Supporting information

NIR-Laser-Controlled Drug Release from DOX/IR-780-Loaded Temperature-Sensitive-Liposomes for Chemo-Photothermal Synergistic Tumor Therapy

Fei Yan [1, 3, *], Wanlu Duan [2, *], Yekuo Li [2, ✉], Hao Wu [3], Yuli Zhou [4], Min Pan [1], Hongmei Liu [3], Xin Liu [1] and Hairong Zheng [1, 3, 5, ✉]

[1] Paul C. Lauterbur Research Center for Biomedical Imaging, Institute of biomedical and Health Engineering, Shenzhen Institutes of Advanced Technology, Chinese Academy of Sciences, Shenzhen, China

[2] Department of Ultrasound, Guangzhou General Hospital of Guangzhou Military Command, Guangzhou, China

[3] Department of Ultrasonography, The Third Affiliated Hospital of Southern Medical University (Academy of Orthopedics Guangdong Province), Guangzhou, China

[4] Department of Ultrasonography, Shenzhen People's Hospital & Second Clinical Medical College of Jinan University, Shenzhen, China

[5] Shenzhen Key Laboratory of Nanobiomechanics, Shenzhen Institutes of Advanced Technology, Chinese Academy of Sciences, Shenzhen, China

✉ Corresponding Authors: Hairong Zheng and Yekuo Li

E-mails: hr.zheng@siat.ac.cn and yekuoli@163.com.

Telephone: +86 755 86392284, Fax: +86 755 96382299

*Both authors contributed equally to this manuscript.

Supplementary methods:

S1. In vivo NIR imaging and dye distribution

The imaging studies were performed when tumor volumes of mice reached to about 100 mm^3. The tumor-bearing mice were randomly divided into two groups (n = 3) and intratumorally injected with free IR-780 or DITSL, respectively. The NIR images of mice were obtained at predetermined time using the ex/in vivo imaging system (Maestro, USA) with a 704 nm excitation wavelength and 745 nm filter. To further demonstrate how the dye is getting redistributed post release induced by laser irradiation, we examined the fluorescence areas of IR-780 distribution before and after laser irradiation. The areas were calculated through tracing around the fluorescence regions.

S2. Toxicologic assessment of DITSL

Hemolytic test and histopathologic examination were used for toxicologic assessment of DITSL. For the hemolytic test, 200 μl of blood with anticoagulant was mixed with 300 μl saline containing different doses of DITSL in a 0.5 ml of Eppendorf tube. 5 min later, the mixture were centrifuged for 20 min at 100 g. The normal saline was used as negative control and the distilled water as positive control. For the histopathologic examination, three heathy mice were intravenously injected with 150 μl of DITSL (1 mg/ml). 24 h later, these mice were sacrificed by standard decapitation. The major organs, including heart, liver, spleen, lung and kidney, were harvested, fixed with formalin and embedded in paraffin. 7-μm sections were cut with a paraffin slicing machine, followed by staining with hematoxylin-eosin (H&E) dyes.

Supplementary figures:

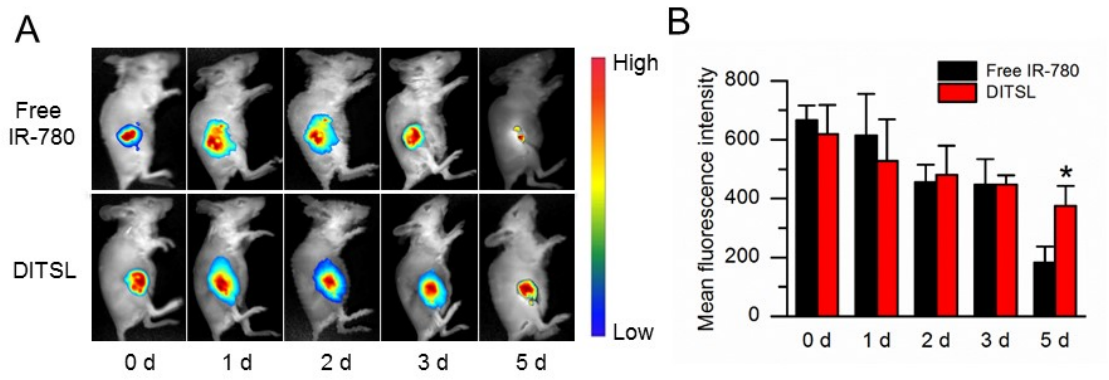

Figure S1. The in vivo fluorescence imaging of tumor-bearing mice injected with free IR-780 or DITSL. (A) The representative fluorescence images of tumor-bearing mice at 0, 1, 2, 3, or 5 days after injected with IR-780 or DITSL. (B) The quantitative analysis of fluorescence intensities from these mice.

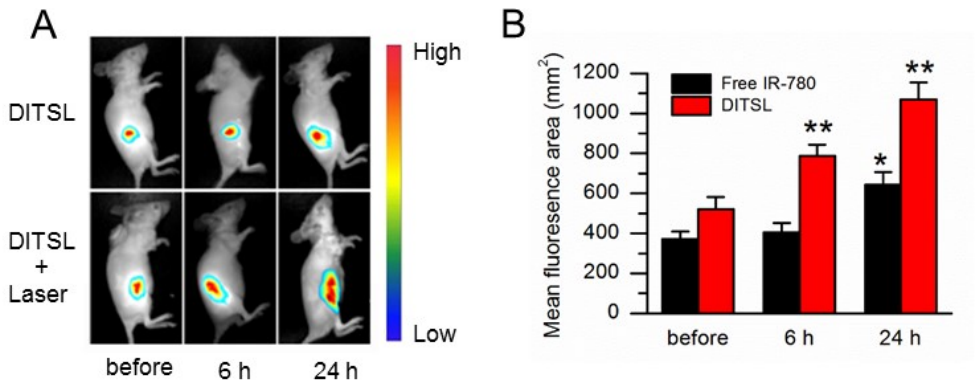

Figure S2. The fluorescence distribution post release induced by laser irradiation. (A) The representative fluorescence images of tumor-bearing mice injected with DITSL or DITSL + laser irradiation at different times. (B) The quantitative analysis of fluorescence areas from these mice.

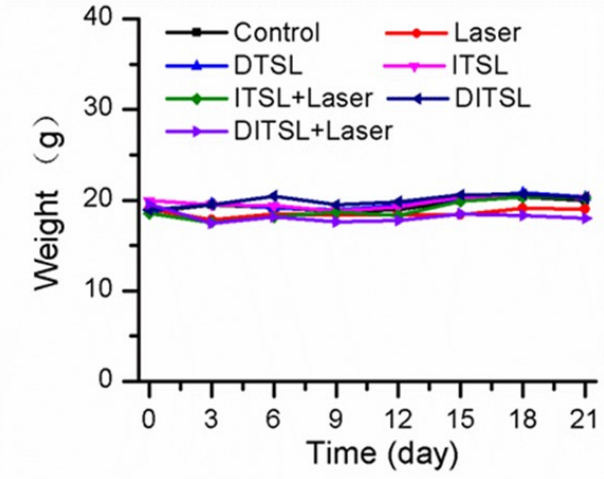

Figure S3. Body weights of mice at different time points after various treatments.

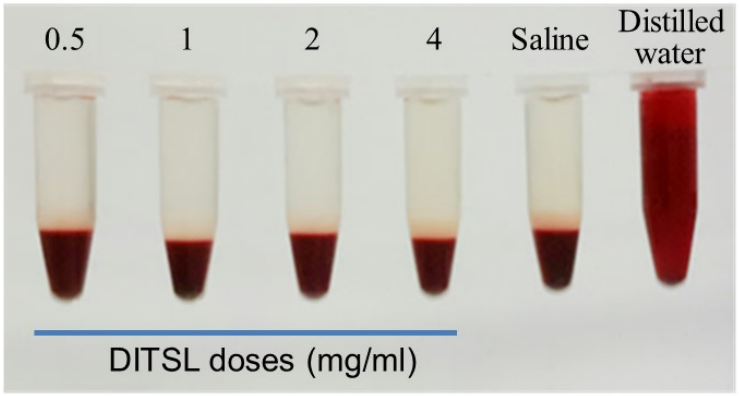

Figure S4. Hemolytic test on the DITSL. The final concentrations of DITSL were 0.5 mg/ml, 1 mg/ml, 2 mg/ml, 4 mg/ml. Saline and distilled water without DITSL were used as negative control and positive control, respectively.

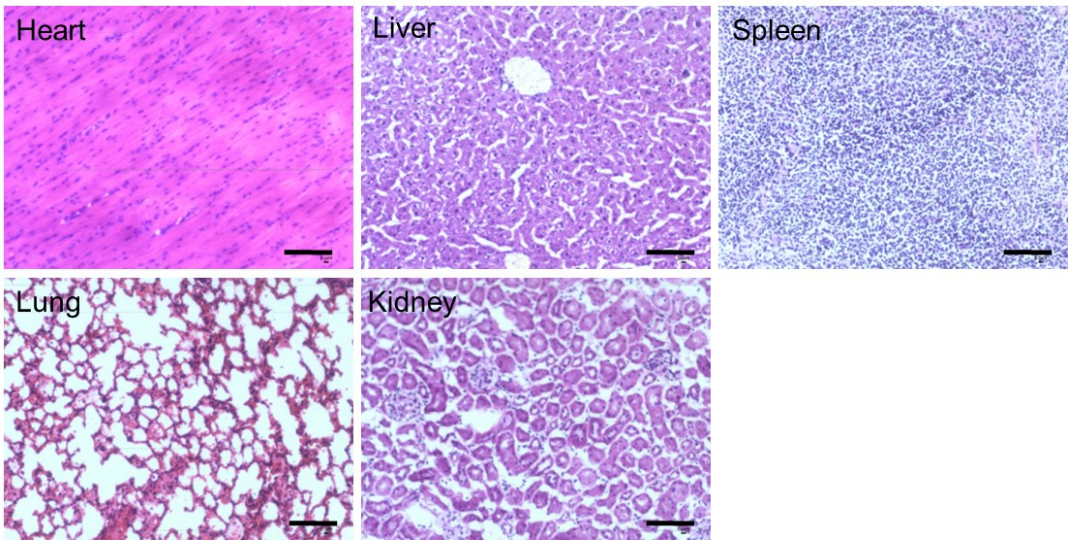

Figure S5. H&E stained images of major organs of mice received with DITSL (scale bar, 50 μm).