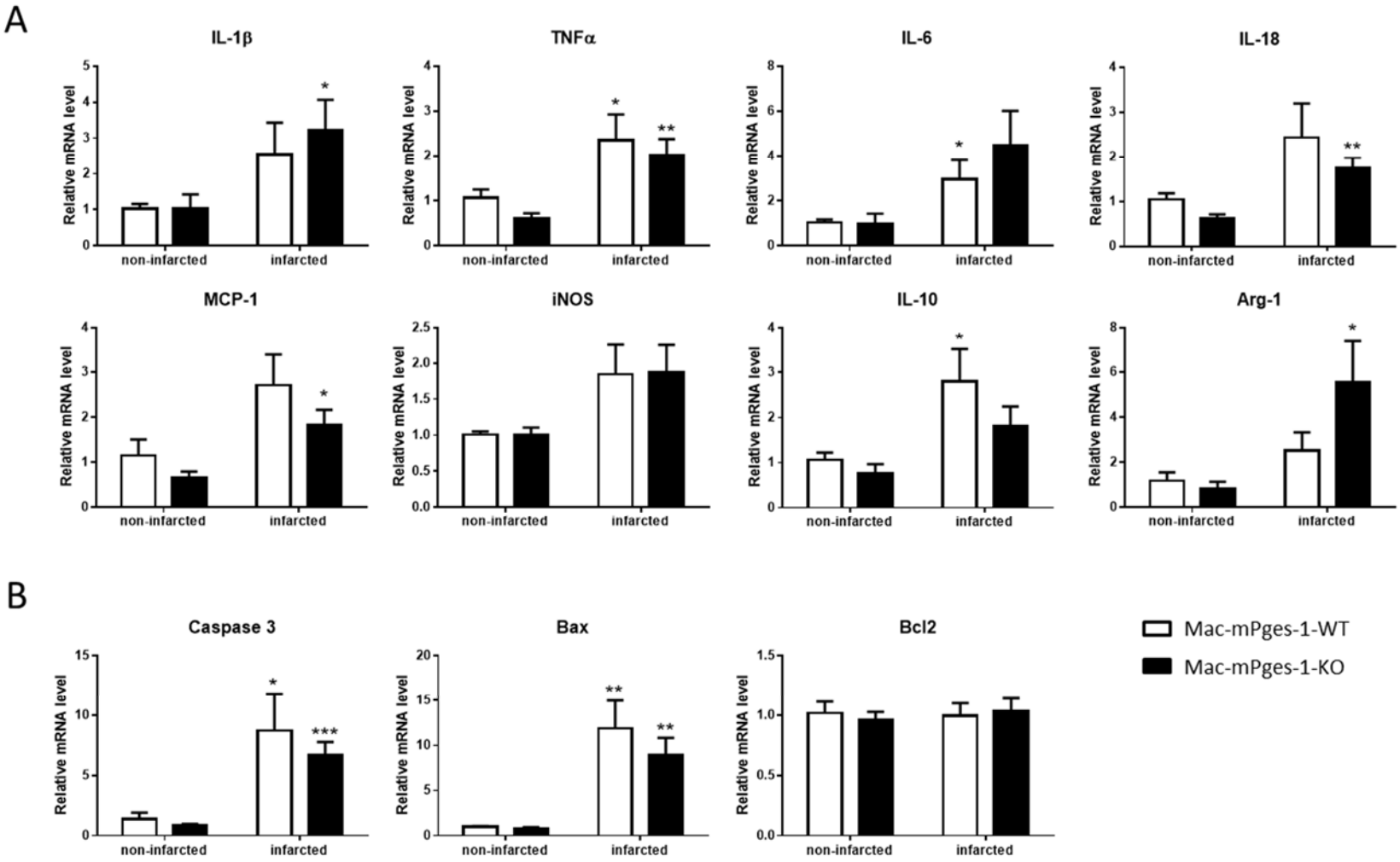

**Figure 5**