



## ARTICLE

# PPAR- $\gamma$ alleviates the inflammatory response in TNF- $\alpha$ -induced fibroblast-like synoviocytes by binding to p53 in rheumatoid arthritis

Xiao-feng Li<sup>1,2</sup>, Shu-qin Yin<sup>1,2,3</sup>, Hao Li<sup>1,2</sup>, Ying-li Yang<sup>1,2</sup>, Xin Chen<sup>1,2</sup>, Biao Song<sup>4</sup>, Sha Wu<sup>1,2</sup>, Yuan-yuan Wu<sup>1,2</sup>, Hua Wang<sup>5</sup> and Jun Li<sup>1,2</sup>

Rheumatoid arthritis (RA) is characterized by synovial inflammation, synoviocyte expansion and damage to cartilage and bone. We recently reported that peroxisome proliferator-activated receptor (PPAR)- $\gamma$  inhibited the proliferation and activation of fibroblast-like synoviocytes (FLS), and was downregulated in RA synovial. In this study we investigated the role of PPAR- $\gamma$  in RA and the underlying mechanisms. Adjuvant-induced arthritis (AIA) was induced in rats; from D15, AIA rats were orally administered pioglitazone (30 mg·kg<sup>-1</sup>·d<sup>-1</sup>) or rosiglitazone (4 mg·kg<sup>-1</sup>·d<sup>-1</sup>) for 14 days. Collagen-induced arthritis (CIA) was induced in wild-type and *Ppar- $\gamma$ <sup>+/-</sup>* mice. We showed that the expression of PPAR- $\gamma$  was significantly reduced, whereas that of TNF- $\alpha$  was markedly increased in human RA FLS. In CIA mice, knockdown of PPAR- $\gamma$  expression (*Ppar- $\gamma$ <sup>+/-</sup>*) aggravated the ankle inflammation. Similarly, T0070907 (a PPAR- $\gamma$  antagonist) or *si-PPAR- $\gamma$*  promoted the activation and inflammation of TNF- $\alpha$ -induced FLS in vitro. On the contrary, administration of PPAR- $\gamma$  agonist pioglitazone or rosiglitazone, or injection of *ad-Ppar- $\gamma$*  into the ankle of AIA rat in vivo induced overexpression of PPAR- $\gamma$ , reduced the paw swelling and inflammation, and downregulated activation and inflammation of FLS in RA. Interestingly, injection of *ad-Ppar- $\gamma$*  into the ankle also reversed the ankle inflammation in *Ppar- $\gamma$ <sup>+/-</sup>* CIA mice. We conducted RNA-sequencing and KEGG pathway analysis, and revealed that PPAR- $\gamma$  overexpression was closely related to p53 signaling pathway in TNF- $\alpha$ -induced FLS. Co-IP study confirmed that p53 protein was bound to PPAR- $\gamma$  in RA FLS. Taken together, PPAR- $\gamma$  alleviates the inflammatory response of TNF- $\alpha$ -induced FLS by binding p53 in RA.

**Keywords:** rheumatoid arthritis; fibroblast-like synoviocytes; inflammatory response; PPAR- $\gamma$ ; adjuvant-induced arthritis; collagen-induced arthritis

*Acta Pharmacologica Sinica* (2023) 44:454–464; <https://doi.org/10.1038/s41401-022-00957-9>

## INTRODUCTION

Rheumatoid arthritis (RA) is an inflammatory and systemic autoimmune disease characterized by synovial inflammation, synoviocyte expansion, and damage to cartilage and bone [1]. The average prevalence of RA is estimated at 0.5%–1.0% worldwide and is 2–3 times more common in women than men. During the pathogenesis of RA, the synovial layer will increase from 3 to 20 layers, which is associated with an increase in fibroblast-like synoviocytes (FLS) [2] and the secretion of metalloproteinases. Crosstalk between inflammation and chemokines leads to RA progression by activating FLS [3]. Recent research has shown that FLS promote arthritis severity, and lining FLS can also contribute to bone erosion and cartilage damage in RA [4]. Moreover, TNF- $\alpha$ , which is mainly derived from activated macrophages, is one of the activators of FLS in the early stages of RA [5, 6]. In RA, after FLS activation, the cells become invasive, directly encroaching upon articular cartilage and digesting it by producing matrix metalloproteinases (MMPs) and secreting

interleukin (IL)-6 and chemokines [5, 7]. Thus, to treat RA, it is vital to inhibit FLS activation and inflammation.

Recently, we reported that peroxisome proliferator-activated receptor (PPAR)- $\gamma$  expression was downregulated in RA synovial tissue and inhibited the proliferation and activation of FLS in adjuvant-induced arthritis (AIA) via the Wnt/ $\beta$ -catenin signaling pathway [8]. PPAR- $\gamma$  activation by 15-deoxy-delta (12,14)-prostaglandin J (2) (15d-PGJ (2)) can induce synoviocyte apoptosis in AIA [9]. PPAR- $\gamma$  has been widely reported to be an anti-inflammatory target, and a large number of agonists have been designed [10, 11]. Agonists of this receptor suppress Th17 immunity by inhibiting glutaminolysis/2-HG/H3K4me3 and GSH/ROS signaling by GLS1 [12]. Additionally, PPAR- $\gamma$  mediates the vasculoregenerative program to reverse pulmonary hypertension by binding to p53 [13]. These results indicate that PPAR may be crucial in inflammation; however, the exact mechanism of aberrant PPAR- $\gamma$  expression in RA and its relationship with inflammation and FLS activation are unclear.

<sup>1</sup>Inflammation and Immune Mediated Disease Laboratory of Anhui Province, Anhui Institute of Innovative Drugs, School of Pharmacy, Anhui Medical University, Hefei 230032, China; <sup>2</sup>The Key Laboratory of Anti-inflammatory and Immune Medicines, Ministry of Education, Hefei 230032, China; <sup>3</sup>Pharmacy Department, Chizhou People's Hospital, Chizhou 247000, China; <sup>4</sup>Pharmacy Department, The First Affiliated Hospital of Anhui Medical University, Hefei 230022, China and <sup>5</sup>Department of Oncology, The First Affiliated Hospital of Anhui Medical University, Hefei 230022, China

Correspondence: Hua Wang (wanghua@ahmu.edu.cn) or Jun Li (lj@ahmu.edu.cn)

These authors contributed equally: Xiao-feng Li, Shu-qin Yin, Hao Li

Received: 24 March 2022 Accepted: 10 July 2022

Published online: 2 August 2022

In this study, we stimulated FLS with TNF- $\alpha$  to simulate the inflammatory microenvironment of synovial cells in joints and used a rat AIA model, a collagen-induced arthritis (CIA) and a PPAR- $\gamma$ <sup>+/-</sup> CIA mouse model to investigate the critical role and underlying mechanism of PPAR- $\gamma$  in RA. Our results clearly showed that PPAR- $\gamma$  plays an essential role by binding to p53 to alleviate the inflammatory response of TNF- $\alpha$ -induced FLS in RA.

## MATERIALS AND METHODS

### Synovium and blood collection

The synovium of RA patients ( $n = 8$ ) and osteoarthritis (OA,  $n = 8$ ) patients undergoing total joint replacement were collected from the Department of Orthopedics of the Second Affiliated Hospital of Anhui Medical University (Hefei, China). The blood of RA patients ( $n = 22$ ) was collected from the Department of Rheumatology, the First Affiliated Hospital of Anhui Medical University (Hefei, China). Monocytes were separated from the blood of RA patients with a Human Peripheral Blood Mononuclear Cell Isolation Kit (Tianjin Hao Yang Biology, China). During the course of the experiment, all patients gave written, informed consent, and experimental protocols involving human subjects were approved by the Biomedical Ethics Committee of Anhui Medical University (Approval no. 20200970). RA patient information is shown in Supplementary Table S1.

### Animals

PPAR- $\gamma$ <sup>+/-</sup> mice were designed by GemPharmatech (Nanjing, China) with CRISPR/Cas9. The gRNA sequences of the *Ppar- $\gamma$*  were 5'-GTCTGTGGGGATAAAGCATC-3' and 5'-CTCGGCACTCAATGGC CATG-3'. Sprague-Dawley rats (80–120 g) were obtained from the Experimental Animal Center of Anhui Medical University (Certificate No. 20200498). All animals were cared for according to the guidelines provided by the Center for Developmental Biology of Anhui Medical University. Experiments were conducted in accordance with the regulations of the Experimental Animal Administration and were approved by Anhui Medical University's subcommittee on animal care (Approval No. 20200963).

AIA model rats were injected with complete Freund's adjuvant (0.1 mL/100 g body weight; Chondrex, Redmond, WA, USA) under the left hind paw [8, 14–16]. On the 14th day, AIA rats were intragastrically administered pioglitazone (30 mg·kg<sup>-1</sup>·d<sup>-1</sup>, Tonghua Maoxiang Pharmaceutical Co., Ltd., Tonghua, China) or rosiglitazone (4 mg·kg<sup>-1</sup>·d<sup>-1</sup>, Jiangsu Yellow River Pharmaceutical Co., Ltd., Yancheng, China) for 14 days. The adenovirus carrying the rat *Ppar- $\gamma$*  gene (*ad-Ppar- $\gamma$* ) or negative control gene (*ad-NC*) (Hanbio, Shanghai, China) was intra-articularly injected into the hind knee (0.1 mL). Secondary (right) hind paw swelling was measured on Days 15, 18, 21, 24, 27 and 30 by a toe volume measuring instrument (Chengdu Taimeng Software Co. Ltd, Chengdu, China).

For the CIA model, 10-to-14-week-old mice (Certificate No. 202133877) were immunized with 0.1 mL of an emulsion (2 mg/mL chicken type II collagen emulsified in 5 mg/mL complete Freund's adjuvant, Chondrex) at the tail root. After 21 days, the mice were boosted with a 0.1 mL emulsion by subcutaneous injection. The adenovirus carrying the mouse *Ppar- $\gamma$*  gene (*ad-Ppar- $\gamma$* ) or the negative control gene (*ad-NC*) (Hanbio, Shanghai, China) was intra-articularly injected into the ankle (0.1 mL) on Day 21. In all models, arthritis was scored in the wrists and ankles as previously described: 0 = normal; 1 = minimal or moderate erythema and mild swelling; 2 = marked erythema and severe swelling, digits not yet involved; and 3 = maximal erythema and swelling, digits involved.

### FLS culture

Cells were harvested from RA synovium, separated and cultured in DMEM (HyClone, UT, USA) containing 20% (v/v) FBS (PAN-Biotech

GmbH, Adenbach, Germany) with normal penicillin and streptomycin (Beyotime, Shanghai, China) at 37 °C and 5% CO<sub>2</sub>. FLS were used for experiments between passages 4 and 8.

### Histopathology

Samples were fixed in paraformaldehyde for 48 h, and the sectioned synovium was subjected to hematoxylin and eosin (H&E) staining, immunohistochemistry (IHC), and immunofluorescence (IF) analysis and photographed by CaseViewer software (3DHISTECH, Budapest, Hungary).

### Enzyme-linked immunosorbent assay (ELISA)

Rat and mouse serum were collected from centrifuged blood, and the human cell supernatant of treated RA FLS was collected at 48 h. Based on the manufacturer's protocol, ELISA was used to measure the levels of IL-6, IL-1 $\beta$  and TNF- $\alpha$  in mouse serum, TNF- $\alpha$  and IL-6 levels in rat serum and IL-6 and IL-8 levels in human FLS. The kit information can be found in the Supplementary Information.

### Immunocytochemical analysis of FLS

PPAR- $\gamma$  antibodies (Abcam, Cambridge, UK) were used overnight to detect FLS expressing TNF- $\alpha$ . The FLS were covered with DAPI (Beyotime, Shanghai, China) for 10 min and then photographed by an epifluorescence microscope (BX-51; Olympus, Tokyo, Japan).

### Gene silencing and overexpression

The PPAR- $\gamma$  small interfering RNA (*PPAR- $\gamma$ -siRNA*, GenePharma, Shanghai, China), overexpression plasmid (*PPAR- $\gamma$ -pcDNA3.1*, Hanbio) or *P53-siRNA* (Hanbio) was transfected into FLS by Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA). The sequences of the sense and antisense oligonucleotides are shown in Supplementary Table S2. After being cultured Opti-MEM for 8 h, the transfected FLS were cultured in complete medium for 48 h at 37 °C.

### Real-time quantitative PCR (RT-qPCR)

TRIzol reagent (Invitrogen) was used to extract total RNA, which was reverse transcribed into cDNA with PrimeScript<sup>TM</sup> RT Master Mix (Takara, Kyoto, Japan). RT-qPCR was performed with a SYBR<sup>®</sup> Green Premix Pro Taq HS qPCR Kit (AG, Changsha, China). The primers for RT-qPCR were synthesized by Sangon Biotech (Shanghai, China), and the sequences are shown in Supplementary Table S3.

### Western blotting (WB)

Total proteins were extracted with RIPA buffer (Beyotime), denatured by boiling, separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis, blocked with primary antibodies and incubated overnight. The antibodies were used at a 1:500 dilution. PPAR- $\gamma$  and tissue inhibitor of metalloproteinase (TIMP)-1 antibodies were provided by Abcam; P53, MMP-3 and MMP-9 antibodies were provided by Cell Signaling Technology (Danvers, MA, USA); and IL-6, IL-1 $\beta$  and  $\beta$ -actin antibodies were provided by Bioworld, Shanghai, China. Immobilon Western Chemiluminescent HRP Substrate (Millipore) was used to incubate the protein bands with horseradish peroxidase-conjugated goat anti-rabbit or anti-mouse antibodies for 1 h. Then, the blots were photographed with a ChemiDoc<sup>MP</sup> Imaging System (Bio-Rad).

### RNA sequencing (RNA-seq)

After being infected with the human PPAR- $\gamma$  overexpression adenovirus (*ad-PPAR- $\gamma$* , Hanbio), RA FLS were examined by RNA-seq at LC-bio Technologies (Hangzhou) Co., Ltd. (Hangzhou, China).

### Paw swelling analysis

After AIA rats were subcutaneously administered complete Freund's adjuvant in the left hind paw, the amount of swelling in the right hind paw was measured by a toe volume measuring

instrument (Chengdu Taimeng Software Co. Ltd) on Days 15, 18, 21, 24, 27 and 30.

#### Wound-healing assay

After the cells reached 60%–80% confluence in a 24-well plate, the FLS were treated with *PPAR- $\gamma$ -siRNA* or *PPAR- $\gamma$ -pcDNA3.1* for 24 h. Then, the cells were scratched with a pipette tip, fixed with methanol, stained with crystal violet for 24 h, and viewed under an Olympus BX-51 microscope.

#### Coimmunoprecipitation (Co-IP) assay

RA FLS were examined by Co-IP according to the manufacturer's protocol (Wanlei bio, Shenyang, China). Anti-PPAR- $\gamma$  and anti-P53 were purchased from Cell Signaling Technology.

#### Statistical analysis

SPSS v13.0 software was used to analyze the data, which are presented as the mean  $\pm$  standard deviation. We performed one-way analysis of variance or RMANOVA with a post hoc Dunnett's test to determine statistical significance. *P* values < 0.05 were considered statistically significant in all analyses.

## RESULTS

PPAR- $\gamma$  and TNF- $\alpha$  expression showed opposite trends in RA FLS. As a first step in investigating the pivotal role of PPAR- $\gamma$  in RA, we collected the synovium from RA and OA patients and found inflammatory cell infiltration in the synovium in RA (Fig. 1a). Moreover, PPAR- $\gamma$  expression was downregulated in RA FLS, as shown by IHC (Fig. 1a), which was confirmed by WB and RT-qPCR (Fig. 1b). PPAR- $\gamma$  expression was also downregulated in AIA and CIA synovial tissue (Supplementary Fig. S1a). PPAR- $\gamma$  levels were reduced in FLS stimulated with inflammatory factors such as IL-1 $\beta$ , IL-6, IL-17A, TNF- $\alpha$ , IFN- $\gamma$ , and lipopolysaccharide (Fig. 1c). Moreover, the expression of PPAR- $\gamma$  and TNF- $\alpha$  in the RA synovium showed opposite trends, and there was some overlap, as shown in Fig. 1d. PPAR- $\gamma$  expression was also downregulated in TNF- $\alpha$ -induced RA FLS (Supplementary Fig. S1b). *PPAR- $\gamma$*  mRNA levels were negatively correlated with RF, ESR, DAS28-ESR and DAS28-CRP in RA PBMCs (*P* < 0.05) (Fig. 1e). However, *PPAR- $\gamma$*  mRNA levels were negatively correlated with anti-CCP and CRP levels (*P* > 0.05) (Supplementary Fig. S1c). Surprisingly, PPAR- $\gamma$  mRNA levels were also negatively correlated with *TNF- $\alpha$* , *IL-1 $\beta$* , *IL-6*, *IL-8* and *IL-17A*, in addition to *IL-10* (*P* < 0.05) (Supplementary Fig. S1c).

#### Knockout of PPAR- $\gamma$ promoted TNF- $\alpha$ -induced FLS activation and inflammation in RA

Next, we established PPAR- $\gamma$ -knockout (*Ppar- $\gamma$ <sup>+/-</sup>*) mice via GemPharmatech with CRISPR/Cas9. WB showed that PPAR- $\gamma$  protein expression was reduced in *Ppar- $\gamma$ <sup>+/-</sup>* mice (Fig. 2a). Joint scoring showed that CIA *Ppar- $\gamma$ <sup>+/-</sup>* mice had exacerbated swelling in the wrists and ankles (Fig. 2b). Moreover, H&E staining suggested that inflammatory cell infiltration and hyperplasia of the synovium were increased in CIA *Ppar- $\gamma$ <sup>+/-</sup>* mouse ankle joints (Fig. 2c). TNF- $\alpha$  expression was also upregulated in the synovium of *Ppar- $\gamma$ <sup>+/-</sup>* mice (Fig. 2d). Moreover, the secretion of IL-1 $\beta$ , IL-6 and TNF- $\alpha$  was increased in CIA *Ppar- $\gamma$ <sup>+/-</sup>* mouse blood.

We also downregulated the expression of PPAR- $\gamma$  by T0070907 or *si-PPAR- $\gamma$*  in TNF- $\alpha$ -induced RA FLS (Supplementary Fig. S2a). WB and RT-qPCR showed that IL-1 $\beta$  and IL-6 expression was upregulated by T0070907 or *si-PPAR- $\gamma$*  in TNF- $\alpha$ -induced RA FLS (Supplementary Figs. S2a and S2b). Moreover, IL-6 and IL-8 secretion was upregulated by T0070907 or *si-PPAR- $\gamma$*  in FLS (Supplementary Fig. S2c). Notably, the expression of the proinflammatory cytokines *IL-1 $\beta$* , *IL-6* and *IL-8*, the chemokines C-C motif chemokine ligand (*CCL-2*, *CCL-3* and *CCL-8*), and MMPs (*MMP-3* and *MMP-9*) in TNF- $\alpha$ -induced FLS was increased by T0070907 or *si-PPAR- $\gamma$* , as shown by WB and RT-qPCR, except for

*IL-10* and *TIMP-1* (Supplementary Figs. S2b and S2d). Moreover, T0070907 or *si-PPAR- $\gamma$*  increased the migration of FLS exposed to TNF- $\alpha$  in RA, as shown by wound-healing assays (Supplementary Fig. S2e). Thus, PPAR- $\gamma$  knockout promoted TNF- $\alpha$ -induced FLS activation and inflammation in RA.

#### PPAR- $\gamma$ overexpression inhibited inflammation and FLS activation in RA

The PPAR- $\gamma$  agonists pioglitazone and rosiglitazone and the *PPAR- $\gamma$ -pcDNA3.1* overexpression vector were used to overexpress PPAR- $\gamma$  in RA FLS. WB and RT-qPCR revealed that PPAR- $\gamma$  expression was increased (Fig. 3a, b). The levels of the proinflammatory cytokines (*IL-1 $\beta$* , *IL-6* and *IL-8*) were suppressed (Fig. 3a, b) in TNF- $\alpha$ -induced FLS, whereas *IL-10* levels were increased by pioglitazone, rosiglitazone or *PPAR- $\gamma$ -pcDNA3.1*. In addition, PPAR- $\gamma$  overexpression reduced IL-6 and IL-8 secretion (Fig. 3c), as well as the expression of *CCL-2*, *CCL-3*, and *CCL-8* (Fig. 3b). Moreover, PPAR- $\gamma$  overexpression downregulated the mRNA and protein expression of *MMP-3* and *MMP-9*, whereas *TIMP-1* levels were upregulated (Fig. 3b, d), and the migration of FLS was decreased, as shown by a wound-healing assay (Fig. 3e).

To test the effects of PPAR- $\gamma$  overexpression on synovial tissues in vivo, AIA rats were intragastrically treated with pioglitazone and rosiglitazone and injected with *ad-Ppar- $\gamma$*  in the knees (Fig. 4a). PPAR- $\gamma$  expression was upregulated by pioglitazone, rosiglitazone and *ad-Ppar- $\gamma$*  in the synovial lining, as shown by WB, HIC and IF analysis (Fig. 4b, c and Supplementary Fig. S3a). Moreover, IL-1 $\beta$ , IL-6 and TNF- $\alpha$  levels were decreased by PPAR- $\gamma$  overexpression, as shown by WB and ELISA (Fig. 4b, d). Moreover, treatment reduced inflammation and infiltration in the AIA synovium (Fig. 4c), as well as paw swelling (Fig. 4e).

Next, we injected *ad-Ppar- $\gamma$*  into the ankles of *Ppar- $\gamma$ <sup>+/-</sup>* mice with CIA (Fig. 5a). After *ad-Ppar- $\gamma$*  treatment, the joint scores showed ameliorated swelling in the wrists and ankles of *Ppar- $\gamma$ <sup>+/-</sup>* CIA mice (Fig. 5b). H&E staining showed that the infiltration of inflammatory cells and hyperplasia of the synovium were reduced by *ad-Ppar- $\gamma$*  in *Ppar- $\gamma$ <sup>+/-</sup>* CIA ankle joints (Fig. 5c). Moreover, TNF- $\alpha$  expression was also downregulated in *Ppar- $\gamma$ <sup>+/-</sup>* CIA mouse synovium (Fig. 5d). IL-1 $\beta$ , IL-6 and TNF- $\alpha$  expression was reduced by *ad-Ppar- $\gamma$*  in *Ppar- $\gamma$ <sup>+/-</sup>* CIA mouse blood (Fig. 5e). In short, by overexpressing PPAR- $\gamma$  in RA, FLS inflammation and activation were inhibited.

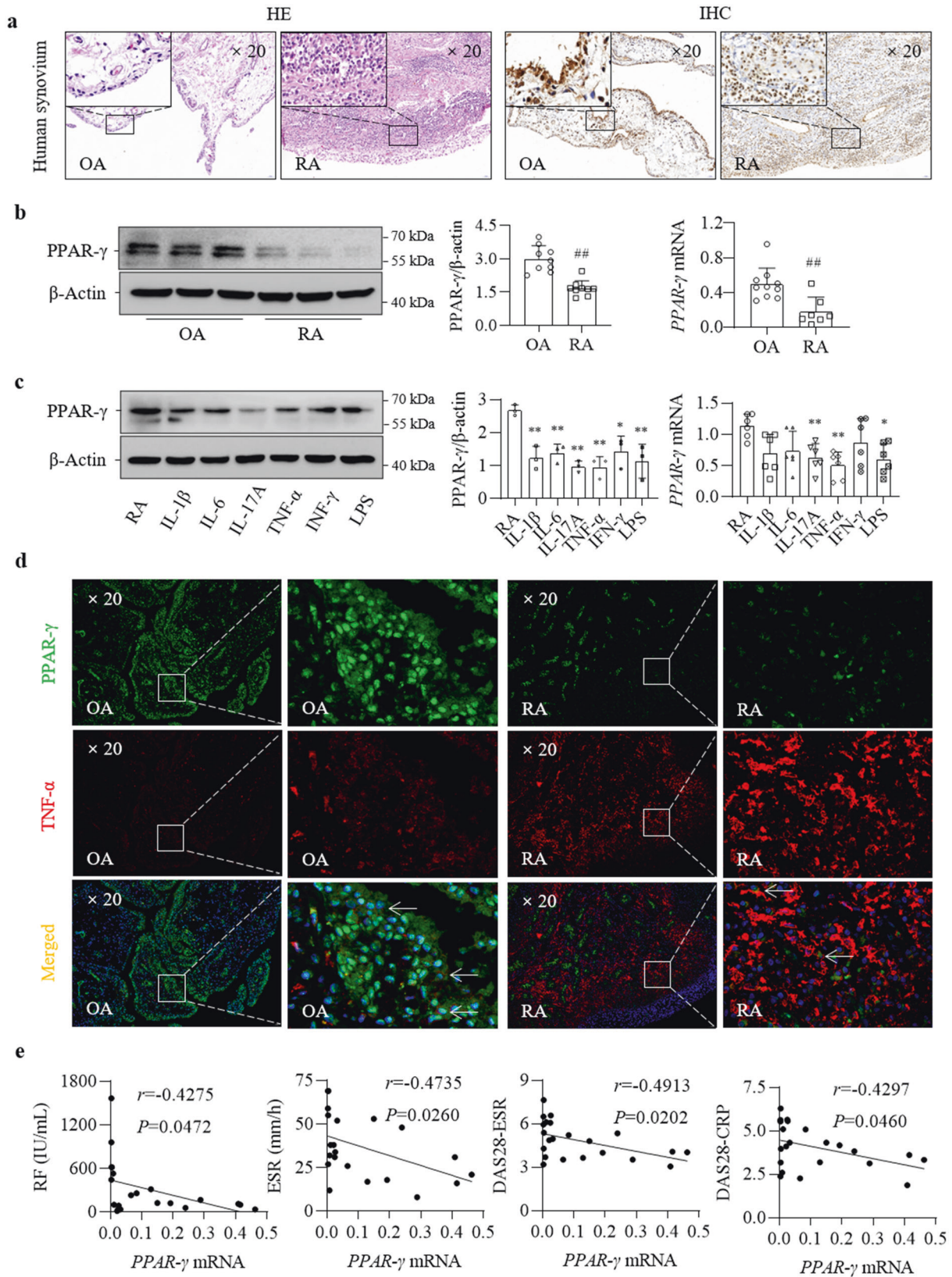
#### PPAR- $\gamma$ bound to p53 in TNF- $\alpha$ -induced FLS

To assess the mechanism of PPAR- $\gamma$  in TNF- $\alpha$ -induced FLS, we examined the gene expression profiles of FLS with *ad-PPAR- $\gamma$*  by RNA-seq. After infection with human *ad-PPAR- $\gamma$* , 101 genes were significantly downregulated, and 170 genes were significantly upregulated in TNF- $\alpha$ -induced FLS (Fig. 6a, b). We also found that many inflammatory factors, chemokines and MMPs were reduced by *ad-PPAR- $\gamma$* , such as *IL-21*, *IL-26*, *CCL13*, *CCL16* and *MMP28* (Fig. 6c). KEGG pathway analysis showed that PPAR- $\gamma$  overexpression was closely related to the p53 signaling pathway in FLS (Fig. 6d). Co-IP showed that the p53 protein bound to PPAR- $\gamma$  in FLS (Fig. 6e). PPAR- $\gamma$  overexpression with *ad-PPAR- $\gamma$*  enhanced p53 expression and reduced p-p53 expression in TNF- $\alpha$ -induced FLS. On the other hand, silencing p53 with *p53-siRNA* did not change the mRNA level of *PPAR- $\gamma$* ; however, silencing p53 increased chemokine (*CCL-2*, *CCL-3* and *CCL-8*) and proinflammatory cytokine (*IL-1 $\beta$* , *IL-6* and *IL-8*) levels (Supplementary Fig. S4a). Thus, PPAR- $\gamma$  alleviated the inflammatory response of TNF- $\alpha$ -induced FLS by binding to p53 in RA (Supplementary Fig. S4b).

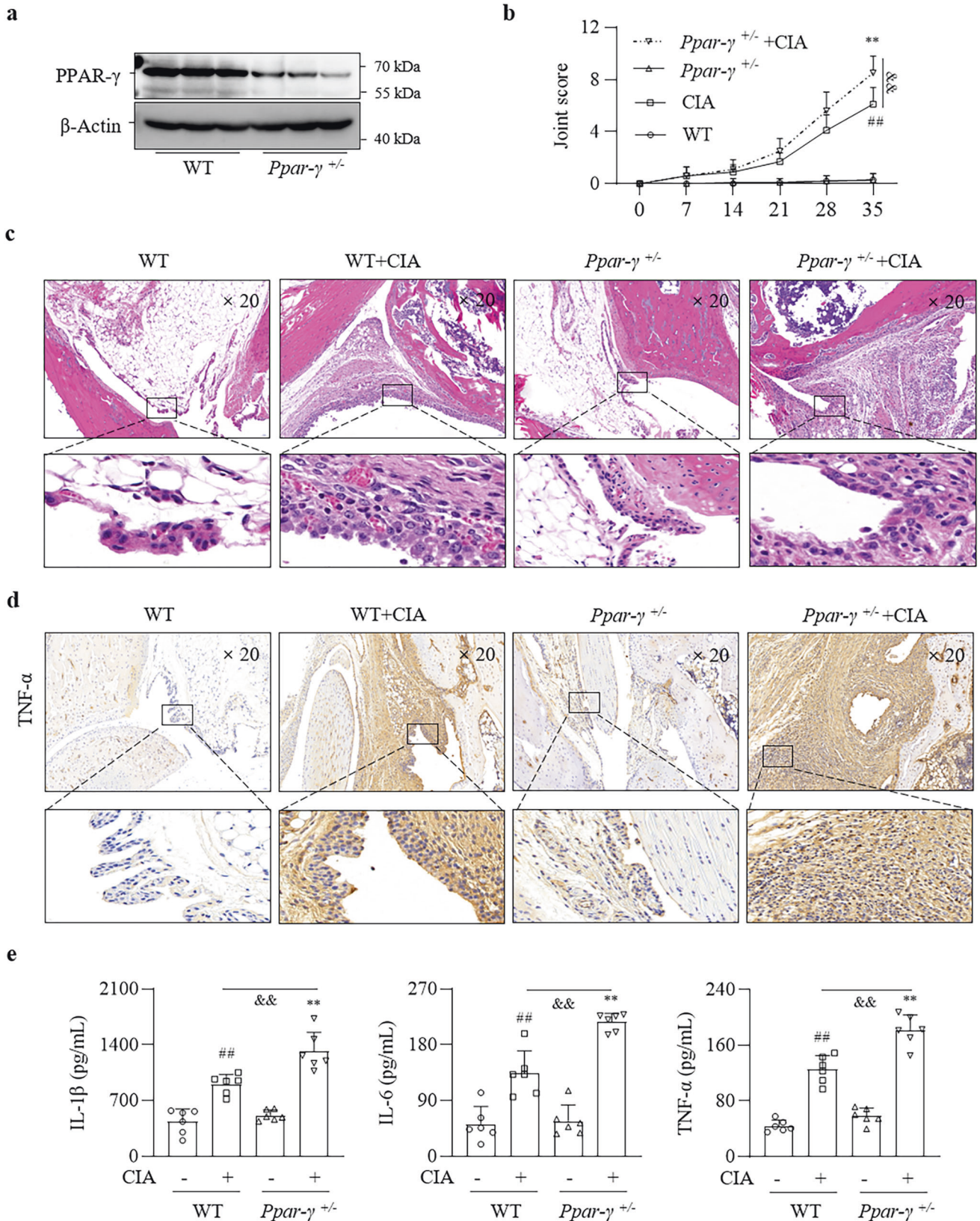
## DISCUSSION

In this study, we confirmed that PPAR- $\gamma$  expression was downregulated in human RA FLS. We also found that knocking out PPAR- $\gamma$  expression increased ankle inflammation in *Ppar- $\gamma$ <sup>+/-</sup>* CIA



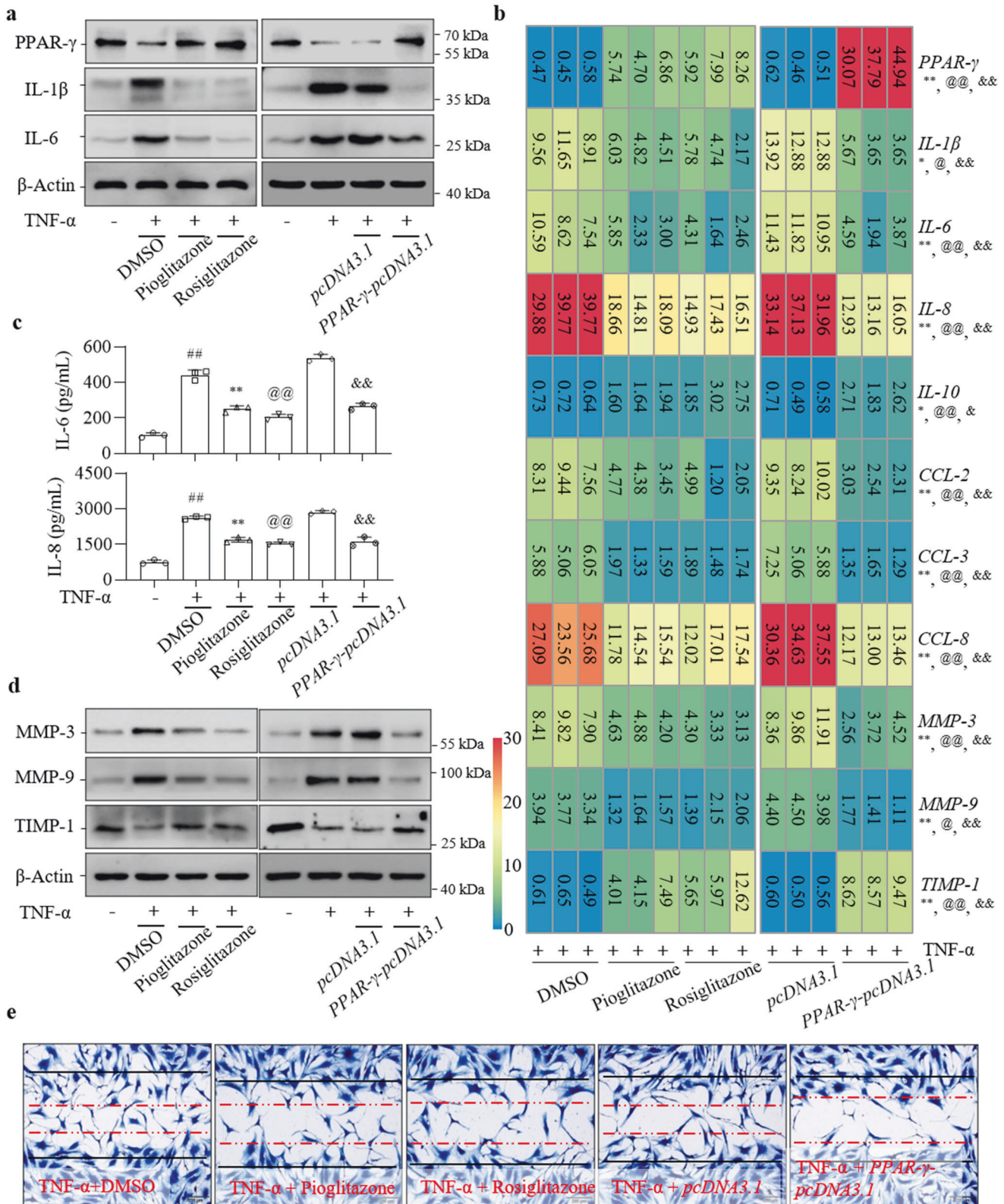


**Fig. 1** PPAR- $\gamma$  and TNF- $\alpha$  expression showed opposite trends in RA FLS. **a** Representative images showing PPAR- $\gamma$  expression in the synovium by H&E staining and IHC (20 $\times$ ). PPAR- $\gamma$  levels were examined by WB and RT-qPCR (**b**) after treatment with IL-1 $\beta$  (2 ng/mL), IL-6 (5 ng/mL), IL-17A (10 ng/mL), TNF- $\alpha$  (10 ng/mL), IFN- $\gamma$  (10 ng/mL), and lipopolysaccharide (LPS; 1  $\mu$ g/mL) (**c**). **d** PPAR- $\gamma$  and TNF- $\alpha$  expression in the synovium was analyzed by IF analysis (20 $\times$ ). **e** Correlation analysis of PPAR- $\gamma$  mRNA and RF, ESR, DAS28-ESR and DAS28-CRP levels in RA PBMCs. The values represent the mean  $\pm$  SD of 3 different samples. ## $P$  < 0.01 vs. OA group; \* $P$  < 0.05, \*\* $P$  < 0.01 vs. RA group.

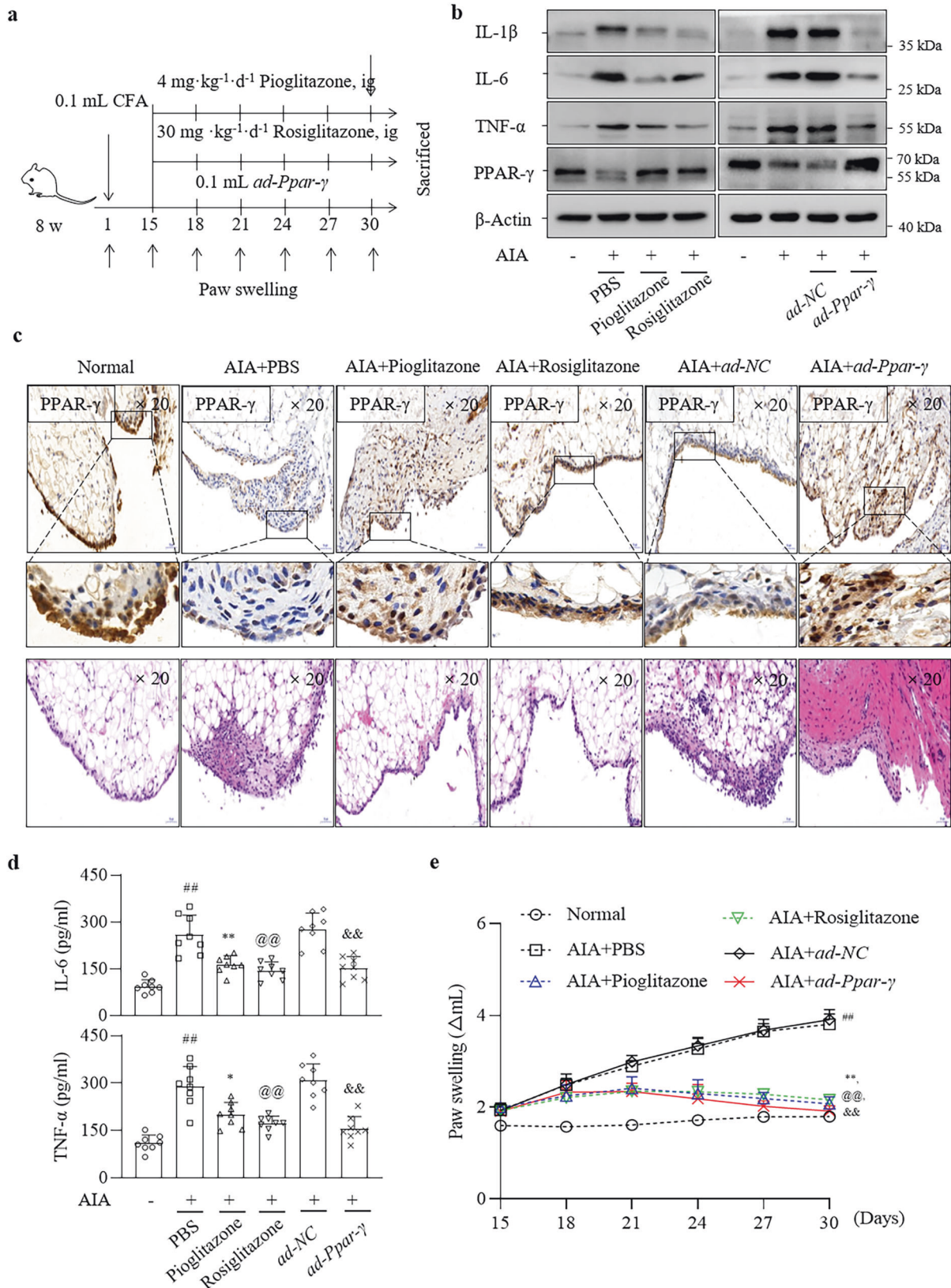


**Fig. 2** *Ppar- $\gamma$ <sup>+/-</sup>* CIA mice exhibited exacerbated joint inflammation. **a** PPAR- $\gamma$  expression in the mouse synovium. **b** Mouse joint scores. **c** Representative images showing H&E staining of the synovium (20 $\times$ ). **d** Representative images showing TNF- $\alpha$  expression by IHC (20 $\times$ ). **e** IL-1 $\beta$ , IL-6 and TNF- $\alpha$  protein levels in mouse blood were examined by ELISA. The values represent the mean  $\pm$  SD of 10 different samples.  $\#\#P < 0.01$  vs. WT group;  $\ast\ast P < 0.01$  vs. *Ppar- $\gamma$ <sup>+/-</sup>* group;  $\&\&P < 0.01$  vs. CIA group.



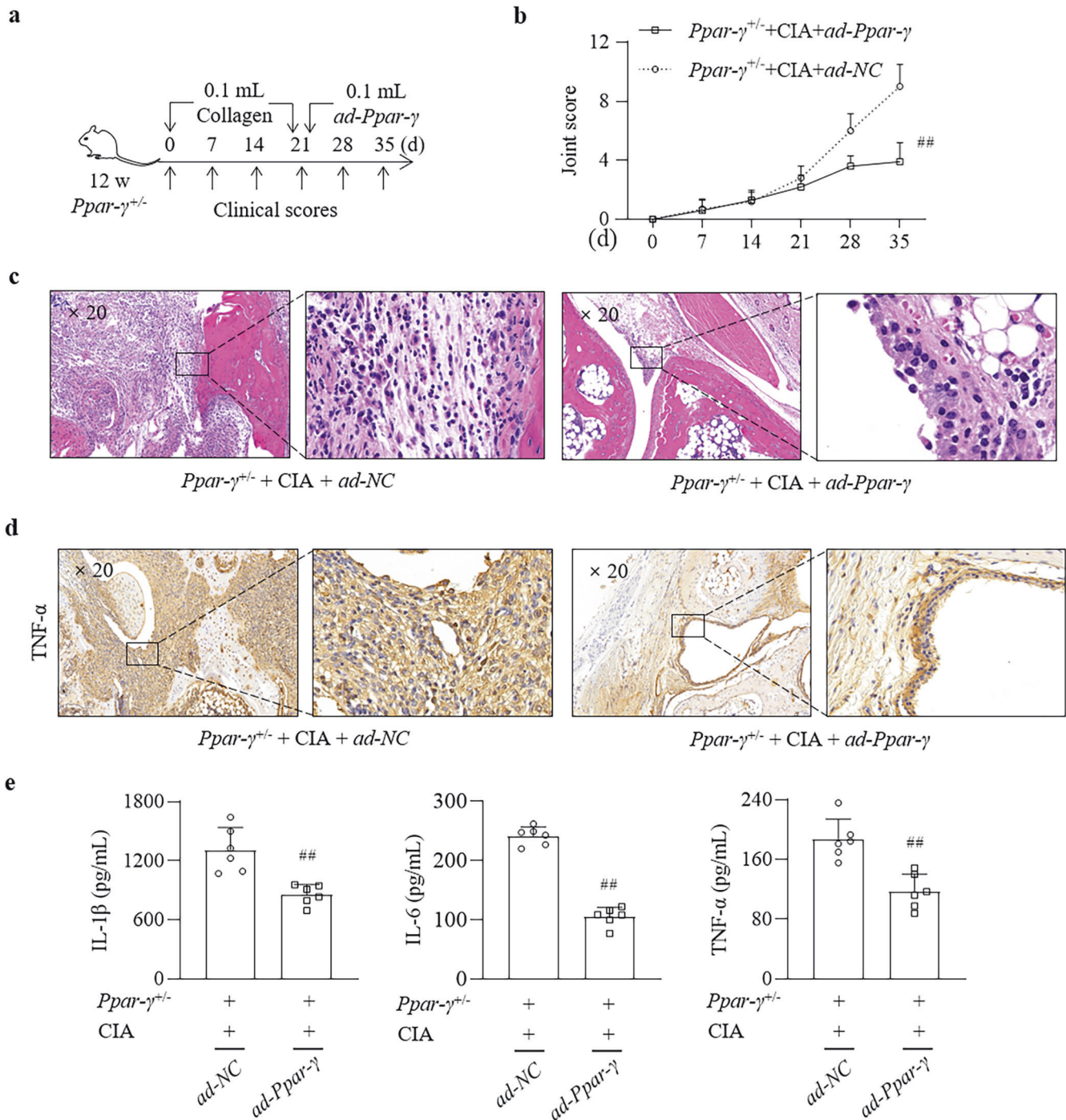


**Fig. 3** PPAR- $\gamma$  overexpression inhibits inflammation and activation of TNF- $\alpha$ -induced FLS in RA. **a** PPAR- $\gamma$ , IL-1 $\beta$  and IL-6 protein expression in FLS was analyzed by WB. **b** PPAR- $\gamma$ , IL-1 $\beta$ , IL-6, IL-8, IL-10, CCL-2, CCL-3, CCL-8, MMP-3, MMP-9 and TIMP-1 mRNA expression in FLS was analyzed by RT-qPCR. **c** IL-6 and IL-8 protein levels in RA FLS supernatant were examined by ELISA. **d** MMP-3, MMP-9 and TIMP-1 protein expression was analyzed by WB. **e** The migration of FLS was examined by wound-healing assays (20 $\times$ ). The values represent the mean  $\pm$  SD of 3 different samples. ## $P$  < 0.01 vs. RA group; \* $P$  < 0.05, \*\* $P$  < 0.01, @ $P$  < 0.05, @@ $P$  < 0.01 vs. TNF- $\alpha$  group; && $P$  < 0.01 vs. pcDNA3.1 group.



**Fig. 4 PPAR- $\gamma$  overexpression suppresses inflammation in the joints of AIA rats.** **a** The schematic shows the delivery method of pioglitazone, rosiglitazone and *ad-Ppar- $\gamma$*  in AIA rats. **b** IL-1 $\beta$ , IL-6, TNF- $\alpha$  and PPAR- $\gamma$  protein expression in the synovium of rat knee joints was analyzed by WB. **c** Representative images of PPAR- $\gamma$  in the synovium were examined by IHC and H&E staining (20 $\times$ ). **d** IL-6 and TNF- $\alpha$  protein levels in rat blood examined by ELISA. **e** Paw swelling. The values represent the mean  $\pm$  SD of 10 different samples. ## $P$  < 0.01 vs. normal group; \* $P$  < 0.05, \*\* $P$  < 0.01, @ $P$  < 0.05, @@ $P$  < 0.01 vs. AIA group; & $P$  < 0.05, && $P$  < 0.01 vs. *ad-NC* group.



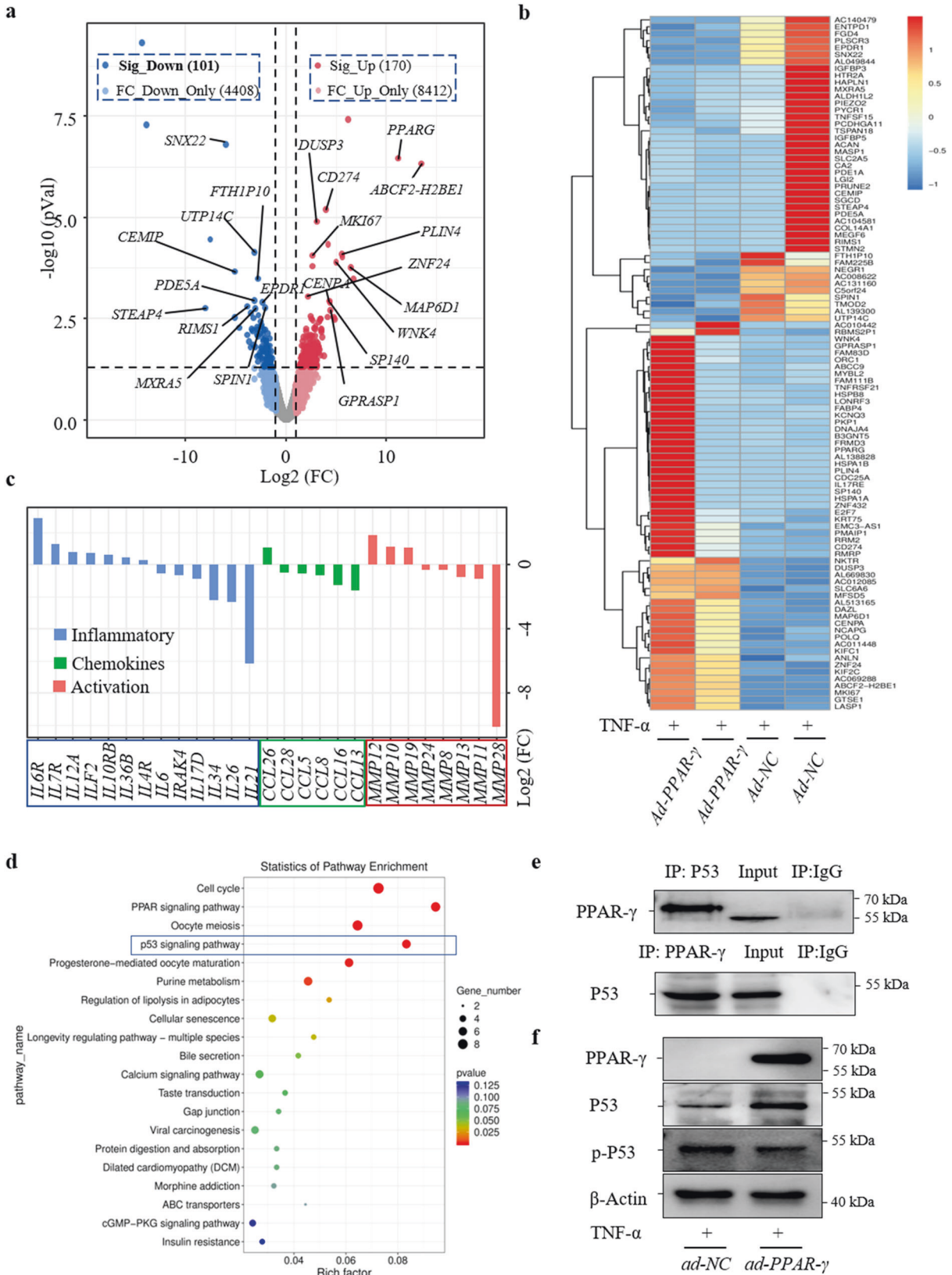


**Fig. 5 PPAR- $\gamma$  overexpression suppresses inflammation in the joints of *Ppar- $\gamma$ <sup>+/-</sup> CIA mice.*** **a** The schematic shows the delivery of *ad-Ppar- $\gamma$*  in *Ppar- $\gamma$ <sup>+/-</sup> CIA mice. b Joint scores in CIA mice. **c** Representative images showing H&E staining in the synovium (20 $\times$ ). **d** Representative images showing TNF- $\alpha$  expression by IHC (20 $\times$ ). **e** IL-1 $\beta$ , IL-6 and TNF- $\alpha$  protein levels in mouse blood were examined by ELISA. The values represent the mean  $\pm$  SD of 10 different samples. ##*P* < 0.01 vs. *Ppar- $\gamma$ <sup>+/-</sup> CIA group.**

mice and promoted RA FLS activation and inflammation in the presence of T0070907 or *si-PPAR- $\gamma$* . Moreover, PPAR- $\gamma$  overexpression reduced paw swelling and inflammation after intragastric administration of pioglitazone and rosiglitazone and the injection of *ad-Ppar- $\gamma$*  in AIA rats in vivo and decreased FLS activation and inflammation in RA. Moreover, mouse *ad-Ppar- $\gamma$*  ameliorated ankle inflammation in *Ppar- $\gamma$ <sup>+/-</sup> CIA mice. Accordingly, the p53 protein bound to PPAR- $\gamma$  in RA FLS. Thus, PPAR- $\gamma$  alleviates the inflammatory response of TNF- $\alpha$ -induced FLS by binding to p53 in RA.*

There is crosstalk between macrophages and FLS in the RA synovium [17, 18]. TNF- $\alpha$  derived from macrophages can activate FLS, leading to inflammation and FLS exacerbation in the occurrence and development of RA [19]. Activated FLS infiltrate and recruit monocytes and macrophages through the release of proinflammatory cytokines, chemokines, cell adhesion molecules and extracellular matrix proteins in RA [20, 21]. FLS are not targeted with therapeutics; however, inhibiting cytokines and chemokines produced by FLS is a potential treatment strategy for RA. PPAR- $\gamma$  was shown to preserve OA by promoting demethylation in mice [22]. We previously reported





**Fig. 6** PPAR- $\gamma$  binds to p53 in TNF- $\alpha$ -induced FLS. **a** Volcano plot showing gene expression alterations in TNF- $\alpha$ -induced FLS treated with ad-PPAR- $\gamma$ . **b** A heatmap showing representative significantly altered genes ( $P < 0.05$ ) in TNF- $\alpha$ -induced FLS treated with ad-PPAR- $\gamma$ . **c** A histogram showing inflammatory cytokine, chemokine and MMP genes in FLS. **d** KEGG pathway analysis showed that the p53 signaling pathway participates in PPAR- $\gamma$ -mediated regulation of TNF- $\alpha$ -induced FLS. **e** The p53 protein bound to PPAR- $\gamma$ , as shown by Co-IP. **f** PPAR- $\gamma$ , p53 and p-p53 expression in TNF- $\alpha$ -induced FLS was analyzed by WB.

that PPAR- $\gamma$  overexpression could inhibit FLS proliferation and migration in AIA rats [8]. In the present work, PPAR- $\gamma$  was downregulated in the synovium, and there was a close relationship between PPAR- $\gamma$  and TNF- $\alpha$  expression in the RA synovium and blood in vitro and in vivo. Importantly, PPAR- $\gamma$  mRNA levels were negatively correlated with RF, ESR, DAS28-ESR, DAS28-CRP and TNF- $\alpha$  levels in RA PBMCs ( $P < 0.05$ ). These results suggested that PPAR- $\gamma$  might be involved in the inflammatory response in FLS induced by TNF- $\alpha$ .

To clarify the role of PPAR- $\gamma$  in RA, we investigated FLS activation, inflammation and chemokine production in the presence of TNF- $\alpha$  in RA. During the development of RA, FLS activation is characterized by invasion and migration, and these cells contact cartilage and induce osteoclast formation through activation. Similarly, various cytokines produced by FLS and macrophages form a complex network and exert physiological effects through overlapping and mutual regulation in RA [23, 24]. Chemokine-induced FLS activation has been shown to exacerbate inflammation and bone destruction [19]. In the present study, Ppar- $\gamma^{+/-}$  mice were established with CRISPR/Cas9. We found that Ppar- $\gamma^{+/-}$  CIA mice had exacerbated swelling of the wrists and ankles and increased inflammatory cell infiltration and hyperplasia of the synovium. Moreover, TNF- $\alpha$  expression was upregulated in Ppar- $\gamma^{+/-}$  mouse synovium and blood in vivo. Knocking out PPAR- $\gamma$  expression with T0070907 or si-PPAR- $\gamma$  promoted FLS activation (MMP-3 and MMP-9), inflammation (IL- $\beta$ , IL-6 and IL-8) and chemokine production (CCL-2, CCL-3 and CCL-8) in the presence of TNF- $\alpha$  in RA in vitro. Thus, PPAR- $\gamma$  knockdown intensified TNF- $\alpha$ -induced FLS activation and the inflammatory response in RA.

PPAR- $\gamma$  overexpression by pioglitazone, rosiglitazone or PPAR- $\gamma$ -pcDNA3.1 reduced the activation, inflammation and chemokine expression of TNF- $\alpha$ -induced FLS in RA in vitro. Moreover, inflammatory cell infiltration into the synovium and secondary swelling was reduced by pioglitazone, rosiglitazone and ad-Ppar- $\gamma$  in AIA rats in vivo, while serum levels of inflammatory cytokines (IL-1 $\beta$ , IL-6 and TNF- $\alpha$ ) were decreased. Moreover, after ad-Ppar- $\gamma$  administration in Ppar- $\gamma^{+/-}$  mice, swelling of the wrists and ankles, inflammatory cell infiltration and hyperplasia of the synovium were reversed, and inflammatory cytokines were reduced in CIA mice. Therefore, PPAR- $\gamma$  overexpression relieved TNF- $\alpha$ -induced FLS activation and the inflammatory response in RA.

In this study, we examined the gene expression profiles of FLS with ad-PPAR- $\gamma$  by RNA-seq and found that 101 genes were significantly downregulated and 170 genes were significantly upregulated in RA. TNF- $\alpha$  is one of the factors that prevents self-injury, and it can also cause the pathogenesis of RA. In this study, RA FLS were activated by TNF- $\alpha$  to simulate the inflammatory environment of synovial tissues and examine the specific mechanism of PPAR- $\gamma$ . We found that PPAR- $\gamma$  overexpression was closely related to the p53 signaling pathway in FLS by KEGG pathway analysis. Hennigs *et al.* showed that PPAR- $\gamma$  mediated the vasculoregenerative program to reverse pulmonary hypertension via p53 [13]. Moreover, the p53 protein was shown to bind to PPAR- $\gamma$  by Co-IP, and this binding was reduced by ad-PPAR- $\gamma$ . Proinflammatory cytokines and chemokines were increased by p53-siRNA in TNF- $\alpha$ -induced FLS. Consistently, p53 regulates macrophage polarization [25] and promotes inflammatory signals by suppressing IL-1 $\beta$  expression [26]. On the other hand, PPAR- $\gamma$  plays an important role in type 2 diabetes, fat synthesis and inflammation. We hypothesize that patients with clinical complications of RA and diabetes can be treated with NSAIDs combined with PPAR- $\gamma$  agonists such as pioglitazone to control the disease. However, we lack such conditions. Taken together, our results indicate that PPAR- $\gamma$  alleviates the inflammatory response of TNF- $\alpha$ -induced RA FLS by binding to p53.

In conclusion, TNF- $\alpha$ , which is one of the activators of FLS, plays a key role in the development of RA. We found that PPAR- $\gamma$  could inhibit FLS activation and inflammatory factors through the p53 pathway, thus improving the immune response of patients with

RA. Based on these findings, therapeutic strategies targeting PPAR- $\gamma$  may be effective in preventing the development of RA.

## ACKNOWLEDGEMENTS

This study was supported by grants from the National Natural Science Foundation of China (No. 82002269), the Anhui Provincial Science and Technology Major Project (No. 8212929035), the China Postdoctoral Science Foundation (No. 2020M671839), and the Postdoctoral Science Foundation from Anhui Medical University (No. BSH201902).

## AUTHOR CONTRIBUTIONS

XFL performed all experiments, analyzed the data, and wrote the manuscript. SQY and HL helped to collect the RA and OA synovial tissue samples. YLY, SW, YYW, XC, and BS helped to isolate FLS and assisted with WB, RT-qPCR analysis. HW and JL conceived the study and revised the manuscript. All authors approved the final manuscript.

## ADDITIONAL INFORMATION

**Supplementary information** The online version contains supplementary material available at <https://doi.org/10.1038/s41401-022-00957-9>.

**Competing interests:** The authors declare no competing interests.

## REFERENCES

- Smolen JS, Aletaha D, Barton A, Burmester GR, Emery P, Firestein GS, et al. Rheumatoid arthritis. *Nat Rev Dis Prim.* 2018;4:18001.
- de Oliveira PG, Farinon M, Sanchez-Lopez E, Miyamoto S, Guma M. Fibroblast-like synoviocytes glucose metabolism as a therapeutic target in rheumatoid arthritis. *Front Immunol.* 2019;10:1743.
- Muntyanu A, Abji F, Liang K, Pollock RA, Chandran V, Gladman DD. Differential gene and protein expression of chemokines and cytokines in synovial fluid of patients with arthritis. *Arthritis Res Ther.* 2016;18:296.
- Croft AP, Campos J, Jansen K, Turner JD, Marshall J, Attar M, et al. Distinct fibroblast subsets drive inflammation and damage in arthritis. *Nature.* 2019;570:246–51.
- Loh C, Park SH, Lee A, Yuan R, Ivashkiv LB, Kalliolias GD. TNF-induced inflammatory genes escape repression in fibroblast-like synoviocytes: transcriptomic and epigenomic analysis. *Ann Rheum Dis.* 2019;78:1205–14.
- Ross EA, Devitt A, Johnson JR. Macrophages: the good, the bad, and the gluttony. *Front Immunol.* 2021;12:708186.
- Bottini N, Firestein GS. Duality of fibroblast-like synoviocytes in RA: passive responders and imprinted aggressors. *Nat Rev Rheumatol.* 2013;9:24–33.
- Li XF, Sun YY, Bao J, Chen X, Li YH, Yang Y, et al. Functional role of PPAR-gamma on the proliferation and migration of fibroblast-like synoviocytes in rheumatoid arthritis. *Sci Rep.* 2017;7:12671.
- Kawahito Y, Kondo M, Tsubouchi Y, Hashiramoto A, Bishop-Bailey D, Inoue K, et al. 15-deoxy-delta(12,14)-PGJ(2) induces synoviocyte apoptosis and suppresses adjuvant-induced arthritis in rats. *J Clin Invest.* 2000;106:189–97.
- Ju Z, Su M, Hong J, Kim E, Jung JH. Anti-inflammatory effects of an optimized PPAR-gamma agonist via NF-kappaB pathway inhibition. *Bioorg Chem.* 2020;96:103611.
- Liu Y, Wang J, Luo S, Zhan Y, Lu Q. The roles of PPARgamma and its agonists in autoimmune diseases: a comprehensive review. *J Autoimmun.* 2020;113:102510.
- Miao Y, Zheng Y, Geng Y, Yang L, Cao N, Dai Y, et al. The role of GLS1-mediated glutaminolysis/2-HG/H3K4me3 and GSH/ROS signals in Th17 responses counteracted by PPARgamma agonists. *Theranostics.* 2021;11:4531–48.
- Hennigs JK, Cao A, Li CG, Shi M, Mienert J, Miyagawa K, et al. PPARgamma-p53-mediated vasculoregenerative program to reverse pulmonary hypertension. *Circ Res.* 2021;128:401–18.
- Li XF, Chen X, Bao J, Xu L, Zhang L, Huang C, et al. PTEN negatively regulates the expression of pro-inflammatory cytokines and chemokines of fibroblast-like synoviocytes in adjuvant-induced arthritis. *Artif Cells Nanomed Biotechnol.* 2019;47:3687–96.
- Li XF, Song B, Xu QQ, Yin SQ, Chen X, Xu L, et al. Over-expression of PTEN suppresses the proliferation and migration of fibroblast-like synoviocytes in adjuvant-induced arthritis. *Cell Physiol Biochem.* 2019;52:1446–62.
- Li XF, Wu S, Yan Q, Wu YY, Chen H, Yin SQ, et al. PTEN methylation promotes inflammation and activation of fibroblast-like synoviocytes in rheumatoid arthritis. *Front Pharmacol.* 2021;12:700373.



17. Nygaard G, Firestein GS. Restoring synovial homeostasis in rheumatoid arthritis by targeting fibroblast-like synoviocytes. *Nat Rev Rheumatol*. 2020;16:316–33.
18. Wu YY, Li XF, Wu S, Niu XN, Yin SQ, Huang C, et al. Role of the S100 protein family in rheumatoid arthritis. *Arthritis Res Ther*. 2022;24:35.
19. Zhang Y, Aldridge J, Vasileiadis GK, Edebo H, Ekwall AH, Lundell AC, et al. Recombinant adiponectin induces the production of pro-inflammatory chemokines and cytokines in circulating mononuclear cells and fibroblast-like synoviocytes from non-inflamed subjects. *Front Immunol*. 2020;11:569883.
20. Cao C, Wu F, Niu X, Hu X, Cheng J, Zhang Y, et al. Cadherin-11 cooperates with inflammatory factors to promote the migration and invasion of fibroblast-like synoviocytes in pigmented villonodular synovitis. *Theranostics*. 2020;10:10573–88.
21. Frey O, Huckel M, Gajda M, Petrow PK, Brauer R. Induction of chronic destructive arthritis in SCID mice by arthritogenic fibroblast-like synoviocytes derived from mice with antigen-induced arthritis. *Arthritis Res Ther*. 2018;20:261.
22. Zhu X, Chen F, Lu K, Wei A, Jiang Q, Cao W. PPARgamma preservation via promoter demethylation alleviates osteoarthritis in mice. *Ann Rheum Dis*. 2019;78:1420–9.
23. Umar S, Palasiewicz K, Volin MV, Zanotti B, Al-Awqati M, Sweiss N, et al. IRAK4 inhibitor mitigates joint inflammation by rebalancing metabolism malfunction in RA macrophages and fibroblasts. *Life Sci*. 2021;287:120114.
24. Choi BY, Choi Y, Park JS, Kang LJ, Baek SH, Park JS, et al. Inhibition of Notch1 induces population and suppressive activity of regulatory T cell in inflammatory arthritis. *Theranostics*. 2018;8:4795–804.
25. Mukhopadhyay S, Antalis TM, Nguyen KP, Hoofnagle MH, Sarkar R. Myeloid p53 regulates macrophage polarization and venous thrombus resolution by inflammatory vascular remodeling in mice. *Blood*. 2017;129:3245–55.
26. Ubertini V, Norelli G, D'Arcangelo D, Gurtner A, Cesareo E, Baldari S, et al. Mutant p53 gains new function in promoting inflammatory signals by repression of the secreted interleukin-1 receptor antagonist. *Oncogene*. 2015;34:2493–504.

Springer Nature or its licensor holds exclusive rights to this article under a publishing agreement with the author(s) or other rightsholder(s); author self-archiving of the accepted manuscript version of this article is solely governed by the terms of such publishing agreement and applicable law.