Long non-coding RNA 01126 activates IL-6/JAK2/STAT3 pathway via serving as a sponge of miR-655-3p in periodontitis

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Research Article

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Abstract

Background

Periodontitis is a serious threat to oral quality of life and overall health. Although our previous studies confirmed that long intergenic non-coding RNA 01126 (LINC01126) is aberrantly expressed in periodontitis tissues, there are few reports on the pathogenesis of LINC01126 in periodontitis. Our study investigated the biological functions of LINC01126 in periodontitis and the potential mechanism.

Results

An inflammatory model of human gingival fibroblasts (HGFs) was successfully established. LINC01126 silencing can alleviate lipopolysaccharide (LPS) induced cell inflammation, reduce cell apoptosis, and promote cell migration. As a "sponge" for miR-655-3p, LINC01126 inhibits its binding to mRNA of IL-6, thereby promoting inflammation progression and JAK2/STAT3 pathway activation. qRT- PCR, WB, and IHC results of clinical tissue samples further confirmed that miR-655-3p expression was down-regulated and IL-6/JAK/STAT3 was abnormally activated in periodontitis tissues.

Conclusions

Our results indicate that LINC01126, as an endogenous competitive RNA (ceRNA) of miR-655-3p, can promote IL-6/JAK3/STAT3 pathway activation, thereby promoting periodontitis pathogenesis. And this is the first study of miR-655-3p in inflammatory periodontal diseases. Our study reveales a new pathogenesis of periodontitis, and provides a new strategy for preventing and treating periodontitis.

Background

Periodontitis is a chronic infectious disease with high incidence in the population, which seriously harms the oral health-related quality of life[1]. Porphyromonas gingivalis (Pg) and Actinobacillus actinomycetemcomitans (Aa) are the most dominant bacteria in the lesion or active site during periodontitis development[2]. Most periodontitis patients have a chronic, long-term disease process, accompanied by bleeding gums, loose teeth, weak bite, and other symptoms[3]. Clinically, basic periodontal treatment (including supragingival scaling and root planning) is the key measure for controlling dental plaque and delaying periodontitis progression[3, 4]. However, there is currently no cure for periodontitis that results in complete physiological healing of periodontal tissues. At present, studies on periodontitis pathogenesis mainly focus on dental plaque[5], immune regulation[6], and genetic susceptibility[7]. Numerous studies have demonstrated a close association between general health and periodontitis, including heart diseases, diabetes, Alzheimer's disease, and adverse pregnancy[8–12]. As a

result, elucidating the pathogenic mechanism of periodontitis is critical for preventing and treating periodontitis and systemic diseases.

Gingival tissue, an important part of periodontal tissue, is involved in the earliest periodontal inflammatory response and maintains an inflammatory state throughout the whole process of periodontitis occurrence and development. Inflammatory gums are often associated with changes in color and texture, and the infiltration of inflammatory cells is significantly increased. Human gingival fibroblasts (HGFs) are the main cells in gingival tissue, and their physiological function determines the health condition of periodontal tissues[13]. Healthy HGFs can participate in connective tissue matrix synthesis, promote the healing of inflammatory periodontal tissues, and play a crucial role in the reconstruction, regeneration, and physiological function of periodontal support tissue[14–16]. Toll-like receptors (TLRs) on the surface of HGFs are activated in response to the invasion of periodontal pathogens and are involved in immune regulation[17].

Long non-coding RNAs (lncRNAs), with a length of more than 200 ribonucleotides and lacking a complete open reading frame (ORF), have great potential for maintaining stem cell differentiation and pluripotency, and their involvement in various biological processes has been extensively reported, including epigenetic processes, transcriptional regulation, gene silencing, and chromatin modification[18, 19]. MicroRNA, encoded by endogenous genes, often target to the mRNA sequence of the target gene or are sponged by lncRNAs, thereby regulating the target gene's expression and forming “lncRNAs-microRNAs-mRNAs” network[20]. By increasing researches on lncRNAs, some have been gradually introduced into oral diseases. Researches have demonstrated that lncRNAs regulate osteogenic differentiation, cell apoptosis, and alveolar bone metabolism of periodontal cells. Jia et al. found that lncRNA-ANCR down-regulation could promote the proliferation of periodontal ligament stem cells (PDLSCs) and osteogenic differentiation of PDLSCs by upregulation of osteogenic differentiation-related genes[21]. LncRNA PCAT1 acts as a molecular sponge during PDLSCs osteogenic induction, thereby regulating BMP2 expression[22]. In addition, cell apoptosis can be regulated by lncRNA-POIR/miR-182/FoxO1 regulatory network in inflammatory microenvironment[23]. These results all suggest that lncRNAs may regulate periodontitis progression by regulating bone metabolism, cell proliferation, and apoptosis of periodontal tissues.

Previous microarray analysis has revealed that long intergenic non-coding RNA 01126 (LINC01126, located on 2p21) is a discrepant expressed lncRNA between gingival tissues of periodontitis patients and healthy controls. Additionally, the results confirmed that LINC01126 promotes periodontitis progression through miR-518a-5p/HIF-1α/MAPK pathway[24]. However, the biological role of LINC01126 in the inflammatory process of gingival tissue remains unknown. Therefore, the biological roles of LINC01126 in human HGFs under inflammation microenvironment were studied. As expected, the results showed that IL-6 expression was closely related to LINC01126. Additional studies confirmed that LINC01126 could act as a miR-655-3p sponge, thus regulating IL-6/JAK2/STAT3 signaling pathway in periodontitis progression.

Results

Proinflammatory genes were up-regulated in inflammatory model in vitro, while LINC01126 silencing significantly alleviates LPS-induced cellular inflammation

HGFs were cultured with inflammatory medium containing LPS with a concentration of 1 ng/mL. All proinflammatory genes were significantly up-regulated with statistical significance (Fig. 1A-D). IL-6 expression increased tenfold in LPS-stimulated HGFs than in the control group. IL-4 expression, an anti-inflammatory cytokine, decreased after inflammatory stimulation (Fig. 1E). Meanwhile, it was confirmed that LINC01126 expression increased under inflammatory microenvironment (Fig. 1F). These results indicated a successful establishment of a cellular inflammatory model of HGFs.

Further, we screened the siRNA sequences of LINC01126. As depicted in Fig. 1G, si-LINC01126_002 had the best silencing effect and thus was used in our experiments. Then, the expression of proinflammatory genes following LINC01126 silencing was verified. The results indicated that expressions of IL-1β, IL-6 and MMP-1 were statistically decreased after silencing, particularly on the second day (Fig. 1H-J). However, expression of TNF-α in HGFs with LPS stimulation was not affected by LINC01126 silencing (Fig. 1K). Among all proinflammatory genes, it was found that IL-6 expression decreased most significantly. However, LINC01126 silencing had no obvious effect on IL-4 in inflammatory microenvironment (Fig. 1L). These findings imply that silencing LINC01126 can reduce the expression level of proinflammatory genes, particularly IL-6.

LINC01126 silencing has no significant effect on cell proliferation, but promotes cell migration and reduces LPS-induced apoptosis

To further elucidate the biological function of LINC01126 in HGFs, Cell viability assay manifested that LINC01126 silencing had no statistical significance on HGFs proliferation (Fig. 2A). However, wound healing experiments and flow cytometry showed that silencing LINC01126 could increase the migration of HGFs and inhibited LPS-induced cell apoptosis (Fig. 2B-D).

LINC01126 silencing inhibits the activation of IL-6/JAK2/STAT3 pathway by down-regulating IL-6 expression under inflammatory microenvironment

Given that silencing LINC01126 can significantly decreased IL-6 expression under inflammatory stimulation, additional studies were conducted to determine whether LINC01126 can affect IL-6-related signaling pathway, called IL-6/JAK2/STAT3 pathway. RT-qPCR results manifested that expression of IL-6, JAK2, and STAT3 in HGFs under inflammatory stimulation were decreased with statistical significance after LINC01126 silencing (Fig. 3A-C). Figure 3D demonstrated that LINC01126 was successfully silenced. In addition, WB further confirmed that protein expressions of IL-6, p-JAK2, and p-STAT3 were dramatically down-regulated in the silencing groups (Fig. 3E). These results indicated that silencing LINC01126 inhibited the activation of JAK2/STAT3 pathway by reducing IL-6 expression.

LINC01126 acts as a sponge for miR-655-3p

It was predicted that miR-655-3p contains a binding site of LINC01126 (Fig. 4A). Therefore, LINC01126 was predicted to be a "sponge" for miR-655-3p. FISH manifested that LINC01126 was expressed in the cytoplasm and nucleus of HGFs (Fig. 4B). LINC01126 expression in cytoplasm provided additional support for our hypothesis. Dual-luciferin reporter assay was used to further confirm the direct binding of miR-655-3p to LINC01126. The data indicated that miR-655-3p mimics transfection decreased the relative luciferase activity of the LINC01126-WT groups, but had no significant difference in LINC01126-MUT group (Fig. 4C). In addition, as the duration of inflammatory culture increased, miR-655-3p expression was significantly down-regulated in HGFs (Fig. 4D). After LINC01126 silencing, miR-655-3p expression returned to the level of the control group (Fig. 4E). Meanwhile, LINC01126 expression decreased after miR-655-3p overexpression (Fig. 4F). The successful miR-655-3p mimics transfection was further confirmed (Fig. 4G). All these results indicate that miR-655-3p could be sponged by LINC01126.

MiR-655-3p targets to mRNA of IL-6 and inhibits IL-6/JAK2/STAT3 signaling pathway under inflammatory stimulation

IL-6 expression was significantly down-regulated in HGFs following LINC01126 silencing. Consequently, it was hypothesized that miR-655-3p could target mRNA of IL-6 and thus regulate its expression. Additionally, the binding ability of miR-655-3p and IL-6 mRNA was confirmed by searching online database (Fig. 5A). The dual luciferase reporter assay confirmed that fluorescence intensity in the IL-6-WT group was markedly decreased after being co-transfected with miR-655-3p mimics, but no statistical difference was observed in IL-6-MUT group (Fig. 5B). Besides, IL-6 expression was significantly decreased in miR-655-3p mimics transfection group (Fig. 5C). These results confirmed the direct targeting ability of miR-655-3p to IL-6. Meanwhile, miR-655-3p overexpression could inhibit mRNA expression of JAK2 and STAT3 and protein expression of IL-6, p-STAT3, and p-JAK2 (Fig. 5D-F). The above results manifested that miR-655-3p could inhibit IL-6/JAK2/STAT3 signaling pathway in inflammatory microenvironment via targeting mRNA of IL-6.

MiR-655-3p is down-regulated in inflammatory gingival tissues, while IL-6/JAK2/STAT3 pathway is abnormally activated

Clinical gingival tissue samples were collected (n = 12). As expected, miR-655-3p expression was markedly down-regulated in inflammatory periodontal tissues than that of normal periodontal tissues (Fig. 6A). LINC01126, IL-6, JAK2, and STAT3 expressions were statistically up-regulated (Fig. 6B-E). LINC01126 expression was consistent with previous lncRNA microarray analysis. WB further confirmed that protein expressions in inflammatory periodontal tissues were markedly up-regulated (Fig. 6F). H&E staining revealed that inflammatory gingival tissues were infiltrated by a mass of inflammatory cells, which were rarely seen in healthy controls (Fig. 6G). IHC staining of gingival tissues also confirmed that expression levels of IL-6, T-STAT3, p-STAT, T-JAK2, and p-JAK2 in inflammatory gingival tissues were much higher than those in healthy tissues (Fig. 6H-L). Combined with previous results, it was manifested that miR-655-3p has anti-inflammatory effect in periodontitis, whereas IL-6/JAK2/STAT3 pathway is abnormally activated during periodontitis progression.

Discussion

In recent years, increasing studies have focused on long non-coding RNAs, which may be aberrantly expressed in many diseases and are involved in epigenetic and transcriptional regulation[25, 26]. According to the 4th National Oral Health Survey of China, the periodontal health rate of Chinese adults is less than 10%, and the prevalence of periodontitis is significantly correlated with age groups[27]. Due to severe situation of periodontitis in the population, it is urgent to reveal pathogenic mechanisms underlying lncRNAs in periodontal diseases. Existing researches indicate that lncRNA-ANCR, PCAT1, and POIR may regulate periodontal cell proliferation, osteogenic differentiation, and bone metabolism[21–23]. In previous studies, clinical tissue samples were obtained for lncRNA microarray analysis, which identified a long non-coding RNA with the most significant expression difference between inflammatory periodontal tissue and healthy controls, namely LINC01126. It has demonstrated that LINC0116 is critical in promoting periodontitis pathogenesis under hypoxia[24]. However, whether LINC01126 regulates proinflammatory gene expression and its specific mechanism has not been investigated.

Periodontitis aggravated gingival tissue disorder and cellular function impairment[28]. The biological function of HGFs is an important indicator of physical condition of gingival tissues, and their migration is critical for gingival tissue restoration under inflammation microenvironment[29, 30]. CCK-8 assay and wound healing experiments revealed that LINC01126 silencing had no obvious effect on cell viability of HGFs. However, cell migration results indicated that LINC01126 expression inhibited the migration of HGFs in the inflammatory microenvironment, thus reducing its resistance to inflammation and tissue repair ability. In addition, numerous studies have demonstrated that lncRNAs, such as lncRNA ATB[31], MALAT1[32], and GAS5[33], have regulatory effects on apoptosis, promoting apoptosis by activating apoptosis-related signaling pathways. Generally, the greater the number of apoptotic cells, the fewer cells can perform normal biological functions, disrupting the body's self-repair and immune defenses[34]. In terms of apoptosis detection, it was found that LINC01126 silencing could reduce LPS-induced apoptosis of HGFs, implying that lncRNAs play a role in apoptosis.

LncRNAs have been shown to regulate the occurrence and development of inflammatory diseases[35, 36]. Maninjay K et al. found that LINC-EPS regulated inflammatory response at the transcriptional level, inhibiting inflammation progression[37]. LncRNA-Mirt2 inhibits the NF-κB and MAPK pathways activation and limits the production of pro-inflammatory cytokines through negative feedback[38]. In addition, lncRNAs can participate in inflammatory progression by regulating macrophage oxidative stress and metabolism[39, 40]. Our study found that LINC01126 silencing significantly reduced expression of proinflammatory cytokines in LPS-induced HGFs, particularly IL-6. As a result, additional studies on IL-6 were conducted to elucidate the role of LINC01126 in inflammation. Through online database comparison, it was found that miR-655-3p contains binding sites for LINC01126 and IL-6, which is an important premise for our study on "endogenous competitive RNA". According to current reports, miR-655-3p has primarily focused on cancer-related fields, where miR-655-3p plays an anti-tumor role by targeting related genes[41, 42]. To our knowledge, miR-655-3p has not been reported in mechanistic studies of

inflammation-related diseases. Through dual luciferin report assay, it was confirmed that miR-655-3p had a targeted effect with LINC01126 and IL-6. Moreover, it was confirmed that miR-655-3p expression in inflammatory group was dramatically lower than that of controls and was negatively correlated with LINC01126. Additionally, these findings indicate that there exists a mechanism of "competing endogenous RNAs" between LINC01126/miR-655-3p/IL-6, which is an important pathway for inflammation regulation in periodontitis.

IL-6 is mainly expressed in fibroblasts and activated T cells, and has received extensive attention, especially in the study of chronic inflammation, autoimmune disease, and tumors[43–45]. During periodontitis progression, HGFs are stimulated by bacteria-derived LPS, resulting in increased expression of various proinflammatory genes, particularly IL-6. Increased IL-6 leads to IL-6 receptor activation, which then activates IL-6-related cell signaling pathways, especially IL-6/JAK2/STAT3 pathway[46]. According to most current studies, aberrant activation of JAK2/STAT3 signaling pathway promotes disease progression and is detrimental to the control of diseases, including liver cancer[47], inflammation[48] and neurological disease[49]. In our study, it was observed that the expressions of IL-6, p-JAK2, and p-STAT3 were abnormal up-regulated in HGFs after LPS induction, and the effect of LPS stimulation was partially reversed following transfection with miR-655-3p mimics. In addition, IL-6, p-JAK2, and p-STAT3 expression levels in periodontitis tissues were far higher than those in normal tissues through IHC and WB. These data indicate that IL-6/JAK2/STAT3 signaling pathway is abnormally activated in inflammatory periodontal tissues, thus exerting a pro-inflammatory effect and regulating various cell biological behaviors as previous reports[50].

Our results revealed an important mechanism of LINC01126 in periodontitis regulation. As a "sponge" of miR-655-3p, LINC01126 obstructs the binding between miR-655-3p and IL-6, thus promoting the activation of IL-6/JAK2/STST3 pathway (Fig. 7). It is worth mentioning that the regulatory mechanism of LINC01126 in the nucleus of HGFs remains unclear. Recently, Chen et al. are the first to identify that lncRNA-SLERT regulates protein conformation and polymer state through RNA chaperone mechanism, thus maintaining normal nucleolus morphology and function[51]. For further speculation, LINC01126 may directly or indirectly serve as a molecular chaperone to regulate target genes through a whole new mechanism, in addition to performing its biological function in the nucleus. Moreover, studies *in vivo* should be conducted to corroborate our current findings. In conclusion, our study proposes a new mechanism of LINC01126 in regulating periodontitis, which is also the first report on the biological role of miR-655-3p in inflammatory periodontal diseases and may provide a new strategy for preventing and treating periodontitis.

Conclusions

In conclusion, LINC01126, as an endogenous competitive RNA (ceRNA) of miR-655-3p, can promote IL-6/JAK3/STAT3 pathway activation, thereby promoting periodontitis pathogenesis.

Methods

Gingival tissues collection and cell culture

Gingival tissues were obtained from patients in the clinic. HGFs were isolated from healthy gingival tissues collected from teeth during tooth extraction. The obtained gingival tissue was rinsed with PBS solution at least three times. After cutting up the tissues in EP tubes, they were digested with Type I collagenase (Sigma, USA) in a 37 ℃ water bath for 30 min and were shaken every five minutes. Followed by centrifugation, precipitated tissue blocks were evenly spread on Petri dish for 12 h before adding culture medium. Cell culture medium of HGFs was composed of 89% (v/v) α-MEM (Gbico, Shanghai, China), 10% (v/v) fetal bovine serum (FBS, VivaCell, ShangHai, China), and 1% (v/v) penicillin/streptomycin (Hyclone, USA). Human embryonic kidney (293T) were cultured in DMEM (Gbico, Shanghai, China) with 10% (v/v) FBS (VivaCell, Shanghai, China) and 1% (v/v) penicillin/streptomycin (Hyclone, USA). Both 293T cells and HGFs were cultured at 37 ℃ with 21% O_2.

The establishment of cell inflammation model

For establishing a model of inflammatory HGFs, a complete culture medium containing a 1 ng/mL concentration of lipopolysaccharide (LPS) (Sigma, USA) was used as the inflammatory medium. The inflammatory medium was added to the 6-well plate when cell density reaches about 60%. Before harvesting, HGFs were cultured with inflammatory medium for 1, 2, and 3 days.

The silencing of LINC01126 in HGFs

Three diffierent siRNAs (si–LINC01126_001, si–LINC01126_002 and si–LINC01126_003) of LINC01126 were designed and synthesized by Guangzhou RiboBio Co.,LTD (**Table S1**). Transfection experiments were performed in 6-well plate when cell density reached 80%. HGFs were transfected with siRNA or si-NC at a concentration of 50 nM using lipo3000 (Thermo Fisher Scientific, USA). HGFs were harvested 24 h later after transfection of synthesized sequences. LINC01126 silencing was verified using quantitative real-time PCR.

Cell transfection

HGFs were seeded in 6-well plates with 2×10^5 cells per well. HGFs were transfected with small interfering RNA from negative control (si-NC) or screened small interfering RNA from LINC01126 (si-LINC01126). MiR-655-3p mimics or mimics-NC (RiboBio, Shanghai, China) were transfected in the same manner with a concentration of 50 nM. And complete medium was substituted by an inflammatory medium 24 h later after cell transfection. Cell samples were harvested at the appointed time.

Cell viability assay

Cell Counting Kit-8 (CCK-8) kit (Bioss, Beijing, China) was utilized to perform cell viability assay. HGFs were seeded in 96-well plates with 10^3 cells per well. Following cell attachment, the selected si-LINC01126

sequence was utilized to transfect HGFs with a concentration of 50 nM. Cell viability was measured at 0, 1, 3, and 5 days after transfection, and OD values were measured at 450 nm using a SpectraMax ID5 Multi-Mode Microplate Reader (Molecular Devices, USA).

Wound healing assay

2×10^5 cells were seeded in 6-well plates. Briefly, simulated wounds were created with a 200 μ sterile tip on a single layer of HGFs when cells reached approximately 90% confluency. Following that, HGFs were rinsed with sterile PBS three times to rinse off the scraped cells. Finally, HGFs were cultured with fresh opti-MEM medium (Gbico, Shanghai, China). After 24 h, the healing of scratches was observed and photographed for analysis.

Flow cytometry

When the density reached approximately 80%, cell transfection was performed as before. After 24 h of si-LINC01126 transfection, HGFs were cultured with inflammatory medium for 48 h. HGFs were then collected for apoptosis detection through flow cytometry. All operations were performed following manufacturer's instructions.

Western blot (WB) assay

For the cell samples, the cells were lysed on ice by RIPA containing 1% PMSF (Beyotime, Shanghai, China). For the tissue samples, the tissues were grinded in Freezer Mixer (Jingxin, Shanghai, China) with RIPA. After protein transmembrane and blocking, the PVDF membranes were incubated with primary antibodies overnight, including GAPDH (Zenbio, Chengdu, China, #200306-7E4), IL-6 (Zenbio, Chengdu, China, #347023), STAT3 (ABclonal, Wuhan, China, #A1192), phospho-STAT3 (Zenbio, Chengdu, China, #310019), JAK2 (ABclonal, Wuhan, China, #A11497), and phospho-JAK2 (ABclonal, Wuhan, China, #AP0631). The immunoblots were detected by chemiluminescence (Beyotime, Shanghai, China).

Quantitative real-time PCR (qRT-PCR) analysis

The purity and concentration of total RNA were detected by NanoDrop-2000 after the extraction of total RNA using RNAiso plus (Takara, Japan). Then, reverse transcription of mRNA and microRNA was performed by using PrimeScript™ Master Mix (Takara, Japan) and miRNA First Strand cDNA Synthesis (Tailing Reaction) (Sangon biotech, China), respectively. GoTaq® qPCR Master Mix (Promega, America) was used for qPCR according to the experiment instruction. The expression level was evaluated using a method of $2^{-\Delta\Delta CT}$. The primers of the target genes were shown in **Table S2**.

Fluorescence in situ hybridization (FISH)

Fluorescence in situ hybridization Kit specific for LINC01126 was designed and synthesized by Guangzhou RiboBio Co., LTD. HGFs were washed with PBS for 5 min and fixed in 4% paraformaldehyde for 10 min when their cell density reached about 60–70%. HGFs were then permeabilized by sterile PBS

containing 0.25% Triton X-100 for 10 min under 4 ℃, and rinsed with sterile PBS at room temperature. Following that, pre-hybridization was performed under 37 ℃ for 30 min. After that, the probes for LINC01126, U6, and 18S were added into a hybridization solution, and the cells were cultured under 37 ℃ overnight in the dark. Finally, the nuclei were stained with DAPI, and photographs were taken using a fluorescence microscope. U6 and 18S were acted as internal references for nucleus and cytoplasm, respectively.

Dual-luciferase reporter assay

293T cells were used in this experiment. The wide or mutant type of 3'-UTR of LINC01126 and IL-6 was cloned into pmiR-RB-Report™ vector (**Figure S1**) (Ribo, China), a vector specifically designed for detecting the combining capacity of miRNA. The plasmids, mimics NC or miR-655-3p mimics, were transfected into 293T cells using Lipo3000 reagent (Thermo Fisher Scientific, USA). Plasmids and mimics NC or miR-655-3p mimics were transfected at 2.5 μg/mL and 50 nM, respectively. The luciferase activity was measured by using Dual-Luciferase Reporter Assay System (Promega, USA) after 48 h. Finally, the activity of firefly luciferase gene (hRluc) and renilla luciferase (hLuc+) was detected. The ratio of hRluc activity to hLuc + activity is the relative luciferase activity.

H&E staining and Immunohistochemistry staining

Periodontitis tissues and healthy controls were obtained from department of oral and maxillofacial surgery of Stomatological Hospital affiliated to Chongqing Medical University. After fixed in 4% paraformaldehyde for 24 h, gradient dehydration and paraffin embedding were performed. Following that, serial sections were performed and used for H&E and IHC staining. Hematoxylin-Eosin/HE Staining Kit (Solarbio, Beijing, China) was utilized for H&E staining. Immunohistochemistry (IHC) staining was conducted with antibodies specific for IL-6 (1:200, Zenbio, Chengdu, China, #347023), phospho-STAT3 (1:100, Zenbio, Chengdu, China, #310019), STAT3 (1:200, ABclonal, Wuhan, China, #A11497), phospho-JAK2 (1:200, ABclonal, Wuhan, China, #AP0631), and JAK2 (1:100, ABclonal, Wuhan, China, #A11497). H&E and IHC staining were performed according to the protocol.

Statistical analysis

Experimental results were analyzed and processed using CytExpert, Image Lab, GraphPad Prism (8.0), Adobe Photoshop and Image J. One-way ANOVA or the independent-sample T-test was used to compare the differences between groups. Data were presented as mean ± SEM compared with the control groups. And $p < 0.05$ was regarded as statistically significant. All experiments were repeated at least three times.

Declarations

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Availability of data and materials

The corresponding author can provide all the original data in our manuscript upon reasonable request.

Ethics approval and consent to participate

The research was approved by Research Ethics Committee of The Affiliated Hospital of Stomatology, Chongqing Medical University (Project No. CQHS-REC-2020 (LSNo.79)).

Competing interests

The authors declare no conflict of interest.

Consent for publication

All volunteers provided informed consent.

Authors' contributions

This study was designed by XNZ and ST. Majority of the work is finished by ST. ST and XNZ analysed and processed the data. ST, XNZ and JL wrote the original draft. YY, ZJG, ZXW, LMP and YZZ involved in critical reviewing of the manuscript. The final manuscript was reviewed by all authors and approved for submission.

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Abbreviations

LINC01126
long intergenic non-coding RNA 01126
LncRNAs
long non-coding RNAs
HGFs
human gingival fibroblasts
PDLSCs
periodontal ligament stem cells
LPS
lipopolysaccharide
TLRs
toll-like receptors
ORF
complete open reading frame
FBS
fetal bovine serum

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Figures

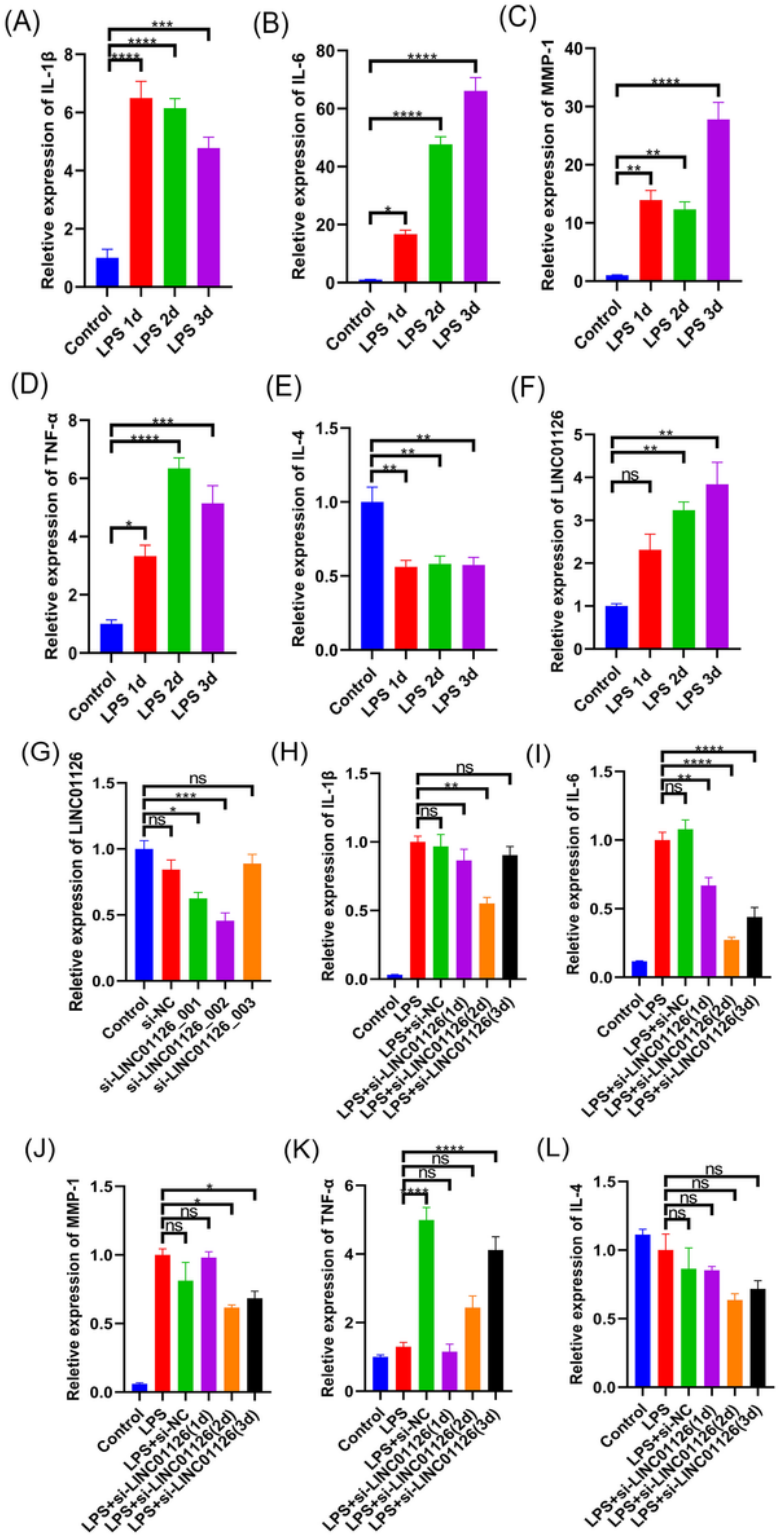

Figure 1

Proinflammatory genes were up-regulated in inflammatory model in vitro, while LINC01126 silencing significantly alleviates LPS-induced cellular inflammation. All the experimental groups were cultured in inflammatory medium for 1,2,3 days, respectively (A-D) the mRNA expression of proinflammatory cytokines in LPS-inducd groups and the corresponding controls. (E) Expression of anti-inflammatory cytokine IL-4 under inflammatory microenvironment and the corresponding controls. (F) Expression of

LINC01126 in inflammatory HGFs and the corresponding controls. After LINC01126 was silenced for 24h, HGFs in the experimental groups were cultured under inflammatory induction for 1, 2 and 3 days, respectively. (G)Three different siRNAs of LINC01126 and corresponding silencing effects after transfected for 24 h.(H-K) The expression of proinflammatory cytokines after LINC01126 silencing. (L) The expression of anti-inflammatory cytokine IL-4 after LINC01126 silencing. Data were presented as mean ±SEM (*p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001).

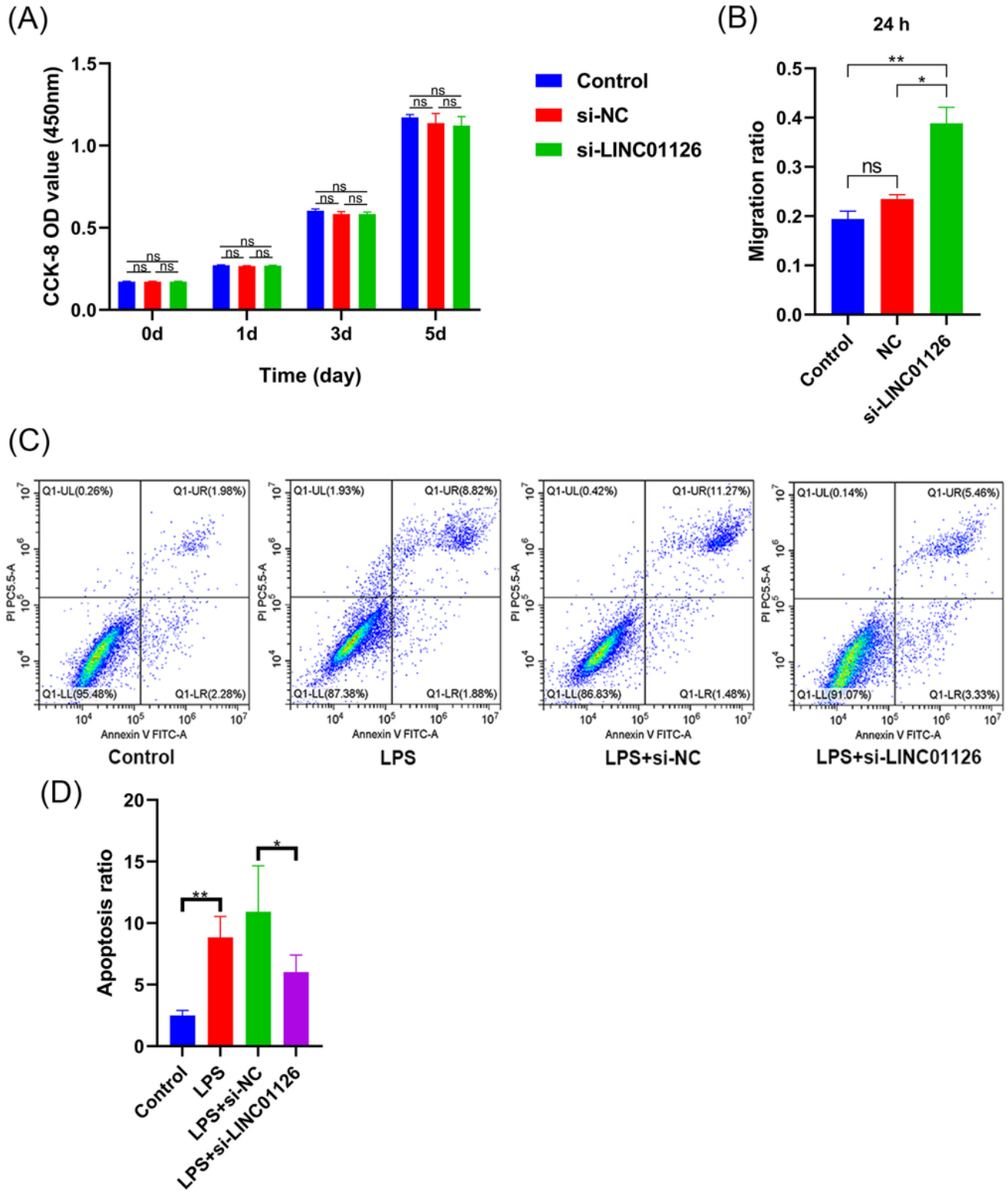

Figure 2

LINC01126 silencing has no significant effect on cell proliferation, but promotes cell migration and reduces LPS-induced apoptosis. (A) Cell vitality of HGFs after LINC01126 silencing was determined by CCK-8 assay. (B) Cell migration ratio of HGFs. (C-D) Apoptosis ratio of HGFs cultured in inflammatory medium for 48 h after LINC01126 silencing. Data were presented as mean ±SEM (*p < 0.05, **p < 0.01).

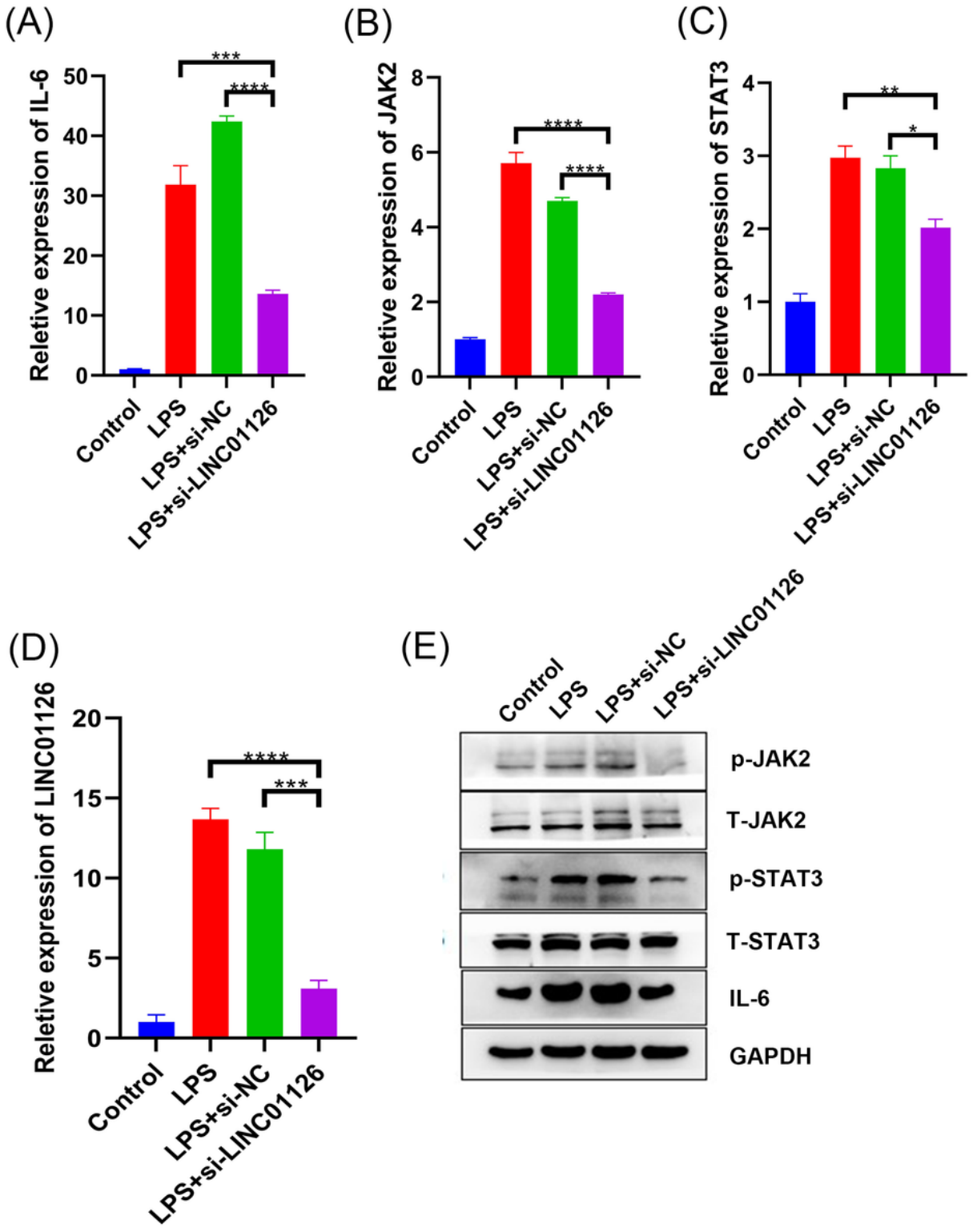

Figure 3

LINC01126 silencing inhibits the activation of IL-6/JAK2/STAT3 pathway by down-regulating IL-6 expression under inflammatory microenvironment. After LINC01126 silencing, all samples were collected after cultured with inflammatory medium for 2 days. (A-C) The expressions of IL-6, JAK2 and STAT3 after LINC01126 silencing and the corresponding controls. (D) The validation of successful LINC01126 silencing after si-LINC01126 transfection. (E) The protein expression level of IL-6/JAK2/STST3 pathway after transfection with si-LINC01126 and the corresponding controls. Data were presented as mean ±SEM (*p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001).

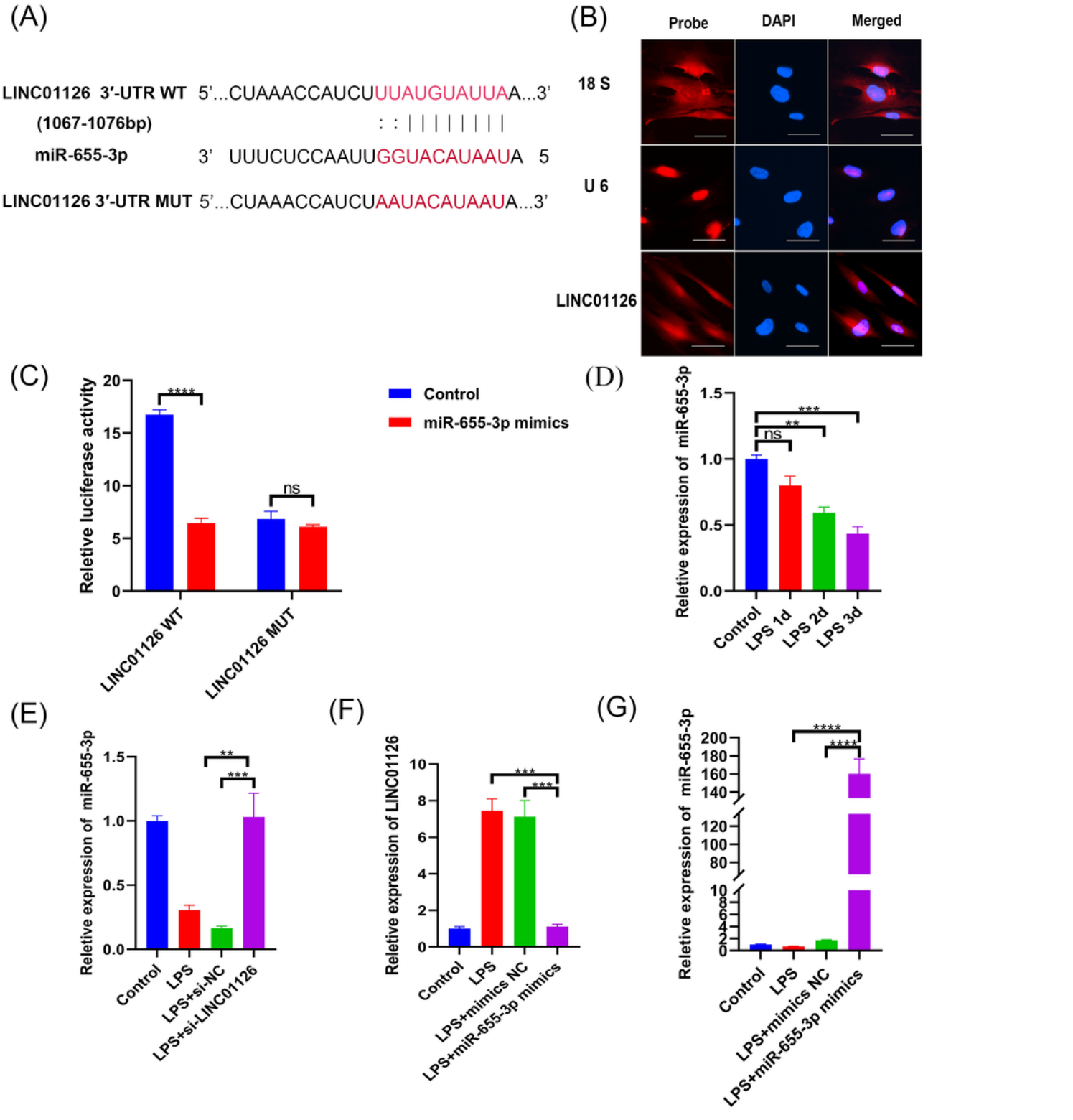

Figure 4

LINC01126 acts as a sponge for miR-655-3p. (A) The predicted bonding site of LINC01126 and miR-655-3p. (B) Probes for U6, 18S and LINC01126 were used for detecting the subcellular location of INC01126 in HGFs (scale bar = 50 μm). (C) The validation of the binding between LINC01126 and miR-655-3p. (D) The miR-655-3p expression of HGFs after cultured with inflammatory medium for 1, 2, 3 days. (E) The miR-655-3p expression of HGFs after si-LINC01126 transfection. (F-G) The LINC01126 and miR-655-3p expression of HGFs after transfected with miR-655-3p mimics. Data were presented as mean ±SEM (**p < 0.01, ***p < 0.001, ****p < 0.0001).

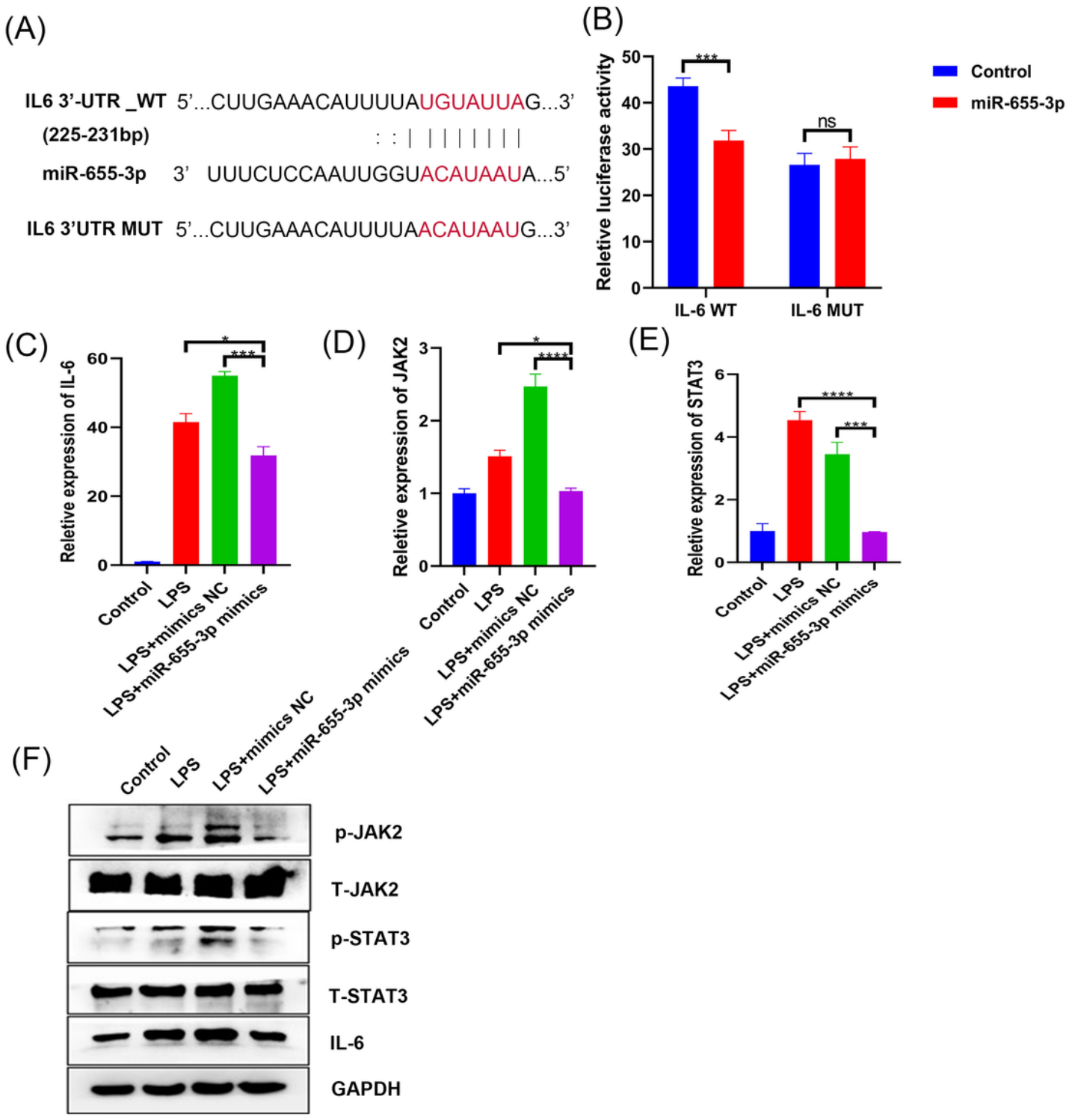

Figure 5

MiR-655-3p targets to mRNA of IL-6 and inhibits IL-6/JAK2/STAT3 signaling pathway under inflammatory stimulation. (A) The predicted bonding zone of LINC01126 and miR-655-3p. (B) Dual-luciferase reporter assay validated the binding between miR-655-3p and mRNA of IL-6. (C-E) The mRNA expression of IL-6, JAK2 and STAT3 in HGFs after transfected with miR-655-3p mimics and the corresponding controls. (F) The protein expression of IL-6/JAK2/STAT3 pathway in HGF after miR-655-3p

mimics transfection and the corresponding controls. Data were presented as mean ±SEM (*p < 0.05, ***p < 0.001, ****p < 0.0001).

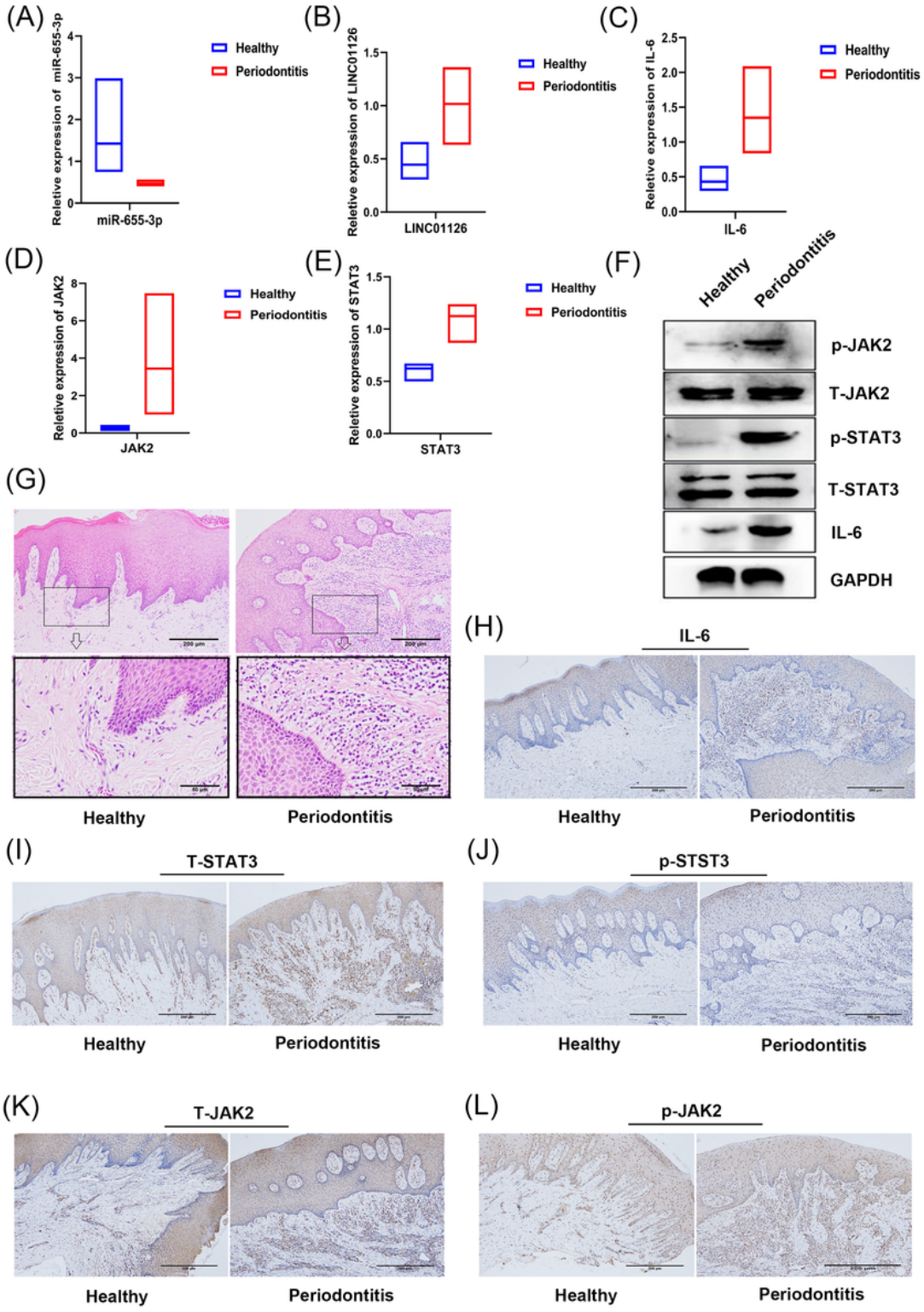

Figure 6

MiR-655-3p is down-regulated in inflammatory gingival tissues, while IL-6/JAK2/STAT3 pathway is abnormally activated. (A-E) The expressions of miR-655-3p, LINC01126, IL-6, JAK2 and STAT3 in periodontitis tissues and healthy controls. (F) The protein expression of IL-6/JAK2/STAT3 pathway in

periodontitis tissues and healthy controls. (G) H&E staining (the scale bar = 200 μm and 50 μm, respectively) confirmed the inflammatory state of periodontitis tissues and the healthy controls. (H-L) The protein expression of IL-6/JAK2/STAT3 pathway in gingival tissues was detected by IHC staining (the scale bar = 200 μm).

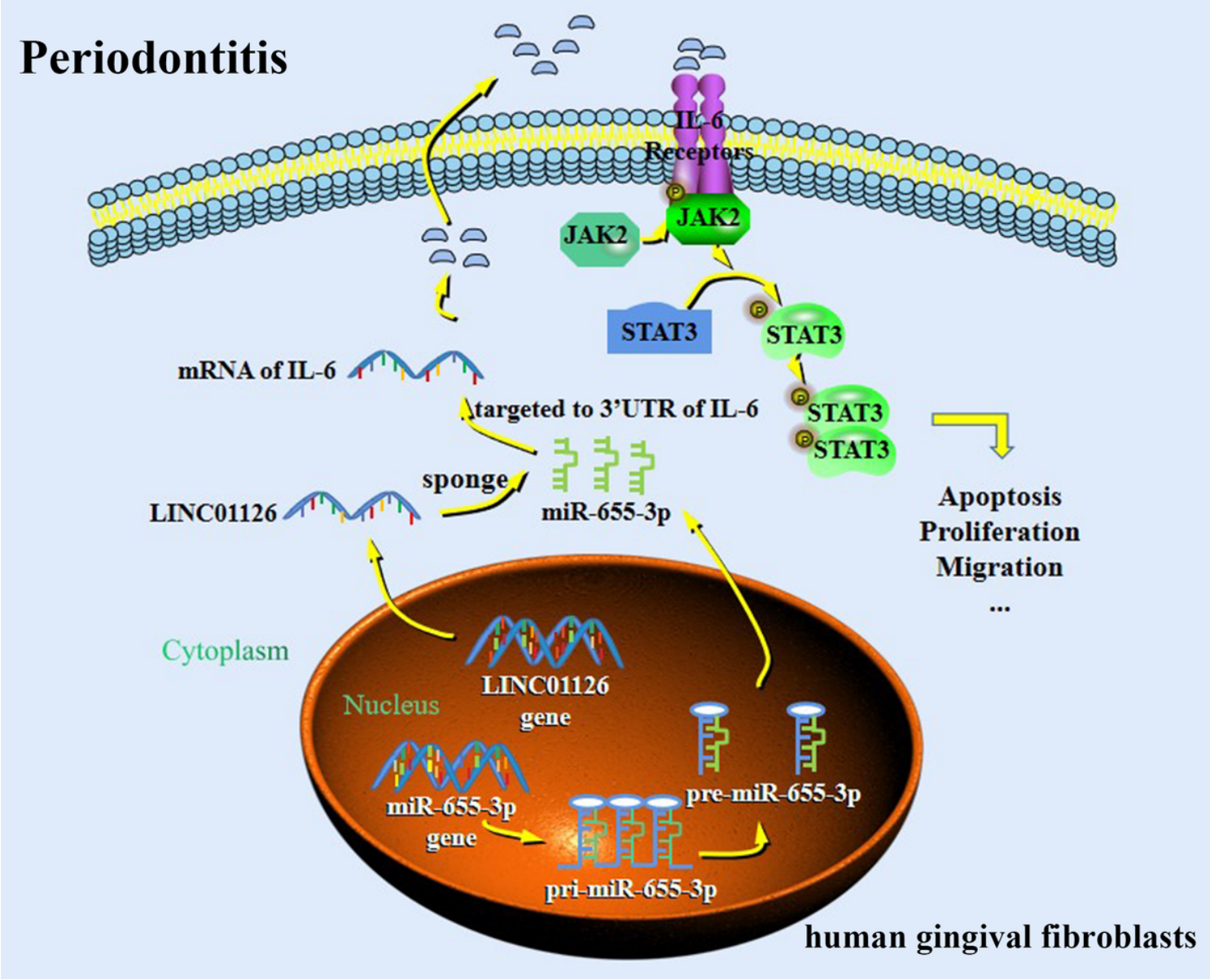

Figure 7

A schematic diagram of the pathogenic mechanism of LINC01126 in periodontitis.

Supplementary Files

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- Supplementarymaterial.docx