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Supplemental Information

Downregulation of Hypoxia-Inducible Factor-1α by RNA Interference Alleviates the Development of Collagen-Induced Arthritis in Rats

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Supplementary Material for

Down-regulation of hypoxia-inducible factor-1α by RNA interference alleviates the development of collagen-induced arthritis in rats

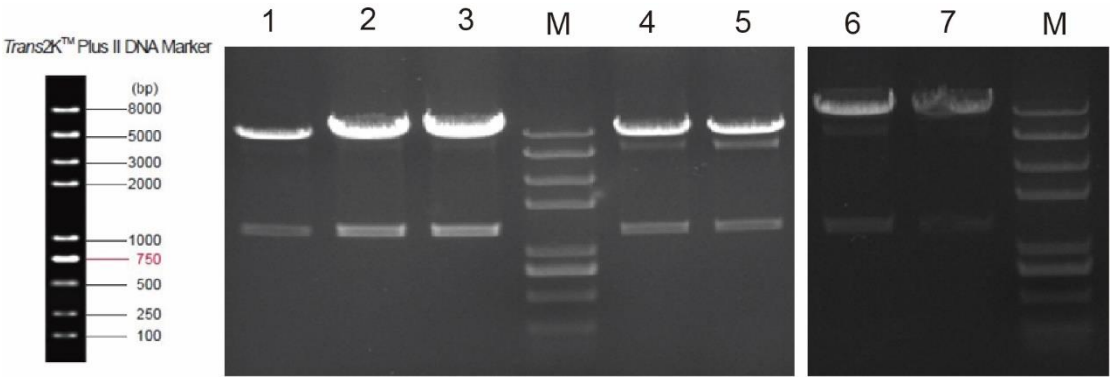

Figure S1. The pLVX-shRNA-HIF-1α plasmid digested and identified by XhoI enzyme. Lane M: Trans2K plus marker, Lane 1,2,3: pLVX-shRNA1-HIF-1α digested by XhoI, Lane 4,5: pLVX-shRNA2-HIF-1α digested by XhoI, Lane 6,7: pLVX-shRNA3-HIF-1α digested by XhoI

Figure S2. Vector plasmid sequencing (A) pLVX-shRNA1-HIF-1α, (B) pLVX-shRNA2-HIF-1α,

(C) pLVX-shRNA3-HIF-1α.

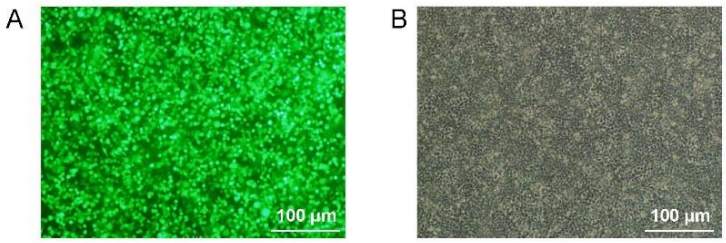

Figure S3. 24 h green fluorescence after transfection，(A) Green fluorescence observation after transfection of 293T cells for 24h, (B) Control non-fluorescent cell

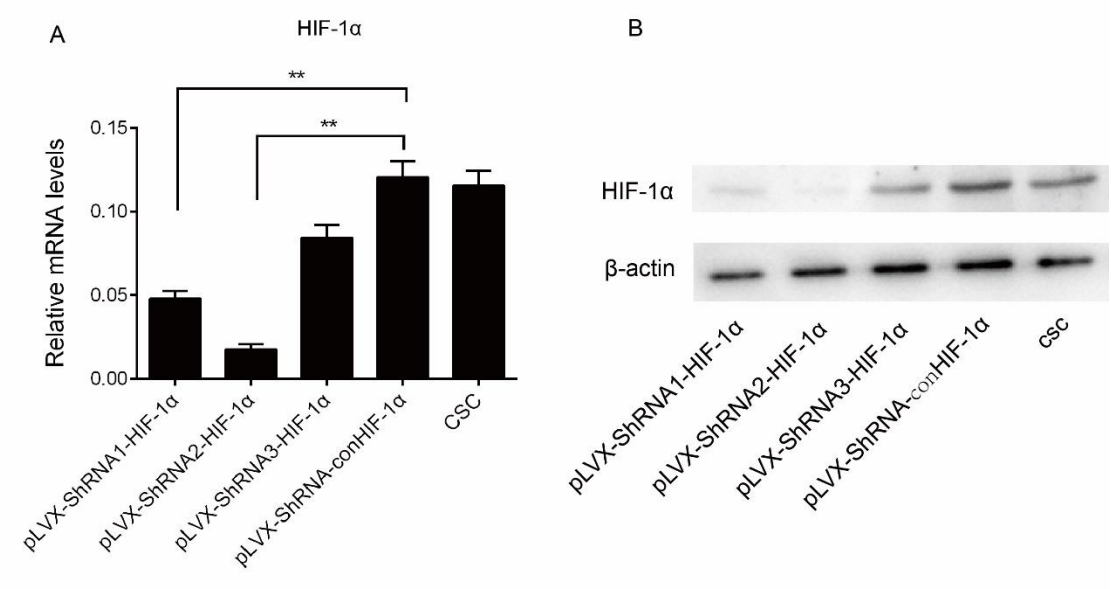

Figure S4. HIF-1α silencing effectiveness analysis, (A) HIF-1α mRNA expression, (B) HIF-1α Protein expression

Methods

shRNA design, viral packaging and its effectiveness screening

(1) We designed 3 pairs of shRNAs that target HIF-1, and the sense and antisense sequences are as follows:

rHIF-lα-shRNA1-F：
5′-GATCCGCAGTGTGGCTACAAGAAACCTTCAAGAGAGGTTTCTTGTAGCCACACTGCTTTTTTCTCGAGG-3′
rHIF-lα-shRNA1-R：
5′-AATTCCTCGAGAAAAAAGCAGTGTGGCTACAAGAAACCTCTCTTGAAGGTTTCTTGTAGCCACACTGCG-3′
rHIF-lα-shRNA2-F：
5′-GATCCGCATTGAAGTTAGAGTCAAGCTTCAAGAGAGCTTGACTCTAACTTCAATGCTTTTTTCTCGAGG-3′

rHIF-lα-shRNA2-R：
5′-AATTCCTCGAGAAAAAAGCATTGAAGTTAGAGTCAAGCTCTCTTGAAGC
TTGACTCTAACTTCAATGCG-3′
rHIF-lα-shRNA3-F：
5′-GATCCGCAGTGACGAAGGACAATATATTCAAGAGATATATTGTCCTTCGT
CACTGCTTTTTTCTCGAGG-3′
rHIF-lα-shRNA3-R：
5′-AATTCCTCGAGAAAAAAGCAGTGACGAAGGACAATATATCTCTTGAATA
TATTGTCCTTCGTCACTGCG-3′

(2) The pLVX-shRNA-HIF-1α plasmid was constructed and extracted in large amounts using a plasmid extraction kit, and then digested and identified by XhoI enzyme (Figure S1).

(3) Vector plasmid sequencing

Sequencing is done by commercial companies (Figure S1)**.**

(4) Fluorescence observation of virus packaging and transfection of 293T cells for 24h (Figure S3)

(5) Detection of the effectiveness of synovial cell silencing HIF-1α

The detailed operation of real time-PCR and western blot analysis are described in the text (Figure S4).