Electronic Supplimentary Information

Rational Design of Multi-Stimuli-Responsive Gold Nanorod-Curcumin Conjugates for Chemo-Photothermal Synergistic Cancer Therapy

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Experimental

Cell cycle analysis

For the flow cytometry of cell cycle arrest experiments, A549, HepG2 and KB cells were seeded in 6-well plates at a density of 1×10^4 cells/well and incubated for 24 h. After harvesting by trypsinization, the cells washed with PBS for three times and fixed with cold 70 % ethanol at 4 °C overnight, followed by centrifugation at 2000 rpm for 5 min to remove the ethanol. Then the fixed cells were dispersed in PBS with 1% Triton-100, 1 mg/mL RNase and 5 mg/mL PI, stained at 37 °C for 30 min. The samples were analyzed by flow cytometer and the percentages of cells in G0/G1, S, and G2/M phases were determined by CellQuest software.

Apoptosis Assay

A549, HepG2 and KB cells were seeded in 6-well plates at a density of 2×10^5 cells/well and incubated for 24 h, respectively. Every cell lines were set into chemotherapy alone and combining therapy groups. The chemotherapy group was treated with Au NRs@Curcumin at 0.2 or 0.4 nM concentration, whereas the cells of combining therapy group was added the same amount of drug and exposed to an 808 nm laser irradiation at 0.7 W/cm^2 for 8 min. After incubating for another 48 h, all the cells were collected and stained with Annexin V: FITC Apoptosis Detection Kit I (BD Pharmingen™) according to the manufacturer's instructions. Samples were measured using a FACS caliber bench-top flow cytometer (Becton Dickinson). FlowJo software

(TreeStar) was used for apoptosis analysis.

Cell viability test

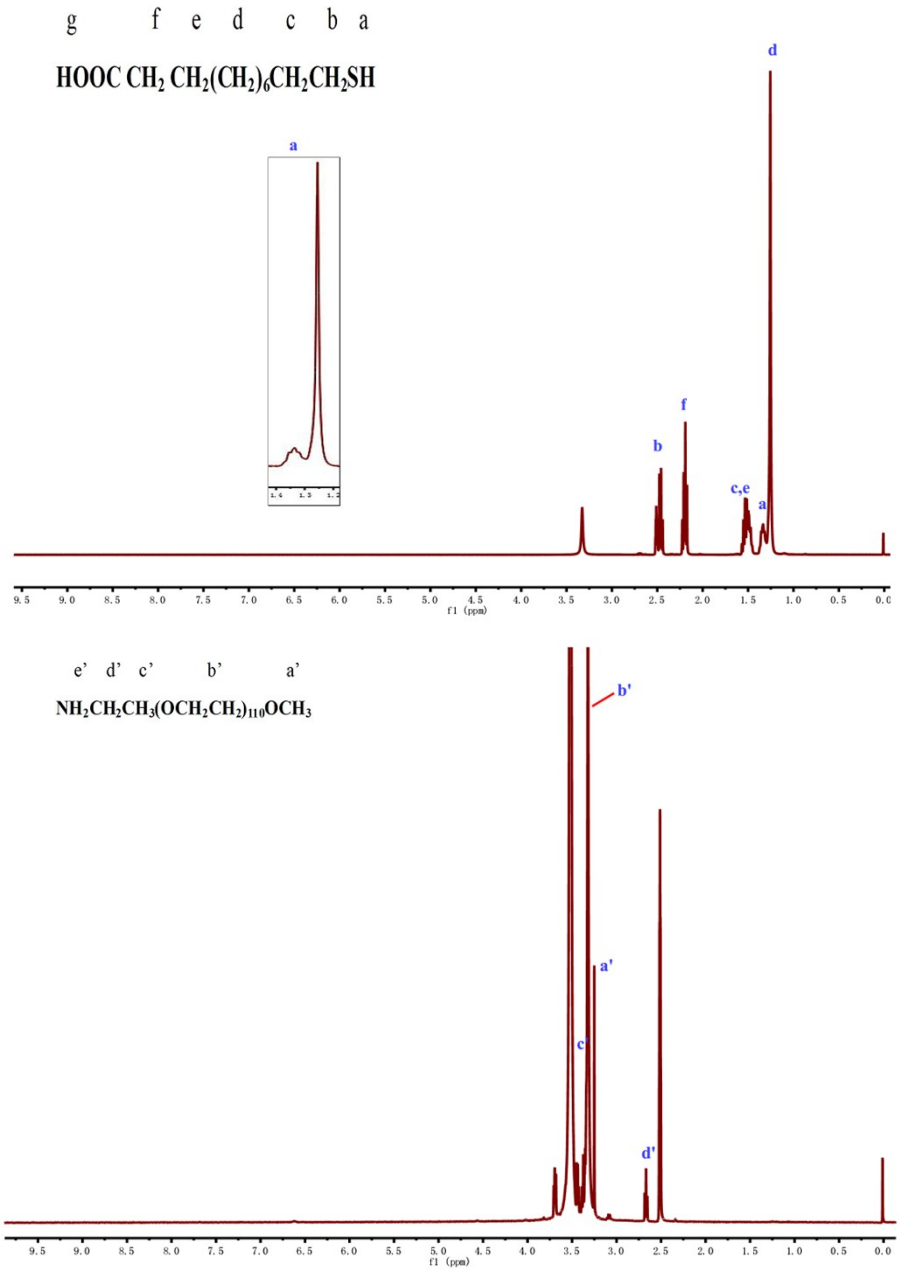
Human liver cell line L-02 was cultured in RPMI 1640 medium supplemented with 10 % fetal bovine serum, penicillin and streptomycin. The cells was seeded in a 96-well cell culture plate at a density 8×10^3 per well in 100 μL medium. After 24 h of seeding, the culture medium was replaced with fresh medium and the cells were exposed to gradient concentrations (5–80 μM, equivalent concentration of curcumin) suspensions of Au NR@Curcumin for 48 h in standard conditions. The experimental medium was discarded and the cells washed with PBS for three times. Then the cells were incubated for further 1~1.5 h with 20 μL of cck-8 solution and 200 μL of culture medium, and the absorbance was measured at 450 nm.

Haemolysis assay

For the haemolysis assay, human whole blood samples were added the fresh EDTA to stabilize and washed with PBS via three rounds of centrifugation (500g, 10 min) to isolate RBCs. Then the samples were treated with different concentrations of Au NR@Curcumin range from 50 to 800 pM on a rocking shaker in an incubator at 37 °C for 3 h. After treatment, the samples were centrifuged at 12000 rpm for 5 min. The absorbance of supernatant was measured at 540 nM against blank using a microplate reader. Total lysis was achieved by adding Triton X-100 (0.05% (v/v)) into wells to lyse all the human RBCs to release haemoglobin into medium.

Table. S1 Phenolic content assay results for unmodified and post-release curcumin.

Sample	Total phenolic content(mg GAE/g)
Curcumin	652.60±11.02
MUA-curcumin conjugates	386.76±5.92
Extracted Curcumin	559.06±12.29



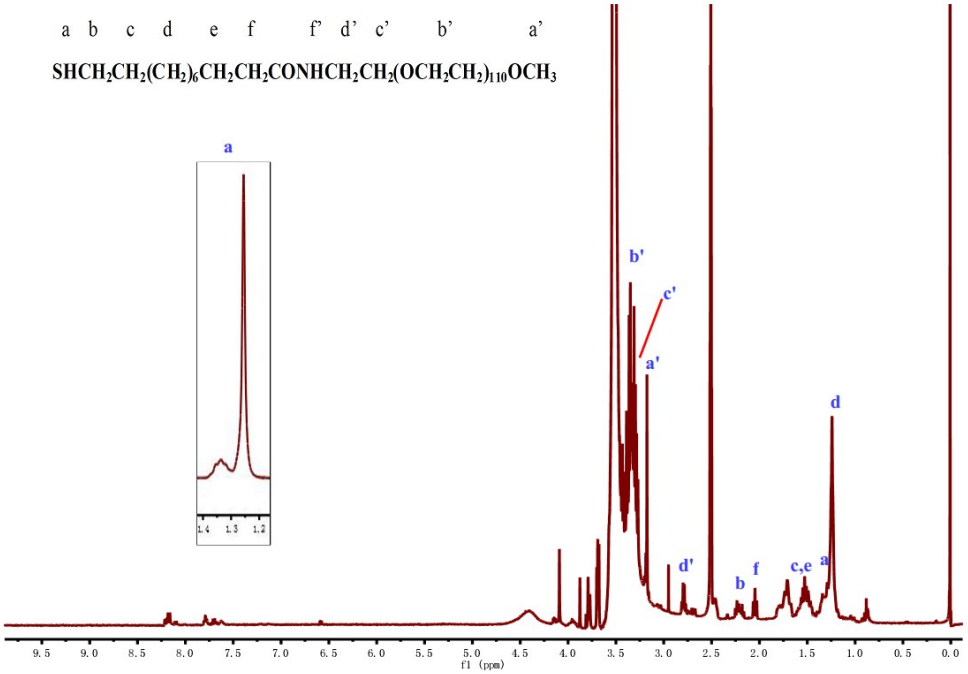


Fig. S1. ^{1}H NMR spectrum of MUA, NH_2-PEG and MUA-PEG.

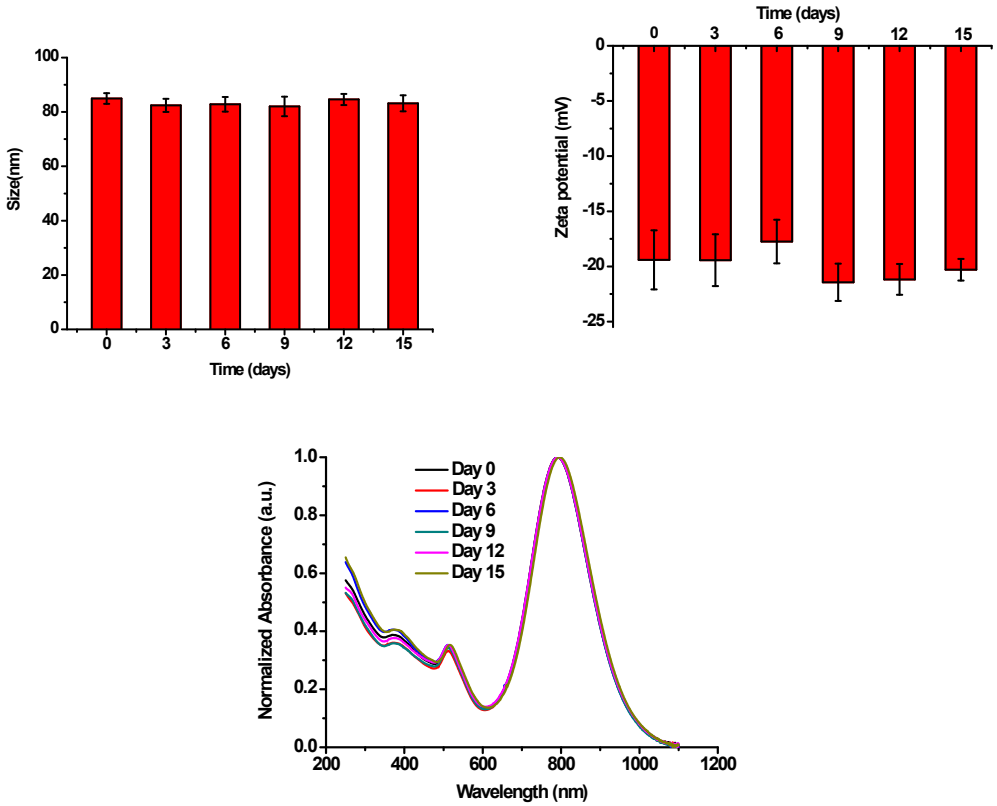


Fig. S2. The stability of Au NRs@Curcumin in PBS. (A) Size (nm) in PBS, (B) Zeta potential (mV) in PBS, (C) The absorption spectroscopy in PBS over 15 days.

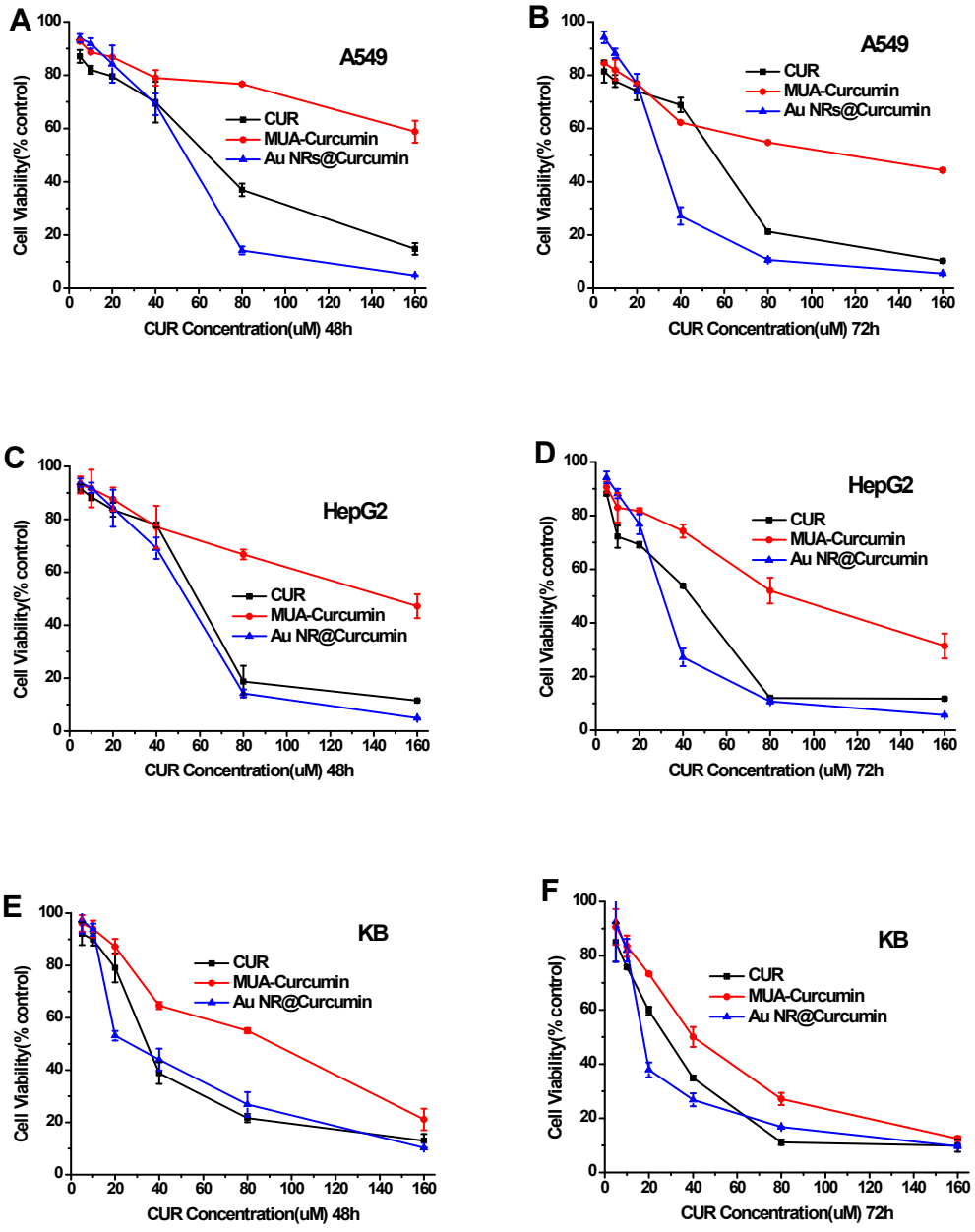
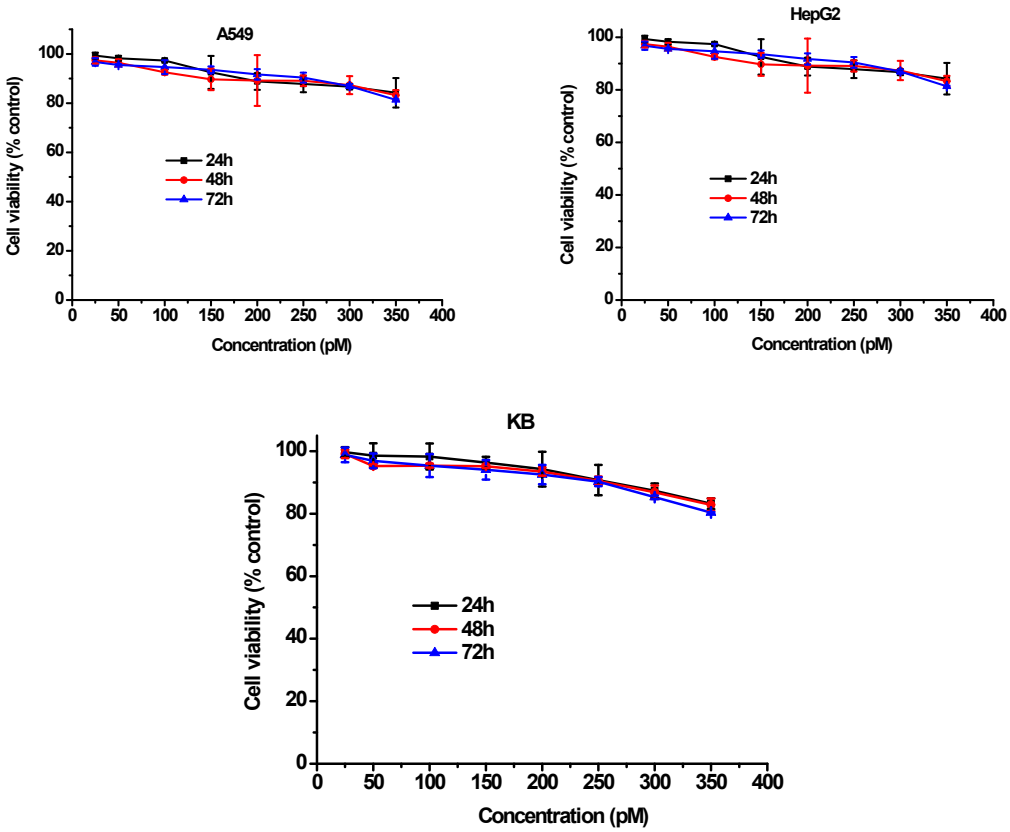


Fig. S3. Cck-8 assay for assessing the viability of A549 incubated for (A) 48 h, (B) 72 h, HepG2 incubated for (C) 48 h, (D) 72 h, KB incubated for (E) 48 h, (F) 72 h, with free curcumin, MUA-curcumin conjugates and Au NR@Curcumin. The results are the means ± SD from three independent experiments. The concentrations of MUA-curcumin conjugate and Au NR@Curcumin are expressed as equivalent curcumin concentration.



(A)

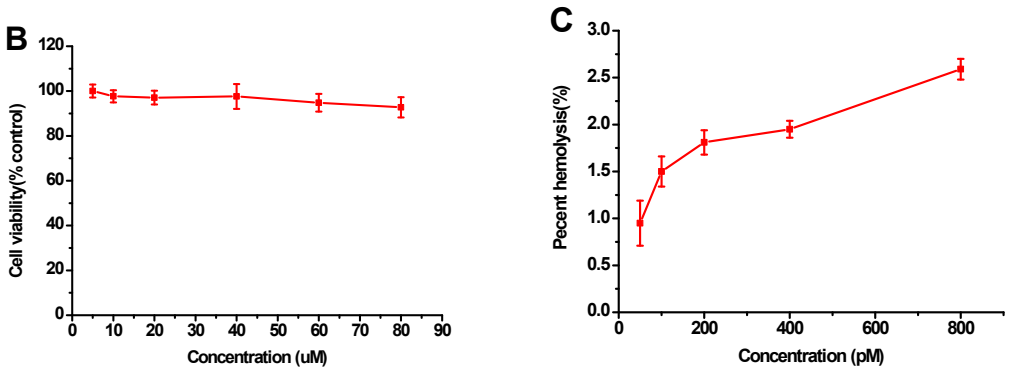


Fig. S4. (A) Cell viability of the Au NR@PEG at different concentrations in three cell lines (A549, HepG2 and KB); (B) Cell viability of the Au NR@Curcumin at different concentrations in human liver cell line L-02; (C) Percent hemolysis of RBCs incubated with different concentrations (50 to 800 pmol) for 3 h at 37°C with agitation. Data were presented as the mean ± SEM of triplicate experiments.

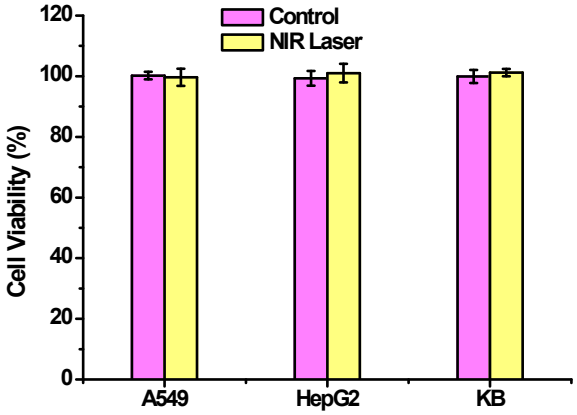


Fig. S5. Cell viability of the NIR groups in three cell lines (A549, KB and HepG2).

Table. S2 Calculated fraction of cell survival by additive interaction of PPTT and chemotherapy using Au NR@Curcumin

Cell line	Au NR@Curcumin concentration(pM)	$f_{combination}$	$f_{additive}$
A549	50	71.83	78.02
	100	45.51	75.23
	200	30.15	65.06
	400	18.26	21.66
HepG2	50	57.24	63.54
	100	46.45	56.71
	200	34.97	43.35
	400	11.78	25.11
KB	50	74.83	75.28
	100	57.64	59.00
	200	25.38	28.52
	400	5.08	7.52

$$f_{\text{additive}} = f_{\text{PPTT}} \times f_{chemotherapy}$$

Where $f_{additive}$ is the fraction of surviving cells by additive interaction of PPTT and chemotherapy, f_{PPTT} is the fraction of surviving cells resulting from PPTT treatment, and $f_{chemotherapy}$ is the fraction of surviving cells resulting from chemotherapy.

When the fraction of surviving cells from the combination treatment, $f_{combination}$, is lower than $f_{additive}$, there is a synergistic effect.

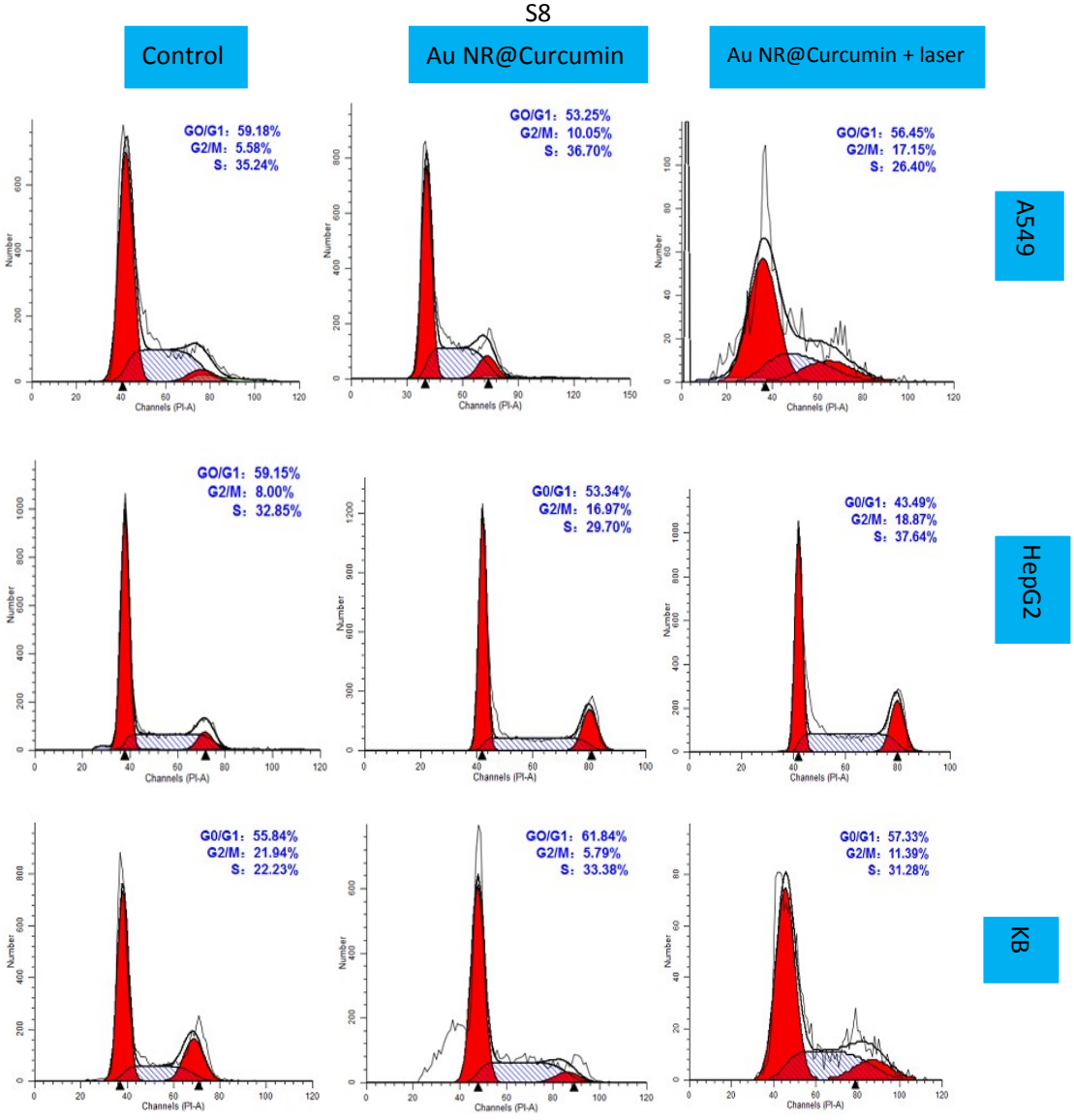


Fig. S6. Cell cycle analysis of A549, HepG2 and KB cells treated with Au NR@Curcumin with or without laser irradiation.