*Electronic Supplementary Information for*

# Cryo-assisted exfoliation of atomically thin 2D $Sb_2Se_3$ nanosheets for photo-induced theranostics

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## 1. Experimental section

### 1.1 Materials

Antimony selenide were purchased from J&K Chemical Co.. Polyvinyl pyrrolidone and N-methyl pyrrolidone were obtained from Sinopharm Chemical Reagent Co.. Deionized water in the experiments was obtained by using a Milli-Q water system.

### 1.2 Exfoliation of PVP-capped $Sb_2Se_3$ nanosheets

Raw $Sb_2Se_3$ powders (100 mg) were soaked into liquid nitrogen for 1h. The cryo-pretreatment powders were immediately transferred into an agate mortar and ground for 40 min for the granular refinement. Then, the $Sb_2Se_3$ powders and PVP (200 mg) were added into 20 mL of N-methyl pyrrolidone (NMP), followed by bath sonication

for 2 h and probe sonication (600 W, 50%, period of 3 s with the interval of 3 s) for 2 h under ice bath, respectively. After centrifugation at 5000 for 15 min to isolate the unexfoliated $Sb_2Se_3$ powders, PVP-capped $Sb_2Se_3$ nanosheets were finally collected by centrifugation of the supernatants at 14000 rpm for 15 min and washed with DI water for 3 times. For cyclic exfoliation, PVP (200 mg) and NMP (20 mL) were added into the sediments containing unexfoliated nanosheets, colloidal $Sb_2Se_3$ nanosheets dispersions could be also effectively exfoliated through another 2 h probe sonication. Such cyclic exfoliation could be repeated for at least 7 times. For comparison, the samples without the cryo-pretreatment of liquid nitrogen were also prepared as a control.

**1.3 Characterization**

The morphology and size of $Sb_2Se_3$ nanosheets was observed by a JEOL high-resolution transmission electron microscope (JEM 2100, Japan). The Zetasizer Nano series (Malvern Instruments Ltd, UK) was used to determine the DLS size and Zeta potentials of $Sb_2Se_3$ nanosheets. The UV-vis-NIR spectra of $Sb_2Se_3$ nanosheets were obtained by a Hitachi F-2700 spectrophotometer, the FTIR spectrum of $Sb_2Se_3$ nanosheets was acquired by a Bruker FTIR spectrometer, the Raman spectrum of $Sb_2Se_3$ nanosheets was acquired by a LabRAM XploRA laser Raman spectrometer, and X-ray photoelectron spectroscopy spectra of samples were measured by a ESCALAB250Xi photoelectron spectrometer.

**1.4 Measurement of photothermal performance of $Sb_2Se_3$ nanosheets**

To evaluate the photothermal performance, deionized water and $Sb_2Se_3$

nanosheets with different concentrations were irradiated for 10 min by a NIR laser (808 nm, 2 W). The dispersion temperature was monitored by an IRT camera (Ti400, Fluke, USA).

To calculate the photothermal conversion efficiency of $Sb_2Se_3$ nanosheets, 1 mL of $Sb_2Se_3$ nanosheets aqueous dispersion (300 μg/mL) was continuously irradiated under the same condition until reaching a steady-state temperature. The laser was then shut off and the temperature decrease process was also recorded. the photothermal conversion efficiency (η) was calculated using equation (1) described by Roper[1]:

$$\eta = \frac{hS(T_{max} - T_{surr}) - Q_s}{I(1 - 10^{-A_{808}})} \quad (1)$$

where $h$ is the heat transfer coefficient, $S$ is the surface area of the container, $T_{max}$ is the maximum system temperature, $T_{surr}$ is the ambient surrounding temperature, $Q_s$ is the heat associated with the light absorbance of the solvent, $I$ is the laser power (2 w) and $A_{808}$ is the absorbance of $Sb_2Se_3$ nanosheets at 808 nm. The value of $hS$ is derived according to equation (2):

$$\tau s = \frac{m_D C_D}{hS} \quad (2)$$

where $\tau_s$ is the sample system time constant, $m_D$ and $C_D$ are the mass (1 g) and heat capacity (4.2 J/ (g. ºC)) of deionized water, respectively. $Q_s$ is measured independently to be 12.6 mW using pure water. In order to get the value of $hS$, we further introduce θ, which is defined as follows:

$$\theta = \frac{T - T_{surr}}{T_{max} - T_{surr}} \quad (3)$$

where $T$ is the solution temperature. Thus, $hS$ can be determined by applying the linear time data from the cooling period vs $-\ln\theta$.

**1.5 Cytotoxicity assay and hemolysis test**

A standard MTT assay was applied to evaluate the cytotoxicity of $Sb_2Se_3$ nanosheets using HUVECs cell line as a model. The cells were seeded into 96-well plates at a density of $1\times10^4$ cells per well. Triplicate wells were treated with indicated concentrations of $Sb_2Se_3$ nanosheets (0, 25, 50, 100, 200, 300, 400, 500 μg/mL) for 24 h. Subsequently, MTT with a final concentration of 1mg/mL was added into the medium, and incubated for 4 h at 37 ºC to allow the formation of formazan. Dimethyl sulfoxide was added to each well to dissolve formazan. The absorbance was measured by microplate reader (Infinite 200 NanoQuant, TECAN) at 490 nm to determine the relative cell viability.

For hemolysis assay, 0.5 mL of fresh blood sample were stabilized by citric acid. Red blood cells (RBCs) were separated by centrifugation (3000 rpm, 10 min), and washed with PBS solution for three times, followed by the dilution into 5 mL of PBS. Afterwards, 0.2 mL of diluted RBC suspension was taken out to mix with 0.8 mL of $Sb_2Se_3$ nanosheets dispersions with different concentrations (0, 25, 50, 100, 200, 300, 400 μg $mL^{-1}$). DI water and PBS solution were used as positive and negative controls, respectively. In addition, pure $Sb_2Se_3$ nanosheets dispersions were used as background control. All the mixtures were kept at 37 °C for 4 hours. After centrifugation (3000 rpm, 10 min), the supernatant were carefully collected for the measurement of absorbance at 541 nm. The hemolysis ratio was calculated using the

following equation:

Percent Hemolysis (%) = $[(A_{sample\text{-}RBCs+}\text{-}A_{sample\text{-}RBCs-})\text{-}A_{negative}]/[A_{positive}\text{-}A_{negative}]\times 100\%$,

where $A_{sample}$, $A_{positive}$ and $A_{negative}$ are the absorbance value of sample groups, positive control and negative control, respectively.

### 1.6 *In vitro* and *in vivo* photoacoustic imaging

Photoacoustic imaging *in vitro* and *in vivo* were both performed under Vevo LAZR-X using 808 nm as the working laser wavelength. For *in vitro* imaging, the photoacoustic signals of different concentrations of $Sb_2Se_3$ nanosheets dispersions (0.1, 0.2, 0.4, 0.6 and 0.8 mg/mL) were recorded, DI water was used as control samples. For *in vivo* photoacoustic imaging of tumor, 4T1 tumor-bearing mice were used as a model. $Sb_2Se_3$ nanosheets dispersion (0.1 mL, 4 mg/mL) was injected into nude mouse through intravenous tail injection. Then photoacoustic images of tumor section were acquired by the photoacoustic scanner at different time periods (0, 2, 4, 8 and 12 h). During the experiment, the body temperature of the mice was maintained by using a water heating system at 37.5 °C.

### 1.7 *In vitro* and *in vitro* photothermal ablation

For *in vitro* photothermal ablation, 4T1 cells were used as model cells. The cells were seeded into 96-well plates at a density of $1\times10^4$ cells per well for 24 h before the experiments. To quantitatively evaluate the photothermal cytotoxicity of $Sb_2Se_3$ nanosheets on 4T1 cells, the cells were incubated with fresh medium containing different concentrations of $Sb_2Se_3$ nanosheets (0, 25, 50, 100, 200, 300, 400, 500 and 800 μg/mL) for 4 h, then irradiated by using a NIR laser (808 nm, 2 W) for different time periods (0, 1, 3 min). The cell viabilities were determined by MTT assay as

described above. For qualitative analysis, the cells were incubated in medium containing $Sb_2Se_3$ nanosheets (250 μg/mL) for 4 h, and then irradiated by a NIR laser (808 nm, 2 W) for 3 min. After the treatment, the cells were stained with calcein acetoxymethyl ester (calcein AM), and visualized under fluorescence microscope (Olympus, Japan).

For *in vivo* photothermal ablation, 4T1-beraing mice were used as animal models. When the tumor volumes were about 50 $mm^3$, the mice were randomly dived into four groups: (i) "PBS", (ii) "$Sb_2Se_3$", (iii) "PBS+laser", (iv) "$Sb_2Se_3$+laser". The mice of group (i) and (iii) were intratumorally injected with 100 μL of PBS while the mice of group (ii) and (iv) were injected with 100 μL of $Sb_2Se_3$ nanosheets dispersions (4 mg/mL). IRT imaging was conducted to monitor the temperature change of tumor during laser irradiation (808 nm, 2 W $cm^{-2}$) by an IRT camera (Ti400, Fluke, USA). After the various treatments, the tumor volume and body weight of each group were measured and monitored for 15 days.

**Statistical Analysis**: The data obtained here are analyzed by student t-tests with a setting significance of $p < 0.05$ (*) and $p < 0.01$ (**).

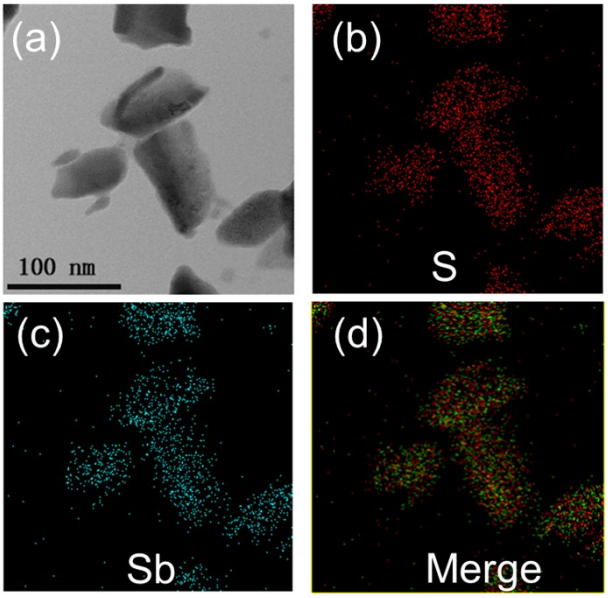


**Fig. S1** EDS mapping of $Sb_2Se_3$ nanosheets. (a) TEM image; (b) elemental Se distribution, (c) elemental Sb distribution, (d) merge picture.

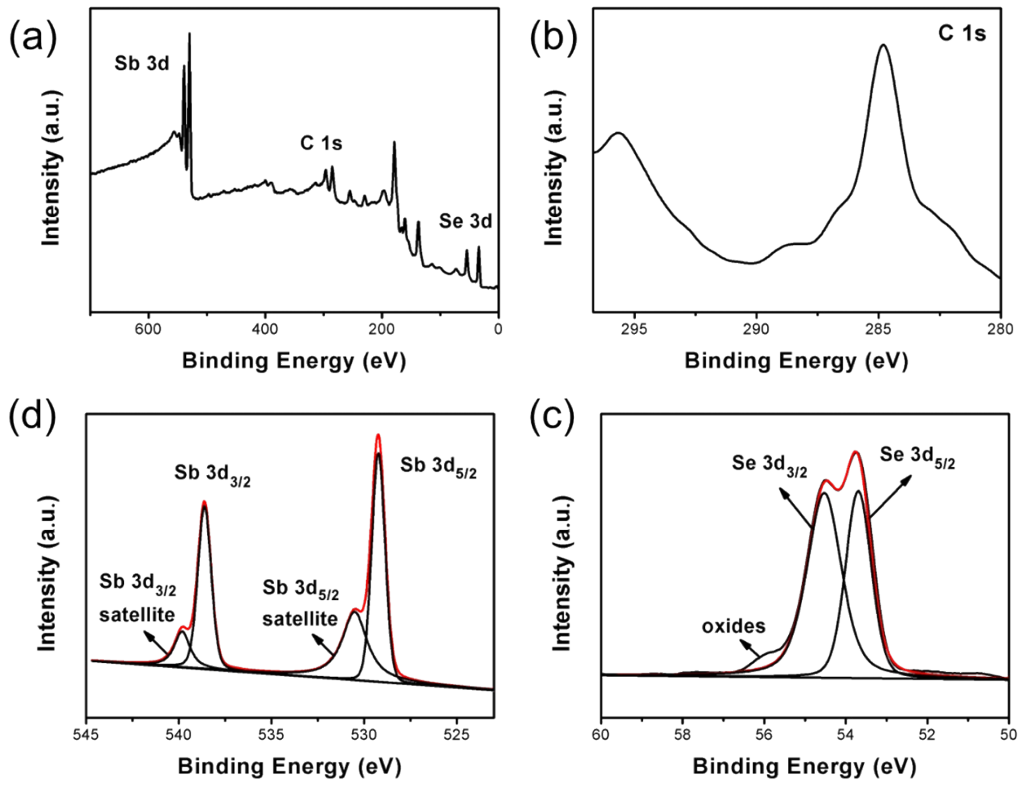


**Fig. S2** XPS spectra of PVP-capped $Sb_2Se_3$ nanosheets. (a) Sum, (b) $C_{1s}$, (c)$Sb_{3d}$, (d) $Se_{3d}$.

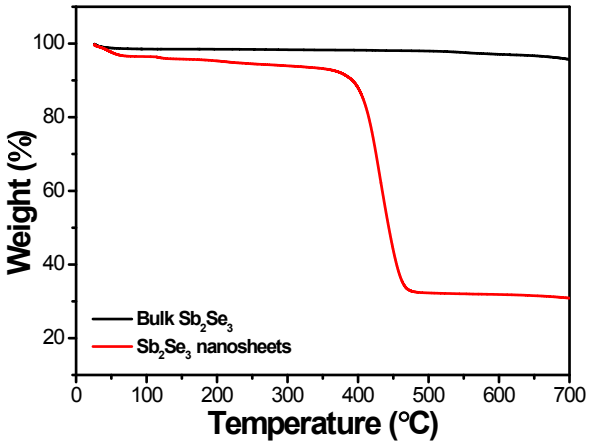


**Fig. S3** Thermogravimetric analysis curves of bulk $Sb_2Se_3$ and $Sb_2Se_3$ nanosheets under $N_2$ atmosphere.

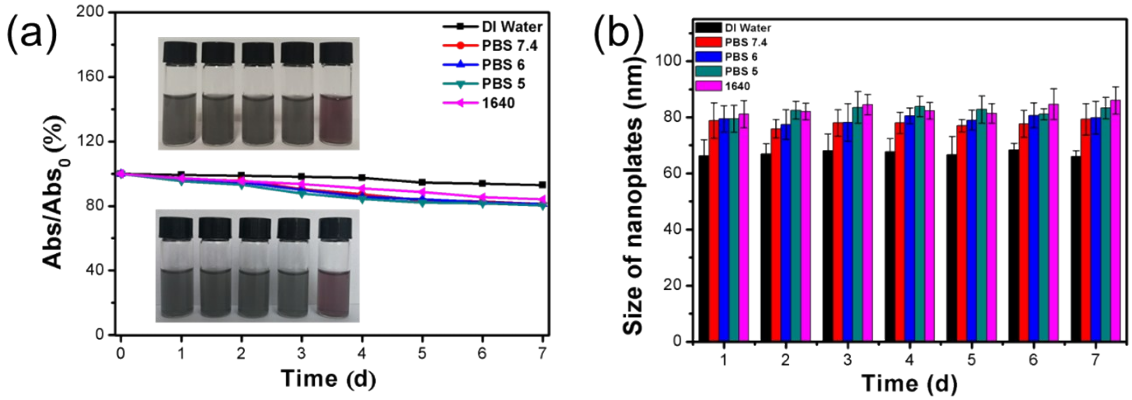


**Fig. S4** Absorbance (a) and DLS size (b) change of $Sb_2Se_3$ nanosheets dispersions in different mediums (DI water, PBS and 1640 cell culture medium) for one week.

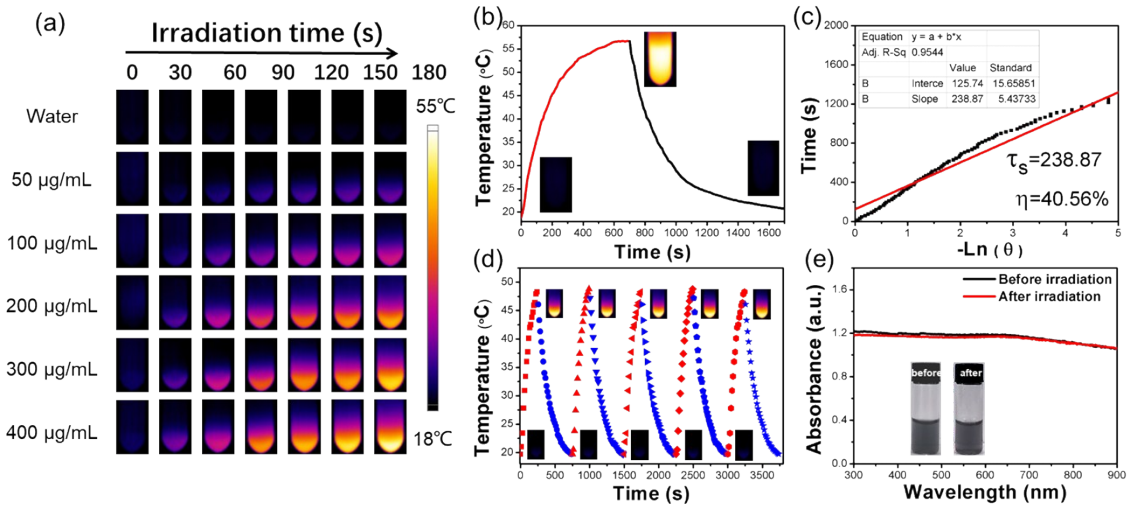


**Fig. S5** (a) The infrared thermal images of $Sb_2Se_3$ nanosheets dispersions under laser irradiation (2 W, 808 nm). (b) The heating/cooling curves of $Sb_2Se_3$ nanosheets with laser on/off. (c) The fitting linear curve of time data vs –lnθ from the cooling period of $Sb_2Se_3$ nanosheets. (d) The heating/cooling curve of $Sb_2Se_3$ nanosheets for repeatedly turning on/off laser irradiation for five cycles. (e) Vis-NIR absorbance spectra of $Sb_2Se_3$ nanosheets before and after irradiation, the inset is the corresponding digital images of $Sb_2Se_3$ nanosheets before and after irradiation.

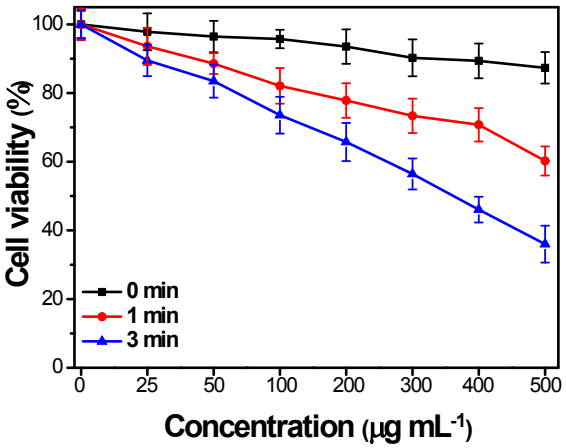


**Fig. S6.** Photothermal ablation of 4T1 cells incubated with different concentrations of $Sb_2Se_3$ nanosheets dispersions under 808-nm laser irradiation (1 W).

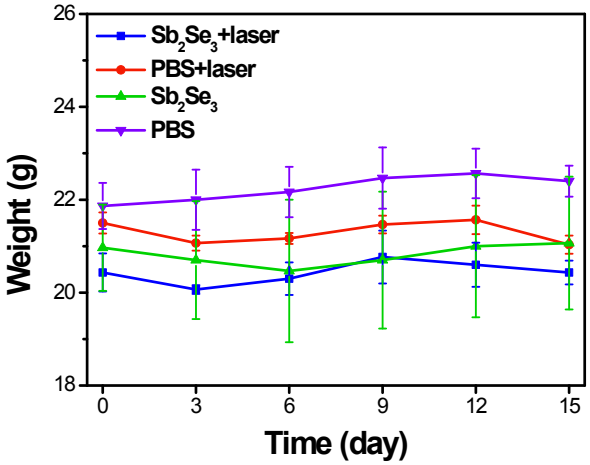


**Fig. S7** Body weight change of mice from different groups as indicated during 15 days.

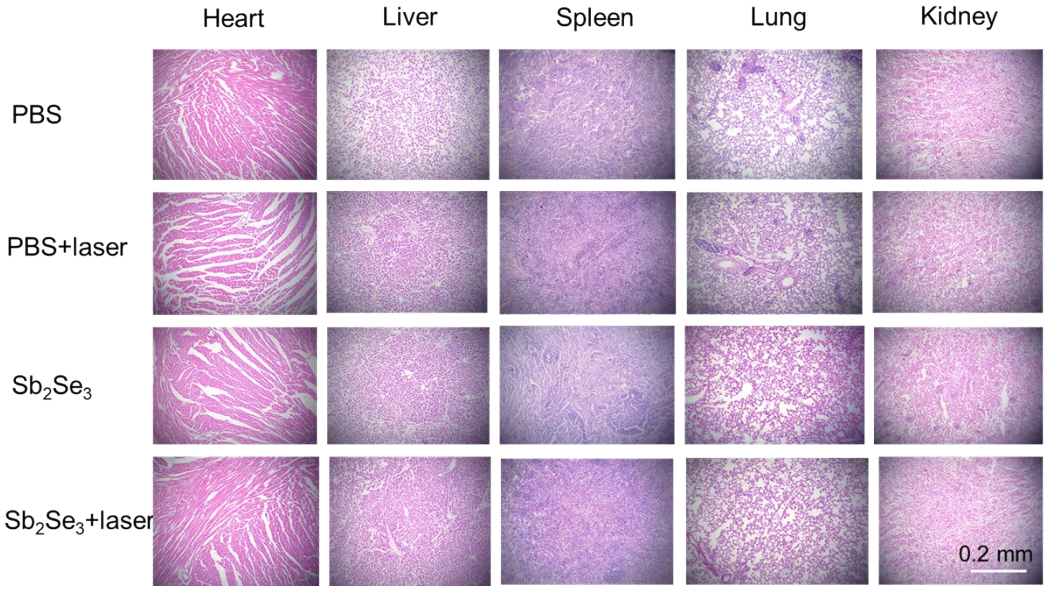


**Fig. S8** Typical images of major organs (herat, liver, spleen, lung and kidney) obtained from different groups as indicated through H&E staining.

## References

1 D. K. Roper, W. Ahn and M. Hoepfner, *J. Phys. Chem. C*, 2007, **111**, 3636.