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ARTICLE

Interleukin-17 upregulation participates in the pathogenesis of heart failure in mice via NF-κB-dependent suppression of SERCA2a and Cav1.2 expression

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Interleukin-17 (IL-17), also called IL-17A, is an important regulator of cardiac diseases, but its role in calcium-related cardiac dysfunction remains to be explored. Thus, we investigated the influence of IL-17 on calcium handling process and its contribution to the development of heart failure. Mice were subjected to transaortic constriction (TAC) to induce heart failure. In these mice, the levels of IL-17 in the plasma and cardiac tissue were significantly increased compared with the sham group. In 77 heart failure patients, the plasma level of IL-17 was significantly higher than 49 non-failing subjects, and was negatively correlated with cardiac ejection fraction and fractional shortening. In IL-17 knockout mice, the shortening of isolated ventricular myocytes was increased compared with that in wild-type mice, which was accompanied by significantly increased amplitude of calcium transient and the upregulation of SERCA2a and Cav1.2. In cultured neonatal cardiac myocytes, treatment of with IL-17 (0.1, 1 ng/mL) concentration-dependently suppressed the amplitude of calcium transient and reduced the expression of SERCA2a and Cav1.2. Furthermore, IL-17 treatment increased the expression of the NF-κB subunits p50 and p65, whereas knockdown of p50 reversed the inhibitory effects of IL-17 on SERCA2a and Cav1.2 expression. In mice with TAC-induced mouse heart, IL-17 knockout restored the expression of SERCA2a and Cav1.2, increased the amplitude of calcium transient and cell shortening, and in turn improved cardiac function. In addition, IL-17 knockout attenuated cardiac hypertrophy with inhibition of calcium-related signaling pathway. In conclusion, upregulation of IL-17 impairs cardiac function through NF-κB-mediated disturbance of calcium handling and cardiac remodeling. Inhibition of IL-17 represents a potential therapeutic strategy for the treatment of heart failure.

Keywords: heart failure; interleukin-17; SERCA2a; L-type calcium channel; calcium transient; NF-κB; transaortic constriction; neonatal cardiac myocytes

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INTRODUCTION

Heart failure is the final stage of various cardiovascular diseases, such as hypertension, cardiac infarction and cardiomyopathy, and represents a major cause of morbidity and mortality worldwide. A host of proinflammatory cytokines, including tumor necrosis factor-α (TNF-α), interleukin (IL)-1, IL-6, IL-8 and IL-18, have been shown to be associated with the development of heart failure [1]. The levels of TNFα and IL-6 are correlated with the severity of heart failure [2]. Antagonization of cytokines was indicated to be an effective strategy for improving cardiac function in heart failure patients. Treatment with the recombinant IL-1 receptor antagonist anakinra alleviates left ventricular remodeling and reduces the left ventricular end-systolic volume index over a 10–14-week follow-up period [2]. Biweekly injections of etanercept, a TNF inhibitor, for 3 months dose-dependently improves left ventricular structure and function in patients with heart failure [3].

Cardiomyocyte contraction is accomplished by the proper handling of intracellular calcium under the coordinated action of calcium-handling proteins. The L-type calcium channel Cav1.2 is responsible for the initiation of Ca^{2+}-induced Ca^{2+} release (CICR), which leads to cardiomyocyte contraction, while relaxation occurs through the reuptake of cytosolic Ca^{2+} into the sarcoendoplasmic reticulum by the sarcoendoplasmic reticulum Ca^{2+}-ATPase SERCA2a [4]. Deregulation of SERCA2a and Cav1.2 during heart failure has been demonstrated to cause abnormal calcium handling and impair cardiomyocyte contraction [5, 6]. Cytokines have been shown to directly regulate cardiac contractile function by altering calcium handling. TNF-α-induced cardiac dysfunction is related to the suppression of SERCA2a gene expression [7]. Exposure of adult rat ventricular myocytes to IL-1 or IL-6 inhibits calcium influx via L-type calcium channels and reduces cell contractility [8]. These studies indicate that cytokines may participate in the pathogenesis of heart failure by interfering with calcium handling.

IL-17 is an interleukin that has been extensively studied and confirmed to play a critical role in various cardiac diseases. The level of IL-17 is increased in heart failure patients compared with

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controls [9]. IL-17 treatment leads to fibrosis, collagen production and apoptosis in ischemic heart failure, and these effects are abolished by an anti-IL-17 neutralizing antibody [9]. Zhou et al. showed that IL-17 induces cardiomyocyte apoptosis and promotes post infarct ventricular remodeling [10]. Our previous study reported that deletion of IL-17 inhibits interstitial fibrosis and improves cardiac function in diabetic mice [11]. However, the contribution of IL-17 to calcium-related cardiac dysfunction remains unknown. Therefore, in this study, we explored the regulatory effect of IL-17 on calcium handling and the development of heart failure.

MATERIALS AND METHODS

Human plasma samples

The study was approved by the institutional review board of Harbin Medical University (IRB3008619) and conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from the participants. Human plasma samples were collected from the Department of Cardiology, the First Affiliated Hospital, Harbin Medical University (Harbin, China). Detailed patient information is shown in Table S1.

Animals

Male C57BL/6 wild-type (WT) mice were purchased from the Animal Center of the Second Affiliated Hospital of Harbin Medical University (Harbin, China). IL-17 Aknockout mice were graciously provided by Prof Zhi-nan Yin (Tianjin University, China) [12]. The mice were kept in an animal room under standard conditions (temperature, 21 ± 1 °C; humidity, 55%–60%) with food and water ad libitum. The use of animals was approved by the Ethics Committee of Harbin Medical University and conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85–23, revised 1996).

Echocardiography

Left ventricular (LV) function was assessed by M-mode recordings taken with an echocardiographic system with an ultrasound machine Vevo2100 (Visualsonics, Ontario, Canada) equipped with a 10 MHz phased-array transducer for as described previously [13]. The measured echocardiographic parameters included LV internal dimension at end-diastole (LVIDd), LV internal dimension at systole (LVIDs), ejection fraction (EF), and fractional shortening (FS). EF was determined automatically by the machine, and FS was calculated according to the following equation: ((LVIDd-LVIDs)/LVIDd) × 100.

Mouse model of heart failure (HF) induced by transaortic constriction (TAC)

The mice were randomly divided into the sham and TAC groups. The mice were anesthetized by injection of avertin (0.2 g· kg^{-1}, ip) for generation of the TAC model. Eachanimal was orally intubated with a 20-gauge tube and ventilated (mouse ventilator, UGO BASILE, Biological Research Apparatus, Italy) at a respiratory rate of 100 breaths/min with a tidal volume of 0.3 mL. The transverse aorta was constricted with a 7–0 silk suture ligature tied firmly against a 27-gauge needle between the carotid arteries. Then, the needle was promptly removed to achieve constriction of 0.4 mm in diameter. For the sham group mice, the animals underwent the same procedures without aortic constriction.

Isolation of adult mouse cardiomyocytes

Adult mouse cardiomyocytes were isolated as described previously [14]. The animals were anesthetized by injection of avertin (0.2 g· kg^{-1}, ip) and 0.1 mL heparin (50 mg· mL^{-1}, ip). The heart was rapidly excised, and the aorta was cannulated on a constant-flow Langendorff apparatus. The heart was digested by perfusion with Tyrode's solution containing 1 mg/mL Type II collagenase powder, protease (0.02 mg· mL^{-1}) and BSA (1 mg ·mL^{-1}). The Tyrode's solution contained (in mM): 123 NaCl, 5.4 KCl, 10 HEPES, 0.33 NaH_2PO_4, 1.0 $MgCl_2$, and 10 glucose (pH adjusted to 7.4 with NaOH). After the tissue became soft, the left ventricle was gently dissected into small chunks and agitated to isolate cardiomyocytes, which were then equilibrated in Tyrode's solution with 200 μM $CaCl_2$ and 1% bovine serum albumin. Single rod-shaped cells with clear cross-striations were used for electrophysiological recording. All solutions were gassed with 95% oxygen and 5% carbon dioxide and warmed to 37 ± 0.5 °C.

Isolation and primary culture of neonatal mouse cardiomyocytes

Neonatal cardiomyocytes were isolated from 3-day-old mice in accordance with the following procedures. Briefly, after dissection, the hearts were washed and minced in 0.25% trypsin. Pooled cell suspensions were centrifuged and resuspended in Dulbecco's modified Eagle's medium (DMEM HyClone, USA) supplemented with 10% fetal bovine serum, 100 U ·mL^{-1} penicillin and 100 μg· mL^{-1} streptomycin. The suspension was incubated in culture flasks for 90 min, which allowed the fibroblasts to preferentially adhere to the bottom of the culture flasks. The neonatal cardiomyocytes were removed from the culture flasks, and the medium was changed. The cultured cells were incubated for 48 h at 37 °C in a humidified atmosphere of 95% oxygen and 5% carbon dioxide before any experimentation.

siRNA cell transfection

siRNAs targeting NF-κB and IL-17 were transfected into cells at a concentration of 50 nM using Lipofectamine 2000 (Invitrogen, Grand Island, NY, USA) according to the manufacturer's instructions. The sequences of the siRNAs target NF-κB are shown in Table S2.

Whole-cell patch clamp recording

The L-type calcium current ($I_{Ca.L}$) in IL-17 knockout and WT cardiomyocytes were recorded using the whole-cell patch clamp technique [15]. The external solution for $I_{Ca.L}$ recording contained (in mM): 120 tetraethylammonium (TEA), 10 HEPES, 1.0 $MgCl_2$, 10 CsCl, 10 glucose, and 1.8 $CaCl_2$, and the pH was adjusted to 7.4 with CsOH. The pipette solution contained (in mM): 120 CsCl, 40 CsOH, 1 $MgCl_2$, 11 EGTA, 5 Mg-ATP, and 10 HEPES and the pH was adjusted to 7.3 with CsOH. Whole-cell currents were sampled at 10 kHz and filtered at 2 kHz. The current amplitude data for each cell were normalized to the cell capacitance (current density, pA/pF), and the current voltage relationship (*I-V* curve) was plotted. Voltage-dependent activation and steady-state inactivation profiles were fitted to the Boltzmann equation.

Measurements of cardiac contractility

Freshly isolated ventricular myocytes were placed in normal Tyrode's solution containing (in mM): 137 NaCl, 5.4 KCl, 0.16 NaH_2PO_4, 10 glucose, 1.8 $CaCl_2$, 0.5 $MgCl_2$, 5.0 HEPES, and 3.0 $NaHCO_3$ (pH adjusted to 7.4 with NaOH) and paced to steady state using a 1 Hz field stimulation. After 20 s of stimulation, the cells showed steady contractions, and video images were acquired using a Flash4.0 LT camera by line scanning (4 ms·$line^{-1}$, C11440–42U, Hamamatsu, Japan). ImageJ was used to measure the cell length under contraction (systolic length) and relaxation (diastolic length). Cardiomyocyte sarcomere shortening (SS), which reflects the contractility of cardiac muscles, was calculated as (diastolic length - systolic length)/diastolic length × 100%. Measurements were taken from more than 20 myocytes from three or more animals from each group.

Measurements of intracellular calcium transients

To measure cellular calcium transients, both freshly isolated cardiomyocytes and primarily cultured cardiomyocytes were incubated with 5 μM Fluo-3 (Invitrogen, Grand Island, NY, USA) and 0.01% Pluronic® F127 (BASF, Florham Park, NJ, USA) in Tyrode's solution for 35 min [8]. To measure intracellular Ca^{2+} transients,

cardiomyocytes were electrically paced at 1 Hz. Images were obtained with an Olympus camera. The amplitude of the intracellular Ca^{2+} transient was calculated as the difference between peak and diastolic calcium levels according to the equation $(F\text{-}F_0)/F_0$ after subtraction of background fluorescence [16]. The calcium transient decay phase time constant (τd) was determined using exponential curve fitting. The experiments were performed at room temperature. Measurements were taken from more than 20 myocytes from three or more animals from each group.

Western blot analysis

Total protein (80 μg) extracted from cardiac tissues was fractionated by SDS-PAGE (8% polyacrylamide gels) and transferred to a nitrocellulose membrane. The membrane was blocked with 5% non-fat milk for 1.5 h at room temperature. The membrane was then incubated with primary antibodies against Cav1.2 (1:500 dilution, Sigma, St. Louis, MO, USA), β-actin (1:20000 dilution, 66009–1-Ig, Proteintech, Chicago, IL, USA), SERCA2a (1:500 dilution, ab3625, Abcam, Cambridge Science Park, UK), calcineurin (1:500 dilution, 2614 S, CST, Boston, USA), p-CAMKII (1:500 dilution, SAB4504356, Sigma, St. Louis, MO, USA), and CAMKII (1:500 dilution, 4436 S, CST, Boston, USA) on a shaker overnight at 4 °C. The membrane was washed with PBS-T 3 times and incubated with secondary antibodies for 1 h at room temperature. Finally, the membrane was rinsed with PBS-T before being scanned by an imaging system (LI-COR Biosciences, Lincoln, NE, USA).

Real-time PCR

Total RNA was extracted by using TRIzol reagent (Invitrogen, Grand Island, NY,USA) according to the manufacturer's protocol. Total RNA (0.5 μg) was reverse transcribed by using Transcript Reverse Transcriptase (GMO Technology, Beijing, China) to obtain cDNA. The RNA levels were determined using SYBR Green I on the ABI 7500 fast Real-Time PCR system (Applied Biosystems, USA), and the relative mRNA expression levels were calculated by the $2^{-\triangle\triangle CT}$ method. The primers are shown in Table S3.

AC16 cell culture

The AC16 human adult ventricular cardiomyocyte cell line was a kindly gift from Dr Dong-mei Zhang from Dalian Medical University. The cells were maintained in DMEM F-12 supplemented with 10% fetal bovine serum. siRNAs targeting NF-κB were transfected into cells at a concentration of 50 nM using Lipofectamine 2000 (Invitrogen, Grand Island, NY, USA) according to the manufacturer's instructions.

Histological staining

For Masson's trichrome staining and hematoxylin and eosin (HE) staining, the hearts were fixed in 4% paraformaldehyde for 24 h, embedded in paraffin and cut into 5 μm-thick slices. Then, the slices were subjected to Masson's trichrome (Solarbio, Beijing, China) or HE staining. The slices were visualized under a microscope (Zeiss).

Measurement of IL-17 levels by ELISA

IL-17 levels were measured by using a human IL-17 (Cat. No. E-EL-H0105c) or mouse IL-17 (Cat. No. E-EL-M0047c) kit from Elabscience (Wuhan, China) according to the manufacturer's instructions.

Statistical analysis

The data are expressed as the mean ± SEM. Comparisons between two groups were analyzed by unpaired Student's *t* test. Data from more than two groups were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons test.

RESULTS

Upregulation of IL-17 expression during heart failure and its association with cardiac function

The change in IL-17 expression was evaluated in a mouse model and heart failure patients. In the TAC-induced heart failure model, the levels of IL-17 in the plasma and cardiac tissue were both increased compared with those in WT mice (Fig. 1a, b). In heart failure patients, the level of plasma IL-17 was higher than that in patients without heart failure (Fig. 1c) and was negatively correlated with EF and FS (Fig. 1d, e). These data imply that IL-17 expression is increased during heart failure and that IL-17 may participate in the development of heart failure.

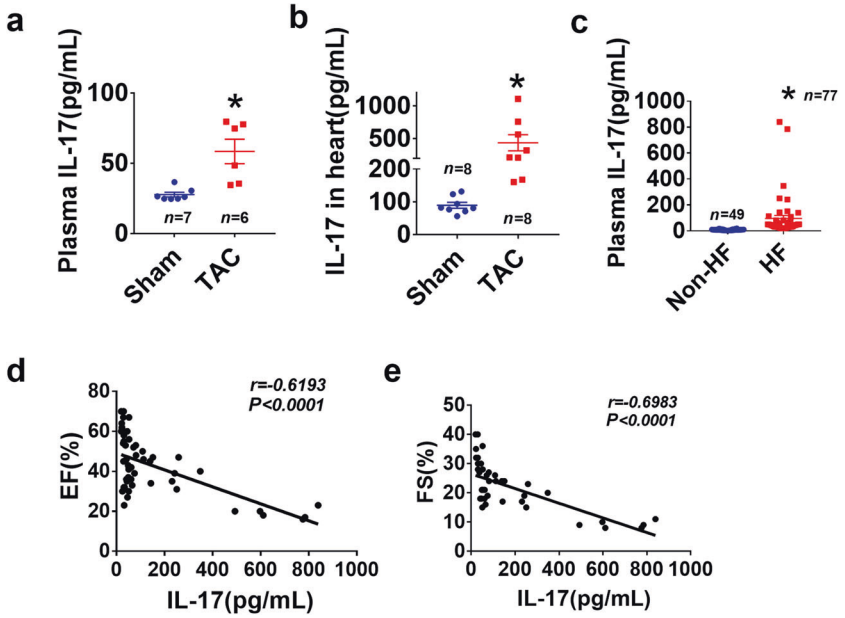

Fig. 1 Changes in the expression of IL-17 during heart failure. a, **b** Level of IL-17 in the plasma and cardiac tissues of mice subjected to TAC. **c** Level of IL-17 in the plasma of heart failure patients. **d**, **e** Correlation of plasma IL-17 levels with cardiac function in heart failure patients. *$P < 0.05$ vs. WT-TAC or non-HF. EF ejection fraction, FS fractional shortening.

Knockout of IL-17 improved the contractility of and calcium handling in isolated ventricular myocytes

To understand the potential role of IL-17 in heart failure, we first explored the contractility of cardiac myocytes isolated from IL-17 knockout mice. The length of ventricular myocytes was significantly increased in IL-17 knockout mice compared with WT controls, indicating mechanical improvement under IL-17 deficiency (Fig. 2a). We then examined intracellular Ca^{2+} handling in isolated cardiomyocytes. The amplitude of the peak systolic Ca^{2+} transient was larger in cardiomyocytes from IL-17 knockout mice than in those from WT controls, and the time course for the decay phase of the Ca^{2+} transient (τd) was shorter in IL-17 knockout

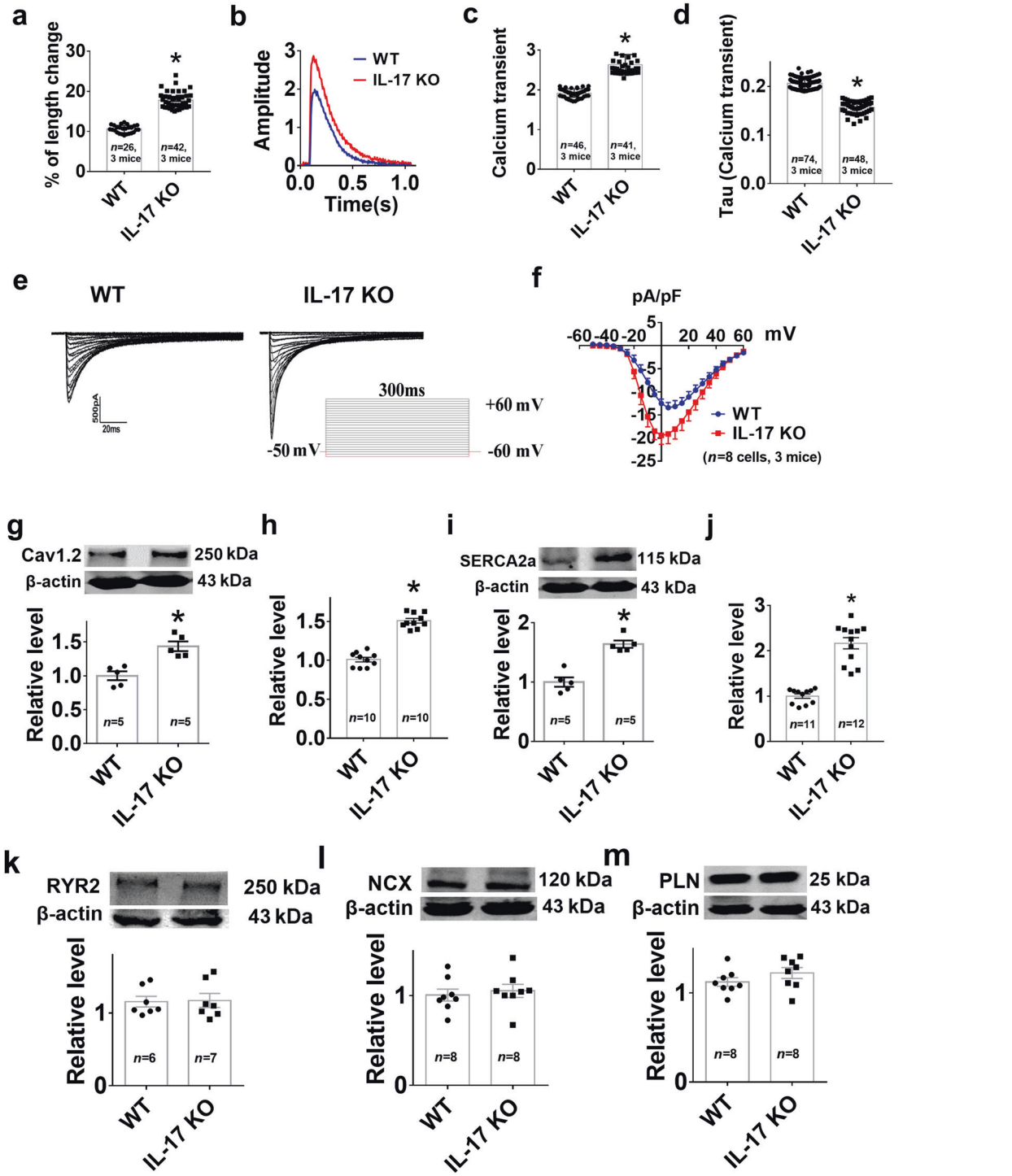

Fig. 2 IL-17 knockout enhanced the contractility of and calcium handling in cardiac ventricular myocytes. a Shortening of isolated single ventricular myocytes. **b** Representative traces of calcium transients in individual isolated ventricular myocytes. **c** Change in the peak calcium transient ($\Delta F/F_0$) in individual isolated ventricular myocytes. **d** Tau value of the recovery phase of the calcium transient. **e** Representative traces of the L-type calcium current (I_{CaL}) in isolated ventricular myocytes. **f** Statistical analysis of the *I-V* relationship of I_{CaL}. **g**, **h** Protein and mRNA levels of Cav1.2. **i**, **j** Protein and mRNA levels of SERCA2a. **k**–**m** Protein expression of RYR2, NCX1 and PLN. RYR2 ryanodine receptor 2, NCX1 sodium/calcium exchanger, PLN phospholamban. WT wild-type, IL-17 KO interleukin-17 knockout. *$P < 0.05$ vs. WT.

mice than in WT mice (Fig. 2b-d). The peak $I_{Ca,L}$ density was significantly increased in ventricular myocytes from IL-17 knockout mice compared with those from WT mice (Fig. 2e, f). Consistently, the protein and mRNA expression of Cav1.2 and SERCA2a were upregulated in the hearts of IL-17 knockout mice compared with those of WT controls (Fig. 2g-j). The protein expression of other calcium-handling proteins, including ryanodine receptor 2 (RYR2), sodium/calcium exchanger (NCX1) and phospholamban (PLN), was not changed (Fig. 2k-m). These data indicated that deletion of IL-17 improved calcium handling and increased cardiomyocyte contraction by upregulating the expression of Cav1.2 and SERCA2a.

IL-17 treatment impaired calcium handling in cultured mouse neonatal cardiomyocytes

The data from IL-17 knockout mice indicated that IL-17 may have a negative impact on cardiac calcium handling. We therefore evaluated the effects of IL-17 on Ca^{2+} transients in cultured cardiomyocytes. Primary cultured cardiomyocytes were treated with 0.1 or 1 ng/mL mouse recombinant IL-17 and then loaded with Fluo-3 and electrically stimulated at 1 Hz to initiate intracellular Ca^{2+} transients. The amplitude of the Ca^{2+} transients was decreased in IL-17 (0.1 or 1 ng/mL)-treated cardiomyocytes relative to control cardiomyocytes (Fig. 3a, b). The time course of the decay phase of the Ca^{2+} transient was prolonged, as reflected by an increase in the decay time constant (τd) (Fig. 3c). Furthermore, the expression of SERCA2a was suppressed at both the protein and mRNA levels by IL-17 (Fig. 3d, e). IL-17 (0.1 or 1 ng/mL) treatment also reduced the current density of $I_{Ca,L}$ (Fig. 3f) and the protein and mRNA expression of Cav1.2 (Fig. 3g, h). These data further confirmed that IL-17 had a regulatory effect on calcium handling through SERCA2a and Cav1.2.

NF-κB mediated the regulatory effect of IL-17 on SERCA2a and Cav1.2 expression

The transcription factor NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) has been reported to be the downstream mediator of the IL-17 signaling pathway [17], which can negatively regulate the transcription of SERCA2a in cardiomyocytes [7] and Cav1.2 in human colonic smooth muscle [18]. These findings drove us to speculate that IL-17 may inhibit the transcription of SERCA2a and Cav1.2 by upregulating NF-κB expression in ventricular myocytes. To test this hypothesis, we first examined the influence of IL-17 on the expression of p50 and p65, the two subunits of NF-κB. We found that the expression of both p50 and p65 was reduced in the hearts of IL-17 knockout mice compared with those of WT mice (Fig. 4a) but increased in cultured cardiomyocytes treated with IL-17 compared with untreated cardiomyocytes (Fig. 4b), indicating that IL-17 can activate NF-κB in cardiomyocytes. We then employed siRNA targeting p50 to knockdown NF-κB. As shown in Fig. 4c, p50 siRNA reduced p50 protein expression. Transfection of cultured cardiomyocytes with p50 siRNA increased the mRNA and protein expression of SERCA2a and Cav1.2 (Fig. 4d, e). Consistently, knockdown of p50 increased the calcium transient amplitude and reduced the decay constant of the calcium transient in cultured cardiomyocytes (Fig. 4f-h). These data indicated that IL-17 negatively regulated calcium handling via NF-κB-mediated suppression of SERCA2a and Cav1.2 transcription.

Regulatory effect of IL-17 on SERCA2a and Cav1.2 expression in cultured human AC16 cells

Next, we explored whether IL-17 regulate calcium handling in human AC16 cells, a cardiomyocyte cell line. Consistently, IL-17 treatment reduced the mRNA and protein expression of SERCA2a and Cav1.2 (Fig. 5a, b) while increasing the expression of p50 and

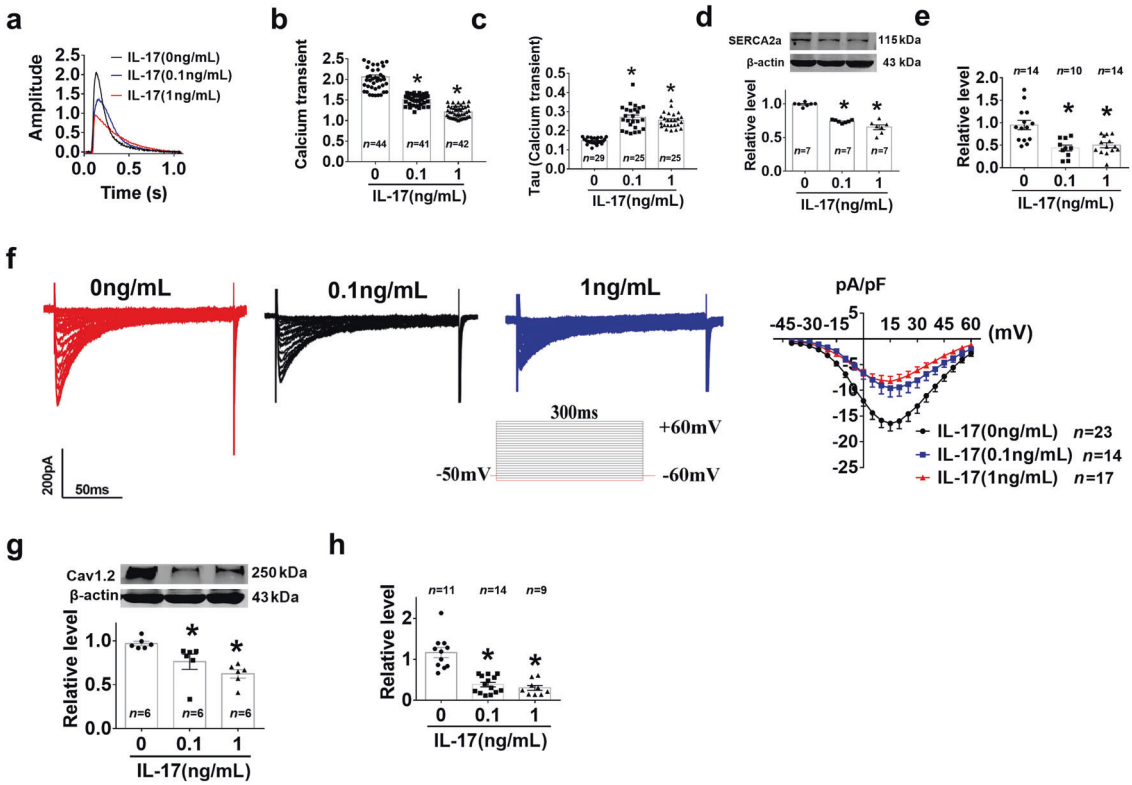

Fig. 3 Effects of IL-17 treatment on calcium handling in cultured neonatal mouse cardiomyocytes. a Representative traces of calcium transients. **b** Changes in the peak calcium transient. **c** Tau value of the recovery phase of calcium transients. **d**, **e** Protein and mRNA levels of SERCA2a. **f** Representative traces and the *I-V* relationship of the L-type calcium current (I_{CaL}). **g**, **h** Protein and mRNA levels of Cav1.2. *$P < 0.05$ vs. control (0 ng· mL^{-1}).

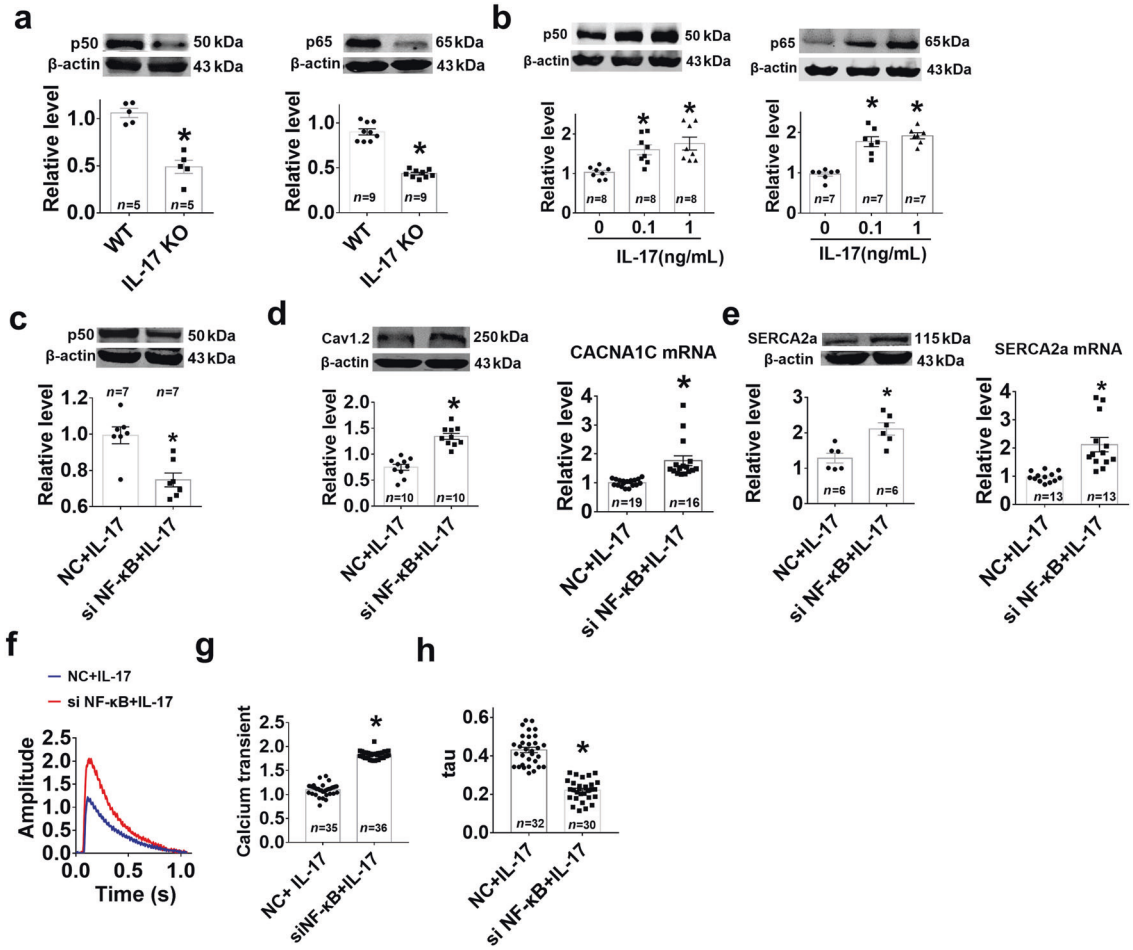

Fig. 4 NF-κB mediated the regulatory effect of IL-17 on Cav1.2 and SERCA2a. a Protein expression of p50 and p65 in the hearts of IL-17 knockout mice. **b** Protein expression of p50 and p65 in cultured neonatal cardiomyocytes treated with IL-17. **c** Knockdown of NF-κB by siRNA against p50. **d** Protein and mRNA expression of Cav1.2 after knockdown of NF-κB. **e** Protein and mRNA expression of SERCA2a after knockdown of NF-κB. **f–h** Representative traces, peak change ($\Delta F/F_0$), and Tau value of the recovery phase of calcium transients following p50 knockdown. $^*P < 0.05$ vs. WT, control (0 ng· mL^{-1}), or NC + IL-17.

p65 (Fig. 5c). We then employed siRNA targeting p50 to knockdown p50 expression (Fig. 5d). In agreement with the data obtained from mouse cardiomyocytes, knockdown of p50 in human AC16 cells led to the upregulation of SERCA2a and Cav1.2 mRNA and protein expression (Fig. 4e, f). These data implied that IL-17 could also regulate SERCA2a and Cav1.2 expression via NF-κB in human cardiomyocytes.

Effects of IL-17 knockout on cardiac function and calcium handling in mice with TAC-induced heart failure

Based on the finding that IL-17 impairs the contractility of cardiomyocytes, we asked whether deletion of IL-17 can increase cardiac contractility and improve the cardiac function of the failing heart. To answer this question, we established a pressure overload-induced heart failure model induced by TAC for 8 weeks in WT and IL-17 knockout mice. Both EF and FS were significantly decreased in WT TAC mice compared with WT controls 8 weeks after TAC, indicating the successful establishment of the heart failure model. Knockout of IL-17 alleviated the deterioration of cardiac function induced by TAC (Fig. 6a, b). The intracardiac hemodynamic data showed that the +d*p*/d*t* max and -d*p*/d*t* max were both reduced in WT TAC mice compared with controls and were partially restored by knockout of IL-17 (Fig. 6c). Moreover, the length of calcium transient amplitude in ventricular myocytes isolated from WT TAC mice were reduced compared with those of ventricular myocytes isolated from sham control and increased in myocytes isolated from IL-17 knockout mice (Fig. 6d, e). The increase in the tau value of calcium transients in ventricular myocytes isolated from WT TAC mice was prevented by knockout of IL-17 (Fig. 6e). Consistent with the alterations in calcium handling, knockout of IL-17 reversed the reduction in I_{caL} and the downregulation of SERCA2a and Cav1.2 expression (Fig. 6f-h). Interestingly, both p50 and p65 were increased in the hearts of WT TAC mice compared with those of sham controls, and this increase was inhibited by knockout of IL-17 (Fig. 6i). These data indicated that knockout of IL-17 prevented the increase in NF-κB expression in TAC-induced heart failure, which in turn led to the suppression of SERCA2a and Cav1.2 expression downregulation and improvements in calcium handling and cardiac function.

Effects of IL-17 knockout on TAC-induced cardiac remodeling in mice

We then explored the influence of IL-17 knockout on cardiac remodeling induced by TAC. Histological examination showed that knockout of IL-17 alleviated cardiac hypertrophy and the increase in the cross-sectional area of cardiac myocytes induced by TAC (Fig. 7a, b). Interstitial fibrosis accompanied by cardiac hypertrophy was also mitigated by knockout of IL-17 (Fig. 7c). The intracellular calcium-mediated signaling pathway has been shown to regulate cardiac hypertrophy. Knockout of IL-17 inhibited the increase in the resting intracellular calcium concentration in ventricular myocytes isolated from TAC mice (Fig. 7d). The

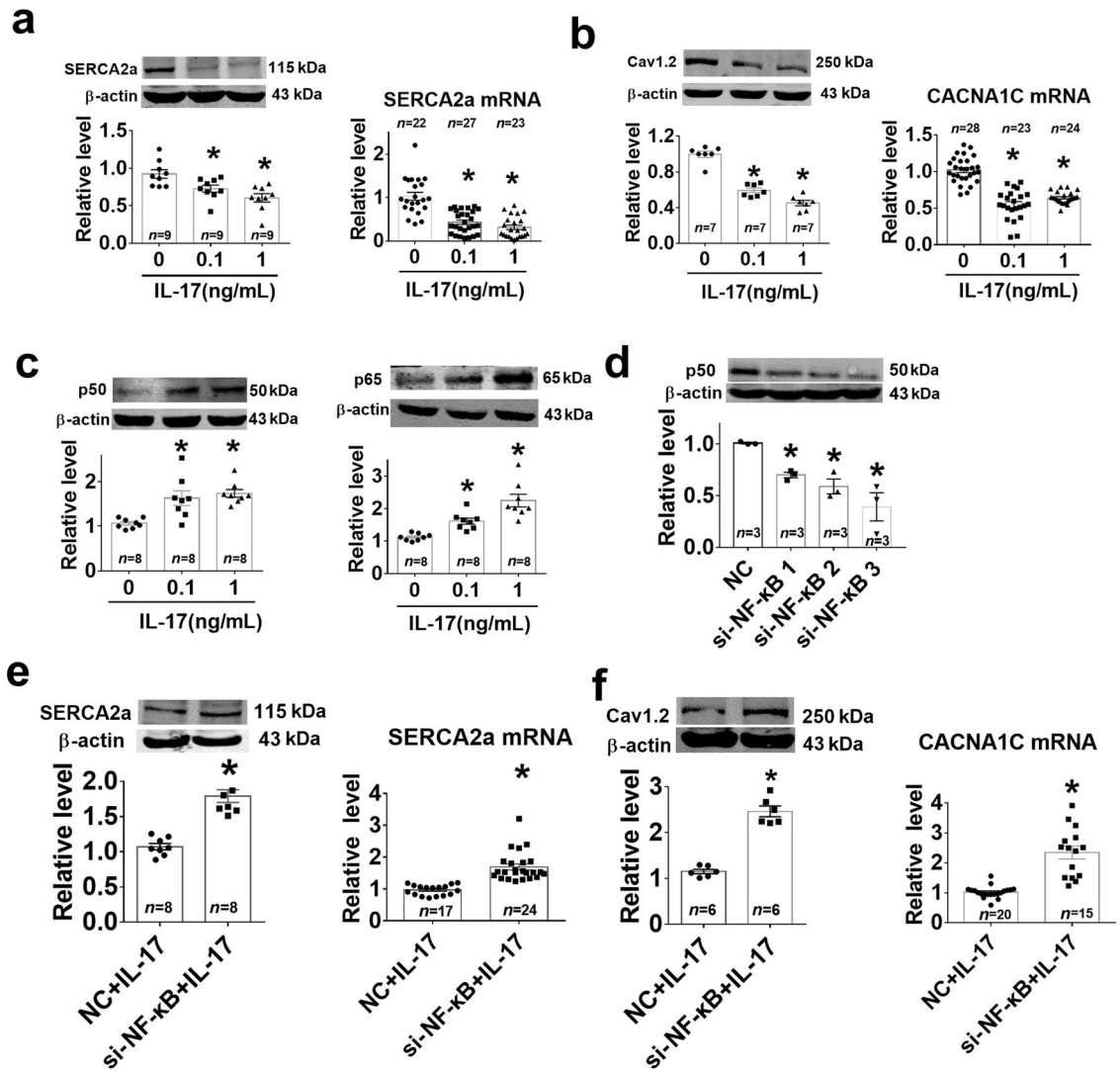

Fig. 5 Regulatory effect of IL-17 on SERCA2a and Cav1.2 expression in cultured human AC16 cells. a, **b** Protein and mRNA expression of SERCA2a in cultured AC16 cells treated with IL-17. **c** Protein expression of p50 and p65 in cultured AC16 cells treated with IL-17. **d** Protein level of p50 in cultured AC16 cells treated with siRNAs against p50. **e** Protein and mRNA expression of SERCA2a and Cav1.2 in cultured AC16 cells following knockdown NF-κB with siRNA targeting p50. $^{*}P < 0.05$ vs. control (0 ng·mL^{-1}), NC (negative control) or NC + IL-17.

increase in the expression of CAMKII, p-CAMKII and calcineurin in hypertrophic hearts was suppressed by the deletion of IL-17 (Fig. 7e-g). In addition, the survival rate of IL-17 knockout TAC mice was significantly increased compared with that of WT controls (Fig. 7h). These data indicated that deletion of IL-17 could not only increase the contractility of cardiomyocytes but also prevent hypertrophic remodeling, both of which contribute to improving the cardiac function of failing hearts.

DISCUSSION

In this study, we discovered that IL-17 participates in the pathogenesis of heart failure by altering calcium handling in cardiomyocytes. Specifically, deletion of IL-17 increased the contractility of cardiomyocytes by upregulating the expression of SERCA2a and Cav1.2. Treatment of cardiomyocytes with IL-17 inhibited calcium transients and downregulated the expression of SERCA2a and Cav1.2 by activating NF-κB. In addition, deletion of IL-17 prevented the development of heart failure induced by TAC, as manifested by increased calcium handling and suppressed activation of the calcium-related hypertrophic signaling pathway. These findings support the notion that IL-17 may be a potential target for the development of agents against heart failure.

Proinflammatory cytokines have been established as important regulators in the development of heart failure. In this study, we found that plasma IL-17 levels were upregulated in patients with heart failure compared with controls and were positively correlated with the severity of cardiac functional impairment, indicating that IL-17 may be a critical regulator of the development of heart failure. Cytokines affect the contractility of cardiomyocytes and cardiac function by altering calcium handling. Treatment with TNF-α and IL-1beta for 3 decreased the amplitude of the spatially averaged Ca^{2+} transients and the associated contraction of isolated ventricular myocytes [19]. Green smith DJ demonstrated that 50 ng/mL TNF-α reduces the amplitude of calcium transients by 31% and systolic shortening by 19% [20]. Cao et al. showed that IL-2 (200 U/mL) treatment reduces the amplitude of calcium transients elicited by electrical stimulation and caffeine [21]. Consistently, in this study, we found that IL-17 treatment suppressed the amplitude of calcium transients, while deletion of IL-17 increased calcium transients and the contraction of cardiomyocytes.

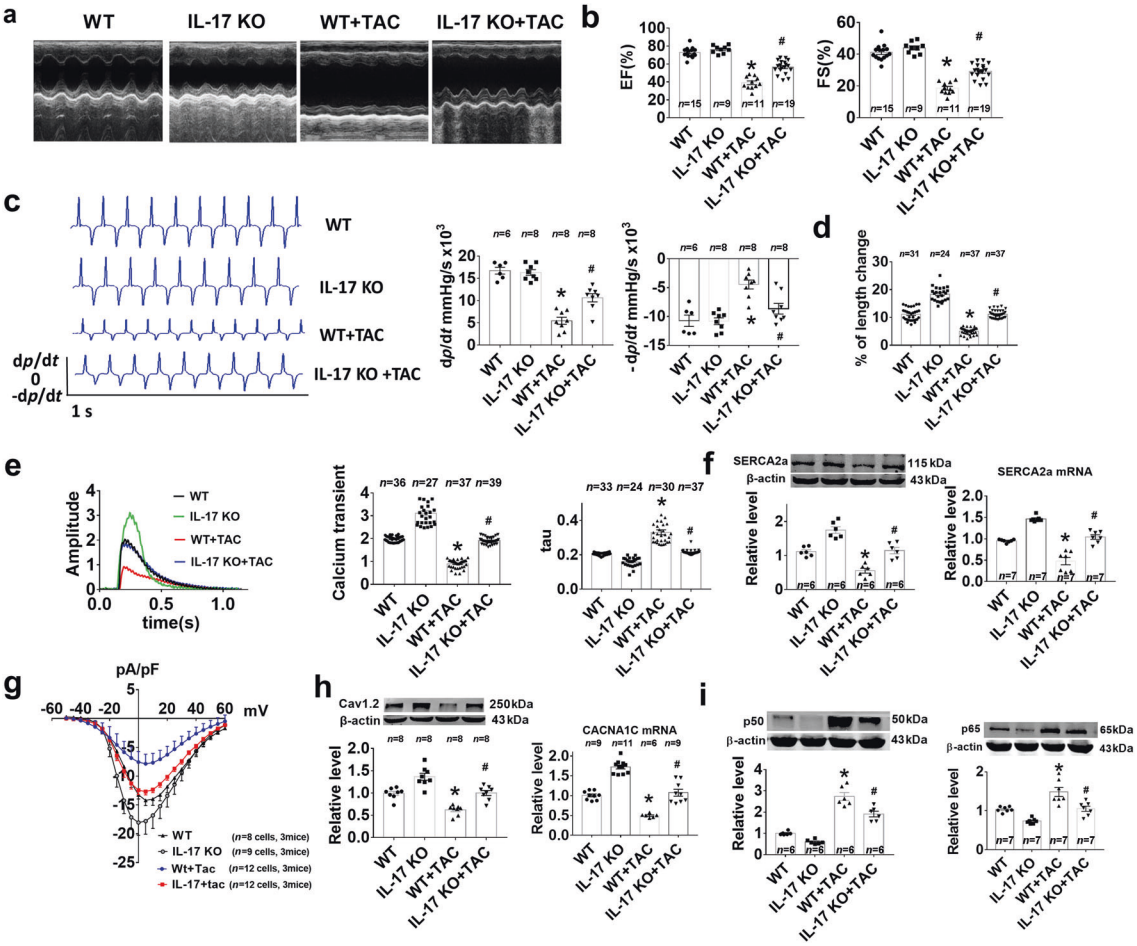

Fig. 6 Effects of IL-17 knockout on cardiac function and calcium handling in mice with TAC-induced heart failure. a Representative cardiac echocardiographic pictures of mice in each group. **b** EF and FS of the hearts determined by echocardiography. EF ejection fraction, FS fractional shortening. **c** Representative traces, +d*p*/d*t* max and -d*p*/d*t* max of intracardiac pressure. **d** Shortening of individual isolated ventricular myocytes. **e** Peak calcium transient and Tau value of the recovery phase of calcium transient in isolated cardiomyocytes. **f** Protein and mRNA expression of SERCA2a in the heart. **g** *I-V* relationship of I_{CaL}. **h** Protein and mRNA expression of Cav1.2 in the heart. **i** Protein expression of p50 and p65 in the heart. TAC transaortic constriction. $^{*}P < 0.05$ vs. WT, $^{\#}P < 0.05$ vs. WT + TAC.

Studies have shown that the regulatory effect of cytokines on calcium transients is mediated mainly by L-type calcium channels, which initiate calcium transients, and SERCA2a, the pump responsible for the reuptake of cytoplasmic calcium and relaxation. For example, the decrease in calcium transients induced by IL-2 results from reduced Ca^{2+} uptake by the SR [22]. The effects of TNF-α on calcium transients are associated with the inhibition of SERCA2a and a decrease in the L-type calcium current [7, 20, 23]. Chronic IL-1β treatment decreases the I_{CaL} density in cardiomyocytes [14]. In this study, we found that IL-17 inhibited the L-type calcium current by reducing the expression of Cav1.2, the pore forming subunit of the L-type calcium channel, and the expression of SERCA2a but had no effect on other calcium-handling proteins, including RYR2, NCX1 and PLN. These findings explain the effects of IL-17 on calcium transients.

Activation of the transcription factor NF-κB is a key event in the IL-17 signaling cascade [24], which has been shown to negatively regulate the transcription of SERCA2a and Cav1.2. Tsai et al. showed that TNF-α inhibits the transcription of SERCA2a by promoting the binding of NF-κB to the promoter region of SERCA2a in cardiomyocytes [7]. Shi et al. reported that activation of NF-κB by tumor necrosis factor alpha inhibits the expression of Cav1.2 in human colonic circular smooth muscle cells and their contractile response to acetylcholine [18]. These studies drove us to explore whether the inhibition of SERCA2a and Cav1.2 expression by IL-17 is mediated by the activation of NF-κB. The data showed that IL-17 treatment increased the expression of p50 and p65, the main subunits of NF-κB, and that knockdown of p50 abrogated the inhibitory effect of IL-17 on SERCA2a and Cav1.2, indicating that IL-17 can alter the transcription and expression of ion channels.

In addition to the functionally regulating cardiac contractility in cardiomyocytes, cytokines are also involved in abnormal structural alterations. IL-1β treatment leads to hypertrophic growth of neonatal rat ventricular myocytes and increased mRNA expression of atrial natriuretic factor and beta-myosin heavy chain (beta-MHC) [25]. Genetic deletion of IL-6 attenuates TAC-induced LV hypertrophy and dysfunction [26]. In addition, intracellular calcium-related signaling pathways have been shown to be critical in the development of cardiac hypertrophy. Calcium oscillations can activate the calcineurin/NFAT pathway and CAMKII pathway, which trigger the hypertrophic growth of cardiomyocytes [27, 28]. It is a reduction, but not the increase, in I_{CaL} that activates the hypertrophic signaling pathway and leads to cardiac hypertrophy [29, 30]. A reduction in I_{CaL} can increase the diastole calcium concentration, which mediates the activation of the hypertrophic signaling pathway [27]. This finding indicates that IL-17 may also influence cardiac hypertrophy due to its effects on calcium handling. As expected, we observed that knockout of IL-17 mitigated cardiac hypertrophy induced by TAC, and this effect was accompanied by a reduced resting

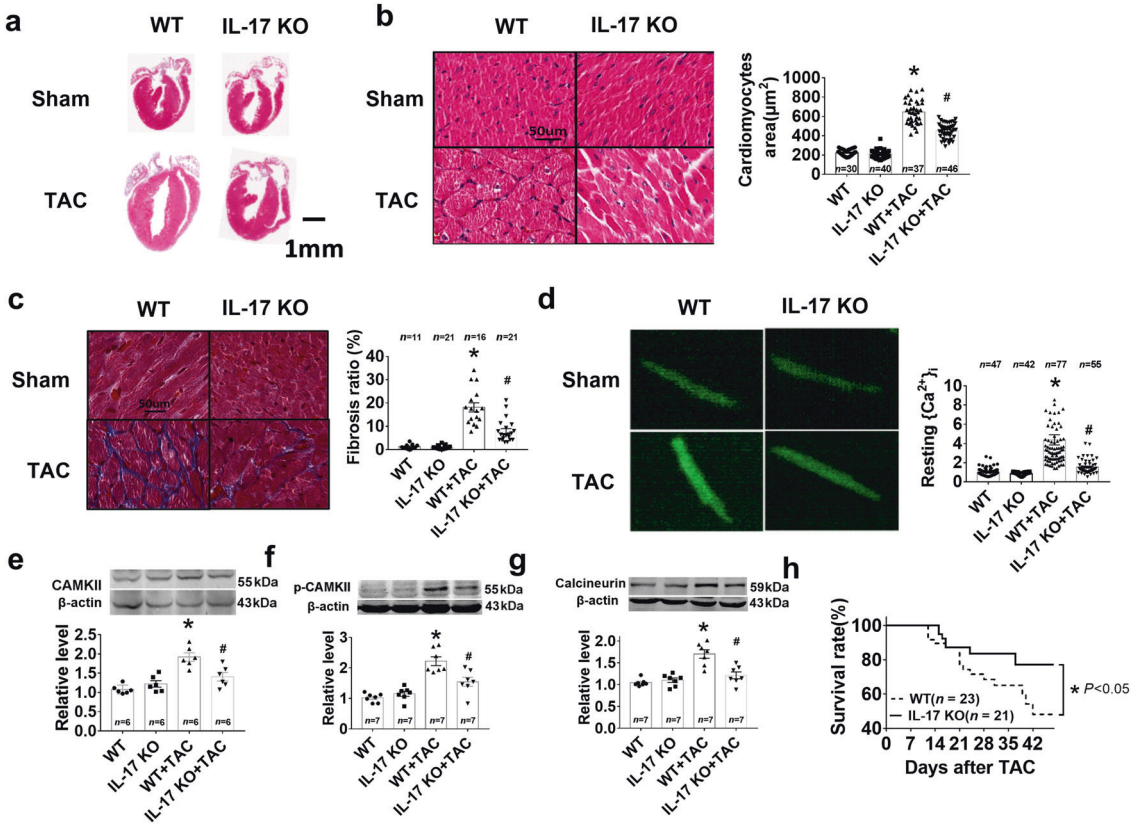

Fig. 7 Effects of IL-17 knockout on TAC-induced cardiac remodeling in mice. a Histological pictures of hearts from each group. **b** Representative H&E staining of ventricular sections and statistical analysis of the cross-sectional area of cardiomyocytes. **c** Interstitial fibrosis visualized by Mason's staining. **d** Resting intracellular calcium concentration. **e**–**g** Protein expression of CAMKII, p-CAMKII and calcineurin. **h** Survival rate of mice subjected to TAC. WT wild-type, IL-17 KO interleukin-17 knockout, WT + TAC, wild-type mice subjected to transaortic constriction; IL-17 KO + TAC, IL-17 knockout mice subjected to transaortic constriction. $^{*}P < 0.05$ vs. WT, $^{\#}P < 0.05$ vs. WT + TAC.

intracellular calcium concentration and suppression of CAMKII, p-CAMKII and calcineurin expression. Long-term upregulation of Cav1.2 in cardiac myocytes has been shown to cause cardiomyopathy [29]. In our study, although knockout of IL-17 increased I_{CaL}, the concomitant upregulation of SERCA2a offset the potential increase in the resting intracellular calcium concentration, which explains why IL-17 knockout alleviated cardiac hypertrophy even though it increased I_{CaL}.

A series of clinical trials have been carried out to evaluate the clinical effects of cytokine-based therapy against cardiovascular disease [31]. Both negative and positive results have been obtained. For example, a clinical trial of infliximab and a TNF-α monoclonal antibody showed that TNF-α antagonism does not improve the clinical condition of patients with moderate-to-severe chronic heart failure [32]. A recent study showed that the IL-1β neutralizing antibody canakinumab reduced hospitalization for heart failure and the composite of hospitalization for heart failure and heart failure-related mortality in a population of patients with prior myocardial infarction and elevation of high-sensitivity C-reactive protein levels [33]. Considering that IL-17 deletion exerts both functional and structural benefits on the failing heart, it may be worthwhile to explore the therapeutic potential of IL-17 blockade in heart failure patients.

CONCLUSION

In conclusion, IL-17 participates in the development of heart failure by impairing the functional contraction and structural remodeling of cardiomyocytes by suppressing SERCA2a and Cav1.2 expression through NF-κB. Deletion of IL-17 improves cardiac function and alleviates the hypertrophic growth of failing hearts, implying that IL-17 represents a promising therapeutic target for the development of agents to treat heart failure.

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AUTHOR CONTRIBUTIONS

GLX and DSL performed experiments, analyzed data, and prepared the paper. ZYW, YL, JMY, CZL, XDL, JDM, MMZ, YJL and YL helped perform experiments and collect data. BFY and ZWP designed the project, oversaw the experiments and prepared the paper.

ADDITIONAL INFORMATION

The online version of this article (https://doi.org/10.1038/s41401-020-00580-6) contains supplementary material, which is available to authorized users.

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