lnc-SAMD14-4 can regulate expression of the COL1A1 and COL1A2 in human chondrocytes

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Osteoarthritis (OA) is the most common motor system disease in aging people, characterized by matrix degradation, chondrocyte death, and osteophyte formation. OA etiology is unclear, but long noncoding RNAs (lncRNAs) that participate in numerous pathological and physiological processes may be key regulators in the onset and development of OA. Because profiling of lncRNAs and their biological function in OA is not understood, we measured lncRNA and mRNA expression profiles using high-throughput microarray to study human knee OA. We identified 2,042 lncRNAs and 2,011 mRNAs that were significantly differentially expressed in OA compared to non-OA tissue (>2.0- or <-2.0-fold change; p<0.5), including 1,137 lncRNAs that were upregulated and 905 lncRNAs that were downregulated. Also, 1,386 mRNA were upregulated and 625 mRNAs were downregulated. QPCR was used to validate chip results. Gene Ontology analysis and the Kyoto Encyclopedia of Genes and Genomes was used to study the biological function enrichment of differentially expressed mRNA . Additionally, coding-non-coding gene co-expression (CNC) network construction was performed to explore the relevance of dysregulated lncRNAs and mRNAs. Finally, the gain/loss of function experiments of lnc-SAMD14-4 was implemented in IL-1β-treated human chondrocytes. In genel, this study provides a preliminary database for further exploring lncRNA-related mechnisms in OA.

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ABSTRACT

Background

Osteoarthritis (OA) is the most common motor system disease in aging people, characterized by matrix degradation, chondrocyte death, and osteophyte formation. OA etiology is unclear, but long noncoding RNAs (lncRNAs) that participate in numerous pathological and physiological processes may be key regulators in the onset and development of OA. Because profiling of lncRNAs and their biological function in OA is not understood. Here we report a new lncRNA-lnc-SAMD14-4 and explored its role and potential ability in osteoarthritis development.

Methods

The human cartilage tissues were obtained from patients. lncRNA and mRNA expression profiles were analyzed using the Affymetrix human lncRNA chips. Gene ontology (GO) enrichment and KEGG pathway analysis of mRNAs were conducted to identify the related biological modules and pathologic pathways. The co-expression analysis was used to predict the interaction between the lncRNA-mRNA couple associated with PI3K-Akt pathway. Real-time PCR was conducted to validate the expression pattern of lncRNA and mRNA. The gain/loss of function experiments of lnc-SAMD14-4 was implemented in IL-1β-treated human chondrocytes to explored its role in osteoarthritis.

Results

(1) In this study, total 2042 lncRNAs were detected significantly different expression in OA compared to non-OA tissue. Total 2011 mRNA was detected significantly different expression in OA compared to non-OA tissue. (2) Go analysis was used to investigate the effect of all differentially expressed gene on pathogenesis of OA. (3) 27 pathways corresponded to up-regulated mRNAs and 94 pathways corresponded to down-regulated mRNAs were determined by KEGG pathway analysis. (4) The result of qRT-PCR was consistent with microarray profiling

analysis. (5)The lnc-SAMD14-4 mediated *COL1A1* and *COL1A2* expression in IL-1β-treated human chondrocytes.

Discussion

This study provides a preliminary database for further exploring lncRNA-related mechnisms in OA. The study also revealed that lnc-SAMD14-4 may perform a significant role in the development of osteoarthritis.

Introduction

Osteoarthritis (OA) is common in aging populations and is a global health burden [1, 2]. OA arises from cartilage matrix degradation, chondrocyte death, and osteophyte formation[3, 4]. The pathology of OA is fairly well understood, and multiple therapies exist, from corticosteroids and nonsteroidal anti-inflammatory drugs to treat symptoms [5, 6], experimental stem cell therapies to treat specific forms of OA, and biologics to address specific inflammatory mediators such as cytokines. OA may also be treated with joint replacement surgery, and this is common in the elderly. Even so, better drugs are always desired.

Recently, gene regulation functions of long non-coding ribonucleic acids (lncRNAs) in OA have been studied. LncRNAs are non-protein-coding transcripts (>200 nucleotides) that participate in numerous pathological and physiological processes[7-9]. Some lncRNAs have been defined as key regulators of the onset and development of OA[10-15]. For instance, overexpression of HOTAIR may cause OA by inducing chondrocyte apoptosis[10, 16]. Fu's group used microarray and bioinformatics to identify 4,714 differentially expressed lncRNA in non-OA cartilage compared to knee OA patients[17]. Liu's group used microarray to identify 153 lncRNAs differently expressed in OA and designated a specific lncRNA CIR to have an important function in degradation of the chondrocyte matrix[14]. Differences in lncRNA expression links with physiological processes often arise between laboratories which use high-throughput screening, and therefore, data about lncRNA in the pathogenesis of OA is needed to reach consensus about which contribute to OA. To address this, we measured differentially expressed lncRNAs and mRNAs using high-throughput microarray to study human knee OA and compared these data to non-OA samples.

Materials and methods

Specimen collection

A Kellgren-Lawrence (KL) system was used to measure OA severity [18]. OA cartilage (KL grades 3 or 4) were collected from 19 patients undergoing total knee joint replacement due to severe OA, and normal cartilage (KL grades 0) were collected from 11 patients (trauma, *thromboangiities obliterans,* or osteosarcoma, or limb amputation) without history of rheumatoid arthritis or OA. Informed consent was signed by all patients and additional informed consent was obtained for publication of subject age and sex. This study was approved by the Ethics commission of the 163rd Central Hospital of the Chinese People's Liberation Army (IRB. [2015] 001). Patient data appear in Supplementary Table 1. Clinical specimens were divided for QPCR and for array analyses.

lncRNA and mRNA microarray analysis

Quality tests, sample labeling, and hybridization to human WT lncRNA microarray (Affymetrix) were conducted by Oebiotech Co (Shanghai, China) (Supplement Figure 1), their detailed definition can be found at [19, 20], The Genechip included 63,542 non-coding and 27,134 coding transcripts. Images were taken with an Affymetrix Scanner3000, and data normalization and probe filtering were conducted using an Affymetrix GeneChip Expression Console (version 4.0, Affymetrix). lncRNA-mRNA expression between OA and non-OA groups was identified as a $\geq$2.0 fold-change (FC) for up-regulation or a $\leq$-2.0 fold-change for down-regulation and $p \leq 0.05$. Afterwards, the GO and KEGG analyses were used to identify roles of differentially expressed mRNAs with respect to GO terms or pathways. Their detailed definition can be found at[21, 22]. Then, a Volcano plot and hierarchical clustering were used to show distinctions in gene expression patterns among samples. Finally, a coding-non-coding gene co-expression (CNC) network was drawn using Cytoscape software.

The microarray data are available through the GEO database with accession number GSE113825.

RNA extraction and quantitative PCR

An RNeasy Lipid Tissue Mini Kit (Qiagen, Germany) was used to extract total RNA according to kit directions. A NanoDrop ND-1000 spectrophotometer was used to measure RNA quality and purity. SDS-PAGE was used to measure RNA integrity. Total RNA from samples was reverse transcribed into cDNA using an All-in-one First-Strand cDNA Synthesis Kit (GeneCopoeia Inc, Rockville, MD) according to the user manual. QPCR primers were designed and synthesized at the Shanghai Sangon Company (Supplementary Table 2). qPCR was conducted using AceQ qPCR SYBR Green Master Mix (Vazyme Biotech Co, China) on a Bio-Rad CFX ConnectTM Real-Time PCR detection system (Bio-Rad Germany). Furthermore, qPCR was performed according to the manual and included 10 μl SYBR Master Mix, 0.4 μl forward primer (10 μM), 0.4 μl reverse primer (10 μM), 0.4 μl ROX Reference Dye 1, 2 μl cDNA, and 6.8 μl MiliQ water. The qPCR reaction conditions were pre-denaturation at 95 °C for 5 min, followed by 95 °C for 10 sec, and 60 °C for 30 sec for 40 cycles, and a final extension at 95 °C for 15 sec, 60 °C for 60 sec, and 95 °C for 15 sec. The $2^{-\Delta\Delta Ct}$ method was used to assess gene expression as previous study[23]. Each experiment was conducted in triplicates.

Cell culture and treatment

Human primary chondrocytes were available from CHI Scientific, Inc(China). The cells were incubated into DMEM(Gibco, USA) medium supplemented with 14% FBS(Gibco, USA). The culture was cultivated at 37℃ in a 5% CO_2 incubator and the medium was changed every 3 days. All study were conducted using primary chondrocytes from passages 1-4. Cells were treated without or with 10 ng/ml human recombinant interleukin-1 beta(IL-1β)(Sigma-Aldrich, St Lousis, MO, Germany) for 12h, 24h and 48h.

Cell transfection

The overexpression vectors of lnc-SAMD14-4(pcDNA3.1^{+}- lnc-SAMD14-4) and blank vector

115 pcDNA3.1(pcDNA3.1^{+}- vector) were transfected to human primary chondrocytes using Lipofectamine
116 3000(Invitrogen, USA). Three independent specifically targeting siRNA, including si-lnc-SAMD14-4(1#, 2# and
117 3#), and control siRNA were designed and purchased from Ribo Biotechnology Co. Ltd.(GuangDong, China). These
118 siRNA were transfected to human primary chondrocytes to conduct gain/loss of function of lnc-SAMD14-4.

119 **Statistical analysis**

120 Differences in expression of lncRNA-mRNA between OA and non-OA groups was assessed with a Student's *t*
121 test. GO and KEGG pathways were assessed using Fisher's exact test. Other data were analyzed using one-way
122 ANOVA and multiple groups were compared using Bonferroni's method. GraphPad Prism 6.0 (GraphPad Software
123 San Diego,CA) was applied for data analysis and drawing. Data are shown as means ± SD, and $p<0.05$ was regarded
124 to be statistically significant.

125 **Results**

126 **Analysis of differentially expression lncRNAs-mRNAs in OA**

127 We found 2,042 lncRNAs significantly differently expressed in OA compared to non-OA tissue and these data
128 appear in Supplementary Table 3. The most upregulated lncRNA was lnc-SAMD14-4 and the most downregulated
129 lncRNA was lnc-MSMP-2. The top 15 most dysregulated(down or up) lncRNAs are listed in Table 1.

130 Data for mRNA expression in OA revealed 2,011 mRNAs were dysregulated in OA compared to non-OA
131 cartilage tissue and 1,386 mRNA were upregulated and 625 mRNAs were downregulated (Supplementary Table 4).
132 Among these mRNAs, MMP-13 was the most up-regulated protein coding RNA, and this regulated articular
133 cartilage degradation[24]. MYOC was the most strongly down-regulated and the top 15 up-regulated or down-
134 regulated mRNAs appear in Table 2. Data for differentially expressed genes (including lncRNA and mRNA) appear
135 in Fig. 1A-C.

Gene Ontology (GO) analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis of dysregulated mRNAs

The most enriched GOs targeted by up-regulated mRNAs were extracellular matrix organization (GO:0030198), extracellular exosome (GO:0070062), and collagen binding (GO:0005518) in biological process, cellular process, and molecular function (Fig. 2A-C, Supplementary Table 5-7). Protein stabilization (GO:0050821), cilium (GO:0005929), and heparan sulfate proteoglycan binding (GO:0043395) were the most enriched GOs associated with down-regulated transcripts (Fig. 2D-F, Supplementary Table 8-10).

We identified 27 pathways with up-regulated mRNAs and the enriched pathway was cytochrome P450 (Supplementary Table 11). Then, 94 pathways corresponded to down-regulated mRNAs according to KEGG pathway analysis and the top enriched pathway was focal adhesion (Supplementary Table 12). The top 20 pathways targeted with down-regulated and up-regulated transcripts appear in Fig 3A and B.

LncRNA-mRNA co-expression network

The co-expression networks are of biological interest since co-expressed genes are controlled by the same transcriptional regulatory program, functionally related, or members of the same pathway or protein complex[25, 26]. We and others confirmed that apoptosis was involved in OA pathogenesis [27-30], and that the PI3K-Akt signaling pathway was important for apoptosis[31]. Since we found 74 dysregulated mRNAs enriched in the PI3K-Akt signaling pathway (Supplementary Table 13), we analyzed these dysregulated mRNA and identified 1,974 correlations for lncRNA-mRNA interaction pairs that satisfy $p<0.01$ and Pearson correlation coefficients>0.95. (Supplementary Table 14). Then, the top 500 of 1,974 lncRNA-mRNA pairs were used to draw the network (Supplementary Table 15). These 500 lncRNA-mRNA pairs including 57 mRNA and 363 lncRNA, and 362 pairs had a positive association; the rest were negatively associated. The co-expression network showed that one lncRNA

157 correlates with many mRNAs and vice versa (Fig. 4). Some lncRNA-mRNA pairs included the most dysregulated
158 mRNA and lncRNA. For example, *COL1A1* (mRNA, FC=175.43033) and *COL1A2* (mRNA, FC=85.32694) were
159 strongly associated with lnc-SAMD14-4 (lncRNA, FC= 86.11047). besides, all the LncRNA-mRNA co-expressed
160 pairs with Pearson correlation coefficients>0.98

161 **Verification of lncRNAs and mRNAs expression by qRT-PCR**

162 To confirm microarray profiling analysis, four lncRNAs (lnc-SAMD14-4, lnc-MARCKS-7, lnc-MSMP-2
163 and lnc-NPVF-4) and four mRNAs (*SERPINF1*, *COL1A1*, *MYOC* and *PAK3*) were selected and measured using
164 qRT-PCR for 53 clinical specimens (34 OA and 19 normal specimens). Data for qRT-PCR agree with chip profiling
165 analysis (Fig. 5).

166 **Prediction of novel lncRNAs targeting**

167 In this study, we selected the most dysregulated lncRNA（lnc-SAMD14-4） as the potential lncRNA for
168 function research. To analysis the gene coding-potential of lnc-SAMD14-4, the open-reading frames(ORFs) and
169 codon-substitution frequency(CSF) were measured according to the former research[14, 32]. The ORF
170 Finder(National Center for Biotechnology Information) fail to predict a ORFs of more than 300nt. Furthermore, we
171 Blasted all the positive-sense strand ORFs, which is marked by "+" in the figure, and no highly homologous protein
172 was found among those short peptide(Supplement Figure 2). On the other hand, the PhyloCSF analysis of lnc-
173 SAMD14-4 was negative(Supplement Figure 3). These all revealed that lnc-SAMD14-4 have no potential to encode
174 any functional protein product.

175 **COL1A1，COL1A2 and lnc-SAMD14-4 were highly expressed in the IL-1β-treated human primary**
176 **chondrocytes.**

177 According our co-expression network analysis, COL1A1 and COL1A2 were strongly associated with lnc-

SAMD14-4. Thus we speculated that lnc-SAMD14-4 may contribute to the osteoarthritis by interacting with COL1A1 and COL1A2. IL-1β-treated human primary chondrocytes was used to fit the process of human osteoarthritis in the previous study[17, 33]. The expression levels of *COL1A1*，*COL1A2* and lnc-SAMD14-4 were examined by QPCR in the IL-1β-treated human chondrocytes, we found that *COL1A1* and *COL1A2* were upregulation after IL-1β-treated for 24h and 48h. Furthermore, lnc-SAMD14-4 were also upregulation by IL-1β in time-dependent(Fig. 6). These results demonstrate that there seemed to be a positive correlation between lnc-SAMD14-4 and COL1A1, COL1A2 expression induced by IL-1β. Hence, the human primary chondrocytes were treated with or without IL-1β for 48h in the follow-up experiments.

lnc-SAMD14-4 was involved in IL-1β-induced COL1A1 and COL1A2 expression in the human primary chondrocytes.

For further discussing the function of lnc-SAMD14-4 in the IL-1β-induced *COL1A1* and *COL1A2* activities. We performed gain/loss of function experiments. As shown in Figure 7A, lnc-SAMD14-4 overexpression promotes the expression of *COL1A1* and *COL1A2* in the human primary chondrocytes with or without IL-1β-treated($p<0.05$).

To explore the knockdown effects of lnc-SAMD14-4 on *COL1A1* and *COL1A2*. We designed and transfected three independent si-lnc-SAMD14-4(1#, 2# and 3#) in human primary chondrocytes. The strongest silencing effects were observed in si-lnc-SAMD14-4(2#) and it's used for subsequent experiments(Data were not show). The knockdown of lnc-SAMD14-4 significantly decreased the expression of *COL1A1* and *COL1A2* (Figure 7B). These data revealed an important lncRNA may closely relate to the COL1A1 and COL1A2 expression in osteoarthritis.

Discussion

To our knowledge, there has been some study that focuses on the lncRNA expression profikes in the osteoarthritis, for instance, Fu's group identified 4,714 lncRNAs and Liu's group identified 152 lncRNAs that were differentially

199 expressed in OA cartilage compared with controls. Pearson's group further identified lncRNAs related to the
200 inflammatory response in human hip OA cartilage using primary chondrocytes isolated from hip articular cartilage
201 and IL-1β induction to simulate OA. But the roles of lncRNAs in osteoarthritis were far from full understood and we
202 think it's essential to get more data about the role of lncRNA in OA

203 In the present study, We measured expression profiles lncRNAs and mRNAs using high-throughput microarray
204 and bioinformatics to study human knee OA and compared this to non-OA tissue by set a standards filter of FC $\geq$
205 2.0, $p<0.05$; Because our work used Affymetrix and previous studies used Arraystar, which are different platforms,
206 comparing lncRNA data is problematic.

207 Using these chips, we identified 2,042 dysregulated lncRNAs and 2,011 differentially expressed mRNAs in OA
208 samples compared to non-OA tissues, including *COL1A1, COL1A2, MMP-13*, *MMP-9*, and *TNFAIP6*, which are
209 associated with cartilage biology and OA. Studies with the top 4 dysregulated lncRNAs and mRNAs, except for
210 *MMP13* mRNA *which* has already been proved to be related to the chondrocyte biological processes or
211 osteoarthritis[34-38], verified that qRT-PCR data for 53 specimens (34 OA and 19 normal specimens) were
212 consistent with chip analysis.

213 Next, GO analysis and pathway analysis were used to study biological function enrichment of differentially
214 expressed mRNA as described aboved. For instance, the most enriched GOs targeted by up-regulated mRNAs were
215 extracellular matrix organization (GO:0030198), extracellular exosome (GO:0070062), and collagen binding
216 (GO:0005518) in biological processes, cellular processes, and molecular function. Functions of the extracellular
217 matrix and collagen binding have been accepted to be important to the onset and progression of OA [39, 40].

218 A LncRNA-mRNA co-expression network was built to identify associations between LncRNA and mRNA.
219 The fundamentals of co-expression network analysis is that if the expression pattern of mRNAs and lncRNAs shows

an opposite or identical correlativity to the other, it is possible that there are some interaction between the lncRNA-mRNA. We noticed that some most dysregulation lncRNA or mRNA made a lncRNA-mRNA paris, among these, lnc-SAMD14-4 (lncRNA, FC= 86.11047) was strongly associated with *COL1A1* (mRNA, FC=175.43033) and *COL1A2* (mRNA, FC=85.32694). Type I collagen is a triple helix consisting of two pro-alpha1(I) chains encoded by two *COL1A1* genes and one pro-alpha2(I) chain encoded by one *COL1A2* gene. Type I collagen is the most abundant collagen of the human body present in scar tissue and it's also regarded as the end product when tissue heals by repair[41], It's reported that Type I collagen are rare in normal chondrocytes but higher levels of Type I collagen was detected in osteoarthritis cartilage, especially in late stages of osteoarthritis[42], it's reasonable to hypothesis lnc-SAMD14-4 may promote *COL1A1* gene and *COL1A2* gene and involved in the pathogenesis of OA. To verify our thoughts, Il-1β-treated human chondrocytes was used to imitated the gene change in the process of osteoarthritis as previous study[17, 43]. Ours study indicated a positive correlation between lnc-SAMD14-4 and *COL1A1* gene and *COL1A2* gene in Il-1β-treated human chondrocytes as well as osteoarthritis patients cartilage. Thus, our study suggested lnc-SAMD14-4 might play a key role in the pathogenesis of osteoarthritis by promote *COL1A1* gene and *COL1A2* gene.

Conclusions

Generally speaking, we used the Affymetrix chips and bioinformatics to explore the differential expression lncRNAs between OA articular cartilage and non-OA articular cartilage, Moreover, our study revealed that lnc-SAMD14-4 may promote *COL1A1* gene and *COL1A2* gene and involved in the pathogenesis of OA, which provides a potential therapeutic target for treatment.

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Supplementary Tables

Supplementary Table 1: Patient data

Supplementary Table 2: qPCR primers used in this study

Supplementary Table 3: Differentially expressed lncRNA in OA and non-OA groups

Supplementary Table 4: Differentially expressed mRNA in OA and non-OA groups

Supplementary Table 5: Biological processes found with GO enrichment analysis for up-regulated mRNA in OA and non-OA groups

Supplementary Table 6: The cellular process of Go enrichment analysis for up-regulated mRNA in OA and non-OA groups

Supplementary Table 7: Molecular functions for Go enrichment analysis of up-regulated mRNA in OA and non-OA groups

Supplementary Table 8: Biological processes for GO enrichment analysis of down-regulated mRNA in OA and non-OA groups

Supplementary Table 9: Cellular process for GO enrichment analysis of down-regulated mRNA in OA and non-OA groups

Supplementary Table 10: Molecular functions for GO enrichment analysis of down-regulated mRNA in OA and non-OA groups

Supplementary Table 11: Pathway analysis of up-regulated mRNA in OA and non-OA groups

Supplementary Table 12: Pathway analysis of down-regulated mRNA in OA and non-OA groups

Supplementary Table 13: Dysregulated mRNAs enriched in the PI3K-Akt signal pathway

Supplementary Table 14: The lncRNA-mRNA interaction pairs based on the PI3K-Akt signal pathway

Supplementary Table 15: Top 500 correlations of lncRNA-mRNA interaction pairs based on the PI3K-Akt signal pathway

Supplementary Figure 1: The quality control of total RNA. (A) the SDS-PAGE gels of total RNA. (B) The electrophoresis pattern of SDS-PAGE of total RNA. (C) the analysis of SDS-PAGE of total RNA, RNA Integrity Number(RIN)＞7 represent the quality of RNA can satisfy the study requirements.

Supplementary Figure 2: Prediction of putative proteins encoded by lnc-SAMD14-4 using ORF Finder.

Supplementary Figure 3: The codon substitution frequency scores(CSF) of lnc-SAMD14-4.

Table 1: Most regulated lncRNAs in this study

Table 2: Most regulated mRNAs in this study

Fig. 1. Volcano plot and hierarchical clustering of lncRNA and mRNA differential expression profiles between non-OA and OA groups for 10 cartilage tissues. (A) Volcano plot of dysregulated lncRNA and mRNA (dots denotes gene). Grey spots represent $p>0.05$; green spots represent $p\leq0.05$ and fold-change <2. Red spots represent up-regulated genes ($p\leq0.05$ and fold-change ≥2.0), and blue spots represent up-regulated genes ($p\leq0.05$ and fold-change $\leq$-2.0). Heatmap of differentially expressed lncRNA (B) and mRNA (C). Colors denote relative expression: red represents high relative expression; green represents low relative expression.

Fig. 2. GO analysis of abnormal mRNAs between OA and non-OA groups. Enriched GO terms targeted by up-regulated mRNAs (A-C) and down-regulated mRNAs (D-F) according to biological processes, cell components, and molecular function ($p<0.05$ as statistically significant according to a Fisher's exact test).

Fig. 3. KEGG pathway analysis of abnormal mRNAs between OA and non-OA groups. Top 20 pathways targeted

by down-regulated (H) and up-regulated (I) transcripts ($p<0.05$ was statistically significant according to a Fisher's exact test).

Fig. 4. LncRNA-mRNA co-expression network. Green node represents mRNA; red node represents lncRNA. Line indicates a co-expression relationship between lncRNA-mRNA.

Fig. 5. qPCR and microarray data. Values are means ± SD (n=3), and *$p<0.05$ was statistically significant compared to the non-OA group.

Fig. 6. The mRNA levels of *COL1A1*，*COL1A2* and lnc-SAMD14-4 were detected by QPCR in the IL-1β-treated human primary chondrocytes. The mRNA expression was normalized to GAPDH expression and conversion by $2^{-\Delta\Delta Ct}$. Values are means ± SD (n=3). Data were analyzed using one-way ANOVA followed by Dunnett-*t* test. *$p<0.05$ was statistically significant compared to the control group.

Fig. 7. Effects of lnc-SAMD14-4 suppression/overexpression on the relative expression levels of *COL1A1*，*COL1A2* in IL-1β-treated human primary chondrocytes detected by QPCR. Data were analyzed using one-way ANOVA followed by Dunnett-*t* test. *$p<0.05$ was statistically significant compared to the control group.

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Figure 1

Volcano plot and hierarchical clustering of lncRNA and mRNA differential expression profiles between non-OA and OA groups for 10 cartilage tissues.

(A) Volcano plot of dysregulated lncRNA and mRNA (dots denotes gene). Grey spots represent $p>0.05$; green spots represent $p < 0.05$ and fold-change <2. Red spots represent up-regulated genes ($p < 0.05$ and fold-change > 2.0), and blue spots represent up-regulated genes ($p < 0.05$ and fold-change < -2.0). Heatmap of differentially expressed lncRNA (B) and mRNA (C). Colors denote relative expression: red represents high relative expression; green represents low relative expression.

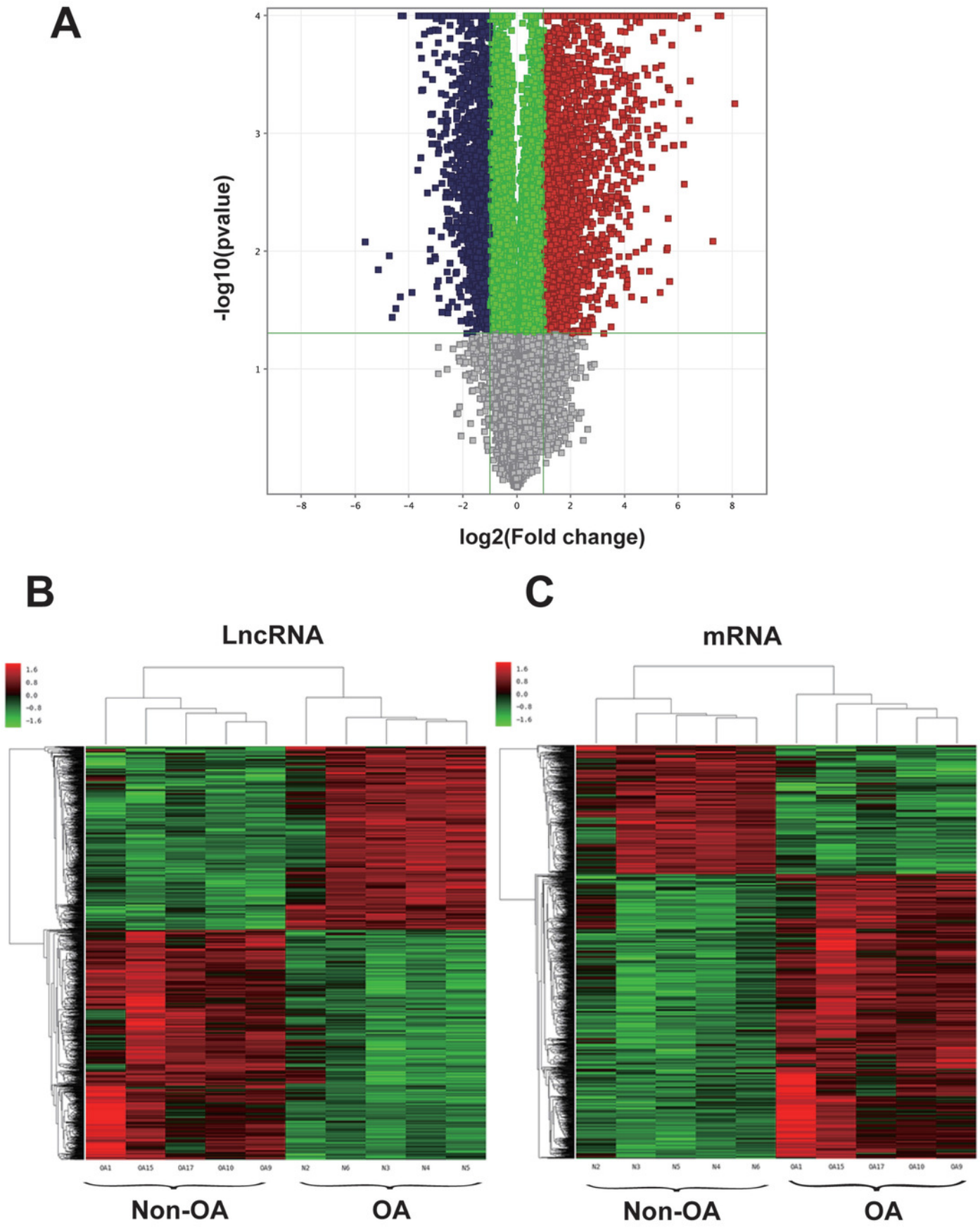

A
-log10(pvalue)
log2(Fold change)
B
LncRNA
Non-OA
OA
C
mRNA
Non-OA
OA

Figure 2

Go analysis of abnormal mRNAs between OA and non-OA groups.

Enriched Go terms targeted by up-regulated mRNAs (A-C) and down-regulated mRNAs (D-F) according to biological processes, cell components, and molecular function ($p<0.05$ as statistically significant according to a Fisher's exact test).

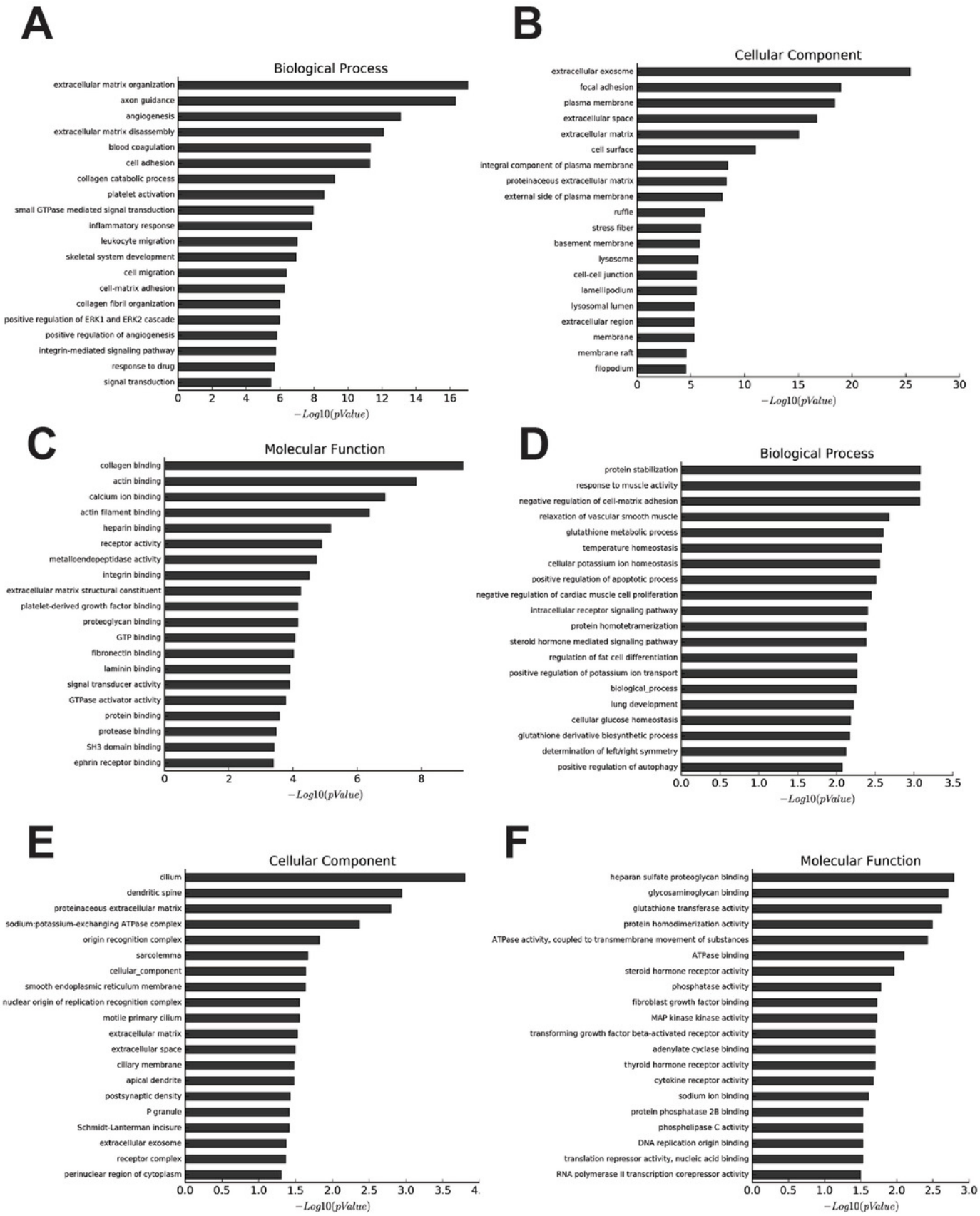

A
Biological Process
extracellular matrix organization
axon guidance
angiogenesis
extracellular matrix disassembly
blood coagulation
cell adhesion
collagen catabolic process
platelet activation
small GTPase mediated signal transduction
inflammatory response
leukocyte migration
skeletal system development
cell migration
cell-matrix adhesion
collagen fibril organization
positive regulation of ERK1 and ERK2 cascade
positive regulation of angiogenesis
integrin-mediated signaling pathway
response to drug
signal transduction
0 2 4 6 8 10 12 14 16
−Log10(pValue)
B
Cellular Component
extracellular exosome
focal adhesion
plasma membrane
extracellular space
extracellular matrix
cell surface
integral component of plasma membrane
proteinaceous extracellular matrix
external side of plasma membrane
ruffle
stress fiber
basement membrane
lysosome
cell-cell junction
lamellipodium
lysosomal lumen
extracellular region
membrane
membrane raft
filopodium
0 5 10 15 20 25 30
−Log10(pValue)
C
Molecular Function
collagen binding
actin binding
calcium ion binding
actin filament binding
heparin binding
receptor activity
metalloendopeptidase activity
integrin binding
extracellular matrix structural constituent
platelet-derived growth factor binding
proteoglycan binding
GTP binding
fibronectin binding
laminin binding
signal transducer activity
GTPase activator activity
protein binding
protease binding
SH3 domain binding
ephrin receptor binding
0 2 4 6 8
−Log10(pValue)
D
Biological Process
protein stabilization
response to muscle activity
negative regulation of cell-matrix adhesion
relaxation of vascular smooth muscle
glutathione metabolic process
temperature homeostasis
cellular potassium ion homeostasis
positive regulation of apoptotic process
negative regulation of cardiac muscle cell proliferation
intracellular receptor signaling pathway
protein homotetramerization
steroid hormone mediated signaling pathway
regulation of fat cell differentiation
positive regulation of potassium ion transport
biological_process
lung development
cellular glucose homeostasis
glutathione derivative biosynthetic process
determination of left/right symmetry
positive regulation of autophagy
0.0 0.5 1.0 1.5 2.0 2.5 3.0 3.5
−Log10(pValue)
E
Cellular Component
cilium
dendritic spine
proteinaceous extracellular matrix
sodium:potassium-exchanging ATPase complex
origin recognition complex
sarcolemma
cellular_component
smooth endoplasmic reticulum membrane
nuclear origin of replication recognition complex
motile primary cilium
extracellular matrix
extracellular space
ciliary membrane
apical dendrite
postsynaptic density
P granule
Schmidt-Lanterman incisure
extracellular exosome
receptor complex
perinuclear region of cytoplasm
0.0 0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.
−Log10(pValue)
F
Molecular Function
heparan sulfate proteoglycan binding
glycosaminoglycan binding
glutathione transferase activity
protein homodimerization activity
ATPase activity, coupled to transmembrane movement of substances
ATPase binding
steroid hormone receptor activity
phosphatase activity
fibroblast growth factor binding
MAP kinase kinase activity
transforming growth factor beta-activated receptor activity
adenylate cyclase binding
thyroid hormone receptor activity
cytokine receptor activity
sodium ion binding
protein phosphatase 2B binding
phospholipase C activity
DNA replication origin binding
translation repressor activity, nucleic acid binding
RNA polymerase II transcription corepressor activity
0.0 0.5 1.0 1.5 2.0 2.5 3.0
−Log10(pValue)

Table 1(on next page)

Most regulated lncRNAs in this study

GeneSymbol	Regulation	Type	Fold change(abs)	*P*-value
lnc-SAMD14-4	Up	Exonic	86.11047	0.00036
lnc-MARCKS-7	Up	-------	80.51785	0.0000923
lnc-SAMD14-5	Up	Exonic	76.39162	0.0000161
lnc-AP3S2-2	Up	Exonic	57.44485	0.001265177
lnc-CASD1-2	Up	Exonic	56.14244	0.001261528
lnc-TSPAN13-4	Up	Exonic	50.915787	0.00000633
lnc-PPM1N-1	Up	Exonic	47.80361	0.009312962
lnc-TRPC4-2	Up	Exonic	47.43022	0.009825662
lnc-C1orf196-1	Up	Linc	40.125366	0.0000202
lnc-SMAD5-7	Up	Exonic	39.585285	0.022567188
lnc-TSPAN13-5	Up	Exonic	37.4391	0.00000077
lnc-SFRP1-2	Up	Exonic	36.96935	0.001035282
lnc-HJURP-12	Up	Exonic	36.914413	0.000862
lnc-TEX2-2	Up	Exonic	33.92366	0.000572
lnc-MS4A6E-1	Up	Exonic	31.000843	0.001087416
lnc-MSMP-2	Down	Exonic	22.78652	0.030858
lnc-NPVF-4	Down	Linc	18.66038	0.0000169
lnc-CBLL1-4	Down	Exonic	14.84985	0.022533
MLLT4-AS1	Down	-------	12.8248	0.002052
lnc-NPVF-10	Down	-------	12.68418	0.0000278
lnc-ASZ1-3	Down	Exonic	11.32834	0.000425
SAMD12	Down	-------	9.023141	0.006789
lnc-NPVF-5	Down	-------	9.018275	0.000095
lnc-IRGM-3	Down	Exonic	8.909008	0.001227
lnc-ERCC5-4	Down	Linc	8.883492	0.000686
lnc-SSR3-6	Down		8.876818	0.000349
lnc-DLGAP1-9	Down	Exonic	8.776601	0.001021
lnc-SSR3-5	Down	Antisense	8.354682	0.000208
lnc-CHI3L1-1	Down	Exonic	8.350381	0.011126

lnc-OXNAD1-6	Down	Antisense	8.14148	0.0000128

1

2

Figure 3

KEGG pathway analysis of abnormal mRNAs between OA and non-OA groups.

Top 20 pathways targeted by down-regulated (H) and up-regulated (I) transcripts (p <0.05 was statistically significant according to a Fisher's exact test).

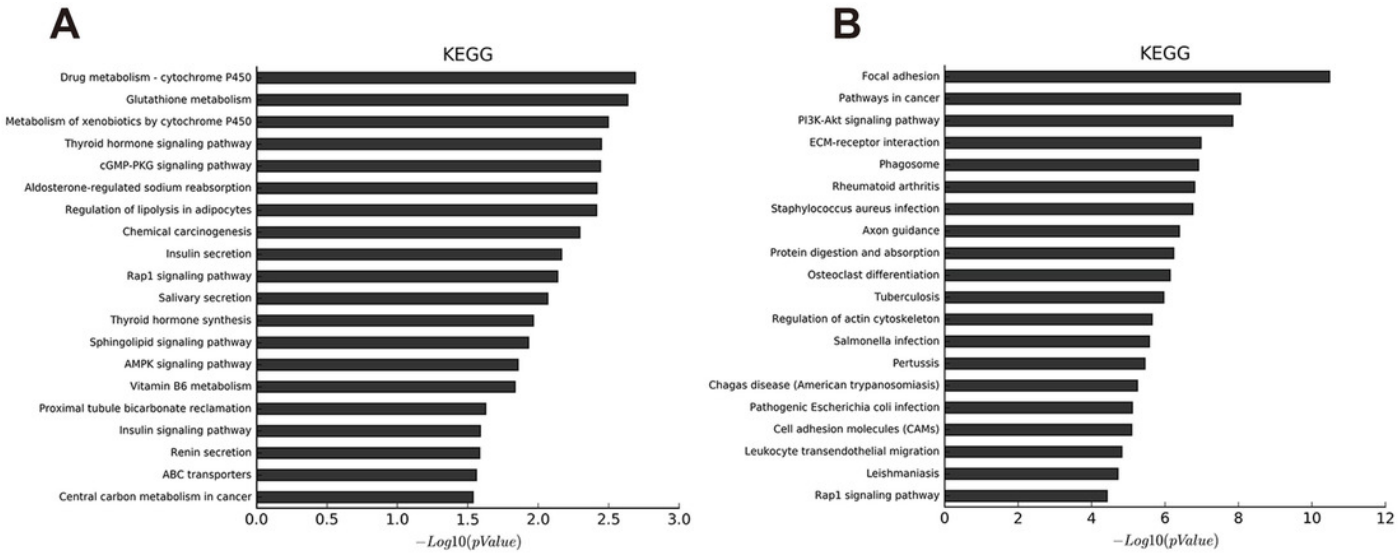


Figure 4

LncRNA-mRNA co-expression network.

Green node represents mRNA; red node represents lncRNA. Line indicates a co-expression relationship between lncRNA-mRNA.

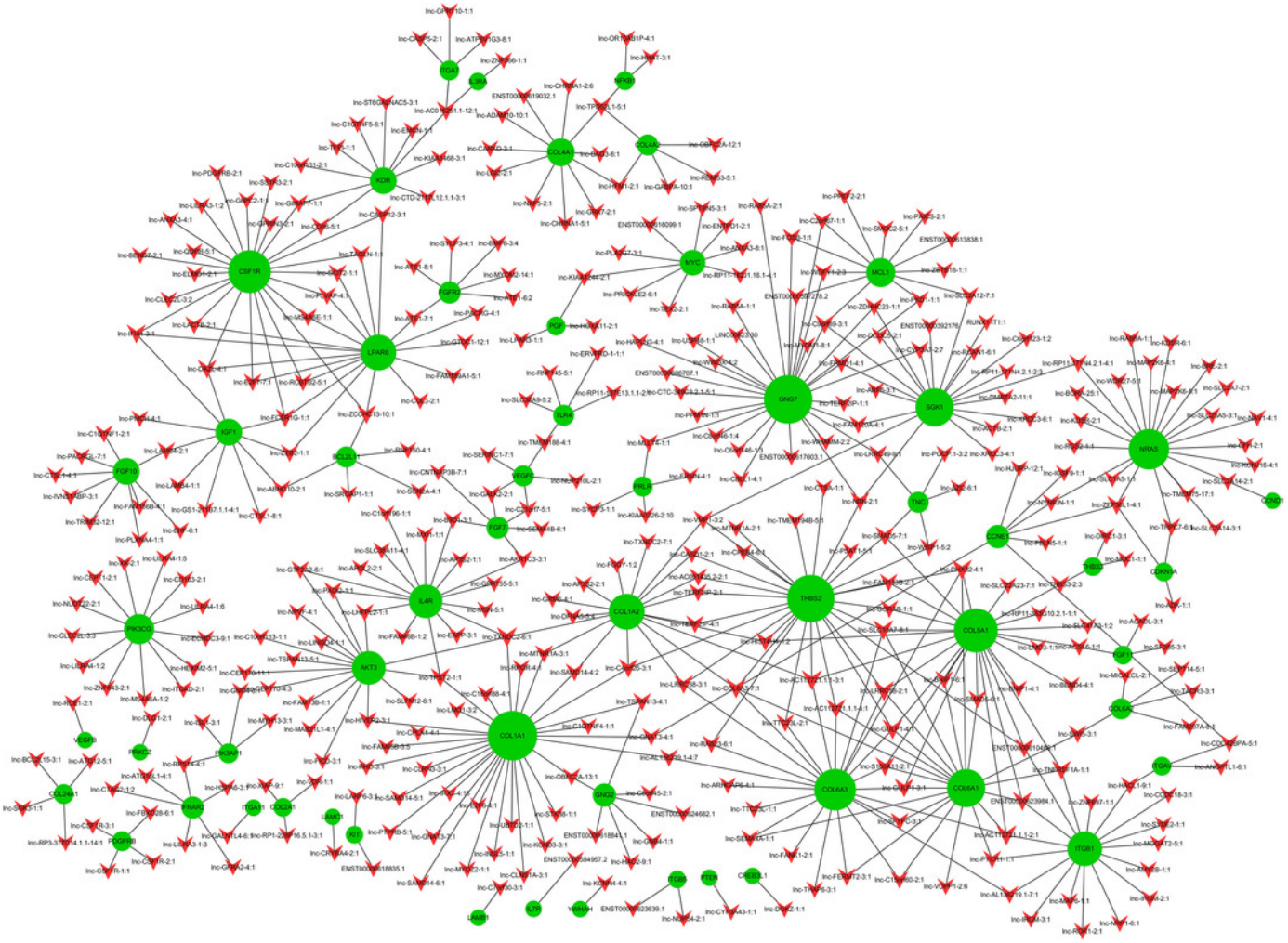


Figure 5

qPCR and microarray data.

Values are means + S D (n=3), and *p<0.05 was statistically significant compared to the non-OA group

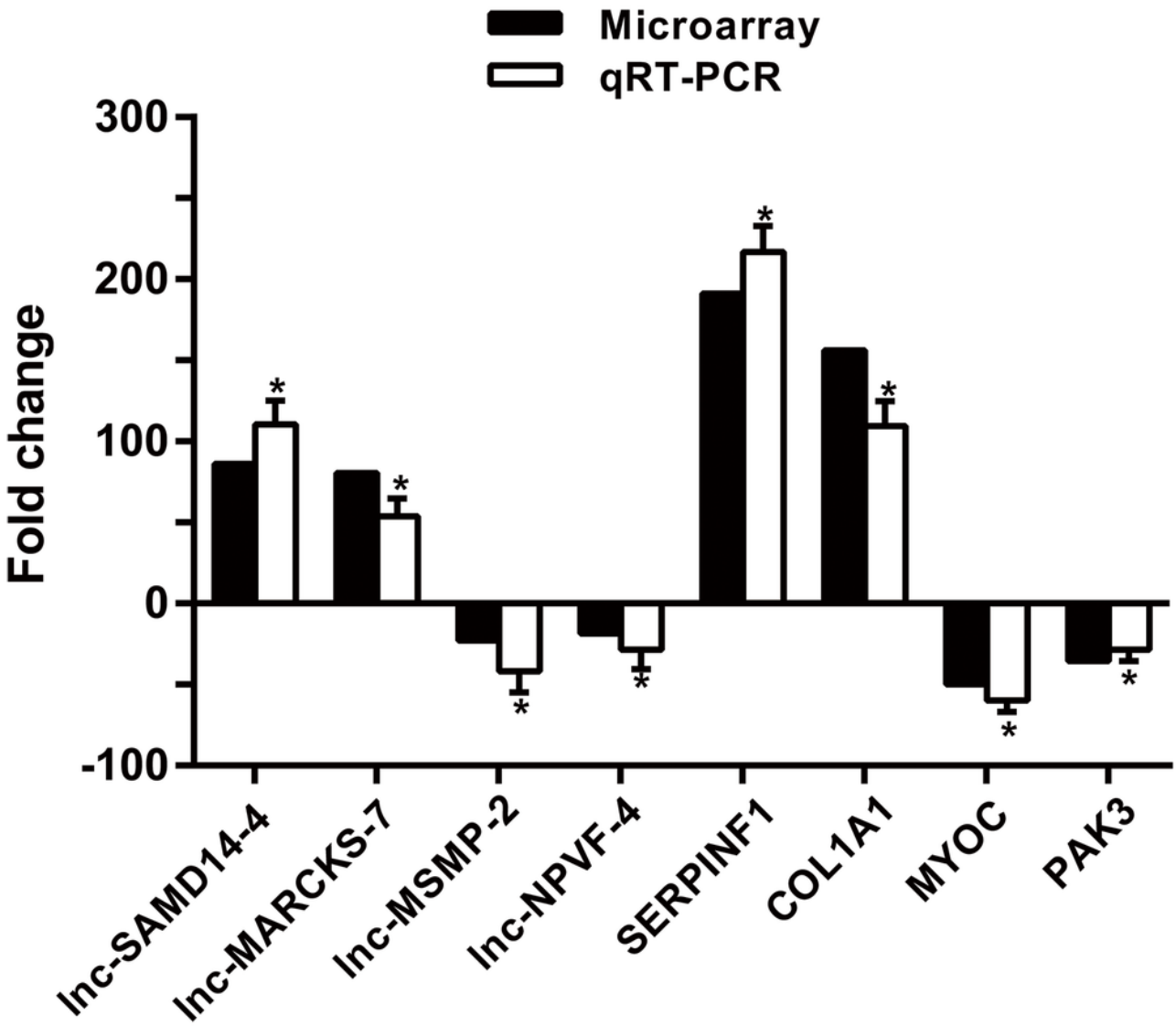


Table 2(on next page)

Most regulated mRNAs in this study

GeneSymbol	Regulation	Fold change(abs)	P-value
MMP13	Up	272.535	0.000564
SERPINF1	Up	191.17508	0.000000141
COL1A1	Up	175.43033	0.0000199
POSTN	Up	156.12422	0.008303139
LRRC15	Up	105.57993	0.000129
COL1A2	Up	85.32694	0.000776
THBS2	Up	74.47372	0.002686227
FNDC1	Up	72.167336	0.001257106
RP11-429G19.3	Up	69.320656	0.000105
MS4A4A	Up	64.19533	0.000564
FPR3	Up	56.89943	0.0000552
MSR1	Up	53.758953	0.000178
AHR	Up	52.798218	0.0000266
TNFAIP6	Up	51.144848	0.018054951
PLXDC1	Up	51.0092	0.00043
MYOC	Down	49.693558	0.008422094
PAK3	Down	35.395626	0.014492353
APOD	Down	26.913952	0.011042964
MSMP	Down	24.774714	0.036745384
SLC26A4	Down	20.350153	0.024579711
AC003090.1	Down	19.897524	0.0001
CYP4F11	Down	12.0151615	0.001603253
CTTNBP2	Down	11.738498	0.000434
ABCA5	Down	10.876956	0.000144
ZMAT1	Down	9.586397	0.000137

1

Figure 6

The mRNA levels of *COL1A1*、*COL1A2* and lnc-SAMD14-4 were detected by QPCR in the IL-1β-treated human primary chondrocytes

The mRNA expression was normalized to GAPDH expression and conversion by $2^{-\Delta\Delta Ct}$. Values are means + SD (n=3). Data were analyzed using one-way ANOVA followed by Dunnett-*t* test. **p*<0.05 was statistically significant compared to the control group.

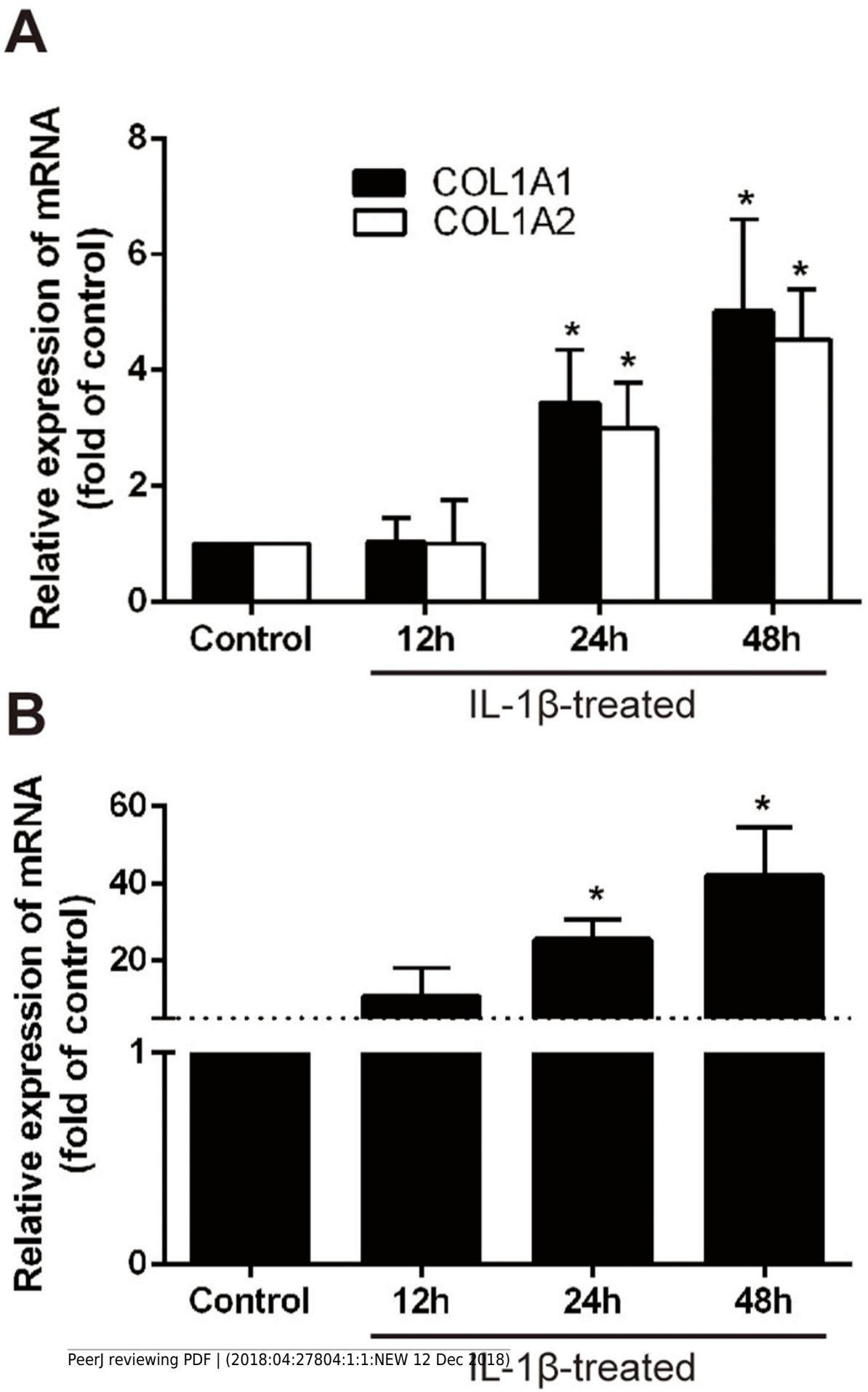
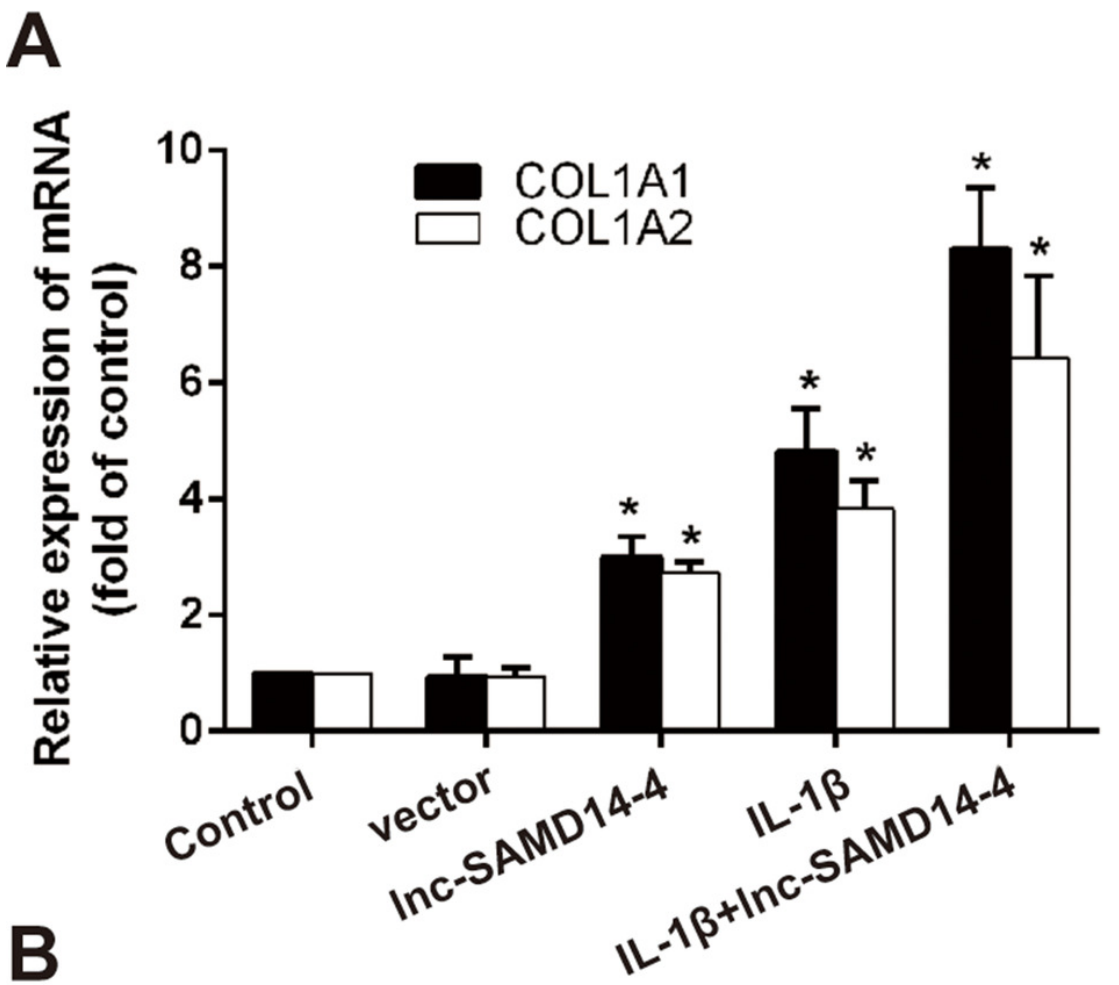
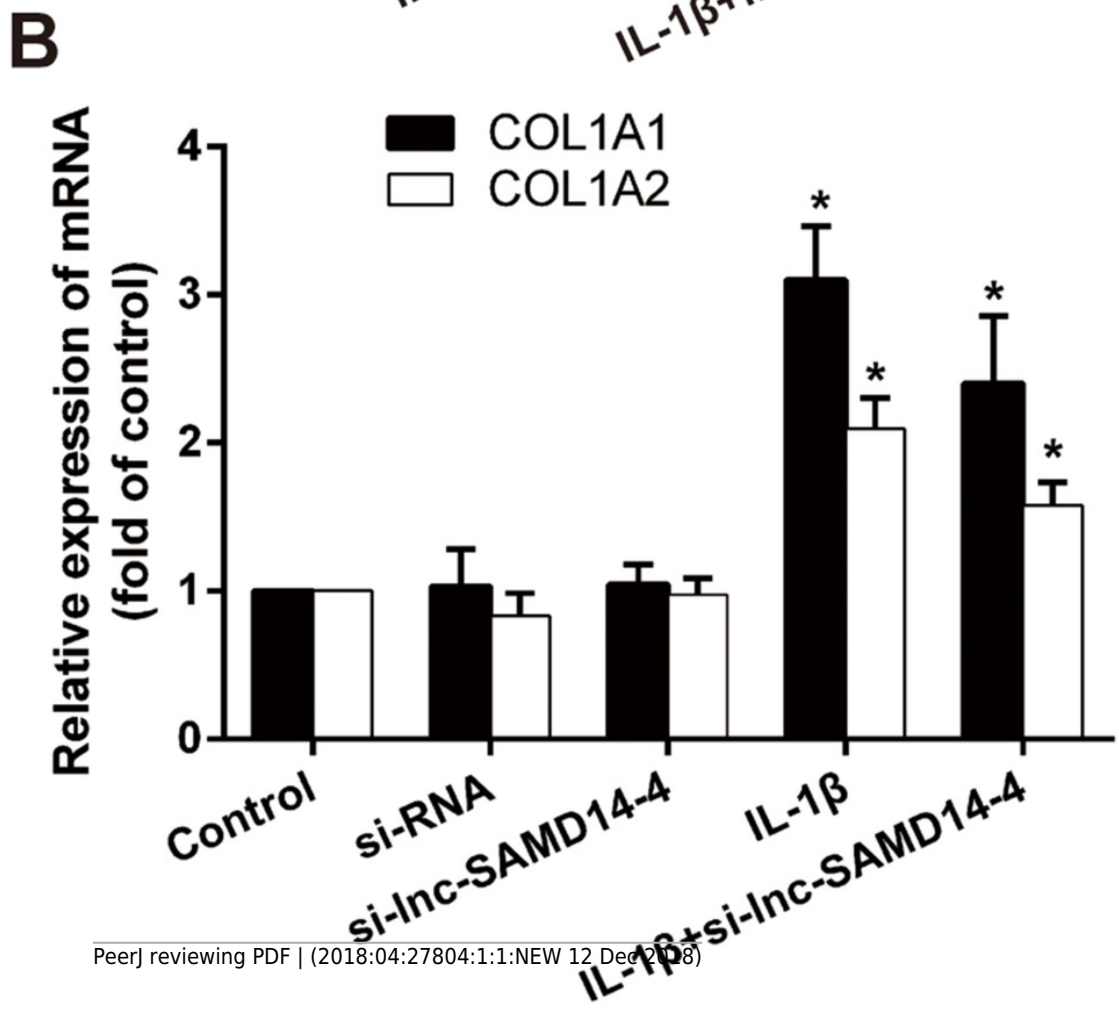

A
Relative expression of mRNA (fold of control)
8
6
4
2
0
COL1A1
COL1A2
*
*
*
*
Control
12h
24h
48h
IL-1β-treated
B
Relative expression of mRNA (fold of control)
60
40
20
1
0
*
*
Control
12h
24h
48h
IL-1β-treated

Figure 7

Effects of lnc-SAMD14-4 suppression/overexpression on the relative expression levels of *COL1A1*、*COL1A2* in IL-1β-treated human primary chondrocytes detected by QPCR

Data were analyzed using one-way ANOVA followed by Dunnett-*t* test. **p* <0.05 was statistically significant compared to the control group.


A
Relative expression of mRNA (fold of control)
COL1A1
COL1A2
0
2
4
6
8
10
*
Control
vector
lnc-SAMD14-4
IL-1β
IL-1β+lnc-SAMD14-4


B
Relative expression of mRNA (fold of control)
COL1A1
COL1A2
0
1
2
3
4
*
Control
si-RNA
si-lnc-SAMD14-4
IL-1β
IL-1β+si-lnc-SAMD14-4