Supporting Information

A Magnetically Responsive Drug-loaded Nanocatalyst with Cobalt-involved Redox for the Enhancement of Tumor Ferrotherapy

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The acquired transmission electron microscope (TEM) images of Fe_3O_4 nanoparticles, Co nanoparticles, Lips@Fe_3O_4, and Co-Lips are shown in Fig. S2-S5 (ESI). A UV-Vis spectrum indicated the presence of characteristic peaks of PTX and Fe_3O_4 in PTX/Co-Lips@ Fe_3O_4, which proved the successful loading of PTX and Fe_3O_4 (Fig. S6, ESI).

We used the disappearance of colour (blue) and of the characteristic peak (665 nm) of methyl blue (MB) as an indicator of the generation of •OH. As shown in Fig. S7 (ESI) and Fig. S8 (ESI), the Fe (II) and Co (II) ions can react with H_2O_2 to form •OH via Fenton reaction. Additionally, we used the appearance of colour (orange-red) and of the characteristic peak (508 nm) of O-phenanthroline to indicate the generation of Fe (II) ions. As shown in Fig. S9 (ESI), the Fe (II) was generated by decomposition of Fe_3O_4 nanoparticles following addition of GSH.

Experimental Section

Materials. Soya lecithin was obtained from Shanghai Advanced Vehicle Technology L.T.D. Co., Ltd (Shanghai, China). Paclitaxel was purchased from Shanghai Yuanye Biotechnology Co., Ltd (Shanghai, China). $FeCl_2 \cdot 4H_2O$, $FeCl_3 \cdot 6H_2O$ and cholesterol were obtained from Tianjin Damao Chemical Instruments Supply Station (Tianjin, China).

Synthesis of Co nanoparticles. The anionic surfactant (sodium bis2-ethylhexyl sulfonate) was dissolved in isooctane at a concentration of 0.27 mM. Then, $CoCl_2$ and $NaBH_4$ were dissolved in this solution at a certain concentration to obtain two kinds of microemulsions. Then, the two kinds of microemulsions were mixed and the solution turned from light pink to black. The colloid is agglomerated with acetone and water as a flocculent. The excess surfactant was removed by filtration and deionized water washing, and the pure Co nanoparticles were obtained and stored at 4°C for reserve.

Synthesis of Fe_3O_4 nanoparticles. First, $FeCl_3 \cdot 6H_2O$ and $FeCl_2 \cdot 4H_2O$ were dissolved in 15 mL deionized water in a molar ratio of 1:1, and 0.3 g PEG-2000 was added to modify Fe_3O_4 nanoparticles to make the nanoparticles more hydrophilic. Then,

the above iron salt solution was placed in ultrasonic cleaning apparatus, and the ultrasonic temperature was set at 60°C with mechanical agitation, and 1 mol/mL NaOH solution was slowly added to generate magnetic Fe_3O_4 nanoparticles. The reaction was complete until the solution turned pure black, then the black suspension was placed in an 80°C water bath and stirred for 15 min. The black suspension was placed on the magnet for separation, the supernatant was dumped to precipitate Fe_3O_4 particles, and the deionized water and anhydrous ethanol were used to wash the particles alternately for three times. Dry the end product thoroughly in a 70°C oven and store it in a dryer for use.

Synthesis of PTX/Co-Lips. First, soybean lecithin, cholesterol and paclitaxel (PTX) with a mass ratio of 50:5:1 were dissolved in anhydrous ethanol and placed on a rotary evaporator for 2 h under reduced pressure to form a uniform lipid film on the round bottom flask. Then, 200 mL of Co nanoparticles, 10 μL Twin-80 and 9800 μL phosphate buffer (PBS, pH = 6.5) were added to the round bottom flask and rotated for hydration. Finally, uniform liposomes were obtained from the liposome suspension by ultrasound 10 min. Liposome solutions are removed from unpackaged reagents by means of a glucan gel (Sephadex G-150) and stored at 4°C for use.

Synthesis of PTX/Co-Lips@Fe_3O_4 nanoparticles. Fe_3O_4 solution (1 mg/mL) was dripped into the prepared PTX/Co-Lips (1:2 volume ratio), stirred gently for 2 h, and then centrifuged and purified to remove the unmodified Fe_3O_4 based on the particle size. Finally, Fe_3O_4 modified nanoparticles PTX/Co-Lip@Fe_3O_4 were obtained.

•OH generation. 1.0 mM GSH, 8 mM H_2O_2 and 1 mL PTX/Co-Lips@Fe_3O_4 were added to 25 mM $NaHCO_3$ buffer solution for 30 min at 37 °C. After centrifugation, add 10 μg/mL methyl blue (MB) to the supernatant and determine the absorbance of MB at 665 nm.

PTX release. 1 mL of the sample was put into the dialysis bag, and then the dialysis bag was placed into a 200 mL dialysate beaker with or without GSH and H_2O_2, and continuously stirred under a 37 °C water bath shaker. The sample was taken out of the beaker at a predetermined time interval, 2 mL of methanol was added to demulsification overnight, and the sample was centrifuged at 1000 RPS for 30 min. Finally, the

absorbance of the sample was determined with a spectrophotometer. The standard curve was calibrated with a known concentration of PTX solution and the drug content was analyzed.

Magnetic field response. In order to observe the response of the prepared target nanoliposomes to the magnetic field, the target sample was added into the small glass bottle in this experiment. After 10 min of response, the concentration of PTX/Co-Lip@Fe_3O_4 nanoparticles towards the direction of the magnetic field (D10 × 5 mm, N35) was observed to evaluate its targeting performance.

Cellular uptake and cytotoxicity. The antitumor effect of all formulations was studied by HeLa cells using the MTT assay. The cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) containing 10% fetal bovine serum and 1% penicillin/streptomycin at 37 °C containing 5% CO_2. HeLa cells were cultured in a 6-well plate for 24 hours, the samples were added to the plate. The cells were then washed with cold PBS three times and fixed with 4% paraformaldehyde for 10 min. Finally, the cells were observed under an inverted fluorescence microscope.

Antitumor effect and MRI *in vivo*. Kunming mice (5∼7 weeks old, female) were inoculated subcutaneously with U14 cells in the right flank of mice. When the tumor size reached about 100 mm^3, the mice were injected 200 μL of all the samples for 12 days. The tracking effect of PTX/Co-Lips@Fe_3O_4 was assessed using MRI images of tumor-bearing mice. Mice with U14 tumors were intravenously injected with 200 μL PTX/Co-Lips@Fe_3O_4 samples, scanned with an MRI system, and then obtained MRI images at different time points. *In vivo* MRI was performed at the Siemens 3.0t MR Leonardo 3682 workstation. Experimental parameters of T_2 rapid recovery of spin echo sequence are set as follows: repeat time (TR) = 1800 ms, echo time (TE) = 110 ms, slice thickness = 2.0 mm, resolution = 1.5×1.0×2.0 mm, field of view (FOV) = 65×65 mm^2. After the experiment, the mice were sacrificed by breaking spinal marrow under ether anesthesia, the main organs (heart, liver, spleen, lung, and kidney) and tumor tissues were excised for H&E staining. All experiments involving animals were performed in accordance with the statute of Experimental Animal Ethics Committee of Yanshan University.

"All in One" Strategy	
Theranostics in One System	**Enhancements of Ferrotherapy in One System**
1. Tumor Targeting (Magnetic field)	1. Depletion of GSH by Fe_3O_4
2. Ferrotherapy Fe (II)-based Fenton reaction	2. Fe (III) to Fe (II) by Co-involved Redox
3. Chemotherapy (PTX)	3. Generation of Co (II) by Co-involved Redox
	4. Magnetic field

Fig. S1 Schematic illustration of "All in one" strategy in this work.

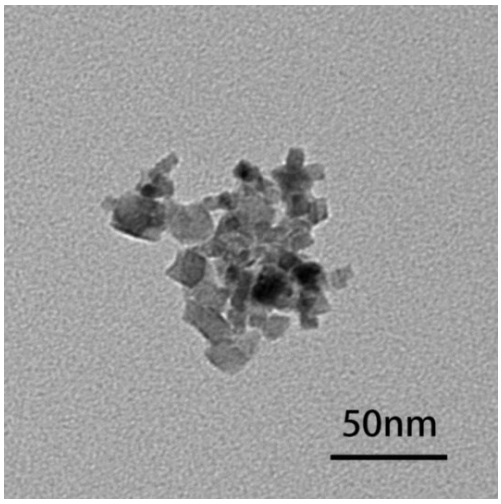

Fig. S2 TEM image of Fe_3O_4 nanoparticles.

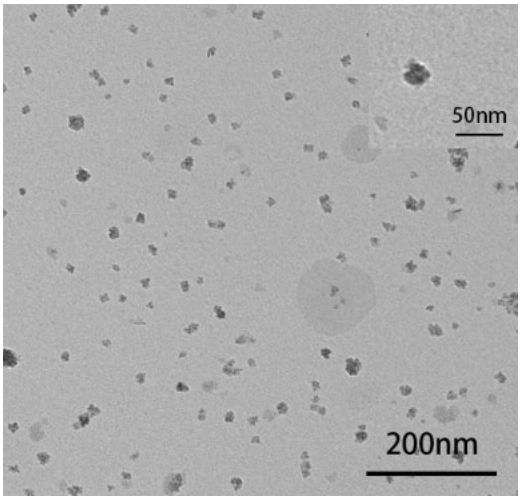

Fig. S3 TEM image of Co nanoparticles.

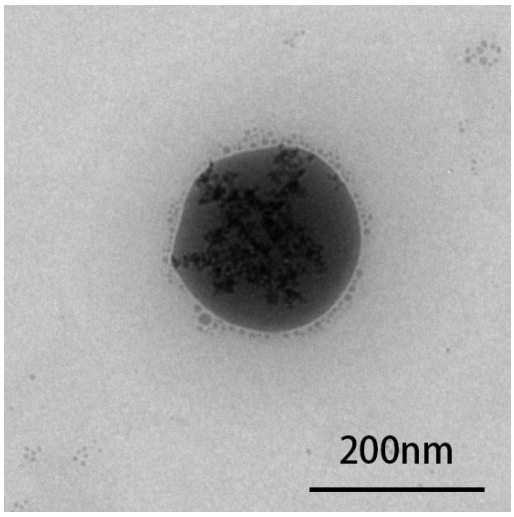

Fig. S4 TEM image of Lips@Fe_3O_4.

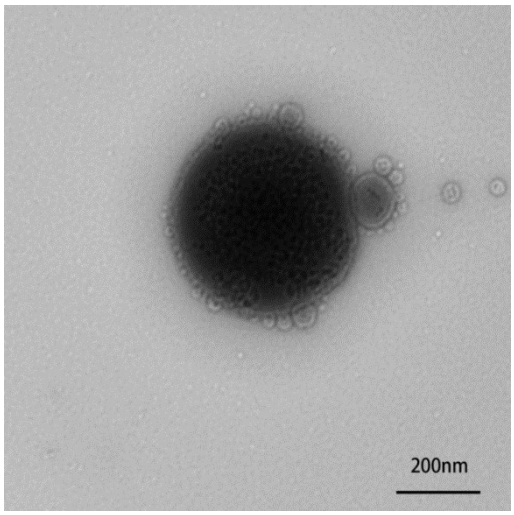

Fig. S5 TEM image of Co-Lips.

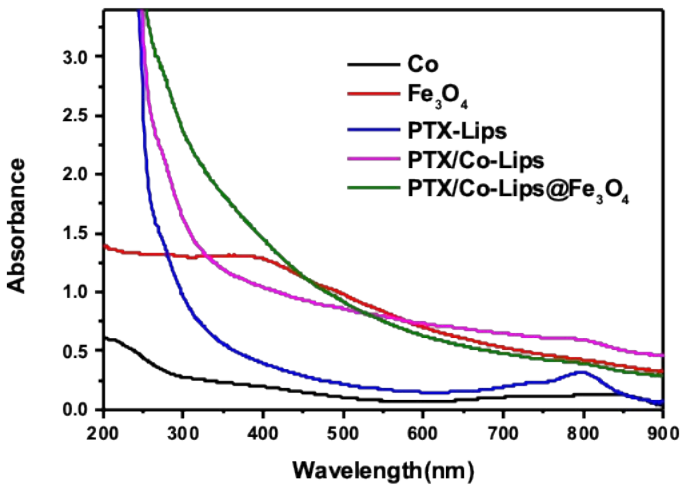

Fig. S6 UV-Vis spectrum of all tested groups (Co, Fe_3O_4, PTX-Lips, PTX/Co-Lips and PTX/Co-Lips@Fe_3O_4).

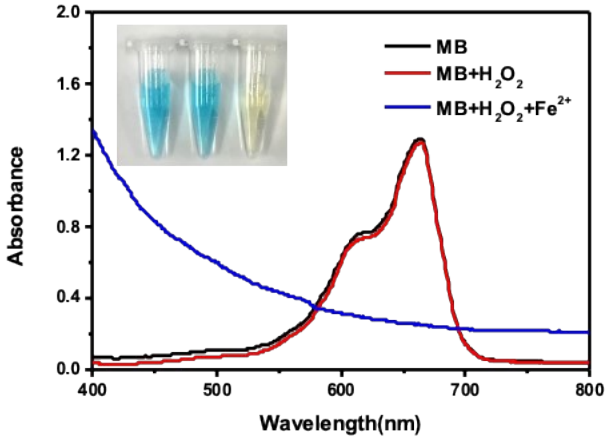

Fig. S7 UV-Vis spectrum of MB with H_2O_2 and Fe (II).

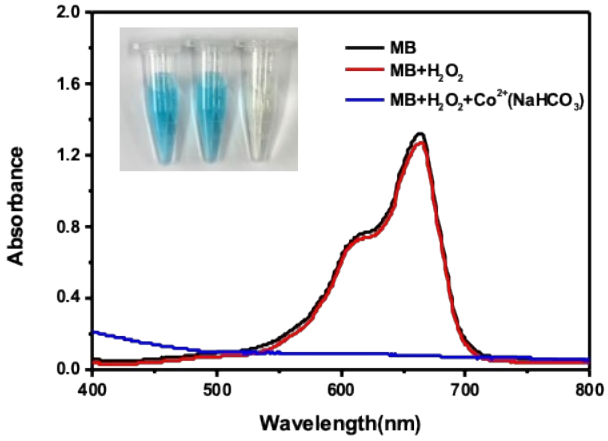

Fig. S8 UV-Vis spectrum of MB with H_2O_2 and Co (II).

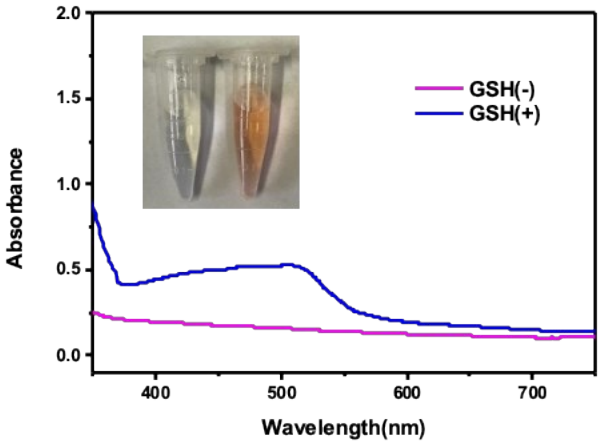

Fig. S9 UV-Vis spectrum of O-phenanthroline + Lips@Fe_3O_4 treated with GSH (+, -).

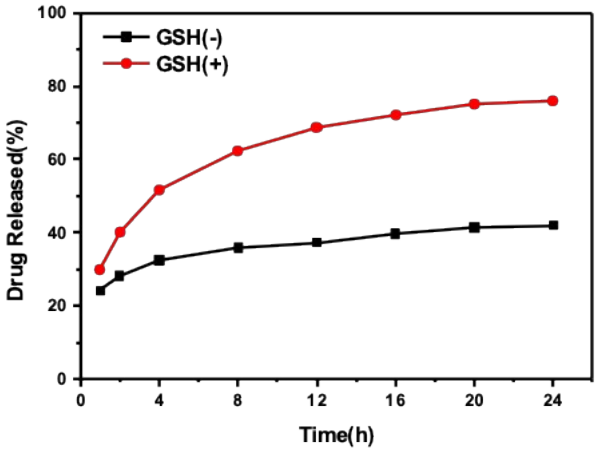

Fig. S10 Drug release profiles of PTX/Co-Lips@Fe_3O_4 with/without GSH.

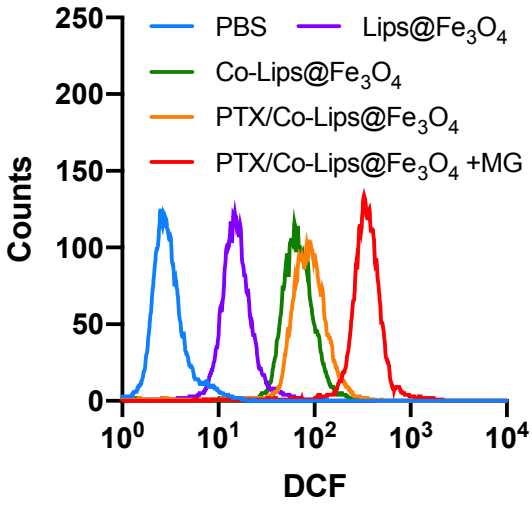

Fig. S11 Flow cytometry analysis of DCF-positive HeLa cells treated with PBS, Lips@Fe_3O_4, Co-Lips@Fe_3O_4 and PTX/Co-Lips@Fe_3O_4 with and without MG.

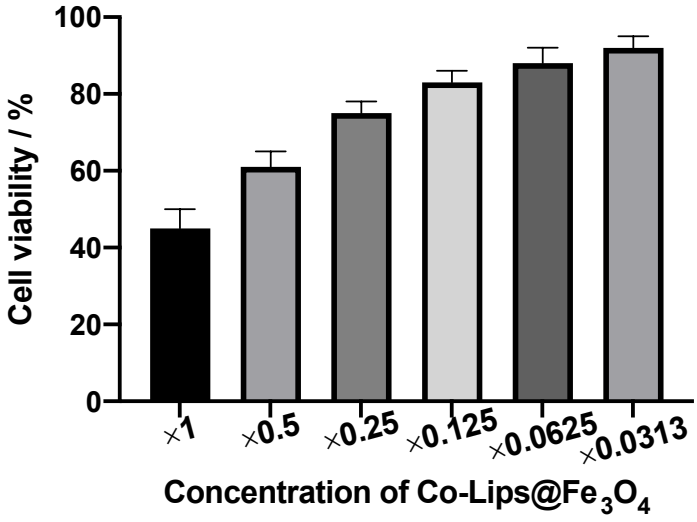

Fig. S12 Cell viability of HeLa cells treated with Co-Lips@Fe_3O_4 with different concentration.

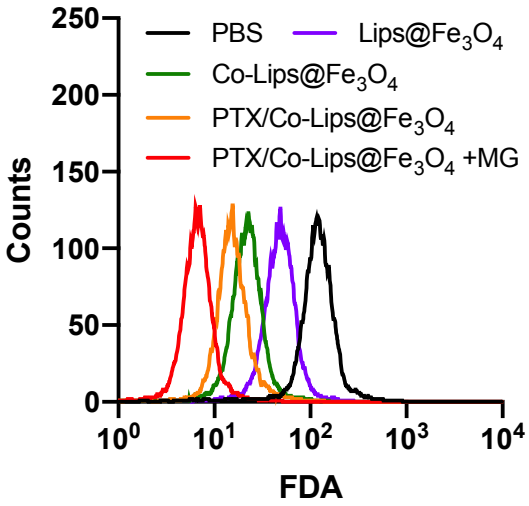

Fig. S13 Flow cytometry analysis of PDA-positive HeLa cells treated with PBS, Lips@Fe_3O_4, Co-Lips@Fe_3O_4 and PTX/Co-Lips@Fe_3O_4 with and without MG.

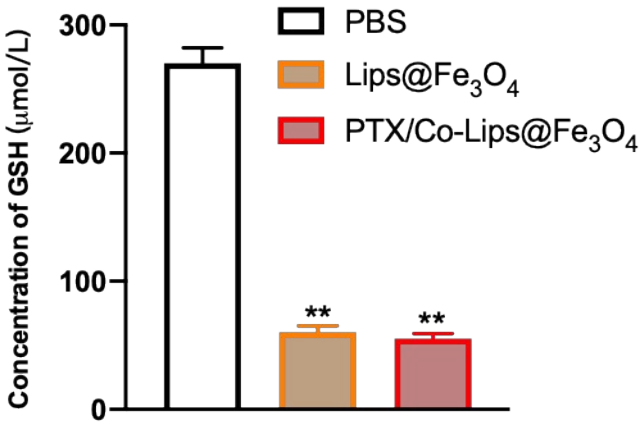

Fig. S14 Concentration of intracellular GSH of PBS, Lips@Fe_3O_4 and PTX/Co-Lips@Fe_3O_4 (**P<0.01 compared with PBS group).

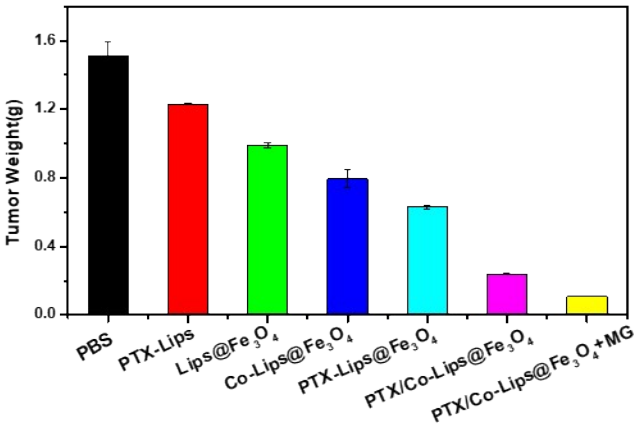

Fig. S15 Tumor weight of all tested groups (PBS, PTX-Lips, Lips@Fe_3O_4, Co-Lips@Fe_3O_4, PTX-Lips@Fe_3O_4, PTX/Co-Lips@Fe_3O_4 and PTX/Co-Lips@Fe_3O_4 with MG).