Electronic Supplementary Information (ESI)

Janus Nanozymes-drug Nanosystems for Synergistic Anti-inflammatory Treatment of Nasal Polyps

Ziming Liang,[a] Qian Xiu,[b] Leichao Zhang,[c] Yuzhou Gao,[d] Shengnan Li,[a] Lingyu Zhang,*[a] Xiangjun Chen,[a] Lu Li,*[a] and Chungang Wang*[a]

a Department of Chemistry, Northeast Normal University, 5268 Renmin Street, Changchun, Jilin, P. R. China, 130024

b Department of Otolaryngology-Head and Neck Surgery, China-Japan Union Hospital, Jilin University, Changchun, China

c Department of Pathology, China-Japan Union Hospital of Jilin University, Changchun, Jilin, 130033

d Suzhou Institute of Biomedical Engineering and Technology Chinese Academy of Sciences, No. 88, Keling Road, Suzhou New District, Jiangsu Province, PR China

E-mail: zhangly827@nenu.edu.cn, lil106@nenu.edu.cn, wangcg925@nenu.edu.cn.

Experimental Section

Materials. Tetraethyl orthosilicate (TEOS ≥ 98 %), polyacrylic acid (PAA, Mw ≈ 1800), lipopolysaccharide (LPS) and hydrogen tetrachloroaurate ($HAuCl_4 \cdot H_2O$) were purchased from Sigma (USA). Isopropyl alcohol (IPA), sodium hydroxide (NaOH), cerium nitrate hexahydrate ($Ce(NO_3)_3 \bullet 6H_2O$, 99.95 %) and aqueous ammonia solution were purchased from Sinopharm Chemical Reagent Beijing Co., Ltd. DSS, nitro blue tetrazolium (NBT) and salicylic acid (SA) were purchased from Energy Chemical. Hydrogen peroxide (H_2O_2, 30 %) was purchased from Beijing Chemical Works. 2',7'-dichlorofluorescein diacetate (DCFH-DA) was purchased from

Beyotime Chemical Reagent Co., Ltd.

Characterization. Transmission electron micrographs (TEM) were taken by JEOL-2100F transmission electron microscope under a 200 kV accelerating voltage. UV-Vis spectra were recorded at room temperature on a Japan JASCO V-570 spectrometer fluorescence spectrophotometer. X-ray photoelectron spectra (XPS) were measured by an ECSALAB 250 using non-mono-chromatized Al-Kα radiation. N_2 sorption analysis was performed by an intelligent gravimetric analyzer Autosorb-iQ (Quantachrome). Confocal laser scanning microscopy (CLSM) was observed by means of an Olympus Fluoview FV1000. Flow cytometric (FCM) analysis was performed by BD FACSCanto™ II Flow Cytometry. Inductively coupled plasma atomicemission spectroscopy (ICP-AES) was measured with Leeman ICP-AES Prodigy.

Synthesis of Au-PAA JNPs. First, the monodisperse 60 nm Au NPs were prepared by reducing tetrachloroauric acid with formaldehyde (37 wt %). All the as-synthesized Au NPs were centrifuged and dispersed into 20 mL deionized water. Then, aqueous PAA solution (0.2 g mL^{-1}, 100 μL) and $NH_3 \cdot H_2O$ (2 M, 150 μL) were added and stirred for 0.5 h. After that, 60 mL of IPA was dropped into the mixture to obtain the Au-PAA JNPs under magnetic stirring.

Synthesis of Au-$Ce(OH)_3$ JNPs. 30 mg of Cerium(Ⅲ) nitrate hexahydrate ($Ce(NO_3)_3 \cdot 6H_2O$, dissolved in 500 μL of water) was added to the suspension of as-synthesized Au-PAA JNPs under vigorous magnetic stirring for 12 h to obtain the Au-$Ce(OH)_3$ JNPs.

Synthesis of (Janus Au-$Ce(OH)_3$)@SiO_2 NPs. The Au-$Ce(OH)_3$ JNPs were washed with water twice and then dispersed into the mixed 20 mL of deionized water and 60 mL of IPA. The pH value of suspension was adjusted to 8.5 with $NH_3 \cdot H_2O$ solution. Then, TEOS solution (20 % in IPA, 500 μL) was added with agitation for 12 h at room temperature to obtain (Janus Au-

$Ce(OH)_3$)@SiO_2 NPs. Thereafter, the resulting NPs were collected by centrifugation and washed with deionized water for three times to remove the excess precursors. The NPs were then placed in a vacuum drying chamber for 12 h.

Synthesis of monodisperse Au-CeO_2 JNPs and surface modification. The (Janus Au-$Ce(OH)_3$)@SiO_2 NPs were calcined at 600 °C for 2 h to obtain monodisperse (Janus Au-CeO_2)@SiO_2 NPs. The resulting JNPs were dispersed in water (4 mL) and then etched with aqueous NaOH (2 M, 200 μL) at 50 °C for 24 h. Thereafter, the Au-CeO_2 JNPs were collected by centrifugation and washed with deionized water for several times**.** After washing, 1 mL of PEG ($M_W \approx 5000$, 10 mg mL^{-1} in H_2O) was added and sonicated for 30 min. After that, the mixed solution was stirred overnight and the PEG modified Au-CeO_2 JNPs were collected after washing twice to remove the excess PEG-SH.

Synthesis of CeO_2 NPs. In a 500 mL conical flask, a PAA aqueous solution (0.2 g mL^{-1}, 1 mL) and $NH_3 \cdot H_2O$ (2 M, 1 mL) were added in deionized water (100 mL) and ultrasonically dispersed for 30 min. After that, IPA (400 mL) was dripped to the flask under magnetic stirring to form a suspension. Subsequently, 350 mg $Ce(NO_3)_3 \cdot 6H_2O$ was added into the suspension under magnetic stirring for 12 h to obtain the $Ce(OH)_3$/PAA-NH_4 NSs. The obtained $Ce(OH)_3$/PAA-NH_4 NSs were centrifuged and washed several times by deionized water and anhydrous ethanol and finally dried at 50 °C for 24 h. Lastly, sample obtained in the previous step were calcined at 600 °C for 2 h to obtain CeO_2 NPs.

Scavenging of $O_2^{\cdot -}$. $O_2^{\cdot -}$ scavenging efficiency of Au-CeO_2 JNPs was assayed by measuring the inhibition ratio of the photoreduction of nitro blue tetrazolium (NBT). The Solutions containing riboflavin (20 μM), methionine (13 mM), NBT (75 μM), and Au-CeO_2 JNPs (12.5 μg/mL or 25

μg/mL) were prepared in PBS (pH=7.4). CeO_2 NPs (12.5 μg/mL), Au NPs (5.3 μg mL^{-1}), CeO_2 NPs (7.2 μg mL^{-1}), and cocktail (mixture of Au NPs and CeO_2 NPs) were used as compared group. The compounds were illuminated under ultraviolet radiation for 15 min at 25 ^oC. After irradiation, the absorbance of compounds was detected at once. The mixtures including riboflavin, methionine, and NBT were known as negative control. The mixtures including riboflavin, methionine, and NBT after irradiation were known as positive control. The whole operations were executed in dark.

Scavenging of H_2O_2. The elimination of H_2O_2 was detected by monitoring its characteristic absorbance at 240 nm using UV-visible absorption spectroscopy. The Au-CeO_2 JNPs (12.5 μg/mL or 25 μg/mL), CeO_2 NPs (12.5 μg/mL), Au NPs (5.3 μg mL^{-1}), CeO_2 NPs (7.2 μg mL^{-1}) and cocktail were mixed with H_2O_2, and then the ultraviolet absorption intensity of the mixture was scanned with time. According to the change of ultraviolet absorption intensity, the change of H_2O_2 concentration could be obtained, thereby the removal effect of Au-CeO_2 JNPs on H_2O_2 was acquired.

Scavenging of ·OH. The ·OH was generated through Fenton reaction of 1.8 mM $FeSO_4$ and 5 mM H_2O_2 for 10 min. Salicylic acid (1.8 mM) reacted with ·OH for 30 min to generate 2,3-dihydroxybenzoic acid, which had characteristic absorbance in 510 nm. When treated with Au-CeO_2 JNPs (12.5 μg/mL or 25 μg/mL), CeO_2 NPs (12.5 μg/mL), Au NPs (5.3 μg mL^{-1}), CeO_2 NPs (7.2 μg mL^{-1}) and cocktail, the amount of ·OH scavenged was obtained by measuring the absorbance of 2,3-dihydroxybenzoic acid at 510 nm.

Drug loading and releasing in vitro. For DSS loading, 1 mL of free DSS (2 mg mL^{-1}) was incubated with Au-CeO_2 JNPs (2 mg) in deionized water at room temperature for 24 h. DSS

loaded Au-CeO_2 JNPs were obtained by centrifugation and washed with deionized water twice to remove the DSS adsorbed on the surface. The amount of original DSS and all the supernatants were determined by measuring the absorbance at 282 nm in UV-Vis spectrophotometer. The DSS loading efficiency (LE %) can be calculated by Equation (1):

$$\text{LE (\%)} = \frac{Abs_{(original\ DSS)} - Abs_{(residual\ DSS)}}{Abs_{(original\ DSS)}} \times 100\% \quad (1)$$

Release profile was assessed at 37 ^oC in phosphate buffered saline (PBS, pH= 7.4). The DSS-loaded Au-CeO_2 JNPs in PBS were placed in a centrifuge tube, which was kept releasing in a 37 ^oC bath without other conditions. The supernatant was carefully extracted by centrifuging and resuspended in 1 mL of fresh PBS. DSS concentration in the supernatant was determined by UV-Vis spectrophotometer at the absorbance of 282 nm.

Cytotoxicity study. The cytotoxicity of Au-CeO_2 JNPs on HNECs was carried out by MTT assays. The HNECs were seeded into 96-well plates and incubated in DMEM culture medium containing 10 % fetal bovine serum (FBS) under the atmosphere of 5 % CO_2 at 37 ^oC for 24 h. After the HNECs adhered to the wells, the initial DMEM was substituted by serum-free medium containing Au-CeO_2 JNPs at various concentrations for 24 h. The cell viability was determined by Equation (2):

$$\text{Cell viability} = \frac{Abs_{(test\ cells)}}{Abs_{(control\ cells)}} \times 100\ \% \quad (2)$$

Isolation and culture of HNECs. HNECs were freshly isolated from patients with Chronic rhinosinusitis with nasal polyps (CRSwNP), who had given their written informed consent in accordance with a study protocol approved by the Ethics Committee of China-Japan Union Hospital. Briefly, the nasal polyps was digested using 0.2 % pronase in culture medium at 4 ^oC for

16 hours for dissociation of the mucosal epithelial cells. After digestion, the dissociated cells were washed with PBS, followed by the centrifuge (1000 r, 5 min). The cell pallet was resuspended with culture medium (BEBM supplemented with BEGM SingleQuots) and plated on a 35 mm culture dish at 37 ^{o}C for 0.5 h to remove fibroblasts. Cells were then proliferated for seven to ten days on collagen-coated 3.5 cm^2 dishes (Thermo Scientific, Suzhou, China) in bronchial epithelial cell basal medium (BEGM), and then subjected to fetal bovine serum starvation. Once confluent, cells were trypsinized and re-seeded evenly on 3.5 cm^2 dishes (Thermo Scientific, Suzhou, China) coated with human collagen type IV (Sigma, Saint Louis, USA). When cells reached 80 % confluence, they were resuspended in BEGM and cultured for further usage. The expression levels of CK-18, Epcam and α-SMA were detected by PCR to identify the HNECs. The PCR primer sequences for CK-18, Epcam, α-SMA and β-actin are as follows:

CK-18 Forward	TCAGCAGATTGAGGAGAGCA
CK-18 Reverse	GAGTCCAGGTCGATCTCCAA
Epcam Forward	CTGCCAAATGTTTGGTGATG
Epcam Reverse	GCTCTCATCGCAGTCAGGAT
α-SMA Forward	CGTGTTGCCCCTGAAGAGCAT
α-SMA Reverse	ACCGCCTGGATAGCCACATACA
β-actin Forward	GAGCACAGAGCCTCGCCTTT
β-actin Reverse	ATCCTTCTGACCCATGCCCA

Intracellular ROS scavenging. The level of intracellular ROS was detected by using DCFH-DA dye. DCFH-DA is a small organic molecule without a fluorescent signal that can be taken up into the cytosol by cells and subsequently hydrolyzed by intracellular esterases to produce DCFH.

DCFH can react with intracellular ROS to produce DCF with fluorescence signal (excitation wavelength at 488 nm; emission wavelength at 520 nm), and the intracellular ROS level can be obtained by detecting the fluorescence intensity of DCF. The specific process was as follows: HNECs were incubated in a 24-well plate for 24 h and then added LPS (1 μg/mL, 500 μL) to increase intracellular ROS level. After 30 min, the cells were washed for three times with DMEM and then cultured with CeO_2 NPs, Au-CeO_2 JNPs, DSS-loaded Au-CeO_2 JNPs (12.5 μg/mL), Au-CeO_2 JNPs (25 μg/mL) or free DSS for one hour. Then, the cells were washed for three times with DMEM and added DCFH-DA (0.01 mM, 500 μL), which was dissolved in serum-free DMEM, and after incubation for 30 min, the medium was washed off. Cell nucleus was dyed with Hoechst for confocal laser scan imaging. For the quantitative results, HNECs were monitored with flow cytometry by detecting the mean fluorescence intensity (MFI) of DCFH-DA fluorescence. Moreover, the ROS scavenging ability of Au-CeO_2 JNPs was also measured in Hela cells. The process of experiment was similar to that in the treatment of HNECs. Additionally, the fluorescence intensity was quantitatively measured by ImageJ software.

Detection of SOD and MDA. To determine the change in oxidative stress as showed by the SOD activity (U/mL) and MDA content (nmol/mL) after LPS and Au-CeO_2 JNPs treatment, the supernatant was collected and tested by the SOD and MDA kits.

Reverse transcription-polymerase chain reaction (RT-PCR). HNECs were incubated in a 6-well plate. After 24 h, the medium was removed, and the cells were cultured with LPS dissolved in culture medium. After 24 h, 12.5 μg/mL of CeO_2 NPs, 12.5 μg/mL of Au-CeO_2 JNPs, free DSS and 12.5 μg/mL of DSS-loaded Au-CeO_2 JNPs were added for another 6 hours, respectively. Then, a RNA aqueous kit was used to isolate RNA from HNECs. For single-stranded cDNA synthesis, 1

μg (for cell lines) of total RNA was reverse transcribed using the RT-Systems supplied by Promega (Promega Corporation, Madison, USA). Quantitative real-time RT-PCR was carried out on a Master Cycler (Eppendorf). The PCR of cytokines IL-1β and IL-6 were performed by using the SYBR Premix Ex Taq kit (TaKaRa Biotechnology, Dalian, China) with appropriate primers constructed from published sequences. Relative gene expression was calculated by using the comparative CT method. A 0 h hypoxia or normoxia exposure sample was used as a calibrator. GAPDH was used as a housekeeping gene for normalization, and a no template sample was used as a negative control. The PCR primer sequences are as follows:

IL-1β Forward	ACGAATCTCCGACCACCACT
IL-1β Reverse	GGCAGGGAACCAGCATCTTC
IL-6 Forward	ACACAGACAGCCACTCACCT
IL-6 Reverse	TTCTGCCAGTGCCTCTTTGC

CT imaging of Au-CeO_2 JNPs. The CT images of Au-CeO_2 JNPs samples in PBS with various Au concentrations (0, 0.56, 1.12, 2.34, 5.46 and 8.88 mg mL^{-1}) were obtained by using a SIEMENS SOMATOM Sensation 64 with a tube voltage of 120 kV, an electrical current of 280 mA, and a slice thickness of 1 mm. Phantom images were treated by using a standard image viewer application to measure the mean HU variation of the acquired image depending on the Au concentration.

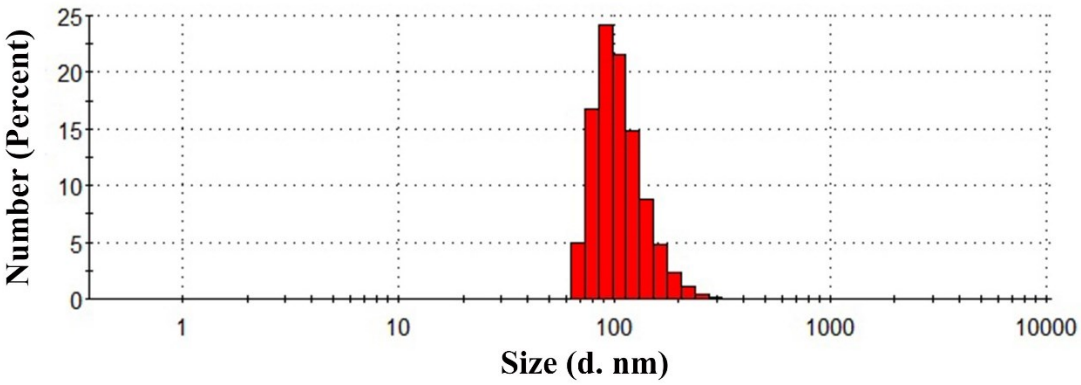

Figure S1. DLS size distribution of the nanoparticles.

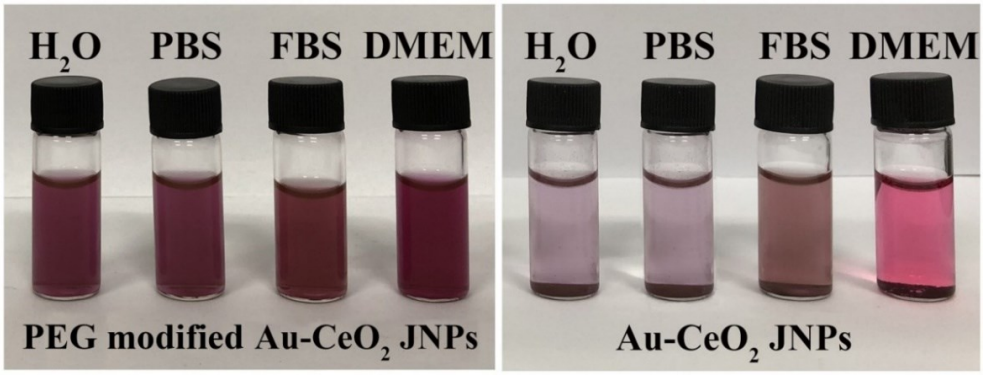

Figure S2. Photographs of Au-CeO_2 JNPs modified with and without PEG in H_2O, PBS, FBS and culture medium (DMEM) for 24 h, respectively.

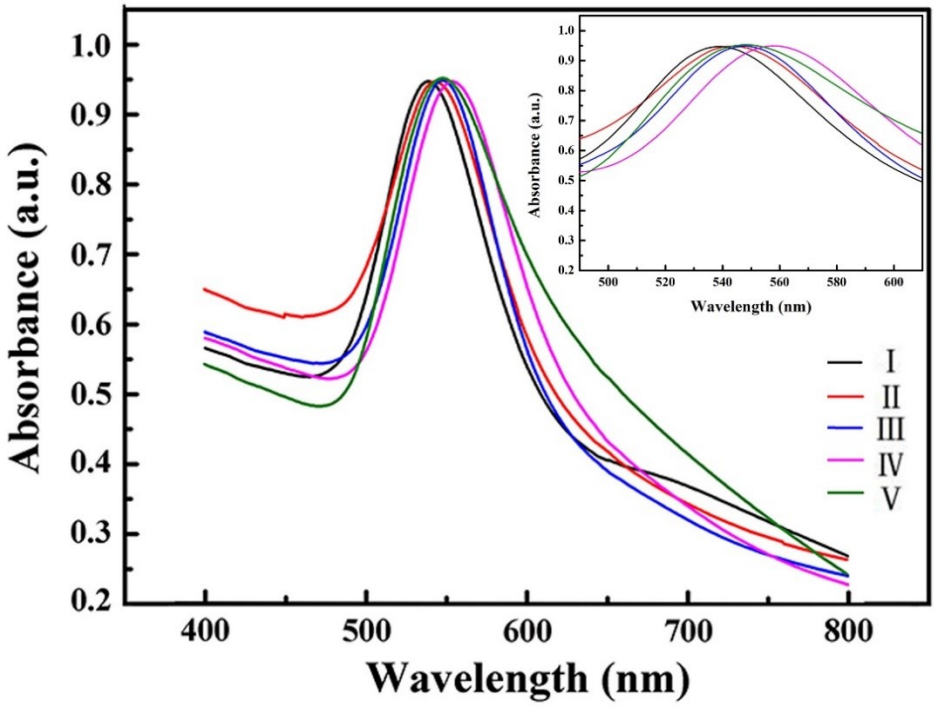

Figure S3. UV-Vis spectra of (I) Au NPs, (II) Au-PAA JNPs, (III) Au-$Ce(OH)_3$ JNPs, (IV) (Janus Au-$Ce(OH)_3$)@SiO_2 NPs and (V) Au-CeO_2 JNPs.

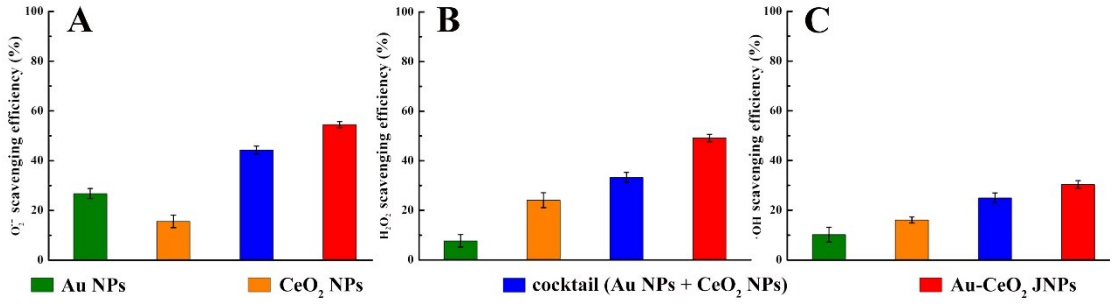

Figure S4. The scavenging efficiency of Au NPs, CeO_2 NPs, cocktail (mixture of Au NPs and CeO_2 NPs), and Au-CeO_2 JNPs to (A) $O_2^{\cdot -}$, (B) H_2O_2, and (C) ·OH.

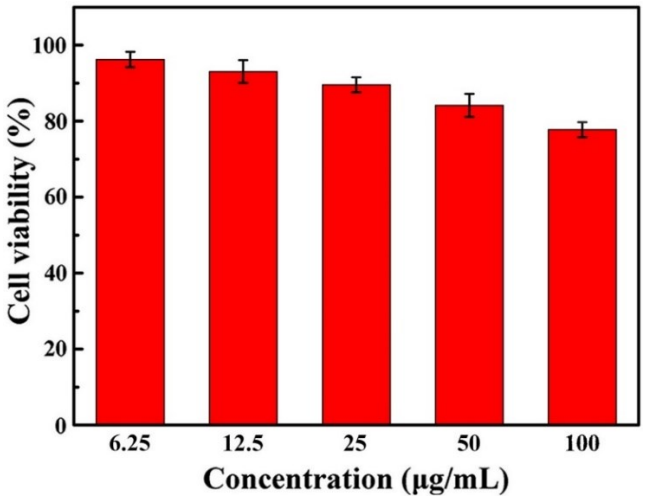

Figure S5. Cytotoxicity of HNECs after incubation with various concentrations of Au-CeO_2 JNPs for 24 h.

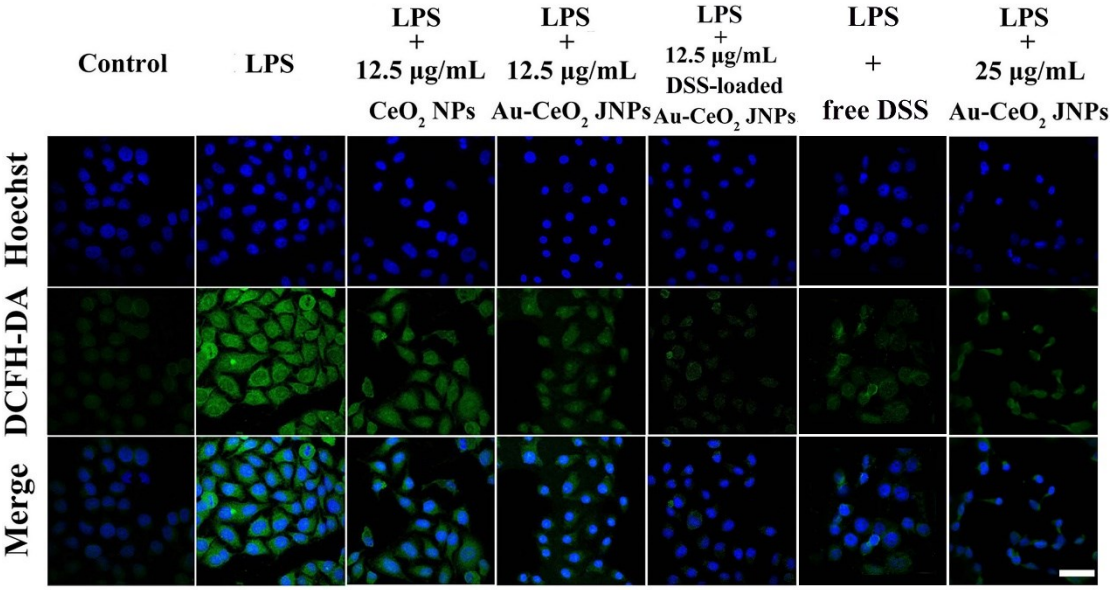

Figure S6. Confocal fluorescence images of ROS levels in LPS-treated Hela cells upon various treatments using DCFH-DA as the ROS probe. Scale bar was equal to 25 μm.

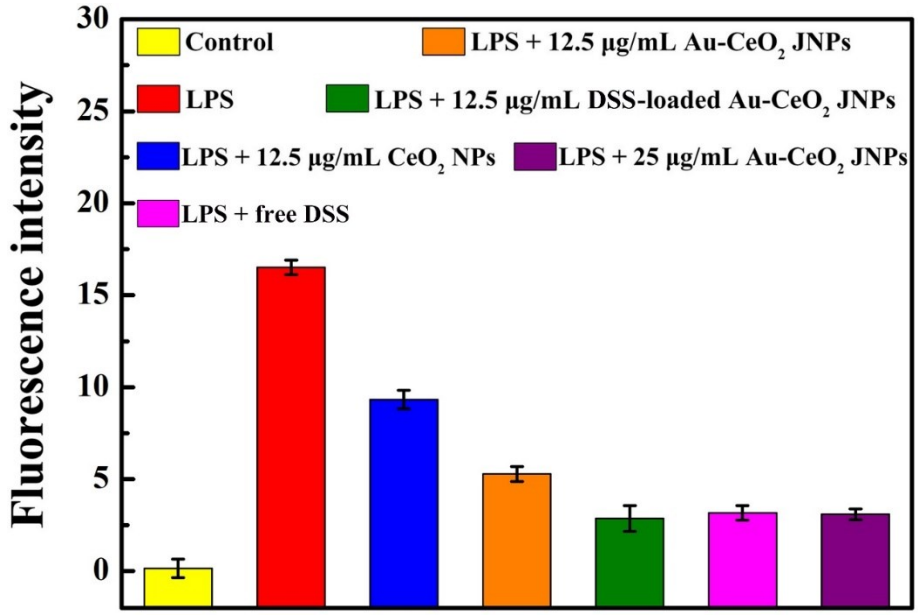

Figure S7. The corresponding quantitative information for confocal fluorescence images of ROS levels in LPS-treated HNECs.

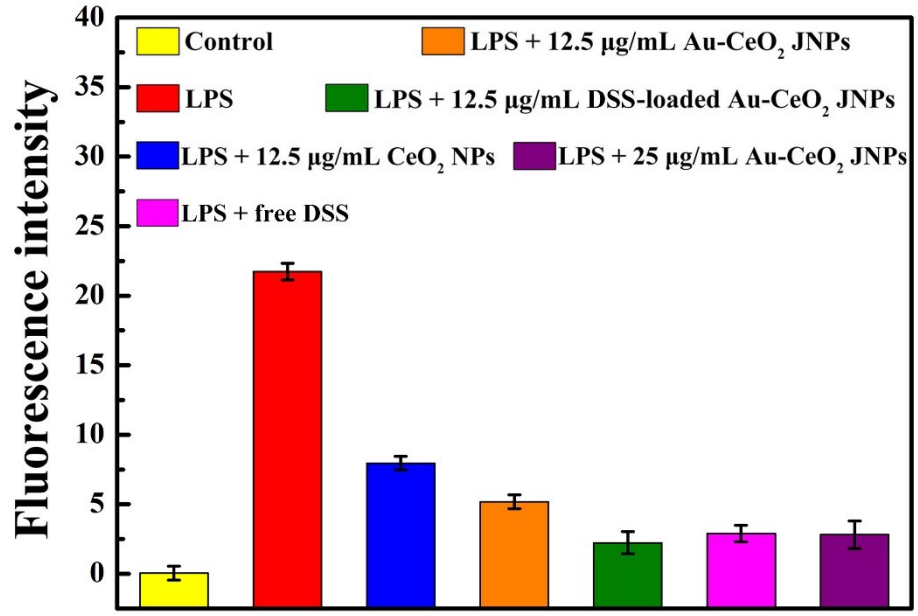

Figure S8. The corresponding quantitative information for confocal fluorescence images of ROS levels in LPS-treated Hela cells.