Supporting Information

Reversal of cisplatin chemotherapy resistance by glutathione-resistant copper-based nanomedicine via cuproptosis

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Materials. Copper chloride dihydrate ($CuCl_2 \cdot 2H_2O$), dimethyl sulfoxide (DMSO), and sodium hydroxide (NaOH) were purchased from Kelong Chemical Reagent Factory (Chengdu, China). *Cis*-Diaminodichloroplatinum (CDDP) was purchased from Shanghai yuanye Bio-Technology Co., Ltd (China). Fluorescein isothiocyanate isomer I (FITC) was purchased from Tokyo Chemical Industry (Japan). RPMI 1640 medium, EMEM medium, trypsin, fetal bovine serum (FBS) and penicillin-streptomycin solution were obtained from GIBCO®. GSH and GSSG assay kit and Annexin V-FITC apoptosis detection kit were supplied by Beyotime Biotechnology Co., Ltd (Shanghai, China), and FractionPREP™ cell fractionation kit was obtained from AMSBIO LLC (California, USA). NPLOC4 (Cat# DF13194, RRID: AB_2846154), Phospho-Histone H2A.X (Ser139, Cat# AF3187, RRID: AB_2834619), FDX1 antibody (Cat# DF7950, RRID: AB_2841351), β-actin (Cat# AF7018, RRID: AB_2839420) and COX IV (Cat# AF5468, RRID: AB_2837951) antibodies were purchased from Affinity Biosciences Ltd (Jiangsu, China). RIPA lysis buffer and BCA protein quantification kit were obtained from Beijing Biosharp®. Mitochondrial protein extraction kit was provided by Beijing Solarbio®.

Cell culture. Human non-small cell lung cancer A549 and CDDP resistant A549/DDP cells were incubated in RPMI 1640 medium containing 10% FBS and 1% penicillin/streptomycin. A549/DDP cells were obtained by gradient incubation of A549 cells with 500-1000 ng/mL of CDDP in complete medium.[1,2] Similarly, human cervical cancer (HeLa), mouse embryonic fibroblast (NIH/3T3), and mouse fibroblast (L-929) cell lines were cultured in DMEM complete medium. All the above cells were cultivated in a humidified incubator with 5% CO_2 at 37 °C, and grown to about 80% confluence before splitting or harvesting.

Cell morphology observations. To examine the morphology of dying cells, A549 and A549/DDP

cells were seeded in the 6-well plates at about 50% confluency. The cells were then treated with CDDP (10 μM for A549 cells; 30 μM for A549/DDP cells), CuET (500 nM), or CuET NPs (917 nM) for 24 h. Phase-contrast cell images were captured on an Olympus IX71 microscope.

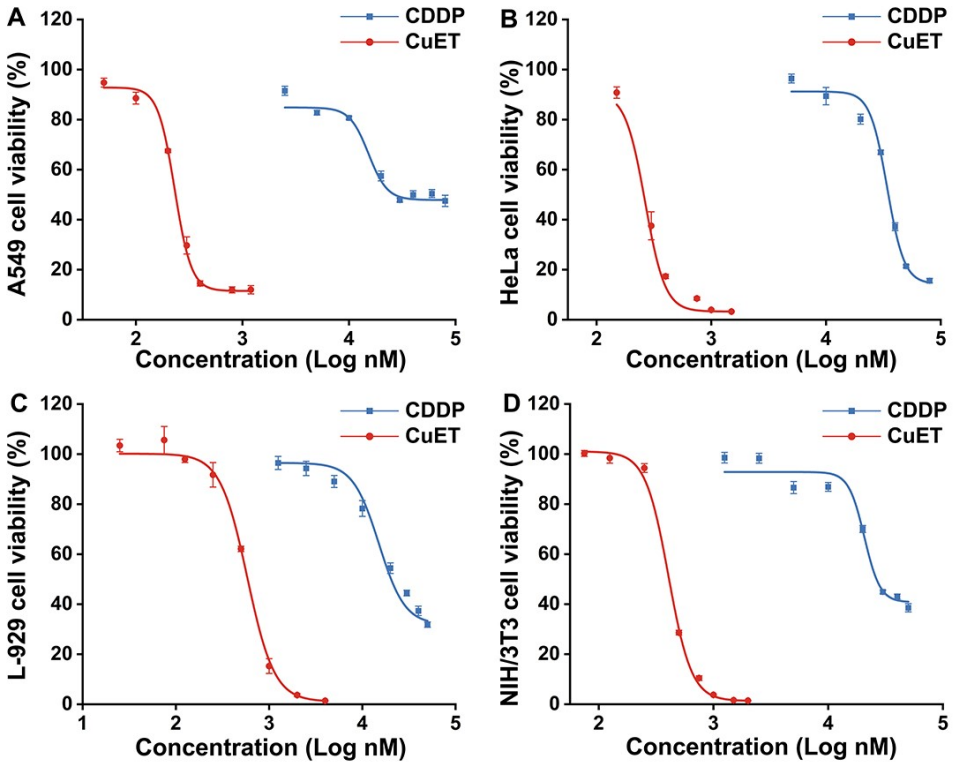

Fig. S1 Cytotoxicity results of CDDP and CuET against cancer (A, B) and normal (C, D) cells.

Table S1 Half-inhibitory concentrations of CDDP and CuET against different cell lines.

IC_{50}	A549	A549/DDP	HeLa	L-929	NIH/3T3
CDDP (μM)	15.2	49.0	34.1	15.1	30.7
CuET (nM)	230.8	358.6	265.1	584.0	405.8

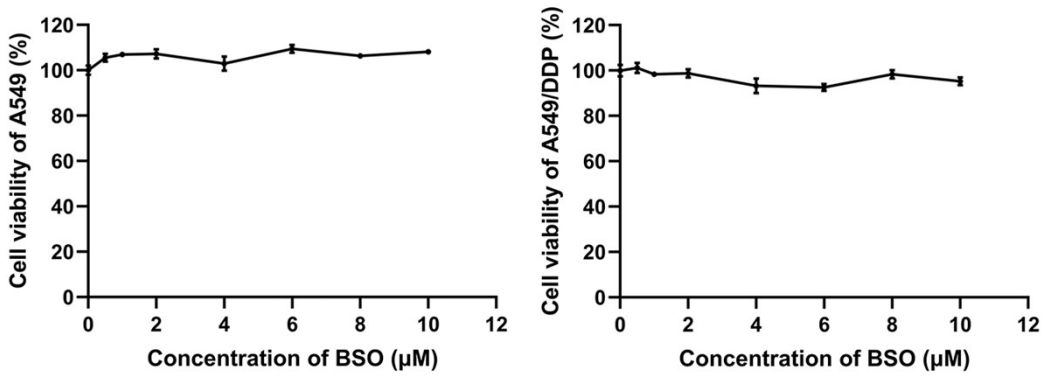

Fig. S2 Viability of A549 and A549/DDP cells treated with different concentrations of BSO.

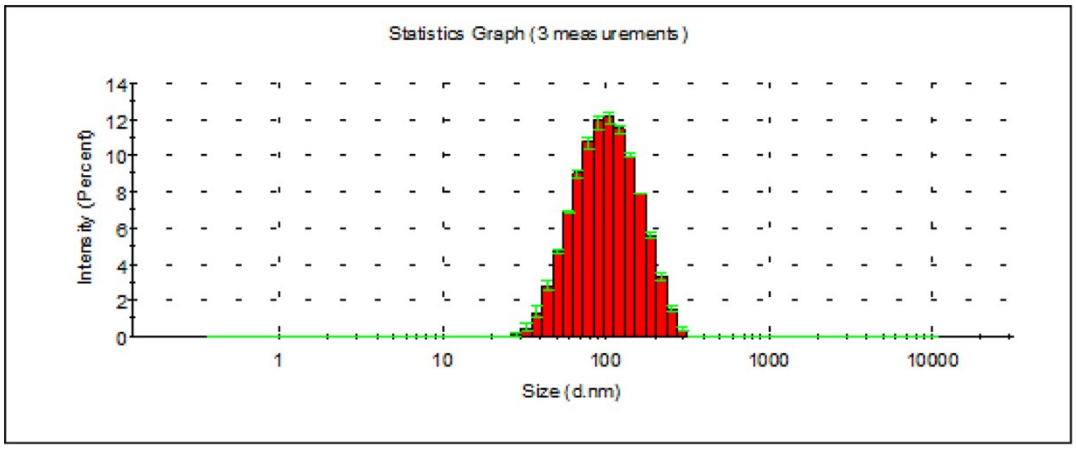

Fig. S3 Hydrodynamic diameter of CuET NPs measured by dynamic light scattering.

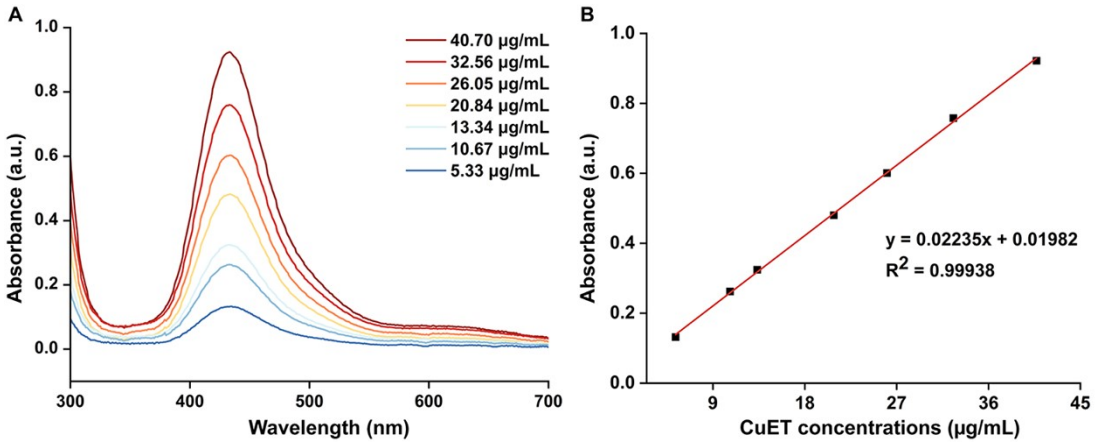

Fig. S4 UV-vis absorption spectra (A) and fitted standard curve (B) of CuET.

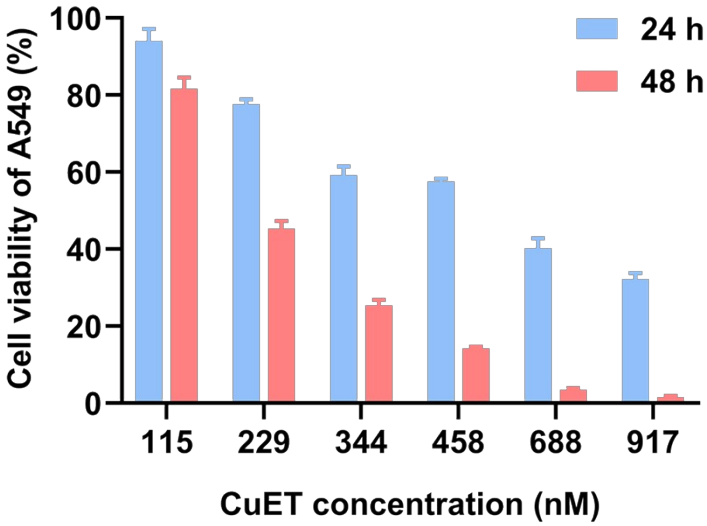

Fig. S5 Viability of A549 cells treated with CuET NPs for different incubation times.

Table S2 Half-inhibitory concentrations of CuET NPs with different incubation times.

IC_{50} (nM)	A549	A549/DDP
24 h	526.6	524.4
48 h	213.8	273.1

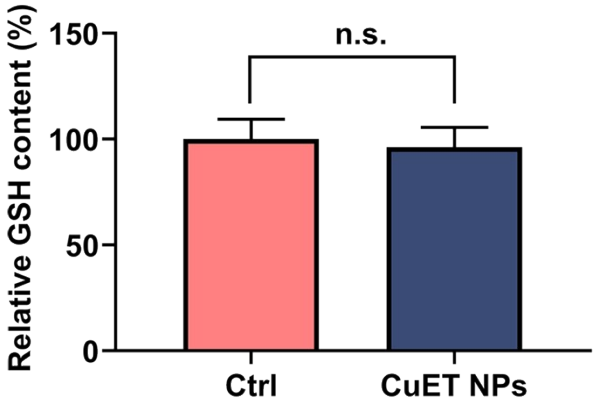

Fig. S6 Relative GSH content in A549/DDP cells treated with CuET NPs. n.s. $P > 0.05$.

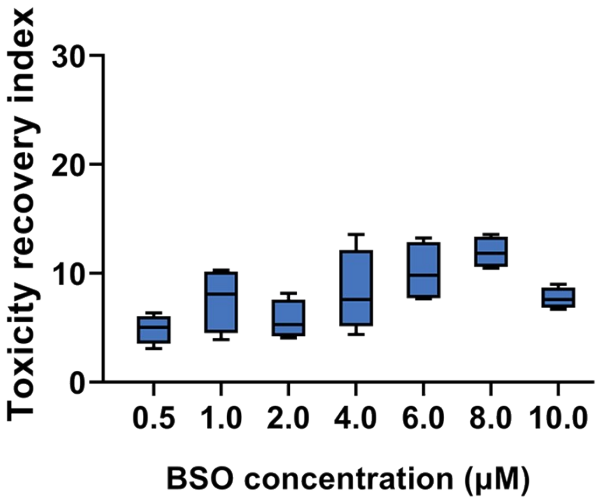

Fig. S7 Toxicity recovery index of CuET NPs under different concentrations of BSO treatment in A549/DDP cells.

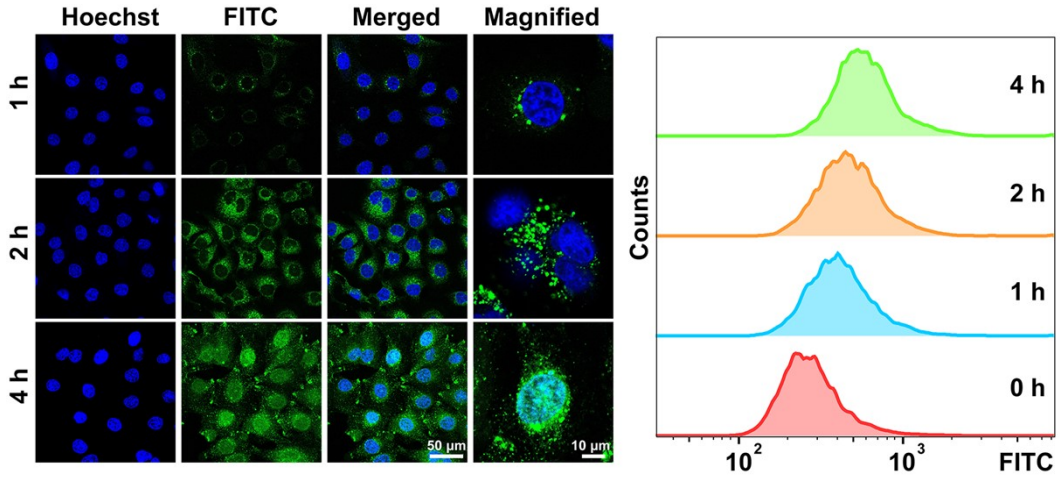

Fig. S8 CLSM and flow cytometry analysis of cellular endocytosis of FITC-labeled CuET NPs by A549 cells at varying treatment durations.

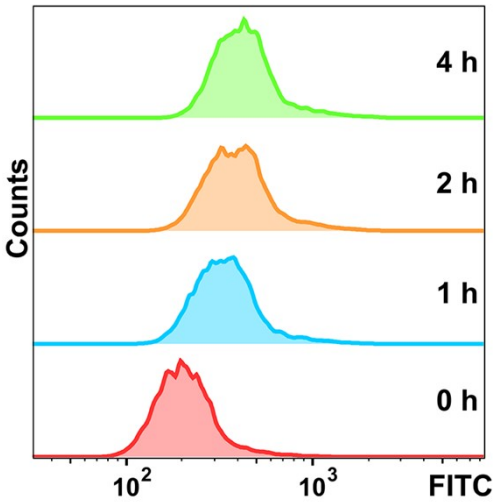

Fig. S9 Flow cytometry analysis of cellular endocytosis of FITC-labeled CuET NPs by A549/DDP cells at varying treatment durations.

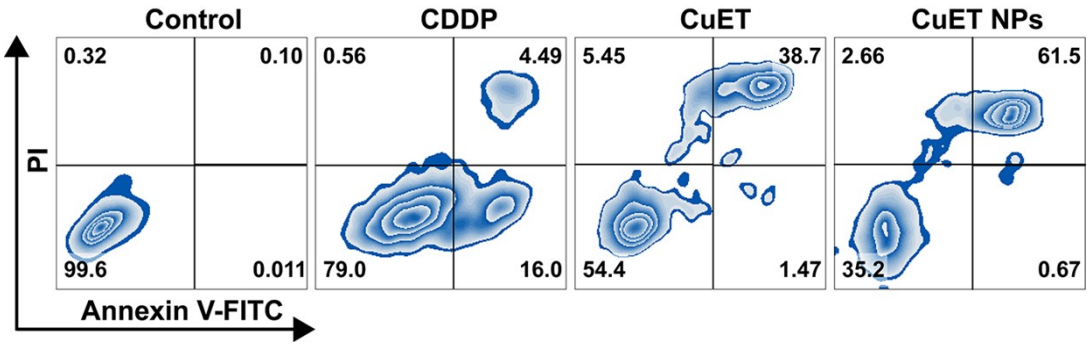

Fig. S10 Flow cytometry results of annexin V-FITC/propidium iodide double-stained A549 cells.

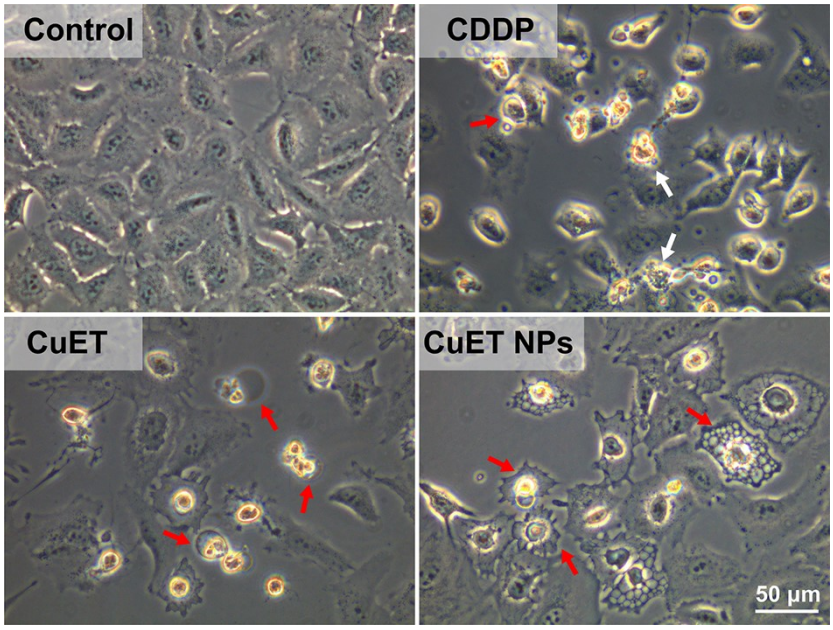

Fig. S11 A549 cell observations by a microscope after different treatments for 6 h. White arrows indicate apoptotic cells with apoptotic bodies, and red arrows indicate cytolytic death.

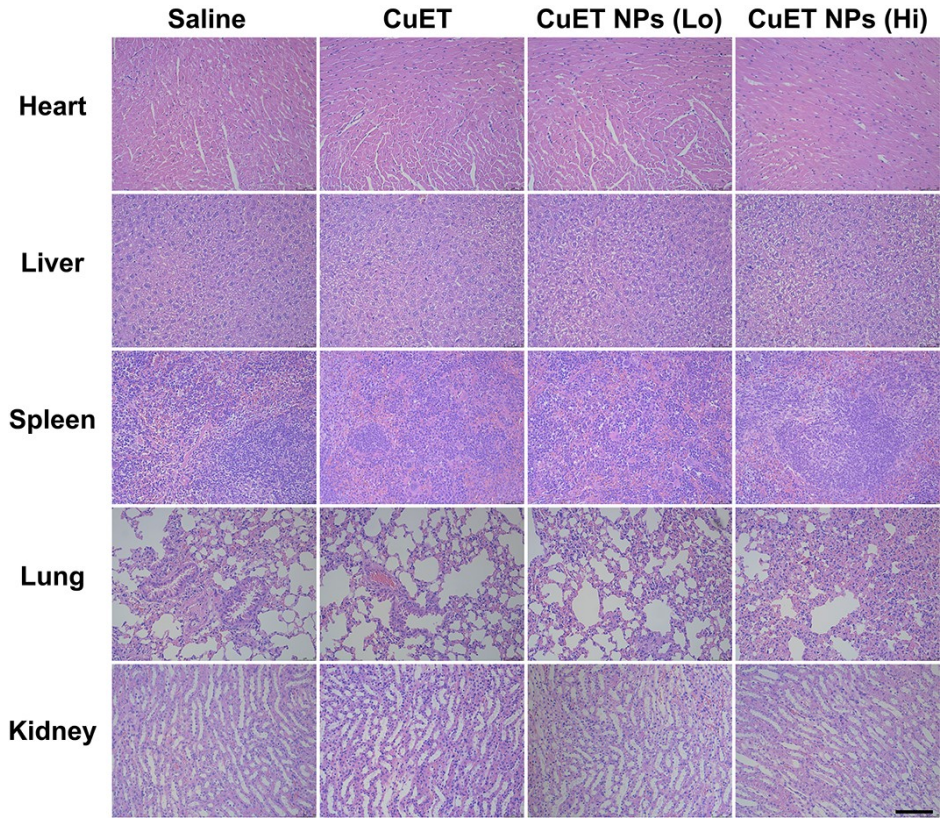

Fig. S12 H&E staining images of main organs in different groups on day 16. Scale bar: 100 μm.

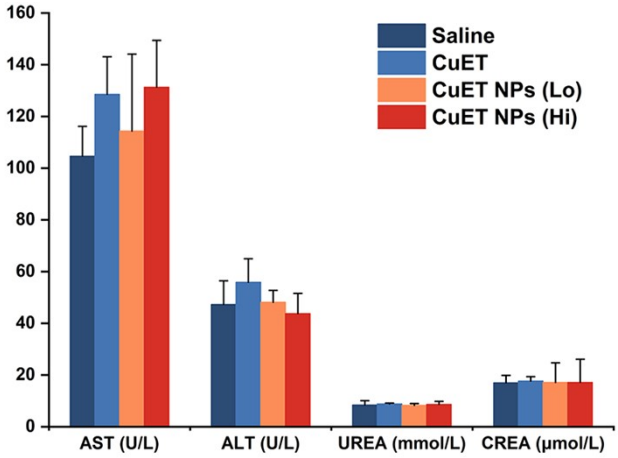

Fig. S13 Hepatic and renal functions of A549/DDP tumor-bearing mice.

References

1 M. P. Barr, S. G. Gray, A. C. Hoffmann, R. A. Hilger, J. Thomale, J. D. O'Flaherty, D. A. Fennell, D. Richard, J. J. O'Leary and K. J. O'Byrne, *PLoS One*, 2013, **8**, e54193.

2 V. Janson, B. Andersson, P. Behnam-Motlagh, K. G. Engström, R. Henriksson and K. Grankvist, *Cell. Physiol. Biochem.*, 2008, **22**, 45-56.