Supporting Information

Photothermal Controlled Generation of Alkyl Radical from Organic Nanoparticles for Tumor Treatment

Rui Xia,[†,‡]Xiaohua Zheng,[†,‡] Xiuli Hu,[*,†] Shi Liu,[†] and Zhigang Xie[*,†,‡]

[†]State Key Laboratory of Polymer Physics and Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun, Jilin 130022, P. R. China

[‡]University of Science and Technology of China, Hefei 230026, P. R. China

Corresponding Authors

* E-mail: lily@ciac.ac.cn (X. H.).

* E-mail: xiez@ciac.ac.cn (Z. X.).

Table of Contents

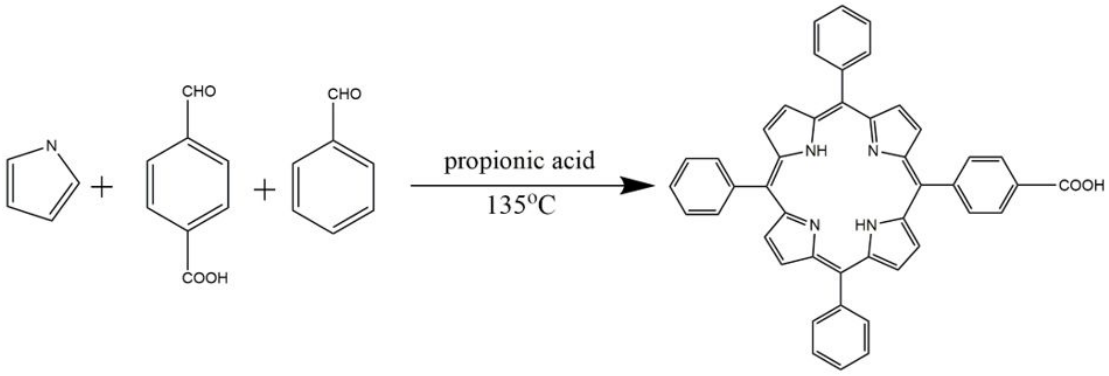
minutes at different point of time.

Figure S16. The corresponding weight changes in eight groups of mice in 14 days. (L = 638 nm laser irradiation at 1 W/cm^2 for 10 min)

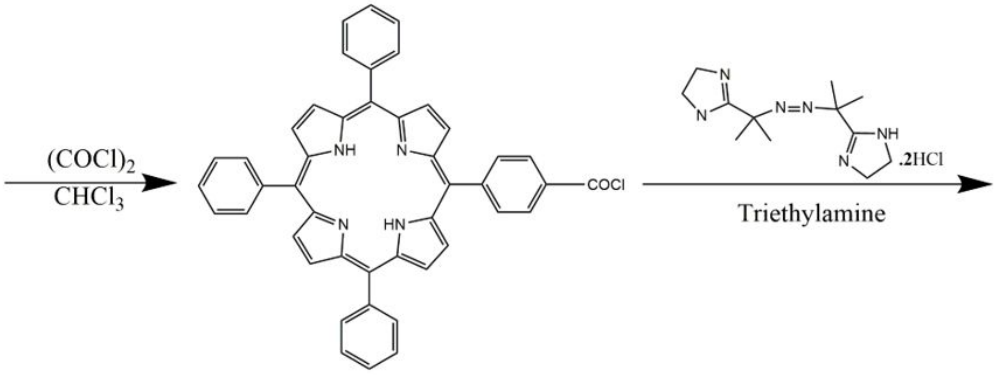
Figure S17. A) WBC, RBC, PLT and B) ALT, C) AST, D) UREA, E) CREA, F) UA levels in the blood of eight groups of mice.

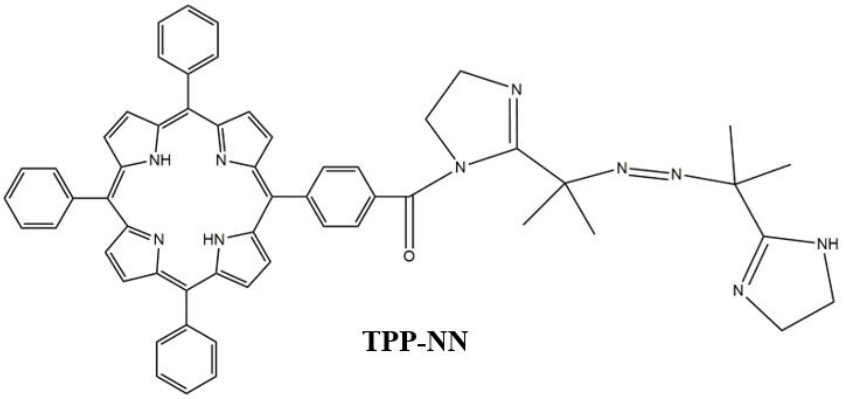
Experimental Section

Synthesis of TPPCOOH and TPP-NN



TPP-COOH





TPP-NN

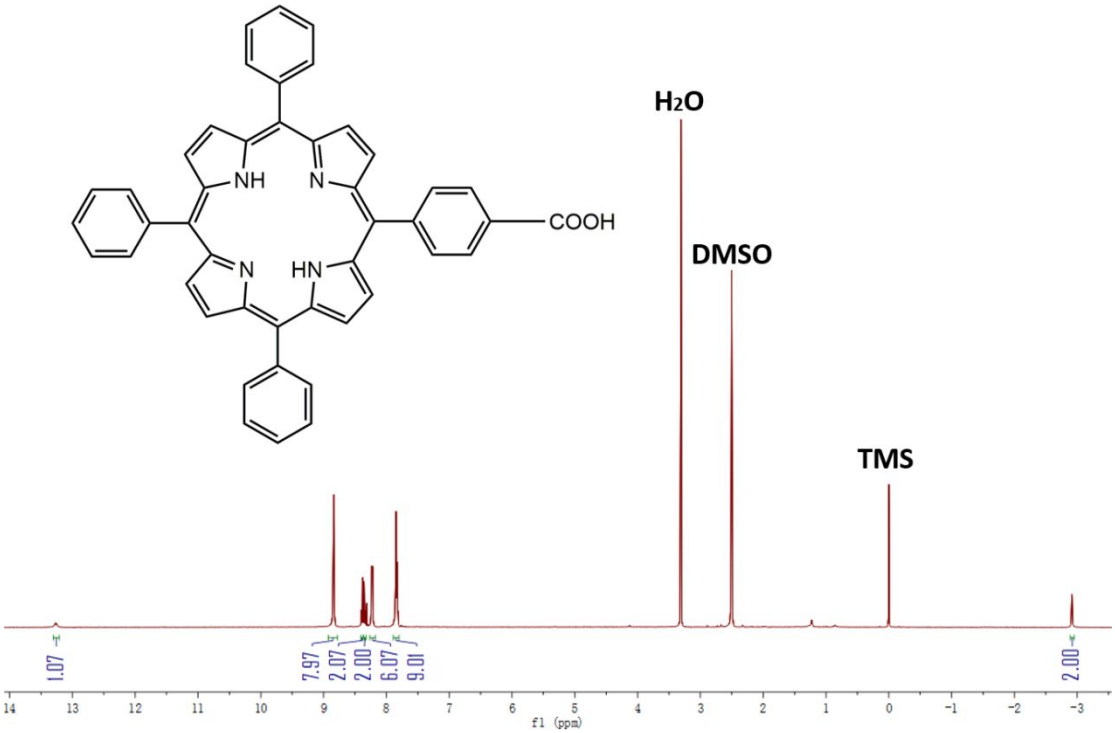


Figure S1. [1]H NMR spectrum of TPPCOOH in DMSO.

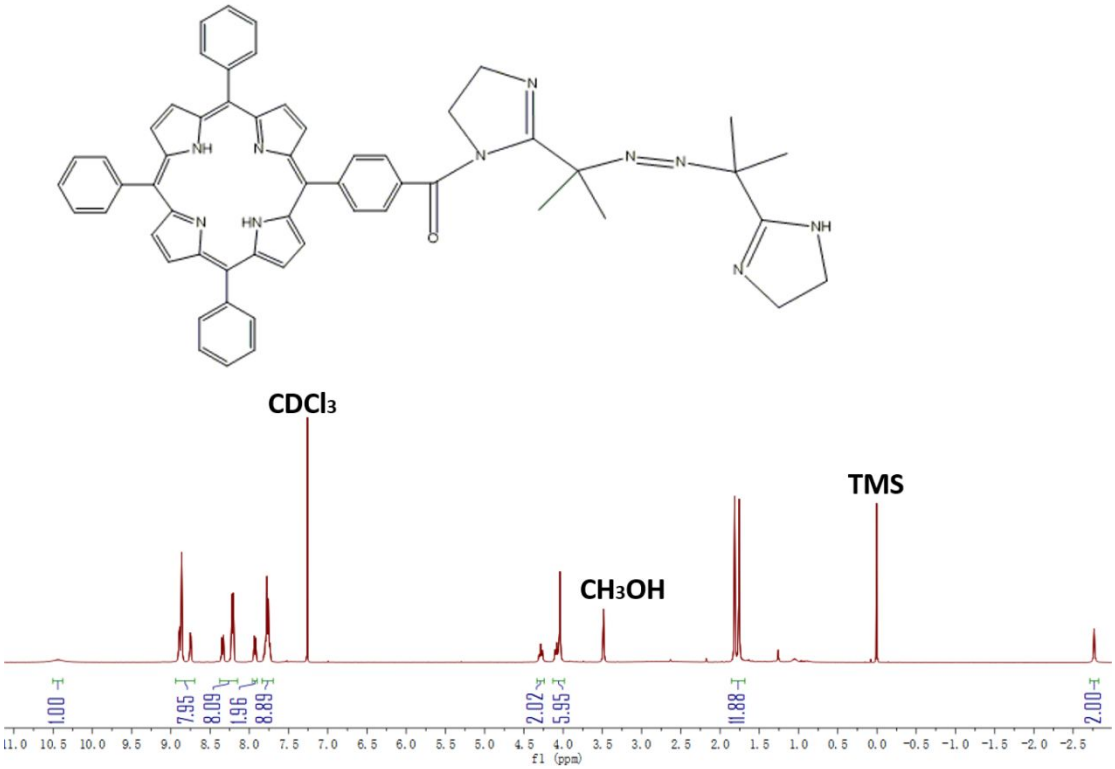


Figure S2. [1]H NMR spectrum of TPP-NN in $CDCl_3$.

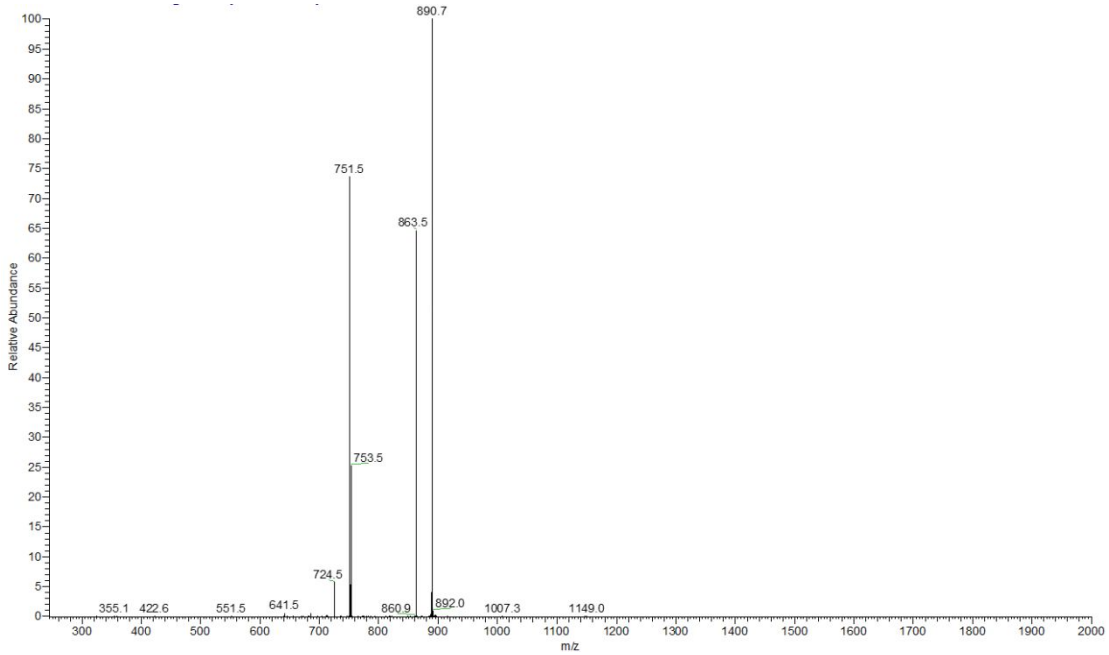


Figure S3. ESI mass spectrum of TPP-NN.

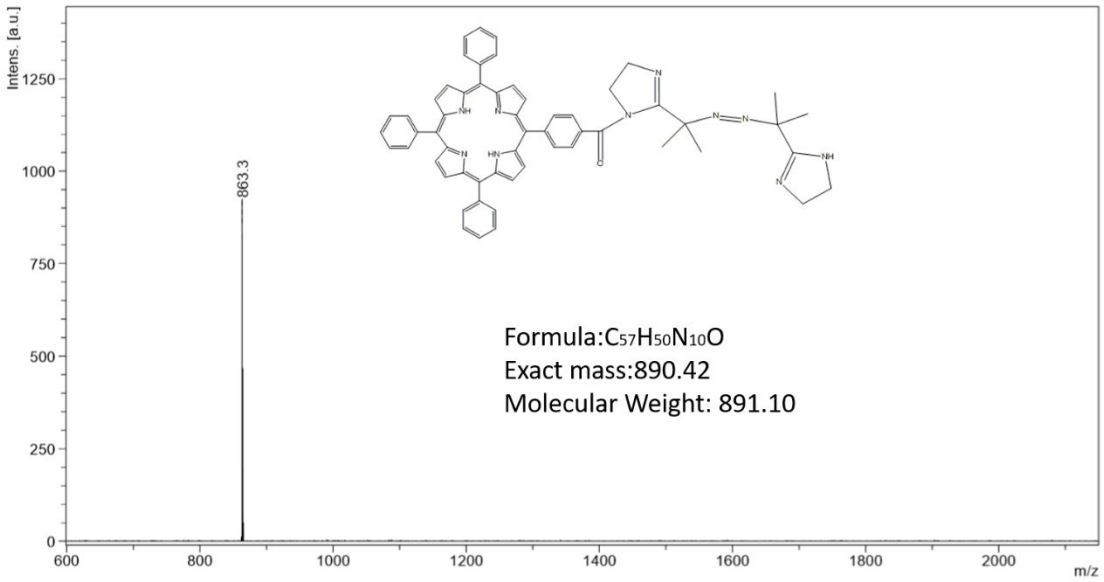


Figure S4. MALDI-TOF mass spectrum of TPP-NN.

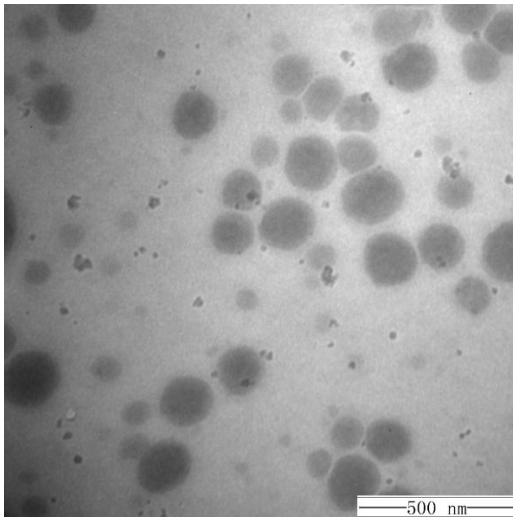


Figure S5. TEM image of TPP-NN NPs in large scale.

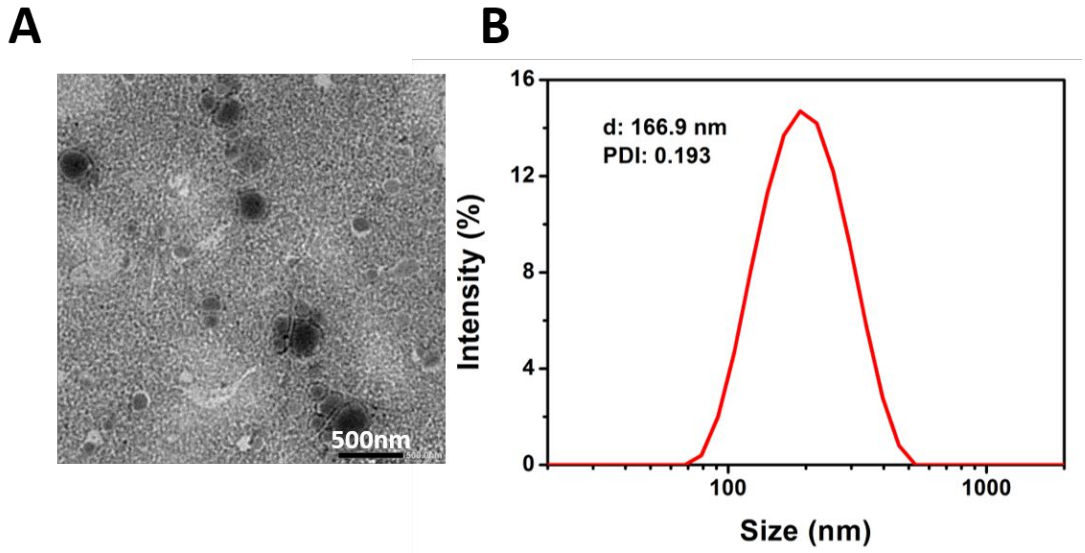


Figure S6. A) TEM image and B) DLS of TPP-NPs.

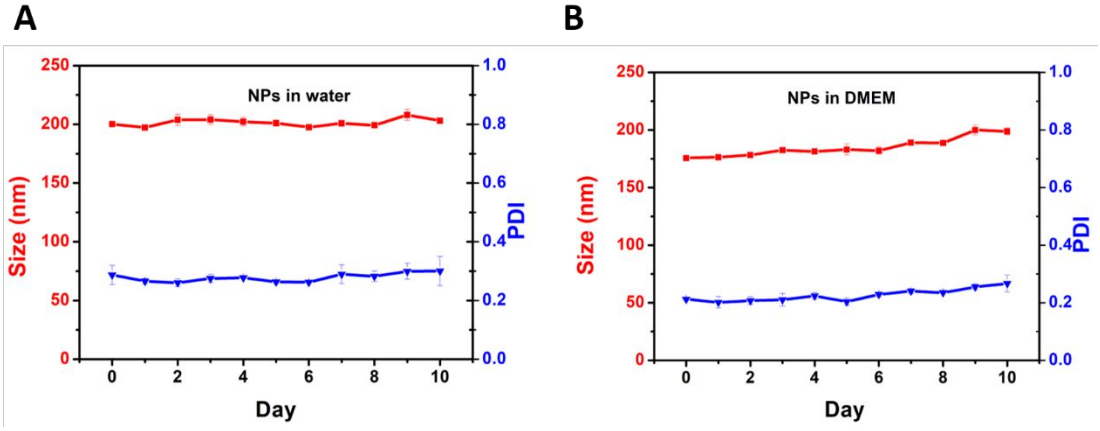


Figure S7. DLS change of TPP-NN NPs in (A) water and (B) DMEM for 10 days.

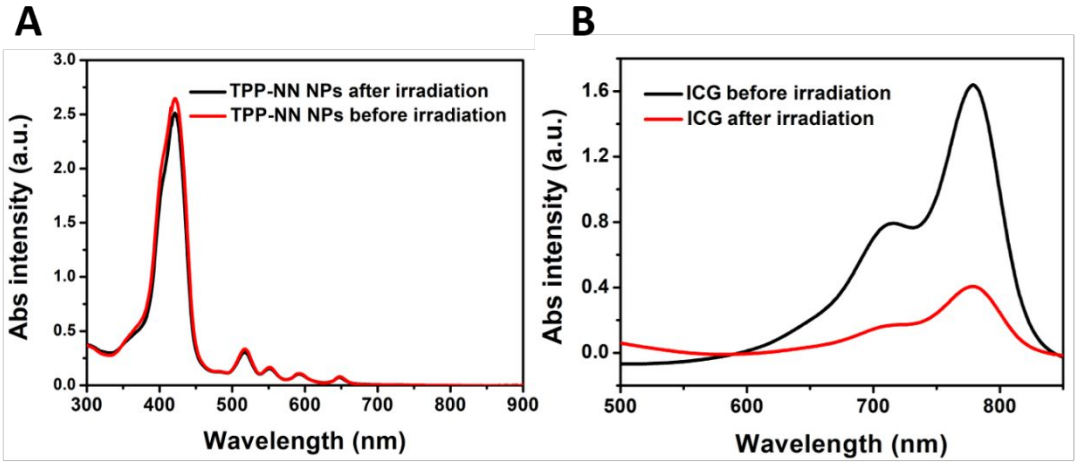


Figure S8. Absorption spectra of A) the TPP-NN NPs and B) indocyanine green (ICG) before and after 638 nm laser irradiation for 10 min with a power density of 1W/cm^2.

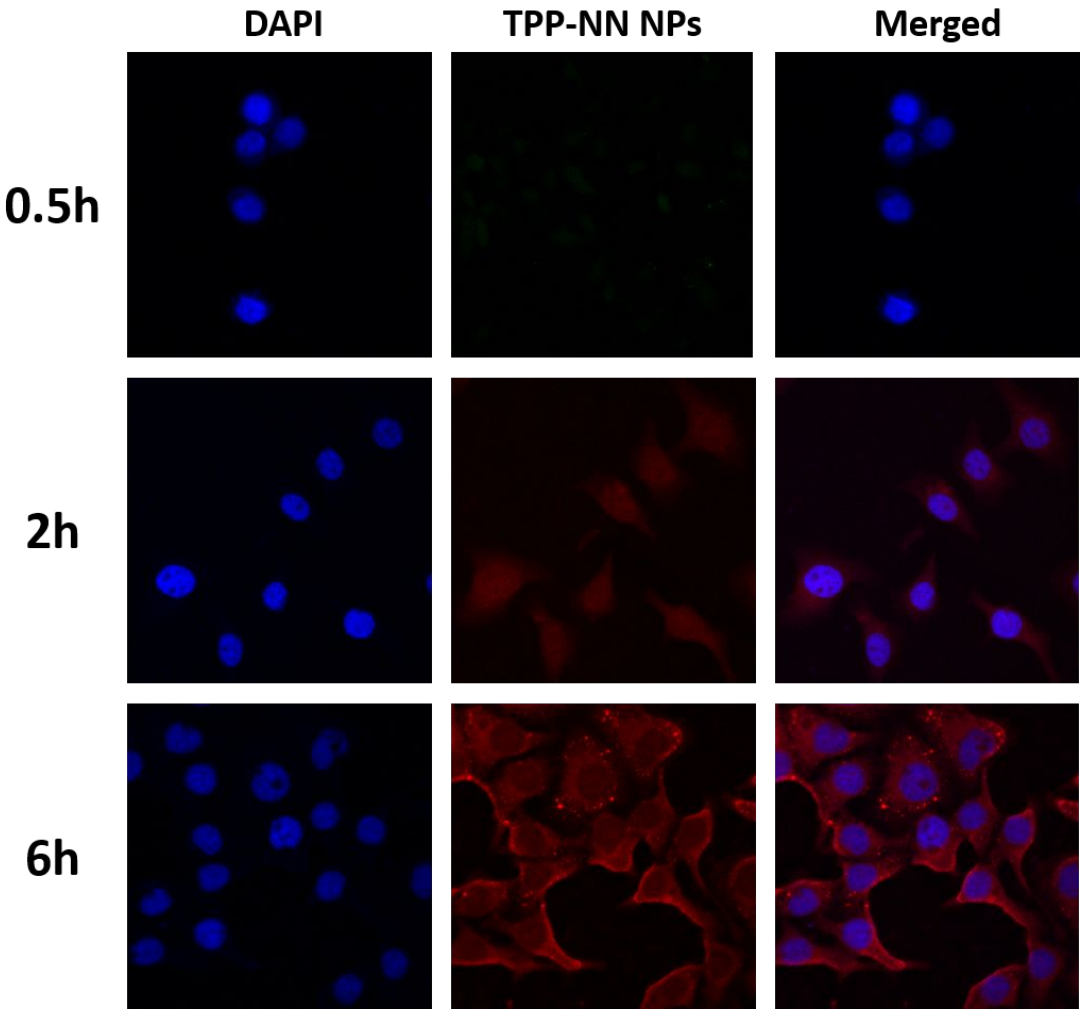


Figure S9. CLSM images of HepG2 cells incubated with TPP-NN NPs for 0.5, 2 and

6h at 37°C, respectively. For each panel, the cell nuclei stained by DAPI (blue), fluorescence of TPP-NN (red), and overlays of both images.

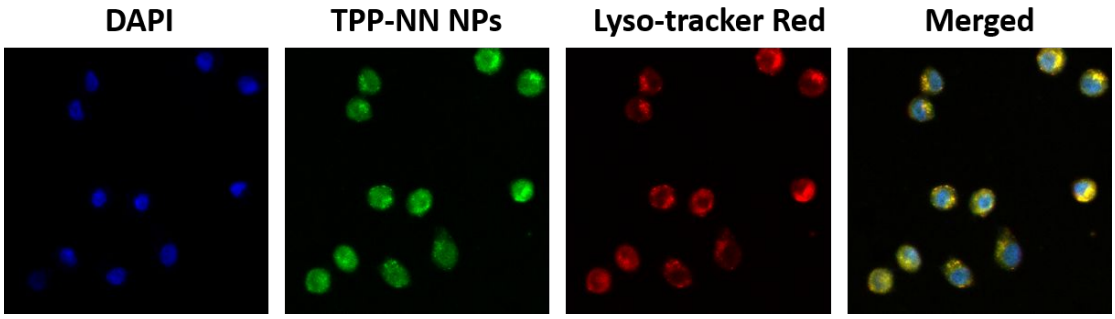


Figure S10. CLSM images of HepG2 cells incubated with TPP-NN NPs and Lyso-tracker Red. For each panel, cell nuclei stained by DAPI (blue), fluorescence of TPP-NN (green), fluorescence of lysosomal stained by Lyso-tracker Red (red), and overlays of three images.

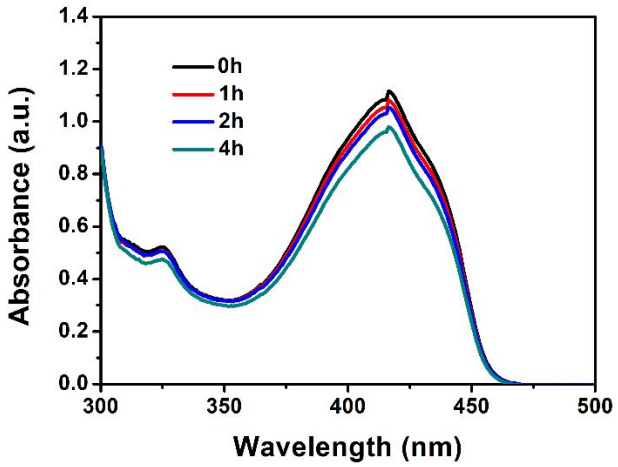


Figure S11. Absorption spectra of DPBF at different time points at 45 °C.

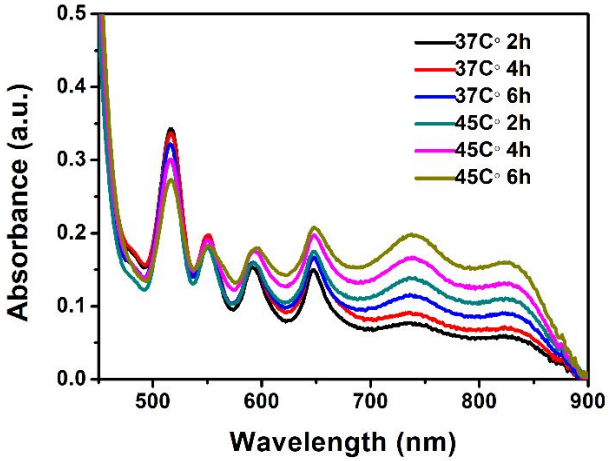


Figure S12. Generation of $ABTS^{+\cdot}$ between ABTS and TPP-NN NPs at different point of time and temperatures.

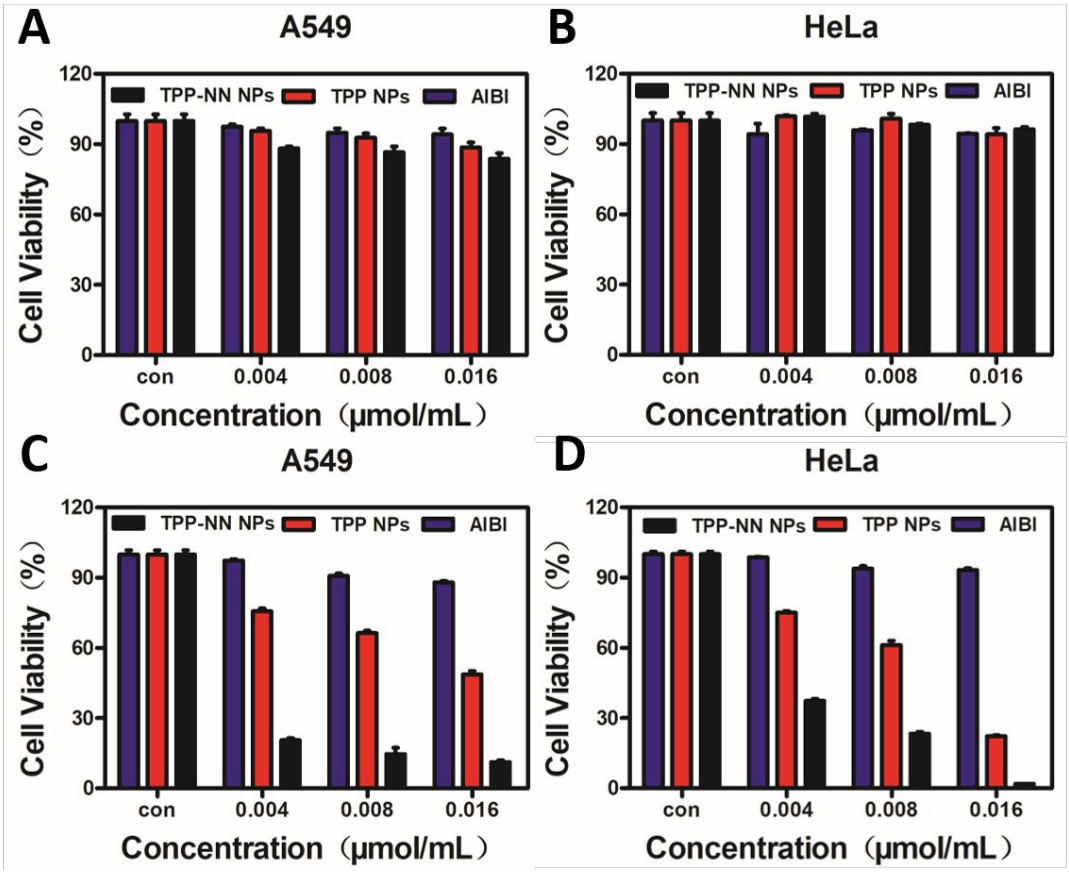


Figure S13. Cytotoxicity of A549 and Hela cells treated with TPP-NN NPs, TPP NPs

and AIBI without (**A, B**) or with (**C, D**) 638 nm laser irradiation for 5 min.

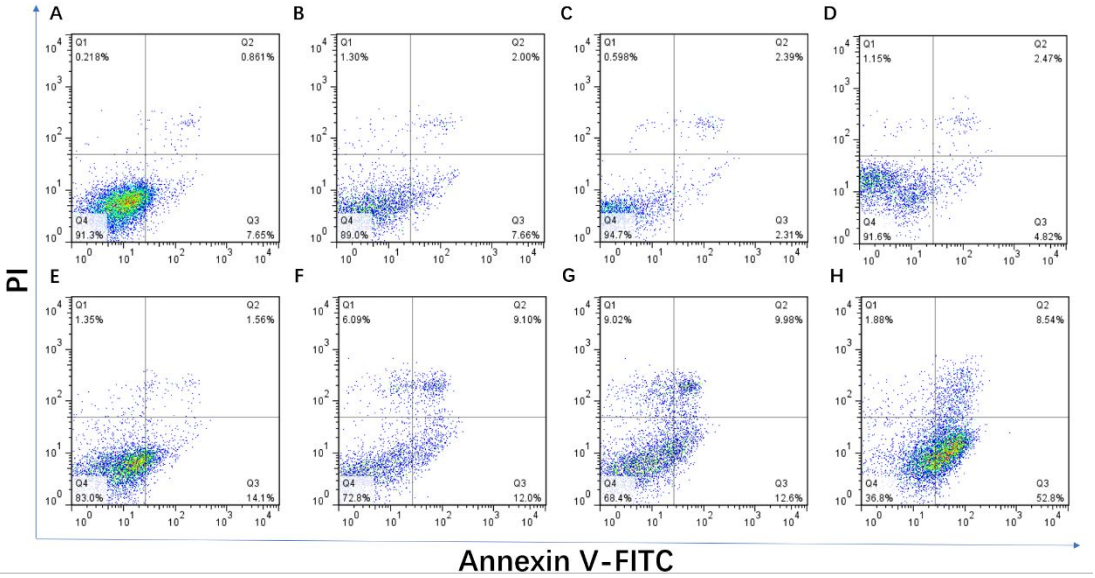


Figure S14. Cell apoptosis assay of HepG2 cells treated with A) PBS, B) AIBI, C) TPP NPs, D) TPP-NN NPs, E) PBS+ Laser, F) AIBI+ Laser, G) TPP NPs+ Laser, H) TPP-NN NPs+ Laser. The cells with laser were irradiated with 638 nm laser for 5 min with a power density of 1 W/cm^2.

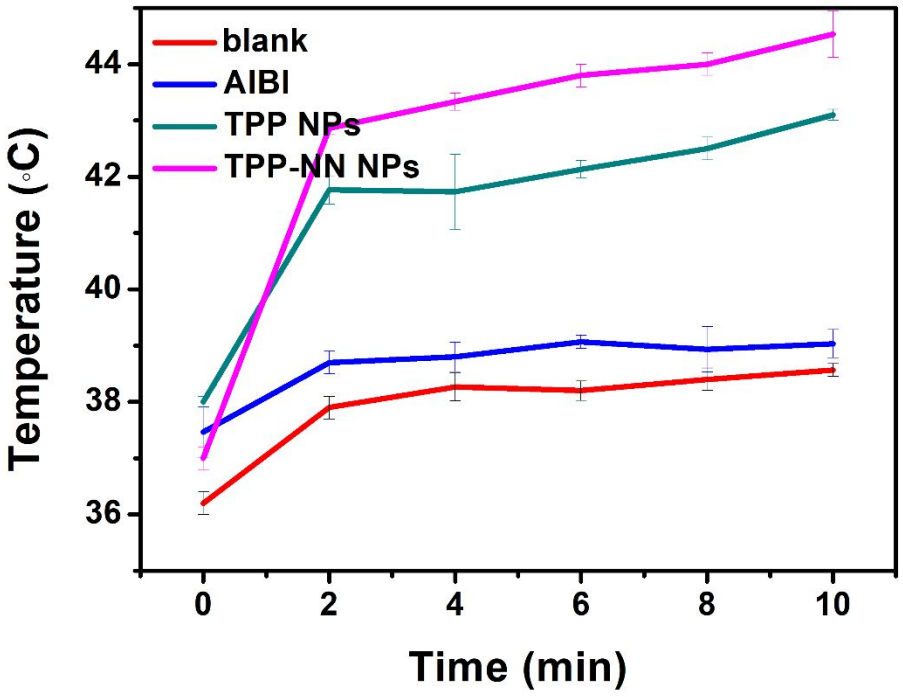


Figure S15. Temperature plot of four groups of mice with laser irradiation for 10 minutes at different point of time.

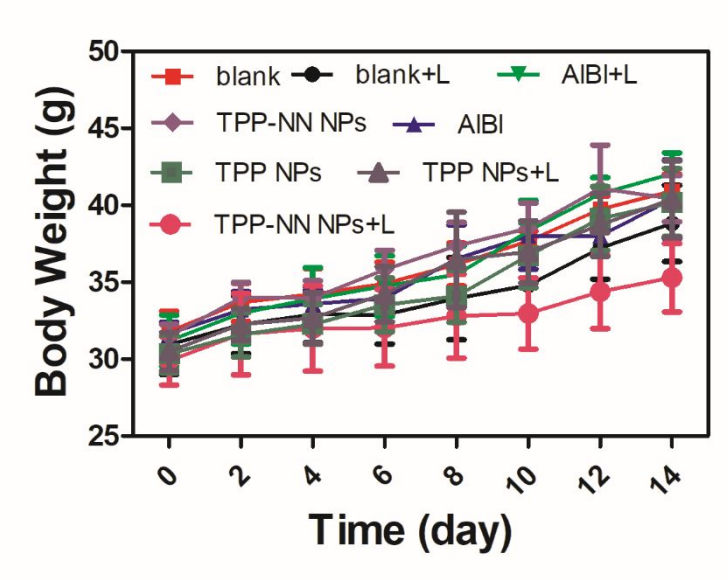


Figure S16. The corresponding weight changes in eight groups of mice in 14 days. (L = 638 nm laser irradiation at 1 W/cm^2 for 10 min).

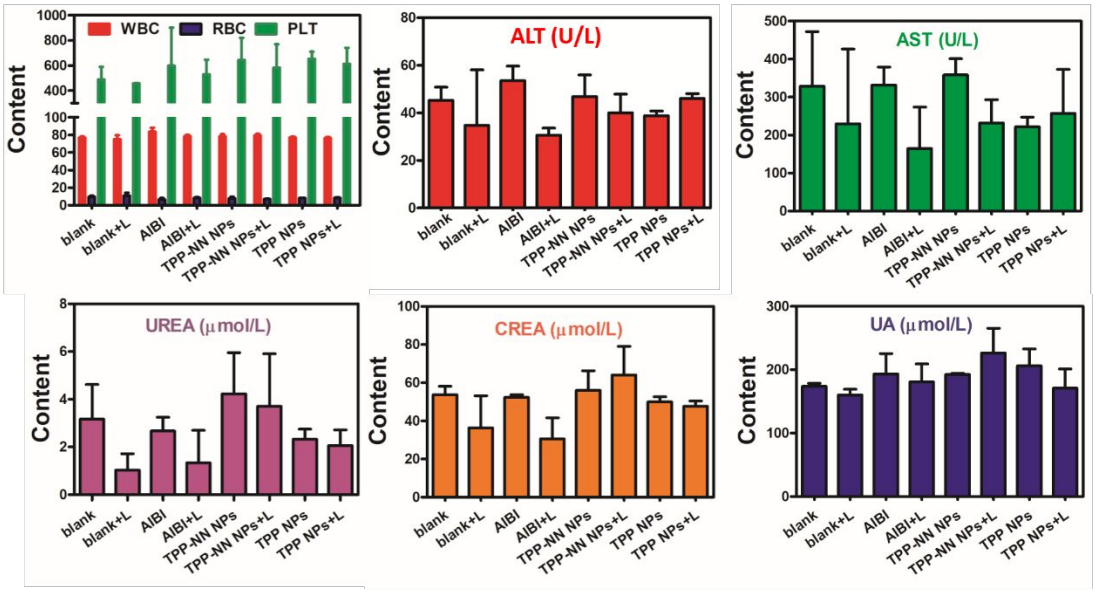


Figure S17. A) WBC, RBC, PLT and B) ALT, C) AST, D) UREA, E) CREA, F) UA levels in the blood of eight groups of mice. (L = 638 nm laser irradiation at 1 W/cm^2 for 10 min).

Experimental Section

Preparation of NPs: NPs were prepared via a reported method. TPP-NN (2 mg) and Poloxamer (6 mg) dissolved in THF (3 mL) were added dropwise into 10 mL deionized water and stirred for 24 hours following by dialyzed for 48 h to move the residual THF. TPP NPs were also prepared with the same method. NPs were stored at 4 ^{o}C for further use.

Photothermal Property and Photothermal Conversion Efficiency of TPP-NN NPs: With a 638 nm laser irradiation (1 W/cm^2) for 5 min, the temperature-rising curve of TPP-NN NPs (0.168 μmol/mL) and deionized water were recorded. TPP-NN NPs with different concentrations were irradiated under a 638 nm laser (1 W/cm^2) for 5 minutes. TPP-NN NPs (0.168 μmol/mL) were irradiated by a 638 nm laser with different laser intensity (0.2,0.4,1.0,2.0 W/cm^2) for 5 min. All the data were recorded by a thermocouple feeler every 10 s. Photothermal conversion efficiency of TPP-NN NPs was calculated according to a previous work.

Free radical generation measurements: The free radical from TPP-NN was measured by the reaction between TPP-NN solution and DPBF solution in DMF. The mixture was kept at 45 °C and protected from light irradiation for 1, 2 and 4 h. Then the absorbance of the mixture was recorded.

Intracellular Free radical Detection: The intracellular free radical was measured by reacting with 2',7'-dichlorofluorescein diacetate (DCFHDA) probe. Briefly, HepG2 cells were separately incubated with TPP NPs, AIBI, TPP-NN NPs (0.008

μmol/mL) for 6 h at 37 °C following by washing with PBS for five times to move out residue materials in culture medium, followed by heated to 45 °C and kept for 10 min or by irradiation with 638 nm laser for 10 min. Then DCFHDA was added and cells were incubated in an incubator for another 20 minutes, then they were washed. Free radical level intracellular was tested by fluorescent imaging with CLSM. For the hypoxic group, cells were incubated in a container with the gas pressure ratio of 5:2:93 corresponding to CO_2, O_2 and N2.

Intracellular toxicity study on HepG2 cells: HepG2 cells were inoculated on three 96-well plates in DMEM overnight. Then they were respectively treated with TPP NPs, AIBI, TPP-NN NPs with different concentrations (0.004, 0.008, 0.016 μmol/ml). After incubation for 6 hours, they were treated in different ways. For the control group, cells were incubated for 24 h at 37 °C without any treatment, the culture medium were removed and 200 μL fresh DMEM were added to each well. After 4 h incubation, we removed the culture medium and added dimethyl sulfoxide (DMSO, 200 μL) to each well. Cell viabilities were detected by the microplate reader. This group was taken as a control. For the normoxic group, cells were irradiated by 638 nm laser for 5 min then incubated in normal condition for another 24 h. Following they were disposed the same as the first plate. As to hypoxic group, cells were incubated the whole process under hypoxic conditions (containing 2 % oxygen, 5 % carbon dioxide, 93 % nitrogen) and the others were the same as the normoxic group.

Calcein-AM/PI Studies: Calcein-AM/PI studies were monitored to further verify the intracellular toxicity of TPP-NN NPs. Dead cells can be stained by PI (red) and live cells with calcein-AM (AM) (green). Cells were divided into eight groups and respectively treated with PBS, AIBI, TPP NPs, TPP-NN NPs (0.008 μmol/mL) with or without laser irradiation. Four of them were irradiated by 638 nm laser for 5 min. The rest of the groups were used as control. After 24 h of further cultivation, they were stained by a mixture of these two dyes for 20 min in dark. Then the samples were observed under a microscope and imaged.

Cell Apoptosis Studies: HepG2 cells were incubated in a 24-well plate for 24h and then divided into eight groups. Following they were respectively treated with PBS, AIBI, TPP NPs, TPP-NN NPs (0.004 μmol/mL). After 6 hours of cultivation, PBS were added to wash the medium clean and then fresh medium were added. Four groups of them were irradiated for five minutes by a 638 nm laser (1 W/cm^2), then they were incubated for another 24 h at 37 °C. Following removing the culture medium by washing with PBS for several times and the cells were digested by trypsin containing no EDTA. Eight groups of cells were respectively collected and stained by PI and annexin V-FITC to perform the cell apoptosis results**.**

In Vivo Tumor Suppressor Effect and Biosafety: Kunming strain mouse were obtained from the Hospital of Jilin University. After subcutaneously inoculated with U14 tumor cells and the tumors reached to a volume of 80mm^3, mice were divided into eight groups, including blank, blank + L, AIBI, AIBI + L, TPP NPs, TPP NPs + L, TPP-NN NPs, TPP-NN NPs + L. Then AIBI, TPP NPs, TPP-NN NPs (1.87

μmol/mL, 200 μL for each mouse) were respectively intravenously injected into the corresponding group of mice. Four groups of mice were irradiated for 10 min after 24 h of administration by a 638 nm laser (1 W/cm^2) at tumor site. Growth situation of mice, tumor weight and tumor volume were recorded every two days in two weeks. Then tumor tissue and major organs of the mice (heart, liver, spleen, lung and kidney) were collected for H&E staining.

Blood Biochemistry Assay: After the experiment, eight groups of mice were sacrificed and their blood were respectively collected for the blood biochemistry studies. The samples were analyzed by the automatic biochemical analyzer (Mindray, BS-220) and an automatic blood cell analyzer (ABX MICROS 60). Content of WBC (white blood cell), RBC (red blood cell), ALT (alanine aminotransferase), AST (aspartate aminotransferase), UREA, CREA and UA (uric acid) were recorded.