Supplementary Information

Supramolecular self-assembled gold nanoparticle clusters for synergistic photothermal-chemo tumor therapy

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1. Materials

β-cyclodextrin (β-CD), triethylamine (TEA), dichloromethane (DCM), and trifluoroacetic acid (TFA) were purchased from Sinopharm Chemical Reagent Co. Ltd (Shanghai, China). 2-Naphthaleneacetic acid (2-NAA) and di-tert-butyl dicarbonate were obtained from Aladdin Reagent Co. Ltd (China). 1,8-Diamino-3,6-dioxaoctane (DADO), *N*-hydroxysuccinimide, *N*-(3-Dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDC·HCl), and 1-hydroxybenzotriazole (HOBT) were purchased from Energy Chemical Co. Ltd (China). All other solvents and reagents were purchased from Sinopharm Chemical Reagent Co. Ltd (Shanghai, China).

2. Synthesis of mono-Boc-protected 1,8-diamino-3,6-dioxaoctane (^{m}Boc-DADO)

DADO (5 mL, 34.1 mmol) was dissolved in DCM (15 mL) and stirred in an ice bath under N_2 atmosphere. DCM solution (10 mL) containing di-tert-butyl dicarbonate (7.4 g, 34.1 mmol) and TEA (6.5 mL, 50.2 mmol) was then added dropwise. After stirring at room temperature for 3 h, the solution was extracted with water and brine. The organic phase was collected and evaporated to obtain the ^{m}Boc-DADO. Yield (80%, colorless transparent oily liquid). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 5.21 (s, 1H, -NH), 3.62 (t, 2H, -CH_2), 3.58-3.51 (m, 6H, -CH_2), 3.38-3.25 (m, 2H, -CH_2), 2.91-2.86 (t, 2H, -CH_2), 1.83-1.78 (br b, 2H, -NH_2), 1.44 (s, 9H, -CH_3).

3. Synthesis of *N*-naphthaleneacetyl-1,8-diamino-3,6-dioxaoctane (NAD)

2-NAA (1 g, 5.4 mmol), HOBT (0.73 g, 5.4 mmol), EDC·HCl (2.1 g, 10.8 mmol), and TEA (0.75 mL, 5.4 mmol) were dissolved in DCM (10 mL) and stirred in an ice bath for 1 h to active the carboxyl group.Then, ^{m}Boc-DADO (2 g, 8.1 mmol) was added

into the mixture, which was stirred overnight under N_2 atmosphere. The resulting solution was extracted with water and brine, and the organic phase was collected, dried over anhydrous $MgSO_4$. The product of Boc-NAD was purified by column chromatography (silica gel, ethyl acetate / DCM, 1/1, v/v). Yield (73%, white solid). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.88-7.69, 7.52-7.35 (m, 7H, Ar-H), 5.98 (br s, 1H, -NH), 4.93 (br s,1H, -NH), 3.75 (s, 2H, -CH_2), 3.54-3.32 (m, 10H, -CH_2), 3.24-3.17 (t, 2H, -CH_2), 1.44 (s, 9H, -CH_3).

The amino group of NAD was deprotected by resolving Boc-NAD (1 g, 2.4 mmol) in trifluoroacetic acid (TFA) / DCM (6 mL, 1/1, v/v) solution in an ice bath for 60 min. The solution was then extracted with saturated NaCl solution, dried over anhydrous $MgSO_4$, and concentrated in vacuo to obtain the product. Yield (65%, white solid). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.86-7.72, 7.52-7.38 (m, 7H, Ar-H), 6.32 (br s, 1H, -NH), 3.73 (s, 2H, -CH_2), 3.53-3.35 (m, 10H, -CH_2), 2.79-2.74 (t, 2H, -CH_2), 2.01 (s, 2H, -NH_2).

4. Synthesis of thiol-modified cyclodextrin (CD-SH)

β-CD (50 g, 44.1 mmol) was suspended in deionized water (450 mL), and NaOH solution (80 mL, 2.5 mol/L) was added dropwise into the mixture, which was stirred for 30 min after the solution turned apparent. Acetonitrile solution (6 mL) containing *p*-toluenesulfonylmethyl chloride (10.4 g, 54.6 mmol) was then added to the solution dropwise, and the mixture was stirred at room temperature for 3 h. The precipitate was removed, and the pH of the filtrate was adjusted to 8 with NH_4Cl. The filtrate was placed into a refrigerator of 4 °C overnight to precipitate mono-6-*p*-toluenesulfonyl-CD

(CD-OTs). The crude product was purified by recrystallizing for three times from deionized water. Yield (16%, white crystal).

CD-OTs (3 g, 2.3 mmol) and thiourea (2.5 g, 32.8 mmol) were dissolved in 80% methanol/water solution (200 mL) and refluxed for two days. The resulting solution was concentrated and followed by precipitated with acetone. The obtained white precipitate was dissolved in NaOH solution (10 wt%) and stirred at 50 °C for 5 h. After the reaction, the solution was cooled to room temperature, and the pH was adjusted to 2 with dilute HCl. Trichloroethylene (5 mL, 55.7 mmol) was then added, and the mixture was stirred overnight. The white precipitate was filtered and dissolved into boiling water. The solution was concentrated to 30 mL to remove excess trichlorethylene, and ethanol (70 mL) was added to precipitate the product CD-SH. Yield (6.4%, white crystal). ^{1}H NMR (DMSO-*d6*, 400 MHz) δ: 5.88-5.55 (m, 14H, OH-2,3), 4.92-4.77 (br d, 7H, H-1), 4.55-4.27 (m, 6H, OH-6), 3.79-3.48 (m, 26H, H-3,5,6), 3.45-3.20 (m, overlapping with HDO, OH-2,4), 3.02-2.71 (m, 2H, H-6), 2.04-1.92 (m, 1H, -SH).

Table S1. Regulation of the average size of AuNCs by adjusting the molar ratios of host/guest molecules.

	Molar ratios of β-CD/2-NAA							
	4:1	2:1	4:3	1:1	4:5	2:3	4:7	1:2
Average size (nm)	167.1±22.5	318.0±21.3	195.2±8.3	147.9±5.9	184.4±7.4	268.4±16.2	284.2±13.7	293.4±12.3
PDI	0.497	0.358	0.348	0.213	0.384	0.421	0.442	0.401

Table S2. ζ-potential of AuNPs, AuNPs-CD, and AuNCs.

	AuNPs	AuNPs-CD	AuNCs
ζ-potential (mV)	-41 ± 1.4	-3.2 ± 0.5	-23 ± 2.6

Table S3. Drug encapsulation efficiency (EE) and drug loading lever (DL) of DOX@AuNCs.

	EE (%)	DL (%)
DOX@AuNCs	92.2 ± 3.2	2.9 ± 0.4

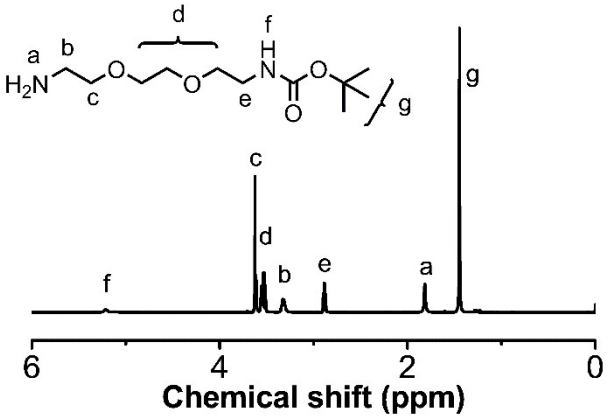

Fig. S1 Chemical structure and ^{1}H NMR spectrum of ^{m}Boc-DADO in $CDCl_3$.

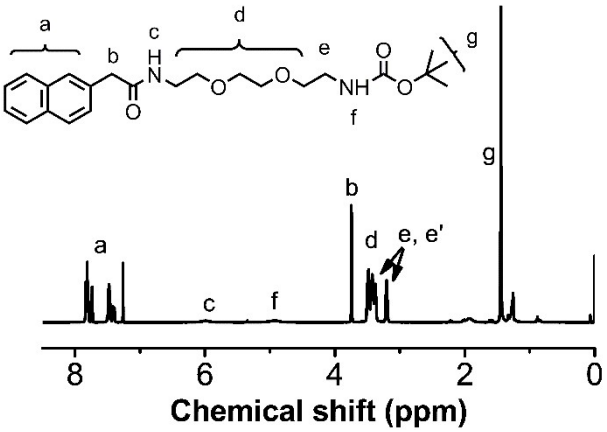

Fig. S2 Chemical structure and ^{1}H NMR spectrum of Boc-NAD in $CDCl_3$.

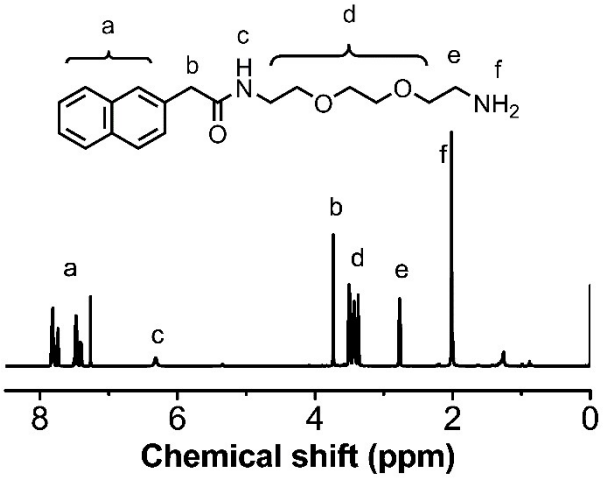

Fig. S3 Chemical structure and ^{1}H NMR spectrum of NAD in $CDCl_3$.

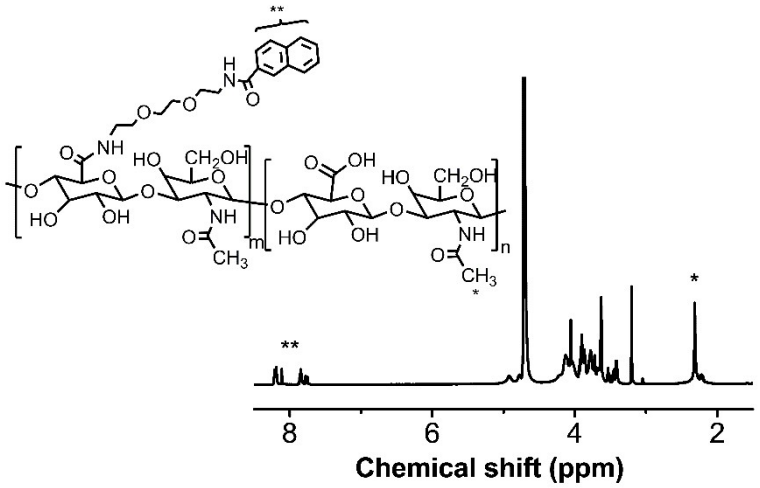

Fig. S4 Chemical structure and ^{1}H NMR spectrum of HAN in CD_3OD/D_2O (1:1).

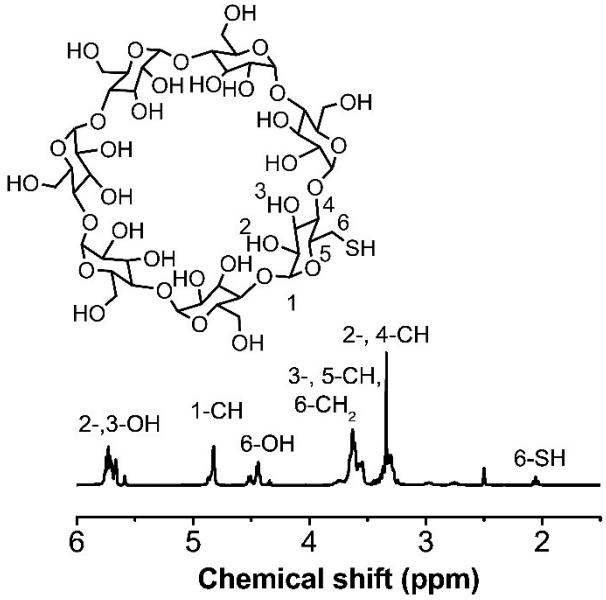

Fig. S5 Chemical structure and ^{1}H NMR spectrum of CD-SH in DMSO-*d6*.

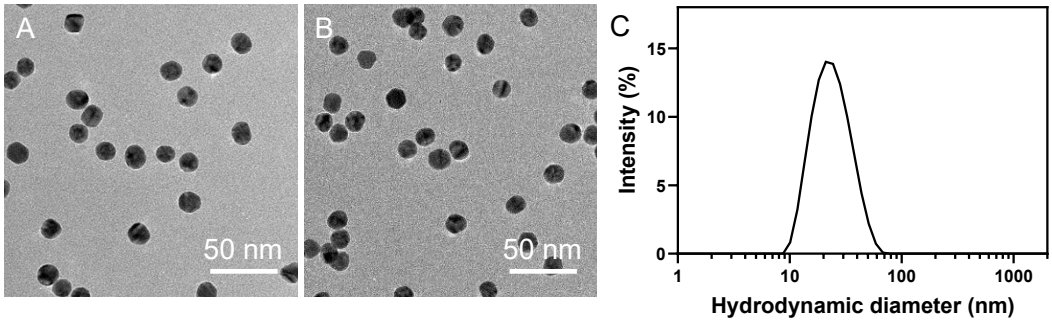

Fig. S6 TEM images of (a) AuNPs and (b) AuNPs-CD. (c) Size distribution of AuNPs-CD.

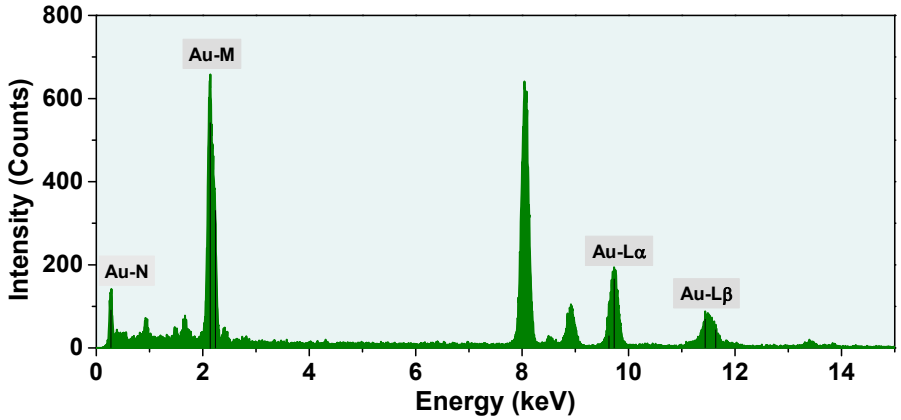

Fig. S7 Energy dispersive X-ray spectroscopy (EDS) spectrum of the AuNCs.

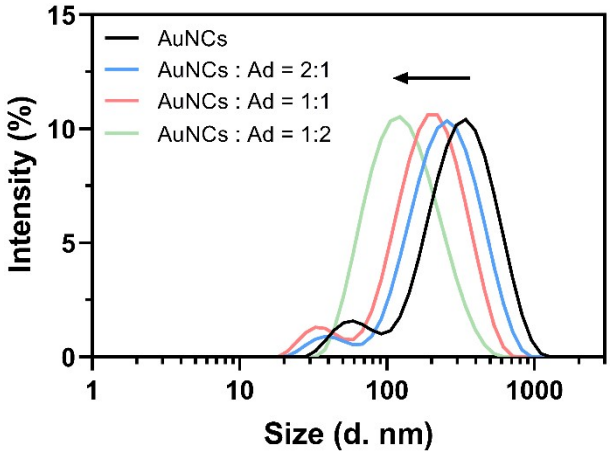

Fig. S8 The size variation of AuNCs with the addition of adamantanamine (Ad).

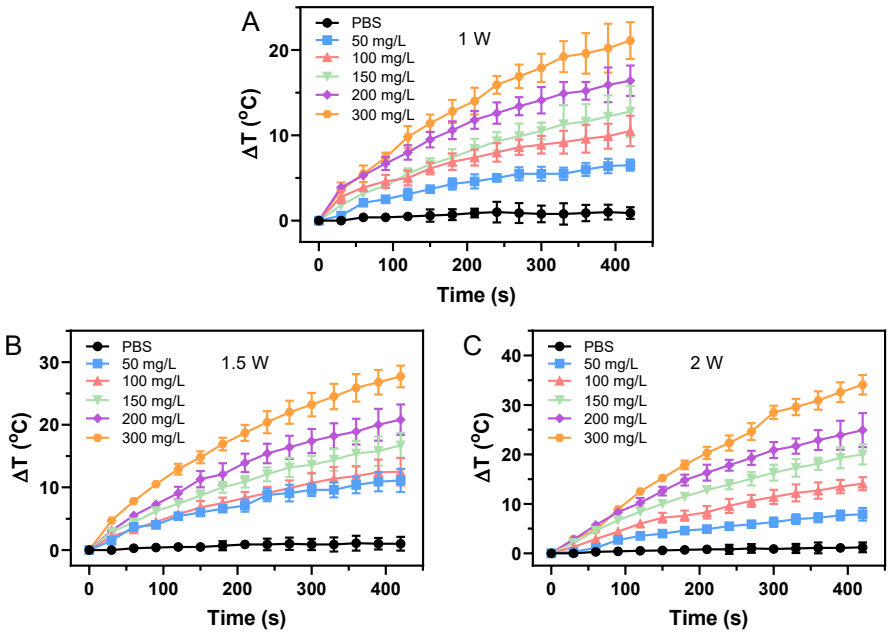

Fig. S9 The NIR-induced hyperthermia of AuNCs with different irradiation times, concentrations, and powers: (a) 1, (b) 1.5, and (c) 2 W/cm^2.

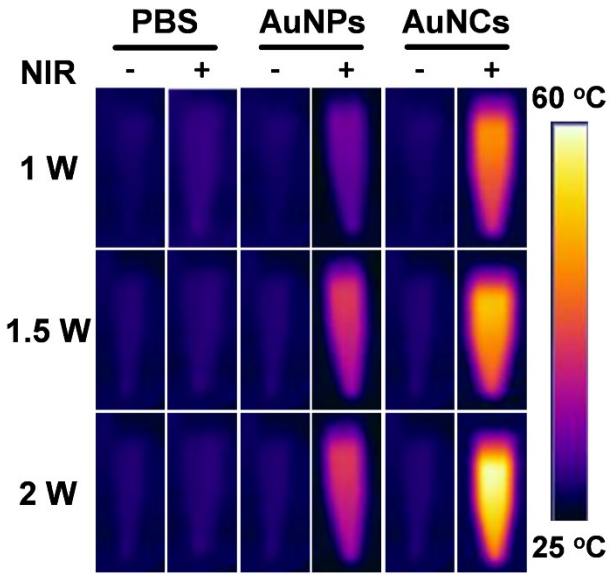

Fig. S10 Real-time thermal images of PBS, AuNPs, and AuNCs (200 mg/L) irradiated with (+) or without (-) the NIR laser (808 nm, 1, 1.5, and 2 W/cm^2 for 5 min).

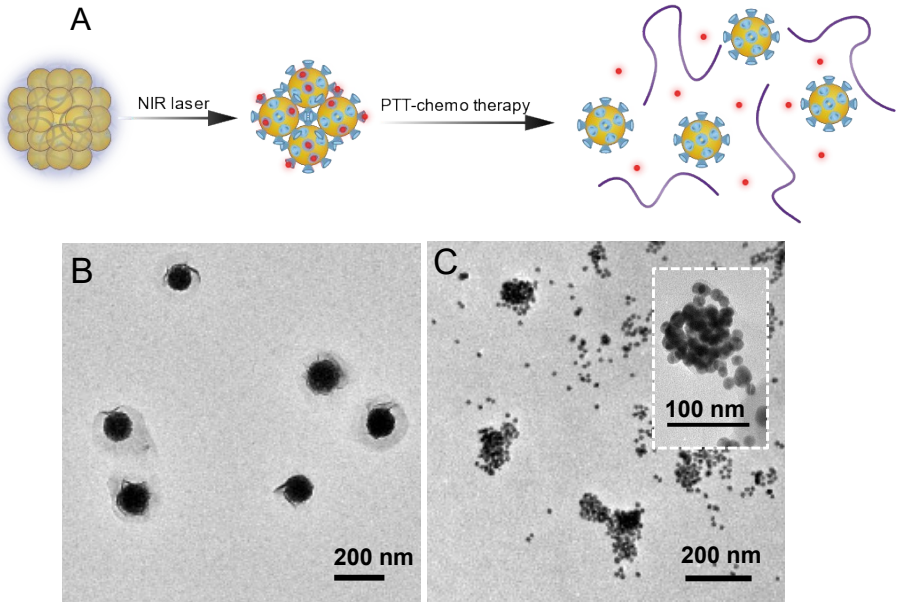

Fig. S11 (A) Schematic illustration of NIR laser-triggered disassembly and accelerated DOX release. TEM images of DOX@AuNCs (B) before and (C) after NIR irradiation for 10 min.

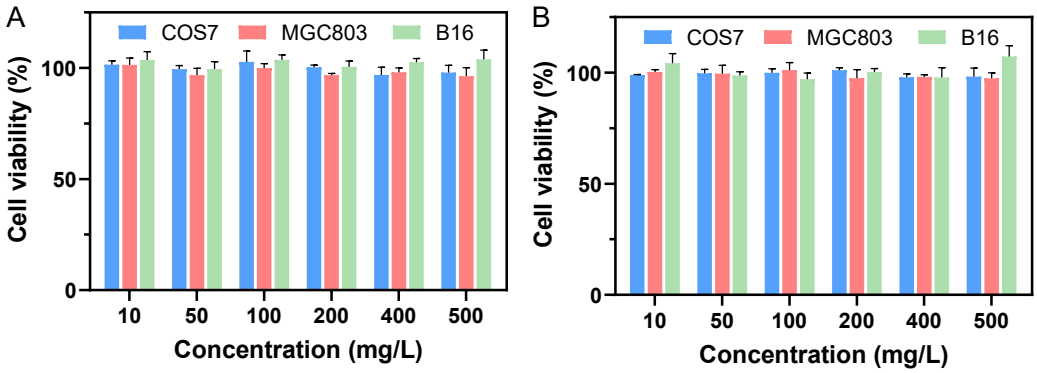

Fig. S12 Cytotoxicity of (A) AuNPs and (B) AuNCs against COS7, MGC803, and B16 cells. Error bar indicated the mean ± SD (n = 3).

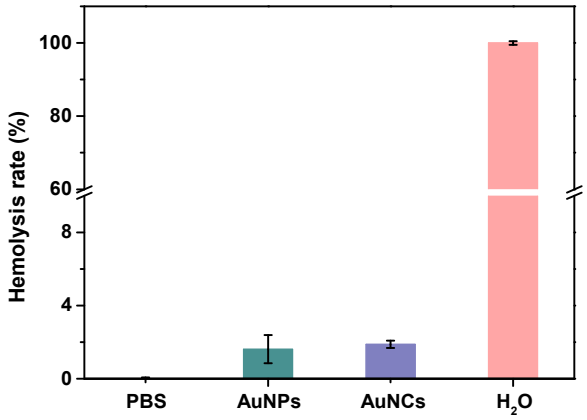

Fig. S13 Hemolysis rates of AuNPs and AuNCs.

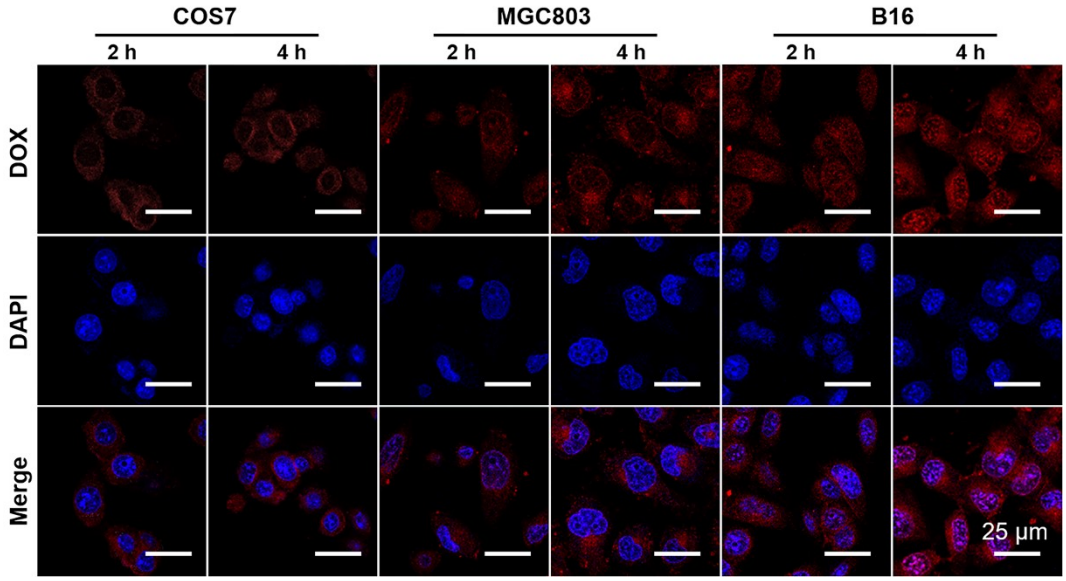

Fig. S14 The CLSM images of COS7, MGC803, and B16 cells incubated with DOX@AuNCs for 2 and 4 h. The fluorescence signals represent the DOX (red) internalized into the cells and nucleus (blue) stained with DAPI.

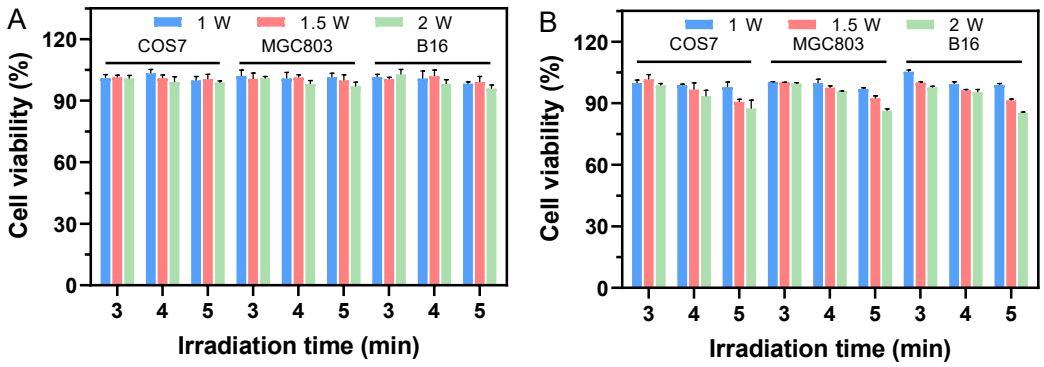

Fig. S15 Photothermal cytotoxicity of (A) NIR light and (B) AuNPs against COS7, MGC803, and B16 cells. Error bar indicated the mean ± SD (n = 3).

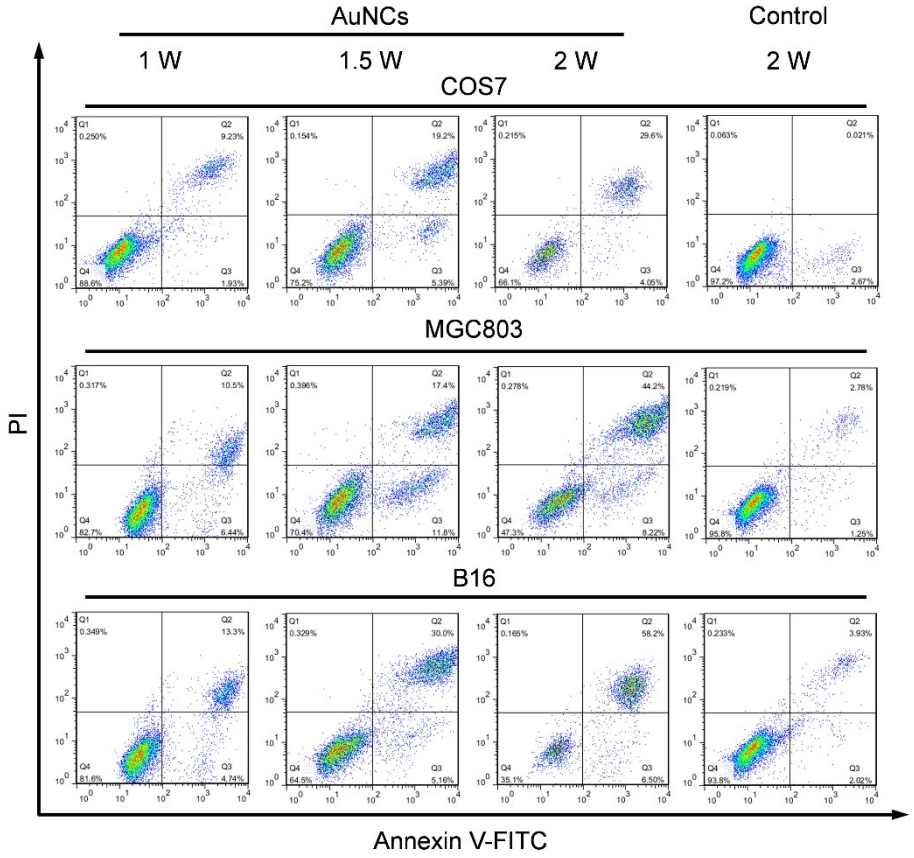

Fig. S16 Flow cytometry analysis of cell apoptosis of COS7, MGC803, and B16 cells incubated with AuNCs (200 mg/L), and then exposed to NIR light for 5 min with different powers (1, 1.5, and 2 W/cm^2). The control represented the cells were incubated without AuNCs but exposed to NIR light.

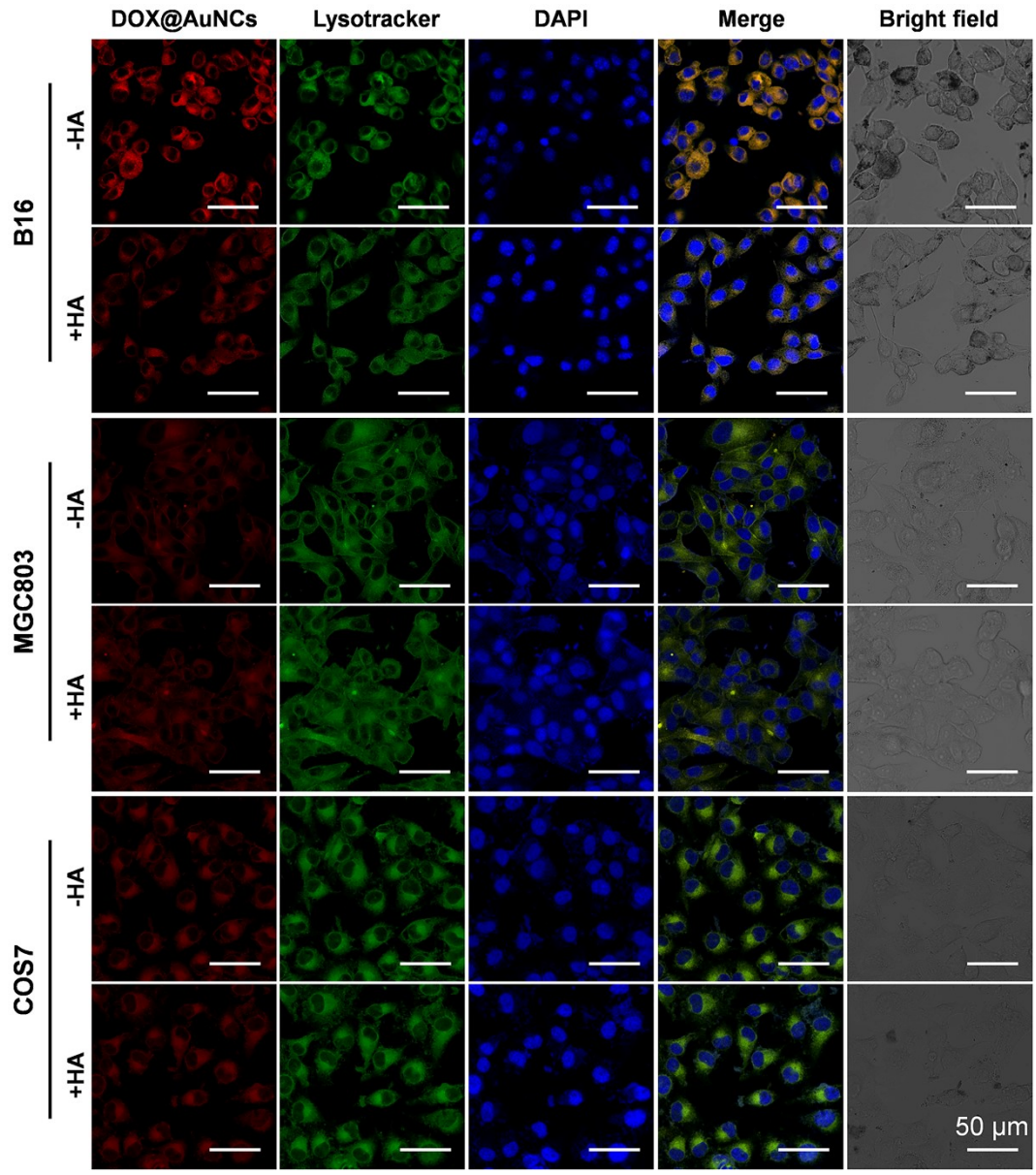

Fig. S17 The CLSM images of B16, MGC803, and COS7 cells incubated with DOX@AuNCs with or without HA pre-treatment.

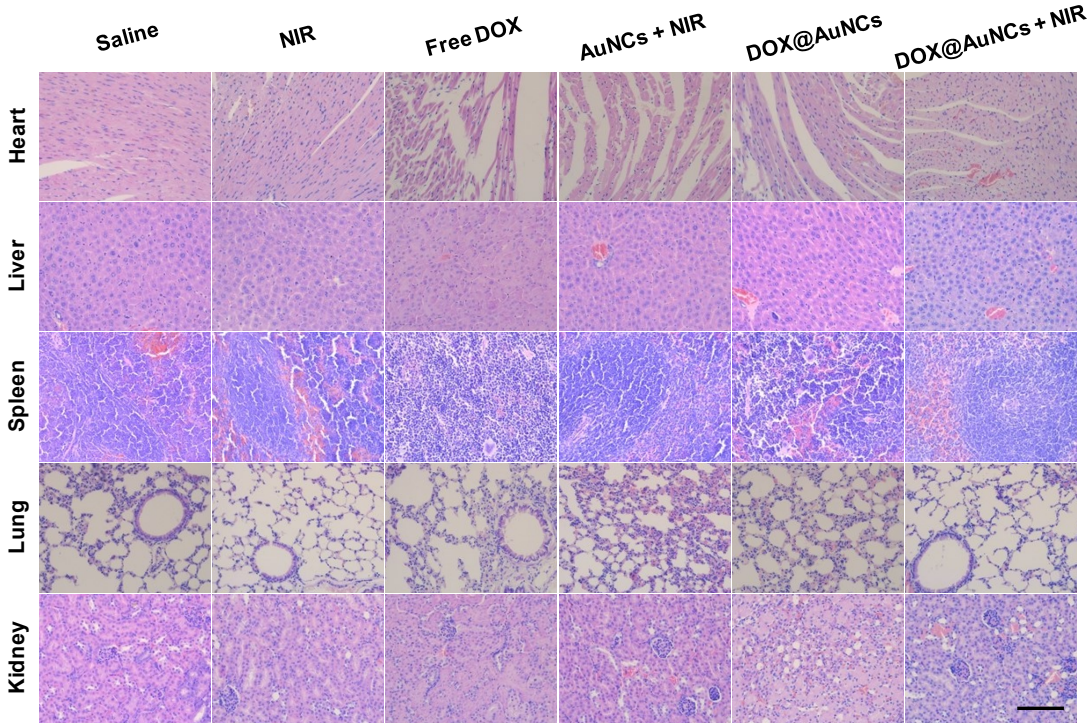

Fig. S18 Representative H&E staining images of major organs including heart, liver, spleen, lung, and kidney collected from tumor-bearing mice after the treatment for two weeks. Scale bar: 100 μm.