Electronic Supplementary Information for

$FeSe_2$ nanosheets as a bifunctional platform for synergistic tumor therapy reinforced by NIR-II light

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Experimental details

Preparation of oleyl amine-polyacrylic acid-$NHNH_2$ (OA-PAA-$NHNH_2$)

Preparation of oleyl amine-poly (acrylic acid-hydrazide *tert*-butylcarbonyl) (OA-PAA-NHNHBoc)

The 300 mg (0.1 mmol) of purified PAA was dissolved in anhydrous DMF (15 mL) at room temperature, and EDC/NHS were used to catalyze the ―COOH group of PAA (EDC/NHS/―COOH molar ratio of 1.5/1.2/1.0) for 45 min at 45 °C. After that, tert-butyl carbazate (436.1 mg, 3.3 mmol) and oleyl amine (220.2 mg, 0.82 mmol) were added dropwise to react with the above-mentioned activated product for 24 h. The resulting mixture was then transferred into a dialysis bag (2000 kDa MWCO) and dialyzed against 3 L of deionized water for 2 days. Deionized water was changed every 8 h. After lyophilization, the product of OA-PAA-NHNHBoc was obtained as a white powder for further H^1 NMR characterization [1].

Preparation of oleyl amine-polyacrylic acid-$NHNH_2$ (OA-PAA-$NHNH_2$)

100 mg of OA-PAA-NHNHBoc was dissolved in 4 mL of TFA and then stirred at 25 °C for 24 h. The resulting solution was evaporated to remove TFA. Light-yellow product was re-dissolved in deionized water for further lyophilization. After lyophilization, the product of OA-PAA-$NHNH_2$ was obtained as a light-yellow powder for further H^1 NMR characterization [2].

Drug release from the OA-PAA-DOX@$FeSe_2$ NSs

OA-PAA-DOX@$FeSe_2$ NSs (200 μg $FeSe_2$) were dispersed in the 1 mL of PBS (pH = 7.4, 6.3 and 5.0). Then, the solution was transferred to a dialysis bag (2000 kDa MWCO) and immersed in a beaker containing 5 mL of PBS (pH = 7.4, 6.3 and 5.0) under constant shaking in dark, respectively. According to the normal body temperature of mice and the tumor temperature of light irradiation after the intratumoral injection of $FeSe_2$, the drug release was conducted at 37 °C and 52 °C to evaluate thermal-enhanced drug release. 300 μL of liquid was taken out at the interval time and replaced with the same volume of PBS immediately. The release sample was determined by the DOX concentration against the calibration curve by fluorescence measurement.

Photothermal performance of $FeSe_2$ NSs

Calculation of the photothermal conversion efficiency

The $FeSe_2$ NSs dispersion (200 μg/mL) was continuously irritated with 1120-nm laser (0.8 W/cm^2) till the temperature reached the steady-state. And then the dispersion allowed to naturally cool to room temperature after turning off the laser.

According to the previous report, the photothermal conversion efficiency of OA-PAA@$FeSe_2$ NSs was calculated as follows [3].

$$\eta = \frac{hA\Delta T_{max,mix} - hA\Delta T_{max,H_2O}}{I(1-10^{-A_\lambda})} = \frac{hA(\Delta T_{max,mix} - \Delta T_{max,H_2O})}{I(1-10^{-A_\lambda})} \quad (1)$$

Where $\Delta T_{max,mix}$ and $\Delta T_{max,H_2O}$ is the changeing temperature of the OA-PAA@$FeSe_2$ NSs dispersion and the solvent at the maximum steady-state temperature, respectively. I is the laser power, A_λ is the absorbance of OA-PAA@$FeSe_2$ NSs at the wavelength of 1120 nm in aqueous solution, and η is the photothermal conversion efficiency of OA-PAA@$FeSe_2$ NSs which means the ratio of absorbed light energy converting to thermal energy.

The value of hA is derived from Equation (2):

$$\tau_S = \frac{m_{H_2O} C_{p,H_2O}}{hA} \quad (2)$$

Where $\frac{m_{H_2O} C_{p,H_2O}}{hA}$ can be calculated by linear relationship of time versus -$ln(\theta)$. m_{H_2O} was 1.5×10^{-3}Kg. C_{p,H_2O} was 4.2×10^3J/Kg·°C.

Cell culture

U87MG cells line was cultured in DMEM culture medium supplemented with 1% (v/v) penicillin, 1% (v/v) streptomycin, and 10% (v/v) fetal bovine serum at 37 °C in 5% CO_2.

The Cellular uptake mechanism evaluation

Firstly, U87MG cells were incubated at 4 °C for 1.5 h to investigate the effect of temperature on cellular uptake. After that, cells were washed by PBS for three times after incubation with OA-PAA@$FeSe_2$ NSs for another 8 h. The uptake amount was

detected by UV-Vis spectroscopy.

To further explore the specific endocytotic pathways, amiloride (20 μM), genistein (200 μM), and chlorpromazine (15 μM) were applied to treat cells for 1.5 h at 37 °C, and then cells were incubated with OA-PAA@$FeSe_2$ NSs for another 8 h. After washed by PBS for three times, the cells were measured by UV-Vis spectroscopy. The uptake amount of U87MG cells which were incubated at 37 °C without any inhibitor was set as 100% control.

***In vitro* cytotoxicity evaluation**

To evaluate the dark-cytotoxicity of OA-PAA@$FeSe_2$ NSs, the standard MTT cell assays were used on U87MG cells. U87MG cells were seeded in 96-well plates at a density of 2×10^4 cells per well and grown in 5% CO_2 at 37 °C overnight. Then the culture medium was removed and 100 μL fresh medium contained different concentrations of OA-PAA@$FeSe_2$ NSs (0, 12.5, 25, 50, 100, and 200 μg/mL) were added to each well. After 8 h incubation, the medium containing $FeSe_2$ was removed and each well was washed thrice with PBS. Then 20 μL of 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) solution (diluted in a culture medium with a final concentration of 0.5 mg/mL) was added to each well and incubated for another 4 h in dark. Afterwards, the supernatant in each well was aspirated, and 150 μL of dimethyl sulfoxide (DMSO) was added to each well. The plate was shaken for 10 min and examined using a microplate reader (Biotek instruments. Inc) at the wavelength of 490 nm. Results were expressed as the percentage of cell viability.

For the light- cytotoxicity evaluation, medium containing non-internalized NSs in each well was removed. Each well was incubated with different concentration of OA-PAA@$FeSe_2$ NSs (0, 12.5, 25, 50,100, 200 μg/mL) for 8 h. After that, each well was washed thrice with PBS, and then exposed to an 1120-nm laser at the energy density of 0.8 W/cm^2 for 5 min. After light exposure, the cells were further incubated for another 4 h in the dark. Followed by removing the medium and the cell viability relative to the control untreated cells was evaluated using MTT assay.

Cellular uptake

The cellular uptake experiment was performed on a dark field microscope

(CytoViva). U87MG cells were incubated with OA-PAA@$FeSe_2$ NSs (100 μg/mL) for 8 h. After that, cells were washed thrice with PBS and imagined with 40x objective.

Animal xenograft model

Female ICR mice (six weeks old) were purchased from the Experimental Animal Center of Nanjing Qinglongshan. The H22 tumor models were successfully built up by subcutaneous injection of mouse cancerous ascites which contained living H22 cells into the left axilla of each mouse. The mice were treated when the tumor volumes approached approximately 60-70 mm^3 after a fortnight.

***In vivo* thermal imaging**

When the size of tumor tissues reached about 60 mm^3, mice bearing tumor were intra-tumoral injected with OA-PAA-DOX@$FeSe_2$ NSs suspension (dose = 5 mg/kg $FeSe_2$) and saline. The thermal imaging of different times after injection was recorded by a R300sr-HD infrared camera at different time points when the tumors were exposed to 1120-nm and 808-nm laser light during 300 s.

Histology examination

Organs fixed in 10% formaldehyde were dehydrated with buffered formalin, different concentration of ethanol, and xylene. Afterward, organs were embedded in liquid paraffin. Hematoxylin and eosin (H&E) were used to stain the sliced organs and tumor tissues (3-5 mm). Stained slices were examined by a microscope.

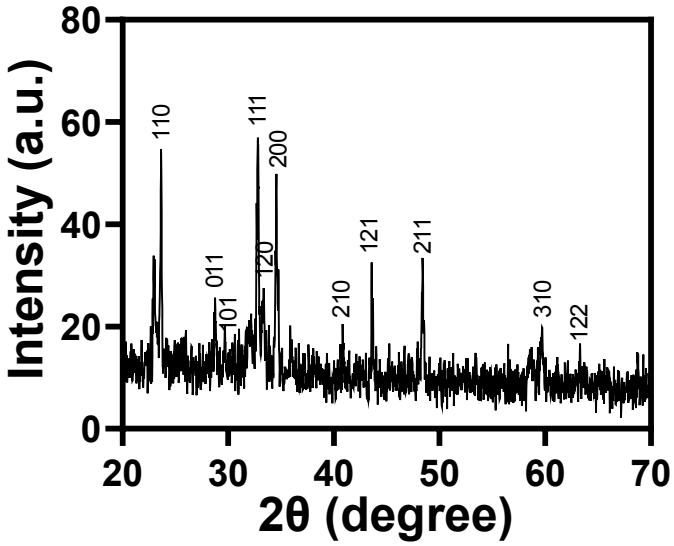

Fig. S1. XRD pattern of pristine $FeSe_2$ NSs

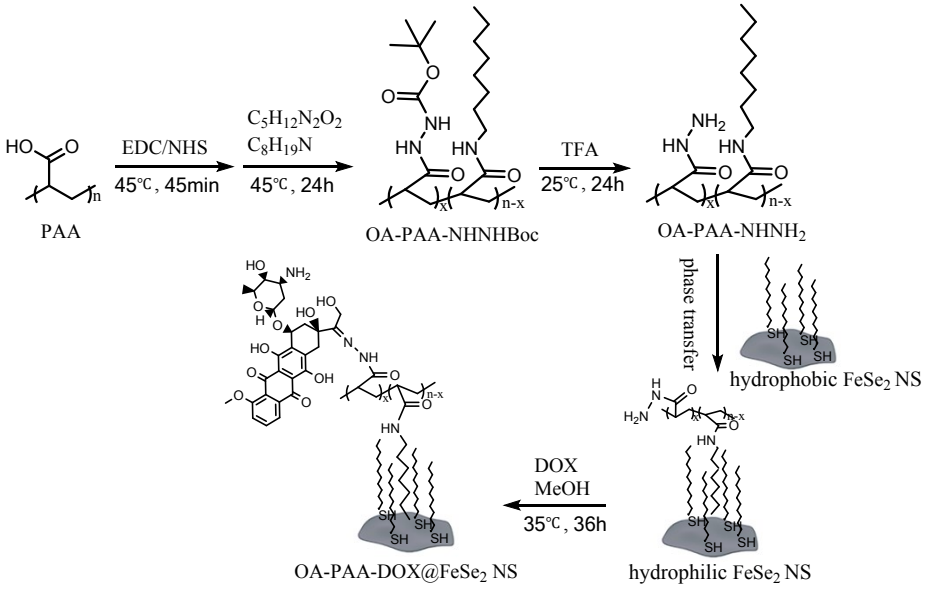

Fig. S2. Schematic presentation of the OA-PAA-DOX@$FeSe_2$ NSs synthesis process.

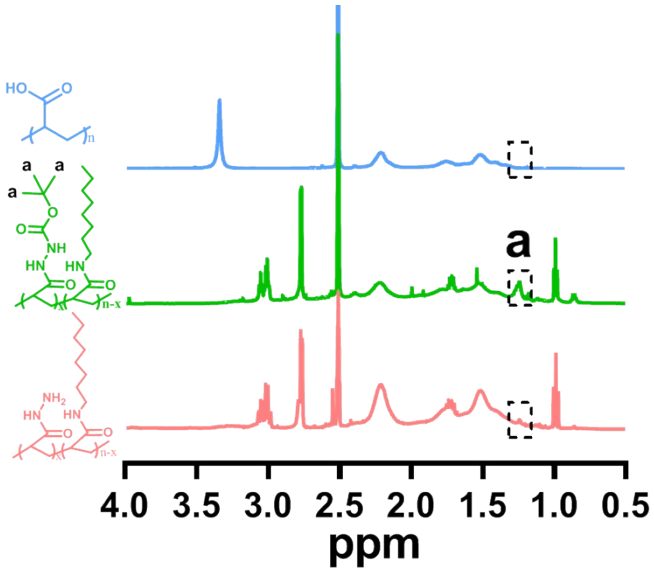

Fig. S3. ^{1}H NMR spectrum of purified PAA, OA-PAA-NHNHBoc, and OA-PAA-$NHNH_2$ polymer.

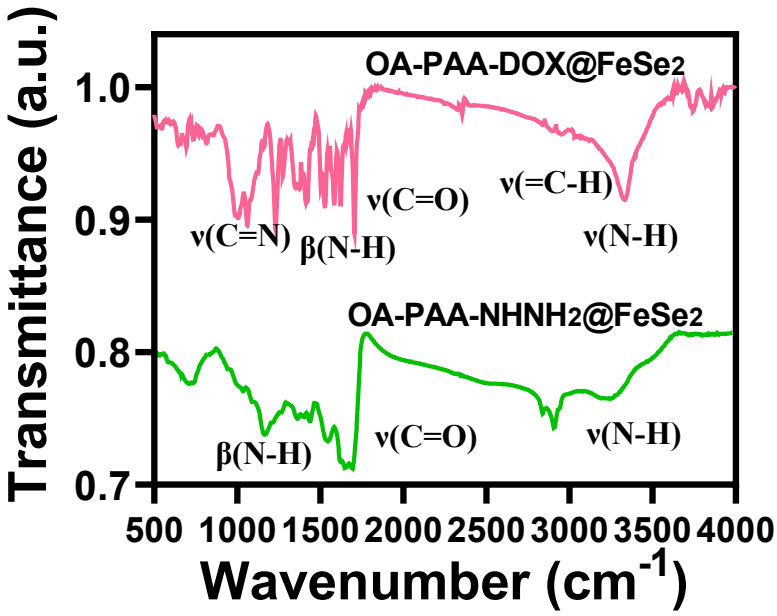

Fig. S4. The FTIR spectra of OA-PAA-DOX@$FeSe_2$ and OA-PAA-$NHNH_2$@$FeSe_2$ NSs.

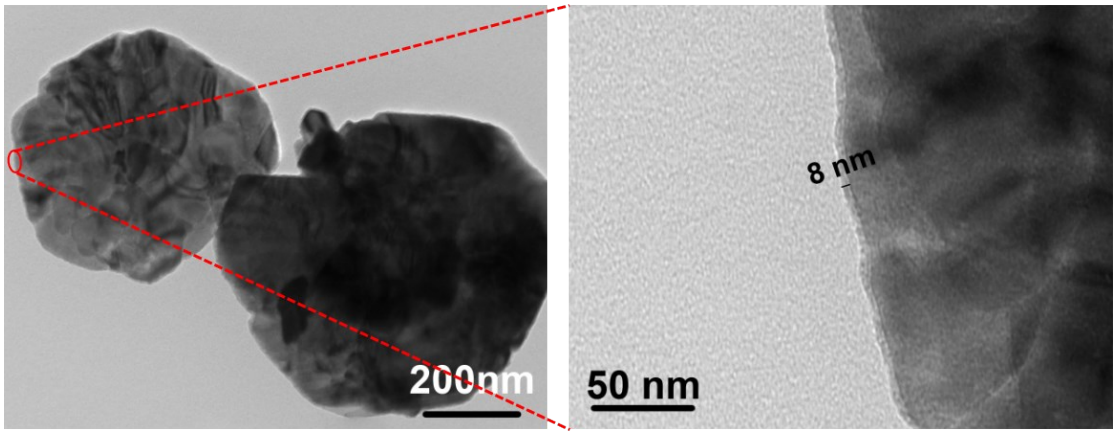

Fig. S5. TEM image of the synthesized OA-PAA-DOX@$FeSe_2$ NSs and partial enlarged view of OA-PAA-DOX@$FeSe_2$ NSs.

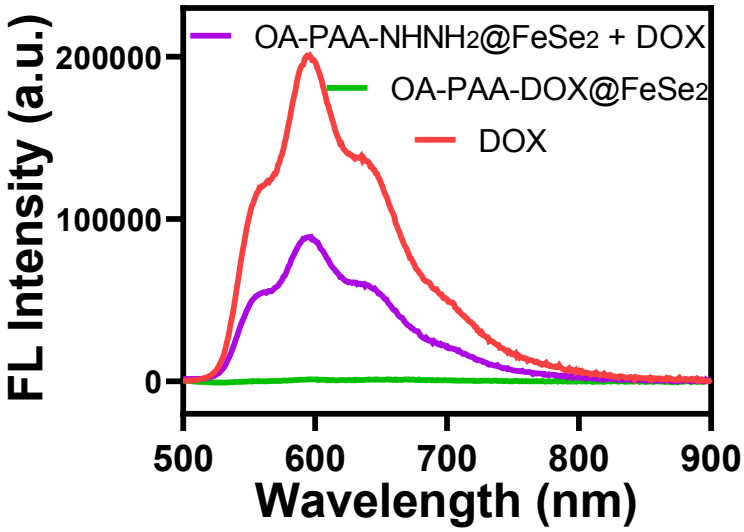

Fig. S6. The fluorescence spectra of DOX, OA-PAA-$NHNH_2$@$FeSe_2$ + DOX and OA-PAA-DOX@$FeSe_2$.

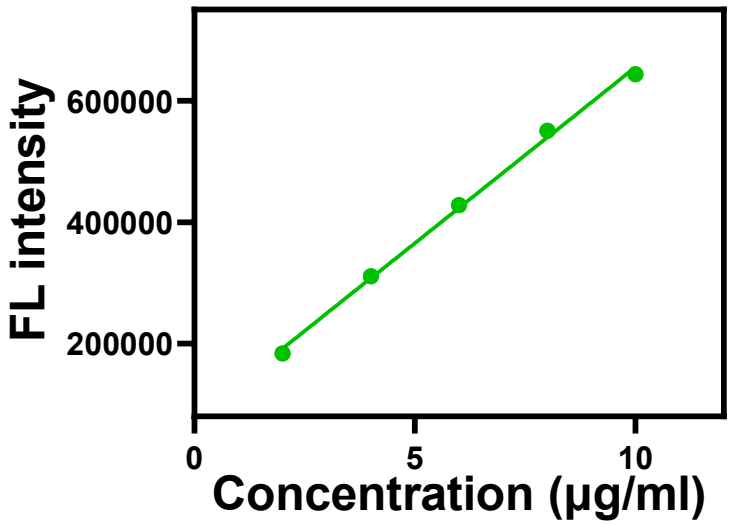

Fig. S7. Calibration curve of DOX.

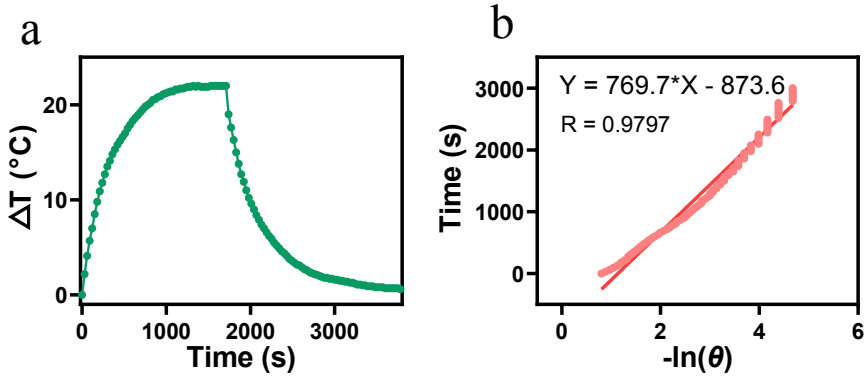

Fig. S8. The photothermal conversion efficiency calculation under 808-nm laser. The temperature increased by 22.0 °C in 1710 s (a), and the τ_s was calculated by the linear relationship of time versus -*ln*(θ) (b).

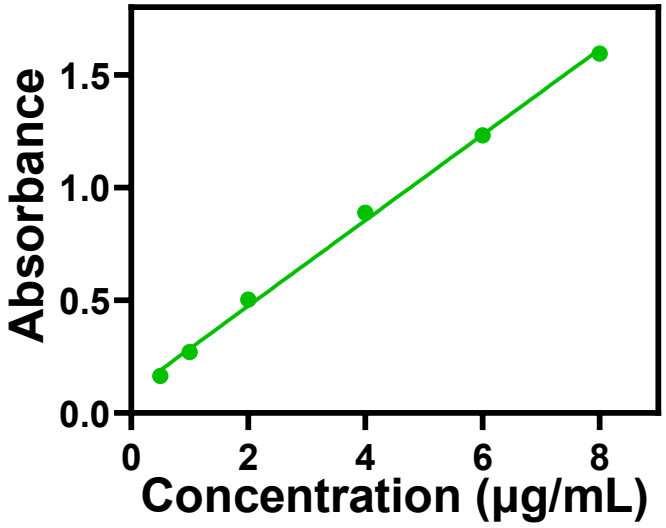

Fig. S9. Calibration curve of MB.

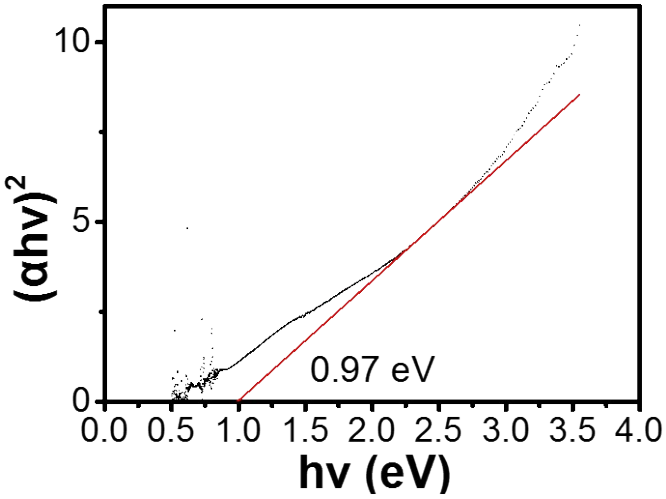

Fig. S10. The corresponding $(Ahν)^2$ versus hν curve.

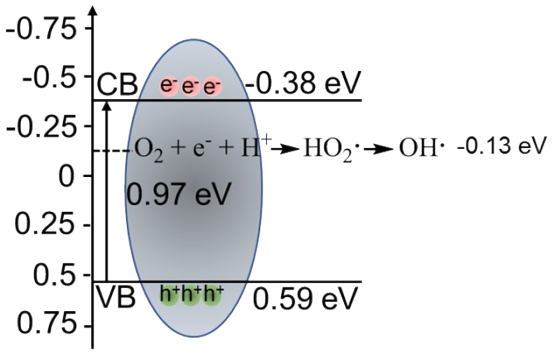

Fig. S11. Measured band structure of the $FeSe_2$ NSs.

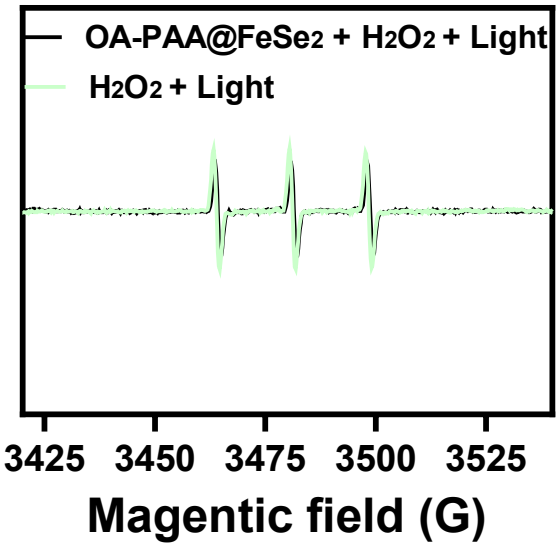

Fig. S12. ESR spectra of TEMP-$^1O_2$.

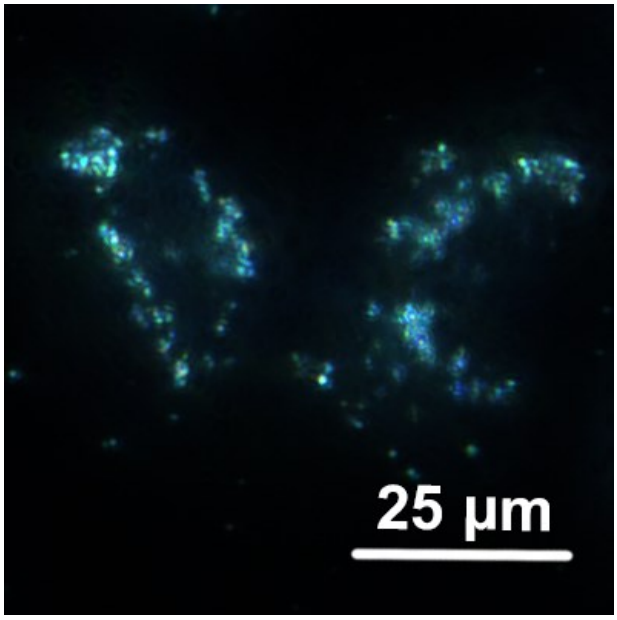

Fig. S13. Dark field microscope image of U87MG cells after 8 h incubation with OA-PAA@$FeSe_2$ NSs.

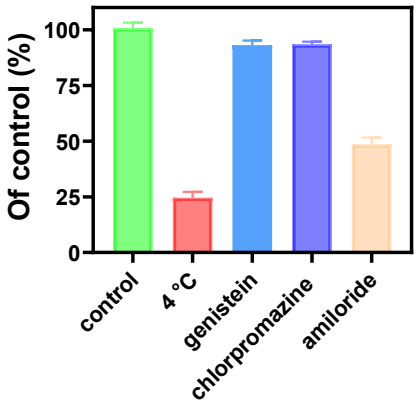

Fig. S14. Cellular uptake mechanism of OA-PAA@$FeSe_2$ NSs in U87MG cells. Data were presented as mean ± SD. (n = 3).

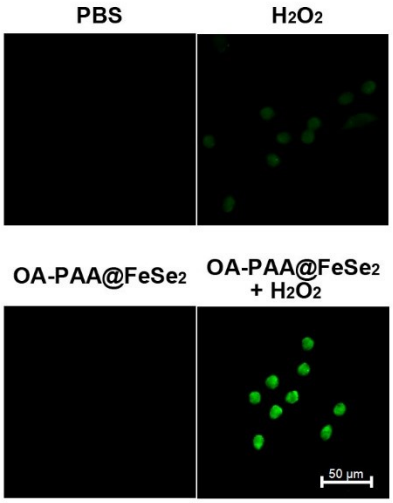

Fig. S15. CLSM images of U87MG tumor cells stained by DCFH-DA in different groups (pH=7.4), including PBS, H_2O_2 only, OA-PAA@$FeSe_2$ NSs only, and OA-PAA@$FeSe_2$ NSs + H_2O_2. All the scale bars are 50 μm.

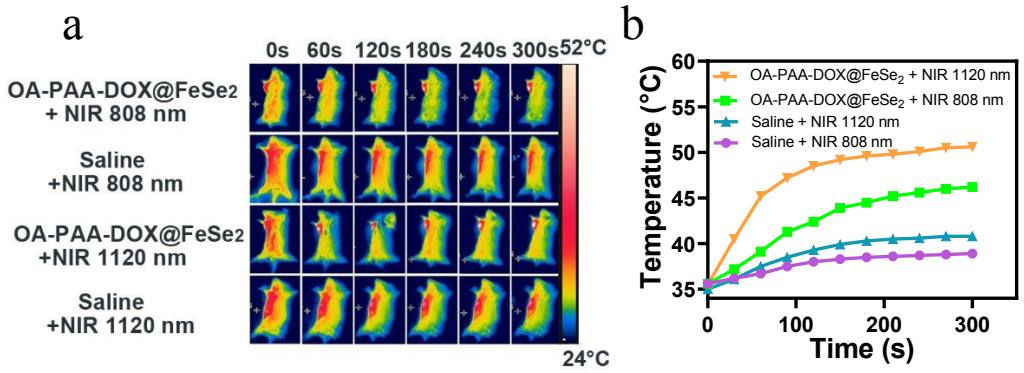

Fig. S16. The thermal imaging pictures (a) and temperature curves (b) of the tumor site after irradiation by 808-nm laser and 1120-nm laser.

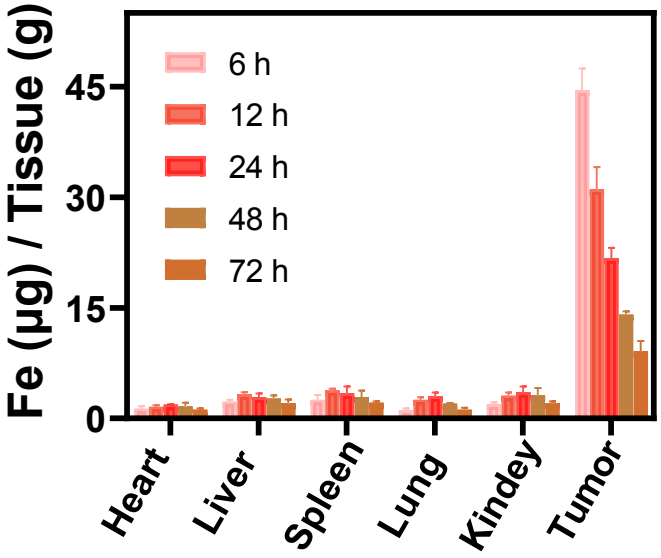

Fig. S17. ICP-MS results of the Fe biodistribution in ICR mice after intratumoral injected with OA-PAA@$FeSe_2$ NSs for different times. The percentages of intratumoral Fe retention were about 74.91% of Fe was reserved at 12 h post injection and 52.48% at 24 h.

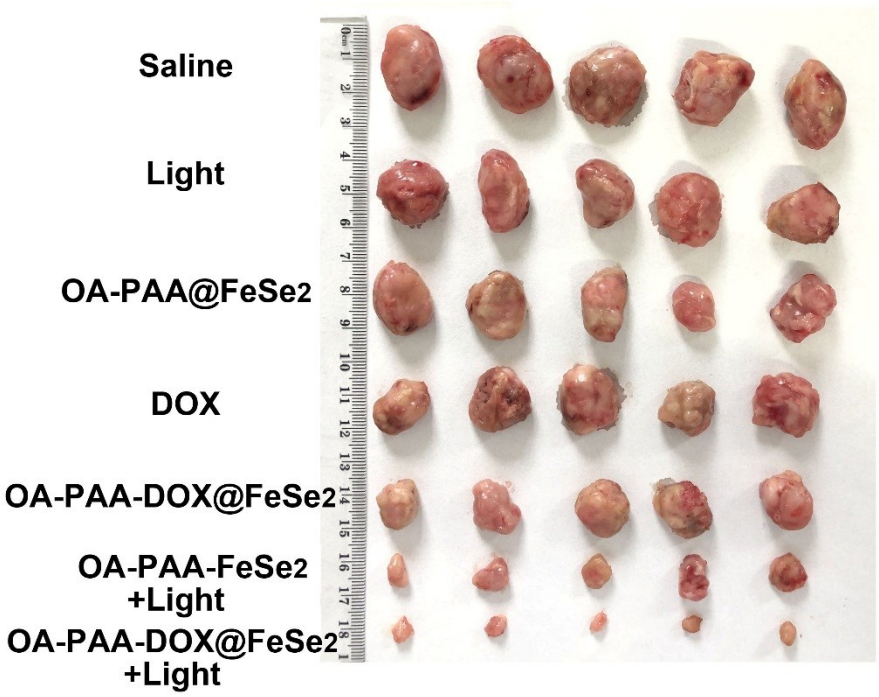

Fig. S18. Digital photos of H22 tumor-bearing mice and dissected tumors from each group on the 15th day.

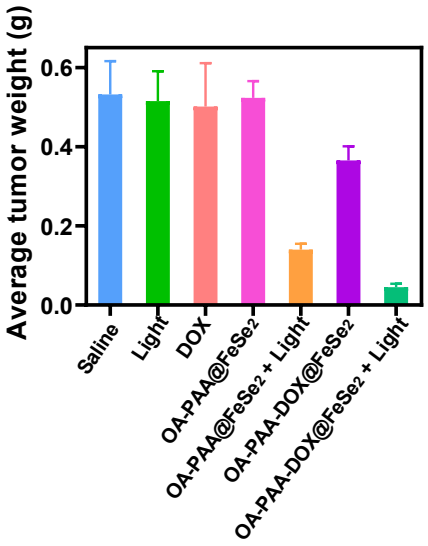

Fig. S19. The average tumor weight of dissected tumors from each group on the 15th day.

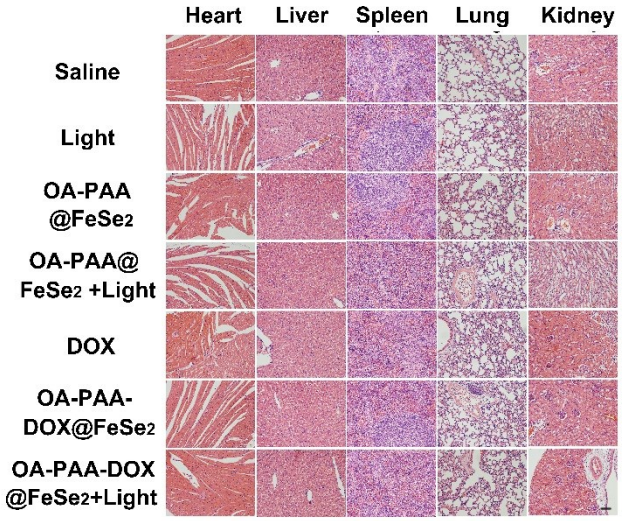

Fig. S20. H&E stained images of heart, liver, spleen, lung, and kidney achieved from various groups after two weeks treatment. (scale bars: 100 μm).

Reference

1. J. Zhang, J. Wang, T. Yan, Y. Peng, D. Xu and D. Deng, *J. Mater. Chem. B*, 2017, **5**, 8152 —8160.
2. W. Chen, L. Su, P. Zhang, C. Li, D. Zhang, W. Wu and X. Jiang, *Polym. Chem.*, 2017, **8**, 6886 —6894.
3. Y. Liu, K. Ai, J. Liu, M. Deng, Y. He and L. Lu, *Adv. Mater.*, 2013, **25**, 1353 —1359.