# Supplementary materials

# Intelligent responsive copper-diethyldithiocarbamate-based multifunctional nanomedicine for photothermal-augmented cancer synergistic therapy

Xiangyu Meng[a,b*], Jiayi Wu[a,c], Zunfu Hu[a,b], Xiuwen Zheng[a,d*]

[a] Key Laboratory of Advanced Biomaterials and Nanomedicine in Universities of Shandong, Linyi University, Linyi 276000, P. R. China

[b] School of Materials Science and Engineering, Linyi University, Linyi 276000, P. R. China

[c] School of Chemistry and Chemical Engineering, Linyi University, Linyi, 276000, P. R. China

[d] Qilu Normal University, Jinan, 250200, P. R. China

*** Corresponding author:**

Dr. Xiangyu Meng, E-mail address: mengxiangyu1@lyu.edu.cn

Dr. Xiuwen Zheng, E-mail address: zhengxiuwen@lyu.edu.cn

## S1 Materials and instruments

### 1. Materials

Doxorubicin hydrochloride (DOX·HCl) were obtained from Beijing Huafeng Pharmaceutical Co., Ltd. (Beijing, China). Polyvinylpyrrolidone (PVP) (Mw = 3.5 kDa) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Sodium diethyldithiocarbamatetrihydrate (DDC-Na), copper chloride aqueous ($CuCl_2$), N, N-dimethylformamide (DMF), triethylamine, and methylene blue (MB) were obtained from Sinopharm Chemical Reagent Co., Ltd. (Nanjing, China). RPMI-1640 medium, 3-(4,5-dimethylthiazol-2-yl)-2,5-di-phenyltetrazolium bromide (MTT), Annexin V-FITC/PI Cell Apoptosis Kit, 4, 6-diamidino-2-phenylindole (DAPI), Calcein-AM/PI, GSH/GSSH detection kit, and 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA) were obtained from Beyotime Biotech. Inc. (Nanjing, China). Breast cancer mice were purchased from Nanjing KeyGen Biotech Co., Ltd.

### 2. Instruments

The morphology of nanoparticles was performed by transmission electron microscopy (TEM) (JEOL JEM 2100F, Japan). Element mapping was performed by scanning electron microscopy (SEM) (FEI Company, Hillsboro, OR, USA). The hydrodynamic diameter was measured by dynamic light scattering (DLS) (NanoBrook Omni, USA). The valence state of Fe and Cu elements in the $Cu(DDC)_2$@Fe/DOX NPs was measured by X-ray photoelectron spectroscopy (XPS) spectrometer (Thermo Scientific, USA). Ultraviolet-visible (UV-vis) absorption spectra were determined with a UV-vis spectrometry (UV-2450, Japan). The crystal form of $Cu(DDC)_2$@Fe/DOX NPs was

measured by an X-ray diffractometer (Smartlab, Rigaku Corporation, Japan). Confocal microscopy (CLSM) imaging was collected by a confocal laser scanning microscope (TCS SP8, Leica, Germany). Animal fluorescence imaging was performed on the IVIS Lumina series III system (PerkinElmer, USA). Cell apoptosis was analyzed by flow cytometry (BD Accuri Flow C6, USA).

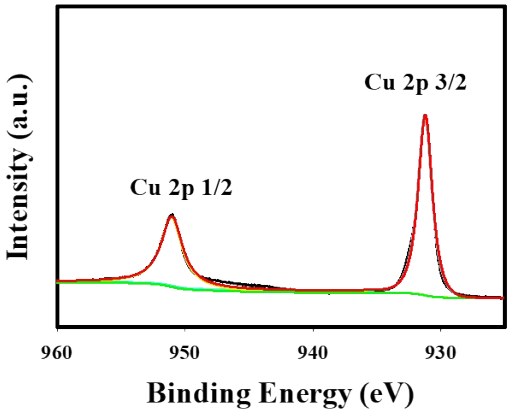


**Fig. S1** High-resolution Cu 2p XPS spectrum.

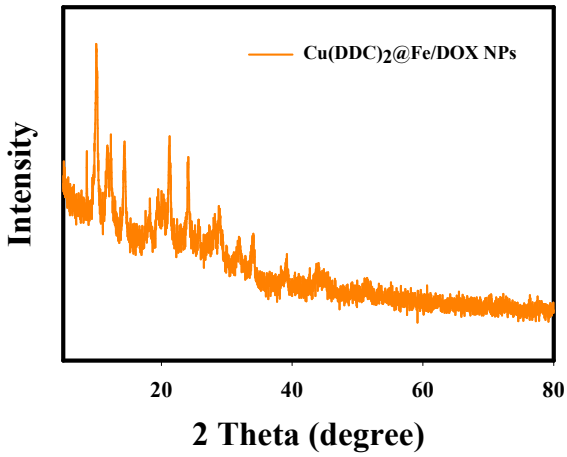


**Fig. S2** XRD pattern of $Cu(DDC)_2$@Fe/DOX NPs.

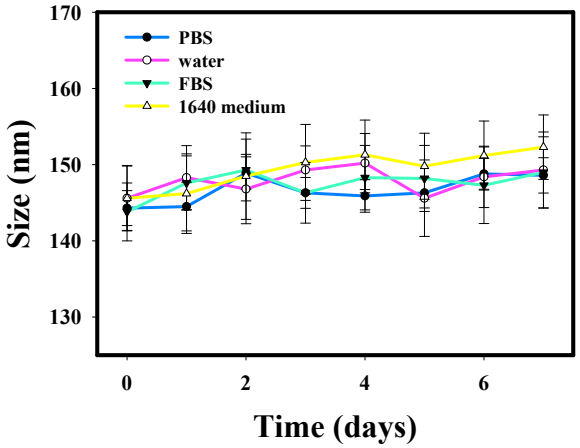


**Fig. S3** The size of $Cu(DDC)_2$@Fe/DOX NPs during 7 days soaking in PBS, water, FBS, and 1640 medium.

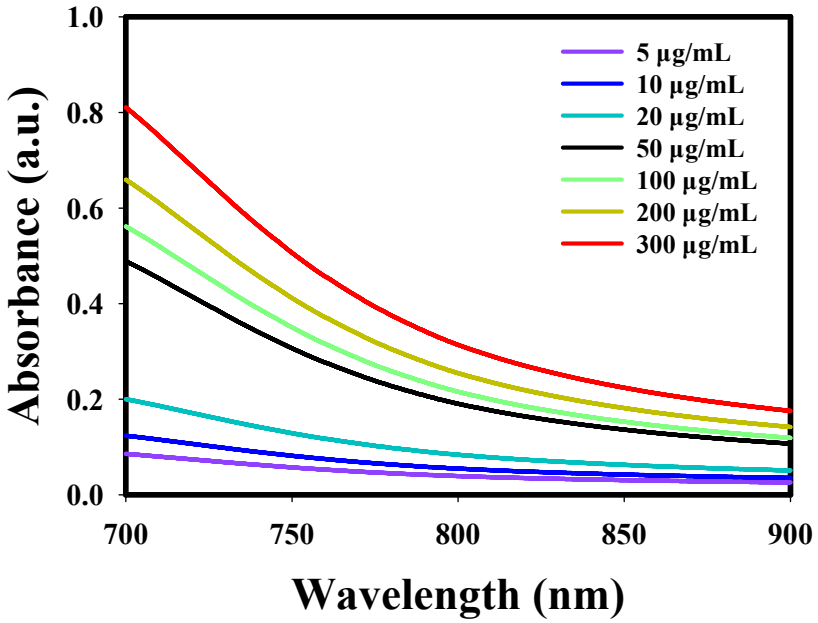


**Fig. S4** UV-vis spectra of aqueous suspensions of dispersed $Cu(DDC)_2$@Fe/DOX NPs at varied concentrations (5, 10, 20, 50, 100, 200, and 300 µg $mL^{-1}$).

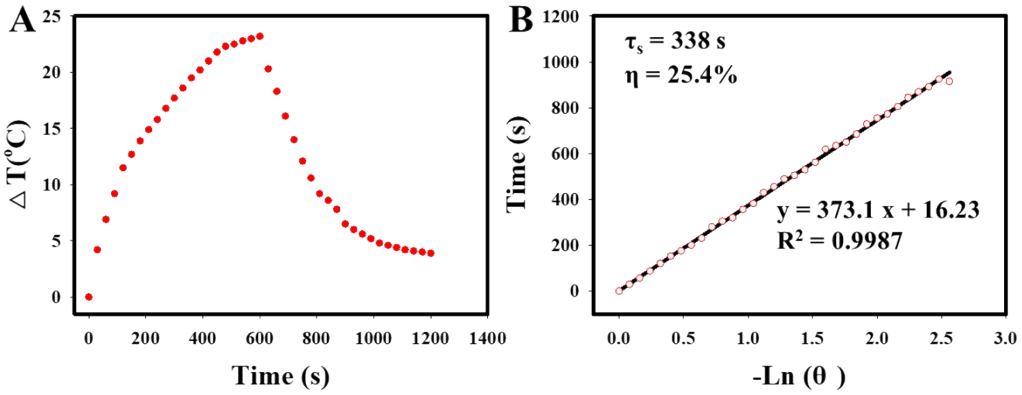


**Fig. S5** (A) Heating and cooling curve of an aqueous dispersion of $Cu(DDC)_2$@Fe/DOX NPs under 808 nm laser irradiation (1.02 W $cm^{-2}$). (B) Calculation of the photothermal-conversion efficiency at 808 nm. The time constant ($\tau_s$) for heat transfer from the system was determined to be $\tau_s$ = 338 s based on the linear time-dependent data ($R^2$ = 0.9987) collected during the cooling period.

The photothermal conversion efficiency ($\eta$) of $Cu(DDC)_2$@Fe/DOX NPs can be calculated as following equations:

$$\eta = [hS(T_{max,\,NPs} - T_{surr}) - Q_{dis}] / [\,I(1 - 10^{-A808})]\times 100\%$$

$$\tau_s = [m_D C_D] / hS$$

$$t = -\tau_s \ln\theta$$

$$\theta = (T - T_{surr}) / (T_{max,\,NPs} - T_{surr})$$

Where $h$ is heat transfer coefficient, $S$ is the irradiated area, and $T_{max,\ NPs}$ is the equilibrium temperature of NPs, $T_{surr}$ is ambient temperature of the surroundings. $I$ is the laser power density, A is absorption of $Cu(DDC)_2$@Fe/DOX NPs at 808 nm, $m$ is the mass of sample, $Q_{dis}$ expresses the heat from light absorbed by the quartz sample cell itself, t is cooling time after irradiation, T is a temperature for NPs solutions at a

constant cooling time, and $\tau_s$ is the sample system time constant. θ represents the dimensionless driving force temperature. [1-3] Where $T_{max, NPs} - T_{surr}$ is 21.2 $^oC$, I is 1.02 W $cm^{-2}$, A808 is 0.335, $\tau_s$ is 338 s. hS can be calculated is calculated to be 12.2 mW $^oC^{-1}$. Therefore, the photothermal conversion efficiency of $Cu(DDC)_2$@Fe/DOX NPs was 25.4%.

References

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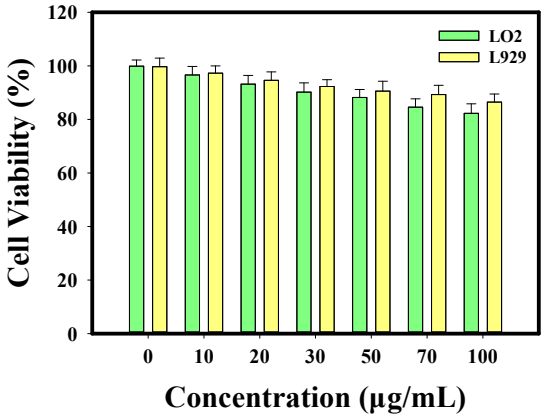


**Fig. S6** The viabilities of normal cells (LO2 and L929) treated with $Cu(DDC)_2$@Fe/DOX NPs (0~100 μg/mL) with or without NIR light irradiation.

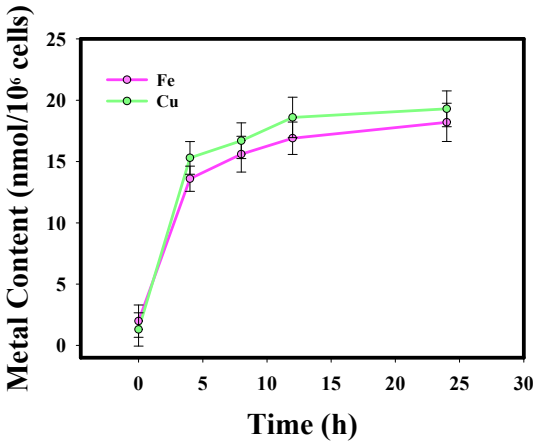


**Fig. S7** The content of Cu ions and Fe ions in cancer cells after being treated with $Cu(DDC)_2$@Fe/DOX NPs over time.

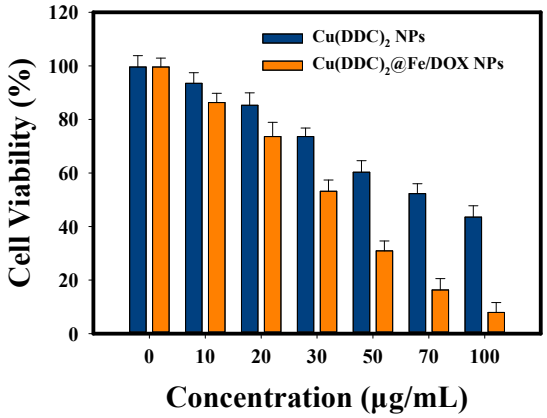


**Fig. S8** The cell viability after MDA-MB-231 cells treatment with different concentrations of $Cu(DDC)_2$ NPs and $Cu(DDC)_2$@Fe/DOX NPs.

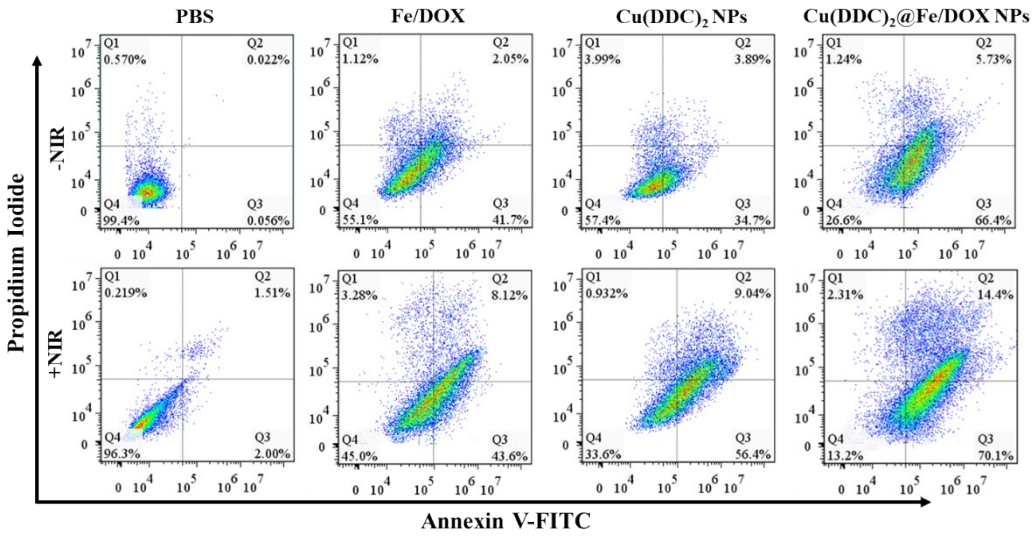


**Fig. S9** Flow cytometry with Annexin V-FITC and propidium iodide (PI) staining was applied to evaluate the apoptosis rates after different treatments.

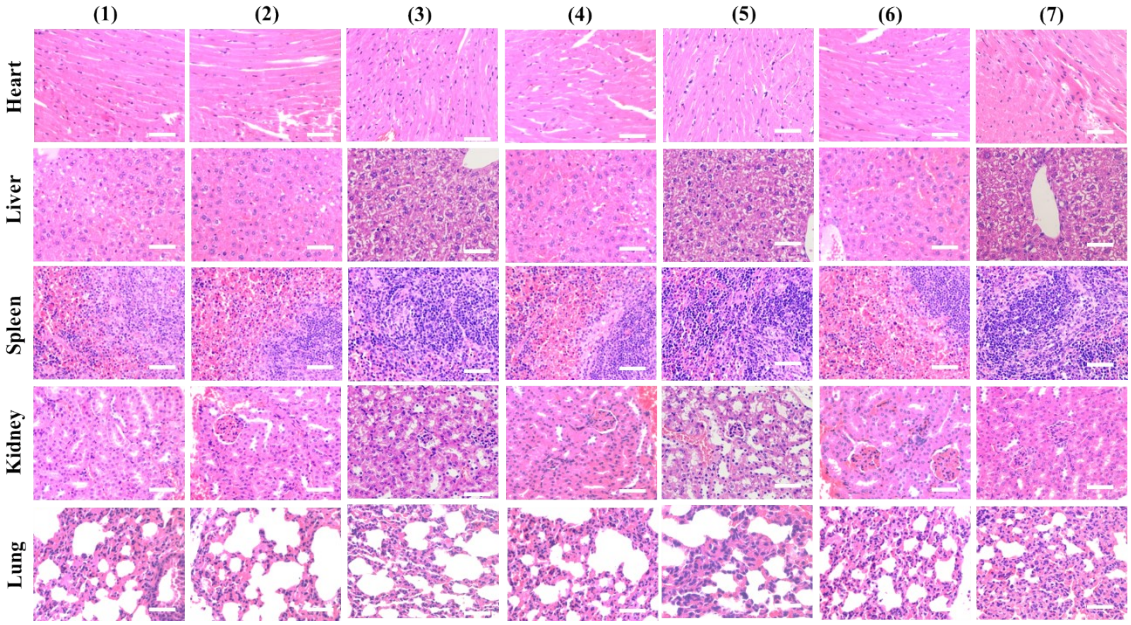


**Fig. S10** H&E staining of the main organs after the mice treated with (1) PBS, (2) Fe/DOX mixture, (3) Fe/DOX mixture + NIR, (4) $Cu(DDC)_2$ NPs, (5) $Cu(DDC)_2$ NPs + NIR, (6) $Cu(DDC)_2$@Fe/DOX NPs, and (7) $Cu(DDC)_2$@Fe/DOX NPs + NIR. Scale bar = 50 μm.

**Table S1.** Hematological and blood biochemistry indices of mice treated with $Cu(DDC)_2$@Fe/DOX NPs or $Cu(DDC)_2$@Fe/DOX NPs + NIR for 24 h. The concentration of $Cu(DDC)_2$@Fe/DOX NPs is 2.0 mg/kg.

| Index | $Cu(DDC)_2$@Fe/DOX NPs | $Cu(DDC)_2$@Fe/DOX NPs + NIR | Blank |
|---|---|---|---|
| WBC ($10^9$/L) | 5.34 ± 1.89 | 5.02 ± 1.68 | 5.56 ± 1.13 |
| RBC ($10^{12}$/L) | 11.23 ± 0.56 | 11.53 ± 0.98 | 11.3 ± 0.74 |
| HGB (g/L) | 155.8 ± 93.6 | 153.5 ± 89.5 | 158.65 ± 99.6 |
| MPV (fL) | 2.23 ± 1.32 | 2.69 ± 1.23 | 2.43 ± 1.30 |
| PLT ($10^9$/L) | 790.3 ± 100.8 | 782.3 ± 88.6 | 800.2 ± 98.5 |
| HCT (%) | 0.50 ± 0.03 | 0.49 ± 0.12 | 0.51 ± 0.05 |
| MCH (pg) | 15.38 ± 0.62 | 15.33 ± 0.42 | 14.96 ± 0.63 |
| NE ($10^9$/L) | 1.89 ± 1.12 | 1.62 ± 1.29 | 1.89 ± 1.10 |
| AST (U/L) | 143.2 ± 36.2 | 135.8 ± 33.2 | 145.6 ± 30.6 |
| ALT (U/L) | 40 ± 3.56 | 43 ± 4.32 | 38 ± 3.22 |
| ALB (g/L) | 21.3 ± 4.32 | 20.8 ± 3.69 | 22.5 ± 4.51 |
| ALP (U/L) | 190 ± 20.3 | 187 ± 25.6 | 194 ± 22.9 |
| TP (g/L) | 36.89 ± 2.65 | 35.43 ± 3.67 | 37.48 ± 2.69 |
| GLO (mmol/L) | 4.61 ± 2.30 | 4.48 ± 1.33 | 4.69 ± 1.56 |
| GREA (μmol/L) | 21.30 ± 3.56 | 20.43 ± 4.57 | 22.01 ± 5.02 |
| BUN (mmol/L) | 7.18 ± 1.03 | 7.87 ± 1.49 | 7.89 ± 1.02 |

**Abbreviations:** WBC, white blood cell count; RBC, red blood cell count; HGB, hemoglobin; MPV, mean platelet volume; PLT, platelet count; HCT, hematocrit; MCH, mean corpuscular hemoglobin; NE, neutrophil count; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALB, albumin; ALP, alkaline phosphatase; TP, total protein; BUN, blood urea nitrogen; CREA, creatinine; GLO, globulin.