Supporting Information

Synthesis temperature-regulated multi-enzyme-mimicking activities of ceria nanozymes

Xiaoli Liu[#a], Jiangjiexing Wu[#*b], Quanyi Liu[c, d], Anqi Lin[b], Sirong Li[b], Yihong Zhang[b], Quan Wang[b], Tong Li[b], Xueying An[e, f], Zijun Zhou[b], Ming Yang[*a, g], Hui Wei[*b, h]

# These authors contributed equally to this work.

a. School of Pharmacy, State Key Laboratory of Southwestern Chinese Medicine Resources, Chengdu University of Traditional Chinese Medicine. Chengdu, Sichuan 611137, China.

b. Department of Biomedical Engineering, College of Engineering and Applied Sciences, Nanjing National Laboratory of Microstructures, Jiangsu Key Laboratory of Artificial Functional Materials, Chemistry and Biomedicine Innovation Center (ChemBIC), Nanjing University. Nanjing, Jiangsu 210023, China.

c. State Key Laboratory of Electroanalytical Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences. Changchun, Jilin 130022, China.

d. University of Science and Technology of China. Hefei, Anhui 230026, China.

e. State Key Laboratory of Pharmaceutical Biotechnology and Jiangsu Key Laboratory of Molecular Medicine, School of Medicine, Nanjing University. Nanjing, Jiangsu 210023, China.

f. Department of Sports Medicine and Adult Reconstructive Surgery, Nanjing Drum Tower Hospital, The Affiliated Hospital of Nanjing University Medical School. Nanjing, Jiangsu 210093, China.

g. Key Laboratory of Modern preparation of Traditional Chinese Medicine, Ministry of Education, Jiangxi University of Traditional Chinese Medicine. Nanchang, Jiangxi 330000, China.

h. State Key Laboratory of Analytical Chemistry for Life Science and State Key Laboratory of Coordination Chemistry, School of Chemistry and Chemical Engineering, Nanjing University. Nanjing, Jiangsu 210023, China.

E-mail: mingyang26@126.com; wujiangjiexing2007@126.com; weihui@nju.edu.cn

Chemicals and materials

Cerium nitrate hexahydrate ($Ce(NO_3)_3 \cdot 6H_2O$), ethylene glycol, ammonia solution, 3,3',5,5'-tetramethylbenzidine dihydrochloride (TMB), para-nitrophenyl phosphate (p-NPP) were obtained from Aladdin. 1,1-diphenyl-2-picrylhydrazyl (DPPH) were obtained from Yuanye bio-technology Co., Ltd. Hydrogen peroxide (H_2O_2, 30%) was supplied by Sinopharm Chemical Reagent Co., Ltd. Glutathione (GSH), glutathione reductase (GR) from baker's yeast (S. cerevisiae), nicotinamide adenine dinucleotide phosphate (NADPH), fetal bovine serum (FBS), and Dulbecco's modified Eagle medium (DMEM) were purchased from Sigma-Aldrich. All chemical reagents were used as received without further purification. All aqueous solutions used in the experiments were prepared with deionized water (18.2 MΩ•cm, Millipore).

Instrumentation

Transmission electron microscope (TEM) images were recorded on a FEI TECNAI F20 TEM (FEI, USA) at an acceleration voltage of 200 kV. The X-ray diffraction (XRD) patterns were obtained with a speed of 2 °/min on a diffractometer (Rigaku Ultima III, Japan) using a Cu Kα radiation. X-ray photoelectron spectroscopy (XPS) was collected using a PHI 5000 VersaProbe (Ulvac-Phi, Japan). Zeta potentials were measured at 25 °C using a Zetasizer Nano ZSP (Malvern, Britain) instrument equipped with a Malvern surface zeta potential cell. All the absorbance data were collected on a Spectra Max M2e microplate reader (Molecular Device, China). UV-visible absorption spectra were recorded on a UV-vis-spectrometer Cary UV-vis 100 (Agilent, USA).

Transmittance

CeO_2 (Ceria_-30, Ceria_0, Ceria_30, Ceria_60, and Ceria_90, 0.025 mL, 2 mg/mL) was treated with H_2O_2 (0.01 mL, 1 M) and then incubated at 37 ℃ for a certain time. The UV-vis spectra of untreated CeO_2, CeO_2 treated with H_2O_2 for 5 min, and CeO_2 treated with H_2O_2 for 7 d were recorded on UV-vis-spectrometer and digital photos.

Glutathione peroxidase (GPx)-mimicking activity of CeO_2

In a typical GPx-mimicking activity measurement, GSH (0.01 mL, 20 mM), NADPH (5 μL, 4 mM), GR (6 μL, 1.7 units), and H_2O_2 (0.005 mL, 8 mM) were sequentially added into 10 mM buffer (pH = 7.4) solution containing CeO_2 nanozymes (Ceria_-30, Ceria_0, Ceria_30, Ceria_60, and Ceria_90). Later, the absorbance at 340 nm of the mixed solution was measured immediately.

Haloperoxidase (HPO)-mimicking activity of CeO_2

The HPO-mimicking activity of ceria nanozymes was determined by oxidized bromination of phenol red solution. The test solution contained ceria nanozymes (50 μg/mL), NH_4Br (25 mM), phenol red (50 μM), H_2O_2 (300 μM), and acetate buffer (200 mM, pH 5.5). After the test solution was mixed 24 h at 37 ℃, the samples were carried out at 590 nm for analysis.

Cell Culture

In the biomedical study, three types of cell lines purchased from Shanghai Institute of Cells, Chinese Academy of Sciences were used. The human bone marrow mesenchymal stem cells (hMSCs) were cultured with F-12 Medium supplemented with 10% FBS and 1% streptomycin/penicillin in atmosphere composed of 5% carbon

dioxide (CO_2) and 95% air at 37 ℃. The MCF-7 cells (breast tumor cells) and C28/I2 cells (human normal chondrocytes) was cultured with high glucose DMEM medium containing 10% FBS and 1% streptomycin/penicillin under air atmosphere supplement with 5% CO_2 at 37 ℃.

Cytotoxicity assay

hMSCs cells were first seeded in 96-well plates with 1×10^4 cells per well and allowed to adhere for 24 h. Then the cells were treated with fresh medium containing ceria nanozymes at different concentrations (12.5, 25, 50, 100, and 200 μg/mL) for another 24 h. At last, each well was washed by phosphate buffer saline for three times. The cell viability was then evaluated by a standard cell counting kit-8 (CCK-8) assay.

Cytoprotection from aging

In a natural aging model, after seeded in 12-well plates at the density of 1×10^5 cells per well for 48 h, hMSCs cells were then treated with fresh medium containing 50 μg/mL Ceria_0 or Ceria_90 nanozymes for 7 days (the same medium was changed after three days.). Afterwards, the original medium was removed and phosphate buffer saline was added to wash each well for three times. The anti-aging effect of ceria nanozymes was subsequently detected through a senescence β-galactosidase staining kit (Cell Signaling Technology, #9860) assay. Meanwhile, the photos were taken and analyzed by optical microscope (EVOS FL Auto 2). The number of aging cells was also counted by ImageJ software with five random photos.

In a D-galactose-induced aging model, the only difference from the natural model was that after seeded in 12-well plates at the density of 1×10^5 cells per well for 48 h,

hMSCs cells were treated with fresh medium containing 10 mg/mL D-galactose (D-gal) and 50 μg/mL ceria nanozymes (Ceria_0 or Ceria_90) for 48 h.

Selective protection for cells

MCF-7 and C28/I2 cells were seeded in 96-well plates with 2×10^4 cells/100 μL in each well for 24 h, then 100 μL fresh medium containing 10 mM H_2O_2, 50 μg/mL ceria nanozymes (Ceria_0, or Ceria_90), and 0.5 mM of 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolium bromide was added into each well and incubated for 24 h. Finally, each well was washed by phosphate buffer saline for three times, and then 200 μL of dimethyl sulfoxide was added to dissolve the resulting crystals. 2 h later, the absorbance of 490 nm was recorded by a microplate reader.

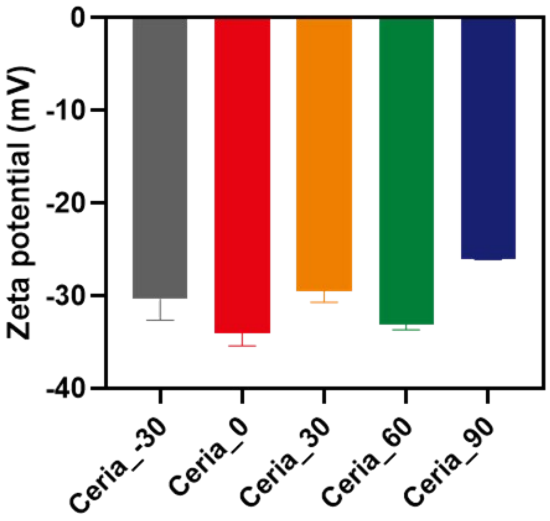


Fig. S1. Zeta potentials of Ceria_-30, Ceria_0, Ceria_30, Ceria_60, and Ceria_90 (n=3).

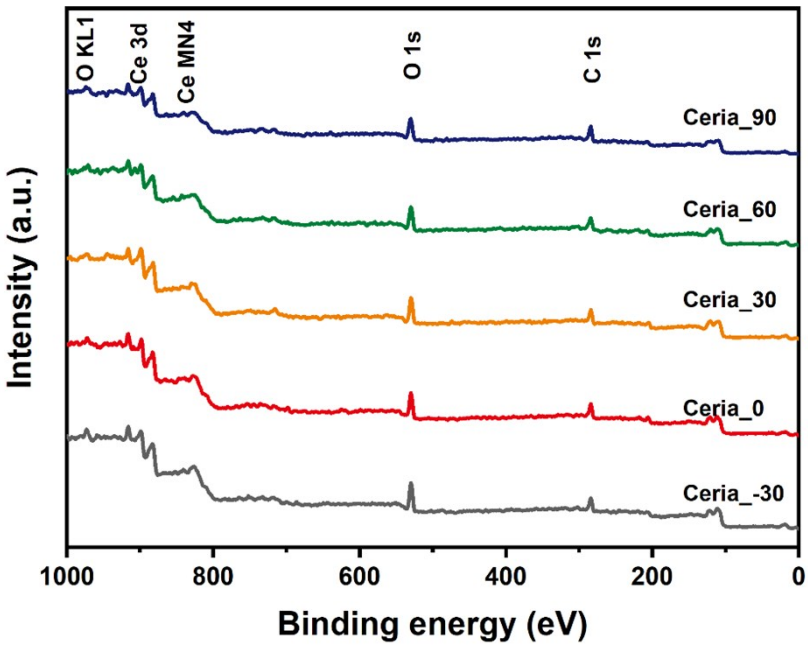


Fig. S2: XPS survey spectra of Ceria_-30, Ceria_0, Ceria_30, Ceria_60, and Ceria_90.

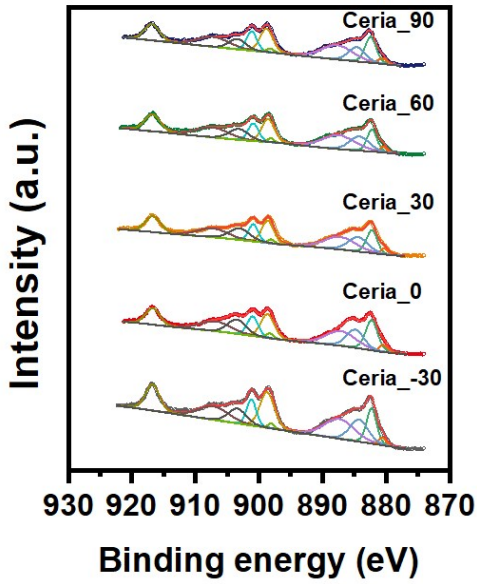


Fig. S3: Ce 3d core level XPS spectra of Ceria_-30, Ceria_0, Ceria_30, Ceria_60, and Ceria_90.

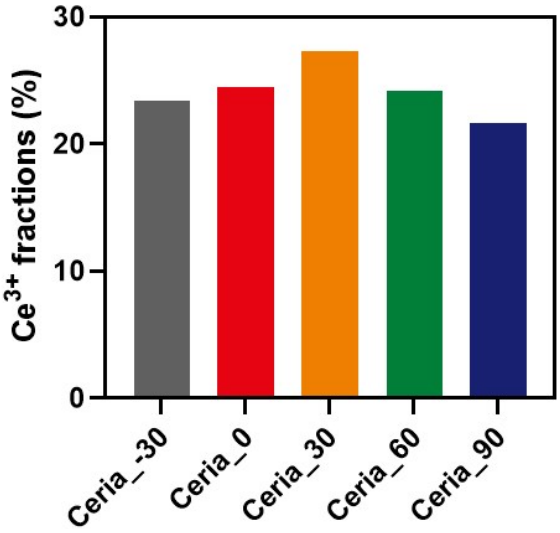


Fig. S4: Ce^{3+} fractions of Ceria_-30, Ceria_0, Ceria_30, Ceria_60, and Ceria_90.

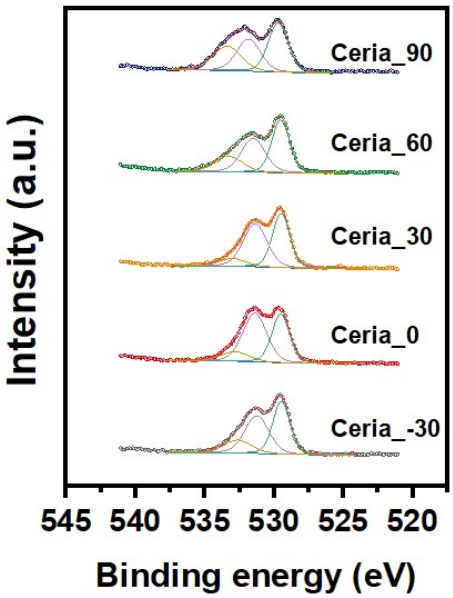


Fig. S5: O 1s core level XPS spectra of Ceria_-30, Ceria_0, Ceria_30, Ceria_60, and Ceria_90.

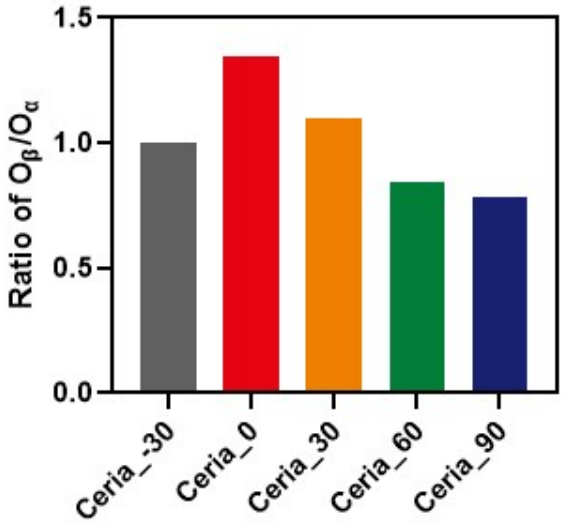
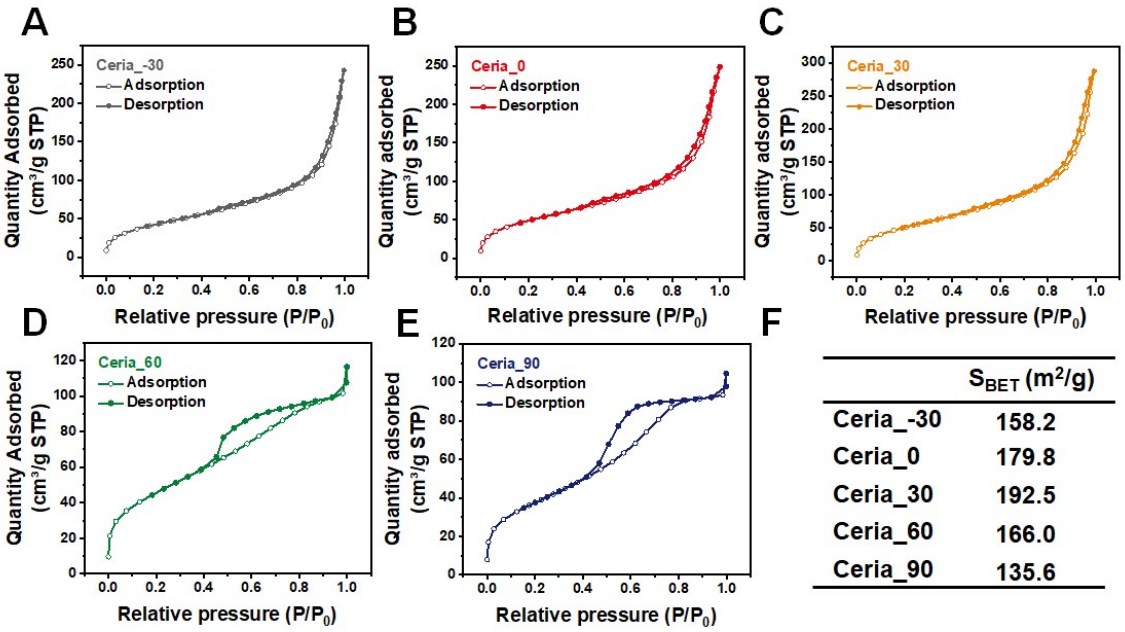


Fig. S6: Ratio of O_β/O_α of Ceria_-30, Ceria_0, Ceria_30, Ceria_60, and Ceria_90 from XPS spectra.



F

	S_{BET} (m^2/g)
Ceria_-30	158.2
Ceria_0	179.8
Ceria_30	192.5
Ceria_60	166.0
Ceria_90	135.6

Fig. S7: BET curves (A-E) and S_{BET} (F) of Ceria_-30, Ceria_0, Ceria_30, Ceria_60, and Ceria_90.

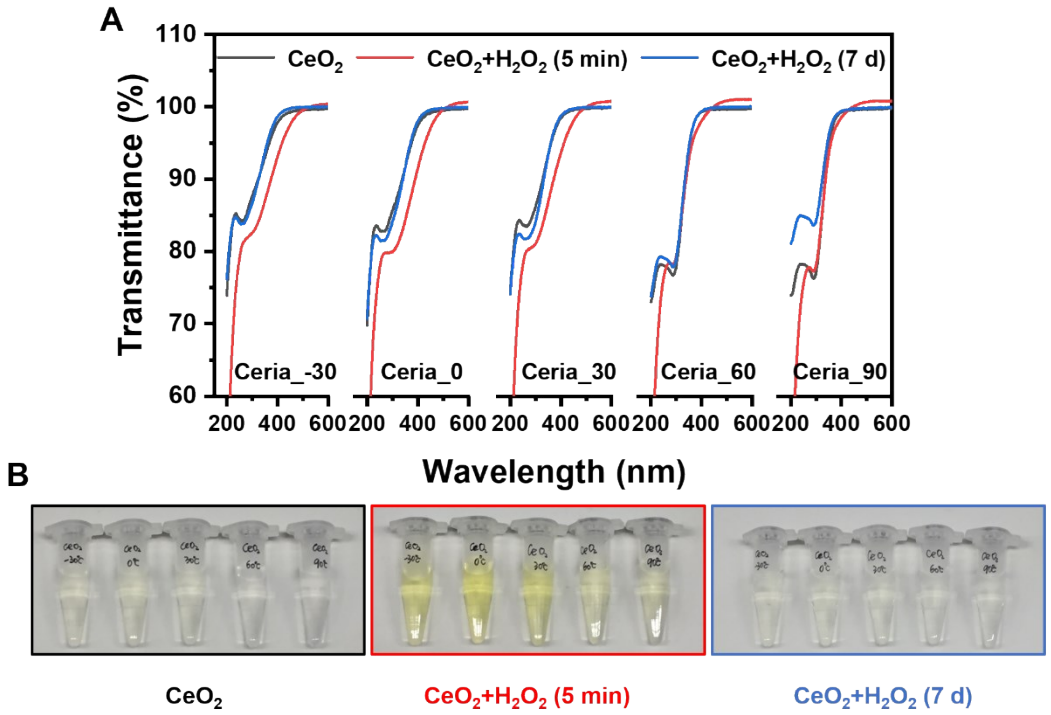


Fig. S8: Autocatalytic activity of CeO_2. (A) Transmittance spectra showing reversible autocatalytic behavior for different CeO_2 upon incubation with H_2O_2 at pH 7.0 (black: control, before addition of H_2O_2; red: 5 min after addition of H_2O_2; blue: 7 days after addition of H_2O_2). (B) Digital photograph of each corresponding assay with different treatments. In each assay, from left to right: Ceria_-30, Ceria_0, Ceria_30, Ceria_60, and Ceria_90.

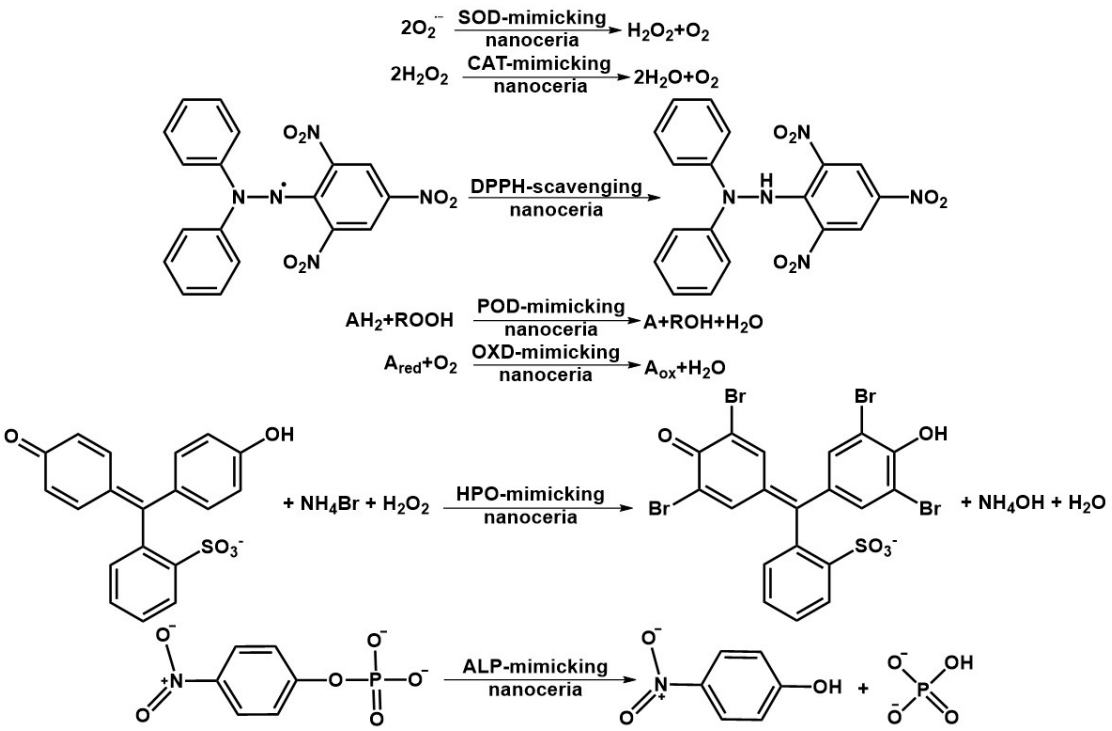


Fig. S9: Scheme of multi-enzyme-mimicking activities catalyzes by ceria nanozymes.

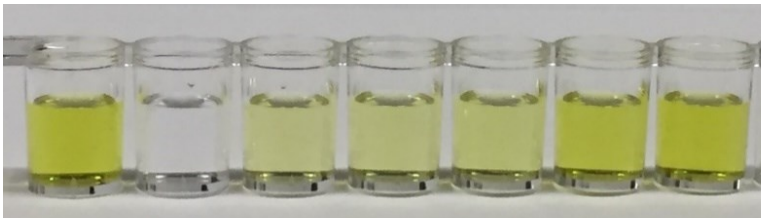


Fig. S10: Digital photograph of the color of each assay with different CeO_2 nanozymes. (WST-1, SOD assay kit)

From left to right: negative control, positive control, Ceria_-30, Ceria_0, Ceria_30, Ceria_60, and Ceria_90.

Fig. S11: Digital photograph of the color of DPPH probes treated with different CeO_2 nanozymes.

From left to right: negative control, Ceria_-30, Ceria_0, Ceria_30, Ceria_60, and Ceria_90.

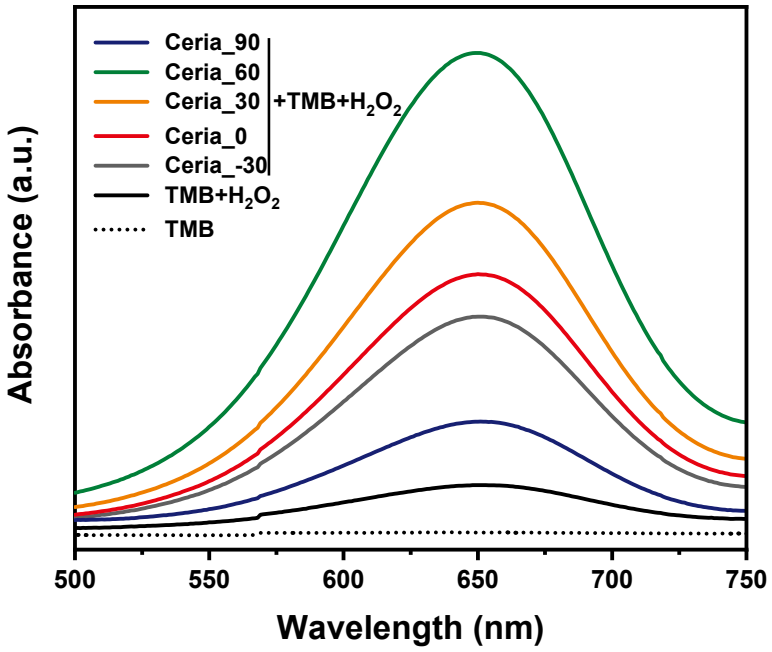


Fig. S12: Visible absorption spectra for the oxidation of TMB catalyzed by different CeO_2 nanozymes with H_2O_2.

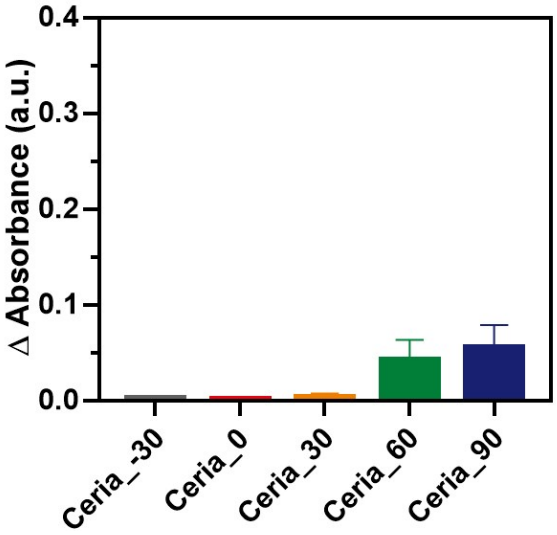


Fig. S13: HPO-mimicking activities of different CeO_2 nanozymes (n=4).

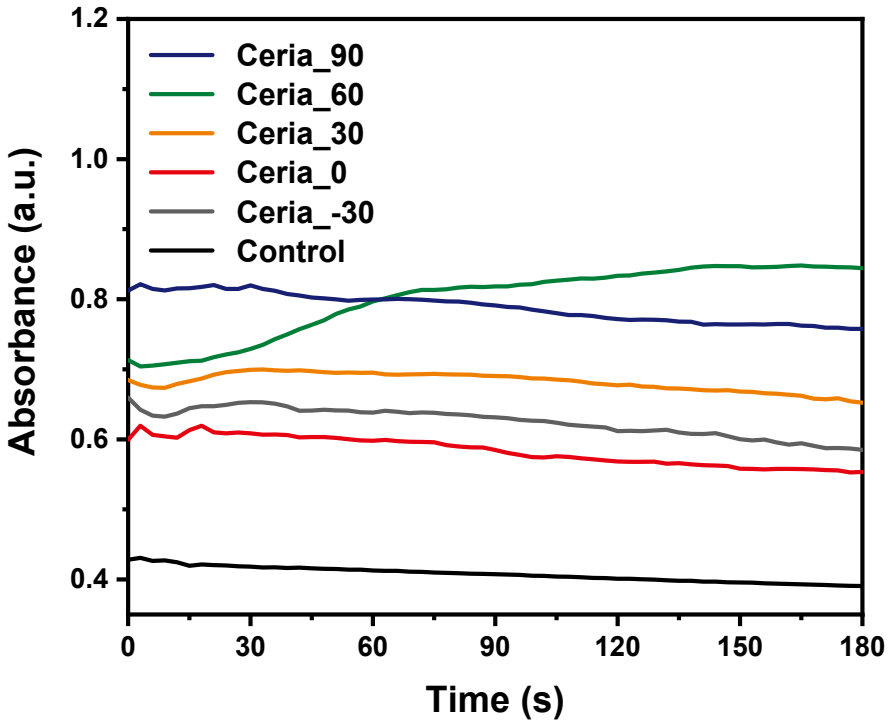


Fig. S14: GPx-mimicking activities of different CeO_2 nanozymes.

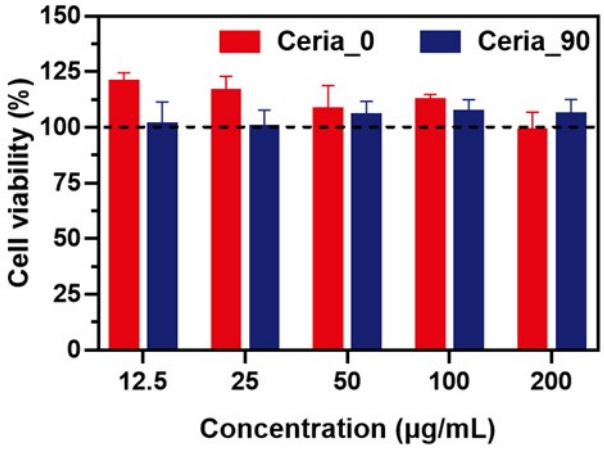


Fig. S15: Cell viability after incubation with different CeO_2 at different concentrations (n=5).

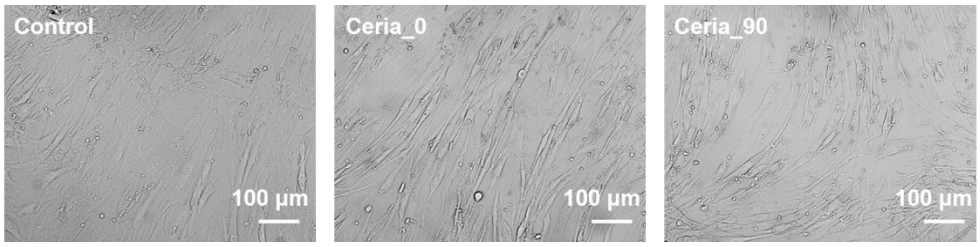


Fig. S16: Photographs of naturally aging cells under different treatments.

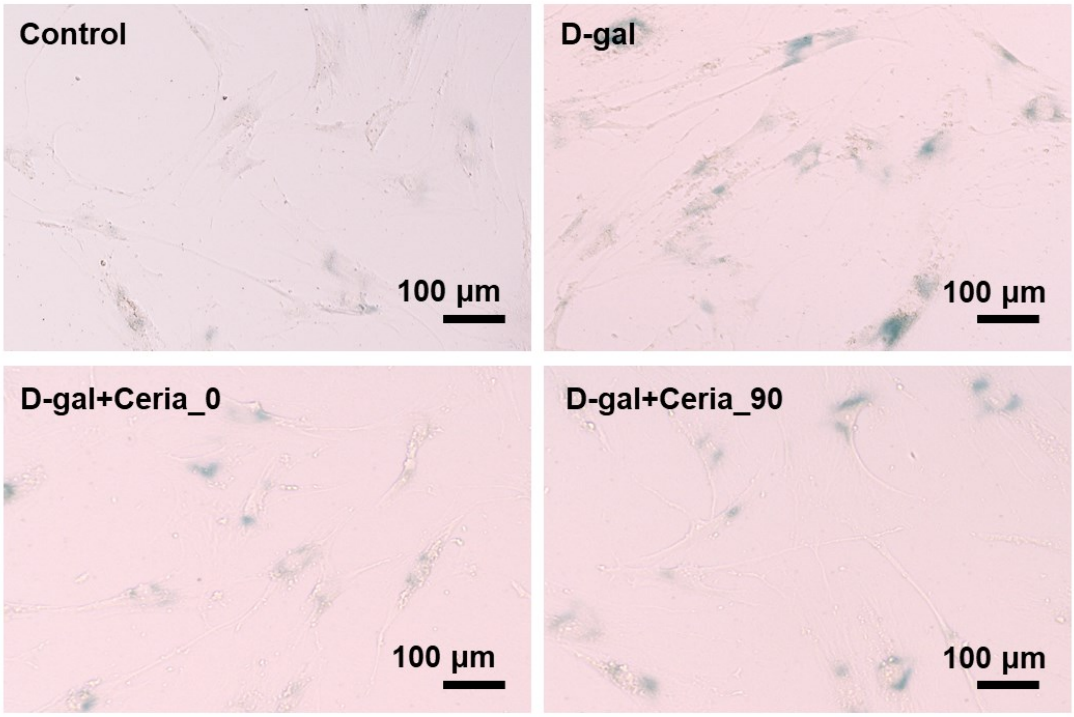


Fig. S17: Photographs of D-gal-induced aging cells under different treatments.

Table S1. Size, zeta potential, Ce^{3+}, Ce^{4+}, and surface oxygen (O_β) of different ceria

	Size (nm)	**Zeta potential (mV)**	**Ce^{3+} %**	**Ce^{4+} %**	**Surface oxygen (O_β) %**
Ceria_-30	2.5	-30.4	23.4	76.6	50.1
Ceria_0	2.6	-34.0	24.5	75.5	57.3
Ceria_30	3.1	-29.5	27.3	72.7	52.3
Ceria_60	3.7	-33.1	24.2	75.8	45.7
Ceria_90	4.2	-26.0	21.7	78.3	43.9