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LncRNA MIAT suppresses inflammation in LPS-induced J774A.1 macrophages by promoting autophagy through miR-30a-5p/SOCS1 axi

Linqian Sun[1], Kun Yang[2], Liqin Wang[1], Si Wu[3], Dawei Wen[1] & Jibo Wang[1]✉

Accumulated data implicate that long noncoding RNA (lncRNA) plays a pivotal role in rheumatoid arthritis (RA), potentially serving as a competitive endogenous RNA (ceRNA) for microRNAs (miRNAs). The lncRNA myocardial infarction-associated transcript (MIAT) has been demonstrated to regulate inflammation. However, the role of MIAT in the inflammation of RA remains inadequately explored. This study aims to elucidate MIAT's role in the inflammation of lipopolysaccharide (LPS)-induced macrophages and to uncover the underlying molecular mechanisms. We observed heightened MIAT expression in LPS-induced J774A.1 cells and collagen-induced arthritis mouse models, in contrast to the expression pattern of miR-30a-5p. Silencing MIAT resulted in increased expression of the inflammatory cytokines IL-1β and TNF-α. Simultaneously, MIAT interference significantly impeded macrophage autophagy, evidenced by decreased expression of autophagy-related markers LC3-II and Beclin-1, alongside increased levels of p62 in LPS-induced J774A.1 cells. Notably, MIAT functioned as a ceRNA, sponging miR-30a-5p and exerting a negative regulatory influence on its expression. SOCS1 emerged as a target of miR-30a-5p, modulated by MIAT. Mechanistically, inhibiting miR-30a-5p reversed the impact of MIAT deficiency in promoting LPS-induced inflammation, while SOCS1 knockdown countered the cytokine inhibitory effect induced by silencing miR-30a-5p. In summary, this study indicates that lncRNA MIAT suppresses inflammation in LPS-induced J774A.1 macrophages by stimulating autophagy through the miR-30a-5p/SOCS1 axis. This suggests that MIAT holds promise as a potential therapeutic target for RA inflammation.

Keywords LncRNA MIAT, Inflammation, Rheumatoid arthritis, miR-30a-5p/SOCS1 axis, Autophagy

Rheumatoid arthritis (RA) is the most common autoimmune, inflammatory disease which induces joint inflammation, potentially leading to permanent joint damage and disability[1]. Despite the intricate pathogenesis of RA, it is widely acknowledged that abnormal immune cell activation and the production of inflammatory mediators are closely associated with RA[2]. Macrophages were found to be widely distributed in the RA lining of the synovium during inflammatory conditions[3], and more importantly, the clinical disease activity is highly correlated with the level of macrophage-derived cytokines in the synovium, such as TNF-α, IL-1β, and IL-6[4]. These cytokines contribute to extended inflammatory responses and protease activation, ultimately resulting in cartilage destruction. There is an imperative need to identify new therapeutic targets to impede the inflammatory response and prevent joint damage in RA.

Recent studies have elucidated the pivotal role of long non-coding RNAs (lncRNAs), transcripts exceeding 200 nucleotides in length, in orchestrating inflammation and immune regulation in autoimmune diseases[5]. Dysregulated quantities of lncRNAs in inflammatory cells, notably macrophages, have been identified and utilised as key clinical diagnostic biomarkers in RA[6]. RA is correlated with the pathogenesis of several diseases, including cardiovascular disease (CVD)[7]. The lncRNA myocardial infarction-associated transcript (MIAT)

[1]Department of Rheumatology & Clinical Immunology, Affiliated Hospital of Qingdao University, Qingdao 266000, China. [2]Medical Research Center, Affiliated Hospital of Qingdao University, Qingdao 266000, China. [3]Department of Infectious Disease, Affiliated Hospital of Qingdao University, Qingdao 266000, China. ✉email: wangjibo2005@126.com

has been demonstrated to exert a critical effect on CVD[8,9]. Recent evidence also suggests a close association between MIAT and inflammation[10–13]. Our prior study indicated that LncRNA MIAT appeared to inhibit the expression of IL-1β, TNF-α, and be suppressed by the NLRP3 inflammasome activation pathway[14]. However, a comprehensive understanding of MIAT's role in the pathogenesis and progression of RA is warranted.

Extensive data have demonstrated that lncRNAs can function as competitive endogenous RNAs (ceRNAs) through miRNA sponging, leading to the suppression of miRNAs in intricate biological processes, such as autoimmune imbalance, inflammatory response, and carcinogenesis[15–17]. miRNAs, common non-coding RNAs involved in regulating autoimmunity and inflammation, can downregulate the expression of targeted mRNAs[18]. Some studies have utilized sequencing and microarray technologies to investigate dysregulated transcripts in specific cells or whole blood from RA patients, identifying functional ceRNAs in RA[19,20]. Indeed, the lncRNA-mediated ceRNA network regulates various cellular processes in RA, encompassing proliferation, invasion, inflammation and apoptosis[21–23]. However, whether lncRNA MIAT acts as a ceRNA to modulate inflammation in RA requires further investigation.

Autophagy is a cellular degradation and recycling process that is highly conserved in all eukaryotes. Autophagy has functions that influence infection, inflammation and immunity. Autophagy plays a protective role in inflammatory diseases by modulating interferon production or inflammasome activation[24]. Recently, continuous study of RA pathogenesis has shown that autophagy also affects RA pathology by participating in synovitis and bone destruction, making autophagy an important regulatory mechanism in RA progression[25]. In addition, lncRNA may regulate autophagy in autoimmune diseases. Results of previous studies demonstrated that LncRNA ZFAS1 regulates the proliferation, apoptosis, inflammatory response and autophagy of fibroblast-like synoviocytes via miR-2682-5p/ADAMTS9 axis in rheumatoid arthritis[26]. However, how lncRNA MIAT regulates autophagy during immune responses remains largely unknown.

The current study aims to elucidate the role of lncRNA MIAT and its mechanism in RA inflammation. Our investigation explores a novel mechanism by which MIAT promotes autophagy to suppress inflammation via the miR-30a-5p/SOCS1 axis in LPS-induced J774A.1 cells, potentially offering new insights into the therapeutic application of lncRNAs in RA.

Results

LncRNA MIAT expresses higher in CIA and LPS-induced J774A.1 macrophages

Firstly, collagen-induced arthritis (CIA) models were established, exhibiting joint redness, swelling, and stiffness (Fig. 1A,B). Arthritis scores were significantly higher in the model group compared to the healthy group starting from day 28 (Fig. 1C). Hematoxylin and eosin (H&E) staining of knee joint synovium revealed a marked increase in the infiltration of inflammatory cells in CIA mice (Fig. 1D). To assess inflammation levels, concentrations of TNF-α and IL-1β were examined using an ELISA kit. The results showed a significant increase in IL-1β and TNF-α levels in CIA serum (Fig. 1E,F), confirming the successful establishment of collagen-induced arthritis models.

For the analysis of MIAT's relative expression, knee joint synovium was collected, revealing higher MIAT expression in the CIA group than in the healthy group (Fig. 1G). To investigate MIAT's association with macrophage inflammation, the murine macrophage cell line J774A.1 was used in vitro. J774A.1 cells were harvested after 24 h of stimulation with 100 ng/ml LPS to induce inflammation. Examination of inflammatory cytokine expression through RT-qPCR and ELISA indicated that LPS significantly upregulated TNF-α and IL-1β levels in J774A.1 cells (Fig. 1H–K). Relative expression of MIAT was notably increased after LPS stimulation compared to controls (Fig. 1L). These findings suggest that MIAT exhibits elevated expression during the inflammation of rheumatoid arthritis (RA).

Knockdown of LncRNA MIAT induces inflammatory response and inhibits autophagy in LPS-induced J774A.1 cells

To investigate the impact of MIAT on the inflammatory response, J774A.1 cells underwent transfection with si-MIAT or si-NC. The most suitable siRNA (siRNA-2) was selected based on the RT-qPCR assay, demonstrating effective inhibition of LncRNA MIAT expression in J774A.1 cells (Fig. 2A). Subsequently, we assessed inflammatory cytokine levels through RT-qPCR and ELISA. The data revealed elevated IL-1β and TNF-α expression in the si-MIAT group compared to the si-NC group (Fig. 2B–D). LPS treatment decreased LC3-II and Beclin-1 expression while significantly increasing p62 expression (Fig. 2E–H). Following si-MIAT transfection, LPS-stimulated J774A.1 cells exhibited reduced secretion of Beclin-1 and LC3-II proteins alongside increased levels of p62 proteins (Fig. 2I–L).

LncRNA MIAT functions as a molecular sponge for miR-30a-5p

Through bioinformatic analyses, we predicted miR-30a-5p as a potential target of MIAT, given its involvement in regulating inflammatory responses. The predicted binding sites between MIAT and the miR-30a-5p sequence are depicted in Fig. 3A. The dual luciferase reporter assay demonstrated a significant repression of luciferase activity in the MIAT WT plasmid by the miR-30a-5p mimic in J774A.1 cells, while no such effect was observed in the MIAT MUT plasmid group (Fig. 3B). The RIP assay further confirmed the interaction between MIAT and miR-30a-5p, with both accumulating in Ago2 antibody precipitates (Fig. 3C). RT-qPCR analysis revealed that MIAT silencing markedly increased miR-30a-5p expression (Fig. 3D). These findings substantiate that MIAT acts as a molecular sponge for miR-30a-5p.

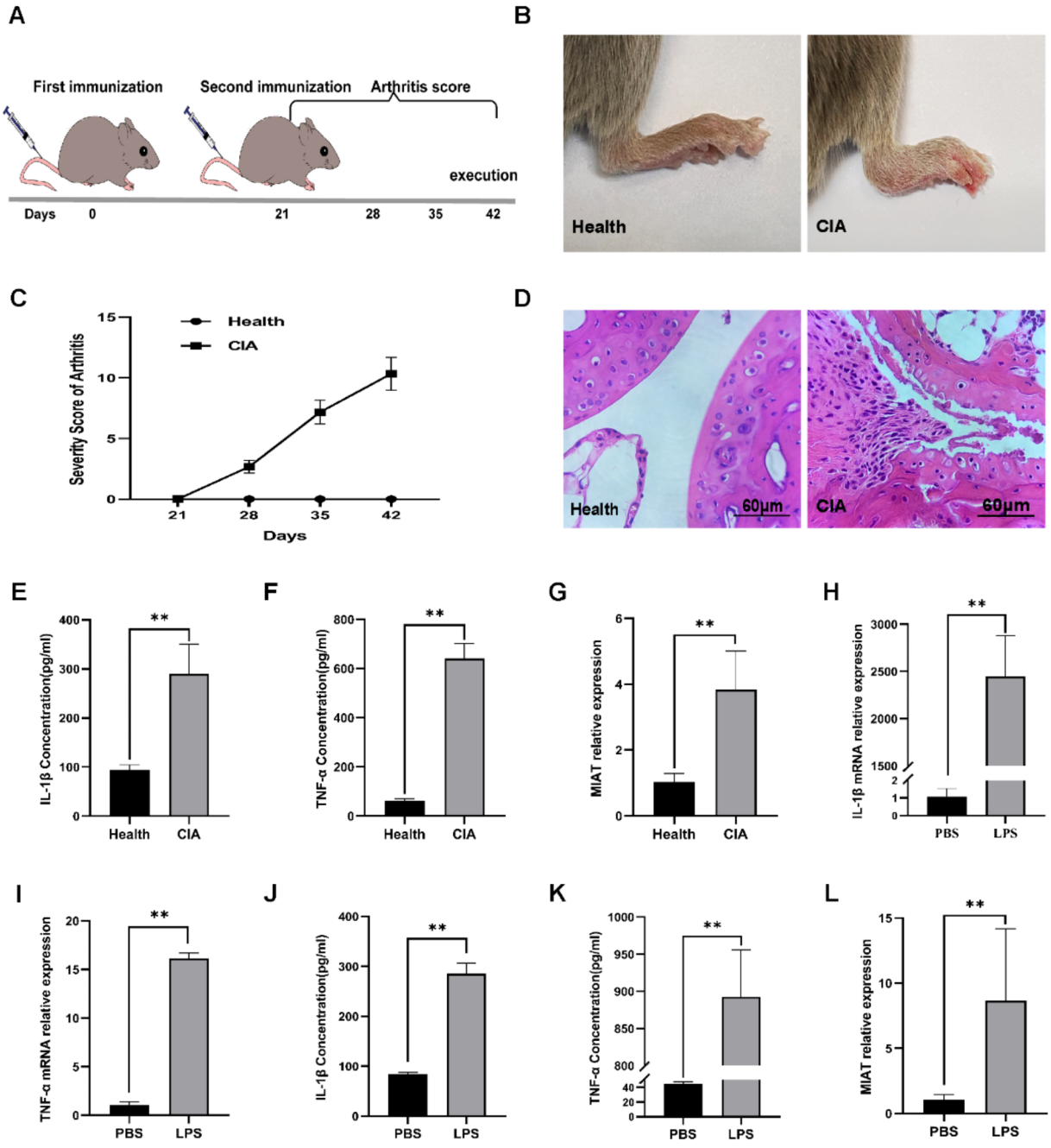

Fig. 1. LncRNA MIAT expression is higher in CIA and LPS-induced J774A.1 macrophages. (**A**) The timeline for constructing CIA mouse models. (**B**) Photographs of healthy and CIA mouse paws ($n=6$ for each group). (**C**) Arthritis severity scores in healthy and CIA mice ($n=6$ for each group). (**D**) Representative micrographs of H&E staining of knee joint tissues from healthy and CIA mice (scale bar = 60 μm; $n=6$ for each group). (**E**,**F**) IL-1β and TNF-α concentrations in serum from healthy and CIA mice ($n=6$ for each group). (**G**) MIAT relative expression measured by RT-qPCR in healthy and CIA mice ($n=3$ for each group). (**H**–**K**) Expression of IL-1β and TNF-α measured by RT-qPCR or ELISA in normal J774A.1 cells and LPS-induced J774A.1 cells. (**L**) Relative expression of MIAT measured by RT-qPCR in normal J774A.1 cells and LPS-induced J774A.1 cells. Data obtained from more than three repeated experiments are presented as mean ± SD, $^{**}P<0.01$.

MiR-30a-5p modulates inflammatory response and autophagy in LPS-induced J774A.1 cells

A significant reduction in miR-30a-5p expression was observed in both CIA mouse and LPS-induced J774A.1 cells (Fig. 4A,B). To elucidate its role in inflammation, we assessed the impact of miR-30a-5p on macrophages using miR-30a-5p mimic or inhibitor. RT-qPCR confirmed the effectiveness of miR-30a-5p mimic or inhibitor in

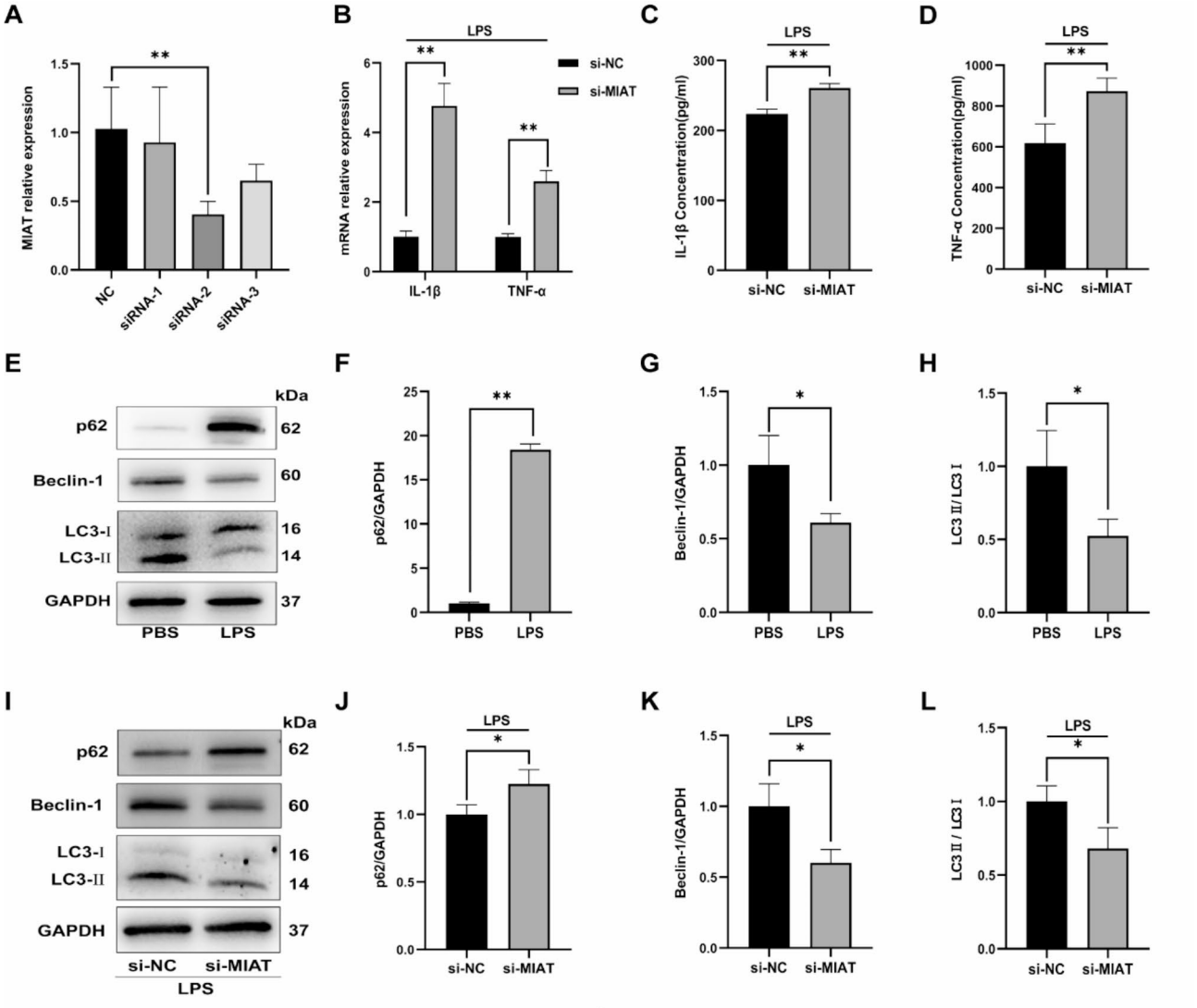

Fig. 2. Knockdown of LncRNA MIAT induces inflammatory response and inhibits autophagy in LPS-induced J774A.1 cells. J774A.1 cells were transfected with si-MIAT or si-NC before LPS stimulation. (**A**) Relative expression of MIAT transfected with si-MIAT measured by RT-qPCR. (**B**) mRNA relative expression of TNF-α and IL-1β measured by RT-qPCR after knocking down MIAT. (**C**,**D**) IL-1β and TNF-α concentration of cell supernatants after knocking down MIAT. (**E**–**H**) Protein expression of LC3, p62, Beclin-1, and GAPDH after LPS stimulation evaluated by western blot analysis. (**I**–**L**) Protein expression of LC3, p62, Beclin-1, and GAPDH after knocking down MIAT detected by western blot analysis. Data represent 3 biological replicates and are expressed as mean ± SD. $^{*}P < 0.05$, $^{**}P < 0.01$.

altering miR-30a-5p expression in macrophages (Fig. 4C,D). Upregulation of miR-30a-5p resulted in increased expression of TNF-α and IL-1β (Fig. 4E–G). Western blot analysis revealed that transfection with miR-30a-5p mimic decreased LC3-II and Beclin-1 expression and increased p62 expression, while the inhibitor had the opposite effect (Fig. 4H–K).

MiR-30a-5p directly interacts with SOCS1

It is widely acknowledged that miRNAs function by suppressing the expression of target genes. To investigate the underlying molecular mechanism of MIAT/miR-30a-5p in immune response regulation under LPS induction, we conducted bioinformatics analysis to identify potential miR-30a-5p targets. The results in Fig. 5A suggested binding sites of miR-30a-5p in the 3′-UTR of SOCS1, indicating SOCS1 as a potential target. The luciferase reporter assay confirmed the predicted binding interactions between miR-30a-5p and SOCS1. Luciferase activity significantly decreased with co-transfection of SOCS1 WT and miR-30a-5p mimic compared to co-transfection with SOCS1 WT and mimic NC, while no change was observed in cells transfected with a mutant SOCS1 binding sequence (Fig. 5B). Additionally, transfection with the miR-30a-5p mimic notably reduced the mRNA and protein expression levels of SOCS1, while the inhibitor had the opposite effect (Fig. 5C–E). SOCS1

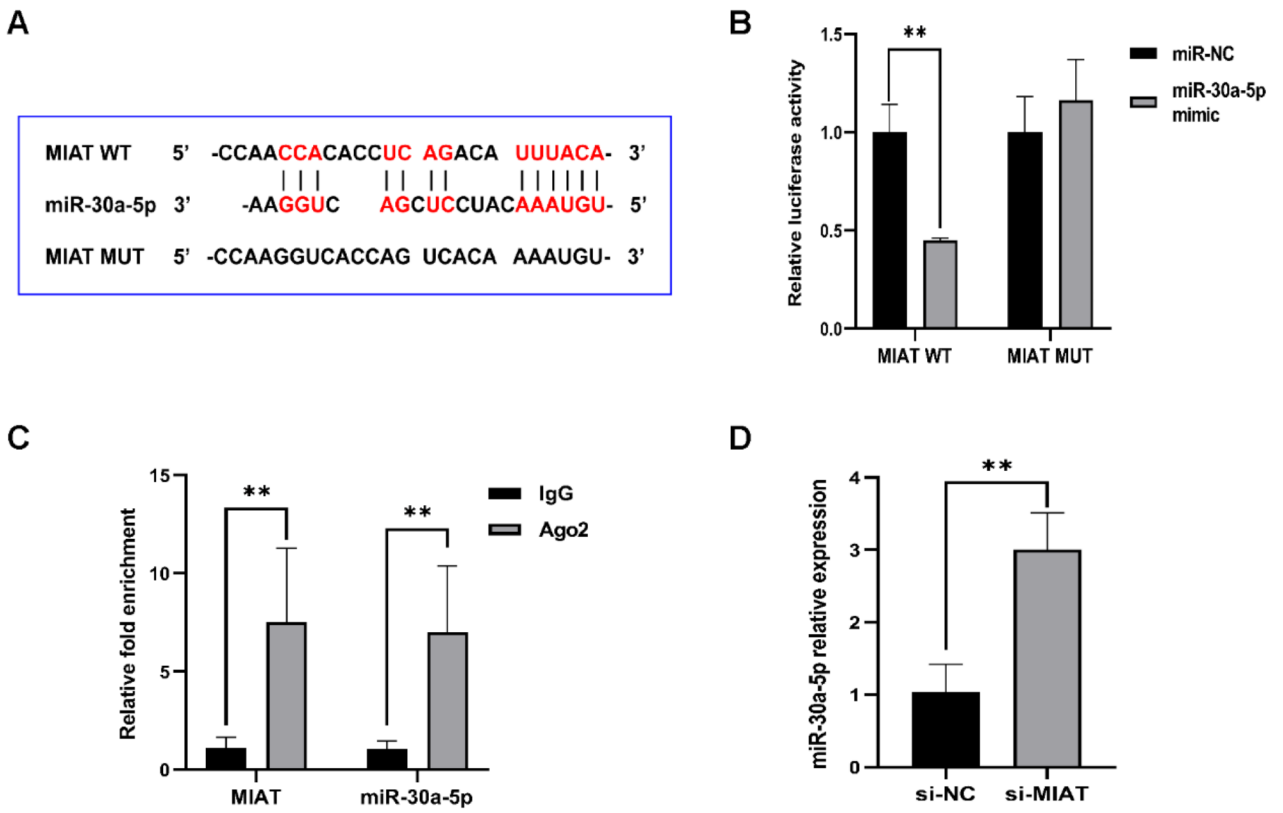

Fig. 3. LncRNA MIAT functions as a molecular sponge for miR-30a-5p. (**A**) The predicted binding sequence between miR-30a-5p and MIAT. (**B**) Interaction between MIAT and miR-30a-5p confirmed by the dual-luciferase reporter assay. (**C**) RIP assay demonstrating binding relationships between MIAT and miR-30a-5p. (**D**) RT-qPCR assay examining miR-30a-5p expression. Data represent 3 biological replicates and are expressed as mean ± SD. $^{**}P < 0.01$.

expression was increased in LPS and CIA groups (Fig. 5F,G). In conclusion, miR-30a-5p downregulates SOCS1 expression by binding to its 3′-UTR.

LncRNA MIAT functions as a ceRNA for miR-30a-5p to regulate SOCS1 expression in J774A.1 cells

The downregulation of SOCS1 significantly increased the expression of IL-1β and TNF-α in macrophages, indicating the pivotal role of SOCS1 in macrophage inflammatory response (Fig. 6A–F). To confirm that LncRNA MIAT acts as a ceRNA for miR-30a-5p to regulate SOCS1 expression, we co-transfected J774A.1 cells with si-MIAT (or si-NC), si-SOCS1, and miR-30a-5p inhibitor. The results from RT-qPCR and western blot analysis demonstrated that the reduction of SOCS1 expression due to LncRNA MIAT knockdown could be reversed by the miR-30a-5p inhibitor in J774A.1 cells. In the si-SOCS1 + miR-30a-5p inhibitor + si-MIAT group, SOCS1 expression was markedly decreased compared to the miR-30a-5p inhibitor + si-MIAT group (Fig. 6G,H). Furthermore, in LPS-induced J774A.1 cells, silencing miR-30a-5p nullified the promoting effect of si-MIAT on the levels of inflammatory factors (TNF-α and IL-1β), which was reversed by the knockdown of SOCS1 (Fig. 6I–L). Similarly, western blot analysis revealed that MIAT knockdown reduced autophagy in J774A.1 cells, characterized by decreased LC3-II and Beclin-1, and increased p62. This effect was partly reversed by the miR-30a-5p inhibitor, while the knockdown of SOCS1 counteracted the functions of the miR-30a-5p inhibitor (Fig. 6M–P). These results demonstrate that MIAT regulates autophagy and the LPS-induced inflammatory response via the miR-30a-5p/SOCS1 axis.

Discussion

Rheumatoid arthritis (RA) is a systemic and chronic inflammatory disorder characterized by persistent synovitis in the joints and systemic inflammatory responses, leading to lifelong disability[27]. Inhibiting immune cells to reduce inflammatory infiltration and joint destruction is the current therapeutic strategy for RA. Conventional medical approaches have shown limited efficacy in managing RA. The advent of gene therapy presents a promising avenue for future RA treatment. Recently, long non-coding RNAs (lncRNAs) have emerged as pivotal players in modulating various cytokines during RA pathogenesis, especially in inflammatory cells such as macrophages[28]. Differentially expressed lncRNAs specific to RA hold potential as diagnostic or therapeutic targets in the future. Notably, MIAT has been linked to inflammation in rheumatoid arthritis[14]. However, the precise mechanism of

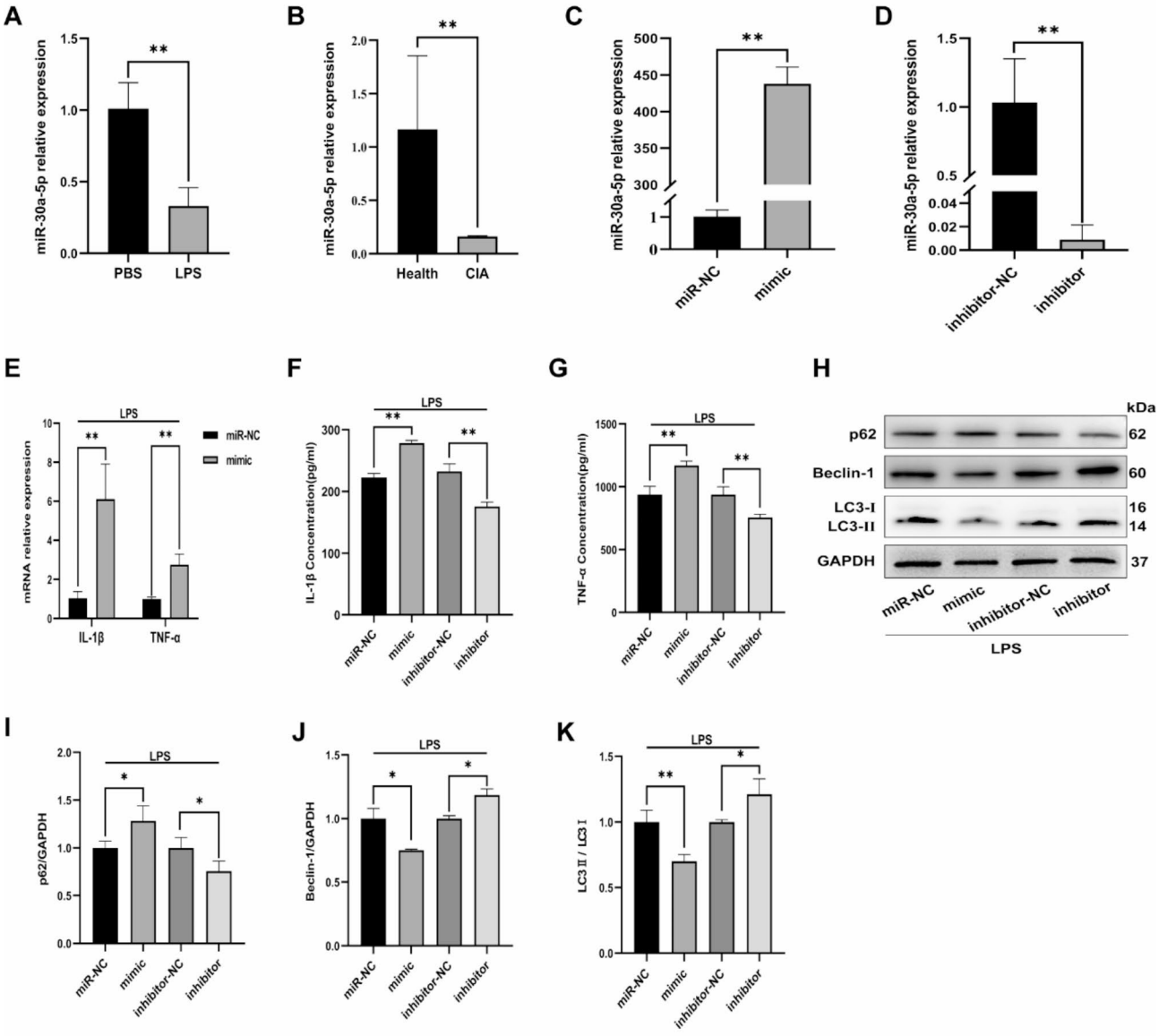

Fig. 4. MiR-30a-5p modulates inflammatory response and autophagy in LPS-induced J774A.1 cells. J774A.1 cells were transfected with miR-30a-5p mimic or inhibitor before LPS stimulation. (**A**,**B**) MiR-30a-5p expression in LPS-induced J774A.1 cells and CIA mice assessed by RT-qPCR. (**C**,**D**) RT-qPCR of miR-30a-5p expression in J774A.1 cells transfected with miR-30a-5p mimic or inhibitor. (**E**–**G**) TNF-α and IL-1β expression measured by RT-qPCR or ELISA. (**H**–**K**) Protein expression of LC3, p62, Beclin-1, and GAPDH detected by western blot analysis. Data from more than three repeated experiments are presented as mean ± SD. $^{*}P < 0.05$, $^{**}P < 0.01$.

lncRNA MIAT in RA inflammation remains incompletely understood. Our study reveals that LncRNA MIAT suppresses inflammation in LPS-induced J774A.1 macrophages by promoting autophagy through the regulation of the miR-30a-5p/SOCS1 axis (Fig. 7), suggesting that MIAT could be a potential therapeutic target for RA inflammation.

Understanding the regulatory mechanisms underlying the inflammatory process is pivotal for effective RA treatment, as inflammation is a key factor in RA pathogenesis. In RA, increased macrophages infiltrate inflamed synovial tissue, contributing to inflammatory lesions and joint damage[29]. Macrophage induced by LPS is a classical cell model for inflammation research. Upon LPS stimulation, macrophages become activated, releasing various cytokines, with excessive release causing extensive tissue damage and pathological changes[30]. In our study, we induced inflammation in J774A.1 macrophages using LPS to mimic the inflammatory microenvironment, aiming to explore the role and underlying mechanisms of MIAT. Our in vitro and in vivo results indicated a significant increase in MIAT expression in LPS-induced J774A.1 cells and collagen-induced arthritis (CIA) mice. Silencing MIAT in LPS-induced cells led to increased expression of TNF-α and IL-1β, consistent with previous findings[12,13]. MIAT may function as a negative regulator of LPS-induced expression of TNF-α and IL-1β. Li et al.

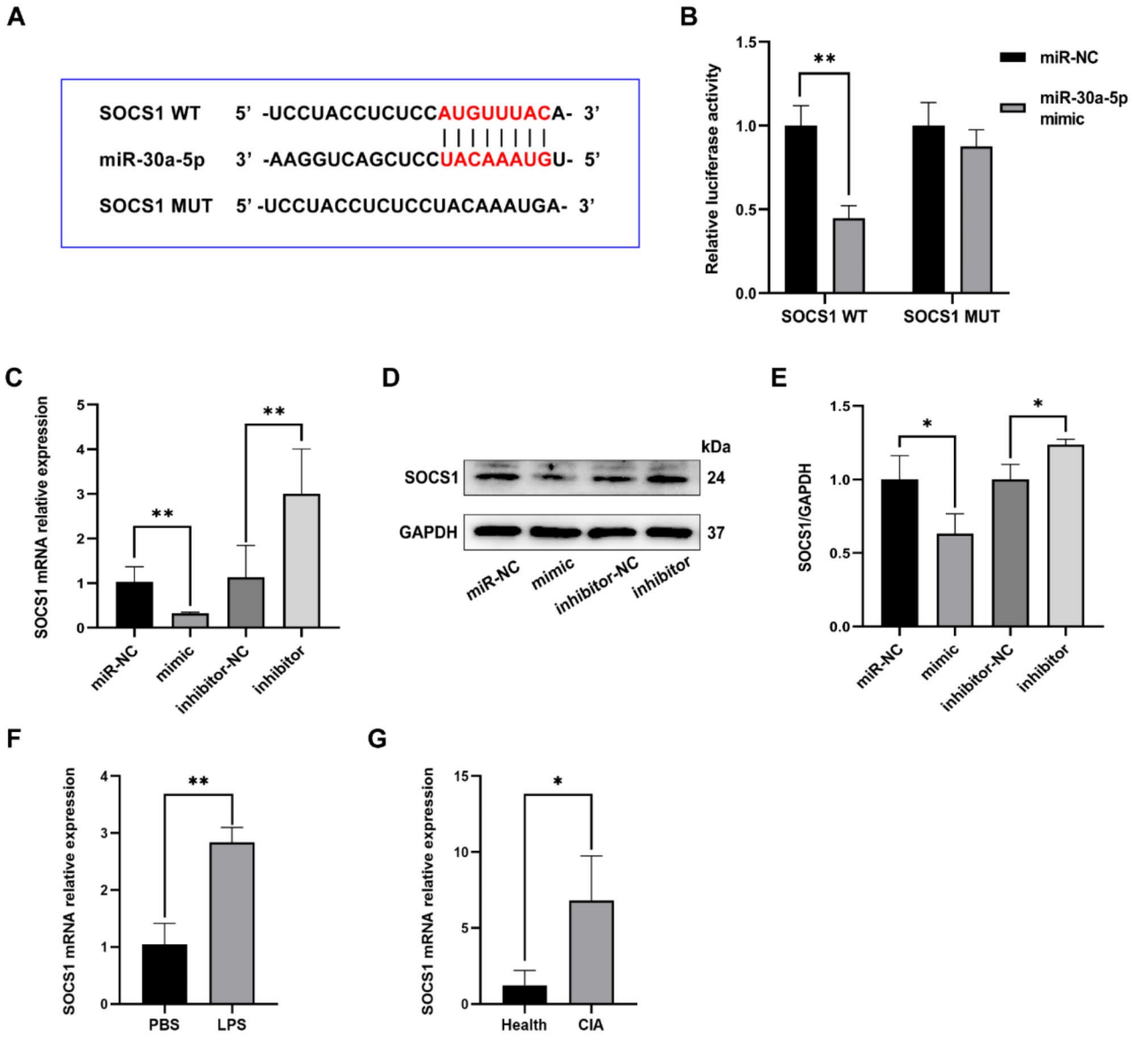

Fig. 5. MiR-30a-5p directly interacts with SOCS1. (**A**) Predicted binding sequence between miR-30a-5p and SOCS1. (**B**) Binding relationship between miR-30a-5p and SOCS1 confirmed by dual-luciferase reporter assays with SOCS1 WT and SOCS1 MUT. (**C**) mRNA relative expression of SOCS1 measured by RT-qPCR. (**D**,**E**) Expression of SOCS1 detected by western blot analysis. (**F**,**G**) SOCS1 expression in LPS-induced J774A.1 cells and CIA mice assessed by RT-qPCR. Data from more than three repeated experiments are presented as mean ± SD. $^{*}P < 0.05$, $^{**}P < 0.01$.

documented increased release of pro-inflammatory cytokines IL-1β, IL-18, and LDH with MIAT knockdown[31]. However, Zhang et al. suggested that the lack of MIAT mitigated high glucose-evoked inflammatory responses in podocytes, leading to reduced release of inflammatory cytokines TNF-α, IL-6, and IL-1β[32]. Discrepancies in our conclusions may stem from differences in cell types and stimulation conditions. Moreover, the regulatory mechanisms of MIAT are intricate, involving gene mutations, copy number alterations, transcription factors, DNA methylation, miRNA, and RNAbinding proteins. Our work shows that the production of pro-inflammatory cytokines significantly elevated under inflammatory conditions, and that MIAT is upregulated to attenuate the expression of TNF-α and IL-1β, which contributes to fine-tune the inflammatory response by compensating for the protective response, may preventing an uncontrolled and potentially deleterious immune response. While our results highlight the positive role of MIAT in RA progression, the exact mechanisms by which MIAT modulates inflammation remain unclear.

As previously highlighted, long non-coding RNAs (lncRNAs) often function as endogenous competitive RNAs (ceRNAs), binding to miRNAs to influence their activity[15–17]. MiRNAs, recognized for their regulatory role in inflammatory processes, are key players in various inflammation-associated diseases[18]. In the context of rheumatoid arthritis (RA), miRNAs contribute significantly to diagnosis, reflecting disease severity, predicting

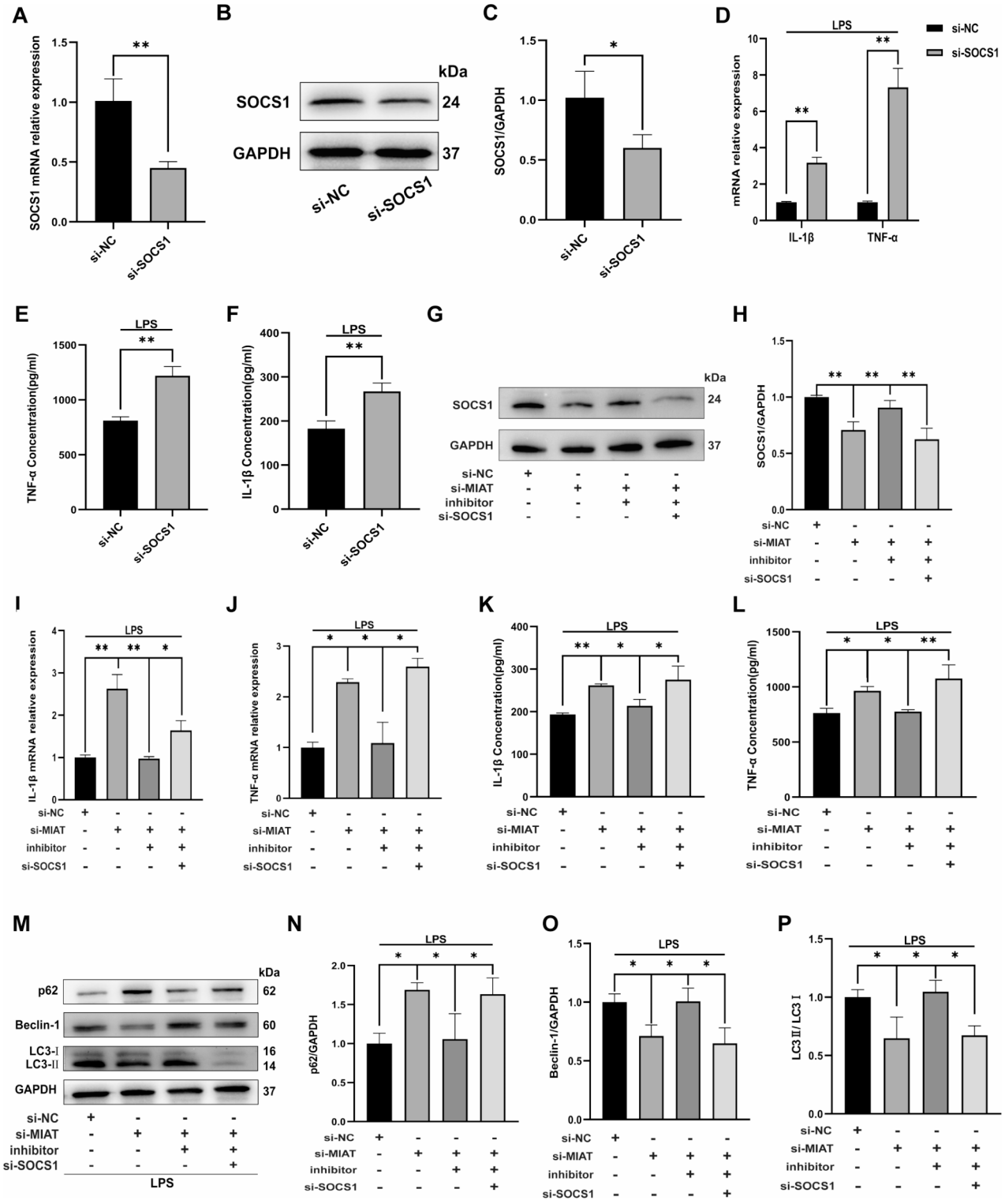

Fig. 6. LncRNA MIAT functions as a ceRNA for miR-30a-5p to regulate SOCS1 expression in J774A.1 cells. (**A**) SOCS1 mRNA levels measured by RT-qPCR in J774A.1 cells transfected with si-SOCS1. (**B**,**C**) SOCS1 protein expression detected by western blot analysis. (**D**–**F**) TNF-α and IL-1β levels measured by RT-qPCR or ELISA. (**G**,**H**) SOCS1 expression detected by western blot analysis in each group. (**I**–**L**) TNF-α and IL-1β levels measured by RT-qPCR or ELISA in each group. (**M**–**P**) Protein expression of LC3, p62, Beclin-1, and GAPDH detected by western blot analysis. Data from more than three repeated experiments are presented as mean ± SD. $^{*}P < 0.05$, $^{**}P < 0.01$.

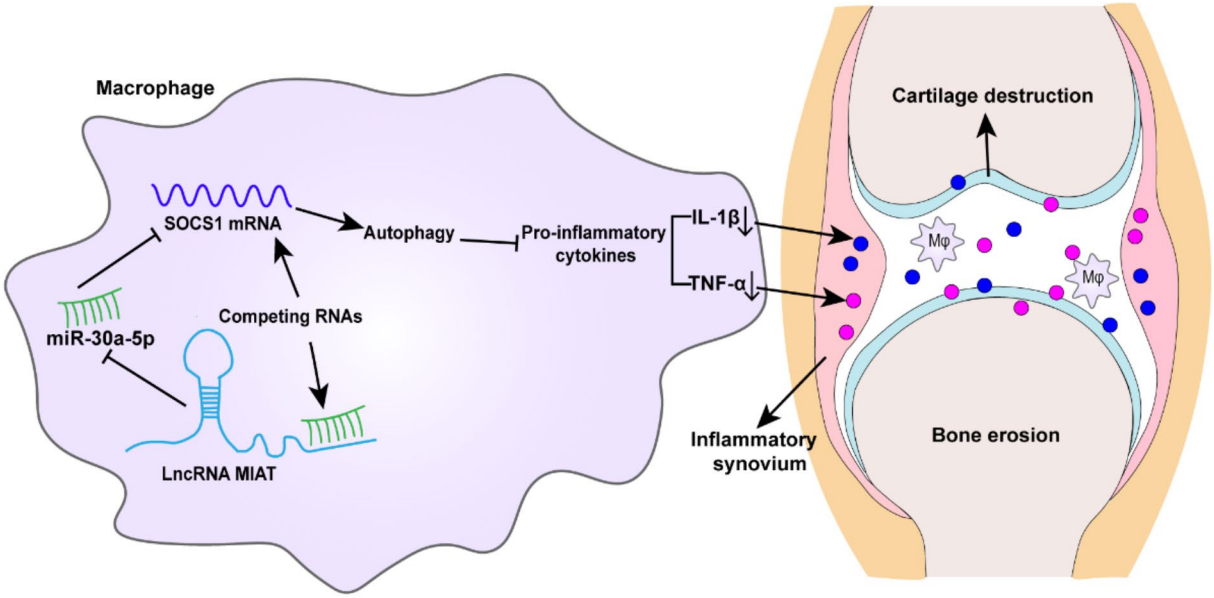

Fig. 7. Potential mechanisms of MIAT inhibits inflammation in LPS-induced J774A.1 macrophages.

treatment responses, and even guiding treatment strategies[33,34]. Our investigation into the molecular mechanisms underlying MIAT-associated modulations in LPS-induced J774A.1 cells employed bioinformatics to identify potential miRNAs interacting with MIAT. Remarkably, miR-30a-5p emerged as a downstream target of MIAT. A recent study implicated miR-30a-5p, the mature form of miR-30a, in exacerbating inflammation and organ damage in a sepsis model[35]. Additionally, the literature indicates that miR-30a-5p is involved in inflammation, as demonstrated by studies revealing its regulatory role in chondrocyte proliferation, cell cycle progression, and inflammation[36,37]. Our findings revealed decreased miR-30a-5p expression in collagen-induced arthritis (CIA) mice and LPS-treated J774A.1 cells, consistent with previous observations of reduced miR-30a levels in synovial tissues from RA patients compared to those with osteoarthritis (OA)[38]. Transfection with miR-30a-5p mimic increased TNF-α and IL-1β expression, in line with prior research[39]. Dual luciferase reporter and RNA immunoprecipitation (RIP) assays further confirmed MIAT's role as a molecular sponge for miR-30a-5p. Notably, our data demonstrated MIAT's negative regulation of miR-30a-5p expression. Furthermore, it has been reported that microRNAs constitute the negative feedback mechanisms controlling the inflammatory response, preventing excessive inflammation, and miR-30a-5p may be compensatory downregulated in CIA mice and LPS-treated cells. Simultaneously, our study reported that MIAT knockdown effects were partially reversed by miR-30a-5p inhibitor, collectively establishing MIAT as a key player in the miR-30a-5p regulatory axis.

Suppressor of cytokine signalling-1 (SOCS1), a member of the SOCS family, assumes a pivotal role in the inflammatory process by inhibiting the generation of pro-inflammatory cytokines[40,41]. Conversely, SOCS1-deficient macrophages exhibit increased production of inflammatory cytokines, including TNF-α, IL-6, and IL-21[42,43]. Mechanistically, DNMT1-mediated SOCS1 hypermethylation results in reduced SOCS1 expression, negatively impacting the JAK2/STAT3 pathway and increasing LPS-induced pro-inflammatory cytokine release in macrophages[44]. Moreover, elevated miR-155 levels have been linked to decreased SOCS1 expression, contributing to increased TNF-α and IL-1β production in RA patients[45]. Our bioinformatics analysis predicted SOCS1 as a target of miR-30a-5p, with luciferase reporter assays confirming direct binding. MiR-30a-5p negatively regulated SOCS1 expression, establishing SOCS1 as a direct target gene of miR-30a-5p in J774A.1 cells. Our data further indicated that MIAT deficiency reduced SOCS1 expression, emphasizing MIAT's collaboration with miR-30a-5p to positively regulate SOCS1 expression. Functional restoration assays confirmed the interplay of MIAT, miR-30a-5p, and SOCS1 in modulating LPS-induced inflammation. Inhibition of miR-30a-5p reversed the pro-inflammatory effect of MIAT deficiency, while SOCS1 knockdown counteracted the cytokine inhibitory effect of miR-30a-5p silencing. These findings underscore the involvement of the MIAT/miR-30a-5p/SOCS1 axis in modulating the anti-inflammatory response in LPS-induced J774A.1 cells.

Autophagy, a cellular process integral to protein and organelle degradation, assumes a pivotal role in sustaining cellular homeostasis. By removing damaged organelles, degrading pro-inflammatory signaling molecules, and regulating the production of inflammatory cytokines, autophagy actively diminishes pro-inflammatory signaling[46,47]. In the context of inflammation, the NLRP3 inflammasome, under inflammatory conditions, binds to and activates MTOR phosphorylation, thereby inhibiting autophagy. This impairment of autophagy exacerbates inflammation by hindering the elimination of pro-inflammatory mediators[48]. In addition, Zhang et

al. found that TFDG protected against collagen-induced arthritis by reducing the inflammation and facilitating anti-inflammatory M2 macrophage polarization through promoting autophagy[49]. MIAT has been reported to promote autophagy in neuronal cells by targeting REDD1 expression[50]. Our study corroborates this by revealing that interference with MIAT restricted macrophage autophagy, evidenced by decreased expression of autophagy-related markers LC3-II and Beclin-1, along with an increase in p62 in LPS-induced J774A.1 cells. Furthermore, miR-30a, identified in previous studies as an inhibitor of autophagy through Beclin-1 gene targeting[38,51], demonstrated an inhibitory effect on autophagy markers LC3, P62, and Beclin-1 in our experiments. Notably, knockdown of MIAT's impact on J774A.1 cell autophagy was partially reversed by miR-30a-5p inhibitor, while SOCS1 knockdown counteracted the effects of miR-30a-5p inhibitor. These results suggest that MIAT regulates macrophage autophagy through the miR-30a-5p/SOCS1 axis. Autophagy has been shown to engage in complex interplay with apoptosis. Generally, autophagy prevents the induction of apoptosis, whereas apoptosis-related caspase activation shuts down the autophagic process. The activation of caspase-3 also can lead to the cleavage of autophagy protein Beclin-1, which results in the inactivation of the autophagic program and cell apoptosis[52]. The dialogue between macrophage autophagy and apoptosis has been reported to influence inflammation. MIAT silencing has been reported to decrease cell viability, proliferation and invasion while enhancing cell senescence and apoptosis[53]. Moreover, with apoptosis, large amounts of pro-inflammatory cytokines are released, thereby exacerbating the inflammatory response. Therefore, we thought that MIAT might promote autophagy and prevent apoptosis thus inhibiting the inflammatory response.

The amplification of NF-κB signaling pathway is one of the major mechanisms involved in the pathogenesis of RA, linked to releasing proinflammatory cytokines and further promoting the disease progression. The NF-κB canonical pathway is activated by proinflammatory cytokines receptors such as TNFR, IL-1 receptor (IL-1R), and some members of toll-like receptor (TLR). LPS signals through TLR4 to induce several distinct signaling pathways in macrophages, which predominantly converge on the activation of NF-κB and its target genes, inducing archetypal proinflammatory cytokines TNF-a, IL-1, and IL-6[54]. Zhou et al. demonstrated that LPS downregulates autophagy in colitis in vitro through the TLR4-MyD88-MAPK signaling pathway, leading to downstream NF-κB activation and subsequent production of pro-inflammatory cytokines and oxidative stress[55]. Dong et al. demonstrated MIAT inhibited the NF-κB pathway and DN progression in vitro via the mediation of miR-182-5p/GPRC5A network[13]. We show that upon cellular stimulation by LPS, MIAT is upregulated and acts as a competitive endogenous RNA to inhibit miR-30a-5p by sponging miR-30a-5p, thereby upregulating the expression of SOCS1, promoting autophagy and decreasing the production of TNF-α and IL-Iβ. Previous studies have demonstrated that high expression of SOCS1 inhibits LPS-induced inflammatory cytokines and NF-κB activation[56]. These findings suggest that MIAT may act as a novel negative feedback regulator of TLR signaling, inhibit the activity of NF-κB, reduce the production of pro-inflammatory cytokines, and prevent excessive inflammatory immune responses.

While our study provides valuable insights, the absence of in vivo experiments remains a limitation. In addition, not only immune cells but also fibroblast-like synoviocytes (FLSs) play an important role in the chronic inflammation and subsequent joint destruction of rheumatoid arthritis. Therefore, future studies in animals should further clarify that whether the treatment works as expected mainly through the cell types investigated in vitro to further consolidate the significance and value of the present study.

In summary, our study unveils MIAT's potential role as a competing endogenous RNA (ceRNA), sponging miR-30a-5p to regulate SOCS1. Inhibiting immune cells to reduce inflammation is the current therapeutic strategy for RA. MIAT's regulatory mechanism promotes autophagy and suppresses cytokine production, offering a promising avenue for inflammation reduction of macrophage in RA. The lncRNA MIAT/miR-30a-5p/SOCS1 axis emerges as a significant player in the development of RA, showcasing MIAT as a potential therapeutic target for RA treatment.

Experimental procedures

Cell culture and LPS treatment

J774A.1 cells (Pricella Biotechnology Co. Ltd., Wuhan, China), a murine macrophage cell line, were cultured in Dulbecco's modified Eagle medium (DMEM, high glucose) (Biological Industries [BI], Kibbutz Beit HaEmek, Israel), supplemented with 10% fetal bovine serum (BI) and 1% penicillin/streptomycin (Pricella). The cells were maintained at 37 °C in a humidified incubator containing 5% CO2. J774A.1 cells were subjected to a 24-hour treatment with 100 ng/ml lipopolysaccharide (LPS) (Sigma-Aldrich Corp., Saint Louis, MO, USA) to induce an inflammatory state.

Cell transfection

SiRNA negative control (si-NC), si-MIAT, si-SOCS1, mimic NC, miR-30a-5p mimic, inhibitor NC, and miR-30a-5p inhibitor were procured from GenePharma (Shanghai, China). Cellular transfection was executed using TransIntro EL Transfection Reagent (TransGen Biotech, Beijing, China) following the manufacturer's instructions. Following a 24-hour transfection, cells were cultured in the presence or absence of LPS for an additional 24 h. After 48 h of transfection, the transfected cells were harvested for subsequent analysis and detection.

RNA extraction and quantitative real-time PCR (RT-qPCR)

Total RNA was extracted from tissues or cells using RNAiso Plus (Takara Biomedical Technology, Beijing, China) in accordance with the protocol. Subsequently, RNA underwent reverse transcription into cDNA using Evo M-MLV RT Kit for qPCR (Accurate Biotechnology [Hunan] Co. Ltd., Changsha, China). The ensuing qPCR was carried out using SYBR Green Premix Pro Taq HS qPCR Kit (Accurate Biotechnology) on a LightCycler 480 system (Roche, Basel, Switzerland) as per the manufacturer's instructions. Amplification conditions comprised

95 °C for 5 s and 60 °C for 30 s, over a total of 40 cycles. Primers for amplification are detailed in Table 1. U6 and GAPDH served as references for miRNAs and mRNAs, respectively. Relative expression was calculated using the $2^{-\Delta\Delta Ct}$ method.

Dual luciferase activity assay

Predicted targets of ncRNAs were identified through lncRNABase and Starbase2.0. Fragments of MIAT containing the predicted binding or mutated sites for miR-30a-5p were cloned into the pmirGLO vector (Promega, Madison, WI, USA) to generate the wild-type or mutant-type MIAT (MIAT WT or MIAT MUT) reporter vector. Similarly, SOCS1 WT and SOCS1 MUT reporter vectors were produced. The reporter vectors, along with miR-30a-5p mimic or mimic NC, were transfected into J774A.1 cells using TransIntro EL Transfection Reagent. After 48 h of co-transfection, luciferase activity was assessed using the Dual-Luciferase Reporter Gene Assay Kit (Yeasen, Shanghai, China), with Renilla luciferase activity normalizing firefly luciferase activity.

Western blot analysis

Total proteins were extracted using RIPA buffer (Solarbio, Beijing, China) supplemented with a protease inhibitor cocktail (MedChemExpress, NJ, USA). Protein concentration was quantified using the BCA protein assay kit (Elabscience, Wuhan, China). Equal protein amounts from each sample underwent electrophoresis on an SDS-PAGE gel and were transferred to PVDF membranes (Millipore Corp., Billerica, MA, USA). Following blocking with 5% non-fat milk, the membranes were incubated overnight at 4 °C with antibodies against LC3B (Cell Signaling Technology, Danvers, MA, USA), GAPDH, SOCS1 (Elabscience, Wuhan, China), Beclin-1, and p62 (Proteintech, Wuhan, China). Subsequently, the membranes were incubated with horseradish peroxidase-conjugated secondary antibodies at room temperature for 1.5 h. Immunostained bands were detected using the chemiluminescence kit (Affinity, Jiangsu, China). Results were expressed as a ratio to GAPDH protein, and band intensity was analysed using ImageJ software (National Institutes of Health, Bethesda, MD, USA).

Animals

Male DBA/1J mice, 6–8 weeks old, were obtained from Charles River Laboratory (Beijing, China) and housed in the Laboratory Animal Center of the Affiliated Hospital of Qingdao University (Qingdao, China) under specific pathogen-free (SPF) conditions. Animal experiments were conducted with approval from the Laboratory Animal Care and Use Committee of the Affiliated Hospital of Qingdao University (Approval No. AHQU-MAL20220902). All procedures adhered to relevant guidelines and regulations and were reported in accordance with ARRIVE guidelines.

Collagen-induced arthritis (CIA) models and disease assessment

Mice received intradermal injections into their tails on day 0 with an emulsified mixture containing complete Freund's adjuvant (2 mg/mL; Chondrex, Washington, DC, USA) and bovine type II collagen (2 mg/mL; Chondrex), and on day 21, a boost immunization containing incomplete Freund's adjuvant (2 mg/mL; Chondrex) and collagen (2 mg/mL; Chondrex). Each paw was evaluated weekly, scored individually from 0 to 4 based on the severity of swelling and erythema. Serum samples, collected on the last day, were used to measure cytokine secretion levels.

Enzyme-linked immunosorbent assay (ELISA)

Serum and cell supernatants were collected to measure cytokine secretion. TNF-α and IL-1β concentrations were determined using ELISA kits (Jingmei Biotech, Jiangsu, China) according to the provided instructions.

Hematoxylin and eosin (H&E) staining

All mice knee joints were fixed in 4% paraformaldehyde, decalcified with 10% EDTA (Servicebio, Wuhan, China), and embedded in paraffin. Fixed tissues were cut into 4-um-thick sections in the sagittal plane, followed by deparaffinization, rehydration, and staining with hematoxylin and eosin. Inflammatory cell infiltration degree was assessed under a microscope with a white light laser system (Olympus, Tokyo, Japan).

RNA immunoprecipitation (RIP) assay

The RNA immunoprecipitation (RIP) assay was conducted employing an RNA immunoprecipitation kit (BersinBio, Guangzhou, China), following the manufacturer's guidelines. Briefly, cells were lysed in RIP lysis buffer, and the resultant cell lysate was incubated with magnetic beads conjugated with either IgG or Ago2

Gene	Forward primer (5'–3')	Reverse primer (5'–3')
GAPDH	ACCCAGAAGACTGTGGATGGC	TCAGATCCACGACGGACACAT
MIAT	AATGGAGAGACCCCGTAGGAA	TGTGGAAGATTGGCCATGAG
IL-1β	TATGAGCTGAAAGCTCTCCACCTC	GCCGTCTTTCATTACACAGGACA
TNF-α	AAATGGCCTCCCTCTCATCAGT	GGTGGTTTGCTACGACGTGG
SOCS1	CTGCGGCTTCTATTGGGGAC	AAAAGGCAGTCGAAGGTCTCG
U6	CGCTTCGGCAGCACATATACTA	GGAACGCTTCACGAATTTGC
miR-30a-5p	GGGGTGTAAACATCCTCGACTG	CCAGTGCAGGGTCCGAGGT

Table 1. Primers used for amplification of RT-qPCR.

antibody (Proteintech, Wuhan, China). RT-qPCR was subsequently employed to assess the expression of MIAT and miR-30a-5p in the precipitates.

Statistical analysis

Statistical analyses were performed using GraphPad Prism 9.0 (Graphpad Software Inc., La Jolla, CA, USA). One-way analysis of variance (ANOVA) was applied to assess differences among multiple independent groups, while the unpaired Student's t-test was employed for comparisons between two groups. Results are presented as mean ± standard deviation (SD) based on at least three repetitions. Statistical significance was defined as $P < 0.05$.

Data availability

All data generated during the current study are available from the corresponding author on reasonable request.

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References

1. Smith, M. H. & Berman, J. R. What Is Rheumatoid Arthritis? *JAMA* **327**, 1194 (2022).
2. Kinne, R. W., Stuhlmüller, B. & Burmester, G. R. Cells of the synovium in rheumatoid arthritis. Macrophages. *Arthritis Res. Ther.* **9**, 224 (2007).
3. Athanasou, N. A. Synovial macrophages. *Ann. Rheum. Dis.* **54**, 392–394 (1995).
4. Ardura, J. A. et al. Targeting macrophages: friends or foes in Disease? *Front. Pharmacol.* **10**, 1255 (2019).
5. Ali, S. A., Peffers, M. J., Ormseth, M. J., Jurisica, I. & Kapoor, M. The non-coding RNA interactome in joint health and disease. *Nat. Rev. Rheumatol.* **17**, 692–705 (2021).
6. Wen, J., Liu, J., Wan, L. & Wang, F. Long noncoding RNA/circular RNA regulates competitive endogenous RNA networks in rheumatoid arthritis: molecular mechanisms and traditional Chinese medicine therapeutic significances. *Ann. Med.* **55**, 973–989 (2023).
7. England, B. R., Thiele, G. M., Anderson, D. R. & Mikuls, T. R. Increased cardiovascular risk in rheumatoid arthritis: mechanisms and implications. *BMJ* **361**, k1036 (2018).
8. Tan, J., Liu, S., Jiang, Q., Yu, T. & Huang, K. LncRNA-MIAT Increased in Patients with Coronary Atherosclerotic Heart Disease. *Cardiol. Res. Pract.* 6280194 (2019). (2019).
9. Yan, B. et al. lncRNA-MIAT regulates microvascular dysfunction by functioning as a competing endogenous RNA. *Circ. Res.* **116**, 1143–1156 (2015).
10. Ye, Z. M. et al. LncRNA MIAT sponges mir-149-5p to inhibit efferocytosis in advanced atherosclerosis through CD47 upregulation. *Cell. Death Dis.* **10**, 138 (2019).
11. Qi, Y. et al. LncRNA-MIAT-Mediated mir-214-3p silencing is responsible for IL-17 production and Cardiac Fibrosis in Diabetic Cardiomyopathy. *Front. Cell. Dev. Biol.* **8**, 243 (2020).
12. Zhou, L. et al. Long non-coding MIAT mediates high glucose-induced renal tubular epithelial injury. *Biochem. Biophys. Res. Commun.* **468**, 726–732 (2015).
13. Dong, Q. et al. Long noncoding RNA MIAT inhibits the progression of diabetic nephropathy and the activation of NF-κB pathway in high glucose-treated renal tubular epithelial cells by the miR-182-5p/GPRC5A axis. *Open. Med.* **16**, 1336–1349 (2021).
14. Wang, Z. et al. LncRNA MIAT downregulates IL-1β, TNF-α to suppress macrophage inflammation but is suppressed by ATP-induced NLRP3 inflammasome activation. *Cell. Cycle* **20**, 194–203 (2021).
15. Mousavi, M. J. et al. Implications of the noncoding RNAs in rheumatoid arthritis pathogenesis. *J. Cell. Physiol.* **234**, 335–347 (2018).
16. Li, L. J. et al. Competitive endogenous RNA network: potential implication for systemic lupus erythematosus. *Expert Opin. Ther. Targets* **21**, 639–648 (2017).
17. Ala, U. Competing endogenous RNAs, non-coding RNAs and diseases: an intertwined story. *Cells* **9**, 1574 (2020).
18. Chen, J. Q., Papp, G., Szodoray, P. & Zeher, M. The role of microRNAs in the pathogenesis of autoimmune diseases. *Autoimmun. Rev.* **15**, 1171–1180 (2016).
19. Zhang, Y. et al. Inferences of individual differences in response to tripterysium glycosides across patients with rheumatoid arthritis using a novel ceRNA regulatory axis. *Clin. Transl Med.* **10**, e185 (2020).
20. Yang, M. et al. Identification of the potential regulatory interactions in rheumatoid arthritis through a comprehensive analysis of lncRNA-related ceRNA networks. *BMC Musculoskelet. Disord.* **24**, 799 (2023).
21. Fu, Q., Song, M. J. & Fang, J. LncRNA OSER1-AS1 regulates the inflammation and apoptosis of rheumatoid arthritis fibroblast like synoviocytes via regulating miR-1298-5p/E2F1 axis. *Bioengineered* **13**, 4951–4963 (2022).
22. Jiang, H., Liu, J., Fan, C., Wang, J. & Li, W. lncRNAS56464.1 as a ceRNA promotes the proliferation of fibroblast–like synoviocytes in experimental arthritis via the wnt signaling pathway and sponges miR–152–3p. *Int. J. Mol. Med.* **47**, 17 (2021).
23. Yan, S. et al. Long non-coding RNA HIX003209 promotes inflammation by sponging miR-6089 via TLR4/NF-κB signaling pathway in rheumatoid arthritis. *Front. Immunol.* **10**, 2218 (2019).
24. Hartleben, B. et al. Autophagy influences glomerular disease susceptibility and maintains podocyte homeostasis in aging mice. *J. Clin. Invest.* **120**, 1084–1096 (2010).
25. Celia, A. I., Colafrancesco, S., Barbati, C., Alessandri, C. & Conti, F. Autophagy in Rheumatic diseases: Role in the Pathogenesis and therapeutic approaches. *Cells* **11**, 1359 (2022).
26. Yang, S., Yin, W., Ding, Y. & Liu, F. Lnc RNA ZFAS1 regulates the proliferation, apoptosis, inflammatory response and autophagy of fibroblast-like synoviocytes via miR-2682-5p/ADAMTS9 axis in rheumatoid arthritis. *Biosci. Rep.* **40**, BSR20201273 (2020).
27. Tofigh, R., Hosseinpourfeizi, M., Baradaran, B., Teimourian, S. & Safaralizadeh, R. Rheumatoid arthritis and non-coding RNAs; how to trigger inflammation. *Life Sci.* **315**, 121367 (2023).
28. Huang, W. et al. LncRNAs and rheumatoid arthritis: from identifying mechanisms to Clinical Investigation. *Front. Immunol.* **12**, 807738 (2022).
29. Udalova, I. A., Mantovani, A. & Feldmann, M. Macrophage heterogeneity in the context of rheumatoid arthritis. *Nat. Rev. Rheumatol.* **12**, 472–485 (2016).
30. Maldonado, R. F., Sá-Correia, I. & Valvano, M. A. Lipopolysaccharide modification in Gram-negative bacteria during chronic infection. *FEMS Microbiol. Rev.* **40**, 480–493 (2016).
31. Li, J. et al. Long noncoding RNA MIAT regulates hyperosmotic stress-Induced corneal epithelial cell Injury via inhibiting the caspase-1-Dependent pyroptosis and apoptosis in Dry Eye Disease. *J. Inflamm. Res.* **15**, 3269–3283 (2022).
32. Zhang, M. et al. Ablation of lncRNA MIAT mitigates high glucose-stimulated inflammation and apoptosis of podocyte via miR-130a-3p/TLR4 signaling axis. *Biochem. Biophys. Res. Commun.* **533**, 429–436 (2020).

33. Chen, X. M. et al. Role of Micro RNAs in the pathogenesis of rheumatoid arthritis: novel perspectives based on review of the literature. *Med. (Baltim).* **94**, e1326 (2015).
34. Churov, A. V., Oleinik, E. K. & Knip, M. MicroRNAs in rheumatoid arthritis: altered expression and diagnostic potential. *Autoimmun. Rev.* **14**, 1029–1037 (2015).
35. Shangxun, Z. et al. ADAR1 Alleviates Inflammation in a Murine Sepsis Model via the ADAR1-miR-30a-SOCS3 Axis. *Mediators Inflamm.* 9607535 (2020). (2020).
36. Zhang, Y. et al. Long noncoding RNA LINC00461 induced osteoarthritis progression by inhibiting miR-30a-5p. *Aging* **12**, 4111–4123 (2020).
37. Liu, X. et al. miR-30a-5p inhibits osteogenesis and promotes periodontitis by targeting Runx2. *BMC Oral Health* **21**, 513 (2021).
38. Xu, K. et al. Reduced apoptosis correlates with enhanced autophagy in synovial tissues of rheumatoid arthritis. *Inflamm. Res. Off J. Eur. Histamine Res. Soc. Al* **62**, 229–237 (2013).
39. Zhang, Y. et al. MicroRNA-30a-5p silencing polarizes macrophages toward M2 phenotype to alleviate cardiac injury following viral myocarditis by targeting SOCS1. *Am. J. Physiol. Heart Circ. Physiol.* **320**, H1348–H1360 (2021).
40. Mortazavi-Jahromi, S. S., Farazmand, A., Motamed, N., Navabi, S. S. & Mirshafiey, A. Effects of guluronic acid (G2013) on SHIP1, SOCS1 induction and related molecules in TLR4 signaling pathway. *Int. Immunopharmacol.* **55**, 323–329 (2018).
41. Wang, D. et al. MiRNA-155 regulates the Th17/Treg ratio by targeting SOCS1 in severe Acute Pancreatitis. *Front. Physiol.* **9**, 686 (2018).
42. Lee, T. L., Yeh, J., Van Waes, C. & Chen, Z. Epigenetic modification of SOCS-1 differentially regulates STAT3 activation in response to interleukin-6 receptor and epidermal growth factor receptor signaling through JAK and/or MEK in head and neck squamous cell carcinomas. *Mol. Cancer Ther.* **5**, 8–19 (2006).
43. Gagnon, J., Ramanathan, S., Leblanc, C. & Ilangumaran, S. Regulation of IL-21 signaling by suppressor of cytokine signaling-1 (SOCS1) in CD8(+) T lymphocytes. *Cell. Signal.* **19**, 806–816 (2007).
44. Cheng, C. et al. SOCS1 hypermethylation mediated by DNMT1 is associated with lipopolysaccharide-induced inflammatory cytokines in macrophages. *Toxicol. Lett.* **225**, 488–497 (2014).
45. Li, X., Tian, F. & Wang, F. Rheumatoid arthritis-associated microRNA-155 targets SOCS1 and upregulates TNF-α and IL-1β in PBMCs. *Int. J. Mol. Sci.* **14**, 23910–23921 (2013).
46. Deretic, V. & Levine, B. Autophagy balances inflammation in innate immunity. *Autophagy* **14**, 243–251 (2018).
47. Netea-Maier, R. T., Plantinga, T. S., van de Veerdonk, F. L., Smit, J. W. & Netea, M. G. Modulation of inflammation by autophagy: consequences for human disease. *Autophagy* **12**, 245–260 (2016).
48. Cosin-Roger, J. et al. Hypoxia ameliorates intestinal inflammation through NLRP3/mTOR downregulation and autophagy activation. *Nat. Commun.* **8**, 98 (2017).
49. Zhang, L. et al. Theaflavin-3,3'-Digallate ameliorates Collagen-Induced Arthritis through Regulation of Autophagy and Macrophage polarization. *J. Inflamm. Res.* **16**, 109–126 (2023).
50. Guo, X., Wang, Y., Zheng, D., Cheng, X. & Sun, Y. LncRNA-MIAT promotes neural cell autophagy and apoptosis in ischemic stroke by up-regulating REDD1. *Brain Res.* **1763**, 147436 (2021).
51. Zhu, H. et al. Regulation of autophagy by a beclin 1-targeted microRNA, miR-30a, in cancer cells. *Autophagy* **5**, 816–823 (2009).
52. Song, S., Tan, J., Miao, Y., Li, M. & Zhang, Q. Crosstalk of autophagy and apoptosis: involvement of the dual role of autophagy under ER stress. *J. Cell. Physiol.* **232**, 2977–2984 (2017).
53. Ghafouri-Fard, S., Azimi, T. & Taheri, M. Myocardial infarction Associated transcript (MIAT): review of its impact in the tumorigenesis. *Biomed. Pharmacother Biomedecine Pharmacother* **133**, 111040 (2021).
54. Dorrington, M. G. & Fraser, I. D. C. NF-κB Signaling in macrophages: Dynamics, Crosstalk, and Signal Integration. *Front. Immunol.* **10**, 705 (2019).
55. Zhou, M. et al. Boosting mTOR-dependent autophagy via upstream TLR4-MyD88-MAPK signalling and downstream NF-κB pathway quenches intestinal inflammation and oxidative stress injury. *EBioMedicine* **35**, 345–360 (2018).
56. Nakagawa, R. et al. SOCS-1 participates in negative regulation of LPS responses. *Immunity* **17**, 677–687 (2002).

Author contributions

Conceptualization, L.S. and K.Y.; methodology, L.W; validation, S.W. and D.W.; formal analysis, L.W., D.W.; investigation, L.S., L.W., S.W.; writing-original draft preparation, L.S.; writing-review and editing, J.W. and K.Y.; project administration and funding acquisition, J.W. All authors have reviewed the final version of the manuscript and approved it for publication.

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Declarations

Competing interests

The authors declare no competing interests.

Additional information

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Correspondence and requests for materials should be addressed to J.W.