

Inflammatory cytokine TNF- α promotes premature senescence of rat nucleus pulposus cells through PI3K/Akt signaling pathway

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Supplementary Table S1. Primers of target genes

Gene	Accession number	Forward (5'-3')	Reverse (5'-3')
β -actin	NM_031144.3	CCGCGAGTACAACCTTCTTG	TGACCCATACCCACCATCAC
Aggrecan	XM_002723376.1	ATGGCATTGAGGACAGCGAA	GCTCGGTCAAAGTCCAGTGT
Collagen II	NM_012929.1	GCCAGGATGCCCGAAAATTAG	CCAGCCTTCTCGTCAAATCCT
p53	XM_008767773.1	CCTTAAGATCCGTGGGCGT	GCTAGCAGTTTGGGCTTTCC
p16	NM_031550.1	TACCCCGATACAGGTGATGA	TACCGCAAATACCGCACGA

Supplementary Table S2. Primary and secondary antibodies used in this study (WB: Western blotting; ICC: Immunocytochemistry)

Antibodies	Purchase information	Host	Application and dilution
Anti- β -actin antibody	Proteintech	Mouse	1:1000 for WB
Anti-Aggregan antibody	Norvus	Mouse	1:200 for ICC
Anti-Collagen II antibody	Abcam	Rabbit	1:200 for ICC
Anti-p53 antibody	Proteintech	Rabbit	1:1000 for WB
Anti-p16 antibody	Norvus	Mouse	1:1000 for WB
Anti-Akt antibody	CST	Mouse	1:1000 for WB
Anti-p-Akt antibody	CST	Rabbit	1:1000 for WB
Goat anti-mouse IgG	ZSGB-Bio, China	Goat	1:2000 for WB and 1:200 for ICC
Goat anti-rabbit IgG	ZSGB-Bio, China	Goat	1:2000 for WB and 1:200 for ICC

Supplementary Figure S1. Effects of PI3K/Akt pathway inhibition on cell viability and matrix biosynthesis of rat nucleus pulposus (NP) cells treated with TNF- α on day 1 and day 3. A: When the inhibitor LY294002 (10 μ M) was added into the culture medium of NP cells in the 10 ng/mL TNF- α group, the activation of the PI3K/Akt

pathway was significantly inhibited. B: There was no significant difference in cell viability before and after inhibiting the activation of the PI3K/Akt pathway in NP cells treated with 10 ng/mL TNF- α . C-E: Inhibitor LY294002 up-regulated gene expression of matrix molecules aggrecan and collagen II (C), increased glycosaminoglycan (GAG) content (D) and increased matrix deposition in rat NP cells on day 1 and day 3 (E). The data are expressed as the mean \pm SD (n=3). *: Indicates a significant difference ($p < 0.05$) between the 10 ng/mL TNF- α group and the 10 ng/mL TNF- α +LY294002 group.

