Electronic Supplementary Information

Vascular disruption agent and phototherapeutics assembled nanoparticles for enhanced tumor inhibition

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EXPERIMENTAL SECTION

Materials

IEICO-4F was purchased from Solarmer Materials Inc. Indocyanine green (ICG), and CA4P was obtained from Energy Chemical. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), Calcein-AM (AM), propidium iodide (PI), O22, and O27 were provided by the Shanghai Zeye Biotechnology Co., Ltd. Singlet Oxygen Sensor Green (SOSG) was obtained from Dalian Meilun Biotechnology Co., Ltd. Annexin V/FITC-7AAD Apoptosis Detection Kit was supplied by Melone Pharmaceutical Co., Ltd. DSPE-PEG_{2000}-NH_2 was procured from Hunan Huateng Pharmaceutical Co. Ltd. Moreover, 2,2,6,6-Tetramethylpiperidine (TEMP) was obtained from Inmochem Co. Ltd.

Instruments

The scanning electron microscopic (SEM) images were captured using a scanning electron microscope (JSM-7610F, JEOL Ltd). UV-vis absorption and fluorescence spectra were measured with Shimadzu UV-2600 and RF-6000 spectrophotometers. Fluorescence cell imaging was performed on an inverted microscope. Zeta potentials were quantified on the zeta-sizer Nano-ZS (Malvern). Electron spin resonance (ESR) spectra were obtained on a BRUKER E500 spectrometer.

Synthesis of IFC NPs

IFC NPs were synthesized using a simple self-assembly method. 20 mg CA4P was added into 20 mL DSPE-PEG-NH_2 (1 mg/mL) aqueous solution. Then, 4 mL **IEICO-4F** (THF, 0.4 mg/mL) was added to the aqueous solution with a syringe under ultrasonic conditions. THF was removed with ultrasound, and then the mixture solution was dialyzed for 24 h to remove the excess CA4P and THF. Eventually, the **IFC NPs** solution was stored at 4 °C. **IEICO-4F NPs** were prepared using the same method (without CA4P added).

Calculation of load concentration

The load concentration (c) of **IEICO-4F** was calculated according to lambert-beer's law (eq. (1)).

$$c = \frac{A_{IEICO-4F}}{k\mathrm{l}} \qquad (1)$$

$A_{\mathbf{IEICO\text{-}4F}}$ is the absorbance of **IEICO-4F** at 850 nm. *k* was the slope of absorbance variation with concentration of **IEICO-4F** at 850 nm. l was the Optical path length. The calculation method of CA4P load concentration is similar to that of **IEICO-4F**.

Evaluation of the photothermal effect

The PCE (η) was calculated based on our previous report.

Detection of $^1O_2$ and •OH

SOSG used to detect $^1O_2$ in the aqueous solution. **IFC NPs** were mixed with SOSG and irradiated with 808nm laser for 5 min, and the fluorescence spectra were recorded every 30s. Two control groups, ICG and H_2O, were set up simultaneously. The $^1O_2$ production of each sample was measured three times under the same conditions. Finally, the $^1O_2$ quantum yield was determined by the formula. Φ_{ICG} was the $^1O_2$ quantum yield of commercial photosensitizer ICG, about 0.8%. K_{ICG} and K_{ps} were the slope of fluorescence intensity changes at 525 nm in ICG and **IFC NPs** groups with the extension of illumination time after the water effect is excluded. A_{ICG} and A_{ps} were the absorbance of ICG and **IFC NPs** at 808 nm.

$$\Phi_{ps} = \Phi_{ICG} \frac{K_{ps}}{A_{ps}} \frac{A_{ICG}}{K_{ICG}}$$

O22 and O27 capture $^1O_2$ and •OH in cells, respectively. Four groups of cells were set: PBS, PBS + laser, **IFC NPs**, and **IFC NPs** + laser. After 24 h of cell culture, **IFC NPs** were added to continue to culture for 4 h. O22 and O27 were added and exposed

to 808 nm laser (1 W/cm^2,10 min) after 30 min. Finally, the cells were washed using PBS to remove O22 and O27, and the fluorescence images of the cells were acquired.

Detection of •OH in ESR

TEMP is used as a •OH capture agent. The mixture solution of **IFC NPs** and TEMP was irradiated using 1 W/cm^2 808 nm laser for 5 min, and then the ESR spectra were recorded within 5 min. **IFC NPs** without laser irradiation were tested as controls.

MTT assay

We incubated 4T1 cells with **IFC NPs** at different concentrations (stepwise dilution) in a 96-well plate for 4 h. Then the cells plate was exposed to laser (808 nm, 10 min each well, 1 W/cm^2) and incubated in darkness for 20 h. An MTT solution diluted in RPMI-1640 (MTT : RPMI-1640 = 1 : 9) was added to the cells, and DMSO was added after 4 h. The dark toxicity to the cells was determined based on the previous method without laser exposure.

Calcein-AM/PI staining

We cultivated 4T1 cells in RPMI-1640 medium in confocal dishes overnight. Four groups of cells were set: PBS, PBS + Laser, **IFC NPs**, and **IFC NPs** + Laser. After the treatment, the cells were stained using Calcein-AM and PI. PBS was washed to remove excess dyes after 30 min. Finally, their fluorescence images were obtained with a fluorescence inversion microscope.

Apoptosis analysis

Cellular apoptosis was assessed with an Annexin V-FITC and 7-AAD Apoptosis Detection Kit. The 4T1 cells in the 96-well plates were treated with **IFC NPs** (8 μg/mL, 808 nm, 1W/cm^2, 5 min). Finally, the cells were stained with apoptosis detection kit and analyzed using flow cytometry.

Animal model

Central South University approved all the animal experiments and operations performed in this work (D2022024). First, BALB/c 3-4 mice (7-8 weeks of age) were selected to develop the tumor model. Then, 4T1 cells were injected subcutaneously into the right back of the mice. The mature tumors on the model mice were stripped, chopped into pieces, and subsequently implanted on the right back of experimental mice to develop the tumor-bearing mice.

Determination of in vivo therapeutic effect

Mice were randomly divided into six groups and treated with PBS, PBS + laser, CA4P, **IEICO-4F NPs** (4.8 μg/mL) +Laser, **IFC NPs** (8 μg/mL), or **IFC NPs** + laser.

Then, the mice were anesthetized and treated (inject 200 μL of corresponding solution). Subsequently, the body weight and tumor volume of mice were noted every two days for 14 days. The tumors were stained with H&E, Ki67, CD31, and TUNEL.

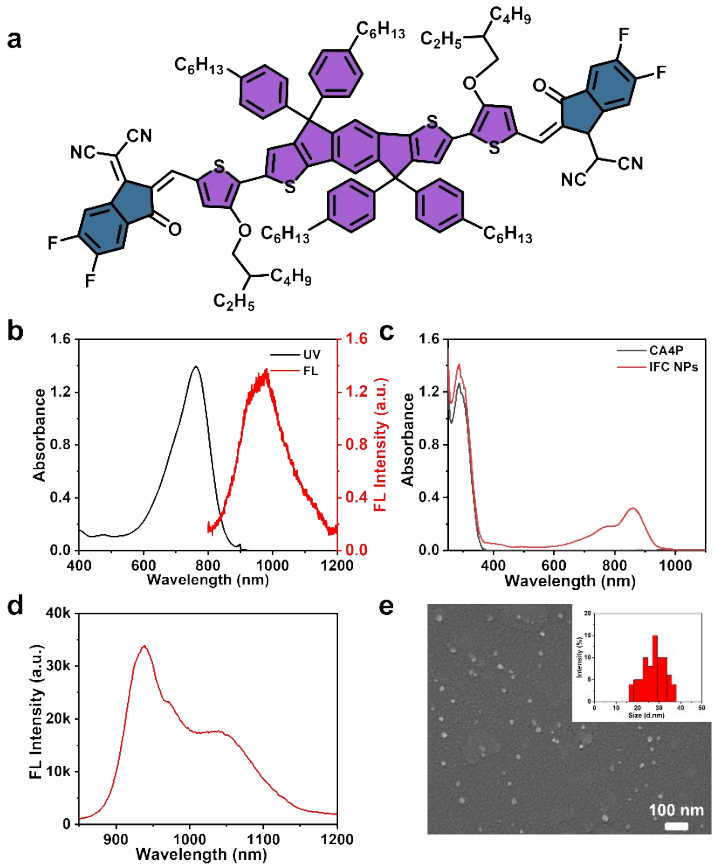

Figure S1. (a) Molecular structure of IEICO-4F. (b) UV-vis absorption and fluorescence spectra of IEICO-4F in THF. (c) UV-vis absorption spectra of IFC NPs and CA4P in aqueous solution. (d) The fluorescence spectrum of IFC NPs in aqueous solution. (e) SEM image and size distribution of IFC NPs (inset).

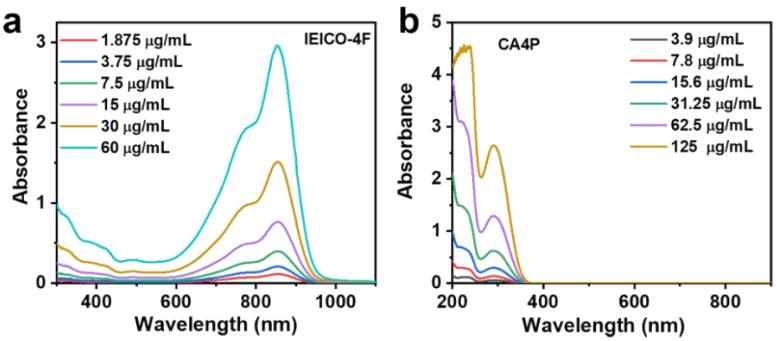

Figure S2. UV-vis absorption spectra of **IEICO-4F** (H_2O) (a) and CA4P (H_2O) (b) with different concentration.

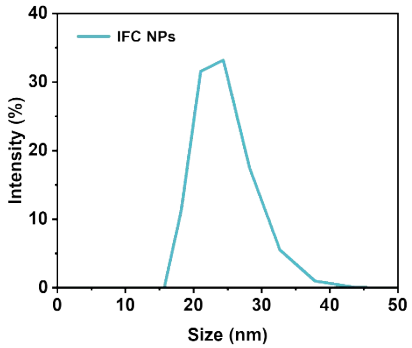

Figure S3 The mean diameters of **IFC NPs** by DLS.

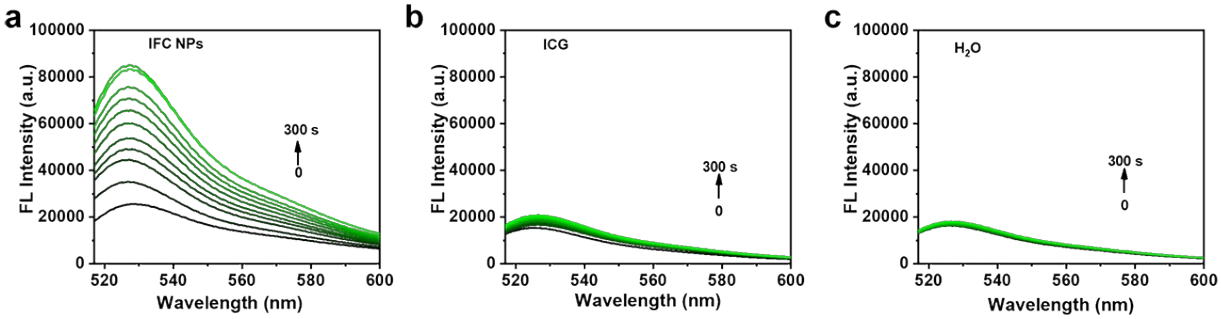

Figure S4. Time-dependent fluorescence spectra of SOSG in the presence of **IFC NPs** (a), ICG(b) and H_2O(c) after 808 nm laser irradiation (0.5 W/cm^2).

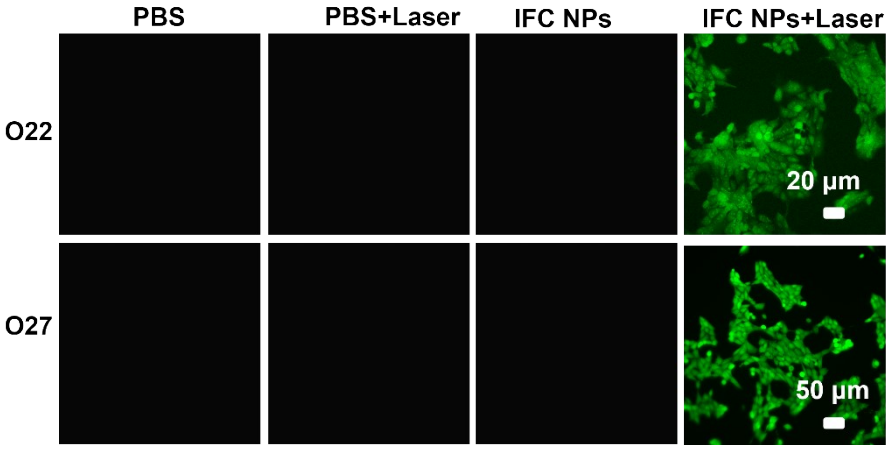

Figure S5. Generation of $^1O_2$ and •OH by **IFC NPs** in 4T1 cells using O22 and O27 as probes, respectively.

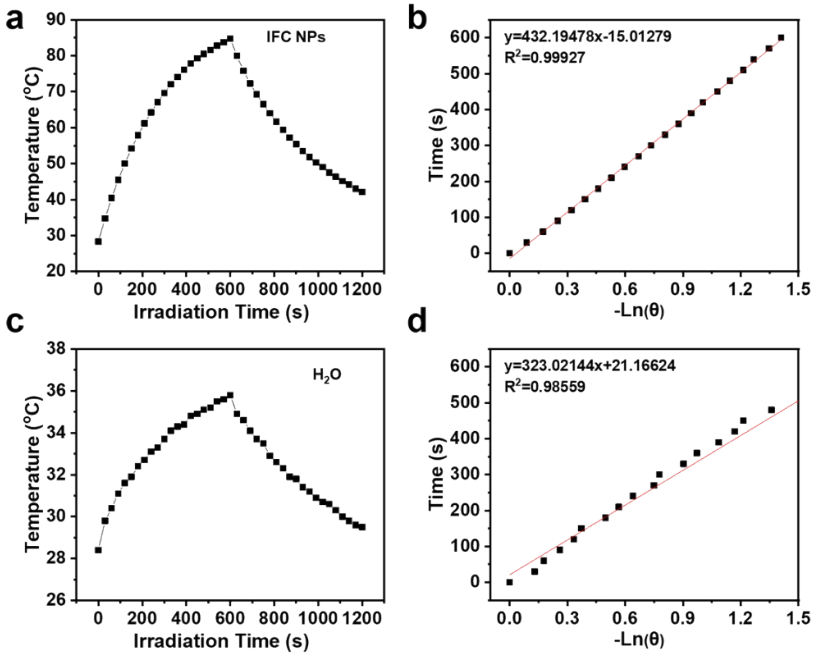

Figure S6. (a) Photothermal efficiency of **IFC NPs** treated with 600 s 808 nm laser irradiation. (b) Time date versus –Lnθ obtained from the cooling stage of (a). (c) photothermal efficiency of H_2O treated with 600 s 808 nm laser irradiation. (d) Time date versus –Lnθ obtained from the cooling stage of (c).

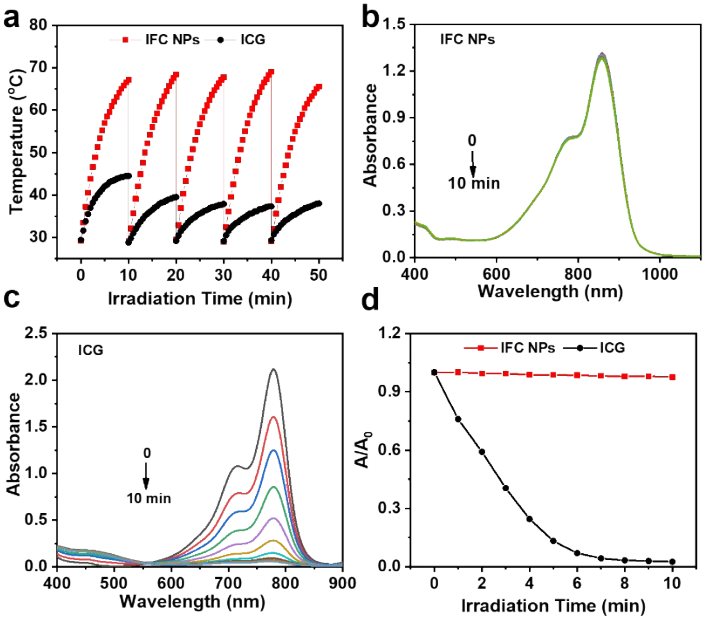

Figure S7. (a) Photothermal stability of **IFC NPs** and ICG for five cycles of on/off 808 nm laser irradiation. The absorption spectra of (b) **IFC NPs** and (c) ICG under the light irradiation (808 nm, 1 W/cm^2). (d) Time-dependent normalized absorbance ratio of **IFC NPs** and ICG at 858 nm and 780 nm, respectively.

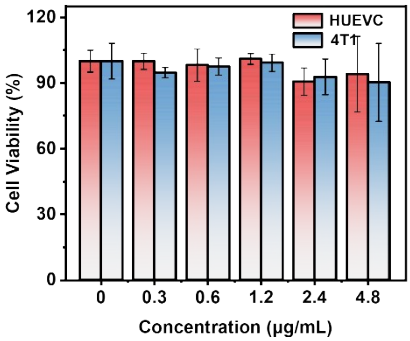

Figure S8. Cytotoxicity of **IEICO-4F NPs** on HUEVC (red) and 4T1 (blue) cells.

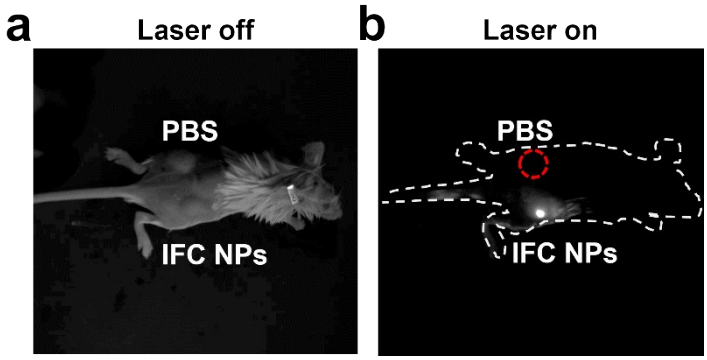

Figure S9. *In vivo* imaging of **IFC NPs.**

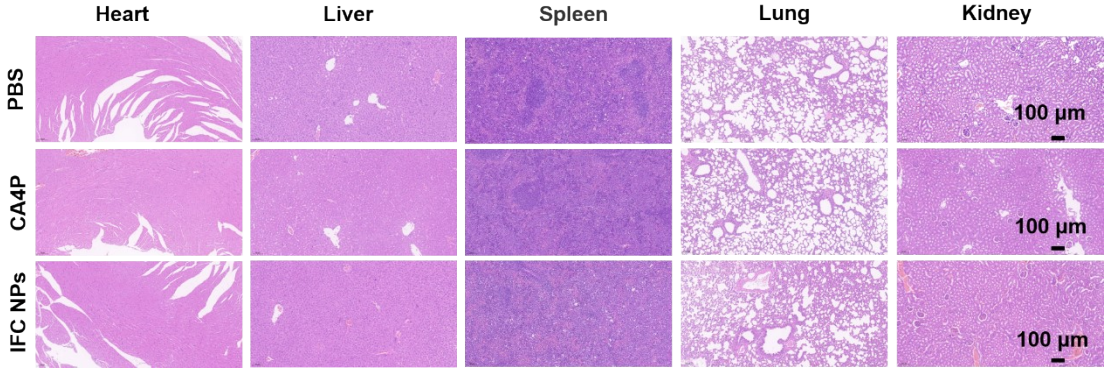

Figure S10. The H&E staining of major organs.