

## Supplementary materials

# Cerium oxide nanoparticles protect against chondrocytes and cartilage explants from oxidative stress via Nrf2/HO-1 pathway in temporomandibular joint osteoarthritis

Liping Xiong<sup>1</sup>, Han Bao<sup>1</sup>, Size Li<sup>1</sup>, Deao Gu<sup>1</sup>, Yuyang Li<sup>2</sup>, Qianwen Yin<sup>1</sup>, Wen Li<sup>2</sup>, Leiying Miao<sup>2\*</sup>, Chao Liu<sup>1\*</sup>

<sup>1</sup>Department of Orthodontics, Nanjing Stomatological Hospital, Medical School of Nanjing University, Nanjing, 210008, China.

<sup>2</sup>Department of Cariology and Endodontics, Nanjing Stomatological Hospital, Medical School of Nanjing University, Nanjing, 210008, China.

### Correspondence:

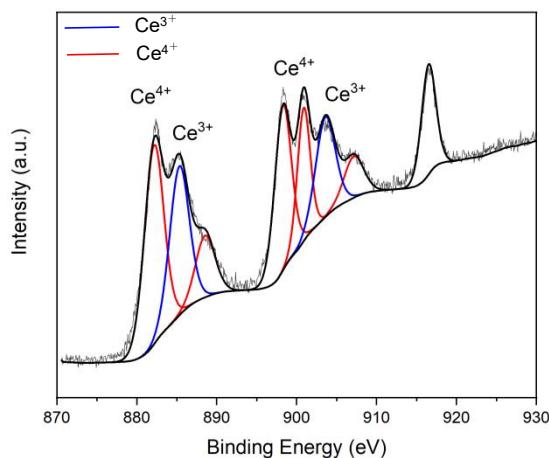
LeiYing Miao, Ph.D, Professor

Email: miaoleiying80@163.com

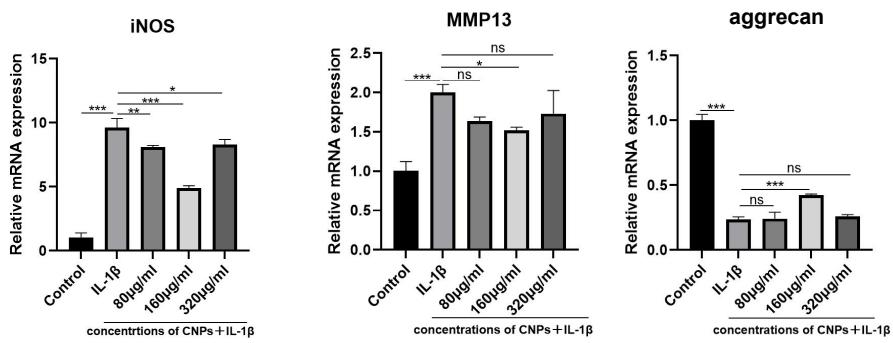
Chao Liu, Ph.D, Professor

Email: dxliuchao@163.com

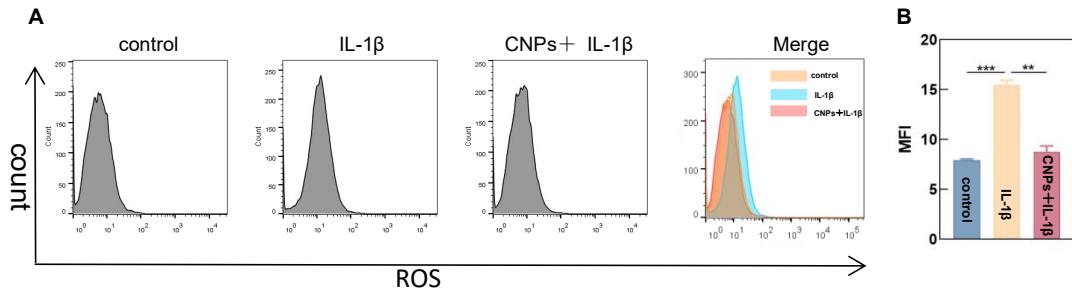
**Keywords:** Cerium oxide nanoparticles; Temporomandibular joint; Osteoarthritis ; Oxidative stress; Nrf2/HO-1 signal pathway; Cartilage explant



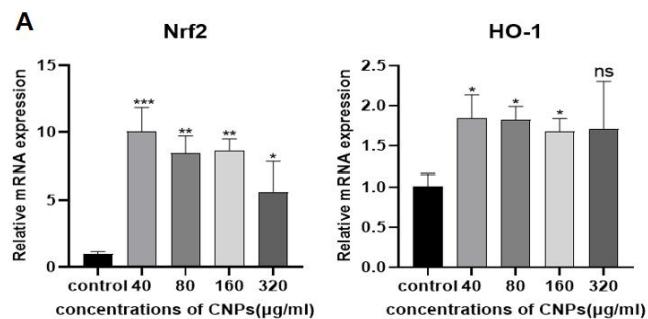
**FigureS1.**XPS spectrum of CNPs.



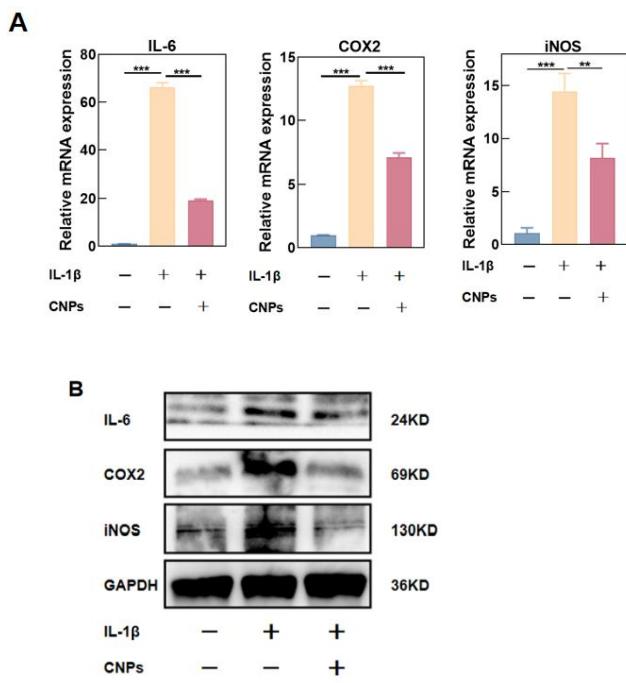
**FigureS2.** Effects of different concentrations of CNPs on iNOS、MMP13 and aggrecan expression levels in inflammatory states. \*P<0.05. \*\*P <0.01. \*\*\*P <0.001 relative to the control group.



**FigureS3.** CNPs reduced the IL-1 $\beta$ -induced oxidative stress in TMJ-OA chondrocytes. (A) Flow cytometry analysis of intracellular ROS levels; (B) Semi-quantitation of intracellular ROS levels detected by flow cytometry. \*\*P <0.01, \*\*\*P <0.001.



**FigureS4.(A)** Different concentrations of CNPs promote the mRNA expression of Nrf2 and HO-1.\*P <0.05,\*\*P <0.01, \*\*\*P <0.001 relative to the control group.



**FigureS5.**(A)Relative mRNA expression of IL-6,COX2,iNOS; (B) protein expression of IL-6,COX2,iNOS.\*\* $P <0.01$ . \*\*\* $P <0.001$ .

**Supplementary Table 1. Primer sequences used in this study.**

Gene	Identification	Sequence (5'-3')
<i>GAPDH</i>	Sense	TGCCACTCAGAAGACTGTGG
	Antisense	TTCAGCTCTGGGATGACCTT
<i>Nrf2</i>	Sense	GGTTGCCCACATTCCCAAATC
	Antisense	CAAGTGACTGAAACGTAGCCG
<i>HO-1</i>	Sense	GAAGAGGGAGATAGAGCGAAACA
	Antisense	CAATCTTCTTCAGGACCTGACC
<i>SOD</i>	Sense	CCTGTTCTACTGCAGTTAGGAA
	Antisense	CATCCTTAGCCTAGTTACACGT

<i>CAT</i>	Sense	AAAAGCTAACCTGTAAAGCACG
	Antisense	CTGTTAAGTGATTGCTAAGCCC
<i>GPx</i>	Sense	AGTGCAGGGTGAATGGTGAG
	Antisense	TCGATGTCGATGGTGCAGAAA
<i>aggrecan</i>	Sense	CCAGAAGGGTCAGGAGAGAAC
	Antisense	AAAGTGTCCAAGGCATCCAC
<i>Coll1</i>	Sense	CAAGATGGTGGCCGTTACTAC
	Antisense	TTAGTCCTTACCGCTTTCCAG
<i>Col2a1</i>	Sense	GGCTCCAGAACATCACCTA
	Antisense	GCCCTCATCTCCACATCATT
<i>MMP13</i>	Sense	ATGTCATAACCATTAGAGCC
	Antisense	ATCCCTTGATGCCATTACCAAG
<i>ADAMTS4</i>	Sense	CCCGGAATGGTGGAAAGTATT
	Antisense	TATGACAAGTGCAGGAGTATG
<i>iNOS</i>	Sense	AGCATCCACGCCAAGAACG
	Antisense	GTCTGGTTGCCTGGAAAAT
<i>COX2</i>	Sense	GAGCTGTAAGAGTCTACTGACC
	Antisense	ACACAGGAATCTTCACAAATGG
<i>IL-6</i>	Sense	TGCACGTGTCAGAAAACAATCTG
	Antisense	CCAGAGCAGATTTCATAAGGC

**Supplementary Table2. Antibodies used in this study.**

MARKER (SPECIES) (Primary antibodies)	DILUTION	DISTRIBUTOR/SOURCE (CATALOGUE NUMBER)
GAPDH Rabbit mAb	1:5000	Abcam;ab181602
Nrf2 Rabbit mAb	1:5000	Proteintech;16396-1-AP
HO-1 Rabbit mAb	1:5000	Abcam;ab68477
aggrecan Rabbit mAb	1:1000	Affinity Biosciences; DF7561
COLI Rabbit mAb	1:1000	Proteintech;14695-1-AP
COLII Rabbit mAb	1:1000	Proteintech;28459-1-AP
MMP13 Rabbit mAb	1:1000	Proteintech;18165-1-AP
ADAMTS4 Rabbit mAb	1:1000	Affinity Biosciences;DF6986
iNOS Rabbit mAb	1:5000	Abcam;ab178945
COX2 Rabbit mAb	1:5000	Abcam;ab15191
IL-6 Rabbit mAb	1:1000	Affinity Biosciences;DF6087