Title: **Telmisartan attenuates the inflamed mesenteric adipose tissue in spontaneous colitis by mechanisms involving regulation of neurotensin/microRNA-155 pathway**

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Key Words: Crohn's disease; mesenteric adipose tissue; neurotensin; microRNA-155; telmisartan

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ABSTRACT

Mesenteric adipose tissue hypertrophy is unique to Crohn's disease while the molecular basis of the crosstalk between MAT and the intestinal inflammation is largely unknown. Telmisartan is an angiotensin II type 1 receptor blocker and a peroxisome proliferator-activated receptor- receptor-γagonist which has beneficial effects on fat distribution and pro-inflammatory adipokine expression. We evaluated the effect of telmisartan upon mesenteric adipose tissue alterations and inflammatory features in IL-10$^{-/-}$ mice. We found treatment with telmisartan significantly ameliorated the severity of colitis in IL-10$^{-/-}$ mice. Additionally, administration of telmisartan was associated with restoration of mesenteric adipose tissue adipocyte morphology and the expression of adipokines. Furthermore, telmisartan treatment suppressed the neurotensin/microRNA-155 pathway in mesenteric adipose tissue from spontaneous colitis which was confirmed by an in vitro study using cultured mesenteric adipose tissue from Crohn's disease patients. Administration of telmisartan showed promising results in spontaneous colitis which was associated with the attenuated mesenteric adipose tissue alteration which at least in part, was associated with its activity in regulation the neurotensin/microRNA-155 pathway. These results support the hypothesis that regulating the abnormal immune response in adipose tissue is an important target for the treatment of Crohn's disease.

KEY WORDS

Crohn's disease; mesenteric adipose tissue; neurotensin; microRNA-155

ABBREVIATIONS

CD, Crohn's disease; DAI, disease activity index; IBD, inflammatory bowel disease; IL-10$^{-/-}$, interleukin-10 deficient; MAT, mesenteric adipose tissue; miR-155, microRNA-155; MPO, myeloperoxidase; PPAR-γ, peroxisome proliferator-activated receptor- receptor-γ; SAA, serum amyloid A; SAT, subcutaneous adipose tissue; UC, ulcerative colitis; WT, wild type.

1. INTRODUCTION

Since the middle of the 20th century, two main forms inflammatory bowel disease (IBD): Crohn's disease (CD) and ulcerative colitis (UC), are increased worldwide [1, 2]. The cause of IBD is relative complex and could be linked to a combination of factors such as gene background, microbiota, immunological mechanisms, and infections [3]. Although most patients with CD are underweight, the ratio of intra-abdominal adipose tissue to total abdominal fat in CD patients is far greater than that in controls [4]. Mesenteric fat have been shown to be an important indicator of intestinal inflammation in CD patients before the concept of adipose tissue as an immune organ.[5] Fat wrapping is considered to be of significant importance and is a common and specific feature of CD which is found to be positively correlated with muscular hypertrophy, fibrosis, transmural inflammation, and clinical disease activity of CD [6, 7], and a high ratio of areas of visceral to subcutaneous fat is a marker of aggressive CD [8]. Mesenteric adipose tissue (MAT) is composed by different cell types such as adipocytes, preadipocytes, macrophages, endothelial cells, fibroblasts, and leukocytes. MAT from patients with CD exhibits a significant interlobular inflammatory infiltrate and the adipocyte diameter is significantly lower than that of healthy subjects [9]. Neuropeptides as well as adipokines including leptin and adiponectin, are hormone-like factors which produced by adipose tissue, can also exert pro- and anti-inflammatory effects and play an important role in the pathogenesis of IBD [2, 4, 10]. In addition, mesenteric adipocyte is demonstrated to be an important source of C reactive protein in CD [11].

Telmisartan, one of the clinically used angiotensin II type 1 receptor blocker, is also a partial agonist of peroxisome proliferator-activated receptor-γ (PPAR-γ) [12]. To date, mounting evidence supports the therapeutic and prophylactic effects of PPAR-γ activation in

multiple animal model with acute/chronic colitis induced by chemical compounds [13, 14], gut pathogens[13], as well as spontaneous colitis in severe combined immunodeficiency (SCID) mice or spontaneous colitis in IL-10-deficient (IL-10$^{-/-}$) mice [15, 16]. Unlike full agonists of PPAR-γ, partial agonists of PPAR-γ are found with the capacity to promote adipocyte differentiation [12, 17]. In addition to have a benefit in the reduction of visceral adipose tissues and the improvement of vascular inflammation [18-20], telmisartan also shows its neuroendocrine characterization by reducing leptin expression and increasing the level of adiponectin [21-23].

Increased expression of leptin in the hypertrophic MAT in CD has been reported and a proinflammatory role for leptin in CD is demonstrated [24]. The leptin responsive region is localized to the regulatory region of the neurotensin gene [25, 26], and leptin can directly induced neurotensin gene expression [27]. In an experimental colitis induced by dextran sodium sulfate (DSS), colonic neurotensin expression is elevated [28]. In addition, increased neurotensin expression is found in adipocytes of MAT of mice with trinitrobenzene sulfonic acid (TNBS)-induced colitis [29]. Additionally, neurotensin can directly stimulate the expression of microRNA-155 (miR-155) [30] which regulates the intestinal immune function [31] and is effective in inhibition of adipocyte differentiation [32-34].

Currently, few reports regarding the action of drugs upon inflammatory alterations in mesenteric MAT from IBD condition [24, 35]. IL-10$^{-/-}$ mice develop spontaneous enterocolitis [36], a process that is characterized by both pathologic T helper type 1 (Th1) and Th17 immune response [37, 38]. The aim of the present study was to elucidate the role of temisartan on MAT alteration in a mouse model with spontaneous colitis, focusing upon the

inflammatory alterations and adipocytokine production as well as the potential mechanisms such as regulation of neurotensin/miR-155 signaling.

2. MATERIALS AND METHODS

2.1. Animals

C3H/HeJBir.IL-10$^{-/-}$ mice were obtained from the Jackson Laboratory (Bar Harbor, Maine) and maintained at the Model Animal Research Center of Jinling Hospital (Nanjing, China). Under these conditions, all IL-10$^{-/-}$ mice eventually developed IBD, moderate to severe colitis in proximal, middle and distal regions were observed as early as 4 weeks old [36, 39]. Age-matched male mice were with established IBD housed in a room with limited access were used for the experiments [40-42]. All animal protocols were approved in advance by the Institutional Ethics Committee of the Jinling Hospital, Medical School of Nanjing University, Nanjing, China.

2.2. Drug and administration protocol

Sixteen-weeks-old IL-10$^{-/-}$ mice with established colitis, which were assigned to the vehicle-treated group and telmisartan-treated group. Age-matched male wild type (WT) mice were used as control group. The IL-10$^{-/-}$ mice were randomized divided into 2 groups to receive either telmisartan in drinking water (3 mg/kg/d) or drinking water alone for 12 weeks. Telmisartan (Sigma-Aldrich, St. Louis, MO) was dissolved in drinking water, and each solution was prepared on the day it was administered. To ensure delivery of the correct telmisartan doses, the drug concentrations were adjusted in the drinking water each week based on the average water consumption and body weights in each group.

2.3. General observation

At weekly intervals, the general conditions and body weight of IL-10$^{-/-}$ mice were observed. Inflammatory bowel disease activity index (DAI) was scored on a weekly basis in each IL-10$^{-/-}$ mice mouse using the numerical system described by Spencer et al [43]. Briefly, the index includes 1 point each for occult blood in stools, rectal prolapse of $<$1 mm, soft stool, and ruffled fur. An extra point was added for diarrhea or severe rectal prolapse ($>$1 mm).

2.4. Colon and fat tissue collection

After 12 weeks, mice were sacrificed under anesthesia. The entire colon tissues were collected and were carefully rinsed with PBS. Colons were weighed and colon length (from the ileocecal junction to the anal verge) were measured for assessment of morphologic change. The entire colon tissues were prepared for histological analysis and further examines. The mass of body fat was measured to evaluate changes in body fat accumulation in mice. The fat tissue of the mice were excised and weighted as described by Araki et al.[44] The weight of the mat and subcutaneous adipose tissue (SAT) were measured separately. Dissection of fat and fat tissue weighting were performed by a single person who was bind to the drug treatment groups. The tissues were then immediately frozen in liquid nitrogen and stored at -80°C until further examination.

2.5. Patient-based study

MAT from surgical specimen of patients with Crohn's colitis were collected with informed consent. All patients underwent colectomy due to medically refractory CD. The diagnosis CD was based on conventional clinical, histopathological and endoscopic criteria [45, 46]. MAT was obtained from those with active CD (n= 6) with activity index >150 and CRP >10. In CD patients, all specimens were taken from MAT next to the intestinal wall and adjacent to the

disease involved intestine. MAT cultures were prepared and cultured as previously reported [47, 48]. Briefly, specimens were gently washed three times in Hanks' balanced salt solution containing 0.2% bovine serum albumin. The pieces of tissue were then incubated in culture medium on sterile tissue culture plates at 37 ◦C in 5% CO2. To explore the direct effects of telmisartan, telmisartan (Sigma-Aldrich, St. Louis, MO) was dissolved in DMSO and added to part of the culture medium within 0.1% of volume with the concentration was 10 μM (we select this concentration treatment based on published work [49] and our pilot technical experiments). After 24-h culture, some tissue samples were collected and fixed in 10% phosphate-buffered formalin and embedded in paraffin for histological studies. Some cultured tissue and the media were immediately frozen in liquid nitrogen and stored -80C until assayed. This study was approved by the Institutional Ethics Committee of the Jinling Hospital, Medical School of Nanjing University, Nanjing, China.

2.6. Histological analysis

The colon samples and MAT near the colon from mice were fixed in bouin's fixative for 24 h and paraffin-embedded. Five-micrometer sections were stained with hematoxylin and eosin for light microscopic examination. Histopathology was evaluated by an investigator who was blinded to the identity of the samples. The colonic inflammatory scores were evaluated as described by Elliott et al [50] . Briefly, the inflammation was scored as 0–4: 0, no change from normal tissue; 1, patchy mononuclear cells infiltrates in the lamina propria; 2, more uniform mononuclear cells inflammation involving both the epithelium and lamina propria; 3, some epithelial and muscle hypertrophy with patchy lymphocytic infiltrates extending into the muscle layers; 4, lesions involved most of the intestinal section. As Zulian et al. described [9],

the adipocyte diameters were measured using optical microscopy with five randomly selected sectional areas.

2.7. Enzyme-linked immunosorbent assay (ELISA) of SAA, MPO, IFN-γ, TNF-α, IL-6 and IL-17

Colon samples, mesenteric adipose tissue, culture media the serum fraction were used for the ELISA analysis. ELISA was performed for detection of SAA, MPO, IFN-γ, TNF-α, IL-6 and IL-17 by using commercial ELISA kit (R&D Systems, Heerbrugg, MN), according to manufacturer's instruction.

2.8. Analysis of gene expression level in mesenteric adipose tissue

Quantization of the gene expression of IL-6, IL-17 neurotensin, miR-155, leptin, adiponectin, F4/80 was performed by quantitative real-time PCR (qRT-PCR). As previously reported [40, 41], total RNA were isolated with Trizol reagent (Invitrogen, Carlsbad, USA), incubated with DNase I and reverse-transcribed with TaKaRa AMV Kit (TaKaRa, Dalian, China) according to manufacturer instruction. Quantitative real-time PCR with SYBR green PCR Master Mix (Applied Biosystems, Foster City, CA, USA) was performed in the MicroAmp Fast Optical 96-Well Reaction Plate (Applied Biosystems) by using the 7300 Fast Real-Time PCR System (Applied Biosystems) for thermal cycling and real-time fluorescence measurements. The PCR cycle protocol consisted of 10 min at 95 °C, and 40 two-step cycles of 15s each at 95 °C and of 1 min at 60 °C. For each sample, all PCR reactions were done in triplicate and product amplification results were normalized to GAPDH or β-actin expression. Threshold cycle (CT) values were recorded as a measure of initial template concentration and the relative fold changes in RNA levels were calculated by the ΔΔCT method. The range for the target which

was relative to a calibrator sample was calculated by $2^{-\Delta\Delta CT}$.

2.9. Statistical Analyses

All data are expressed as the mean ±standard deviation (SD). Statistical significance of evaluated data was tested using Mann-Whitney's U-test and Student's t-test. P-values less than 0.05 were considered to be significant.

3. RESULTS

3.1. Administration of telmisartan improved clinical signs of colitis in animal model

All mice in these groups were survived. The administration of telmisartan resulted in an attenuation of signs of colitis as established by DAI, the level of serum SAA and macroscopical features of colon. In our experiment, the individual DAI which were used to monitor the therapeutic benefit of treatments were assigned one week after the administration of telmisartan. As shown in Figure 1A, telmisartan-treated IL-10$^{-/-}$ mice showed lower mean DAI values since the 8th week after drug administration when compared to untreated IL-10$^{-/-}$ mice. In addition, the SAA protein level in serum was significantly lower in telmisartan-treated mice than those in untreated IL-10$^{-/-}$ mice (Figure 1B). We next examined whether telmisartan was also effective against chronic colitis in IL-10$^{-/-}$ mice. Colon weights of IL-10$^{-/-}$ mice were increased after the development of inflammation; while treatment with telmisartan dramatically decreased this inflammatory parameter (Figure 1C). In addition, our results indicated that telmisartan-treated mice had significantly longer colons than untreated IL-10 deficient mice (Figure 1D). Thus, telmisartan had therapeutic activity in IL-10$^{-/-}$ mice with established colitis.

3.2. Treatment with telmisartan reduces chronic colonic inflammation in established IL-

10$^{-/-}$ mice

In the untreated IL-10$^{-/-}$ mice with chronic colitis, microscopically, the inflammatory infiltrate in the colon contains large numbers of lymphocytes as the predominant cell type. In contrast, administration of telmisartan improved the infiltrate of lymphocytes, restoring the histological appearance of the mucosa and submucosa (Figure 2A). The colonic inflammation scores in the IL-10$^{-/-}$ mice treated with telmisartan significantly improved when compared with the untreated mice (Figure 2B). Additionally, compared with those of untreated mice, the concentration of MPO (Figure 2C), IFN-γ and TNF-α (Figure 2D) in the colonic mucosal tissue were significantly decreased in telmisartan-treated IL-10$^{-/-}$ mice.

3.3. Effect of telmisartan on fat distribution and mesenteric adipose tissue morphology

Our data indicated, the weight of MAT in untreated IL-10$^{-/-}$ mice group was higher than those in the control WT mice group (Figure 3A). Telmisartan treatment decreased MAT weights compared with those in the non-treated IL-10$^{-/-}$ mice (Figure 3A). Interestingly, the ratio of MAT/SAT was higher in the non-treated IL-10$^{-/-}$ mice than in the control wild type mice. However, telmisartan administration significantly decreased the ratio of MAT/SAT in IL-10$^{-/-}$ mice (Figure 3B).

We next observed the morphology of MAT. H&E staining of MAT revealed the presence of fibroblastic, inflammatory cells, and adipocytes in IL-10$^{-/-}$ mice (Figure 3C). Moreover, it was revealed that the adipocytes have a smaller size compared with adipocytes from normal mesenteric adipose tissue in WT mice. Interestingly, our data indicated IL-10$^{-/-}$ mice with telmisartan administration had a significant increased diameter of adipocyte when compared with those untreated (Figure 3D). Significant inflammatory infiltration of lymphatic cells and

macrophages were observed in IL-10$^{-/-}$ mice, however, this was attenuated by telmisartan administration. Indeed, the effect was confirmed by the result of decreased expression of F4/80 (Figure 3E) [29, 51].

3.4. Treatment with telmisartan attenuated the MAT inflammation

In MAT, our in vivo study indicated both IL-6 and IL-17 expression were low in untreated IL-10$^{-/-}$ mice compared with those in WT mice. IL-10$^{-/-}$ mice receiving telmisartan had a reduced IL-6 and IL-17 mRNA expression compared with IL-10$^{-/-}$ mice without telmisartan therapy (Figure 4A). In addition, leptin expression was significantly increased in MAT from IL-10$^{-/-}$ mice compared with control WT mice (Figure 4B). In IL-10$^{-/-}$ mice treated with telmisartan, a reduced leptin expression was found compared with untreated IL-10$^{-/-}$ mice (Figure 4B). In contrast, adiponectin expression was quite low from MAT in IL-10$^{-/-}$ mice compared with WT mice. However, IL-10$^{-/-}$ mice receiving telmisartan displayed a significantly higher adiponectin expression than those without telmisartan treatment (Figure 4B).

It was important to determine whether telmisartan would directly regulate the adipokines expression and cytokines secretion in disease affected MAT from CD. We then determined the effect of telmisartan on the expression of leptin and adiponectin as well as the secretion of IL-6 and IL-17 in mesenteric adipose tissue from patients maintained in short-term organ culture. Similar to the in vivo study, the expression of adiponectin (Figure 4C) was increased and the levels of leptin (Figure 4C), IL-6 (Figure 4D), and IL-17 (Figure 4E) were decreased significantly in MAT cultured with telmisartan.

3.5. Telmisartan therapy repressed the neurotensin/miR-155 signaling in MAT

We next examine the neurotensin/miR-155 signaling pathway in the MAT of IL-10$^{-/-}$ mice. The expression of neurotensin and miR-155 were shown in Figure 5. Our data indicated MAT from untreated IL-10$^{-/-}$ mice exhibited significantly higher level of neurotensin than control mice (Figure 5A). A marked decrease in neurotensin expression was observed in the adipose tissue from telmisartan-treated mice (Figure 5A). Additionally, the level of miR-155, an important target of neurotensin signaling, was determined using quantitative PCR. The expression of miR-155 mRNA was remarkably increased in MAT from untreated IL-10$^{-/-}$ mice compared with those from WT group (Figure 5A). Interestingly, the gene expression of miR-155 in adipose tissue was significantly decreased in IL-10$^{-/-}$ mice receiving telmisartan administration, suggesting telmisartan may have the inhibiting activity in neurotensin/miR-155 signaling pathway.

To examine the direct effect of telmisartan on neurotensin/miR-155 signaling pathway in MAT from CD patient, the levels of neurotensin and miR-155 were detected using real-time PCR. Similarly, the expression of neurotensin and miR-155 mRNA were significantly decreased in MAT cultured with telmisartan when compared with those without telmisartan (Figure 5B), which was consistent with the in vivo study.

4. DISCUSSION

The impact that telmisartan treatment on MAT alterations during intestinal inflammation was not investigated properly. Therefore, the authors decided to investigate this topic for the first time by using an experimental model of spontaneous colitis, in which the MAT alterations were still unknown. We first tried to determine if telmisartan was able to attenuate the colonic inflammation. Our data indicated, telmisartan treatment was associated with improvement of

DAI in those mice receiving telmisartan. Telmisartan was able to significantly reduce the inflammatory score, histopathological alterations, MPO concentration and serum SAA expression. It was reported that, colonic inflammation has been correlated with decreased colon length in rodent models of colitis [52, 53], and the length of colon is widely accepted as a reliable inflammation index of colitis [54]. In this study, we found an increased colon weight and reduced colon length in the colitis animals and telmisartan was able to reverse these pathologic changes. The SAA protein, an acute phase protein showed the closet correlation with CDAI and histological activity [55] which can be a useful marker of inflammation, was significantly lower in telmisartan-treated mice than those in untreated IL-$10^{-/-}$ mice. Furthermore, intestinal mucosal cytokines such as IFN-γ and TNF-α in the IL-$10^{-/-}$ mice were significantly decreased, confirming that telmisartan was effective for animal colitis. In contrast to a previous report which indicated PPAR-γ ligand rosiglitazone significantly delayed the onset of IBD but did not absolutely prevent colitis in the IL-$10^{-/-}$ mice [16], we found in this study that telmisartan could effectively attenuated the established colitis in IL-$10^{-/-}$ mice.

We next verified the ability of telmisartan to attenuate the MAT inflammatory parameters, such as the cytokines secretion as well as the indicator of macrophage infiltration. In agreement with the previously reported results [56, 57], our data demonstrated that telmisartan administration reduced MAT inflammation as well as the cytokines secretion. The weight of MAT and the ratio of MAT to SAT were significantly decreased in IL-$10^{-/-}$ mice receiving telmisartan treatment. Additional inflammatory markers, F4/80 expression, was also inhibited by telmisartan treatment in the animal model, suggesting that telmisartan promoted

the efficient control of MAT inflammation in vivo. Decreased cytokine expression including IL-6 and IL-17 in MAT was observed in vivo study which was also confirmed by the in vitro study, implying telmisartan has its activity in attenuating MAT inflammation during spontaneous colitis.

In CD, adipocyte from creeping fat was smaller than control subjects [9]. To the best of our knowledge, the alteration of MAT in IL-10$^{-/-}$ mice has never been reported. In this study, we found the adipocytes in MAT from this spontaneous colitis animal model are smaller, but the MAT is hyperplasic and more weight than control WT mice. In this study, we also found telmisartan administration was able to restore the morphological changes of mesenteric adipocytes in mice with spontaneous colitis which was indicated as the increased the diameter of adipocyte. Abnormal adiposity is found to be associated with a chronic low inflammatory or altered immune response with increases in several pro-inflammatory cytokines that are attributed to MAT hypertrophy [58, 59]. The potential link between adipose tissue and intestinal inflammation came from the evidence that the levels of the adipokines are increased and correlate with intestinal inflammation in animal colitis models [60-62]. Thus, adipose tissue becomes of interest as a potential target tissue for therapeutic approaches for autoimmune inflammatory disease such as IBD.

In this study, we also evaluated the ability of telmisartan to modify the production of adipokines in MAT during colitis. Telmisartan was able to significantly reduce the production of leptin and to increase the expression of adiponectin in MAT in mice with colitis. Interestingly, similar results were also observed in an in vitro study with cultured MAT explants from CD patients. PPAR-γ has been reported with the ability of increasing the

adiponectin function by inducing the expression of adiponectin and its receptor [63], which was confirmed by our results. It is considered that leptin exerts pro-inflammatory immune effects while adiponectin regulates both pro- and anti-inflammatory responses in IBD [10]. In addition to demonstrating an important pro-inflammatory role for leptin during colitis [61, 64], increased leptin expression is also confirmed in the colon of IBD patients [65]. A low expression of adiponectin was found in creeping fat from experimental animal colitis [66]. Interestingly, adiponectin expression from adipose tissue was decreased significantly in obese subjects who have the similar hypertrophic MAT with CD [67]. In a recent study, methotrexate treatment was found to be associate with the decreased production of inflammatory adipokines in in reactivated animal colitis [68]. Furthermore, addition to control intestinal inflammation, infliximab treatment could restore the adipocyte morphology and PPAR-γ expression [69]. In line with previous reports, our data demonstrated that telmisartan could contribute to the control of intestinal inflammation, modify adipokine production and restore the morphological changes of MAT adipocytes.

As adipokines could regulate the neurotensin expression, we next observed the expression of neurotensin/miR-155 signaling pathway to explore the potential therapeutic mechanisms of telmisartan. Our data indicated telmisartan administration was able to reverse the increase in neurotensin/miR-155 expression in mice with spontaneous colitis. Importantly, our data also demonstrated the direct inhibition effects of telmisartan on neurotensin/miR-155 signaling pathway in vitro. MiR-155 signaling plays an important role in the pathogenesis of MAT inflammation as well as adipocyte differentiation [32-34]. Thus, the re-establishment of adipocyte size could be a direct consequence of reversed expression of neurotensin/miR-155

signaling pathway. Neurotensin/miR-155 signaling pathway plays an important role in the adipose inflammation and adipocyte differentiation. Telmisartan treatment contributed to inhibit the neurotensin/miR-155 signaling pathway in the MAT, suggesting that this pathway could contribute to attenuate the MAT alteration and gut inflammation. Indeed, it was previously demonstrated that neurotensin level is chronically increased in fat during the course of experimental colitis.[29] In the same study, the authors also demonstrated that neurotensin could induce the secretion of IL-6 and the migration of macrophages [29]. The effects of neurotensin on adipose tissue imply that the inflammation observed in MAT of CD patients may be resulted from neuropeptide-adipose interactions [29, 70]. MiRNA-155 is a potential regulator of immune cells and has a key role in autoimmunity. MiR-155 positively regulates the production of TNF-α and is considered to be a potential therapeutic target for treating such autoimmune diseases [71]. MiR-155 is also demonstrated to be involved in a number of biological activities such as an activity in differentiation of adipocyte [32-34]. In this study, we found that telmisartan treatment was associated with the inhibition of neurotensin/miR-155 pathway both in vivo and in vitro. We may thus speculated that telmisartan ameliorated the MAT inflammation and restored the adipocyte change by the mechanisms involving suppression of neurotensin/miR-155 pathway.

Although our data indicated the regulation of neurotensin/miR-155 pathway was associated with the attenuated inflammation of MAT and intestine in telmisartan treated mice, telmisartan may exert its protective effect on colon inflammation by suppression of angiotensin II. In fact, increased expression of angiotensin II has been reported in experimental colitis [72] and patients with CD [73]. In addition, the renin-angiotensin system

was reported to be involved in the pathophysiology of colitis, suggesting antagonism of the renin-angiotensin system such as angiotensin II receptor may be a potential prophylactic strategy for the treatment of human IBD [74, 75]. In fact, angiotensin receptor antagonist is effective in preventing experimental colitis through the blockade of growth factor-beta1 overexpression or via regulation of mucosal vascular addressin cell adhesion molecule 1 [76-79]. Therefore, considering the fact that telmisartan has its function as an angiotensin II type 1 receptor blocker and a PPAR-γ agonist, we cannot rule out the possibility that telmisartan showed its ability in attenuation colon inflammation through regulation of renin-angiotensin system.

In conclusion, treatment with telmisartan could attenuate the spontaneous colitis in an animal model which was associated with the attenuated MAT alteration. In addition, our study indicated telmisartan could act on MAT, reducing the cytokine production and resulting in an ameliorated mesenteric adipose tissue inflammation. We suggest that the telmisartan-induced diminishing of the inflammatory environment of MAT as well as the improved MAT alteration, at least in part, is associated with it activity in regulation of the neurotensin/miR-155 pathway. These results support the concept that regulating the abnormal immune response in mesenteric adipose tissue is an important target for the treatment of IBD.

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Conflict of interest: The authors declare no conflict of interest.

FIGURE LEGENDS

Figure 1. Therapeutic effects of telmisartan on IL-10$^{-/-}$ mice with established spontaneous colitis. Telmisartan-treated IL-10$^{-/-}$ mice showed lower mean DAI values since the 8th week after drug administration when compared to untreated IL-10$^{-/-}$ mice (**A**). The SAA protein level in serum was significantly lower in telmisartan-treated mice than those in untreated IL-10$^{-/-}$ mice (**B**). Telmisartan dramatically decreased colon weights of IL-10$^{-/-}$ mice (**C**). Telmisartan-treated mice had significantly longer colons than untreated IL-10 deficient mice (**D**). Data are presented as means±SD, n=10. *Statistical differences with $P < 0.05$.

Figure 2. Telmisartan reduces chronic colonic inflammation in established IL-10$^{-/-}$ mice. IL-10$^{-/-}$ mice with chronic colitis, microscopically, the inflammatory infiltrate in the colon which was restored by telmisartan (**A**). The colonic inflammation scores in the IL-10$^{-/-}$ mice treated with telmisartan significantly decreased (**B**). Compared with those of untreated mice, the concentration of MPO (**C**), IFN-γ and TNF-α (**D**) in the colonic mucosal tissue were significantly decreased in telmisartan-treated IL-10$^{-/-}$ mice. Data are presented as means±SD, n=6. *Statistical differences with $P < 0.05$.

Figure 3. Telmisartan restores the alteration of fat distribution and mesenteric adipose tissue morphology from IL-10$^{-/-}$ mice. Telmisartan treatment decreased MAT weights compared with those in the non-treated IL-10$^{-/-}$ mice (**A**). Telmisartan administration significantly decreased the ratio of MAT/SAT in IL-10$^{-/-}$ mice (**B**). Telmisartan restores the morphological change of MAT adipocyte from IL-10$^{-/-}$ mice (**C**). IL-10$^{-/-}$ mice with telmisartan administration had a significant increased diameter of adipocyte (**D**). Decrease in expression of F4/80 was observed in telmisartan treated IL-10$^{-/-}$ mice (**E**). All data are presented as means±SD, n=10, *Statistical differences with $P < 0.05$.

Figure 4. Telmisartan attenuates the MAT inflammation in vivo and in vitro. IL-10$^{-/-}$ mice receiving telmisartan had a reduced IL-6 and IL-17 mRNA expression (**A**). A reduced leptin expression and an increased expression of adiponectin was found IL-10$^{-/-}$ mice receiving telmisartan (**B**). The expression of adiponectin (**C**) was increased and the levels of leptin (**C**), IL-6 (**D**), and IL-17 (**E**) were decreased significantly in MAT from Crohn's patients cultured with telmisartan in vitro study. All data are presented as means±SD, n=6-10, * $P < 0.05$ as statistical differences.

Figure 5. Telmisartan therapy repressed the neurotensin/miR-155 signaling n vivo and in vitro. The increased expression of neurotensin and miR-155 in MAT from IL-10$^{-/-}$ mice were inhibited by telmisartan (**A**). In vitro study, the expression of neurotensin and miR-155 mRNA were significantly decreased in MAT from Crohn's patients cultured with telmisartan (**B**). All data are presented as means±SD, n=6-10, * represents $P < 0.05$.

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Figure-1

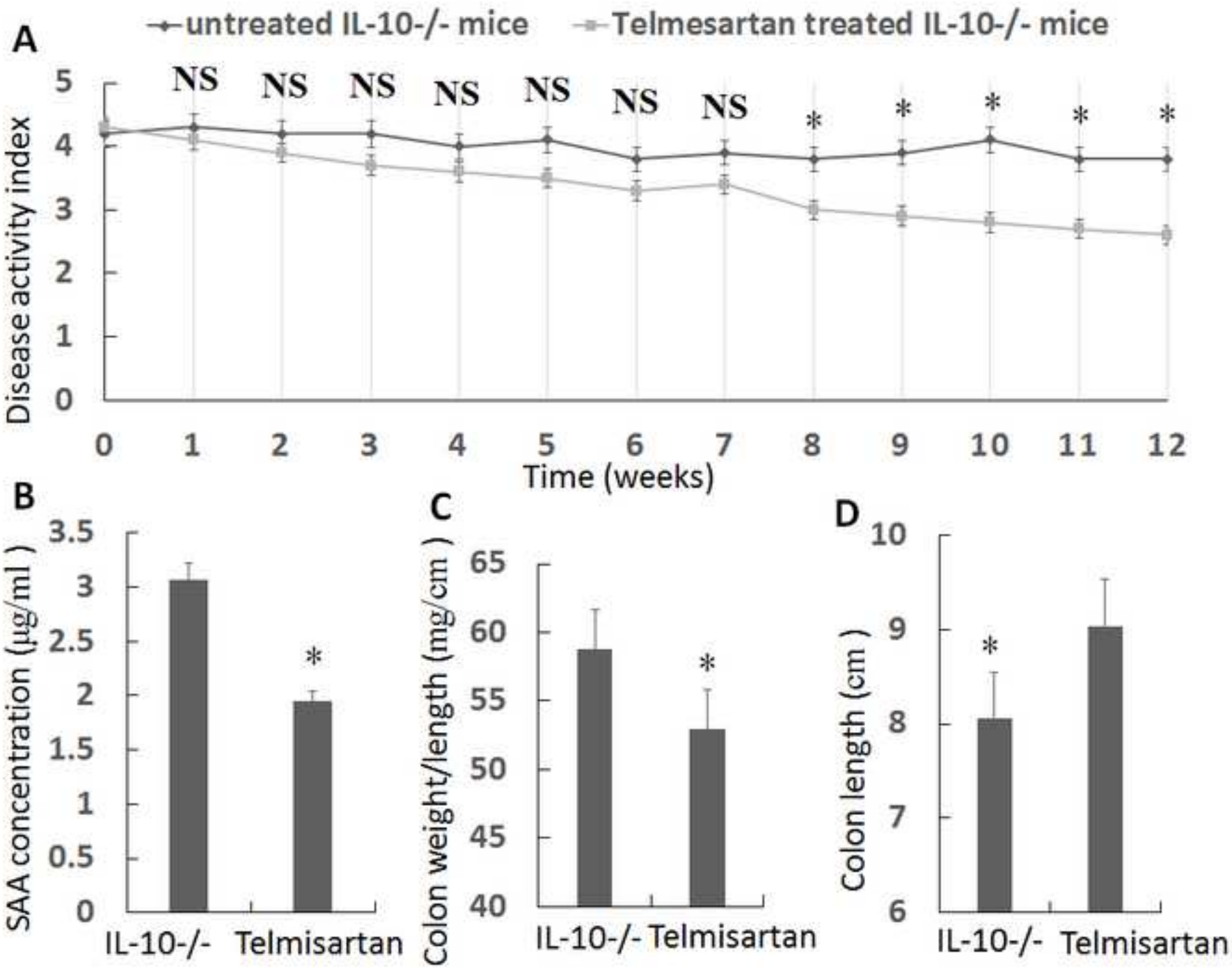

A
untreated IL-10-/- mice
Telmesartan treated IL-10-/- mice
Disease activity index
NS
*
Time (weeks)
B
SAA concentration (μg/ml)
IL-10-/-
Telmisartan
C
Colon weight/length (mg/cm)
IL-10-/- Telmisartan
D
Colon length (cm)
IL-10-/- Telmisartan

Figure-2

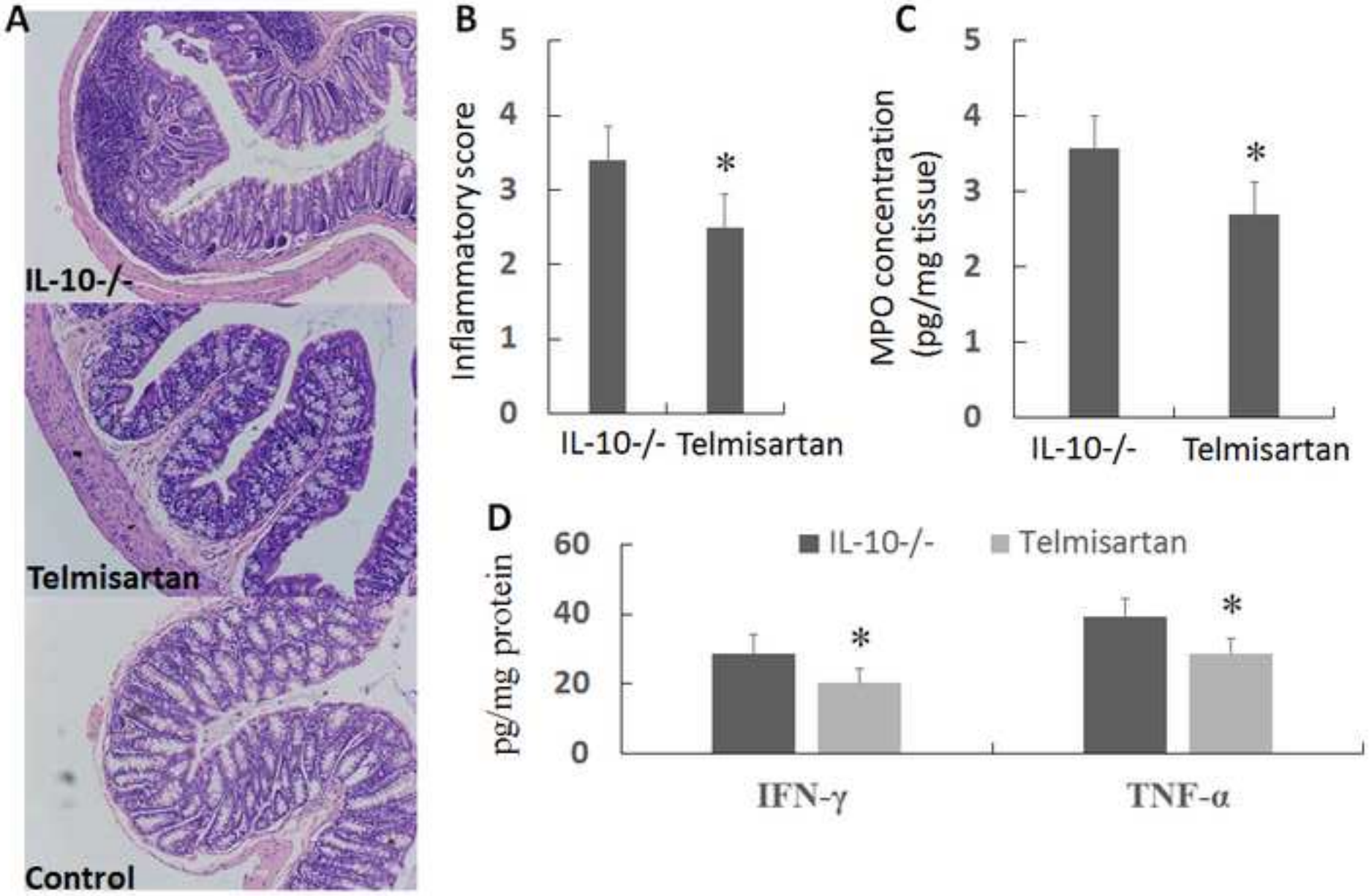

A
IL-10-/-
Telmisartan
Control
B
Inflammatory score
0
1
2
3
4
5
*
IL-10-/-
Telmisartan
C
MPO concentration (pg/mg tissue)
0
1
2
3
4
5
*
IL-10-/-
Telmisartan
D
pg/mg protein
0
20
40
60
IL-10-/-
Telmisartan
*
*
IFN-γ
TNF-α

Figure-3

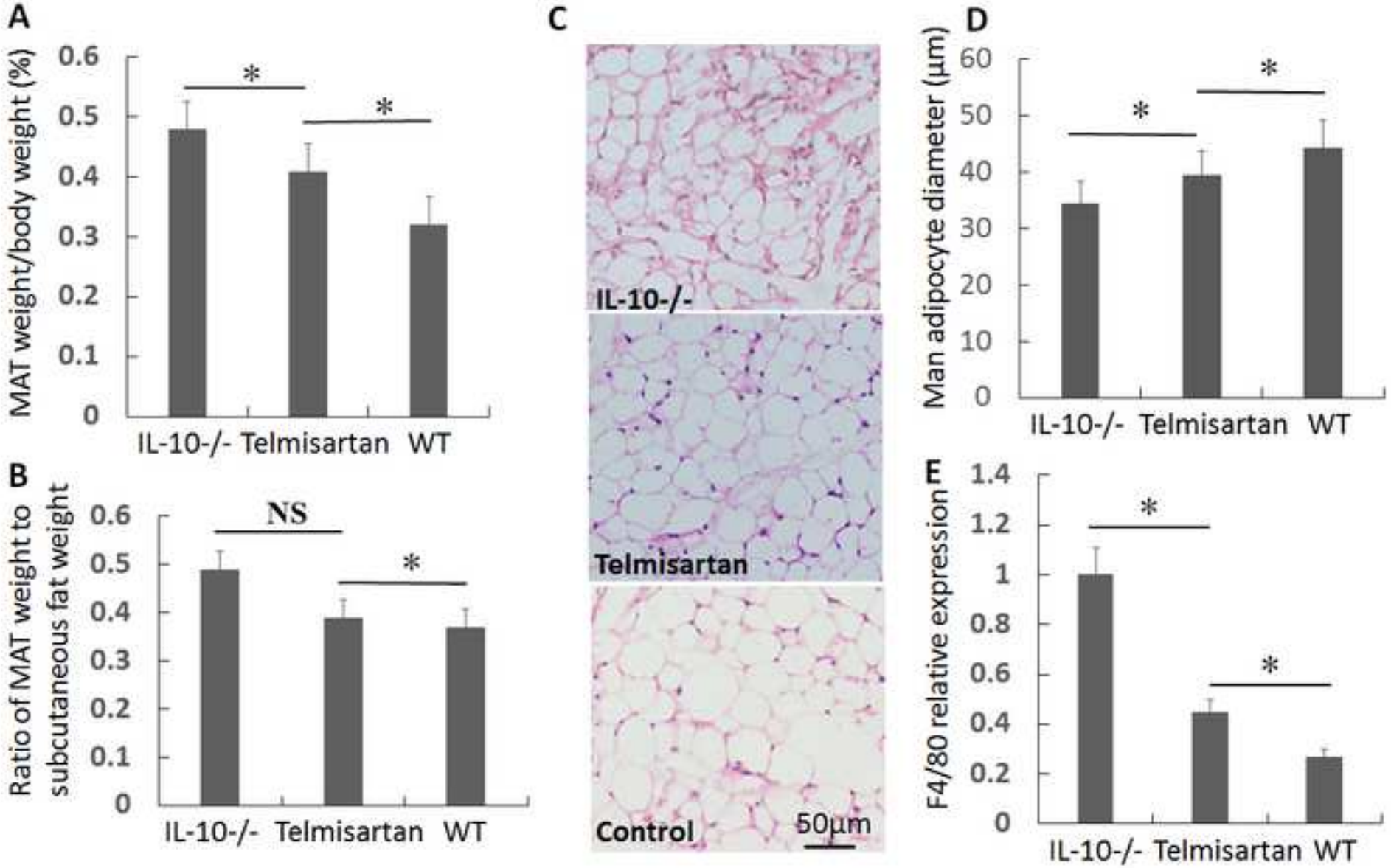

A
MAT weight/body weight (%)
0.6
0.5
0.4
0.3
0.2
0.1
0
*
*
IL-10-/- Telmisartan WT
B
Ratio of MAT weight to subcutaneous fat weight
0.6
0.5
0.4
0.3
0.2
0.1
0
NS
*
IL-10-/- Telmisartan WT
C
IL-10-/-
Telmisartan
Control
50μm
D
Man adipocyte diameter (μm)
60
50
40
30
20
10
0
*
*
IL-10-/- Telmisartan WT
E
F4/80 relative expression
1.4
1.2
1
0.8
0.6
0.4
0.2
0
*
*
IL-10-/- Telmisartan WT

Figure-4

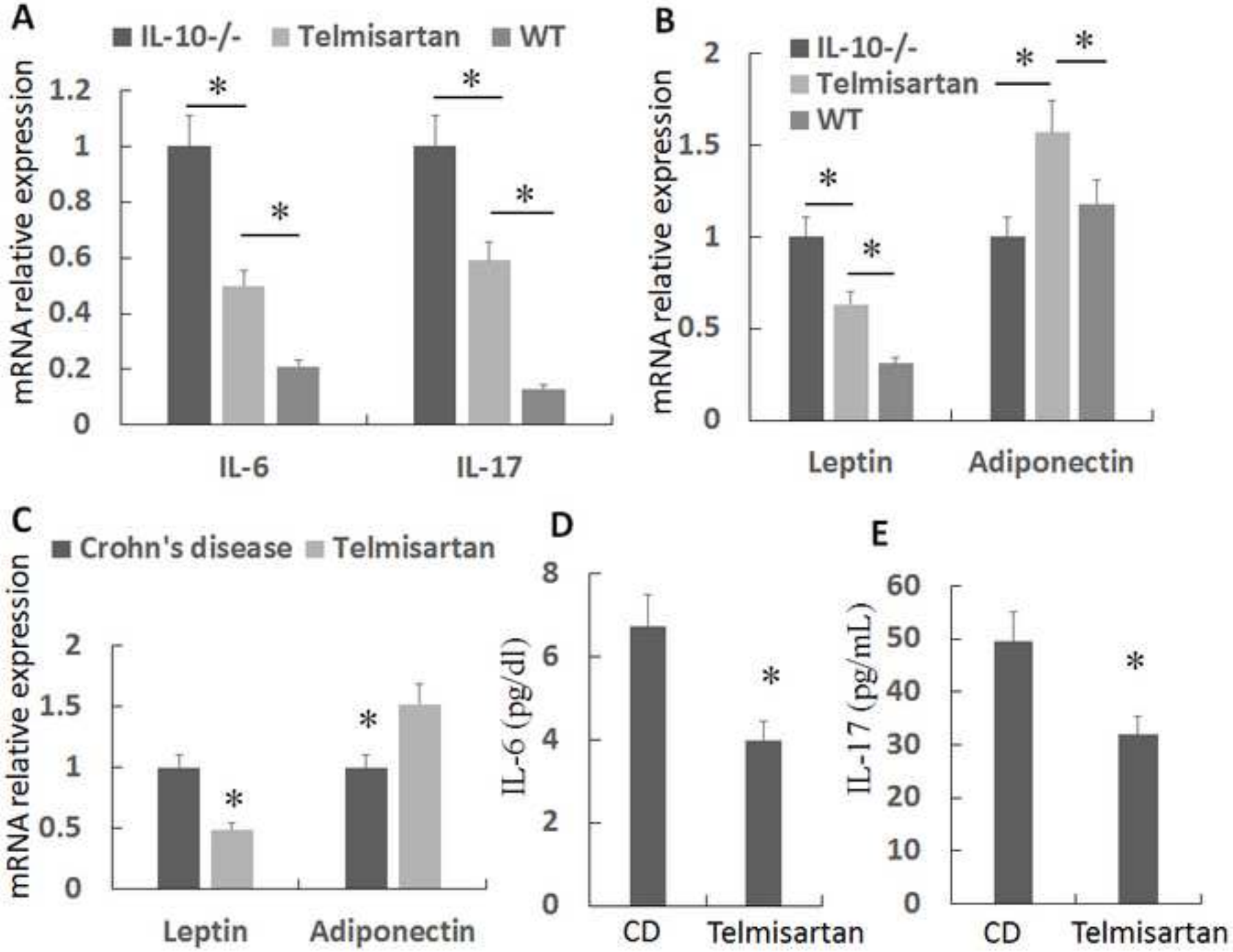
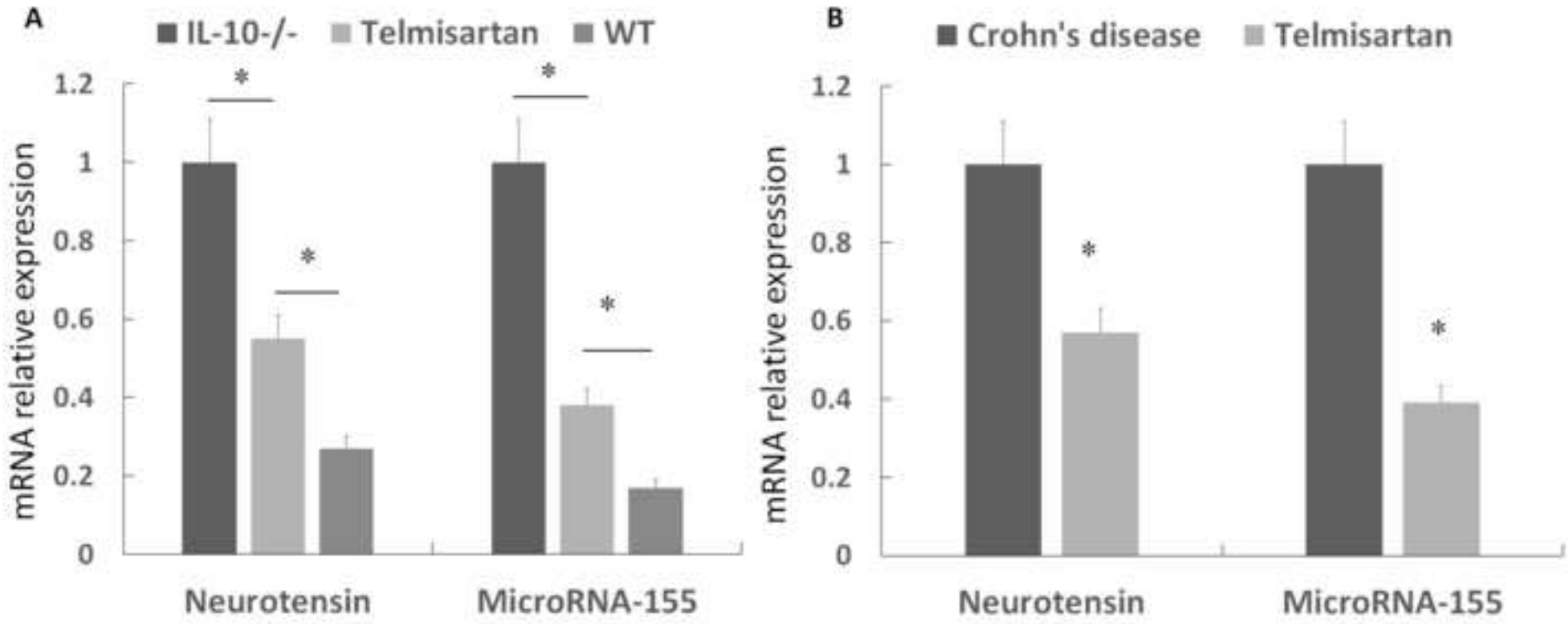

A
IL-10-/-
Telmisartan
WT
mRNA relative expression
1.2
1
0.8
0.6
0.4
0.2
0
*
IL-6
IL-17
B
IL-10-/-
Telmisartan
WT
mRNA relative expression
2
1.5
1
0.5
0
Leptin
Adiponectin
C
Crohn's disease
Telmisartan
mRNA relative expression
Leptin
Adiponectin
D
IL-6 (pg/dl)
8
6
4
2
0
CD
Telmisartan
E
IL-17 (pg/mL)
60
50
40
30
20
10
0
CD
Telmisartan

Figure-5


A
IL-10-/-
Telmisartan
WT
mRNA relative expression
1.2
1
0.8
0.6
0.4
0.2
0
*
*
*
*
Neurotensin
MicroRNA-155
B
Crohn's disease
Telmisartan
mRNA relative expression
1.2
1
0.8
0.6
0.4
0.2
0
*
*
Neurotensin
MicroRNA-155

Graphical Abstract