Journal of Zhejiang University-SCIENCE B (Biomedicine & Biotechnology) in press
www.jzus.zju.edu.cn; www.springer.com/journal/11585
E-mail: jzus_b@zju.edu.cn

Research Article

https://doi.org/10.1631/jzus.B2500477

IL-15 aggravates cardiac ischemia injury via impairing macrophage efferocytosis and driving inflammation

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Abstract: Acute myocardial infarction (AMI) remains a major global health burden, which is characterized by profound cardiac inflammation, apoptotic cell death, and impaired myocardial function. While interleukin-15 (IL-15) has been implicated in immune regulation, its precise role in the pathogenesis of AMI has not been clarified. Therefore, this study sought to delineate the functional role of IL-15 in the progression of AMI, with a particular focus on its influence on macrophage-driven inflammation, efferocytosis, and metabolic reprogramming. IL-15 levels were assessed in AMI patients and murine models. To evaluate the impact of IL-15 on cardiac inflammation, apoptosis, and functional outcomes following AMI, IL-15 and interleukin-15 receptor (IL-15R) α knockout (KO) mouse models were employed. Mechanistic studies were conducted to investigate IL-15-mediated effects on macrophage efferocytosis, polarization and metabolic remodeling, with an emphasis on nuclear factor kappa-B (NF-κB) signaling and glycolytic flux. Elevated IL-15 levels were detected in both the plasma of AMI patients and the cardiac tissues of murine AMI models, correlating with increased disease severity. The genetic deletion of IL-15 or IL-15Rα significantly ameliorated cardiac injury by reducing inflammation and apoptosis while preserving myocardial function. Mechanistic analyses revealed that IL-15 impaired macrophage efferocytosis via MERTK downregulation and promoted M1 polarization via NF-κB pathway activation. Furthermore, IL-15 reprogrammed macrophage metabolism by enhancing glycolytic activity. Ultimately, IL-15 restoration exacerbated cardiac ischemia injury following AMI, serving as a critical regulator of macrophage-mediated inflammation in AMI. These findings highlight the role of IL-15 as a potential therapeutic and prognostic target for mitigating cardiac inflammation and improving myocardial recovery in AMI.

Key words: Myocardial infarction; Inflammatory phenotype; Macrophage function; Metabolic reprogramming; Interleukin-15 (IL-15)

1 Introduction

Acute myocardial infarction (AMI), as a leading cause of global morbidity and mortality, is primarily caused by coronary artery occlusion leading to myocardial ischemia and infarction (Roth et al., 2020). Despite

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Received Aug. 9, 2025; Revision accepted Nov. 15, 2025
Crosschecked xxx. xx, 20xx; Published online xxx. xx, 20xx

advancements in AMI management, the relevant global burden remains significant. There is accumulating evidence that inflammation plays a crucial role in the development of AMI, determining the infarct size and contributing to ventricular remodeling and clinical outcomes (Westman et al., 2016). Sterile inflammation secondary to AMI is characterized by the recruitment and activation of innate and adaptive immune cells, which might facilitate the early complication of cardiac rupture (Li et al., 2022). The process by which macrophages eliminate apoptotic cells, known as efferocytosis, plays a crucial role in averting necrosis and inflammatory responses while simultaneously initiating pro-resolving pathways that promote ongoing efferocytosis (Ngai et al., 2023). However, the precise mechanisms underlying AMI pathogenesis remain unclear, necessitating further investigations to identify novel therapeutic targets that can mitigate AMI-associated mortality and improve clinical prognosis.

Interleukin-15 (IL-15), a member of the IL-2 cytokine family, is a 14-15 kDa protein comprising 114 amino acids, with a 4-α-helical bundle structure (Allard-Chamard et al., 2020). As a pleiotropic cytokine, IL-15 is widely expressed by diverse cell types, including monocytes, macrophages, dendritic cells, fibroblasts, epithelial cells, and skeletal muscle cells (Mortier et al., 2008; Perera et al., 2012; Patidar et al., 2016). Its expression is regulated at three levels: transcriptional, translational and secretory. IL-15 exerts its biological effects in inflammatory response and immunoregulation mainly through binding to its specific IL-15 receptor (IL-15R), which is composed of α, β and γ chains. IL-15 shares receptor components IL-15Rβ and IL-15Rγ with IL-2, while the unique IL-15Rα subunit specifically binds IL-15, conferring ligand specificity. During intracellular signaling, IL-15 binds to the trimer and exerts its activity primarily through the alpha chain (IL-15Rα or CD215) (Mortier et al., 2004).

Existing reports on the effects of IL-15 and its specific receptor on AMI are limited. Genetic variations of IL-15 and higher serum IL-15 levels have been found in patients with either acute coronary syndrome or ischemic stable coronary heart disease (Gokkusu et al., 2010). It was also suggested that epicardial adipose tissue may be a potential source of both IL-15 and IL-15Rα. Furthermore, previous studies have demonstrated that IL-15 was prominently expressed in atherosclerotic lesions of hyperlipidemic mice aortas and human carotid arteries (Wuttge et al., 2001; Dozio et al., 2014). IL-15 could regulate macrophages in the spinal cord and sciatic nerve after chronic constriction injury, and its expression is also regarded as a signal for the activation of macrophages in the sciatic nerve (Gomez-Nicola et al., 2008). However, the precise roles of IL-15 in the macrophage efferocytosis process during AMI and the underlying mechanisms remain unclear. Based on these previous reports, we hypothesized that IL-15, as a pro-inflammatory cytokine, might aggravate cardiac ischemia injury by specifically impairing macrophage efferocytosis and driving inflammation.

This study utilized IL-15 and IL-15Rα knockout (KO) mice as sophisticated genetic tools to delineate the specific contributions of IL-15 to the pathophysiology of AMI. We investigated the molecular mechanisms through which IL-15 contributes to AMI pathogenesis, with the objective of providing mechanistic insights that could guide the development of innovative therapeutic strategies to enhance AMI outcomes.

2 Materials and methods

The study methods are extensively described in the Supplementary Material.

2.1 Human plasma samples

This investigation was performed according to the Declaration of Helsinki, and written informed consent was obtained from all study participants prior to inclusion with approval by the Institutional Ethics Committee of Second Affiliated Hospital of Zhejiang University School of Medicine. Patients who complained of chest pain or distress and underwent coronary angiography at the Second Affiliated Hospital of Zhejiang University School of Medicine from 2018 to 2022 were enrolled. Those who had 100% coronary occlusion were assigned to the AMI group and those who had coronary myocardial bridge (CMB) formed the healthy control (CTL) group. Participants' characteristics were listed in Table S1. Blood samples were collected during coronary angiography and stored for further tests. The study protocol was approved by the institution's ethical committee (2021-0392, Clinical trial no. I2021001496).

2.2 Animals

Male C57BL/6J mice aged 8-10 weeks and neonatal C57BL/6J mice were purchased from Shanghai Slac Laboratory Animal Technology Corporation (Shanghai, China). Global IL-15 KO mice and IL-15Rα KO mice (all with C57BL/6J background) were purchased from Shanghai Model Organisms (Shanghai, China). Mice were housed at room temperature (20 ± 2 °C) with an alternating 12 h light/dark cycle and provided with a standard diet. All animal procedures were conformed to the guidelines from Directive 2010/63/EU of the European Parliament on the protection of animals used for scientific purposes. The study protocol was approved by the institution's ethical committee (AIRB-2021-1012).

2.3 AMI model

Mice were put under 2.0% isoflurane (Sigma-Aldrich, USA) anesthesia and mechanical ventilation (Alcott Biotech, Shanghai, China) assisted by tracheotomy, then subjected to AMI by the ligation of left anterior descending artery. Mice in the Sham group underwent thoracotomy without the ligation of coronary arteries. The animals were placed on a heating pad and watched carefully for the next three hours until they achieved full locomotor behavior. To investigate whether the inhibition of IL-15 protected myocardium from acute ischemic injury, IL-15 KO and IL-15Rα KO mice were established with AMI. To validate the detrimental effect of IL-15 in acute cardiac ischemic injury, IL-15 KO mice and wild-type littermates were used and intraperitoneally injected either with recombinant murine IL-15 (rmIL-15, 50 ug/kg, Peprotech, USA) or PBS daily for 14 days after AMI establishment, respectively. At the end of the experiment, euthanasia was performed using 2% inhaled isoflurane followed by cervical dislocation.

3 Results

3.1 IL-15 is Elevated in AMI Patient Plasma and Murine Hearts

In order to investigate the potential role of IL-15 in AMI, we initially collected serum samples from patients diagnosed with AMI. A total of 243 patients presenting with chest pain or discomfort were enrolled according to the inclusion criteria and underwent coronary angiography. Among them, 121 patients with 100% coronary artery occlusion were classified as the AMI group, while 122 patients with CMB were assigned to CTL group. No significant differences in age, gender or smoking history were observed between the two groups (Table S1). Plasma IL-15 levels were significantly elevated in AMI patients compared to those in CTL subjects (Fig. 1a), and this association remained significant after adjustment for BMI, hypertension, diabetes, and hyperlipidemia in a multivariable logistic regression model (adjusted OR = 1.32, 95% CI: 1.13-1.542, P<0.001; Table S2), indicating a potential association between IL-15 and the pathophysiological processes of AMI.

In order to further elucidate the temporal and spatial expression of IL-15, we employed a mouse model of AMI. Tissue lysates from the border zone of infarcted myocardium revealed a gradual increase in IL-15 levels, peaking on the third day post-AMI and subsequently declining (Fig.1b). Consistently, western blot analysis revealed the same trend in the expression of IL-15. We sought to identify the specific receptor subtype involved in mediating the interaction with IL-15 in this process. IL-15Rα expression was significantly upregulated in the myocardium following AMI, whereas the levels of IL-15Rβ and IL-15Rγ remained unchanged (Fig.1c). Interestingly, although IL-15Rα expression was elevated in the remote myocardium, IL-15 levels were not significantly changed in this region (Figs. S1a-S1c). Immunofluorescence staining further demonstrated that IL-15 was predominantly localized intracellularly in the border zone of the infarcted myocardium in AMI mice (Figs. 1d and 1e). Immunofluorescence staining confirmed a significant co-localization of IL-15 protein with macrophage markers (CD68) in the cardiac tissues of our AMI model, which suggests that macrophages are prominent producers of IL-15 (Fig. 1f). Collectively, these findings highlight the dynamic regulation of IL-15 and IL-15Rα and suggest a potential role for IL-15 in the pathophysiology of AMI.

3.2 IL-15/IL-15Rα Deficiency Reduces Infarct Size and Improves Cardiac Function.

In order to elucidate the role of IL-15 in AMI, IL-15 and IL-15Rα KO mice were generated using gene-editing techniques (Figs. S2a-S2e) and subjected to AMI. Notably, 30% of wild-type (WT) littermates succumbed within one week of AMI, whereas most IL-15 KO and IL-15Rα KO mice survived (Fig. 2a). Echocardiographic analyses revealed that IL-15 KO and IL-15Rα KO mice exhibited significantly higher ejection fraction (EF) and fractional shortening (FS), along with reduced left ventricular internal dimension of

end diastole (LVIDd) compared to WT littermates after AMI (Figs. 2b and 2c; Table S3). Cardiac mass, as reflected by the heart-to-body weight ratio, was lower in IL-15 KO and IL-15Rα KO mice than in WT littermates (Fig. 2d). Histological examination demonstrated that the infarct size was significantly smaller in IL-15 KO and IL-15Rα KO mice on the third day post-AMI compared to WT mice (Figs. 2e and 2f). Additionally, plasma BNP levels, which progressively increased after AMI, were significantly lower in IL-15 KO and IL-15Rα KO mice on the fourteenth day post-AMI (Fig. S3). Taken together, IL-15 and its receptor IL-15Rα are critical mediators in the pathological progression of AMI: the absence of IL-15 or IL-15Rα improved survival, preserved cardiac function, and reduced myocardial damage, suggesting that IL-15 may contribute to cardiac dysfunction.

3.3 Deficiency of IL-15 and IL-15Rα Alleviates Myocardial Apoptosis and Inflammation.

Apoptosis, or programmed cell death, is a crucial mechanism in AMI pathophysiology. The percentage of terminal-deoxynucleoitidyl transferase-mediated nick end labeling (TUNEL)-positive cells in the heart was markedly lower in IL-15 KO and IL-15Rα KO mice than in WT littermates on the third day post-AMI (Figs. 3a and 3b). Western blot analysis further demonstrated reduced cleaved-caspase 3 levels in the border zone of the infarcted myocardium in IL-15 KO and IL-15Rα KO mice (Fig. 3c). To determine whether IL-15 directly influences cardiomyocyte apoptosis, an in vitro hypoxia model was established. Cardiomyocyte apoptosis was induced strongly by hypoxia; however, it was not exacerbated by preconditioning with rmIL-15 apoptosis, as evidenced by TUNEL staining, lactate dehydrogenase activity (Figs. S4a and S4b), and cleaved-caspase 3 levels (Fig. 3d). These findings suggest that IL-15 contributes to hypoxia-mediated cardiomyocyte apoptosis, whereas its pro-apoptotic effects may not be directly mediated through cardiomyocytes.

Inflammation also plays a critical role in tissue damage and repair during MI. We therefore aimed to explore the contributions to IL-15 and IL-15Rα in inflammation-induced myocardial injury. On the day post-AMI, the mRNA levels of pro-inflammatory cytokines *Il-1β* and *Il-6* in the border zone of the infarcted myocardium were significantly reduced in IL-15 KO and IL-15Rα KO mice compared to WT littermates. Conversely, the expression of anti-inflammatory cytokines IL-4 and IL-10 was upregulated in these knockout mice, indicating anti-inflammatory roles for IL-15 and IL-15Rα deficiency (Fig. 3e). Considering that IL-15 exerts its biological functions through IL-15Rα, we next performed immunofluorescence to localize IL-15Rα in the ischemic myocardium. IL-15Rα was predominantly co-localized with macrophages ($CD68^+$) compared to other cell types (Figs. 3f and 3g; Fig. S4c). Moreover, the number of $CD68^+$ macrophages was markedly reduced in the cardiac tissue of IL-15 KO and IL-15Rα KO mice following AMI. (Figs. 3h and 3i). These findings suggest that the interaction between IL-15 and IL-15Rα may serve as a critical driver of macrophage-mediated cardiomyocyte apoptosis during AMI.

3.4 IL-15 Impairs Macrophage Efferocytosis by Suppressing Mertk Expression

Efferocytosis, the process by which phagocytic cells such as macrophages recognize and engulf apoptotic cells, is essential for maintaining tissue homeostasis and resolving inflammation. We hypothesized that IL-15 exacerbates apoptotic cardiomyocyte accumulation by impairing macrophage efferocytosis. Therefore, bone marrow-derived macrophages (BMDMs) were treated with rmIL-15 and their phagocytic activity was assessed. IL-15 treatment significantly reduced macrophage phagocytosis (Figs. 4a and 4b). To confirm these effects in vitro, CD68-stained BMDMs were co-cultured with PKH26-stained apoptotic mouse cardiomyocytes. Flow cytometry revealed a markedly reduced phagocytic rate in rmIL-15-treated macrophages compared to controls (Figs. 4c and 4d), which was further validated by immunofluorescence imaging (Figs. 4e and 4f). To elucidate the relevant mechanism, we examined the expression of genes associated with macrophage efferocytosis. MERTK is a receptor with a critical role in recognizing and clearing apoptotic cells. Treatment with rmIL-15 markedly suppressed MERTK expression in macrophages at both the transcriptional and protein expression levels (Figs. 4g and 4h). Our findings strongly suggest that IL-15 suppressed efferocytosis by downregulating Mertk. To definitively establish this causal relationship, we performed a crucial rescue experiment. As anticipated, IL-15 treatment potently suppressed the endogenous MERTK protein levels in Lenti-Vector cells. In stark contrast, the Lenti-Mertk cells robustly maintained high levels of MERTK expression, successfully overriding the inhibitory signal from IL-15 (Fig 4i). Most critically, we assessed the functional consequences. In Lenti-Vector cells, IL-15 treatment led to a profound suppression of efferocytosis, consistent with our findings in primary macrophages. However, the Lenti-Mertk cells were rendered completely resistant to this

IL-15-mediated functional defect. Despite the presence of IL-15, the Lenti-Mertk cells maintained a high level of efferocytic capacity that was comparable to the untreated controls (Figs 4j and 4k). Collectively, these data unequivocally demonstrate that the downregulation of MERTK is the direct and rate-limiting mechanism by which IL-15 impairs macrophage efferocytosis.

3.5 IL-15 Drives Macrophage M1 Polarization and Activates Nuclear Factor Kappa-B (NF-κB) Signaling.

Beyond efferocytosis, the polarization of macrophages between the pro-inflammatory M1 and the anti-inflammatory M2 states critically influences the inflammatory microenvironment and overall cardiac outcomes. Thus, we sought to determine whether IL-15 affects macrophage polarization and the signaling pathways involved. We therefore assessed the expression of M1 and M2 markers in IL-15 KO and IL-15Rα KO mice. $CD206^+$ macrophages (M2 phenotype) were notably increased in both knockout models, while $iNOS^+$ macrophages (M1 phenotype) were significantly reduced (Figs. 5a and 5b). These observations were confirmed in ex vivo experiments, where macrophages treated with rmIL-15 exhibited elevated levels of M1-associated cytokines (IL-1α, IL-1β, IL-6, IFN-γ, and TNF-α) and reduced levels of M2-associated cytokines (IL-4 and IL-10) in the culture supernatants (Figs. 5c and S5).

Next, we investigated the involvement of the NF-κB signaling pathway, the classical pathway related to inflammatory responses, in IL-15-induced macrophage polarization. Under baseline conditions, p65, a key component of the NF-κB pathway, was primarily localized in the cytoplasm of $CD68^+$ macrophages. However, following rmIL-15 treatment, p65 translocated from the cytoplasm to the nucleus (Fig. 5d), indicating NF-κB activation. To confirm the role of this pathway, gossypin, an NF-κB inhibitor, was used to treat BMDMs alongside rmIL-15 at varying concentrations (Fig. 5e). The treatment of rmIL-15 significantly increased the transcriptional levels of M1-related genes (IL-1α, IL-1β, and IL-6) and decreased the levels of M2-related genes (IL-4 and IL-10). These effects were reversed by gossypin, with the most pronounced inhibition observed at 50 μmol/L (Fig. 5f). To test the IL-15-induced interaction between macrophages and cardiomyocytes and the effect of inhibitor on this interaction, primary cardiomyocytes were treated with the supernatant of IL-15-treated macrophages. The supernatant of macrophages treated with rmIL-15 increased the ratio of cleaved-caspase3/caspase3 in cardiomyocytes under the hypoxic condition, which could be reduced by gossypin (Fig. 5g). These findings suggest that IL-15 promoted the pro-inflammatory M1 polarization of macrophages via the activation of NF-κB signaling pathway, increasing the secretion of proinflammatory cytokines, which amplified cardiomyocyte apoptosis.

3.6 IL-15 Enhances Glycolytic Activity and Metabolic Activation in Macrophages.

Building on the observed effects of IL-15 on macrophage inflammatory responses and efferocytosis, we next investigated whether IL-15 modulates macrophage function via influencing metabolism. Glycolysis, a primary energy source for macrophage activation and inflammatory signaling, was firstly assessed through intracellular and extracellular lactate measurements. The treatment of rmIL-15 significantly increased lactate concentrations both intracellularly and extracellularly in macrophages (Figs. 6a and 6b). To further explore this metabolic shift, we analyzed glycolysis-related gene expression. IL-15 treatment induced the pronounced upregulation of glycolytic genes, including phosphoglycerate kinase 1 (PGK1) and enolase 1 (ENO1), highlighting its role in promoting glycolytic reprogramming (Fig. 6c). PGK1 and ENO1 are key glycolytic enzymes that catalyze critical steps in the glycolytic pathway, directly contributing to ATP production via substrate-level phosphorylation. Cellular ATP levels were also markedly elevated in IL-15-treated macrophages, indicating enhanced metabolic activity (Fig. 6d). These findings suggest that IL-15 promoted glycolytic reprogramming in macrophages, characterized by increased lactate production and ATP generation, thereby contributing to their activation and inflammatory function.

3.7 Restoring IL-15 Exacerbates Cardiac Ischemia Injury After AMI.

In order to confirm and validate the adverse effects of IL-15, exogenous rmIL-15 or vehicle was administered intraperitoneally to IL-15 KO and WT mice once daily for 14 days following AMI. The administration of rmIL-15 significantly increased mortality in both WT and IL-15 KO mice (Fig. 7a). Furthermore, rmIL-15 treatment aggravated cardiac dysfunction in WT mice, as evidenced by reduced EF and FS, along with LVIDd expansion post-AMI. Similarly, IL-15 repopulation in IL-15 KO mice resulted in

impaired EF and FS, though it did not significantly affect LVIDd (Figs. 7b and 7c; Table S4). Moreover, rmIL-15 administration increased the cardiac mass index and resulted in larger infarct sizes in both WT and IL-15 KO mice (Figs. 7d-7f). These findings indicate that restoring IL-15 exacerbates cardiac dysfunction and myocardial injury following AMI, further supporting its detrimental role in AMI pathogenesis.

4 Discussion

This study identified IL-15 as a pivotal regulator in the early-phase pathogenesis of AMI. IL-15 was significantly upregulated in the plasma of AMI patients and the ischemic myocardium of AMI mice, as an independent risk factor for the occurrence of AMI. IL-15Rα, as the receptor for IL-15, was shown to be a key mediator in driving macrophage polarization toward the pro-inflammatory M1 phenotype, exacerbating inflammation, impairing efferocytosis, and increasing myocardial apoptosis, which ultimately contributed to adverse cardiac remodeling and heart failure. These effects were mediated through the activation of NF-κB signaling pathway, which underscores the role of IL-15 as a driver of macrophage pro-inflammatory responses. Beyond these effects, IL-15 was found to modulate macrophage glycolysis, enhancing metabolic activation to support inflammation and other energy-intensive processes. Hence, these new findings allowed us to reach the potential mechanisms discussed below.

Notably, our findings underscore the critical role of IL-15 in the early pathogenesis of AMI following coronary occlusion, establishing IL-15 as a compelling therapeutic target for intervention. AMI is fundamentally an inflammatory condition, as supported by evidence showing that targeting pro-inflammatory cytokines reduces recurrent cardiovascular events in AMI patients (Ridker et al., 2017). A variety of immune cells constitutively express cytokines in the acute phase of AMI, exerting multifaceted biological roles and contributing to inflammation status in the ischemic microenvironment. If effective therapies are not promptly applied after coronary occlusion, severe complications may be presented, such as malignant arrhythmia, heart failure, mitral valve insufficiency, and even cardiac rupture. Even though the incidence of cardiac rupture is relative rare at 1% to 3%, it is almost always fatal as the mortality is nearly up to 100% once it occurs (Hao et al., 2020). Therefore, it has very high clinical significance to investigate the pathogenic mechanisms in the early phase of AMI incidence and figure out the potential targets. Our research demonstrated that the content of human plasma IL-15 still has an independent association with AMI incidence after adjusting for risk factors such as BMI, hypertension, diabetes, and hyperlipidemia.

It is becoming increasingly well recognized that interleukins are involved in the development and early-phase remodeling of AMI. IL-4 and IL-10 has been proved to improve early-phase remodeling by modulating the balance between anti-inflammation and pro-inflammation, whereas IL-17A and IL-32 play pathogenic roles in the inflammation cascade (Zhou et al., 2014; Jung et al., 2017; Xuan et al., 2017; Daseke et al., 2020). IL-33 exhibits biphasic regulatory effects, facilitating significant inflammatory resolution by the fourth day post-AMI while suppressing prolonged inflammation between days four and seven. However, these effects ultimately result in exacerbated cardiac remodeling and an increased risk of cardiac rupture (Ghali et al., 2020). Different from sharing the βγ signaling complex with other interleukins (Patidar, et al., 2016). IL-15Rα binds IL-15 and anchors it to the cell surface, facilitating its presentation and stability in the extracellular microenvironment (Wuttge, et al., 2001). This supports our findings that the expression of IL-15Rα in infarct-border area was upregulated, but no change occurred in the expression of either IL-15Rβ or IL-15Rγ. A previous study has demonstrated that IL-15Rα signaling exerts opposing regulatory effects on IL-17-producing γδ T cells (Colpitts et al., 2015). In the present study, we constructed both IL-15 KO and its specific receptor IL-15α KO mice and investigated their roles in the early-phase after AMI, which might have better clinical translational significance of reducing the risk of heart failure after AMI.

Accumulating evidence underscores the diverse and critical roles of immune cells, including neutrophils, macrophages, eosinophils, mast cells, and dendritic cells, in modulating the ischemic and hypoxic microenvironment (Daseke, et al., 2020; Ghali, et al., 2020; Wei et al., 2020). It has been well-documented that macrophages are involved in ventricular remodeling after AMI (Lambert et al., 2008; Hamada et al., 2014; Jakubzick et al., 2017). The infiltration of macrophages begins at first day and peaks at 3-5th day post-AMI (Yan et al., 2013). The early intervention of inflammation after ischemia may prevent cardiomyocytes against

ischemic injury and improve cardiomyocytes survival in the border zone of infarcted myocardium. In the present study, we noticed that the expression of IL-15 in the border area of infarcted myocardium increased gradually, peaked at the third day post-AMI and then decreased thereafter.

An increasing body of evidence underscores that controlling the macrophage M1/M2 phenotype may represent a novel therapeutic strategy for treating AMI (Van Den Bossche et al., 2016). It has been indicated that promoting macrophage M2 polarization through the exogenous administration of IL-10 leads to the activation of cardiac fibroblasts, a reduction in microenvironmental inflammation, and accelerated wound healing following AMI (Jung, et al., 2017). In the current study, the peak of IL-15 expression was highly consistent with that of the presence of M1 phenotype macrophages. Also, exogenous IL-15 administration promoted the M1 polarization of macrophages, whereas the inhibition of either IL-15 or IL-15Rα intercepted macrophage infiltration and M1 polarization during hypoxia. These results imply the importance of IL-15 signaling in macrophage polarization after AMI.

Targeting the IL-15 signaling pathway could be beneficial in addressing many clinical problems. NF-κB is a key regulator in inflammatory response. It has been reported that IL-15 triggered NF-κB activation in human neutrophils (Mcdonald et al., 1998). Otherwise, NF-κB might be responsible for IL-15 actions on brain microvessel endothelial cells, thus affecting cellular permeability, endocytosis, and intracellular trafficking at the level of the blood-brain barrier (Stone et al., 2011). Consistent with our study, some studies have demonstrated that the downregulation of phosphorylated NF-κB p65 expression led to a decrease in $Ly6C^{low}$ macrophages in the heart (indicated as M1 macrophage), ameliorating isoprenaline-induced cardiac hypertrophy and fibrosis (Li et al., 2020). One study also showed that HMGB1/NF-κB pathway regulates macrophage M2 polarization and alleviates AMI by impeding inflammation, oxidation, apoptosis, and autophagy (Wang et al., 2023). In the present work, we further revealed that IL-15 activated NF-κB signaling pathway in macrophages during ischemic injury by enhancing intracellular NF-κB translocation and phosphorylation.

Existing literature suggests that immune-metabolic reprogramming is critically involved in shaping macrophage phenotypic transitions, providing a mechanistic link between cellular metabolism and immune function (Van Den Bossche, et al., 2016). This underscores the potential of targeting metabolic pathways to modulate macrophage polarization and develop novel therapeutic strategies for AMI. Our findings demonstrated that IL-15 disrupts macrophage metabolic homeostasis by significantly upregulating the expression of key glycolytic enzymes, such as Pgk1 and Eno1, and enhancing the production of downstream metabolites, including lactate and ATP. These results highlight IL-15 as a potential target for therapeutic intervention aimed at restoring metabolic balance and mitigating inflammation. In summary, our findings demonstrate that IL-15 treatment markedly amplified macrophage-mediated inflammation and glycolytic metabolism while impairing efferocytosis, underscoring the multifaceted role of IL-15 in orchestrating macrophage activation and functional reprogramming. These effects collectively exacerbated infarct expansion, heightened myocardial apoptosis, and impaired cardiac function, highlighting their detrimental impact on post-AMI cardiac pathology. Our study focused on the acute inflammatory phase following MI. In contrast, the work by Ameri et al. elegantly demonstrated a beneficial role for IL-15 in the late-phase remodeling stage. In their study, IL-15 treatment significantly increased vessel area density and reduced fibrosis after MI. IL-15 is not the only cytokine that shows a rapid increase in expression in early-stage MI and exacerbates myocardial damage. However, when administered exogenously, it can actually reduce the infarcted area. In mice, TNF-α levels were seen to rise significantly on the first day post-AMI, and the deletion of TNF-α significantly improved myocardial function on the third day post-MI but not on the seventh day (Zhang et al., 2013). In male rats with left anterior descending artery ligation, TNF-α mRNA and protein production were increased in the myocardium on the first day, which were detectable until the 35^{th} day post-AMI (Irwin et al., 1999). In young rat hearts, the injection of TNF-α significantly reduced the extent of myocardial injury after coronary ligation. This protective effect may be related to its activation of the downstream platelet-derived growth factor-B (PDGF-B) pathway (Cai et al., 2003). These studies, combined with our own research, further suggest the complexity of the role of cytokines in the early and late stages of MI.

Despite the promising findings, our study has certain limitations. To investigate the exact roles of IL-15 in myocardial ischemia, we constructed IL-15 and IL-15Rα KO mice and established an AMI model. The mice we used were global KO mice, not conditional KO mice, and these animals were not able to demonstrate the effect of IL-15 on macrophages well. Our future research will employ macrophage-specific IL-15 KO mice using

Lyz2-Cre to directly verify this hypothesis, which will be regarded as the core direction of our subsequent studies. Furthermore, we only reported IL-15 interactions between macrophages and cardiomyocytes after AMI. Future studies must assess the long-term effects of IL-15 inhibition on repair, metabolism, and immune homeostasis. Last but not least, follow-up research examining the molecular pathways linking IL-15 signaling, glycolysis and efferocytosis could provide deeper insights into how metabolic reprogramming facilitates macrophage functions in inflammatory diseases.

5 Conclusions

Our study identified IL-15 as a pivotal mediator in the early-stage pathogenesis of AMI, driving inflammatory responses, disrupting macrophage efferocytosis, and promoting glycolytic reprogramming in macrophages. These mechanisms synergistically contribute to infarct expansion, heighten myocardial apoptosis, and facilitate progressive cardiac dysfunction. Collectively, our findings underscore IL-15 as a promising therapeutic target, which has the potential to attenuate inflammation, limit cardiac injury, and improve clinical outcomes in AMI patients.

Data availability statement

The data supporting this research could be requested from the corresponding author upon reasonable request.

Acknowledgments

This work is supported by the grants from the National Natural Science Foundation of China (82270498, to Xiao-jie XIE, 81570322 to Dr Jun JIANG). We thank Professor Wei ZHU (Department of Cardiology, The Second Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, China) for his instructional advice and technical assistance. Visual abstract was created using resources available by Figdraw (https://www.figdraw.com/).

Author contributions

Lei GUO and Zhe-hui YIN conducted the experiments, analyzed the data and wrote the manuscript draft. Ning ZHANG and Han CHEN provided conceptual advice. Zhuo WANG, Yu-xue HUANG, Ji-niu HUANG, Ya-you YOU, Chen-yun ZHANG, Qin-yi BAO and Shu-xin LEI participated in specific experiments and analyzed the results. Jun JIANG and Xiao-jie XIE designed the study and revised the manuscript. All authors read and approved the final manuscript and, therefore, had full access to all the data in the study and take responsibility for the integrity and security of the data.

Compliance with ethics guidelines

Lei GUO, Zhehui YIN, Ning ZHANG, Han CHEN, Zhuo WANG, Yuxue HUANG, Jiniu HUANG, Yayu YOU, Chenyun ZHANG, Qinyi BAO, Shuxin LEI, Jun JIANG and Xiaojie XIE declare that they have no conflict of interest.

This investigation was performed according to the Declaration of Helsinki and written informed consent was obtained from all study participants prior to inclusion in the study with approval by the Institutional Ethics Committee of Second Affiliated Hospital of Zhejiang University School of Medicine. Participants' study protocol was approved by the Institutional Ethics Committee (2021-0392, Clinical trial no. I2021001496). All animal procedures were conformed to the guidelines from Directive 2010/63/EU of the European Parliament on the protection of animals used for scientific purposes. Animal's study protocol was approved by the Institutional Ethics Committee (AIRB-2021-1012). All institutional and national guidelines for the care and use of laboratory animals were followed.

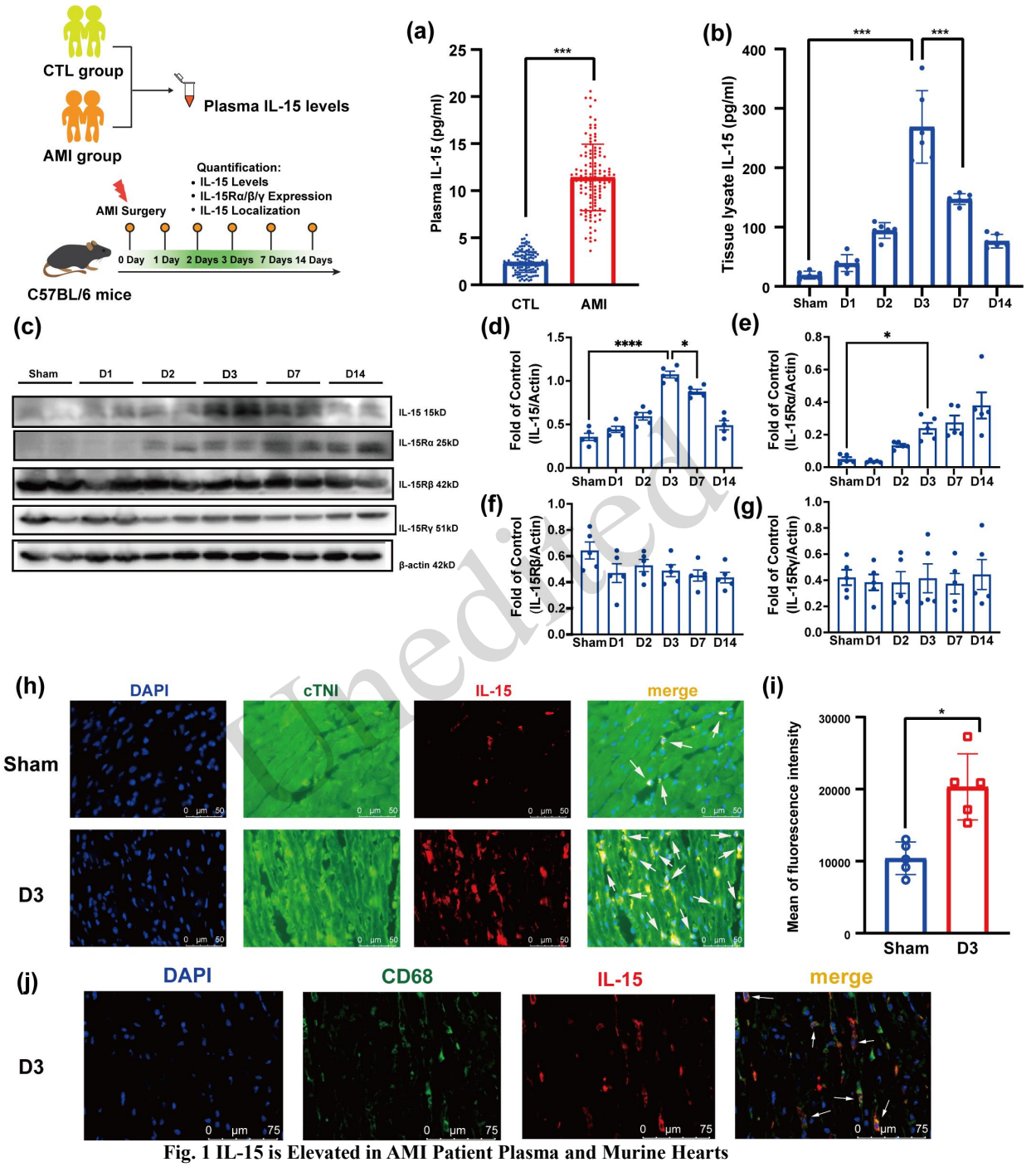

Fig. 1 IL-15 is Elevated in AMI Patient Plasma and Murine Hearts

(a) Plasma IL-15 level in patients with AMI (n=121) and CTL (n=122). (b) IL-15 level in tissue lysate of the infarct-border area of mice myocardium at the different days after AMI. n=6 for each group. (c-g) Expressions of IL-15 and its receptors in the infarct-border area of mouse myocardium at the different days after AMI by western blot (n=5 for each group). (h) Representative immunofluorescence staining of IL-15 (red), cTnI, (green), and DAPI (blue) in the infarct-border area of mouse myocardium at the third day after AMI. Positive staining is indicated as white arrow. (i) Fluorescence intensity analysis of IL-15 in the infarct-border area of mouse myocardium at the third day after AMI (n=5 for each group). (j) Representative immunofluorescence staining of IL-15 (red), CD68 (green), DAPI (blue) in the infarct-border area of mouse myocardium at the third day after AMI. $^{*}P$<0.05, $^{***}P$<0.001.

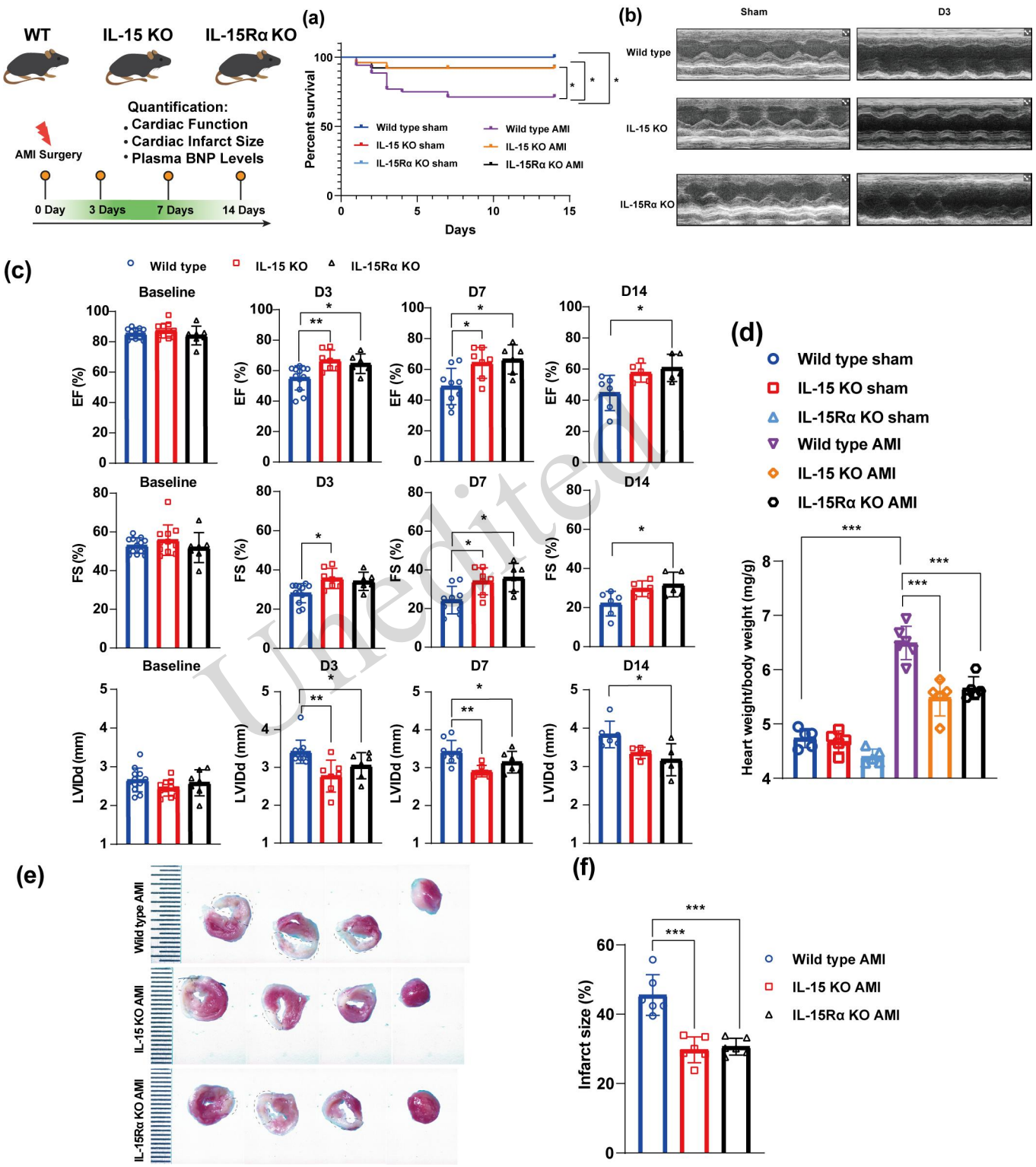

Fig. 2 IL-15/IL-15Rα Deficiency Reduces Infarct Size and Improves Cardiac Function

(a) Kaplan-Meier survival analysis of *Il-15* KO mice with AMI (n=25) or sham (n=12), *Il-15rα* KO mice with AMI (n=26) or sham (n=11), and wild type littermates with AMI (n=52) or sham (n=12). (b) Representative images of M-mode echocardiography in each group. (c) Quantitative echocardiographic analysis of left ventricular EF, FS and LVIDd (n=6-12). (d) Quantitative analysis of cardiac mass index (n=5-6). (e) Representative images of a heart section (1 mm) after TTC staining at the third day after AMI. The infarct size was circled by the dotted line. (f) Quantitative analysis as the percentage of infarct size to the whole heart (n=6 for each group). $^{*}P$<0.05, $^{**}P$<0.01, $^{***}P$<0.001.

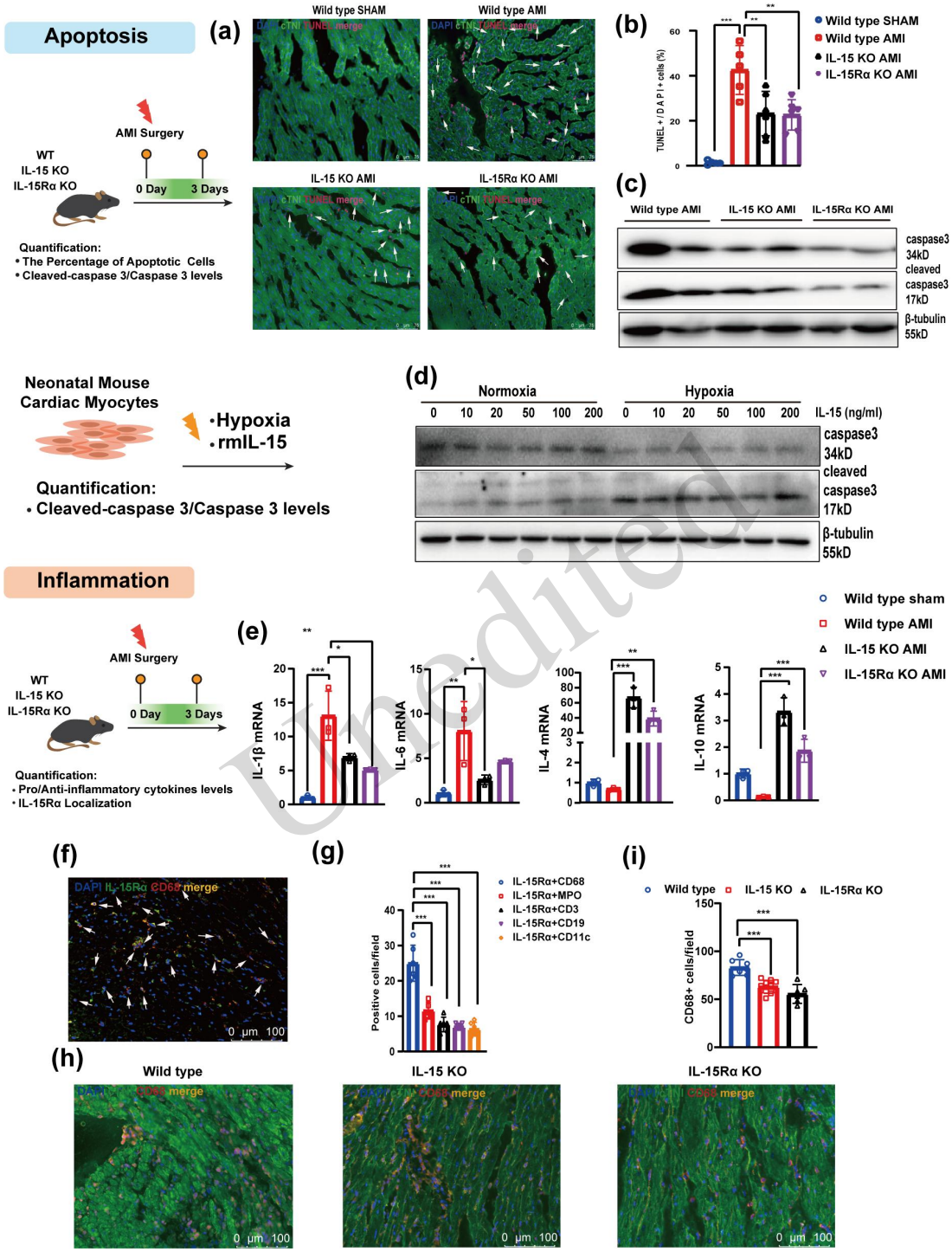

Fig. 3 Deficiency of IL-15 and IL-15Rα alleviates myocardial apoptosis and inflammation

(a) Representative images of heart tissue by TUNEL staining at the third day. TUNEL positive cell is indicated as white arrow. (b) Quantitative analysis of TUNEL positive cells. (c) Expressions of caspase3 and cleaved-caspase3 in the infarct-border area at the third day after AMI. (d) Expressions of caspase3 and cleaved-caspase3 in primary cardiomyocytes under either normoxia or hypoxia, preconditoning with rmIL-15 or vehicle. (e) Expressions of genes in the infarct-border zone at the third day after AMI or sham. (f) Representative immunofluorescence staining of IL-15Rα, CD68 and DAPI in the heart section of wild-type mice. Positive stained cell is indicated by a white arrow. (g) Quantitative analysis of positive con-stained cells per slice (n=5-6). (h) Representative immunofluorescence staining of CD68, cTNI and DAPI in the heart section at the third day after AMI (n=5-10). (i) Quantitative analysis of positive con-stained cells per slice. $^{*}P$<0.05, $^{**}P$<0.01, $^{***}P$<0.001.

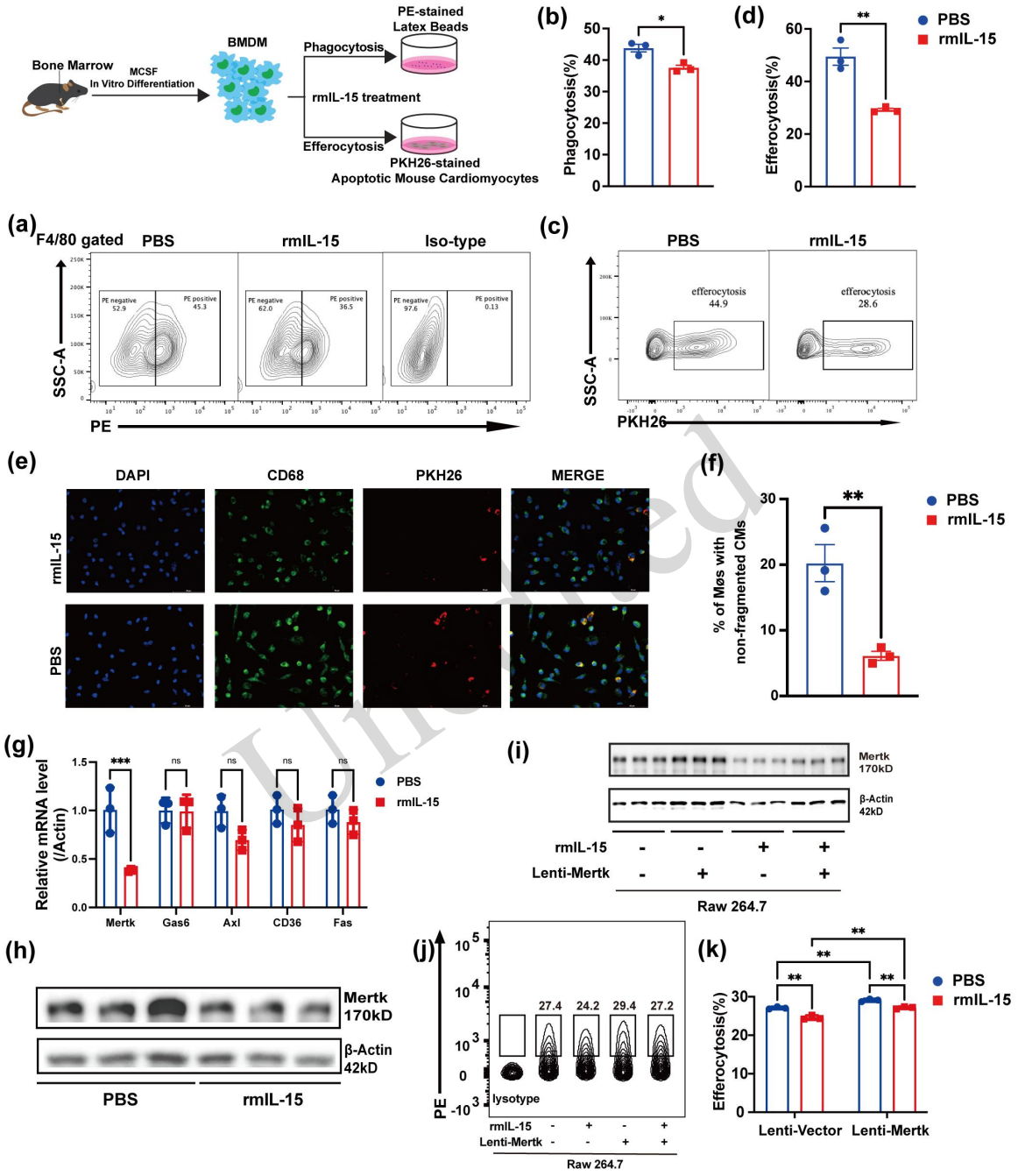

Fig. 4 IL-15 Impairs Macrophage Efferocytosis by Suppressing Mertk Expression

(a) Representative images of flow cytometry analysis of phagocytic activity in BMDMs treated with rmIL-15 or PBS. (b) Quantification of phagocytosis activity, expressed as the percentage of PE-positive macrophages. (c) Representative images of flow cytometry analysis of efferocytosis, measured as the uptake of PKH26-stained apoptotic cardiomyocytes by $CD68^{+}$ macrophages. (d) Quantitative analysis of efferocytosis activity. (e) Representative immunofluorescence imaging of BMDMs co-cultured with apoptotic cardiomyocytes. CD68 (green), PKH26 (red), and DAPI (blue). (f) Quantitative analysis of positive con-stained cells per slice. (g) mRNA levels of efferocytosis-related genes. (h) Western blot of MERTK protein expression. (i) Representative western blot analysis of MERTK protein expression in Lenti-Vector or Lenti-Mertk RAW 264.7 cells treated with PBS or rmIL-15. (j) Representative images of flow cytometry analysis of efferocytosis, measured as the uptake of PKH26-stained cardiomyocytes by Lenti-Vector or Lenti-Mertk RAW 264.7 cells treated with PBS or rmIL-15. (k) Quantitative analysis of efferocytosis activity. $^{**}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$.

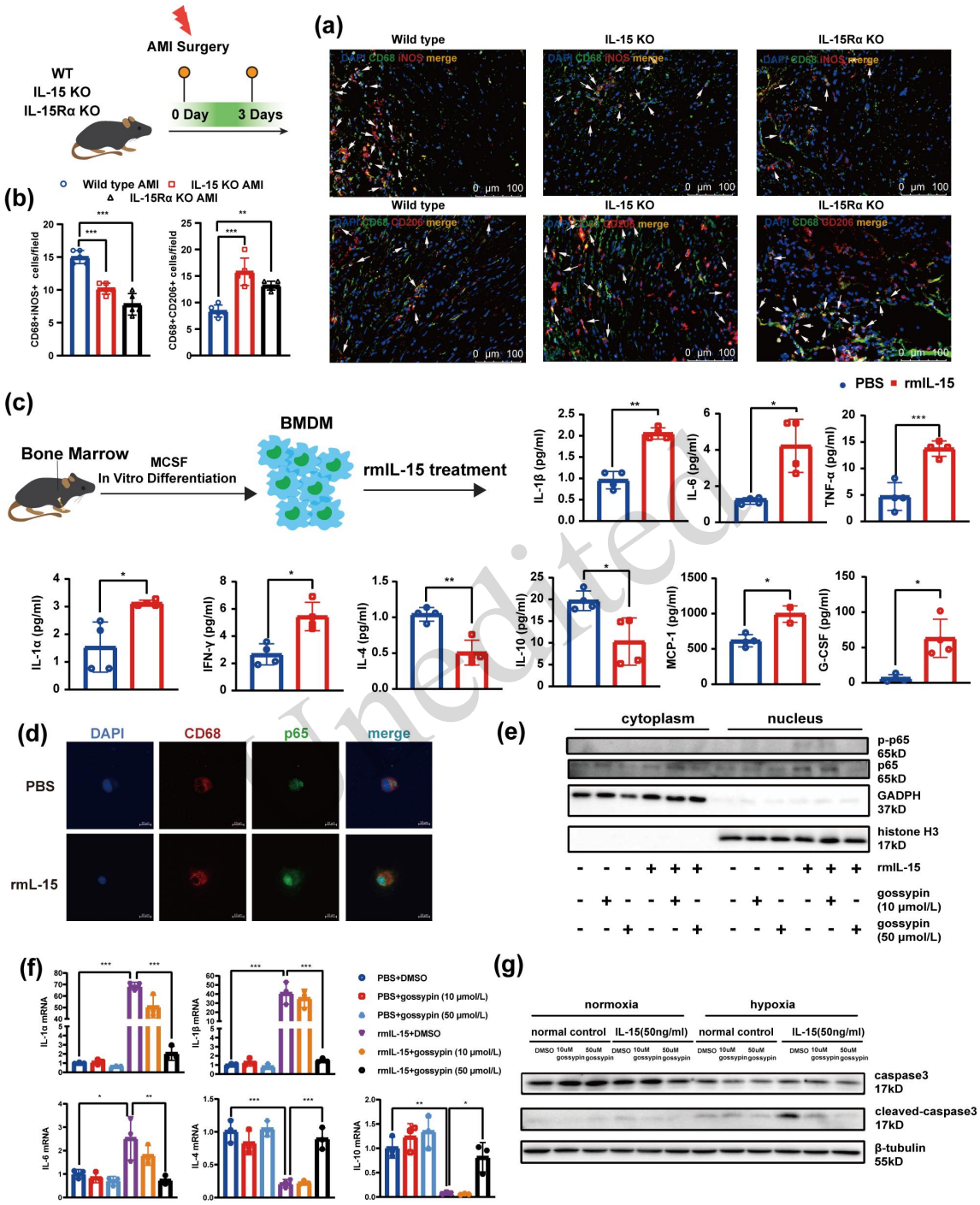

Fig. 5 IL-15 Drives Macrophage M1 Polarization and Activates NF-κB Signaling

(a) Representative immunofluorescence staining of CD68, iNOS, CD206, and DAPI in the heart section of mice at the third day after AMI. Positive stained cell is indicated by a white arrow. (n=5-10). (b) Quantitative analysis of positive con-stained cells per slice. (c) Levels of IL-1α, IL-1β, IL-6, TNF-α, IFN-γ, MCP-1, G-CSF, IL-4, and IL-10 in the supernatant of macrophages by protein microarray assays, incubated with either rmIL-15 (50 ng/mL) or PBS. (n=4 for each group). (d) Representative immunofluorescence staining of CD68, NF-κB p65, DAPI in cultured BMDM. (e) Expressions of NF-κB p65, phosphorylated-p65, GAPDH and histone-H3 by western blot in BMDM incubated with rmIL-15 (0, 50 ng/mL) or/and gossypin (10, 50 μmol/L). (f) Expressions of *Il-1α*, *Il-1β*, *Il-4*, *Il-6*, and *Il-10* mRNA by qPCR in cultured BMDMs, incubated with rmIL-15 (50 ng/mL) and/or gossypin (10, 50 μmol/L). n=3 for each group. (g) Expression of cleaved-caspase3, caspase3 and β-tubulin in primary cardiomyocytes after treatment of supernatant of macrophages exposed to different concentrations of rmIL-15 (0 ng/mL, 50 ng/mL) and gossypin (10 umol/L, 50 umol/L). $^{*}P$<0.05, $^{**}P$<0.01, $^{***}P$<0.001.

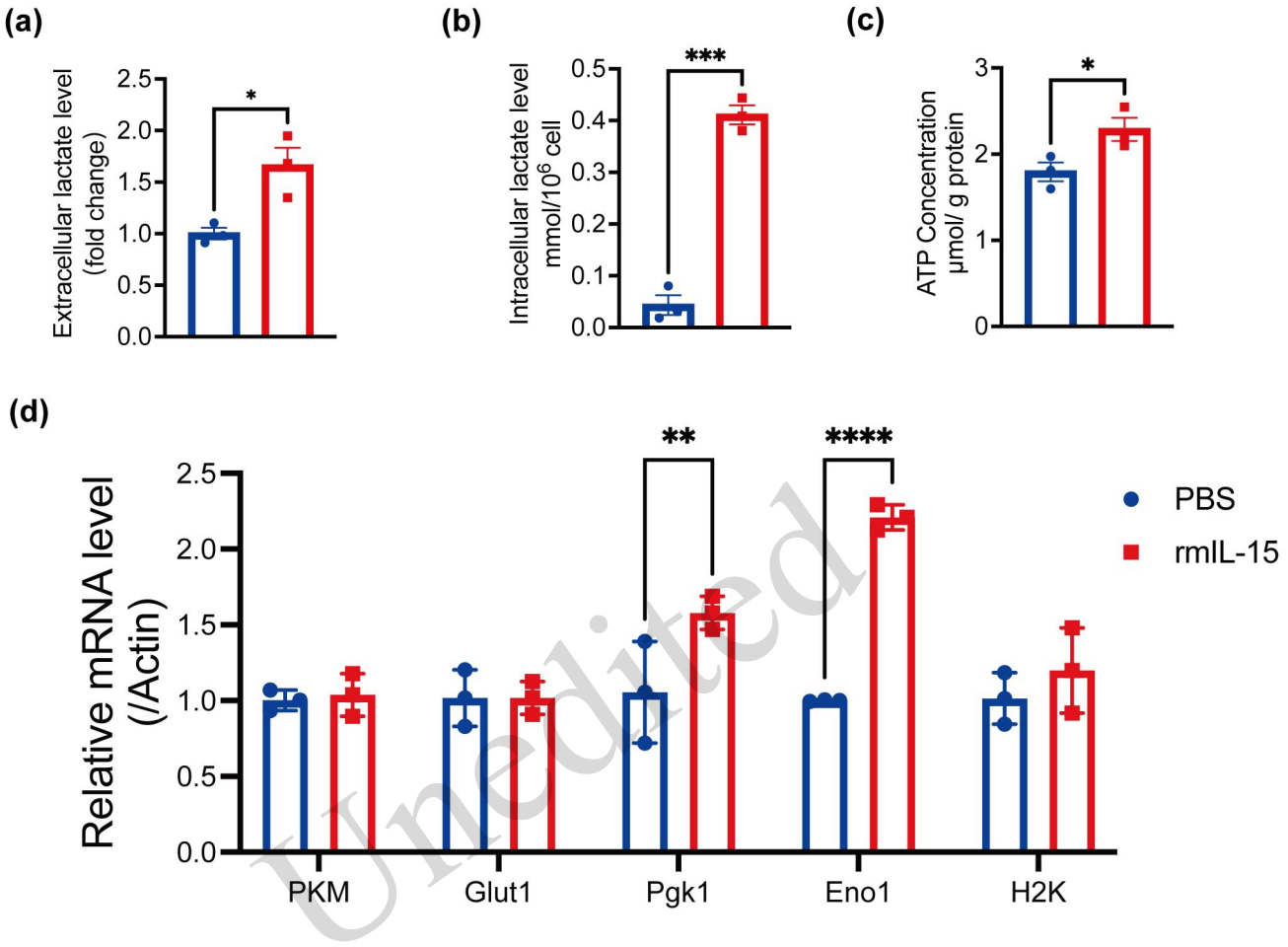

Fig. 6 IL-15 enhances glycolytic activity and metabolic activation in macrophages

(a, b) Levels of extracellular and intracellular lactate in macrophages by ELISA kit, incubated with either rmIL-15 (50 ng/mL) or PBS. (c) Levels of cellular ATP in macrophages by ELISA kit (d) mRNA levels of glycolysis-related genes. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$, $^{****}P<0.0001$.

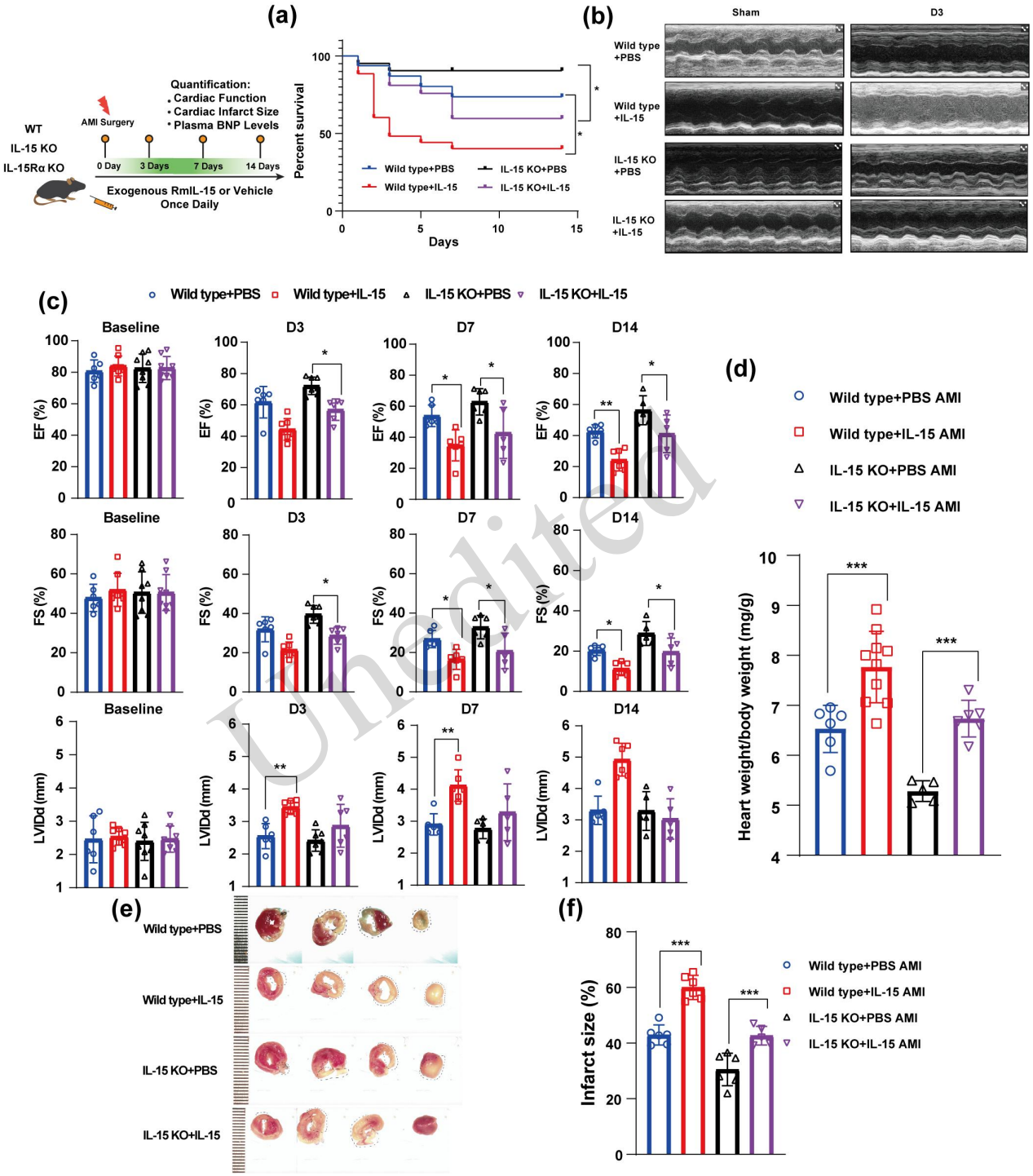

Fig. 7 Restoring IL-15 aggravates cardiac ischemia injury after AMI

(a) Kaplan-Meier survival analysis after AMI of IL-15 KO mice administrated with IL-15 (50 ug/kg, n=21) or vehicle (PBS, n=21), wild-type littermates administrated with IL-15 (50 ug/kg, n=26) or vehicle (PBS, n=16). (b) Representative images of M-mode echocardiography in each group. (c) Quantitative echocardiographic analysis of EF, FS and LVIDd. (n=5-8). (d) Quantitative echocardiographic analysis of cardiac mass index (n=5-6). (e) Representative images of heart section (1 mm) by TTC staining at the third day after AMI. The infarct size was circled by the dotted line. (f) Quantitative analysis as the percentage of infarct size to the whole heart (n=6 for each group). $^{*}P$<0.05, $^{**}P$<0.01, $^{***}P$<0.001.

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Supplementary information

Materials and methods; Tables S1-S4; Figs. S1-S5