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Supplementary information

Photothermal augment stromal disrupting effects for enhanced Abraxane synergy chemotherapy in pancreatic cancer PDX mode

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Experimental

Materials: Molybdenum Selenide ($MoSe_2$) powder was purchased from Aladdin. Abraxane was purchased from Fresenius Kabi. Calcein-AM/PI and Indocyanine green (ICG) was obtained by J&K Scientific. Apoptosis/necrosis detection kit (Annexin V-FITC/PI) and cell counting kit-8 (CCK8) were obtained by Dojindo Laboratories. Ultrapure DI-water was obtained from a Milli-Q Gradient System (18.2 M Ω cm, Millipore, U.S.), which was utilized in experiments.

Preparation of $MoSe_2$ nanosheet and Abraxane @$MoSe_2$

$MoSe_2$ nanosheet was prepared according to previous reported works with slightly modified[1]. Briefly, 75 mg of $MoSe_2$ powder was dispersed into DI-water and stirred for 0.5 h. Afterward, the mixture solution was sonicated in an ice bath at 70% amplitude by using a tip sonication (Yimaneili, 950 W, 25 kHz) for 12 h. Then, the treated mixture solution was centrifuged to remove the large aggregate, and the supernatant was centrifuged. The precipitate was then dispersed into DI-water to obtain the nanosize of $MoSe_2$ (3 mg/mL). Furthermore, to obtain the Abraxane @$MoSe_2$, 25 mg Abraxane was added into the above $MoSe_2$ solution with

stirring overnight. Then, the mixture was centrifuged to remove the unbound Abraxane, and the precipitate contained Abraxane@$MoSe_2$ was dispersed in DI-water at 4°C for further use.

The loading amount of Abraxane in Abraxane@$MoSe_2$ was calculated by an indirect method. The Abraxane@$MoSe_2$ was obtained by mixing the excess Abraxane with $MoSe_2$ (3 mg/mL). The amounts of Abraxane in the Abraxane@$MoSe_2$ were calculated according to the corresponding absorbance at 285 nm for Abraxane from 0.023 to 0.75 μg mL^{-1} ($y = 0.5383x + 0.01453$, $R^2 = 0.99855$). The loading amount of Abraxane was obtained by subtraction method of weight, which Abraxane@$MoSe_2$ ($MoSe_2$, 3 mg/mL; Abraxane, 1.5 mg/mL).

Cell Culture and animals.

BXPC-3 cells, PANC-1 cells (Human pancreatic cancer cell lines) and HUVEC cells (Human Umbilical Vein Endothelial Cells) were purchased from the Cell Bank, Chinese Academy of Sciences (Shanghai, China). All cells were genotyped for identification by the Cell Bank, Chinese Academy of Sciences and were tested to rule out mycoplasma contamination. All cells were incubation in RPMI 1640 (ATCC, Manassas, VA) and mixed with FBS (10%) and penicillin- streptomycin (100 IU mL^{-1}) (Cellgro, Manassas, VA).

Characterization of Abraxane @$MoSe_2$ nanosheet

Transmission electron microscope (TEM) analysis was investigated by JEM-2010 (JEOL, Japan). Atomic Force Microscope was used on Multimodel8 AFM (Bruk, Germany). The UV-vis-NIR spectrum of $MoSe_2$ and Abraxane@$MoSe_2$ were recorded through Spectro Max M5e (Germany). Zeta potential and dynamic light scattering (DLS) assays were conducted at 25°C on the NanoZS. Fourier infrared absorption (FTIR) spectrum was performed on an FTIR spectrometer (Spectrum Two, Perkin-Elmer). XPS with a monochromated Mg Kα radiation (1253.6 eV) was performed to analyze the elements.

Photothermal conversion ability of Abraxane @$MoSe_2$ nanosheets upon the NIR laser irradiation.

The PT conversion effect of Abraxane@$MoSe_2$ was investigated according to previous reported works[2]. Briefly, 1 mL of aqueous solution with Abraxane@$MoSe_2$ were irradiated by 808 nm laser (1, 1.5 or 2 W/cm^2) for 500s, and real-time imaged by IR thermal camera. PT

conversion efficiency *(η)* of Abraxane@$MoSe_2$ was calculated by previous report method[3]. Briefly, where h is the heat transfer coefficient, $T_{max}$is the equilibrium temperature, A is the surface area of the container, T_{Surr} is ambient temperature of the surroundings, $\Delta T_{max} = T_{max} - T_{Surr}$, I is incident laser power (1 W/cm^2), and A_λ is the absorbance of Abraxane@$MoSe_2$ at 808 nm. Q_s is the heat, which is associated with the light absorbance of the solvent, and measured independently to be 25.2 mW through DI-water without any solvent.

(1) $\eta = \frac{hA\Delta Tmax - Q_s}{I(1 - 10^{-A_\lambda})}$

(2) $K_S = \frac{m_D C_D}{hA}$

(3) $t = -K_S Ln\theta$

(4) $\theta = \frac{\Delta T}{\Delta T_{max}}$

According to the previous reported method, the photothermal conversion efficiency (η) of $MoSe_2$@Ab-PTX can be calculated.

$hA = m_D C_D/K_S$=4.2 J/294.54, A_λ=0.6544, K_S=294.54

I = 1 W cm^{-2}; ΔT_{max}=25.5℃

H = ((4.2 J/294.54)*25.5-0.0252)) / (1*(1-$10^{-0.6544}$)=0.3384/0.7784=43.47%

Evaluation of Cellular Uptake of Abraxane @$MoSe_2$

To trace the Abraxane@$MoSe_2$ nanosheet, the Cy5 labeled Abraxane @$MoSe_2$ with red fluorescence was conducted. BXPC-3 cells (2.5 × 10^5 cells/well) were incubation of 35 mm glass-bottom Petri dishes for 24 h and then incubated with Cy5-Abraxane @$MoSe_2$ for different time. Afterwards, the treated cells were washed by PBS buffer solution to remove the free Abraxane@$MoSe_2$. The treated cells were then fixed by paraformaldehyde and staining DAPI, and further imaged by CLSM.

Cell Cytotoxicity and In Vitro Antitumor Efficacy of Abraxane@$MoSe_2$

First, to evaluate the biocompatibility of Abraxane@$MoSe_2$, the Hemolysis Test by mouse red blood was performed according to the previous reported protocol[4]. Next, the cytotoxicity was

evaluated by CCK8. BXPC-3 cells and HUVEC cells were cultured in a 96-well plate with the density of 2×10^4 /well and cultured under a humid atmosphere overnight. All cells were replaced with a new 1640 medium with different concentration of $MoSe_2$ ($MoSe_2$, 10~1000 μg/mL) and co-incubation for 24 h and 48h. Afterwards, 100 μL of fresh culture medium and 10 μL of CCK8 were mixed. The absorbance at 450 nm in each well was recorded by a microplate reader after incubation for 1-3 h at 37°C (Spectra Max M5e, Germany). Cell viability was conducted as above:

The cell viability (%) = $(OD_{sample}-OD_{blank}) / (OD_{control}-OD_{blank}) * 100$.

The OD_{sample} and $OD_{control}$ are the absorbance of treated cells, the freshly medium was as a control. The OD_{blank} was the CCK8 solution at the absorbance at 450 nm. All assays were conducted in five independent experiments.

To investigate the *in vitro* antitumor efficacy of Abraxane@$MoSe_2$, BXPC-3 cells and PANC-1 cells were first incubated in 96-well plates with the density of 2×10^4 / well, and cultured overnight. The cells were then cultured with PBS, a new medium with $MoSe_2$, a new medium with Abraxane, and a new medium with Abraxane@$MoSe_2$ for 24h. Afterwards, the treated cell was irradiated by 808 nm laser for 5 min (1 W/cm^2). Then, the viable cells were measured by CCK8. On the other hand, the cells were incubation of viability/cytotoxicity kit for directly detection of alive and dead cells according to our previous reported protocol[5,6].

To investigate the cell apoptosis/ necrosis induced by Abraxane@$MoSe_2$ upon the laser irradiation. BXPC-3 cells were cultured in 6-well with the density of 1×10^6 cells overnight. Afterwards, the dead cells were removed and then incubated with Abraxane@$MoSe_2$ for 12 h at 37 °C. Then, the cells were washed by buffer solution, and irradiated by 808 nm laser for 5 min (1 W/cm^2). Moreover, the thermal image of treated cells upon the NIR light was recorded by IR thermal camera. Then, the treated cells were cultured in new 1640 medium for 24 h. Afterwards, the treated cells were collected and stained by Annexin V-FITC / PI according to the manufacturer protocol and evaluated by flow cytometry.

Patient-derived xenograft (PDX) mice model

Immunodeficient mice nude mice (BALB/C, male) (5-6 weeks) were obtained by China Wushi, Inc (Shanghai, China). Animal experiment protocols were approved by the Ethics

Committee for Animal Research of 900 Hospital of the Joint Logistics Team. Patient-derived xenograft (PDX) mode was built according to the previous reported method[7-9]. Briefly, PDAC tumor was obtained from fresh PDAC patients (P0) surgical specimens at Fujian Medical University Union Hospital. Tumors were washed thrice and putting in RPMI medium with FBS (10%) and cut into about 0.3 × 0.3 × 0.3 cm pieces. After sterilizing by iodophor and 75% alcohol, the tumors were transferred into the mice back. When the tumors volume was grew up to 500 mm^3, and then transplanted into secondary mice (P2). The expanded tumors can then be cryopreserved or transplanted into (P3) mice.

In Vivo fluorescence imaging and antitumor therapy of Abraxane@$MoSe_2$

To evaluate the bio-distribution of Abraxane@$MoSe_2$, ICG-NHS labeled Abraxane@$MoSe_2$ was intravenous injection into PDX mice mode with the tumor volume (100 mm^3) and imaged by UniNano-NIR II fluorescence imaging system at different time points. Afterwards, the main organs and tumors were isolated from treated mice at 48 h and imaged by UniNano NIR-II imaging system (United Well, China). To quantify the intensity within the main organs and treated mice tumors, ImageJ software was performed. To investigate the antitumor efficacy, the PDAC PDX mice were divided into five groups:

(1) PBS without any treatment (n=5);

(2) PBS exposed to the 808 nm lasers (1 W /cm^2) for 10 min (n=5);

(3) Intravenous injection of Abraxane (100 μL, 1.5 mg/mL) (n=5);

(4) Intravenous injection of Abraxane@$MoSe_2$ 100 μL ($MoSe_2$ 3 mg/mL; Abraxane, 1.5 mg/mL) (n=5);

(5) Intravenous injection of Abraxane@$MoSe_2$ 100 μL. ($MoSe_2$ 3 mg/mL; Abraxane, 1.5 mg/mL) with NIR laser irradiation (1 W /cm^2) for 10 min (n=5).

The NIR laser irradiation timing was at 48 h after intravenous injection. To investigate the photo-chemotherapeutic efficiency of different groups, the tumor volume and body weight were measured by calipers in every 2d following equation:

$V=A*B^2 / 2$

(A and B are the longer and shorter diameter (mm) of the tumor).

To investigate the histological changes of treated tumors in each group, one of the tumor-

bearing mice was collected after different treatments at 48 h. The tumor section was stained through H&E, Ki67 and TUNEL, respectively.

Assessment of CAFs in PDAC

To evaluate the content of CAFs after different treatment, the activated fibroblasts were detected by a double staining through vimentin (acted as a total fibroblasts marker with the ratio of 1: 50) and smooth muscle actin (SMA) (acted as activated fibroblast marker) according to the previous method[10,11]. The secondary antibodies were utilized by Alexa Fluor 488 donkey anti-goat IgG with the ratio of 1: 200 for vimentin, and the Alexa Fluor 546 goat anti-mouse IgG with the ratio of 1: 200 (SMA) [12]. Tumors after received different treatment were stained by DAPI dye. All of the tumor slices were imaged by CLSM (LSM 780 Zeiss).

Long-term toxicity of Abraxane@$MoSe_2$ nanosheet *in vivo*

To investigate the potential toxicity, the treated mouse were sacrificed at 20th day after different treatment as indicated and major organs were fixed in 4% formaldehyde, and the slices were then stained through H&E. Afterwards, the section was imaged by CLSM. To further assess the toxicity of Abraxane@$MoSe_2$, the nanoparticle (5 mg/kg) treated (BALB/c) mice at different time points (0, 2, 7, 14 d) were sacrificed, and blood was collected for biochemical analysis.

Statistical Analysis

The data are analyzed by a one-way of variance (ANOVA) method or the two-tailed paired Student's $*p<0.05$, $***p<0.001$, $****p<0.0001$ was considered as statistically significant. All the data were shown as means ± SD through at least three experiments.

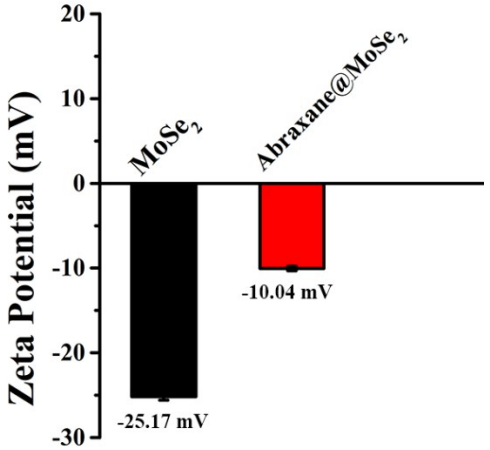

Figure S1. Zeta potential of $MoSe_2$ and Abraxane@$MoSe_2$ in DI-water.

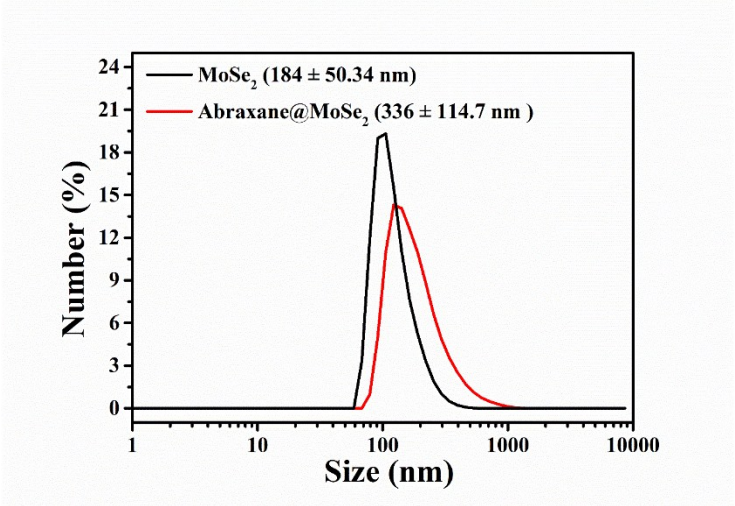

Figure S2. DLS analysis of $MoSe_2$ and Abraxane@$MoSe_2$ in DI-water.

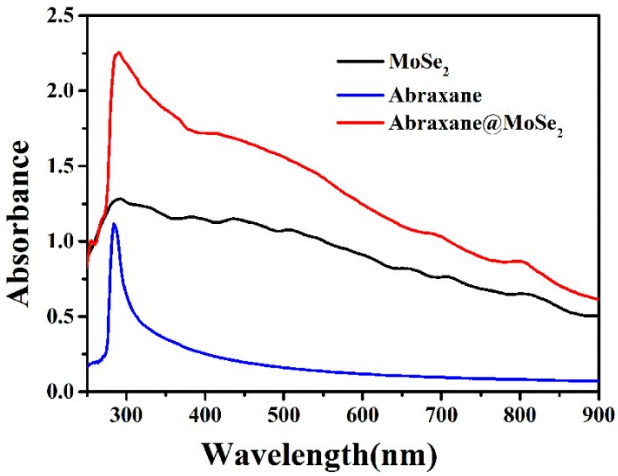

Figure S3. UV-vis-NIR absorbance of $MoSe_2$, Abraxane and Abraxane@$MoSe_2$.

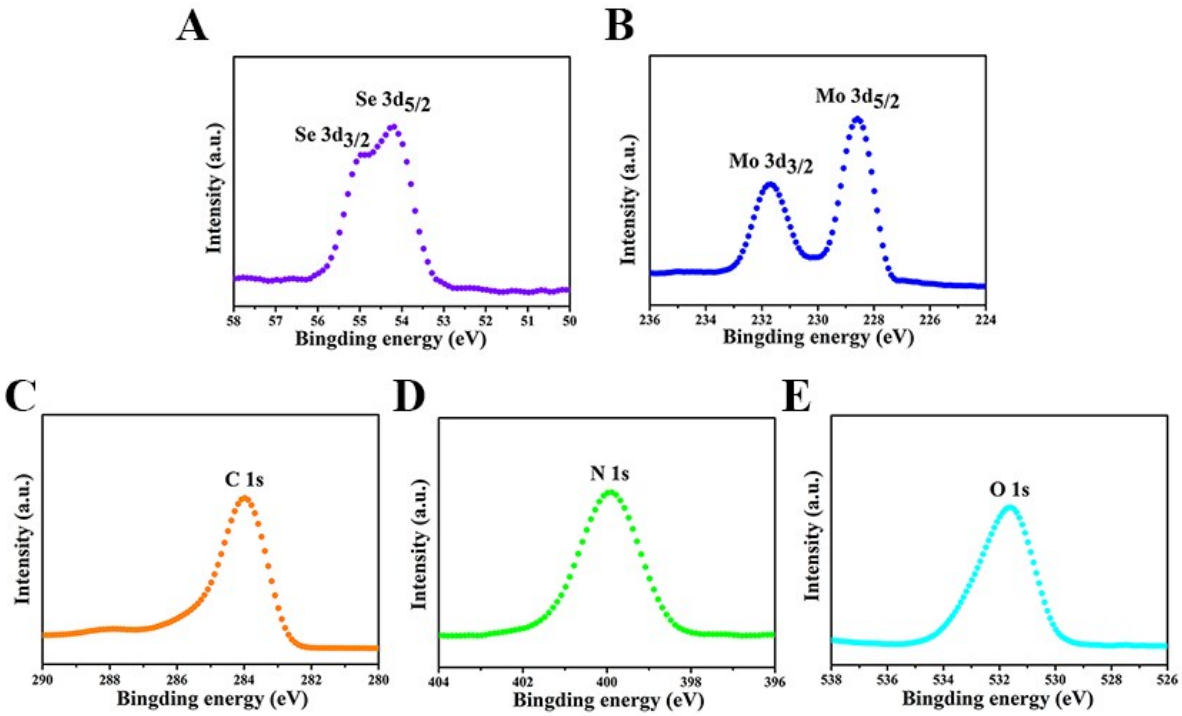

Figure S4. The XPS spectra of A) Se, B) Mo, C) C 1s, D) N 1s, E) O1s in Abraxane@$MoSe_2$.

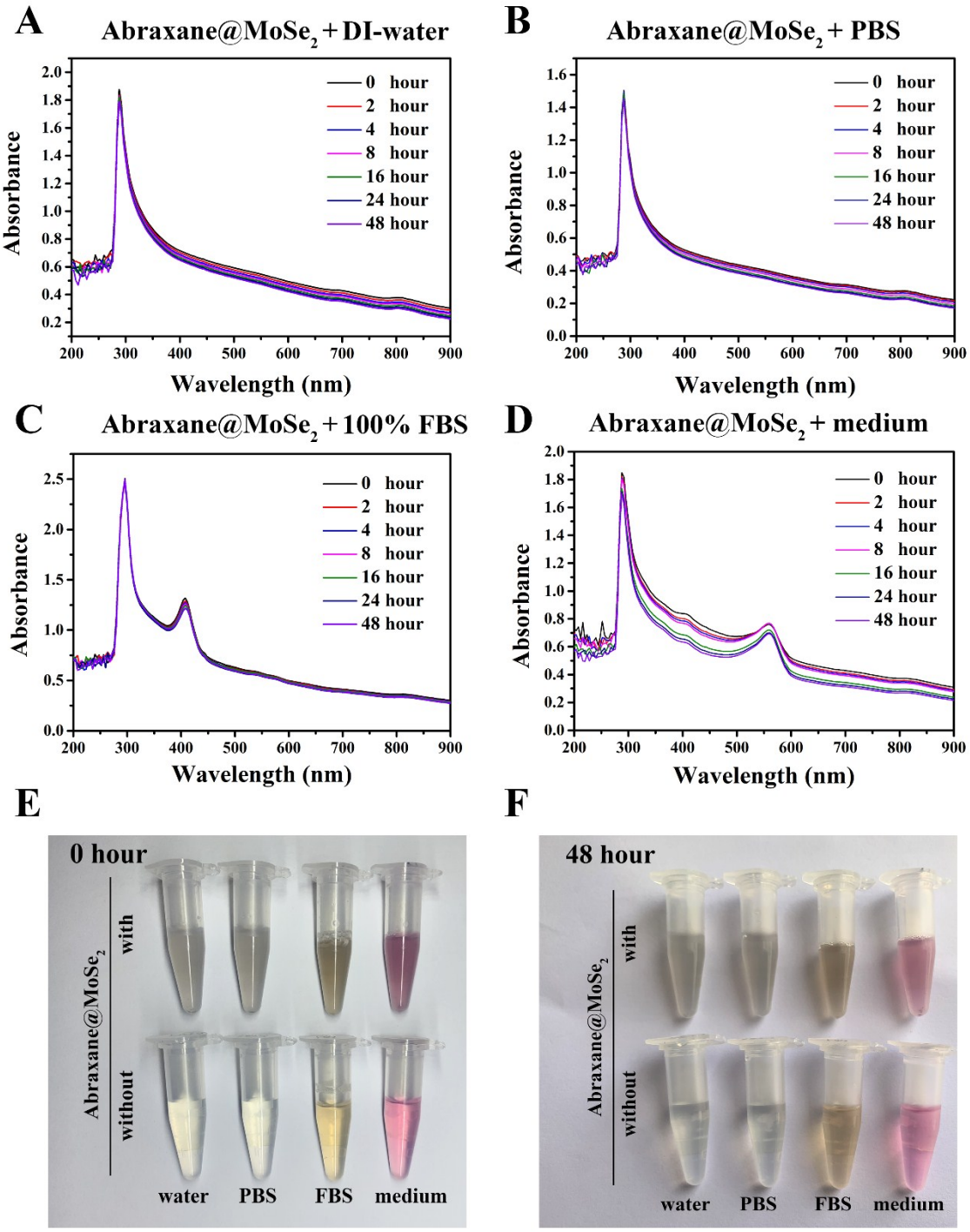

Figure S5. The bio-stability of Abraxane@$MoSe_2$ in (A) water, (B) PBS buffer solution, (C) 100% FBS (fetal bovine serum), and cell culture medium (contained 10% FBS) with different incubation times. The UV-vis-NIR absorption spectra were measured, respectively. (E) and (F) the photograph of different solutions with or without Abraxane@$MoSe_2$ at 0 h and 48 hrs.

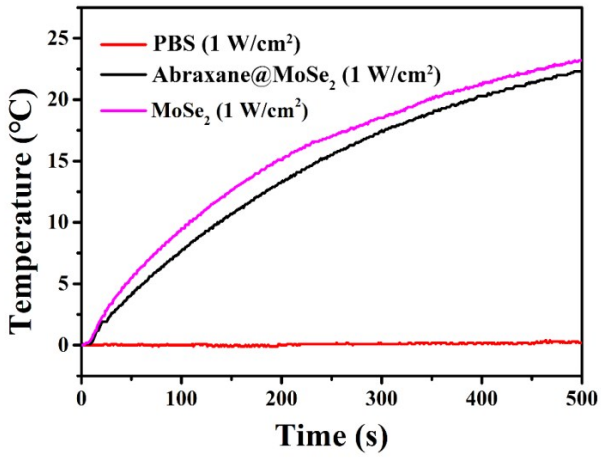

Figure S6. Photothermal heating curves of aqueous dispersions of PBS, free $MoSe_2$ and Abraxane@$MoSe_2$ at power density (1.0 W/cm^2).

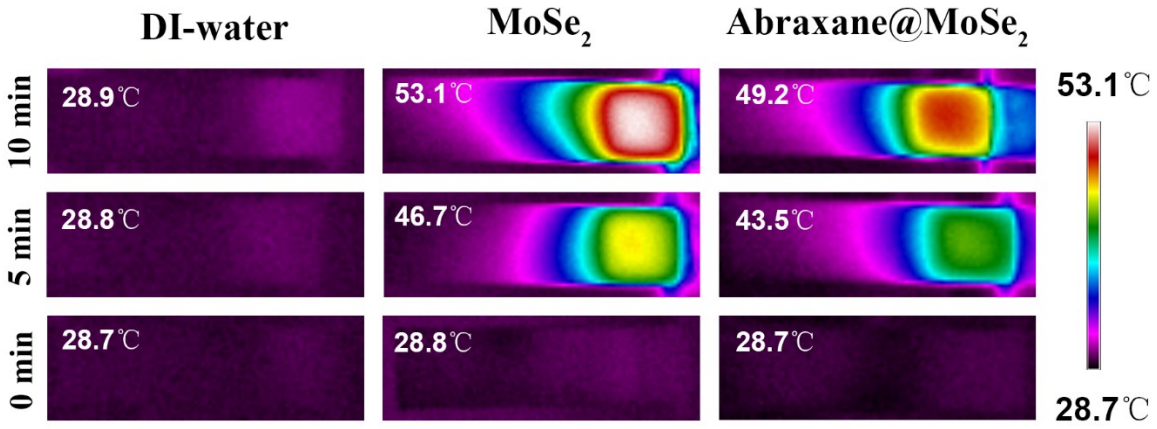

Figure S7. IR thermal image of DI water, $MoSe_2$ and Abraxane@$MoSe_2$.

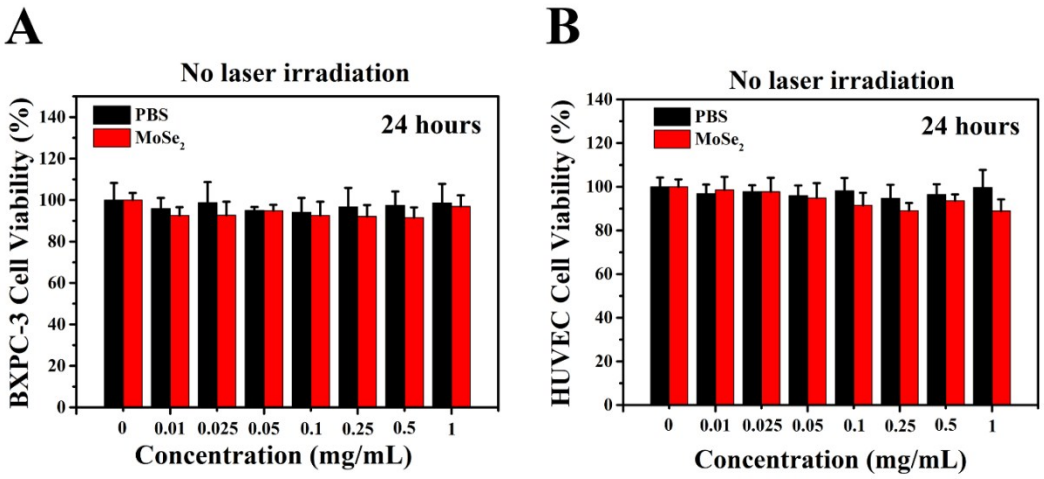

Figure S8. (A) and (B) The cell viability of BXPC-3 cells and HUVEC cells after co-cultured with different concentrations of Abraxane@$MoSe_2$ for 24 h in dark.

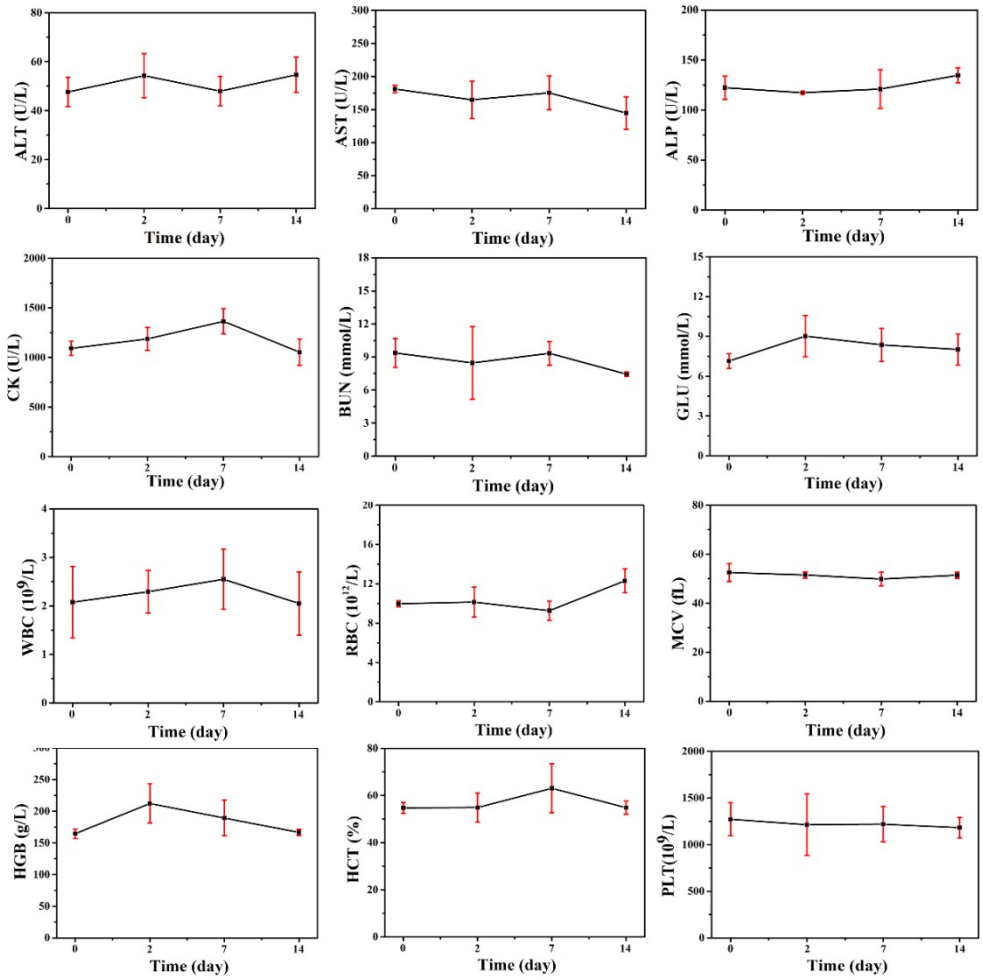

Figure S9. Biochemical analysis of ALT, AST, ALP, CK, BUN, GLU, WBC, RBC, MCV, HGB, HCT and PLT in BALB/c nude at different time after *i. v.* injection of Abraxane@$MoSe_2$ (n= 4).

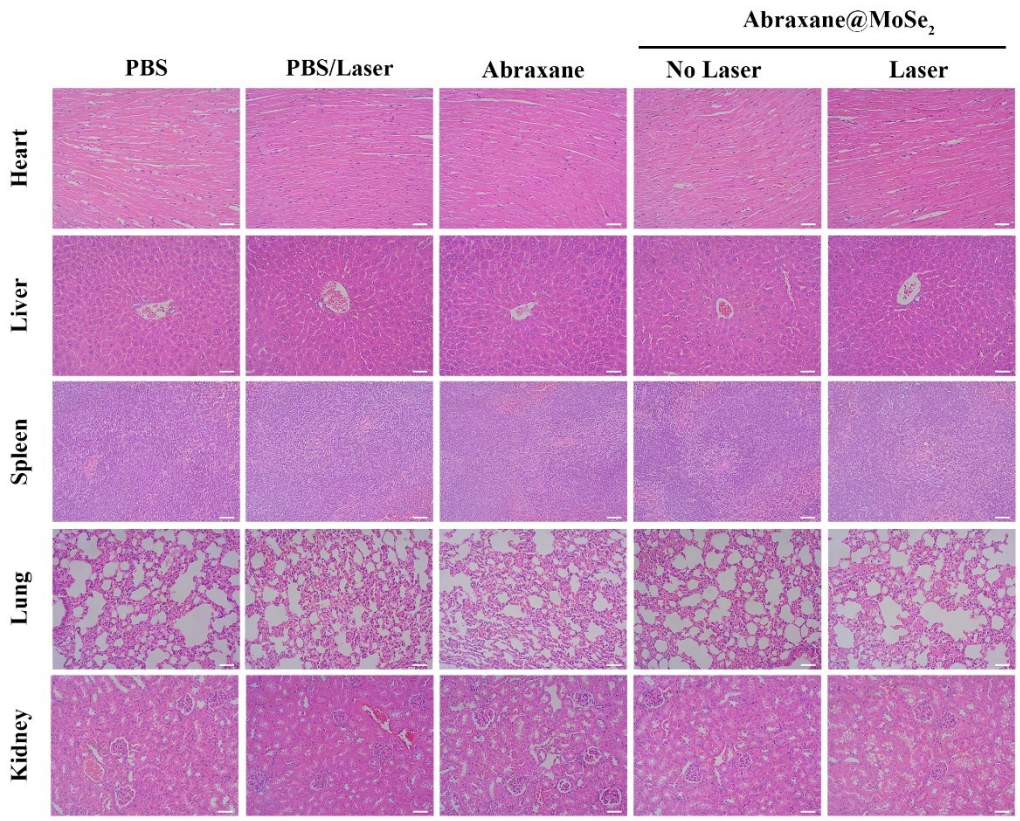

Figure S10. The pathological changes of main organs evaluated by H&E staining which were acquired at 20 d after received different treatment as indicated. (Scale bar: 50 μm).

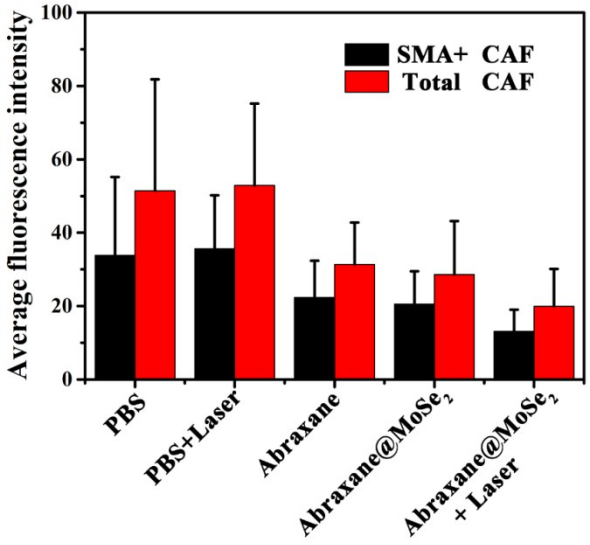

Figure S11. The average fluorescence intensity of α SMA (+) CAF and total CAF after received different treatment as indicated.

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