Supporting Information:

Cyanine-Curcumin Assembling Nanoparticles for Near-Infrared Imaging and Photothermal Therapy

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The cellular experiment procedure in detail.

Cell culture. CT26 cells and HeLa cells were propagated to confluence in DMEM medium supplemented with 100 U/mL penicillin, 100 μg/mL streptomycin and 10% FBS, and maintained at 37^{o}C in a humidified atmosphere of 5% CO_2 for further cell experiments.

In vitro cell imaging. Cells harvested in a logarithmic growth phase were seeded in 6-well plates at a density of 2.5×10^5 cells/well and incubated in DMEM for 24 h. The medium was then replaced by 2 mL of DMEM containing predetermined concentrations of CCNPs and incubated for 1, 4, 6, 8 h at 37°C, and further washed using PBS for 3 times. Then the cells were fixed with 4% of paraformaldehyde solution for 10 min. After that, DAPI was added for another 5 min incubation to locate the nucleus. Later, the cells were washed with PBS and observed using CLSM (Zeiss LSM 700).

Cellular uptake in vitro. The cells were handled as the protocol in In vitro cell imaging experiment before fixed. Then the cells were harvested with trypsinization and followed by the centrifuge. The cells were disrupted under ultrasonication. The Cyc4 was extracted from the cells using ethanol as solvent and followed by the centrifuge. The supernatant further measured

using UV-vis analysis.

Photothermal cytotoxicity in vitro by MTT Assay. Cells harvested in a logarithmic growth phase were seeded in 96-well plates at a density of 8×10^3 cells per well and incubated in DMEM for 24 h. The medium was then replaced by 200 μL of DMEM containing predetermined concentrations of CCNPs and incubated for 8 h. After removing the original medium, 200 μL of DMEM was added in each well. Later, cells were irradiated by the 808 nm laser at a power of 1.5 W/cm^2 for 5 min. After laser irradiation, the cells were incubated for another 24 h, followed by MTT assays to measure the live cells. Cell viabilities were determined by reading the absorbance of the plates at 490 nm with a microplate reader. The cells incubated with DMEM and no irradiation was used as the control. The cell viability (%) $=A_{sample}/A_{control}\times100\%$.

Calcein-AM/PI Test. Cells harvested in a logarithmic growth phase were seeded in 6-well plates at a density of 2.5×10^5 cells/well and incubated in DMEM for 24 h. The medium was then replaced by 2 mL of DMEM containing various concentrations of CCNPs and incubated for 8 h at 37°C. After removing the original medium, 2 mL of DMEM was added in each well. Later, cells were irradiated by the 808 nm laser at a

power of 1.5 W/cm^2 for 5 min. After laser irradiation, the cells were incubated for another 12 h, followed by staining with Calcein-AM/PI for 30 min and imaged with a confocal fluorescence microscope.

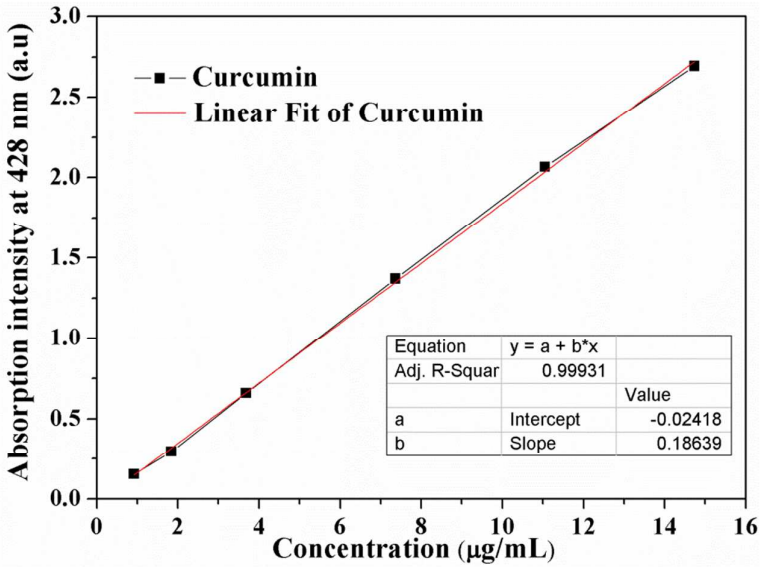

Figure S1. Standard absorbance curve of curcumin. (The absorbance of curcumin molecules at 428 nm (from a mixture of THF and water (v/v =1:1)) as a function of curcumin concentration.)

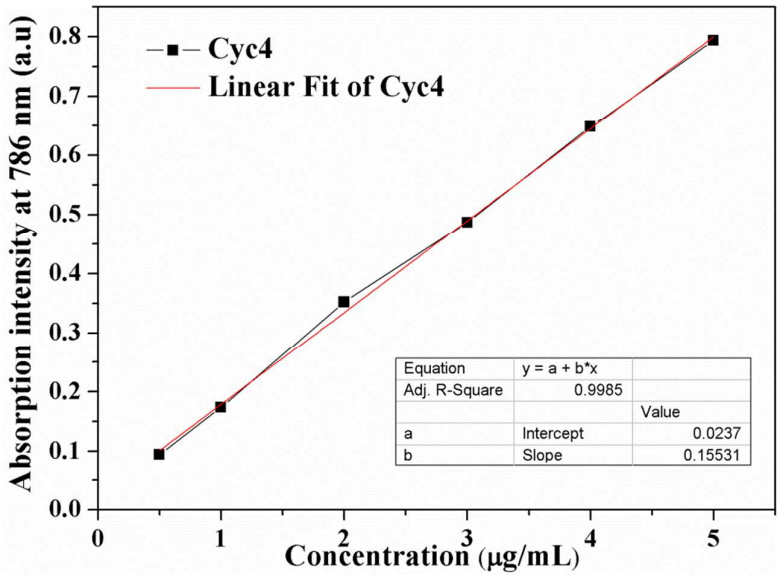

Figure S2. Standard absorbance curve of Cyc4. (The absorbance of Cyc4 molecules at 786 nm (from a mixture of ethanol and water (v/v=4:1)) as a function of Cyc4 concentration.)

Table S1. Composition of the CCNPs

Composition	Curcumin	Cyc4
Weight ratio (wt %)	30.04%	69.96%

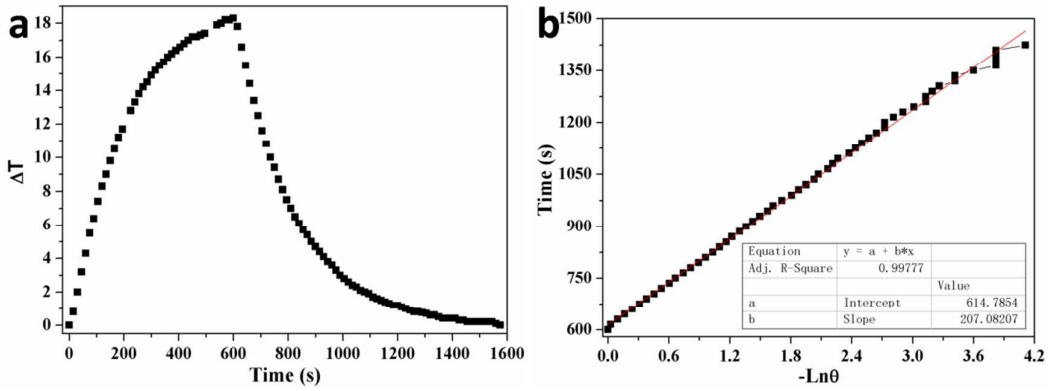

Figure S3. a) The photothermal response of the CCNPs aqueous solution (20 μg/mL) for 600 s with an NIR laser (808 nm, 1.5 W/cm^2) and then the laser was shut off. b) Linear time data versus − ln θ obtained from the cooling period of Figure S3a.

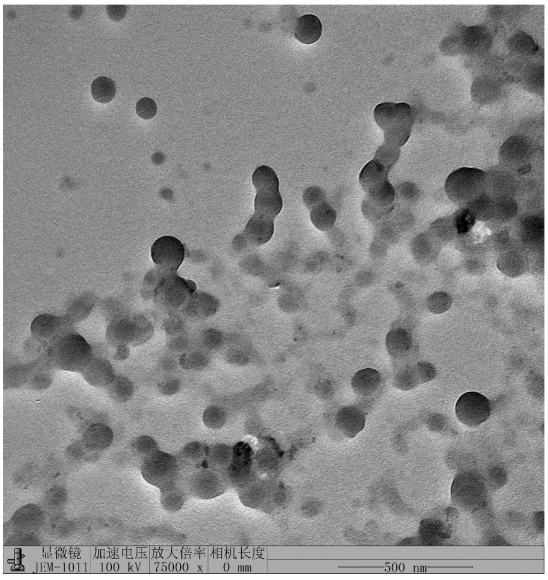

Figure S4.TEM images of CCNPs after four weeks in water.

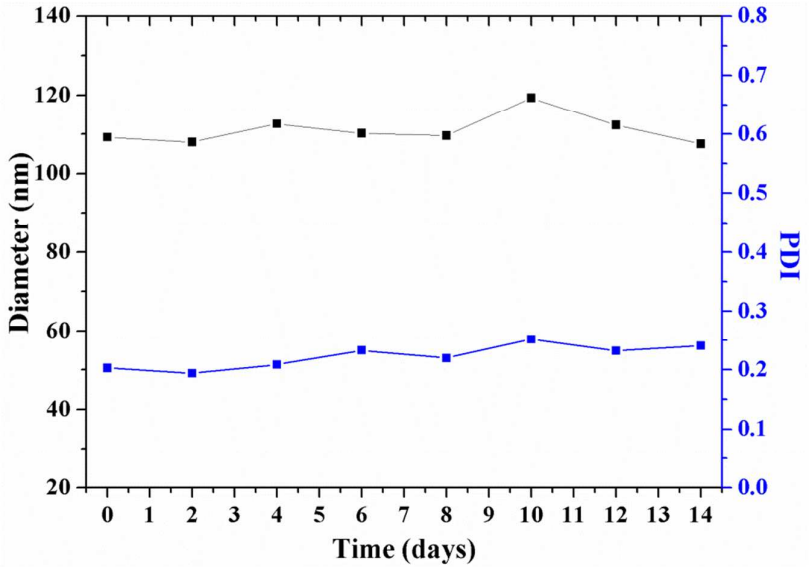

Figure S5. Size-stability of CCNPs dispersed in DMEM supplemented with 10% FBS and 1% penicillin/streptomycin over 14 days.

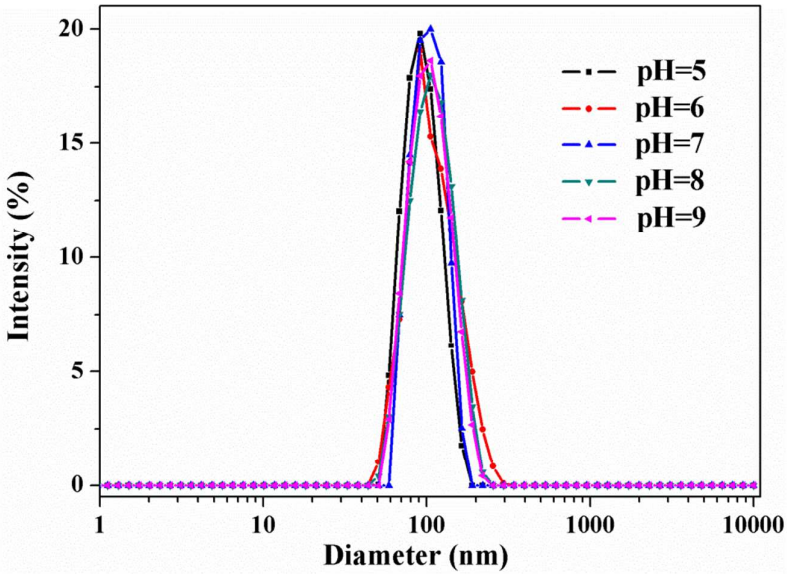

Figure S6. Size of CCNPs in different pH.

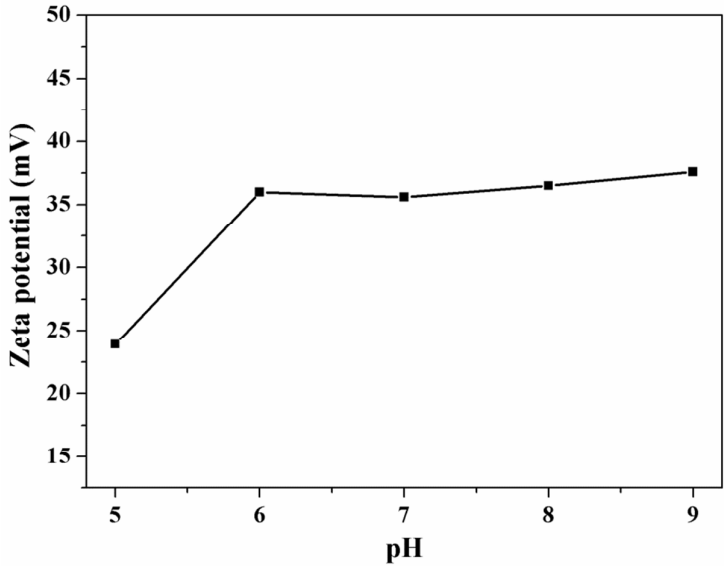

Figure S7. Zeta potential of CCNPs in different pH.

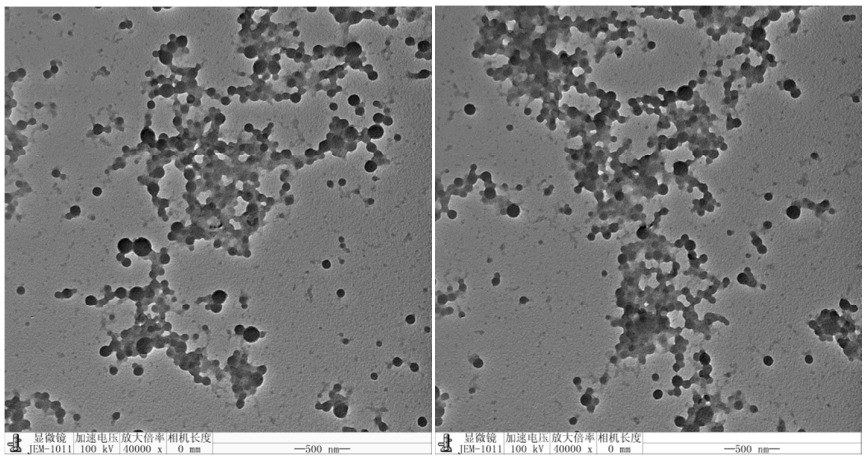

Figure S8. TEM images of CCNPs with or without irradiation (808 nm, 1.5 W/cm^2) for 10 min.

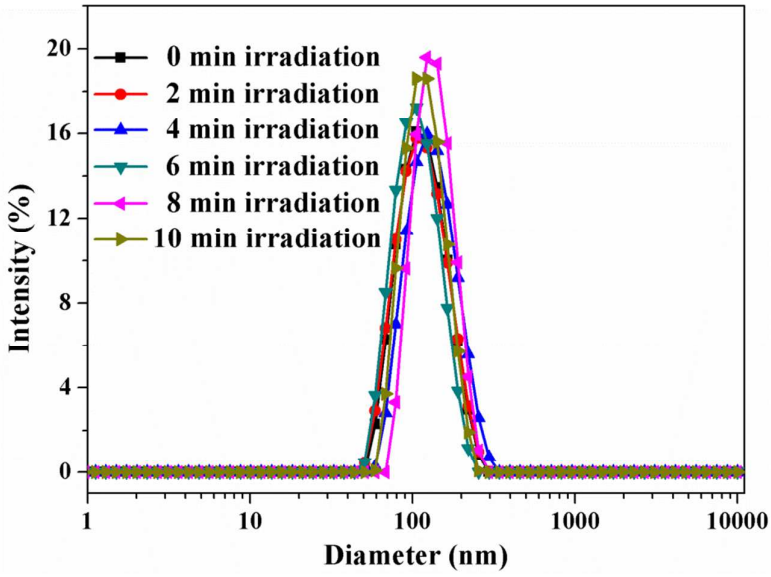

Figure S9. Sizes of CCNPs with different times of irradiation (808 nm, 1.5 W/cm^2)

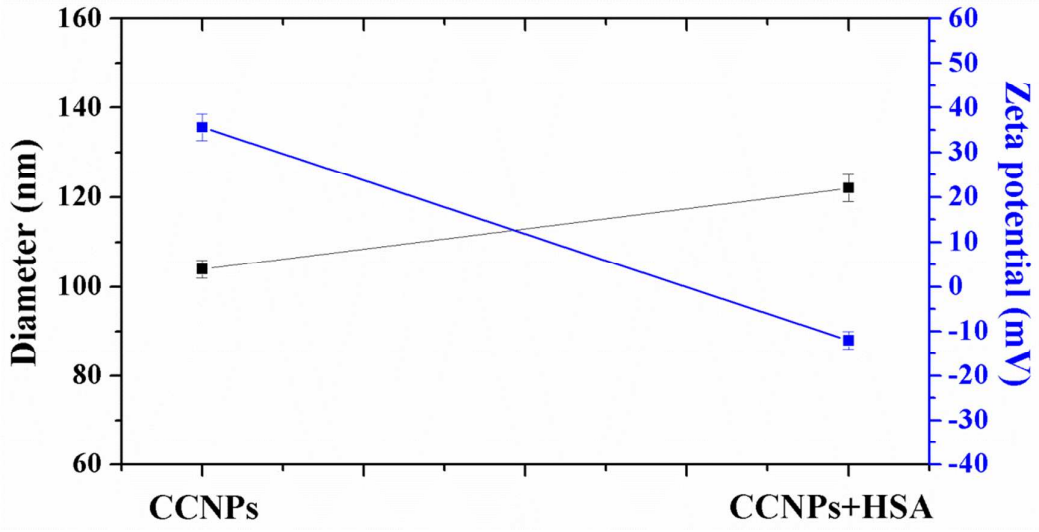

Figure S10. Size and zeta potential of CCNPs incubated with or without 1 mg/mL HSA for 6 h with shaking at 37^{o}C.

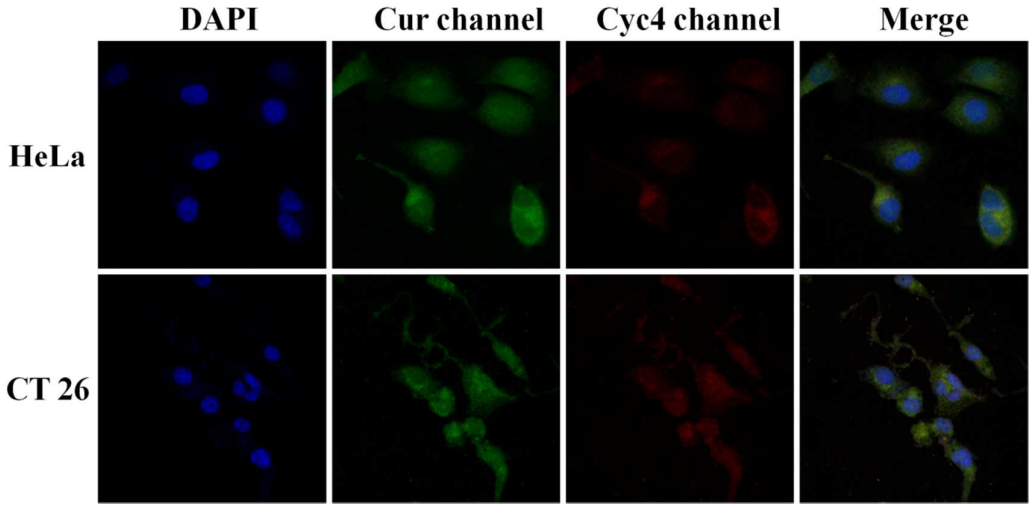

Figure S11. In vitro cell imaging and subcellular localization of CCNPs, monitored by fluorescence imaging in HeLa and CT26 cells.

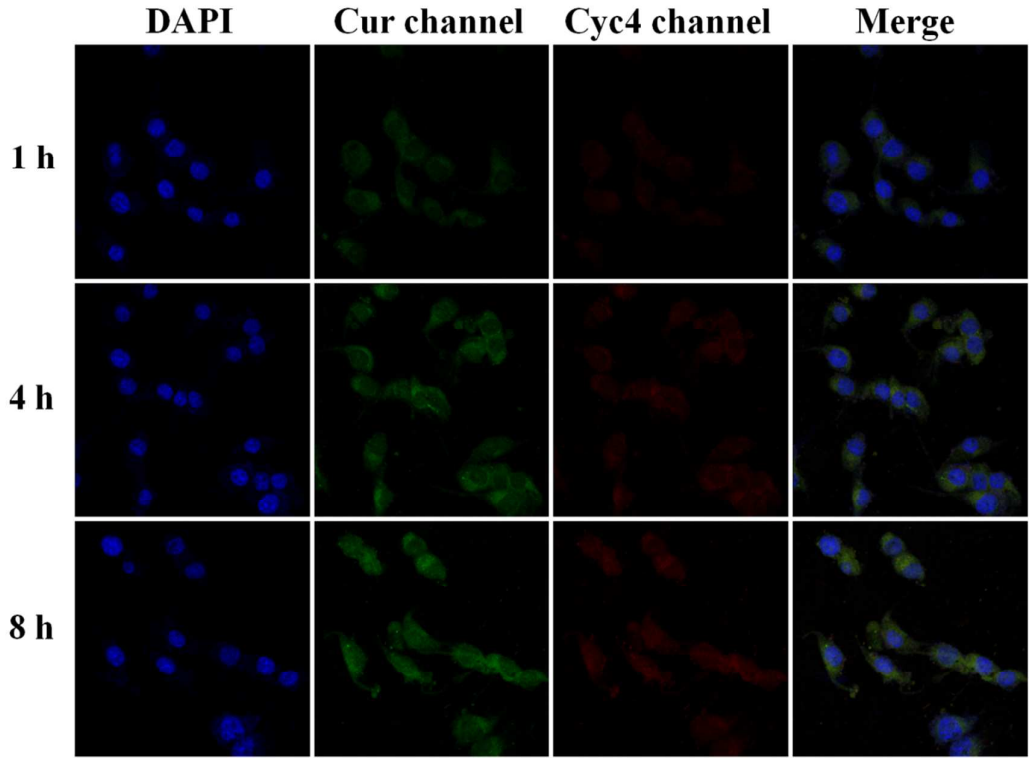

Figure S12. Confocal microscopy images showing changes in the signal of curcumin and Cyc4 in CT 26 cells treated with CCNPs at 1, 4, and 8 h. Different imaging channels are displayed horizontally for each sample (from left to right): DAPI (405 nm excitation), Cur channel (488 nm excitation), Cyc4 channel (639 nm excitation), and merged images.

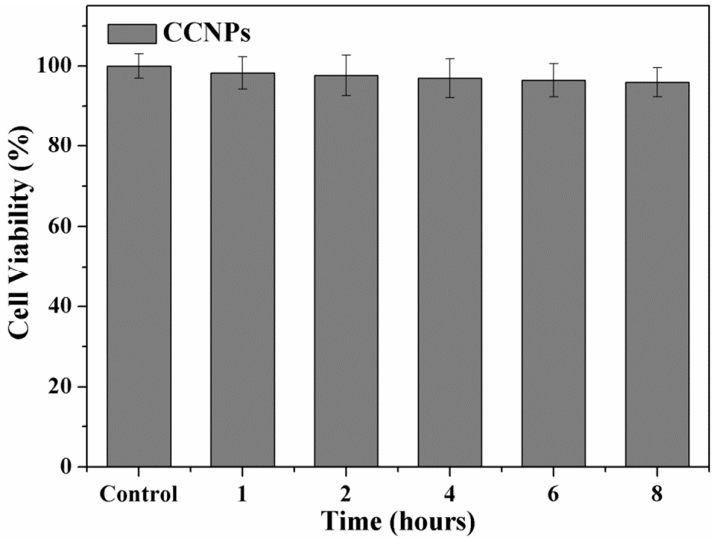

Figure S13. In vitro cytotoxicity of CCNPs incubated in CT26 cells for 1, 2, 4, 6, and 8 h respectively.

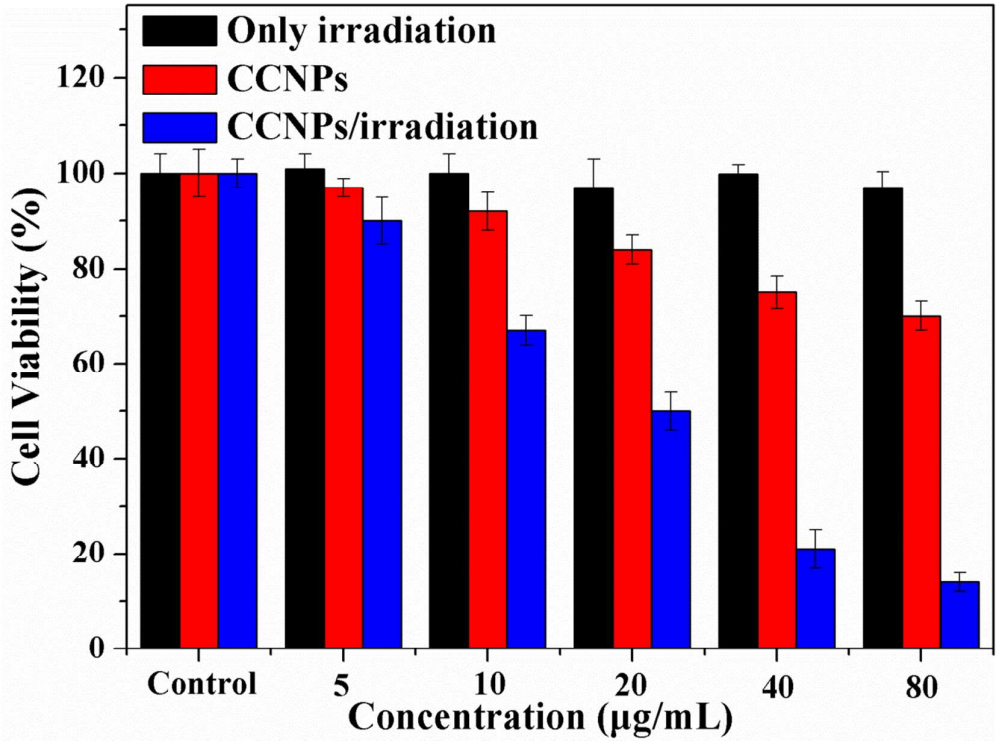

Figure S14. Cytotoxic effects of only irradiation, CCNPs without irradiation, CCNPs with irradiation in CT26 cells with increasing NPs concentrations.

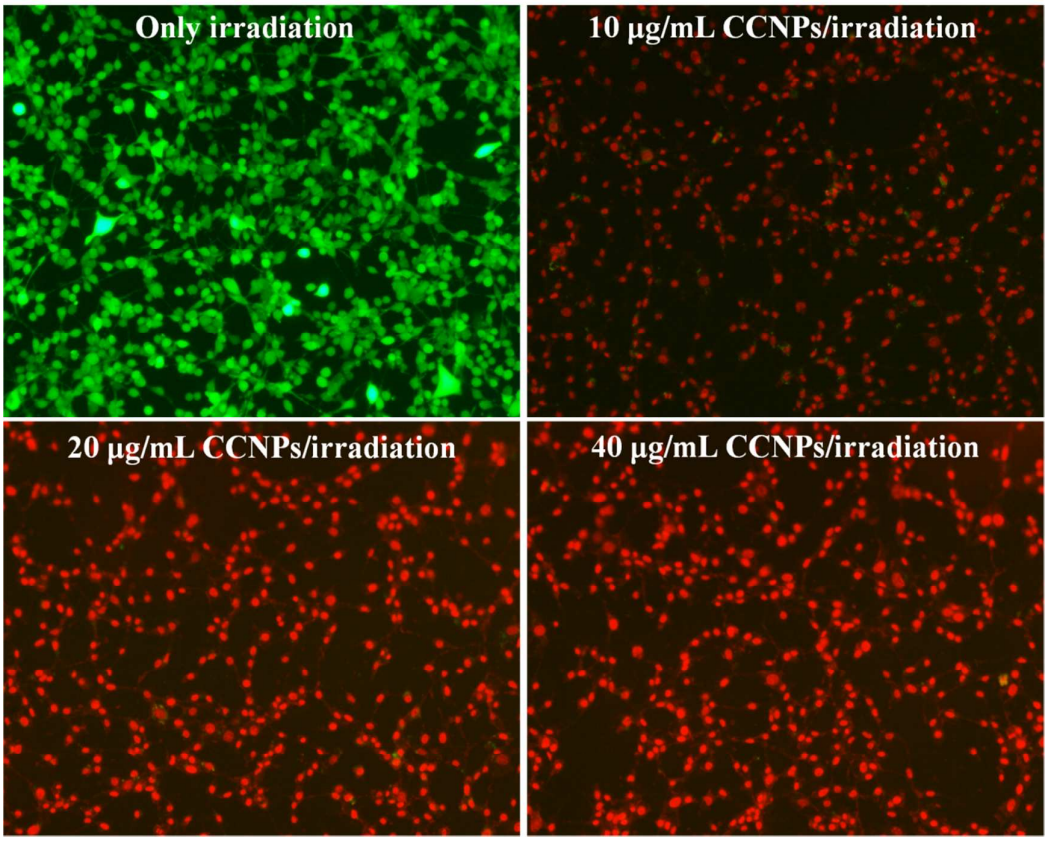

Figure S15. Confocal flurescence images of calcein AM (green, live cells) and propidium iodide (red, dead cells) co-cultured CT26 cells with only irradiation and different concentrations of CCNPs with irradiation.

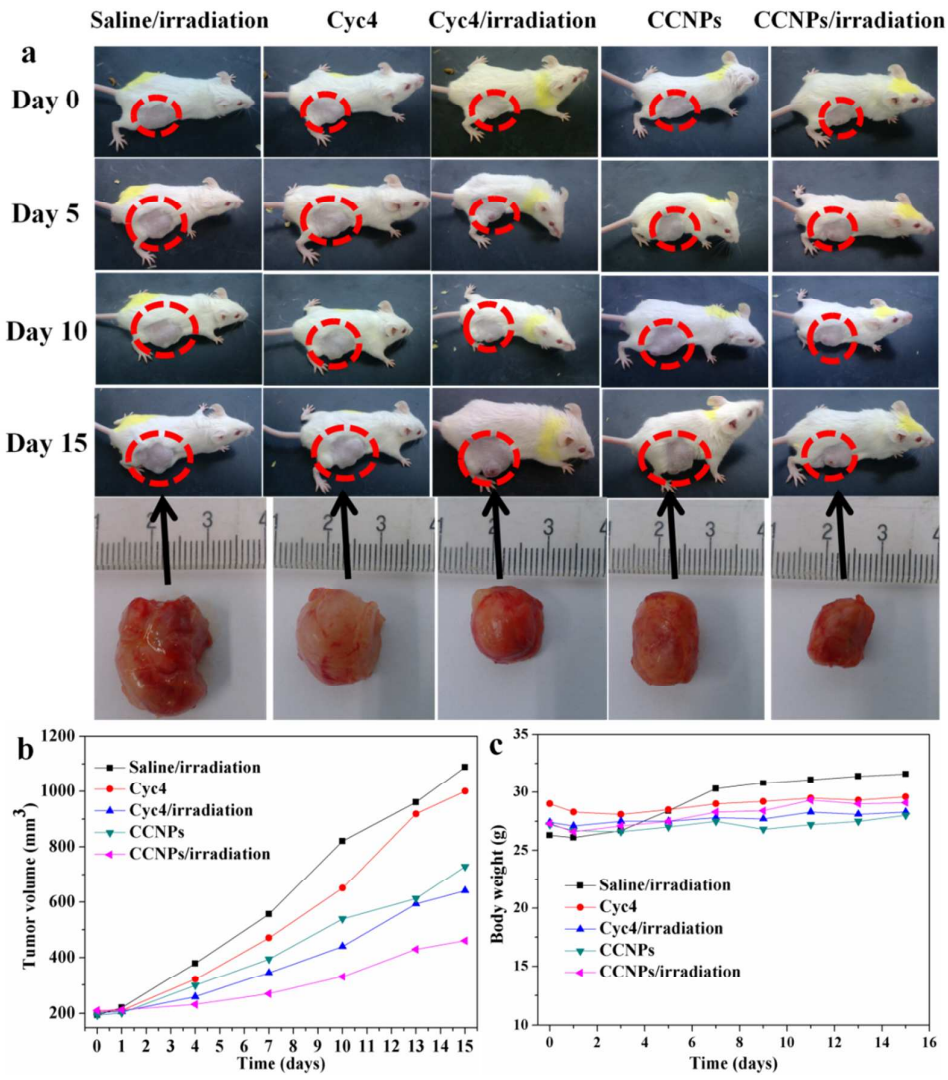

Figure S16. In vivo tumor inhibition of CCNPs in BALB/c mice bearing CT26 tumors after injection. a) Representative photos of mice bearing CT26 tumors and excised tumors on 15 d after treatments. The tumors were marked with red dashed circles. b) Tumor growth inhibition profiles and c) Body weight of changes of the mice bearing CT26 tumors treated with saline, free Cyc4 and CCNPs at the dose of 0.5 mg/kg Cyc4 without or with 808 nm irradiation (5 min, 1.0 W/cm2) in 15 days.

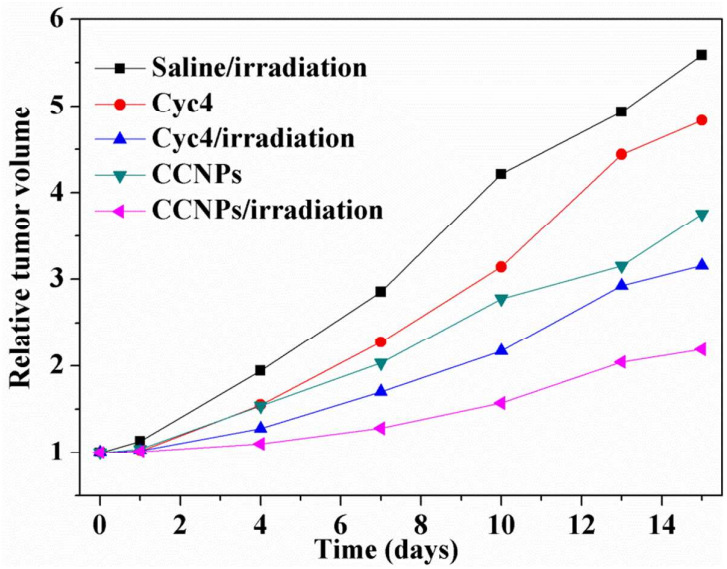

Figure S17. Relative tumor volume in the five mice groups (days 0-15).