


## ORIGINAL ARTICLE

# Cardioprotective cytokine interleukin-33 is up-regulated by statins in human cardiac tissue

Richard Pentz<sup>1</sup> | Christoph Kaun<sup>1</sup> | Barbara Thaler<sup>1</sup> | Stefan Stojkovic<sup>1</sup> | Max Lenz<sup>1</sup> |  
Konstantin A. Krychtiuk<sup>1</sup> | Andreas Zuckermann<sup>2</sup> | Kurt Huber<sup>3,4,5</sup> |  
Johann Wojta<sup>1,5,6</sup> | Philipp J. Hohensinner<sup>1</sup> | Svitlana Demyanets<sup>7</sup> 

<sup>1</sup>Department of Internal Medicine II, Division of Cardiology, Medical University of Vienna, Vienna, Austria

<sup>2</sup>Department of Surgery, Medical University of Vienna, Vienna, Austria

<sup>3</sup>3<sup>rd</sup> Medical Department, Cardiology and Intensive Care Medicine, Wilhelminen Hospital, Vienna, Austria

<sup>4</sup>Medical Faculty, Sigmund Freud Private University, Vienna, Austria

<sup>5</sup>Ludwig Boltzmann Cluster for Cardiovascular Research, Vienna, Austria

<sup>6</sup>Core Facilities, Medical University of Vienna, Vienna, Austria

<sup>7</sup>Department of Laboratory Medicine, Medical University of Vienna, Vienna, Austria

## Correspondence

Svitlana Demyanets, Department of Laboratory Medicine, Medical University of Vienna, Waehringer-Guertel 18-20, A-1090 Vienna, Austria.  
Email: svitlana.demyanets@meduniwien.ac.at

## Funding information

Austrian Science Fund, Grant/Award Number: T445-B11; Association for the Promotion of Research in Arteriosclerosis, Thrombosis and Vascular Biology

## Abstract

Interleukin (IL)-33 is a member of the IL-1 family and is able to act cardioprotective. The aim of this study was to investigate the regulation of IL-33 by 3-hydroxy-3-methylglutaryl-coenzyme-A (HMG-CoA) reductase inhibitors (statins) and bisphosphonates (BPs) in human cardiac tissue. The lipophilic fluvastatin, simvastatin, atorvastatin, and lovastatin as well as the nitrogenous BPs alendronate and ibandronate, but not hydrophilic pravastatin increased IL-33 mRNA and intracellular IL-33 protein levels in both human adult cardiac myocytes (HACM) and fibroblasts (HACF). Additionally, fluvastatin reduced soluble ST2 secretion from HACM. IL-33 was also up-regulated by the general inhibitor of prenylation perillc acid, a RhoA kinase inhibitor Y-27632, and by latrunculin B, but statin-induced IL-33 expression was inhibited by mevalonate, geranylgeranyl pyrophosphate (GGPP) and RhoA activator U-46619. The IL-33 promoter was 2.3-fold more accessible in statin-treated HACM compared to untreated cells ( $P = 0.037$ ). In explanted hearts of statin-treated patients IL-33 protein was up-regulated as compared with the hearts of non-statin-treated patients ( $P = 0.048$ ). As IL-33 was previously shown to exert cardioprotective effects, one could speculate that such up-regulation of IL-33 expression in human cardiac cells, which might happen mainly through protein geranylgeranylation, could be a novel mechanism contributing to known cardioprotective effects of statins and BPs.

## KEYWORDS

bisphosphonates, cardiac cells, interleukin-33, pleiotropic, statins

## 1 | INTRODUCTION

HMG-CoA reductase inhibitors or statins are used for primary and secondary prevention of atherosclerosis-associated cardiovascular events.<sup>1</sup> Besides their lipid-modifying properties, a variety of pleiotropic effects of statins has been discovered: statins function as anti-inflammatory, anti-thrombotic and anti-oxidant agents.<sup>2-5</sup>

Cardiac tissue remodelling is an important factor in the progression of cardiovascular diseases. Statins are considered to positively affect cardiac remodelling by attenuation of myocardial hypertrophy.<sup>2,6</sup> Previous investigations from our group revealed modulating effects of statins on the plasminogen activator/plasmin system,<sup>7</sup> apoptosis,<sup>8</sup> and inflammation-triggered B-type natriuretic peptide<sup>9</sup> in human cardiac cells. These mechanisms are implicated in the

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2018 The Authors. Journal of Cellular and Molecular Medicine published by John Wiley & Sons Ltd and Foundation for Cellular and Molecular Medicine.

organisation of cardiac tissue and, as a consequence, in myocardial hypertrophy.

BPs represent another group of drugs targeting the mevalonate (MVA) pathway and are used for the treatment of osteoporosis and other diseases involving bone resorption.<sup>10</sup> BPs also exert beneficial functions in the cardiovascular system: they attenuate cardiac hypertrophy as well as fibrosis and improve endothelial function in hypertensive rats and mice, and cause regression of atherosclerosis.<sup>11–14</sup> However, the mechanisms underlying the pleiotropic effects of statins and BPs in myocardial tissue remodelling are not yet fully clarified.

IL-33 belongs to the IL-1 family of cytokines.<sup>15</sup> Depending on the experimental model and the pathophysiological context IL-33 can act either pro- or anti-inflammatory.<sup>3,16–20</sup> In the heart, IL-33 is believed to be cardioprotective as it was shown to possess anti-hypertrophic and anti-apoptotic properties in animal models of pressure overload or myocardial infarction, respectively.<sup>21–24</sup>

In this study, we aimed to explore if statins and BPs can influence the expression of IL-33 in human adult cardiac myocytes and fibroblasts, and to reveal the responsible mechanisms. Additionally, we compared IL-33 expression in explanted hearts from statin-treated versus non-statin-treated patients with end-stage HF undergoing heart transplantation.

## 2 | MATERIALS AND METHODS

### 2.1 | Reagents

Stock solutions of atorvastatin (kindly provided by Pfizer, Sandwich, UK), fluvastatin (kindly provided by Novartis, Basel, Switzerland), pravastatin (kindly provided by Bristol-Myers-Squibb, Paris, France), lovastatin and simvastatin (kindly provided by Merck Sharpe and Dome, Ballydine, Ireland) were prepared as described.<sup>25</sup> Such stocks were aliquoted and stored at  $-80^{\circ}\text{C}$ . Alendronate, ibandronate, MVA, GGPP, FPP, squalene, coenzyme Q10, latrunculin B, and perilic acid were purchased from Sigma (St. Louis, MO, USA). Y-27632 and U-46619 were purchased from Cayman Chemical Company (Ann Arbor, MI, USA).

### 2.2 | Cell culture

Primary HACM and HACF were isolated from myocardial tissue obtained from explanted recipients' hearts after heart transplantation and cultivated as described by our group.<sup>8,26,27</sup> For this study, cells were isolated from the hearts of 10 different donors (eight persons >18 years old and two children, mean age  $40.5 \pm 20.8$ ; six males and four females) suffering from different pathologies (three patients with ischaemic cardiomyopathy, five patients with dilated cardiomyopathy (in one case perinatal), one patient with constrictive pericarditis, and one child with an atrioventricular septal defect).

HACM were isolated from ventricular tissue and HACF from the papillary muscle. For HACM isolation, ventricular tissue was

grinded into very small fragments in PBS (pH 7.4) without enzymatic digestion. Red blood cells were removed by rinsing with PBS twice and debris was removed by passing through 40 mm cell strainer (Gibco-Life Technologies, Paisley, UK). The filtrated suspension was centrifuged at 335 g for 10 minutes. Next, the pellet was washed twice with PBS and resuspended in Dulbecco's Minimal Essential Medium (DMEM) supplemented with 10% fetal calf serum (FCS), 100 U/mL penicillin and 100 mg/mL streptomycin (all Cambrex, East Rutherford, NJ, USA). Cells were seeded into a Petri-dish and incubated for 60 minutes at  $37^{\circ}\text{C}$  in a humidified atmosphere of 5%  $\text{CO}_2$ :95% air in order to separate myocytes from fibroblasts by pre-plating. Afterwards the non-attached cells were removed, centrifuged, and washed twice with PBS. The cell pellet was resolved in DMEM supplemented with 10% FCS, 100 U/mL penicillin, 100 mg/mL streptomycin, 10 mg/mL transferrin (Sigma), and 10 mg/mL insulin (Sigma). HACM were seeded at the density of around  $1 \times 10^4$  cells/ $\text{cm}^2$  into fibronectin-coated (Roche, Basel, Switzerland) culture flasks. Furthermore, cells were cultured at  $37^{\circ}\text{C}$  in a humidified atmosphere of 5%  $\text{CO}_2$ :95% air. Only cultures in which >95% of the cells stained positive for cardiac myocyte markers (troponin I [Santa Cruz, Santa Cruz, CA, USA], tropomyosin (Sigma), cardiotin [Chemicon, Temcula, CA, USA] and myocardial muscle actin [Dako, Glostrup, Denmark]) were used in this study. In these cultures contamination with smooth muscle cells, endothelial cells, and fibroblasts as judged by staining for smooth muscle actin (Dako), vWF (Dako) and fibroblast specific antigens (antibody 1B10 and antibody 5B5, both from Dako) was <2%.

For HACF isolation, the explant technique was used. Pieces of myocardial tissue were covered with Medium199 (M199, Sigma) supplemented with 10% FCS, 100 U/mL penicillin and 100 mg/mL streptomycin in a Petri dish. After the explants became adherent, which took 3–5 days, the Petri dish was filled with fresh M199 supplemented with FBS and antibiotics. HACF growing out from the explants were maintained to confluence and cultured at  $37^{\circ}\text{C}$  in a humidified atmosphere of 5%  $\text{CO}_2$ :95% air. >95% of these cells stained positive for fibroblast specific antigen. HACF did neither stain for the cardiac myocyte markers troponin I, tropomyosin, cardiotin, and myocardial muscle actin, nor for the endothelial marker vWF nor for smooth muscle actin ruling out contamination with myocytes, smooth muscle cells, and endothelial cells, respectively. Cells were further cultured in minimum essential medium 199 (M199, Sigma) containing 20% fetal calf serum (FCS), 100 U/mL penicillin, 100 U/mL streptomycin, 0.25  $\mu\text{g}/\text{mL}$  fungizone, and 2 mmol  $\text{L}^{-1}$  L-glutamine (all Cambrex, East Rutherford, NJ, USA) at  $37^{\circ}\text{C}$  in a humidified atmosphere of 5%  $\text{CO}_2$ :95% air. Cells used in this study were between passage 2 and 5. The study has been reviewed and approved by the Ethic Committee of the Medical University of Vienna, Austria.

### 2.3 | Treatment of the cells

HACM and HACF were incubated in M199 containing 0.1% bovine serum albumin (BSA; Sigma) for 24 h prior to treatment with the

respective substance. Thereafter, the medium was replaced with fresh M199 containing 0.1% BSA, and the cells were treated with different statins (atorvastatin, fluvastatin, lovastatin, simvastatin, or pravastatin) at concentrations from 0.5 to 5  $\mu\text{mol L}^{-1}$  or BPs alendronate or ibandronate at concentrations from 1 nmol  $\text{L}^{-1}$  to 1  $\mu\text{mol L}^{-1}$  for time periods up to 72 h. In additional experiments HACM and HACF were treated with fluvastatin, ibandronate, or alendronate in the absence or presence of MVA (100  $\mu\text{mol L}^{-1}$ ), GGPP (10  $\mu\text{mol L}^{-1}$ ), FPP (10 nmol  $\mu\text{mol L}^{-1}$ ), squalene (10 nmol  $\mu\text{mol L}^{-1}$ ), coenzyme Q10 (10 nmol  $\mu\text{mol L}^{-1}$ ), or U-46619 (10 nmol  $\text{L}^{-1}$ ) for 48 h. Moreover, human cardiac cells were treated with Y-27632 at 5  $\mu\text{mol L}^{-1}$  or latrunculin B from 10 nmol  $\text{L}^{-1}$  to 1  $\mu\text{mol L}^{-1}$  for 48 h. The concentrations of the substances used in this study were in the same range as concentrations used in numerous other tissue culture studies, including ours.<sup>3,7-9,25,28,29</sup>

## 2.4 | Human tissue

Human heart tissue was obtained from the left ventricle of explanted hearts from 10 patients (mean age  $53.5 \pm 9.3$ ; nine males; five with statin treatment) undergoing heart transplantation for end-stage HF. Specimens were paraffin-embedded and cut into 5  $\mu\text{m}$  sections for further analysis. All human material was obtained and processed according to the recommendations of the hospital's Ethics Committee.

## 2.5 | Total RNA purification and cDNA preparation

Cells were treated as described, supernatants were removed and total cellular RNA was isolated using High Pure RNA Isolation Kit (Roche, Basel, Switzerland) according to the manufacturer's instructions. Reverse transcription was performed using Transcriptor First Strand cDNA Synthesis Kit (Roche).

## 2.6 | RealTime-PCR

RealTime-PCR was performed using LightCycler<sup>®</sup> TaqMan<sup>®</sup> Master (Roche) according to the manufacturer's instructions. Primers were designed using the Roche UniversalProbeLibrary Assay Design Centre (<http://www.universalprobelibrary.com/>): glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (forward primer: 5'-agccatcatcgcctcagacac-3', reverse primer: 5'-gcccaatcagcaaatcc-3', UPL probe #60; Amplicon Size [bp] 66) – IL-33 (forward primer: 5'-agcaagtggagaacacagc-3', reverse primer: 5'-cttcttggccttctgttg-3', UPL probe #33, Amplicon Size [bp] 74) – sST2 (forward primer: 5'-gggagagatgctactcggag-3', reverse primer: 5'-cgctgctcttctgtatgtt-3', UPL probe #86, Amplicon Size [bp] 68) – ST2L (forward primer: 5'-gaaatactgagactgggtgattat 3', reverse primer: 5'-gaagtgcctcttggctt-3', UPL probe #29, Amplicon Size [bp] 149). The amplification conditions consisted of an initial incubation at 95°C for 10 minutes, followed by 45 cycles of 95°C for 10 seconds, 63°C for 20 seconds and 72°C for 6 seconds and a final cooling to 4°C. Data were analyzed using LightCycler Software Version 3.5 (Roche).

## 2.7 | Promoter accessibility assay

Chromatin accessibility was analysed using a commercially available Chromatin Accessibility Assay Kit (Abcam, Cat.Nr. ab185901) as described.<sup>30</sup> In short, HACM were treated with fluvastatin at 5  $\mu\text{mol L}^{-1}$  or alendronate at 10 nmol  $\text{L}^{-1}$  for 24 h followed by cell lysis and chromatin extraction. Chromatin was thereafter digested using a nuclease mix. Following a DNA cleanup, samples were analysed by qPCR using two IL-33 promoter specific primer (forward primer 1: 5'-gcagtgcagtgggtgttta-3', reverse primer 1: 5'-agaacaattcattactctgcgtgtt-3', UPL probe #9; Amplicon Size [bp] 60; forward primer 2: 5'-cttcaggggcaataacatgc-3', reverse primer 2: 5'-cctcaggcagtctgtcaaa-3', UPL probe #6; Amplicon Size 86 [bp]). Results were calculated by fold enrichment using a ratio of amplification efficiency of nuclease-treated samples over that of untreated nuclease-free control samples as suggested in the protocol.

## 2.8 | Protein detection

Cells were lysed with PBS containing 0.1% Triton X-100 (Sigma). IL-33 protein in cell lysates and sST2 protein in cell culture supernatants were measured by a specific enzyme-linked immunosorbent assay (ELISA) (both R&D Systems, Minneapolis, MN, USA), as described by us previously.<sup>27</sup>

## 2.9 | Immunofluorescence analysis of IL-33 in explanted hearts

Paraffin-embedded sections were deparaffinised and then boiled in target retrieval solution (DAKO North America, Inc, CA) for 10 minutes. Staining with either mouse monoclonal anti-IL-33 (Alexis Biochemicals, Enzo Life Sciences AG, Lausen, Switzerland, dilution 1:200) or with rabbit polyclonal anti-Troponin I (abcam, Cambridge, UK, dilution 1:100) antibodies was performed as described.<sup>27</sup> The IL-33 fraction of the stained myocardial tissue was quantified using ImageJ software. Mean fluorescence intensity (MFI) was compared between patients with or without statin treatment in order to determine the myocardial IL-33 content.

## 2.10 | Statistics

Values are expressed as mean  $\pm$  SD. Data were compared by two-tailed student's *t* test or by ANOVA. Values of  $P \leq 0.05$  were considered significant. Each experiment was performed at least three times with cells obtained from different donors. Representative experiments are shown if not indicated otherwise.

## 3 | RESULTS

### 3.1 | Statins induce IL-33 and reduce sST2 in human cardiac cells

Treatment of HACM and HACF with the lipophilic fluvastatin, simvastatin, atorvastatin, and lovastatin at 5  $\mu\text{mol L}^{-1}$  for 48 h led to a significant increase in IL-33 expression on mRNA (Figure 1A) and

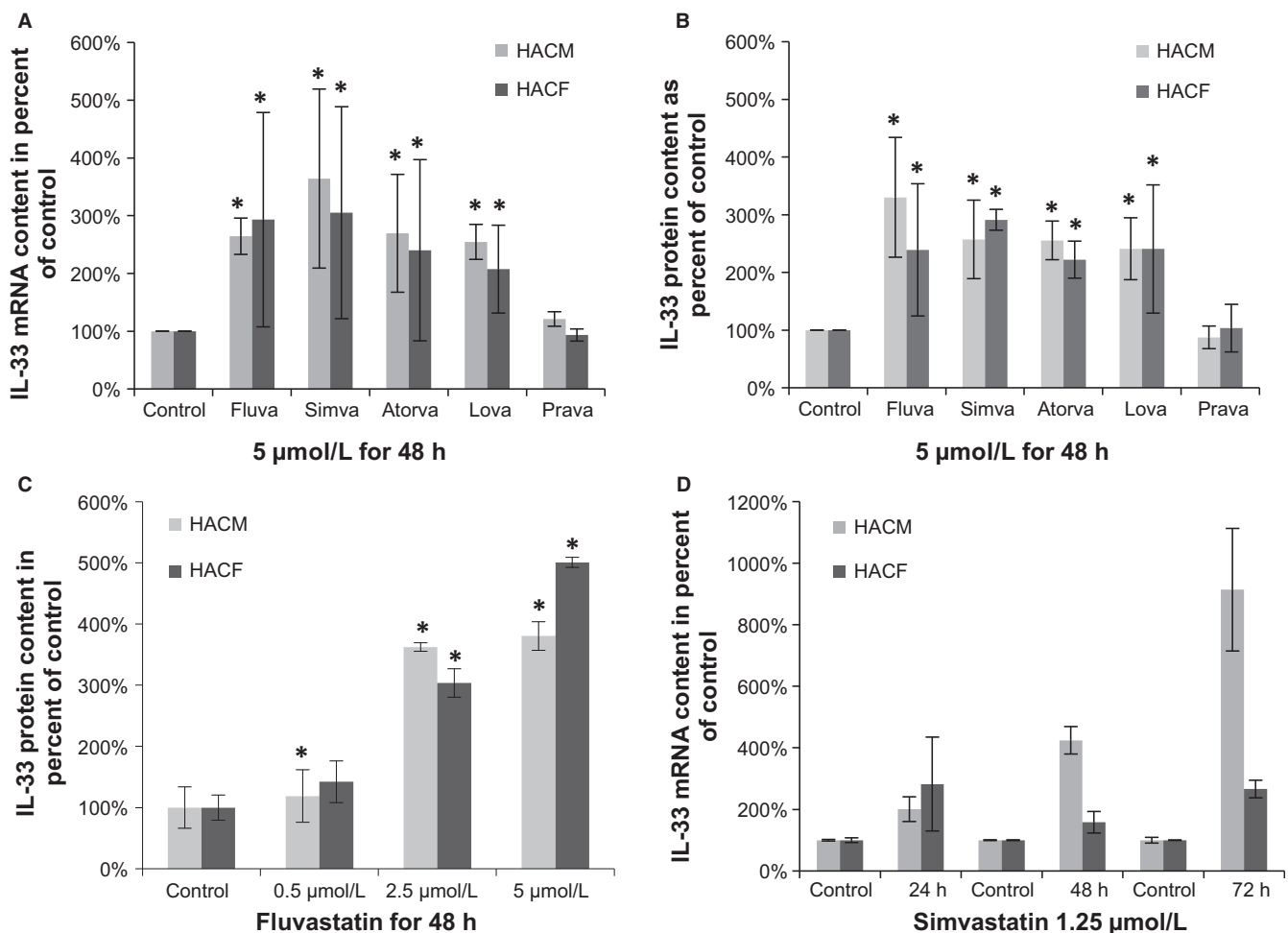
protein levels (Figure 1B). Hydrophilic pravastatin did not change IL-33 expression levels (Figure 1A,B).

This stimulatory effect on IL-33 expression was reproducible in cells derived from different donors' hearts when the cells were treated with fluvastatin at  $5 \mu\text{mol L}^{-1}$  for 48 h (Table 1). IL-33 expression was up-regulated from 1.7- to 3.8-fold in HACM ( $n = 6$  donors) and from 1.5- to 3.2-fold in HACF ( $n = 4$  donors). In a series of experiments where the cells were treated with different concentrations of statins for different time points, we observed that the induction of IL-33 by statins was concentration-dependent in both cell types (Figure 1C) and time-dependent in HACM (Figure 1D). Fluvastatin was effective at  $5 \mu\text{mol L}^{-1}$  and  $2.5 \mu\text{mol L}^{-1}$  in both HACM and HACF, and even at  $0.5 \mu\text{mol L}^{-1}$  in HACM (Figure 1C). Treatment with simvastatin at  $2.5 \mu\text{mol L}^{-1}$  for 24 h, 48 h, and 72 h induced a 3.2-, 8.2-, and 17.4-fold increase of IL-33 mRNA levels in HACM, and a 2.7-, 1.5-, and 2.8-fold increase of IL-33 mRNA levels in HACF, respectively (Figure 1D).

sST2 protein was detectable in cell culture supernatants of HACM in three from five tested donors and its secretion was reduced by fluvastatin at  $5 \mu\text{mol L}^{-1}$  to  $15\% \pm 2\%$  after 24 h and to  $29\% \pm 19\%$  after 48 h of treatment compared to the respective controls (both  $P < 0.05$ ). Fluvastatin also showed a trend towards down-regulation of mRNA specific for sST2 in HACM in five donors tested (to  $85\% \pm 24\%$  after 24 h and to  $62\% \pm 38\%$  after 48 h as compared to the respective controls), but had no influence on the levels of ST2L mRNA (data not shown).

### 3.2 | Statin-induced IL-33 expression is inhibited by the components of the MVA pathway but not by its non-prenyl end-products or FPP

To assess whether the observed effect of statins depends on their blockage of the MVA pathway and to further discriminate, which of the different branches of this pathway are important in this context,



**FIGURE 1** HMG-CoA reductase inhibitors up-regulate IL-33 in HACM and HACF dose- and time-dependently. A and B, HACM and HACF were incubated for 48 h in the absence (control) or presence of fluvastatin (Fluva), simvastatin (Simva), atorvastatin (Atorva), lovastatin (Lova), or pravastatin (Prava) at  $5 \mu\text{mol L}^{-1}$  each. C, HACM and HACF were incubated for 48 h in the absence (control) or presence of fluvastatin at 0.5, 2.5, and  $5 \mu\text{mol L}^{-1}$ . D, HACM and HACF were incubated for 24, 48, or 72 h in the absence (control) or presence of simvastatin at  $1.25 \mu\text{mol L}^{-1}$ . Values for IL-33/GAPDH mRNA (A, D) and IL-33 protein (B, C) are given as % of control, which was set as 100%. A and B show mean  $\pm$  SD from three different donors, each treated in triplicates (the internal replicates were not included in the analysis), \* $P \leq 0.05$  as compared to respective control

**TABLE 1** Effect of fluvastatin on intracellular IL-33 protein in HACM and HACF isolated from different donors

	HACM	HACF
Donor 1	3.3	
Donor 2	2.2	
Donor 3	1.7	
Donor 4	3.8	
Donor 5	3.2	
Donor 6	2.5	
Donor 7		1.5
Donor 8		1.8
Donor 9		3.2
Donor 10		2.9

HACM and HACF isolated from different donors were incubated for 48 h in the absence or presence of fluvastatin at a concentration of  $5 \mu\text{mol L}^{-1}$ . Cells were permeabilised with PBS containing 0.1% Triton X-100, and IL-33 protein in cell lysates was determined as described in "Materials and methods". Values are given in x-fold of control, which was set as 1.0.

we co-incubated human cardiac cells with fluvastatin and different components or end-products of the MVA pathway. Fluvastatin-induced IL-33 expression in HACM and HACF was inhibited by addition of MVA at  $100 \mu\text{mol L}^{-1}$  (Figure 2A,B) or GGPP at  $10 \mu\text{mol L}^{-1}$  (Figure 2C,D), both of which are metabolites downstream of HMG-CoA reductase. However, addition of squalene (Figure 2E) or coenzyme Q10 (Figure 2F), two end-products of the MVA pathway, did not reverse the effect of fluvastatin in human cardiac cells at any concentration used ( $10 \text{ nmol L}^{-1}$ ,  $100 \text{ nmol L}^{-1}$ , or  $1 \mu\text{mol L}^{-1}$  each). Also addition of FPP, an isoprenoid intermediate of the cholesterol pathway, at different concentrations between  $10 \text{ nmol L}^{-1}$  and  $1 \mu\text{mol L}^{-1}$  did not abolish fluvastatin-induced IL-33 protein increase in HACM (Figure 2G).

To confirm that statin-induced IL-33 expression is driven by prevention of protein prenylation, we incubated HACM with perillidic acid, which is a general inhibitor of prenylation.<sup>28</sup> Treatment with perillidic acid at  $100 \mu\text{mol L}^{-1}$  for 48 h induced IL-33 protein 13-fold in HACM (data not shown).

### 3.3 | Bisphosphonates-induced expression of IL-33 is reversed by GGPP but not by mevalonate

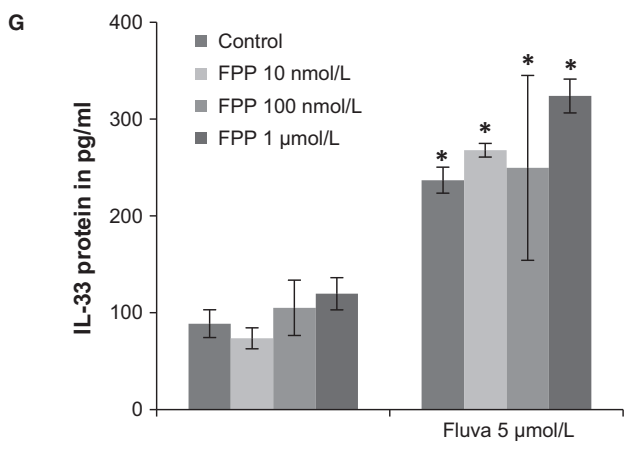
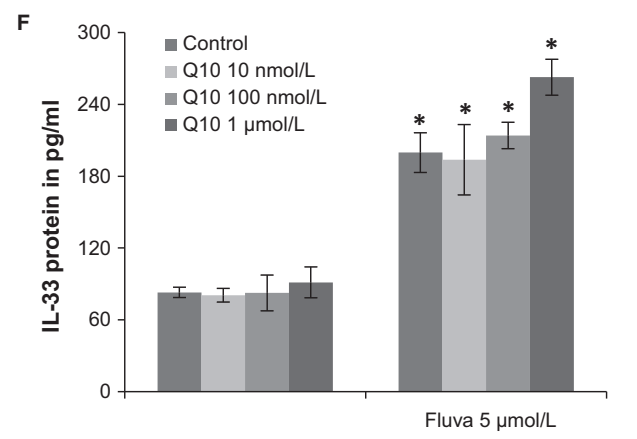
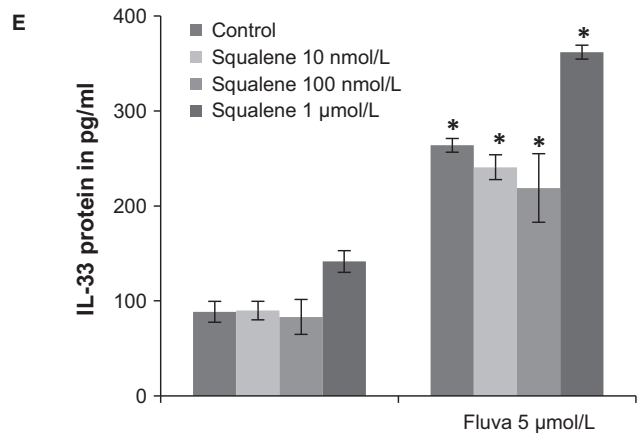
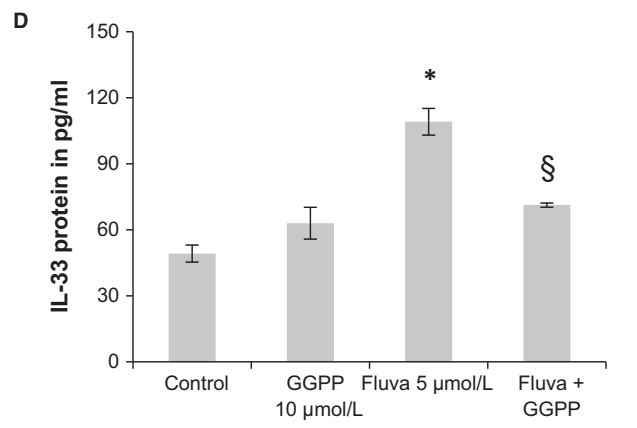
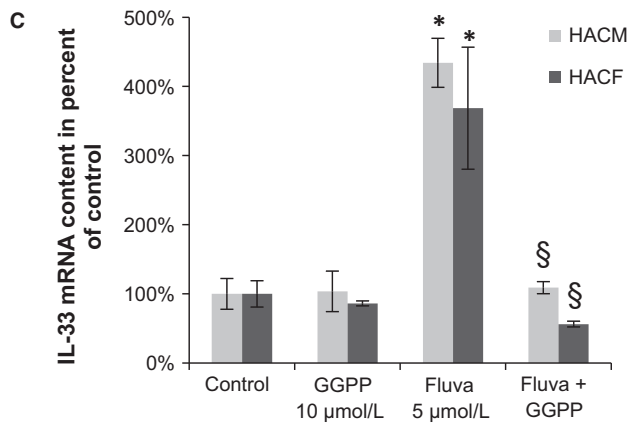
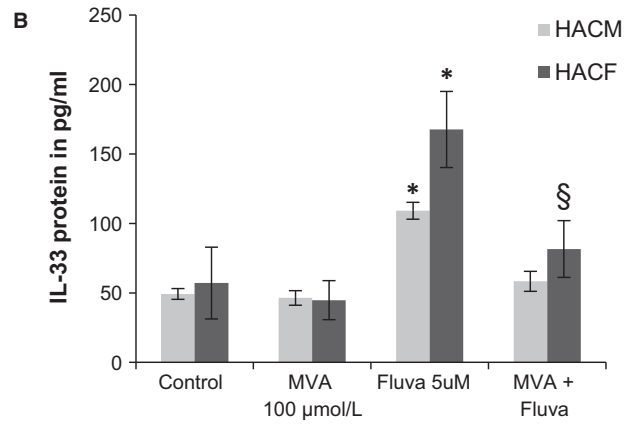
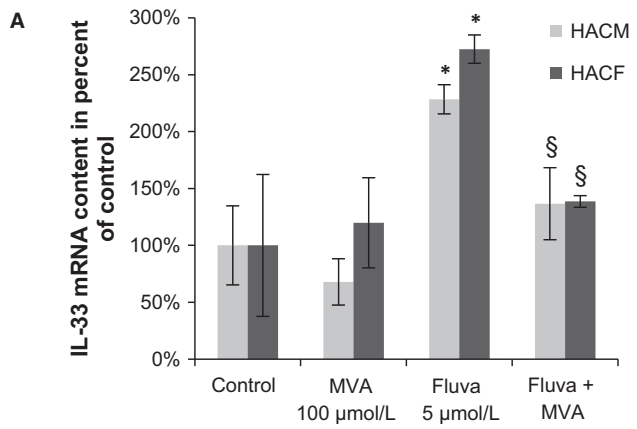
Two different BPs, alendronate and ibandronate, significantly up-regulated IL-33 protein levels in human cardiac cells (Figure 3). It should be noted that the induction of IL-33 protein was evident with concentrations from  $1$  to  $10 \text{ nmol L}^{-1}$  of alendronate and with  $10 \text{ nmol L}^{-1}$ ,  $100 \text{ nmol L}^{-1}$ , and  $1 \mu\text{mol L}^{-1}$  of ibandronate in HACM (Figure 3A,B), whereas in HACF alendronate was effective at a concentration of  $10 \text{ nmol L}^{-1}$  and ibandronate starting from  $100 \text{ nmol L}^{-1}$  (data not shown). Thus, we observed cell type-specific differences in the concentrations of BPs that significantly induced IL-33 in HACM and HACF. Alendronate at  $10 \text{ nmol L}^{-1}$  had no effect on the levels of ST2L and sST2 mRNA or secretion of sST2 in HACM from five different donors between 2 h and 48 h of treatment (data not shown). In contrast to fluvastatin, the BPs alendronate and ibandronate induced IL-33 in HACM even when mevalonate was added at the concentration of  $100 \mu\text{mol L}^{-1}$  (Figure 3A,B). Addition of GGPP had a reversing effect on BPs-induced IL-33 expression in both cell types (data not shown).

### 3.4 | IL-33 is increased upon RhoA kinase inhibition and decreased upon RhoA activation

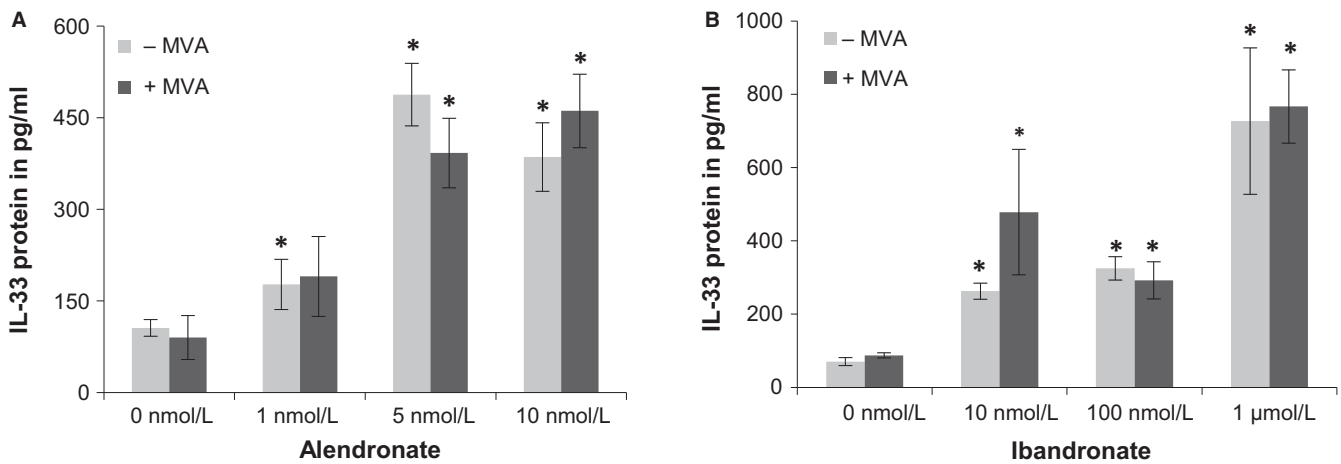
One of the most prominent geranylgeranylated protein is RhoA.<sup>31</sup> We tested if inhibition of ROCK, which is activated by active RhoA, could result in an increase of IL-33 expression. Indeed, treatment with Y-27632, a ROCK inhibitor, at  $5 \mu\text{mol L}^{-1}$  increased IL-33 mRNA levels in HACM to similar extent (approximately 3-fold) as fluvastatin at the same concentration (Figure 4A). In line with these results, the RhoA activator U-46619 at  $10 \text{ nmol L}^{-1}$  significantly decreased both basal and fluvastatin-induced IL-33 protein in HACM (Figure 4B). In HACF, there was a trend to the reduction of fluvastatin-induced IL-33 protein level with U-46619 (data not shown).

RhoA activation results in changes in gene expression via activation of serum response factor (SRF).<sup>32</sup> Treatment of HACM with latrunculin B, which prevents SRF to localise to the nucleus,<sup>33</sup> at concentrations between  $100 \text{ nmol L}^{-1}$  and  $1 \mu\text{mol L}^{-1}$  for 48 h led to a significant up to 15-fold increase of IL-33 protein levels (Figure 4C).

**FIGURE 2** Statin-induced IL-33 is inhibited by mevalonate and GGPP, but not by squalene, coenzyme Q10, or FPP in human cardiac cells. A and B, HACM and HACF were incubated for 48 h in the absence (control) or presence of mevalonate (MVA) alone ( $100 \mu\text{mol L}^{-1}$ ), fluvastatin (Fluva) alone ( $5 \mu\text{mol L}^{-1}$ ), or a combination. C, HACM and HACF or D, HACM were incubated for 48 h in the absence (control) or presence of GGPP alone ( $10 \mu\text{mol L}^{-1}$ ), fluvastatin (Fluva) alone ( $5 \mu\text{mol L}^{-1}$ ), or a combination. E, HACM were incubated for 48 h in the absence (control) or presence of squalene alone ( $10 \text{ nmol L}^{-1}$ ,  $100 \text{ nmol L}^{-1}$ ,  $1 \mu\text{mol L}^{-1}$ ), fluvastatin (Fluva) alone ( $5 \mu\text{mol L}^{-1}$ ), or a combination. F, HACM were incubated for 48 h in the absence (control) or presence of coenzyme Q10 (Q10) alone ( $10 \text{ nmol L}^{-1}$ ,  $100 \text{ nmol L}^{-1}$ ,  $1 \mu\text{mol L}^{-1}$ ), fluvastatin (Fluva) alone ( $5 \mu\text{mol L}^{-1}$ ), or a combination. G, HACM were incubated for 48 h in the absence (control) or presence of FPP alone ( $10 \text{ nmol L}^{-1}$ ,  $100 \text{ nmol L}^{-1}$ ,  $1 \mu\text{mol L}^{-1}$ ), fluvastatin (Fluva) alone ( $5 \mu\text{mol L}^{-1}$ ), or a combination. Values for IL-33/GAPDH mRNA are given as % of control, which was set as 100% (A, C). Values for IL-33 protein are shown in pg/mL (B, D-G). \* $P \leq 0.05$  as compared to respective control;  $\$P \leq 0.05$  as compared to fluvastatin-treated cells







**FIGURE 3** Mevalonate does not reverse bisphosphonates-induced IL-33 in human cardiac cells. A, HACM were incubated for 48 h with medium alone (0 nmol L<sup>-1</sup>), MVA (100 μmol L<sup>-1</sup>) alone, alendronate at 1 nmol L<sup>-1</sup>, 5 nmol L<sup>-1</sup>, or 10 nmol L<sup>-1</sup> alone, or a combination. B, HACM were incubated for 48 h with medium alone (0 nmol L<sup>-1</sup>), MVA (100 μmol L<sup>-1</sup>) alone, ibandronate at 10 nmol L<sup>-1</sup>, 100 nmol L<sup>-1</sup>, or 1 μmol L<sup>-1</sup> alone, or a combination. Values are given as pg/mL. \**P* ≤ 0.05 as compared to respective control

### 3.5 | Epigenetic changes in the promoter region of IL-33 in statin-treated human cardiac cells

Epigenetic changes in the promoter region have the potential to change the expression of genes<sup>34</sup> and statins were shown to induce epigenetic changes in cancer cells.<sup>35</sup> To analyse if statin treatment might have effects on chromatin organisation, we determined the accessibility of the IL-33 promoter in fluvastatin-treated HACM. After treatment of HACM with 5 μmol L<sup>-1</sup> fluvastatin for 24 h, the IL-33 promoter was on average 2.3-fold more accessible in statin-treated compared to untreated cells (*P* = 0.037; Figure 4D). We did not observe a significant change in the IL-33 promoter region after treatment with alendronate (1.4 fold of control, *P* = 0.6).

### 3.6 | Increased IL-33 expression in myocardial tissue of statin-treated patients

In order to support our in vitro data, we additionally examined IL-33 protein level in myocardial tissue from patients undergoing heart transplantation for end-stage HF under statin treatment or not treated with statins. As shown in Figure 5B, myocardial tissue from statin-treated patients express higher level of IL-33 protein compared with patients without statin treatment (Figure 5A, *P* = 0.048). Quantification of MFI of the staining is shown in Figure 5C.

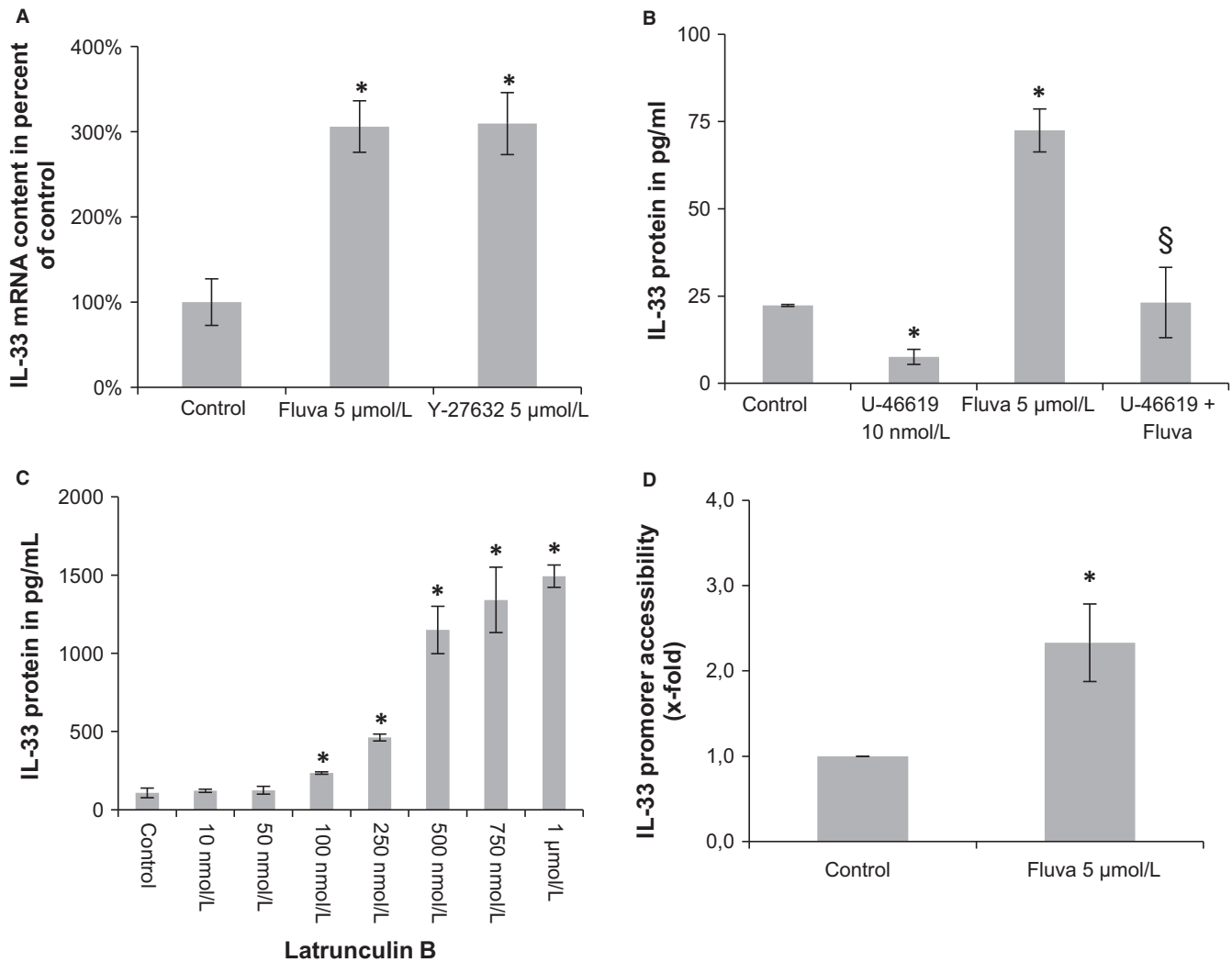
## 4 | DISCUSSION

In our study, we report a novel aspect of action for two widely applied classes of medications: BPs, which are inhibitors of FPP synthase, and HMG-CoA reductase inhibitors (statins). We found that statins as well as BPs increase the cytokine IL-33 in human cardiac myocytes and fibroblasts in vitro. Such induction was observed at

comparable levels with nitrogen-containing BPs alendronate and ibandronate and lipophilic statins simvastatin, fluvastatin, lovastatin, and atorvastatin, but not with hydrophilic pravastatin. The induction of intracellular IL-33 protein as well as mRNA was concentration- and time-dependent, and was reproducible in cells isolated from different donors' hearts. These results are in accordance with previously published results from our group, which showed that pravastatin had no effect on apoptosis or the expression of components of the plasminogen system and osteoprotegerin in human cardiac, endothelial, or smooth muscle cells in vitro.<sup>7,8,25,36</sup>

In order to support our in vitro findings, we analysed IL-33 protein by immunofluorescence in human myocardial tissue from explanted hearts of statin-treated versus non-statin-treated patients with end-stage HF. In accordance with our in vitro data, myocardial tissues from statin-treated patients express higher amounts of IL-33 protein as compared to myocardial tissue obtained from non-statin-treated patients. However, we have to acknowledge that small sample size (5 statin-treated vs. 5 not treated failing hearts) is an important limitation of this set of experiments, and besides statin treatment many factors might affect IL-33 expression in end-stage HF.

Despite having different primary targets, statins and BPs act as inhibitors of the same MVA pathway.<sup>37</sup> Though the main clinical indication for statins is to modify blood lipids and BPs are primarily used to suppress bone resorption, ample evidence is available that these two classes of medications exert also other, so-called pleiotropic, effects.<sup>2,5,6,11</sup> Previous studies have shown that statins inhibit cardiomyocyte hypertrophy in vitro and in vivo.<sup>6,38</sup> The mechanisms involved are an antioxidant effect implicating inhibition of Rac1, the inhibition of Rho kinase, p21ras inactivation and a down-regulation of microRNA-22.<sup>6,39</sup> Clinical studies investigating the effects of statin treatment in patients with HF are contradictory. The CORONA and GISSI-HF trials failed to demonstrate prognostic benefits of



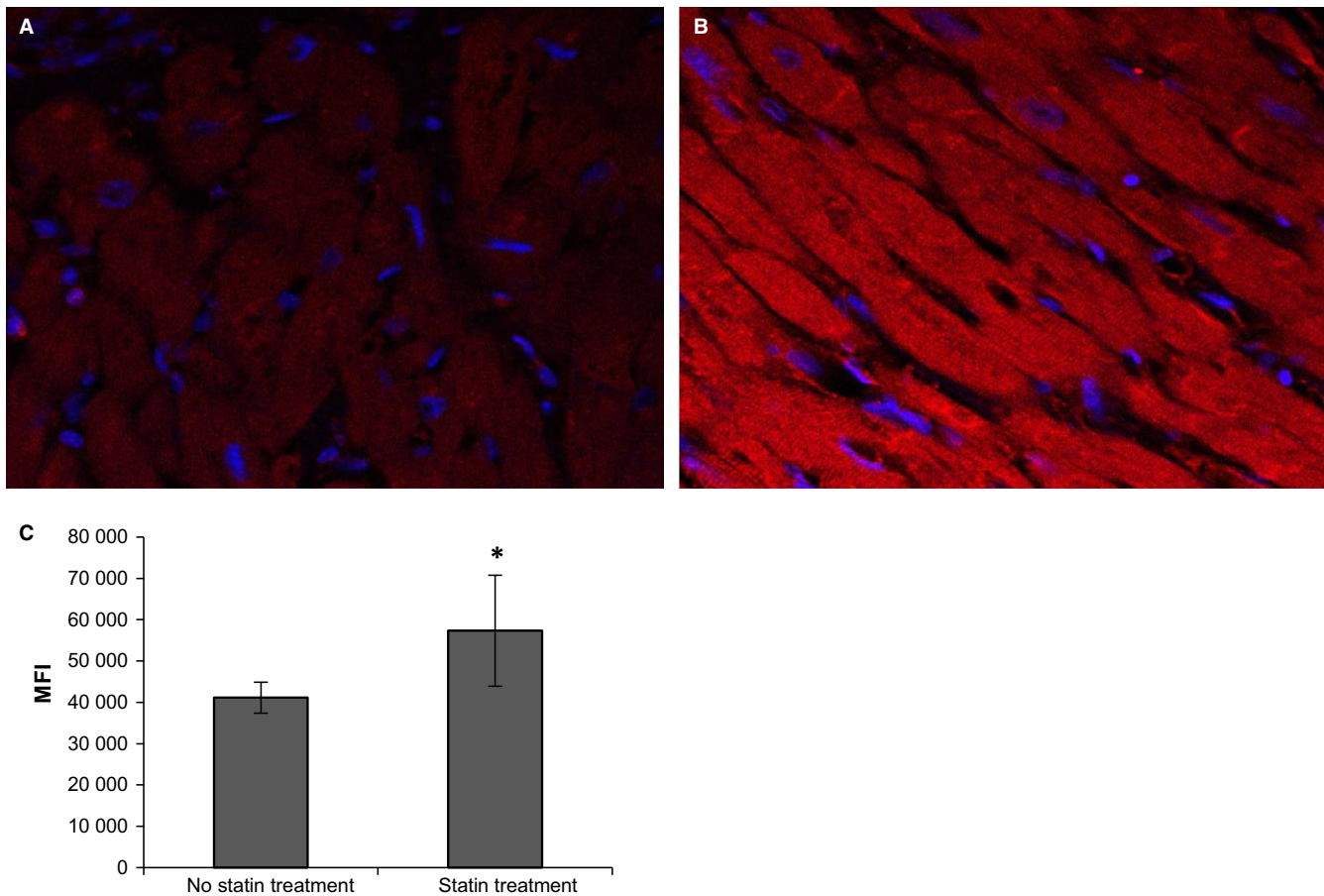
**FIGURE 4** Mechanisms underlying the increase of IL-33 in human cardiac cells. A, HACM were incubated with medium alone (control), fluvastatin (Fluva, 5 µmol L<sup>-1</sup>), or ROCK inhibitor Y-27632 (5 µmol L<sup>-1</sup>). B, HACM were incubated for 48 h in the absence (control) or presence of RhoA activator U-46619 (10 nmol L<sup>-1</sup>) alone, fluvastatin (Fluva, 5 µmol L<sup>-1</sup>) alone or a combination. C, HACM were incubated for 48 h in the absence (control) or presence of latrunculin B at the indicated concentrations. D, HACM were incubated with medium alone (control) or fluvastatin (Fluva, 5 µmol L<sup>-1</sup>) for 24 h. Values for IL-33/GAPDH mRNA are given as % of control, which was set as 100% (A). Promoter accessibility is given as x-fold control (D). Values for IL-33 protein are shown in pg/mL (B, C). \**P* ≤ 0.05 as compared to respective control; §*P* ≤ 0.05 as compared to fluvastatin-treated cells

statins in patients with HF.<sup>40,41</sup> On the other hand, the TOPCAT (Treatment of Preserved Cardiac Function Heart Failure with an Aldosterone Antagonist) trial was associated with improved outcome in patients with HF with preserved ejection fraction,<sup>42</sup> and the CHART-2 (Chronic Heart Failure Registry and Analysis in the Tohoku District 2) study concluded that higher-intensity statin use is associated with beneficial outcome in HF patients with ischaemic heart disease.<sup>43</sup>

Interestingly, an overexpression of FPP synthase, the primary target of BPs, induced cardiac hypertrophy and HF in mice.<sup>44</sup> Furthermore, inhibition of FPP synthase attenuates hypertrophy in neonatal rat cardiomyocytes.<sup>45</sup> Therefore, both statins and BPs can act as anti-hypertrophic agents in different experimental models but the exact mechanisms are not clarified yet.

IL-33 is the most recently identified cytokine of the IL-1 family.<sup>15</sup> Intracellular IL-33 expression was shown in endothelial, epithelial, smooth muscle cells, keratinocytes, and fibroblasts.<sup>15,17,21,27,46</sup> IL-33 is released by necrotic cells during damage or by living cells during mechanical strain.<sup>47,48</sup> We showed previously the intracellular expression of IL-33 in human cardiac myocytes and fibroblasts, and the release of IL-33 during necrosis of these cells.<sup>27</sup> Moreover, the “decoy” receptor for IL-33, sST2, was shown to have predictive value in patients with coronary artery disease and HF<sup>16,49–51</sup> as well as in critically ill patients.<sup>52</sup> In our study presented here, we found that fluvastatin is able to reduce secretion of sST2 from human cardiac myocytes. Interestingly, a recent report showed that sST2 levels were attenuated after 12 months of atorvastatin treatment in patients with HIV.<sup>53</sup> However, rosuvastatin had no effect on the





**FIGURE 5** Increased IL-33 expression in explanted hearts of statin-treated patients as compared to hearts of non-statin-treated patients. A and B, Representative confocal immunofluorescence images of human heart tissue obtained from a non-statin-treated patient (A) and a statin-treated patient (B). Staining for IL-33 (red) and DAPI (blue). Original magnification  $\times 200$ . Scale bar = 20  $\mu\text{m}$ . C, Quantification of the staining was performed as described in “Materials and Methods”, values are presented as mean fluorescence intensity (MFI) and represent mean values of 5 non-statin-treated patients and 5 statin-treated patients  $\pm$  SD respectively.  $*P \leq 0.05$  as compared to non-statin-treated patients

levels of sST2 in patients with HF from the CORONA study though the change in sST2 levels was evaluated after 3 months of rosuvastatin treatment.<sup>54</sup> In the light of our in vitro data about down-regulation of sST2 secretion by fluvastatin, it would be warranted to evaluate a possible influence of long-term statin treatment on sST2 blood levels in clinical studies.

As an inflammatory mediator IL-33 plays a role in a variety of diseases such as bronchial asthma, inflammatory bowel diseases, and rheumatoid arthritis.<sup>16</sup> On the other hand, IL-33 antagonised pro-hypertrophic stimuli in rat neonatal cardiomyocytes and exhibited an anti-hypertrophic effect after transverse aortic constriction (TAC) in mice.<sup>21</sup> Furthermore, IL-33 protected rat neonatal cardiomyocytes from hypoxia-induced apoptosis and improved cardiac function in rats after experimental myocardial infarction.<sup>22</sup> IL-33 also attenuated ischaemia/reperfusion-induced myocardial injury in diabetic mice.<sup>23</sup> Recently, a cardioprotective role of the IL-33/ST2 system was shown in IL-33 knockout (IL-33<sup>-/-</sup>) mice subjected to TAC, which exhibited exacerbated left ventricular hypertrophy, aggravated fibrosis, and impaired survival compared to wild-type littermates after TAC.<sup>24</sup> Therefore, IL-33 has been recognised as a cardioprotective cytokine.

In this context, it should be emphasised that the effects of statins on IL-33 expression in human cardiac cells were never studied before. Only one previous study showed that simvastatin induced IL-33 mRNA expression in human endothelial cells.<sup>55</sup>

We further investigated, which mechanisms are responsible for the increase of IL-33. It was shown previously that the MVA pathway inhibitors simvastatin and ibandronate affect epigenetic regulation in cancer cells.<sup>35</sup> We demonstrate here that the promoter region of IL-33 comprises a more open chromatin structure in fluvastatin-treated cardiac cells compared to untreated cells as analysed by promoter accessibility. Therefore, we suggest that statin-treated human adult cardiac myocytes up-regulate IL-33 expression via epigenetic changes in the IL-33 promoter region. Alendronate did not cause significant changes in the IL-33 promoter region in human cardiac myocytes.

Statins inhibit HMG-CoA reductase, an enzyme located at the apex of the MVA pathway, whereas second- and third-generation aminobisphosphonates such as alendronic acid and ibandronic acid, inhibit FPP synthase.<sup>37</sup> The production of FPP and GGPP occurs in a side branch of the MVA pathway and these substances are attached

to a variety of proteins as post-translational modifications known as protein prenylation (protein farnesylation and geranylgeranylation).<sup>56</sup>

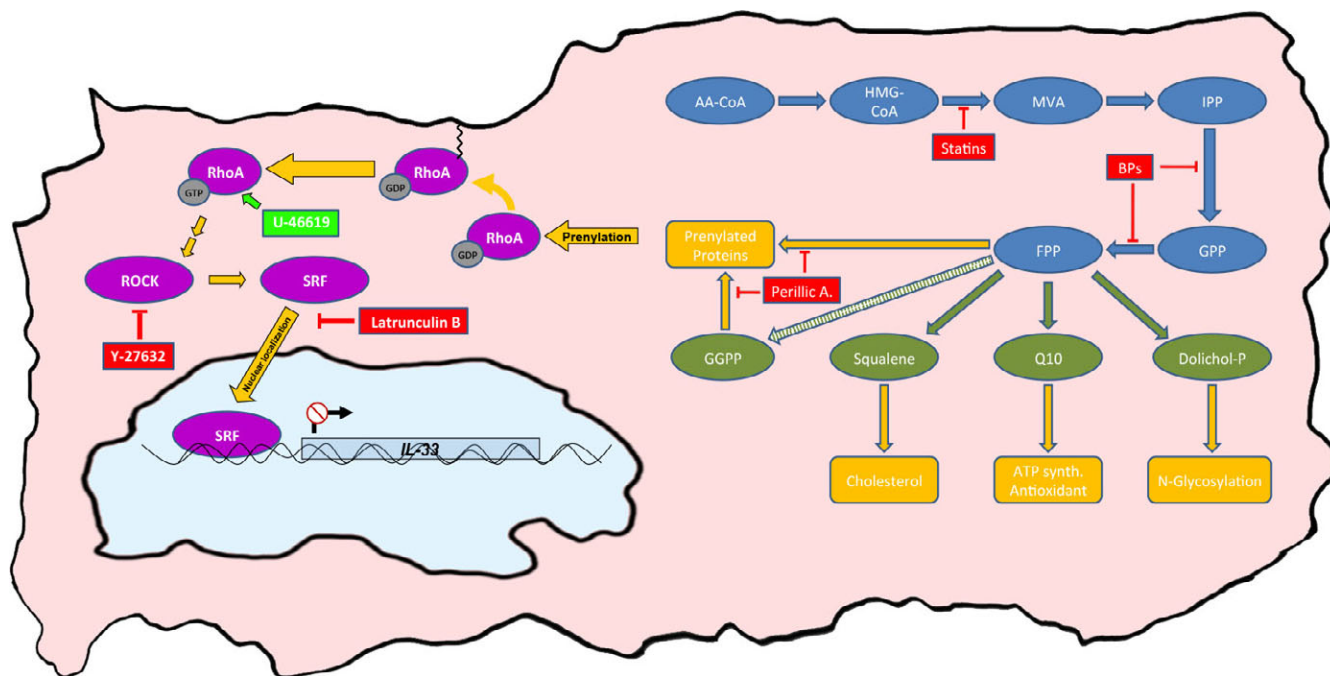
We showed that GGPP abolished IL-33 expression induced by both statins and BPs, whereas only the effect of statins on IL-33 expression was reduced by MVA supplementation. BPs-induced IL-33 induction was not suppressed by MVA, which can be explained by the fact that the BPs' target FPP synthase is downstream of MVA in the MVA pathway. Additionally, we found that squalene, the precursor of cholesterol, or coenzyme Q10, another end-product of the MVA pathway, which is responsible for energy production, did not change the effect of statins on IL-33 expression. This indicates that statin-induced IL-33 expression is not driven by lipid depletion or energetic defects but rather caused by prevention of protein prenylation. The finding that perillic acid, a general inhibitor of protein prenylation via inhibition of both geranylgeranyl transferase 1 and Rab geranylgeranyl transferase, also led to an increase of IL-33 expression in human cardiac myocytes, further supports the notion that the effect of statins and BPs are dependent on the inhibition of this post-translational modification. FPP could not reverse the effect of statins in our study, which led us to conclude that IL-33 induction is dependent on geranylgeranylation but not farnesylation.

RhoA is a prominent geranylgeranylated protein, which has also been associated with the diseases of the cardiovascular system and bone.<sup>31</sup> Statins block the isoprenylation and thus the membrane targeting and functional activation of the Rho family member.<sup>31</sup> It has been shown that fluvastatin reduces RhoA in the membrane fraction

of rat cardiac myocytes.<sup>29</sup> In spontaneously hypertensive rats simvastatin attenuated RhoA activity and myocyte hypertrophy.<sup>57</sup> The most important direct target of RhoA is ROCK. We therefore suspected RhoA and subsequent ROCK inactivation of being responsible for the effect of statins and BPs observed by us in human cardiac cells. We confirmed our hypothesis by treating these cells with the ROCK inhibitor Y-27632. We found that Y-27632 at 5  $\mu\text{mol L}^{-1}$  exerts a similar induction of IL-33 in human cardiac cells as fluvastatin at the same concentration. Moreover, U-46619, a ROCK activator, reduced basal and fluvastatin-induced IL-33 levels in our study. RhoA can regulate muscle specific gene expression through activation of SRF.<sup>31,32</sup> We inhibited nuclear localisation of SRF and thereby its impact on gene expression by latrunculin B and again observed significant and concentration-dependent increase in IL-33 levels in human cardiac myocytes. Taken together, our results suggest that statins up-regulate IL-33 via impaired geranylgeranylation of RhoA and resulting ROCK inhibition, which entails an inactivation of SRF and its effect on gene expression. An overview of the analysed pathways and results is given in Figure 6.

It should be noted that the concentrations of statins and BPs as well as the components of the MVA pathway and specific inhibitors used in this study were in the same range as concentrations of these substances used in numerous other tissue culture studies.<sup>3,7-9,14,25,29,36</sup>

In conclusion, our study showed for the first time that IL-33 could be an important mediator of the pleiotropic effects of statins and BPs in human cardiac tissue. Considering potential safety issues,



**FIGURE 6** Schematic representation of the downstream targets of the MVA pathway, depicting its various intermediates (blue), end products (green) and functional implications (yellow). Prenylation of RhoA with GGPP anchors RhoA to the plasma membrane, where it can be activated by signalling factors. Active RhoA activates ROCK, which triggers activation and translocation of SRF into the nucleus, where it represses the transcription of IL-33. Inhibitors used in this study are depicted in red: statins inhibit HMG-CoA-reductase; BPs inhibit GPP synthase and FPP synthase; perillic acid inhibits protein prenylation; latrunculin B prevents SRF activation; Y-27632 is a ROCK inhibitor. The RhoA activator U-46619 is depicted in green

the feasibility and possible implications of the therapeutic modulation of IL-33 signalling, which is known to act cardioprotective,<sup>21-24</sup> are currently discussed.<sup>58</sup> As statins and BPs represent widely used and standardised classes of drugs for primary and secondary prevention of cardiovascular disease, our results might have impact also in the clinical setting.

## ACKNOWLEDGEMENTS

This work was supported by a grant from the Austrian Science Fund (FWF) to Svitlana Demyanets (grant number T445-B11). The work was also supported by the Association for the Promotion of Research in Arteriosclerosis, Thrombosis and Vascular Biology.

## CONFLICT OF INTEREST

The authors confirm that there are no conflicts of interests.

## ORCID

Svitlana Demyanets  <http://orcid.org/0000-0003-0601-0667>

## REFERENCES

- Stone NJ, Robinson JG, Lichtenstein AH, et al. 2013 ACC/AHA guideline on the treatment of blood cholesterol to reduce atherosclerotic cardiovascular risk in adults: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. *Circulation*. 2014;129:S1-S45.
- Oesterle A, Laufs U, Liao JK. Pleiotropic effects of statins on the cardiovascular system. *Circ Res*. 2017;120:229-243.
- Stojkovic S, Kaun C, Heinz M, et al. Interleukin-33 induces urokinase in human endothelial cells—possible impact on angiogenesis. *J Thromb Haemost*. 2014;12:948-957.
- Eilenberg W, Stojkovic S, Kaidar A, et al. NGAL and MMP-9/NGAL as biomarkers of plaque vulnerability and targets of statins in patients with carotid atherosclerosis. *Clin Chem Lab Med*. 2017;56:147-156.
- Xu X, Zhang A, Halquist MS, et al. Simvastatin promotes NPC1-mediated free cholesterol efflux from lysosomes through CYP7A1/LXRalpha signalling pathway in oxLDL-loaded macrophages. *J Cell Mol Med*. 2017;21:364-374.
- Porter KE, Turner NA. Statins and myocardial remodelling: cell and molecular pathways. *Expert Rev Mol Med*. 2011;13:e22.
- Demyanets S, Kaun C, Maurer G, et al. Statins modulate expression of components of the plasminogen activator/plasmin system in human cardiac myocytes in vitro. *J Thromb Haemost*. 2006;4:476-479.
- Demyanets S, Kaun C, Pfaffenberger S, et al. Hydroxymethylglutaryl-coenzyme A reductase inhibitors induce apoptosis in human cardiac myocytes in vitro. *Biochem Pharmacol*. 2006;71:1324-1330.
- Jarai R, Kaun C, Weiss TW, et al. Human cardiac fibroblasts express B-type natriuretic peptide: fluvastatin ameliorates its up-regulation by interleukin-1alpha, tumour necrosis factor-alpha and transforming growth factor-beta. *J Cell Mol Med*. 2009;13:4415-4421.
- Reid IR, Brown JP, Burckhardt P, et al. Intravenous zoledronic acid in postmenopausal women with low bone mineral density. *N Engl J Med*. 2002;346:653-661.
- Kawahara T, Nishikawa M, Kawahara C, et al. Atorvastatin, etidronate, or both in patients at high risk for atherosclerotic aortic plaques: a randomized, controlled trial. *Circulation*. 2013;127:2327-2335.
- Li L, Chen GP, Yang Y, et al. Chronic inhibition of farnesyl pyrophosphate synthase attenuates cardiac hypertrophy and fibrosis in spontaneously hypertensive rats. *Biochem Pharmacol*. 2010;79:399-406.
- Yang J, Zhu HH, Chen GP, et al. Inhibition of farnesyl pyrophosphate synthase attenuates angiotensin II-induced cardiac hypertrophy and fibrosis in vivo. *Int J Biochem Cell Biol*. 2013;45:657-666.
- Ye Y, Hu SJ, Li L. Inhibition of farnesylpyrophosphate synthase prevents angiotensin II-induced hypertrophic responses in rat neonatal cardiomyocytes: involvement of the RhoA/Rho kinase pathway. *FEBS Lett*. 2009;583:2997-3003.
- Schmitz J, Owyang A, Oldham E, et al. IL-33, an interleukin-1-like cytokine that signals via the IL-1 receptor-related protein ST2 and induces T helper type 2-associated cytokines. *Immunity*. 2005;23:479-490.
- Altara R, Ghali R, Mallat Z, et al. Conflicting vascular and metabolic impact of the IL-33/sST2 axis. *Cardiovasc Res*. 2018. <https://doi.org/10.1093/cvr/cvy166>. [Epub ahead of print]
- Demyanets S, Konya V, Kastl SP, et al. Interleukin-33 induces expression of adhesion molecules and inflammatory activation in human endothelial cells and in human atherosclerotic plaques. *Arterioscler Thromb Vasc Biol*. 2011;31:2080-2089.
- Stojkovic S, Kaun C, Basilio J, et al. Tissue factor is induced by interleukin-33 in human endothelial cells: a new link between coagulation and inflammation. *Sci Rep*. 2016;6:25171.
- Montanari E, Stojkovic S, Kaun C, et al. Interleukin-33 stimulates GM-CSF and M-CSF production by human endothelial cells. *Thromb Haemost*. 2016;116:317-327.
- Stojkovic S, Thulin A, Hell L, et al. IL-33 stimulates the release of procoagulant microvesicles from human monocytes and differentially increases tissue factor in human monocyte subsets. *Thromb Haemost*. 2017;117:1379-1390.
- Sanada S, Hakuno D, Higgins LJ, et al. IL-33 and ST2 comprise a critical biomechanically induced and cardioprotective signaling system. *J Clin Invest*. 2007;117:1538-1549.
- Seki K, Sanada S, Kudinova AY, et al. Interleukin-33 prevents apoptosis and improves survival after experimental myocardial infarction through ST2 signaling. *Circ Heart Fail*. 2009;2:684-691.
- Rui T, Zhang J, Xu X, et al. Reduction in IL-33 expression exacerbates ischaemia/reperfusion-induced myocardial injury in mice with diabetes mellitus. *Cardiovasc Res*. 2012;94:370-378.
- Veeraveedu PT, Sanada S, Okuda K, et al. Ablation of IL-33 gene exacerbate myocardial remodeling in mice with heart failure induced by mechanical stress. *Biochem Pharmacol*. 2017;138:73-80.
- Wiesbauer F, Kaun C, Zorn G, et al. HMG CoA reductase inhibitors affect the fibrinolytic system of human vascular cells in vitro: a comparative study using different statins. *Br J Pharmacol*. 2002;135:284-292.
- Macfelda K, Weiss TW, Kaun C, et al. Plasminogen activator inhibitor 1 expression is regulated by the inflammatory mediators interleukin-1alpha, tumor necrosis factor-alpha, transforming growth factor-beta and oncostatin M in human cardiac myocytes. *J Mol Cell Cardiol*. 2002;34:1681-1691.
- Demyanets S, Kaun C, Pentz R, et al. Components of the interleukin-33/ST2 system are differentially expressed and regulated in human cardiac cells and in cells of the cardiac vasculature. *J Mol Cell Cardiol*. 2013;60:16-26.
- Ferri N, Arnaboldi L, Orlandi A, et al. Effect of S(-) perillidic acid on protein prenylation and arterial smooth muscle cell proliferation. *Biochem Pharmacol*. 2001;62:1637-1645.
- Ogata Y, Takahashi M, Takeuchi K, et al. Fluvastatin induces apoptosis in rat neonatal cardiac myocytes: a possible mechanism of statin-

- attenuated cardiac hypertrophy. *J Cardiovasc Pharmacol*. 2002;40:907-915.
30. Hohensinner PJ, Baumgartner J, Kral-Pointner JB, et al. PAI-1 (plasminogen activator inhibitor-1) expression renders alternatively activated human macrophages proteolytically quiescent. *Arterioscler Thromb Vasc Biol*. 2017;37:1913-1922.
  31. Dai Y, Luo W, Chang J. Rho kinase signaling and cardiac physiology. *Curr Opin Physiol*. 2018;1:14-20.
  32. Hill CS, Wynne J, Treisman R. The Rho family GTPases RhoA, Rac1, and CDC42Hs regulate transcriptional activation by SRF. *Cell*. 1995;81:1159-1170.
  33. Liu HW, Halayko AJ, Fernandes DJ, et al. The RhoA/Rho kinase pathway regulates nuclear localization of serum response factor. *Am J Respir Cell Mol Biol*. 2003;29:39-47.
  34. Rosa-Garrido M, Chapski DJ, Vondriska TM. Epigenomes in Cardiovascular Disease. *Circ Res*. 2018;122:1586-1607.
  35. Karlic H, Thaler R, Gerner C, et al. Inhibition of the mevalonate pathway affects epigenetic regulation in cancer cells. *Cancer Genet*. 2015;208:241-252.
  36. Ben-Tal Cohen E, Hohensinner PJ, Kaun C, et al. Statins decrease TNF-alpha-induced osteoprotegerin production by endothelial cells and smooth muscle cells in vitro. *Biochem Pharmacol*. 2007;73:77-83.
  37. Thurnher M, Gruenbacher G, Nussbaumer O. Regulation of mevalonate metabolism in cancer and immune cells. *Biochim Biophys Acta*. 2013;1831:1009-1015.
  38. Patel R, Nagueh SF, Tsybouleva N, et al. Simvastatin induces regression of cardiac hypertrophy and fibrosis and improves cardiac function in a transgenic rabbit model of human hypertrophic cardiomyopathy. *Circulation*. 2001;104:317-324.
  39. Tu Y, Wan L, Bu L, et al. MicroRNA-22 downregulation by atorvastatin in a mouse model of cardiac hypertrophy: a new mechanism for anti-hypertrophic intervention. *Cell Physiol Biochem*. 2013;31:997-1008.
  40. Kjekshus J, Apetrei E, Barrios V, et al. Rosuvastatin in older patients with systolic heart failure. *N Engl J Med*. 2007;357:2248-2261.
  41. Tavazzi L, Maggioni AP, Marchioli R, et al. Effect of rosuvastatin in patients with chronic heart failure (the GISSI-HF trial): a randomised, double-blind, placebo-controlled trial. *Lancet*. 2008;372:1231-1239.
  42. Tsujimoto T, Kajio H. Favorable effects of statins in the treatment of heart failure with preserved ejection fraction in patients without ischemic heart disease. *Int J Cardiol*. 2018;255:111-117.
  43. Oikawa T, Sakata Y, Nochioka K, et al. Prognostic impact of statin intensity in heart failure patients with ischemic heart disease: a report from the CHART-2 (chronic heart failure registry and analysis in the tohoku district 2) study. *J Am Heart Assoc*. 2018;7:e007524.
  44. Yang J, Mou Y, Wu T, et al. Cardiac-specific overexpression of farnesyl pyrophosphate synthase induces cardiac hypertrophy and dysfunction in mice. *Cardiovasc Res*. 2013;97:490-499.
  45. Ye Y, Mou Y, Bai B, et al. Knockdown of farnesylpyrophosphate synthase prevents angiotensin II-mediated cardiac hypertrophy. *Int J Biochem Cell Biol*. 2010;42:2056-2064.
  46. Zeyda M, Wernly B, Demyanets S, et al. Severe obesity increases adipose tissue expression of interleukin-33 and its receptor ST2, both predominantly detectable in endothelial cells of human adipose tissue. *Int J Obes (Lond)*. 2013;37:658-665.
  47. Luthi AU, Cullen SP, McNeela EA, et al. Suppression of interleukin-33 bioactivity through proteolysis by apoptotic caspases. *Immunity*. 2009;31:84-98.
  48. Kakkar R, Hei H, Dobner S, et al. Interleukin 33 as a mechanically responsive cytokine secreted by living cells. *J Biol Chem*. 2012;287:6941-6948.
  49. Demyanets S, Speidl WS, Tentzeris I, et al. Soluble ST2 and interleukin-33 levels in coronary artery disease: relation to disease activity and adverse outcome. *PLoS ONE*. 2014;9:e95055.
  50. Hauser JA, Demyanets S, Rusai K, et al. Diagnostic performance and reference values of novel biomarkers of paediatric heart failure. *Heart*. 2016;102:1633-1639.
  51. Stojkovic S, Kaider A, Koller L, et al. GDF-15 is a better complementary marker for risk stratification of arrhythmic death in non-ischaemic, dilated cardiomyopathy than soluble ST2. *J Cell Mol Med*. 2018;22:2422-2429.
  52. Krychtiuk KA, Stojkovic S, Lenz M, et al. Predictive value of low interleukin-33 in critically ill patients. *Cytokine*. 2018;103:109-113.
  53. deFilippi C, Christenson R, Joyce J, et al. Brief report: statin effects on myocardial fibrosis markers in people living with HIV. *J Acquir Immune Defic Syndr*. 2018;78:105-110.
  54. Nymo SH, Aukrust P, Kjekshus J, et al. Limited added value of circulating inflammatory biomarkers in chronic heart failure. *JACC Heart Fail*. 2017;5:256-264.
  55. Hot A, Lavocat F, Lenief V, et al. Simvastatin inhibits the pro-inflammatory and pro-thrombotic effects of IL-17 and TNF-alpha on endothelial cells. *Ann Rheum Dis*. 2013;72:754-760.
  56. Molnar G, Dagher MC, Geiszt M, et al. Role of prenylation in the interaction of Rho-family small GTPases with GTPase activating proteins. *Biochemistry*. 2001;40:10542-10549.
  57. Takayama N, Kai H, Kudo H, et al. Simvastatin prevents large blood pressure variability induced aggravation of cardiac hypertrophy in hypertensive rats by inhibiting RhoA/Ras-ERK pathways. *Hypertens Res*. 2011;34:341-347.
  58. Braun H, Afonina IS, Mueller C, et al. Dichotomous function of IL-33 in health and disease: from biology to clinical implications. *Biochem Pharmacol*. 2018;148:238-252.

**How to cite this article:** Pentz R, Kaun C, Thaler B, et al. Cardioprotective cytokine interleukin-33 is up-regulated by statins in human cardiac tissue. *J Cell Mol Med*. 2018;22:6122–6133. <https://doi.org/10.1111/jcmm.13891>