Supplementary Information

$Gd@C_{82}(OH)_{22}$ harnesses inflammatory regeneration for osteogenesis of mesenchymal stem cells through JNK/STAT3 signaling pathway

Jiao Lin,[a,c,#] Rong Cai,[a,c,#] Baoyun Sun,[c,d] Jinquan Dong,[c,d] Yuliang Zhao,[a,c] Qing Miao,[a,b,*] Chunying Chen,[a,c,*]

a. CAS Key Laboratory for Biomedical Effects of Nanomaterials and Nanosafety and CAS Center for Excellence in Nanoscience, National Center for Nanoscience and Technology, Beijing 100190, P. R. China.

b. Division of Paediatric Surgery, Department of Surgery, Children's Research Institute, Medical College of Wisconsin, 8701 W Watertown Plank Rd, Milwaukee, WI 53226, USA.

c. University of Chinese Academy of Sciences, Beijing 100049, P. R. China.

d. CAS Key Laboratory for Biomedical Effects of Nanomaterials and Nanosafety and Key Laboratory for Nuclear Techniques, Institute of High Energy Physics, Beijing 100049, P. R. China.

# Jiao Lin and Rong Cai contributed equally to this work.

*Correspondence authors:

Prof. Chunying Chen

National Center for Nanoscience and Technology, Chinese Academy of Science

No.11, Beiyitiao Zhongguancun

Beijing 100190, China

Email: chenchy@nanoctr.cn

Phone: +86 10 8254 5560

Fax: +86-10-62656765

Prof. Qing Miao,

Division of Paediatric Surgery, Department of Surgery, Children's Research Institute, Medical College of Wisconsin, 8701 W Watertown Plank Rd, Milwaukee, WI 53226, USA

Email: miaoq@nanoctr.cn

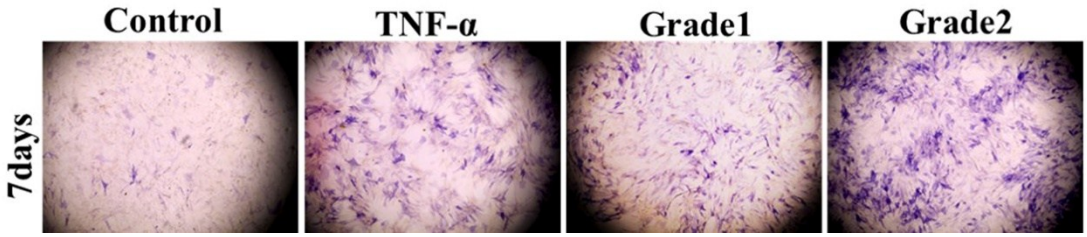

Figure S1. The effects of TNF-α, Grade 1 and Grade 2 on osteogenesis of hMSCs. Alkaline phosphatase (ALP) staining was performed, after hMSCs were induced to osteoblasts with the treatment of 10 ng/ml TNF-α, Grade 1 and Grade 2 for 7 days. Inflammatory microenvironment, Grade 1 and Grade 2.

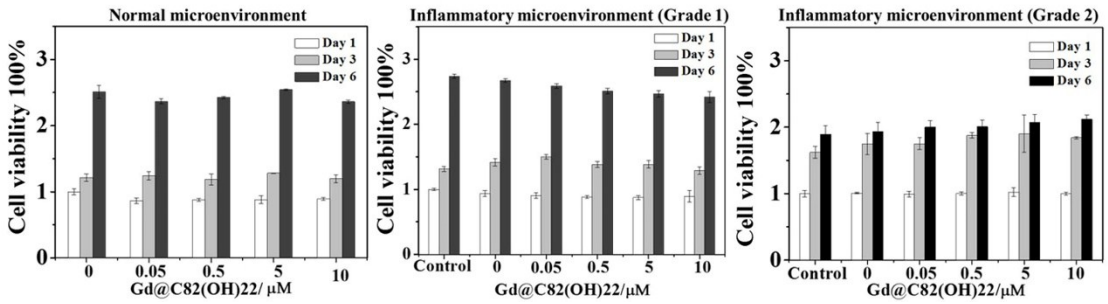

Figure S2. $Gd@C_{82(}OH)_{22}$ have no cytotoxicity of hMSCs in normal

microenvironment (growth medium) and inflammatory microenvironment (Grade 1, Grade 2). After treating hMSCs with 0.05, 0.5, 5, 10 μM Gd@$C_{82}(OH)_{22}$ for 1, 3, 6 days, CCK-8 kits were used to measure the cell viability of hMSCs. Experiments were performed in triplicates. The data was presented as the mean ± standard derivation.

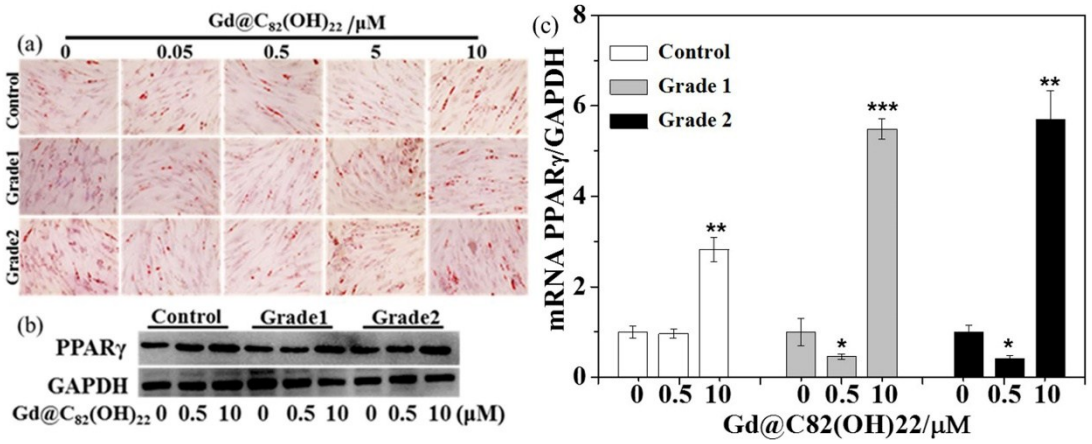

Figure S3. The effect of Gd@$C_{82}(OH)_{22}$ on adipogenesis of hMSCs in Control (adipogenic induction medium, AIM) and inflammatory microenvironment (dilute CM with AIM 50 (Grade 1) and 20 (Grade 2) folds). **(a)** hMSCs were induced to adipocytes with treatment of 0, 0.05, 0.5, 5 and 10 μM Gd@$C_{82}(OH)_{22}$ for 10 days, Oil droplets were stained (first row: AIM; second row: Grade 1; third row: Grade 2). **(b)** PPARγ expression was measured by western blotting after adipogenic differentiation 5 days in different microenvironment. **(c)** PPARγ expression was measured by RT-PCR after hMSCs were induced to adipocytes with treatment of 0, 0.5, 10 μM Gd@$C_{82}(OH)_{22}$ for 5 days. *P<0.05; **P<0.01; ***P<0.001.

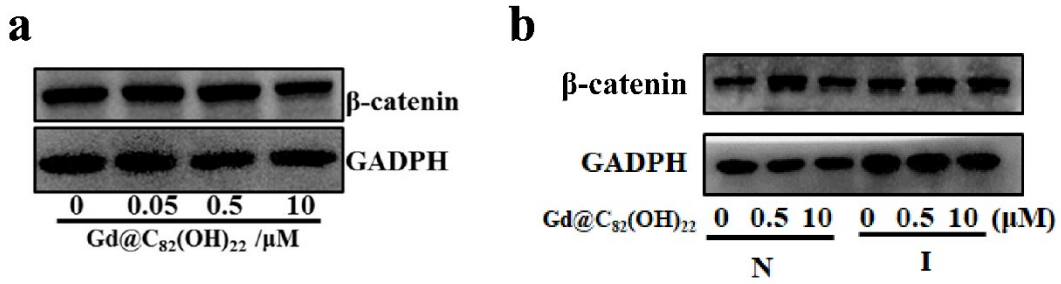

Figure S4. $Gd@C_{82}(OH)_{22}$ regulates osteogenesis by the β-catenin-independent signaling pathway. (a) After 0.05, 0.5 and 10 μM $Gd@C_{82}(OH)_{22}$ treatment in normal microenvironment, the expression of β-catenin of hMSCs were analyzed by western blotting. (b) The role of inflammatory cytokines on the β-catenin expression of hMSCs. N: Normal microenvironment; I: inflammatory microenvironment.

Table S1. Concentration of main inflammatory factors in inflammatory microenvironment

	Inflammatory microenvironment(pg/ml)	
Inflammatory factors	Grade 1	Grade 2
IL-6	1.3±0.1	3.2±0.2
TNF-α	1.1±0.1	2.6±0.1
IL-1β	1.1±0.1	2.8±0.1
IL-10	0.3±0.1	0.9±0.1

Table S2. Primer of specific osteogenic and adipogenic gene

Gene	Upstream primer	Downstream primer
GAPDH	5'-CCTGCACCACCAACTGCTTA-3'	5'- GGCCATCCACAGTCTTCTGAG-3'
PPARγ	5'-GCGAGGGCGATCTTGACAGG-3'	5'- TGATCACCTGCAGTAGCTGCAC-3'
OCN	5'-GGCTCACCCTCCATCACTC-3'	5'- TCCAGCACTGTTTATACCCTCT-3'

OPN	5’-CAGATGCTGTGGCCACATGG-3’	5’- GGTGAGACTCATCAGACTGGTGAG-3’