

Supporting Information for

Dopamine-melanin nanoparticles scavenge reactive oxygen and nitrogen species and activate autophagy for osteoarthritis therapy

Gang Zhong,^a Xueyuan Yang,^b Xianfang Jiang,^c Anil Kumar,^b Huiping Long,^d Jin Xie^{b,}, Li Zheng^{a,*}, Jinmin Zhao^a*

^a Department of Orthopaedics Trauma and Hand Surgery, The First Affiliated Hospital of Guangxi Medical University, Nanning, 530021, China

^b Department of Chemistry, University of Georgia, Athens, Georgia 30602, USA.

^c The College of Stomatology, Guangxi Medical University, Nanning, 530021, China

^d Department of Neurology, Second Affiliated Hospital of Guangxi Medical University, Nanning, 530007, China

* Corresponding author: zhengli224@163.com, jinxie@uga.edu

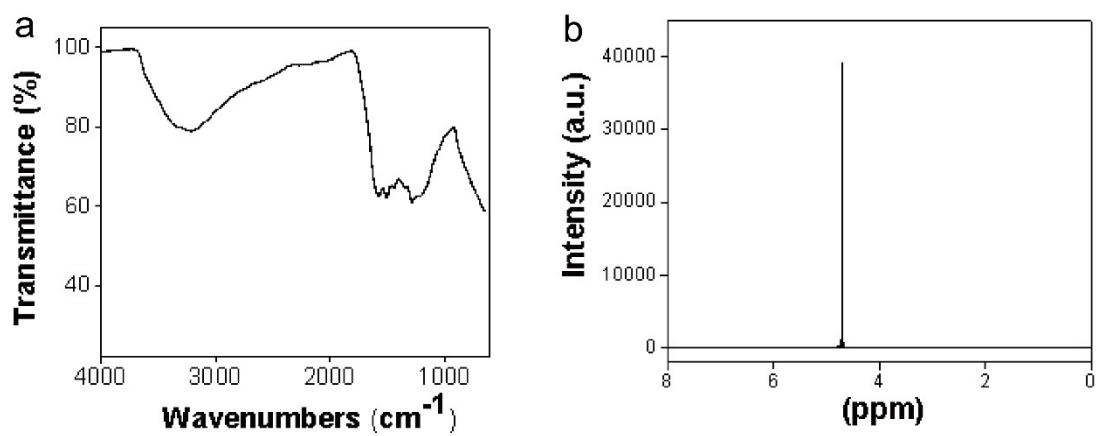


Figure S1. (a) FT-IR spectrum of DM nanoparticles. The peak at $\sim 3200 \text{ cm}^{-1}$ corresponds to the stretching vibrations of -OH and N-H. The peaks at 1574 cm^{-1} and 1281 cm^{-1} were attributed to the C=O and C-O bonds, respectively. The peak at 1442 cm^{-1} corresponds to C=C. (b) ^1H NMR spectrum of DM nanoparticles. The peak at $\sim 4.7 \text{ ppm}$ in the ^1H NMR spectrum is attributed to the D_2O solution.

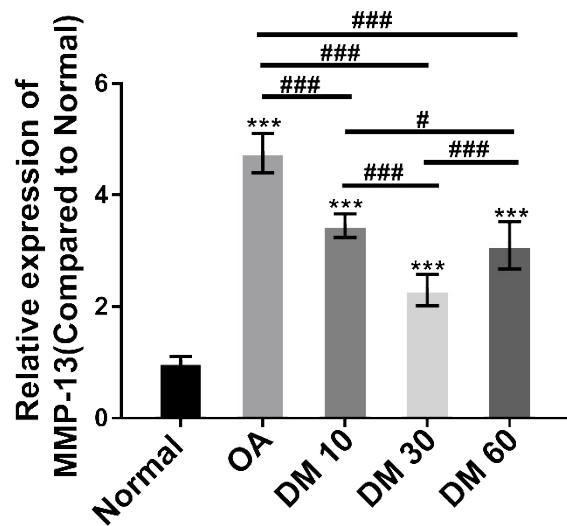


Figure S2. Quantitative analysis immunohistochemical staining of MMP-13 in rat cartilage tissue by Image J. Values are presented as means \pm SD, n=6. * indicates $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$, relative to the normal group. #, indicate, $P < 0.05$; ##, $P < 0.01$; ###, $P < 0.001$, relative to the OA group.

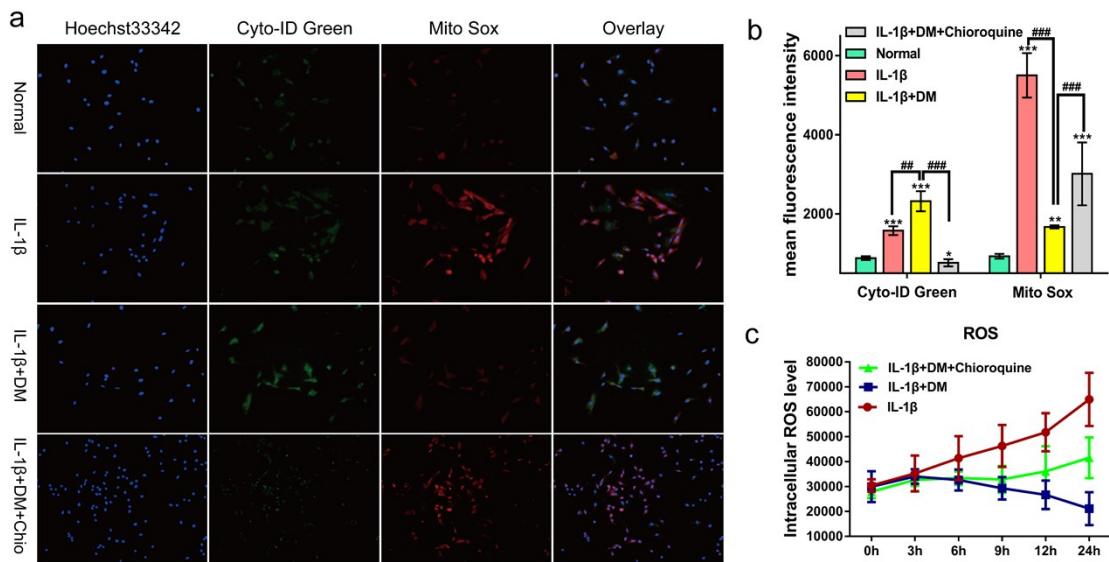


Figure S3. Effect of autophagy on free radical scavenging of DM nanoparticles. (a) Chondrocyte samples concurrently stained with Cyto-ID Green dye and MitoSox Red dye were analyzed by confocal microscopy. (b) The fluorescence intensity of chondrocytes was analyzed by Image J. (c) After treated 30 μ g/mL DM Nanoparticles and/or Chloroquine for 0, 3, 6, 9, 12 and 24 h, the chondrocytes were stained with DCFH for 30 minutes, and the intracellular ROS levels were analyzed on a fluorescence microplate reader. IL-1 β (with 10ng/mL IL-1 β); IL-1 β +DM (with 10ng/mL IL-1 β and 30 μ g/ml DM nanoparticles); IL-1 β +DM+Chloroquine (with 10 ng/mL IL-1 β , 30 μ g/ml DM nanoparticles and 1 μ mol/L Chloroquine). Values are presented as means \pm SD. * indicates $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$, relative to the normal group. #, $P < 0.05$, ##, $P < 0.01$, ###, $P < 0.001$, relative to the IL-1 β +DM group.

Table 1. Primer sequences used in qRT-PCR experiments

Gene name	Forward primer	Reverse primer
GAPDH	5'-AGGGCCCTGACAACCTTTT-3'	5'-AGGGTCTACATGGCAACTG-3'
IL-1 β	5'-TGAGCTGCCAGTGAAATGA-3'	5'-CATGCCACAACAACTGACG-3'
MMP-13	5'-GCCATTACCAGTCTCCGAGG-3'	5'-TACGGTTGGGAAGTTCTGGC-3'
TNF-a	5'-TCAGAGGGCCTGTACCTCAT-3'	5'-GGAGGTTGACCTTGGTCTGG-3'
IL-6	5'-TCTGCGCAGCTTAAGGAGT-3'	5'-CCCAGTGGACAGGTTCTGA-3'
COX-2	5'-ACACTCTATCACTGGCATCC-3'	5'-GAAGGGACACCCTTCACAT-3'
iNOS	5'-GTGTTCCACCAGGAGATGTTG-3'	5'-CTCCTGCCCACTGAGTTCCGTT-3'
LC3	5'-GACGTCACCGGGCGAGTTA-3'	5'-GCTGTACCTCCTTACAGCGG-3'
Beclin-1	5'-TCCGGGCTCCCGAGG-3'	5'-GGGGATGAATCTGCGAGAG-3'
ATG7	5'-TGGTTACAAGCTTGGCTGCT-3'	5'-TCAAGAACCTGGTGAGGCAC-3'