Supporting Information

Methods and materials

Materials

Phosphomolybdic acid hydrates (AR), phosphotungstic acid hydrate (reagent grade), silicomolybdic acid (AR), tungstosilicic acid (AR), and copper nitrate (AR), cesium cabonate were from Sigma Aldrich. Hydrochloric acid (37%) and nitric acid (65%) and ethyl acetate were provided by Merck Pte. All the chemicals above were simply used as received.

Preparation of Cu-POM nanozyme

Taking Cu SAN as an example, a solution of Cs_2CO_3 (0.0375 mol/L) and $Pd(NO_3)_2$ (appropriate amount for the desired weight loading), in 30 mL DI water was added at 1 mL/min to an aqueous solution of tungstosilicic acid (30 mL, 0.025 mol/L) under ice cooling and stirring (1200 rpm) after which a colloidal solution of precipitated formed. Under ice cooling and stirring (1200 rpm), the solution was aged for 5 h and then centrifuged (4 min, 12,200 × g) to obtain a light-blue solid. After washing the solid three times with water, the product was dried. Water was removed by freeze-drying and the catalyst was used without further washing steps. For other Cu-polyoxometalates, the molar amounts of polyoxometalate were kept the same.

Catalyst Characterization

Electrospray ionization time-of-flight mass (ESI-TOF-MS) spectra were obtained from a Bruker MicroTOF-Q system. The samples were directly injected into the chamber at 20 μL·min-1. Typical instrument parameters: capillary voltage, 4 kV; nebulizer, 0.4 bars; dry gas, 2 L·min-1 at 120 °C; *m/z* range, 500 – 3000.

Metal content in the catalyst was determined by iCAP 6000 series inductively coupled plasma optical emission spectrometry (ICP-OES). The catalysts were digested in aqua regia (HCl/HNO_3 = 3:1) at 353 K for 4 h and then diluted with deionized water to a certain volume before analysis. Transmission electron microscopy (TEM) was performed on a JEM 2100F (JEOL, Japan) microscope operated at 200 kV. Size and Zeta potential characterization were measured on Zetaszier Nano-ZS90.

X-ray photoelectron spectra (XPS) were recorded on a VG Escalab MKII spectrometer, using a mono Al Kα X-ray source (hν = 1486.71 eV, 5 mA, 15 kV), and the calibration was done by setting the C1s peak at 284.5 eV. Attenuated total reflectance infrared (ATR-IR) spectroscopy analysis was carried out on a Thermo Scitific Nicolet iS50 FT-IR spectrometer integrated with a diamond ATR accessory. The IR spectra were collected in the spectral range 4000–500 cm^{-1} with a resolution of 4 cm^{-1} and scan time of 32. Cu K-edge XAS analyses were performed with Si(111) crystal monochromators at the BL14W Beam line at the Shanghai Synchrotron Radiation Facility (Shanghai, China). Before the analysis at the beamline, samples were placed into aluminum sample holders and sealed using Kapton tape film. The XAFS spectra were recorded at room temperature using a 4-channel Silicon Drift Detector (SDD) Bruker 5040. Cu K-edge extended X-ray absorption fine structure (EXAFS) spectra were recorded in transmission/fluorescence mode. Negligible changes in the line-shape and peak position of Cu K-edge XANES spectra were observed between two scans taken for a specific sample. The XAS spectra of these standard samples were recorded in transmission mode. The spectra were processed and analyzed by the software codes Athena.

Catalytic activity

SOD activity was determined using a Superoxide Dismutase Assay Kit (S311-10, Dojindo Molecular Technologies), and CAT activity assays of Cu SAN were detected by measuring the oxygen concentration produced. STA and H_2O_2 (different concentrations) were added to 10 mL of PBS, and the oxygen concentration was recorded every 15 s and continuously for 10 min using the dissolved oxygen meter.

Cell Culture

The House Ear Institute-Organ of Corti 1(HEI-OC1) cells were cultivated in high-glucose Dulbecco's Modified Eagle Medium (DMEM), enriched with 10% Fetal Bovine Serum (FBS; Gibco, Cat. No. 10099-141) and ampicillin (1000x concentration; Beyotime, Cat. No. ST008), at a temperature of 37 °C and maintained under a 5% CO2 atmosphere. Different concentrations (0.5, 1, 2, 5, 10, 25, 50, 100 μg/mL) of Cu SAN were dissolved in the DMEM. For cisplatin toxicity test, HEI-OC1 cells were treated with cisplatin (B24464; Yuanye) at 25 μM for 24 h. In the following comparative cell experiments, CTR represented untreated DMEM, CIS represented DMEM with 25

μM cisplatin, Cu SAN represented DMEM contained 2μg/mL Cu SAN. Cu SAN+CIS represented DMEM with 2μg/mL Cu SAN and 25 μM cisplatin.

Organ Culture of Cochlear Explant

On postnatal day 3, cochlea explants from C57BL/6 mice were dissected in PBS and adhered to coverslips coated with cell-Tak (354240; Corning). Subsequently, the explants were cultured in DMEM/F12 (11320033; Gibco) supplemented with 1% N2 (17502-048; Gibco), 2% B27 (17504-044; Gibco), and ampicillin, at 37°C with 5% CO2. For cisplatin toxicity test, the explants were exposed to 25 μM cisplatin for 24 h. In the following comparative tissue experiments, CTR represented untreated DMEM/F12, CIS represented DMEM/F12 with 25 μM cisplatin, Cu SAN represented DMEM/F12 contained 5μg/mL CU. Cu SAN+CIS represented DMEM/F12 with 5μg/mL Cu SAN and 25 μM cisplatin.

Animals

Male C57BL/6 mice (6~8 weeks) were used in the following animal experiments. The mice in the CIS group underwent once-daily intraperitoneal injections of cisplatin at a dosage of 4 mg/kg for four consecutive days, followed by a 10-day recovery period, spanning a total duration of 42 days. Mice in the CTR group received intraperitoneal injections of normal saline. Mice in the Cu SAN+CIS group received intraperitoneal injections of Cu SAN (0.1mg/ml), starting with the injection of cisplatin. Animal experiments and research protocols were in accordance with the Nanjing University Institutional Animal Care and Use Committee (reference number: 2024AE01021). All animal experiments were performed according to the protocols approved by the Animal Ethics and Use Committee.

In Vitro Cell Experiments

The biocompatibility of biomaterials was assessed using the CCK8 assay and calcein-AM/PI staining. Cells were seeded in 96-well plates at a density of 1×10^4 cells per well. After 24 hours of incubation, the cells were subjected to various treatments for an additional 24 hours. Subsequently, CCK-8 reagents were diluted in DMEM (1:10) and added to the wells. After a further 1-hour incubation, the absorbance at a wavelength of 450 nm was measured to determine the results. In

order to obtain a more direct and microscopic assessment of cytotoxicity, the live/dead (calcein-AM/PI) double stain kit was employed. Cells were cultured and treated in the same manner as described above. The kit was then used according to the protocol, and fluorescent images of live (green) and dead (red) cells were observed. Each experiment was performed with five replicates.

Antioxidant Efficiency of Cu SAN

The DCFH-DA (S0033S, Beyotime) was applied to measure the ROS levels in cells. HEI-OC1 were treated with DCFH-DA in the medium for 0.5 h in the cell incubator. After PBS washing three times, the images were visualized by a Leica THUNDER microscope. The cells were analyzed on a BD Accuri C6 flow cytometer analyzer to quantify the results, with FlowJo X Software to deal with the data. Total Superoxide Dismutase Assay Kit (S0101S, Beyotime) and Catalase Assay Kit (KGA7312-100, KeyGEN) were used to detect the level of SOD and CAT according to the instructions respectively.

Measurement of Cu^+ Concentration

All the cell and tissue homogenates supernatant liquids were collected for Copper (Cu) Colorimetric Assay Kit (E-BC-K300-M, E-BC-K775-M, Elabscience) tests afterward to acquire the Cu+ concentration. The supernatant liquids were treated with chromogenic agent for 5min at 37 °C and detected at the wavelength of 580 nm.

Immunofluorescence Staining

Cochleae tissue sections and cochlear explants of mice cochleae were fixed with 4% PFA (G1101, Servicebio) for 1 h at room temperature. The samples were permeabilized with 0.1% TritonX-100 for 30 min and blocked with 10% goat serum at RT for 1 h, then incubated with primary antibody rabbit anti-Myosin7a (1:1000, 25-6790, Proteus BioSciences) at 4°C overnight. The samples were incubated with Alexa Fluor555 labeled secondary antibody (1:500, ab150078, abcam) at room temperature for 1 h. The samples were then mounted with a medium containing DAPI (ab104139, Abcam) for visualization using confocal fluorescent (Zeiss, LSM 900).

Western blotting

Cochleae tissue sections were lysed in RIPA lysis solution (FD009, FUDE) containing the protease inhibitor cocktail (FD1001, FUDE) and centrifuged at 12,000 rpm for 5 minutes at 4°C. The samples were separated in 12% sodium dodecyl sulfate–polyacrylamide gels. The proteins were incubated with primary antibodies(1:100000, β-Actin Rabbit mAb, AC026, ABclonal; 1:1000, FDX1/ADX Rabbit mAb, A20895, ABclonal; 1:1000, DLAT Rabbit mAb, A8814, ABclonal; 1:1000, ALOX5 Rabbit mAb, A2877, ABclonal; 1:1000, HMOX1 Rabbit pAb, GTX101147, GeneTex; 1:1000, GPX4 Rabbit mAb, A11243, ABclonal; 1:1000, iNOS Rabbit pAb, GTX130246, GeneTex; 1:1000, NOX4 Rabbit mAb, A23465, ABclonal; 1:1000, NQO1 Rabbit pAb, GTX100235, GeneTex; 1:1000, SOD1 Rabbit pAb, A0274, ABclonal) overnight at 4°C and the corresponding secondary antibody(1:1000, HRP-labeled Goat Anti-Rabbit IgG(H+L), A0208, Beyotime) for 1 h at room temperature. Finally, the protein signals were detected by the chemiluminescent substrate kit (34580; Thermo Scientific) and then analyzed by Image J software.

RNA extraction and Sequencing

Two cochlear from different groups were harvested for RNA extraction. TRIzol reagent (ET111-01-V2, TransGen) was used to extract total RNA following the manufacturer's instructions. The extracted RNA was sent to the Shanghai Personal Biotechnology Corporation for next-generation sequencing. Differentially expressed genes (DEGs) were detected at $q \leq 0.05$ and $|\log2\_ratio| \geq 1$.

Real-time quantitative polymerase chain reaction (RT-qPCR)

One microgram of total RNA was used as the template for reverse transcription in PCR amplification, with actin serving as the internal reference gene. The relative expression levels of the genes were calculated using the $2^{-\triangle\triangle CT}$ method. The experiment was performed with three biological replicates, and the primers used are listed in Table S1.

Auditory brainstem response (ABR)

ABR recording was conducted using the TDT system (Tucker Davis Technologies, Alachua, FL, USA). Mice were anesthetized with pentobarbital sodium (100 mg/kg). The recording, reference, and ground electrodes were implanted behind the ears and under the scalp. The ABR tests were performed at frequencies of 8, 12, 16, 24, and 32 kHz using the SigGenRZ software, and the

threshold response for each frequency was analyzed and digitized.

In Vivo Biosafety Assessment of Cu SAN

Whole blood, blood serum, and major organs (heart, liver, spleen, lung, and kidney) of the mice were collected for blood chemistry analysis (alanine aminotransferase ALT, aspartate aminotransferase AST, and blood urea nitrogen BUN), complete blood panel (white blood cells WBC, red blood cells RBC, hemoglobin HGB, platelets PLT, lymphocyte percentage Lymph%, granulocyte percentage Gran%, and monocyte percentage Mon%) analysis, and histological examination, respectively.

Supplementary Figure

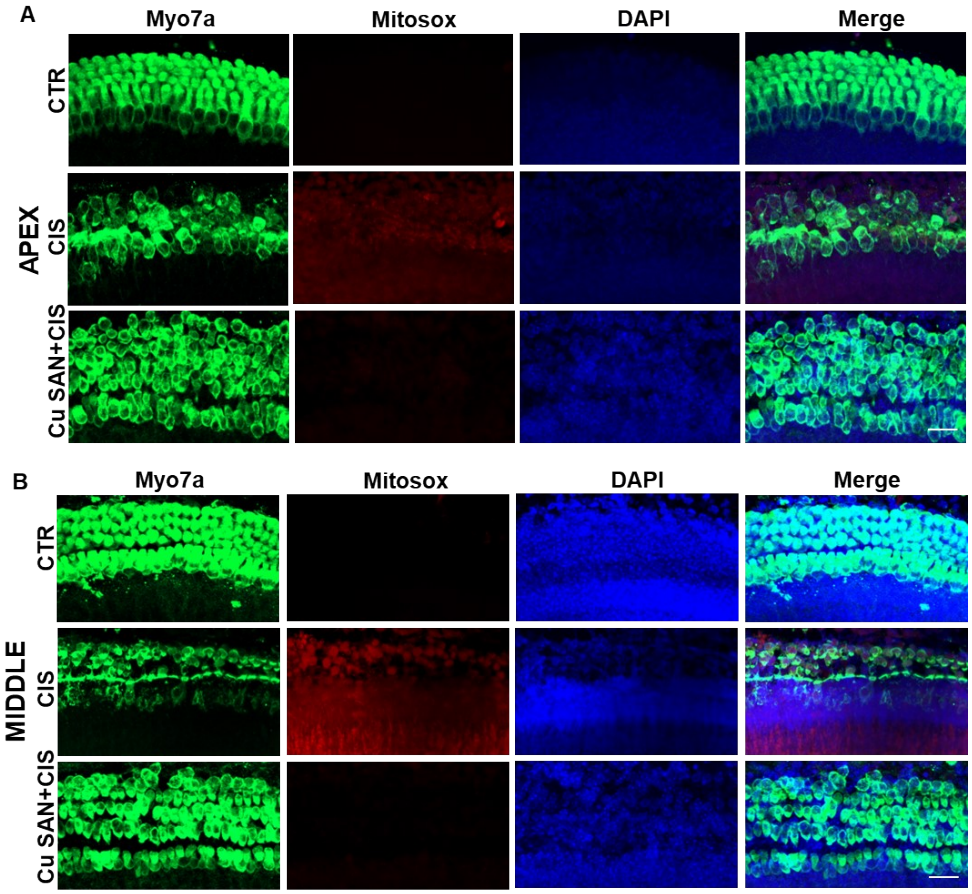

Figure S1. Mitosox red probe analysis for cochlear explants treated with Cu SAN. Immunofluorescence images of apex turns(A) and middle turns(B) in each treatment. Cochlear explants were stained with Myosin 7a (green), Mitosox (red), and DAPI (blue). Scale bars: 20μm.

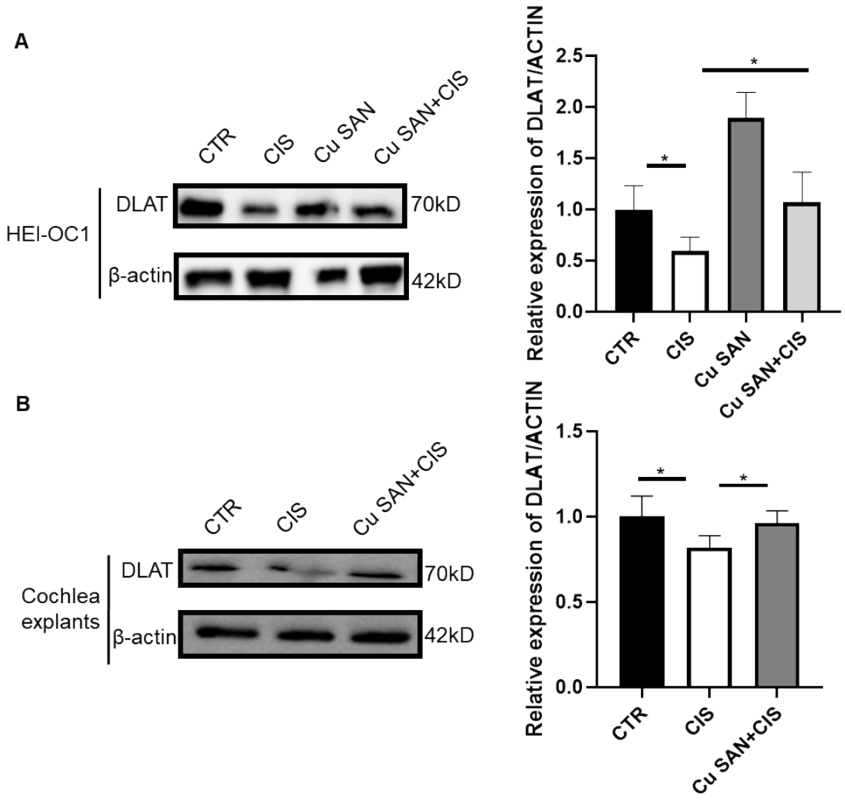

Figure S2. Western blot analysis and quantitative analysis of DLAT in HEI-OC1 cell lines and cochlea explants after different treatments. Data are presented as mean ± SD; n = 5; *p < 0.05.

Supplementary Table

Table S1. The specific primers for qRT-PCR analysis.

Gene	Forward Sequence	Reverse Sequence
Prdx1	TGCCAAGTGATTGGCGCTTCTG	AGCAATGGTGCGCTTGGGATCT
Duox1	GAAGACTGCGTCATCACCACAG	GCTGCGTTGAAACTTCTCTCGG
Gsr	GTTTACCGCTCCACACATCCTG	GCTGAAAGAAGCCATCACTGGTG
Sod1	GGTGAACCAGTTGTGTTGTCAGG	ATGAGGTCCTGCACTGGTACAG
Nox1	CTCCAGCCTATCTCATCCTGAG	AGTGGCAATCACTCCAGTAAGGC
Aox1	GCGAAGCAAGTGAAGGTGGTCT	ACACTCCAGCTTCCGTTCTGAC
Ptgs2	GCGACATACTCAAGCAGGAGCA	AGTGGTAACCGCTCAGGTGTTG
Gpx4	GCCTGGATAAGTACAGGGGTT	CATGCAGATCGACTAGCTGAG
Actin	GGCTGTATTCCCCTCCATCG	GGCTGTATTCCCCTCCATCG