Supplementary information

Sonoporation-assisted micelle delivery in subcutaneous glioma-bearing mice evaluated by PET/Fluorescent bi-modal imaging

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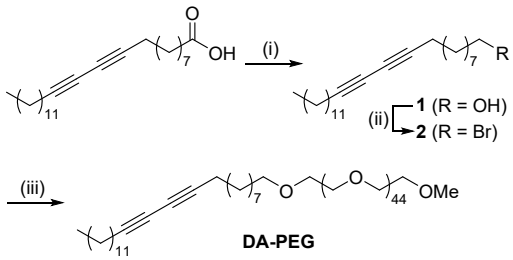
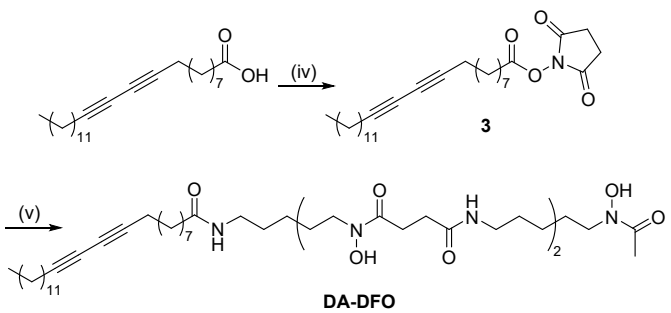


Scheme S1. Synthesis of **DA-PEG**. (i) AlLiH4, Et2O, 0 °C à room temp., 1.5 h, under N2, 81%; (ii) PPh3, CBr4, DCM, room temp., 1 h, under N2, 59%; (iii) NaH, MeO-PEG2000, THF, room temp., 72 h, 40%.



Scheme S2. Synthesis of **DA-DFO**. (iv) EDCI, NHS, DCM, 14 h, room temp., 90%; (v) DFO mesylate, PBS/THF, 48 h, room temp., 51%.

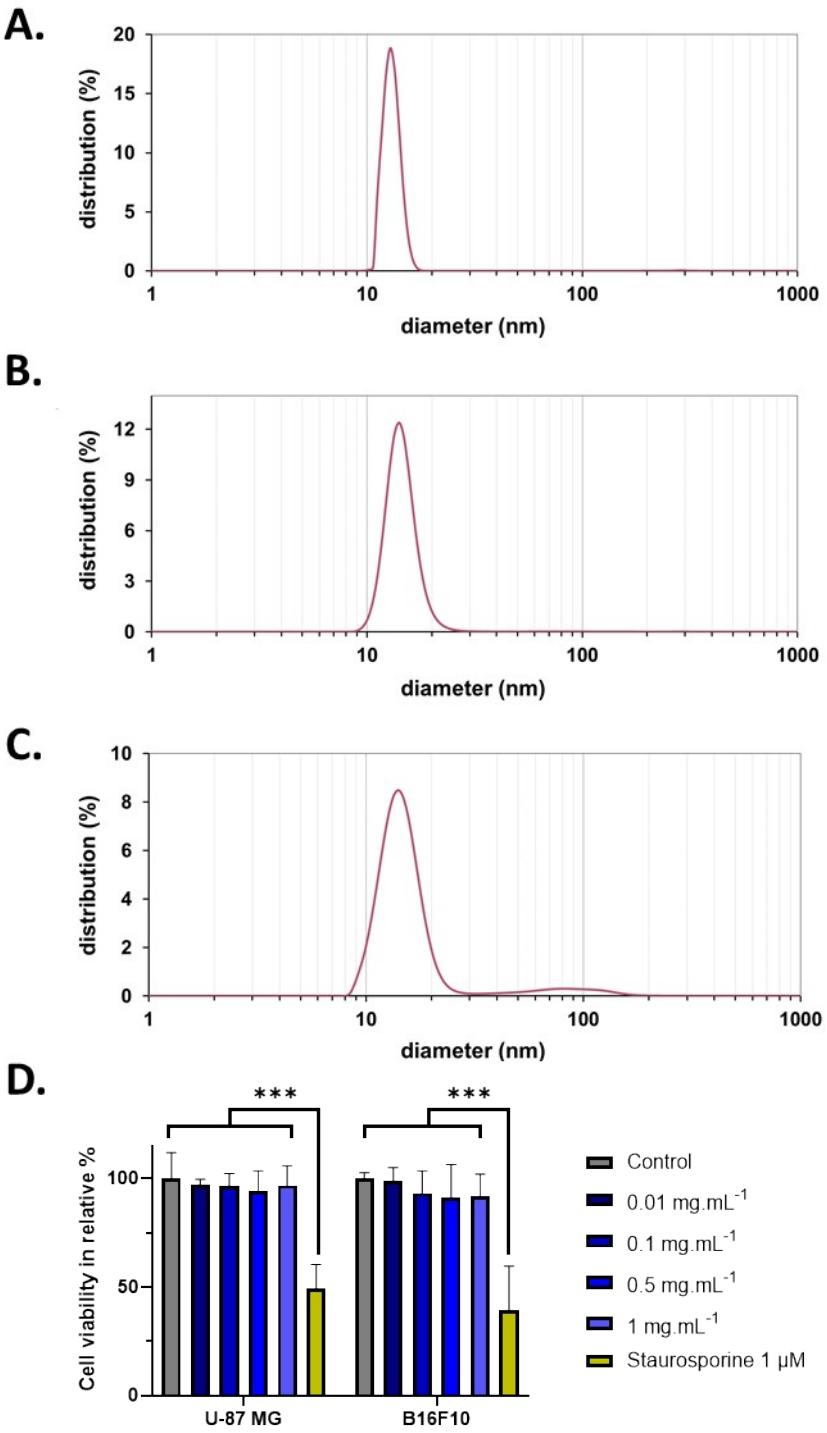


Figure S1. Characterization of the nanoparticles with A-C Dynamic light scattering profiles of **A.** pDA-PEG/DFO micelles; **B.** DiD@pDA-PEG/DFO micelles after purification; **C.** DiD@pDA-PEG/DFO micelles after purification, freeze-drying and resuspension in water. **D.** Toxicity of the DiD@pDA-PEG/DFO determined by MTS assay in different cell lines with U-87 MG and B16F10 (murine melanoma cell line). 8 103 cells per well for each cell lines in 96-well plate were incubated during 24h with different concentration of micelles going to 0.01 to 1 mg mL^{-1}. The control group for this experiment was incubated with phosphate-buffered saline (PBS). To evaluate the impact of toxicity on the tumor cells, staurosporine at a concentration of 1 µM was used as a positive control. Following a 2-hour incubation with the MTS reagent at 37°C, the plate was analyzed using a microplate reader at 490 nm. The control group treated with PBS served as a baseline without any observed toxicity. Cell viability was determined by calculating the percentage of surviving cells relative to the control group. Each experiment was repeated six

times. Notably, no significant impact on cell survival was observed at any concentration of micelles tested. (*** : p < 0,001).

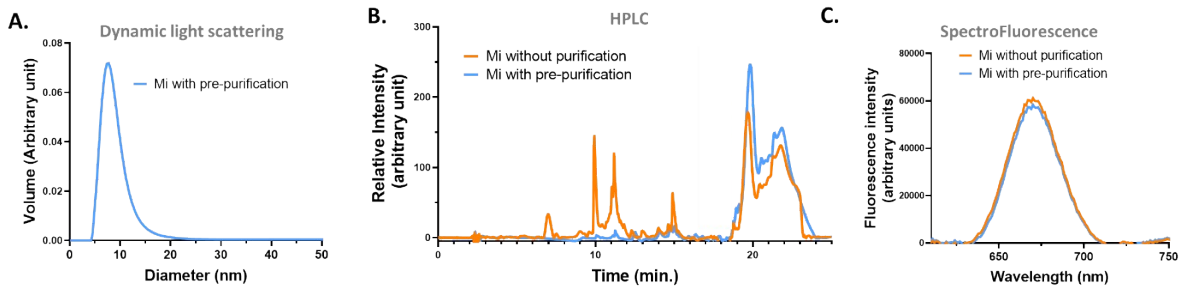


Figure S2. Purification of DiD@pDA-PEG/DFO micelles. A. Hydrodynamic diameter of DiD@pDA-PEG/DFO[^{89}Zr] micelles dispersed in water (10 mg mL^{-1}, H_2O optimal) measured by DLS. B. HPLC chromatograms detected at 280 nm of the crude DiD@pDA-PEG/DFO[^{89}Zr] micelles (orange) or after a pre-first purification step by size exclusion using PD-10 (blue). C. Fluorescent spectra of DiD@pDA-PEG/DFO micelles diluted in water (10 mg mL^{-1}, H_2O optimal) between 606 and 795 nm (λ_{exc} = 575 nm).

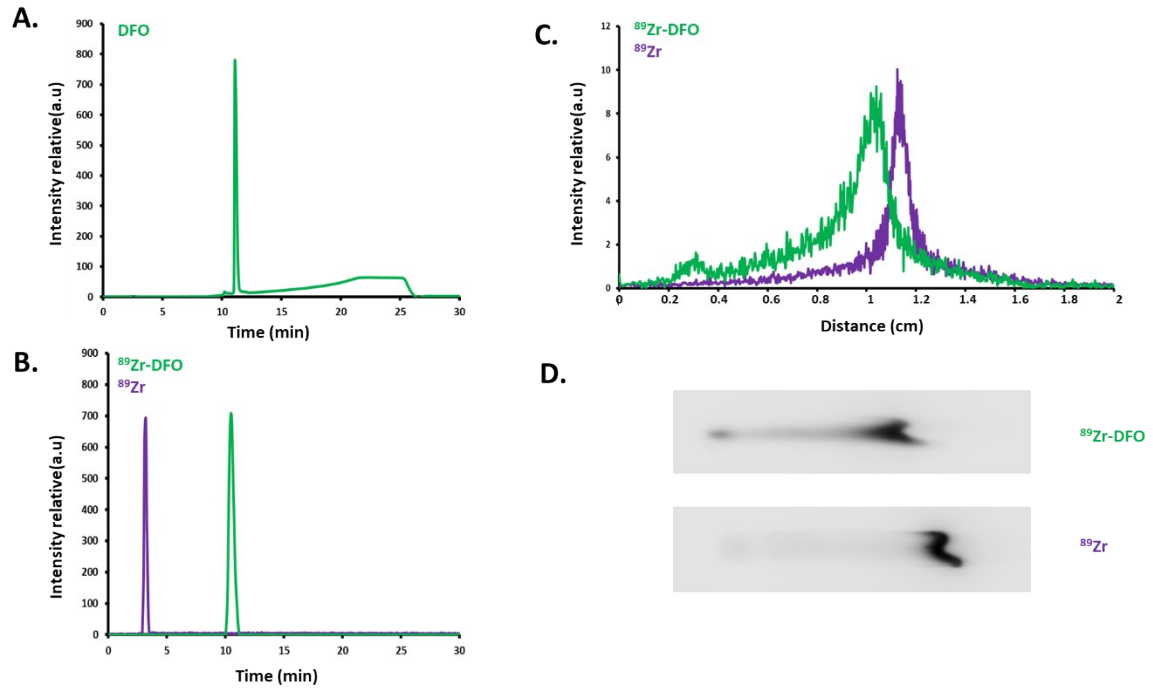


Figure S3. DFO, ^{89}Zr and ^{89}Zr-DFO characterizations. HPLC chromatograms of A. DFO detected at 280 nm or B. ^{89}Zr (violet) and ^{89}Zr-DFO (green) detected at 511 KeV with a retention time of 3 and 10 min respectively. C. Radio-iTLC chromatograms and D. autoradiography of the ^{89}Zr and ^{89}Zr-DFO on iTLC-SG using an eluent of acetonitrile : citric acid (20 mM, adjusted at pH 4.9–5.1 with Na_2CO_3 (2M)) = 10:90 *v*/*v*.

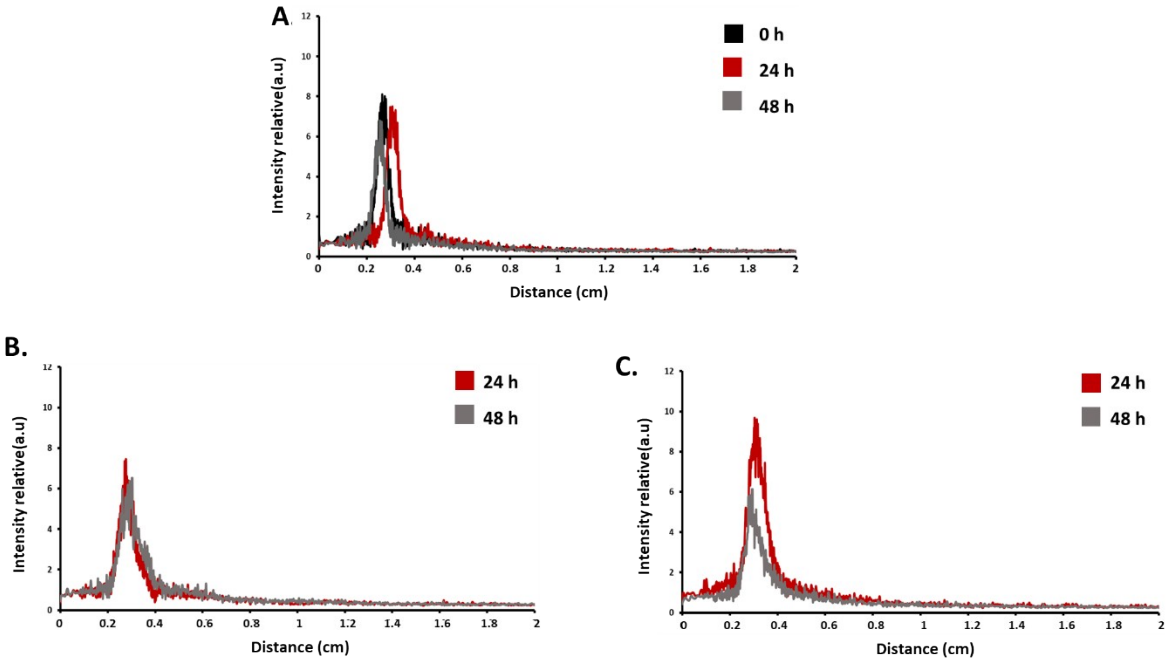


Figure S4. Evaluation of the radiolabeling stability of the DiD@pDA-PEG/DFO[^{89}Zr] micelles incubated at 37 °C in A. PBS, B. mouse plasma and C. mouse serum. Samples were analyzed by radio-iTLC chromatograms obtained right after mixing (0 h) or after 24 h and 48 h of incubations.

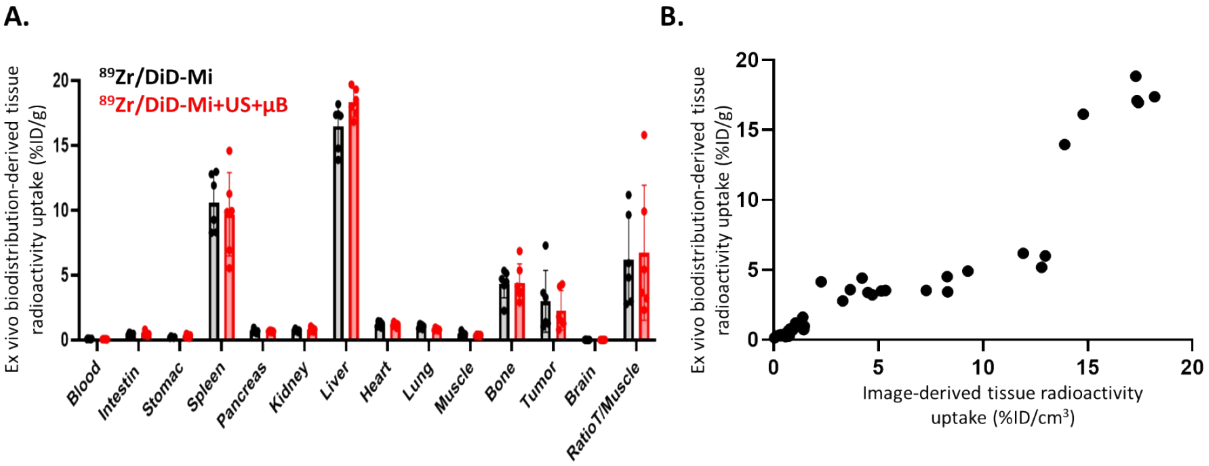


Figure S5. *Ex vivo* analyses obtained one week after DiD@pDA-PEG/DFO[^{89}Zr] micelle injection in mice bearing subcutaneous U87-MG tumors. A. *Ex vivo* biodistribution obtained with or without US exposure. Results are represented as %ID/g mean ± standard deviation. %ID/g = percentage of injected dose per tissue gram B. Correlation between the accumulation of DiD@pDA-PEG/DFO[^{89}Zr] micelles into the main organs obtained by biodistribution *ex vivo* and by PET images analyses. Person test was used for correlation evaluation between the activity measurements obtained by the two methods. Pearson correlation coefficient was determined as $r = 0.94$, $p < 0.0001$ ($n = 6$).

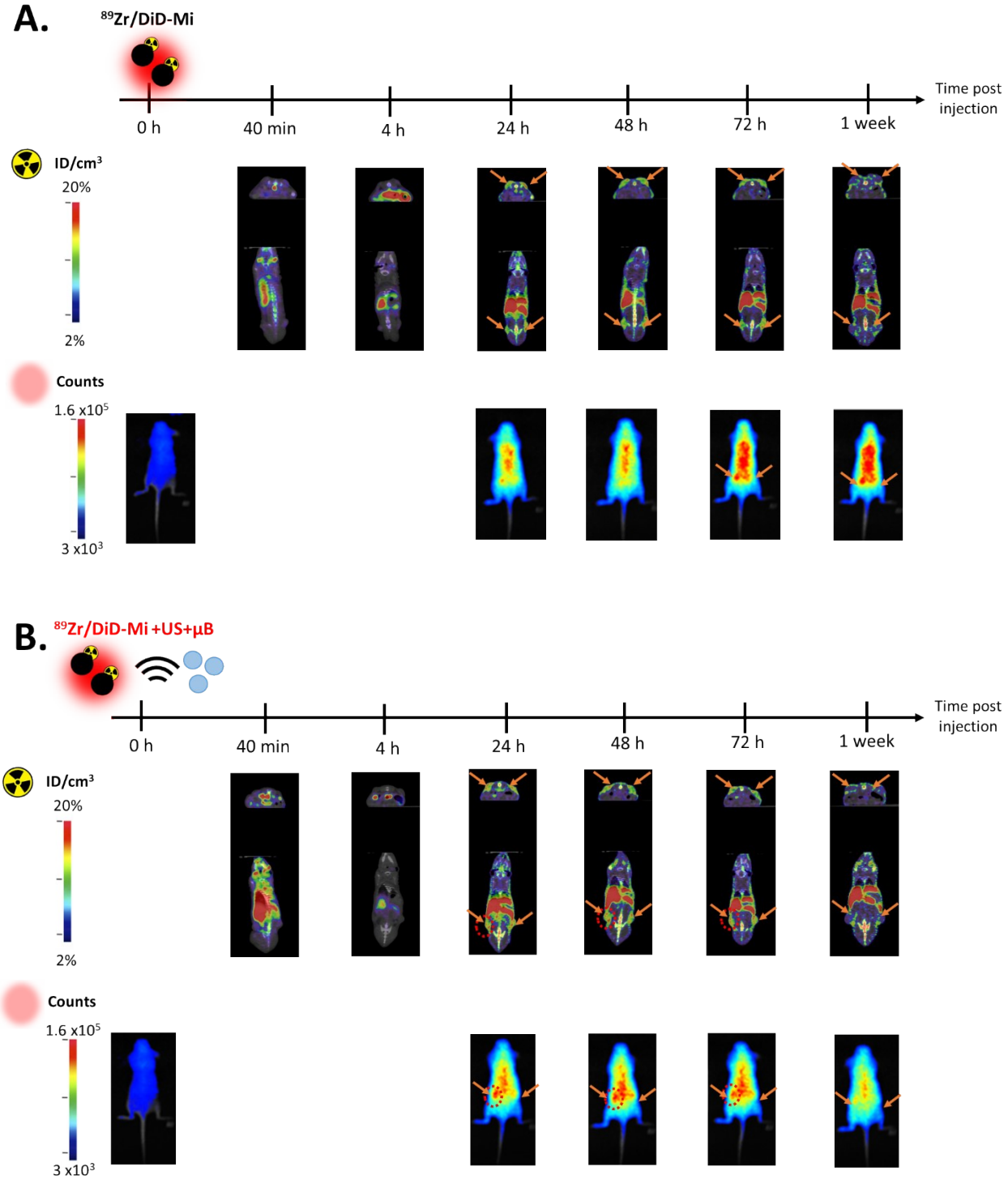


Figure S6. *In vivo* biodistribution of DiD@pDA-PEG/DFO[^{89}Zr] micelles in subcutaneous U87-MG tumor model. PET (Coronal-upper and axial-lower) and optical images obtained from mice A. without or B. with US at different time points (0 h, 40 min, 4 h, 24 h, 48 h, 72 h and 1 week) after injection of DiD@pDA-PEG/DFO[^{89}Zr] micelles (150 µL, 10 mg/mL, 113 µCi). The orange arrows indicate the position of the tumors, and the red dot circle shows the enestatic tumor.

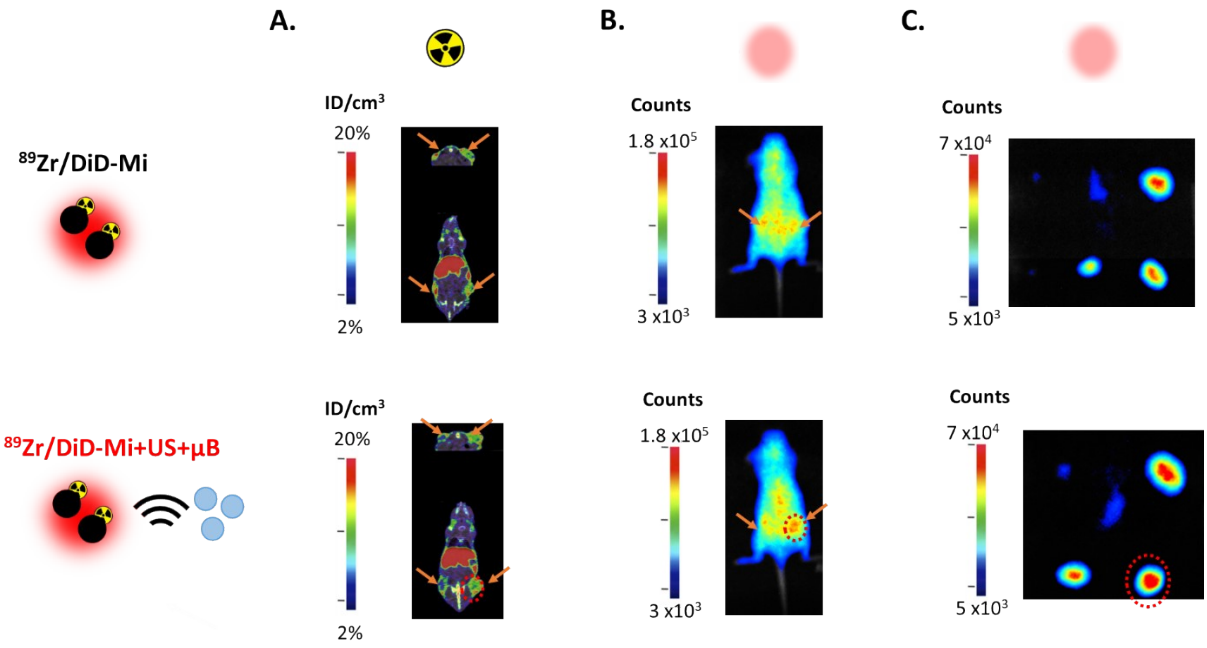


Figure S7. *In vivo associated with ex vivo* biodistribution and the main organs images obtained 72 h after ^{89}Zr/DiD-Mi injection (150 µL, 10 mg/mL, 113 µCi) in mice bearing subcutaneous U87-MG tumors wihout (upper) or with (lower) US exposure. A. Coronal (upper) and axial (lower) images of mice obtained by PET imaging. B. Axial images of mice obtained by optical imaging. C. Images of the main organs obtained by optical imaging (upper from the left to the right = muscle, spleen and liver, lower = tumors).The orange arrows indicate the position of the tumors and the red dot circle shows the enestatic tumor.

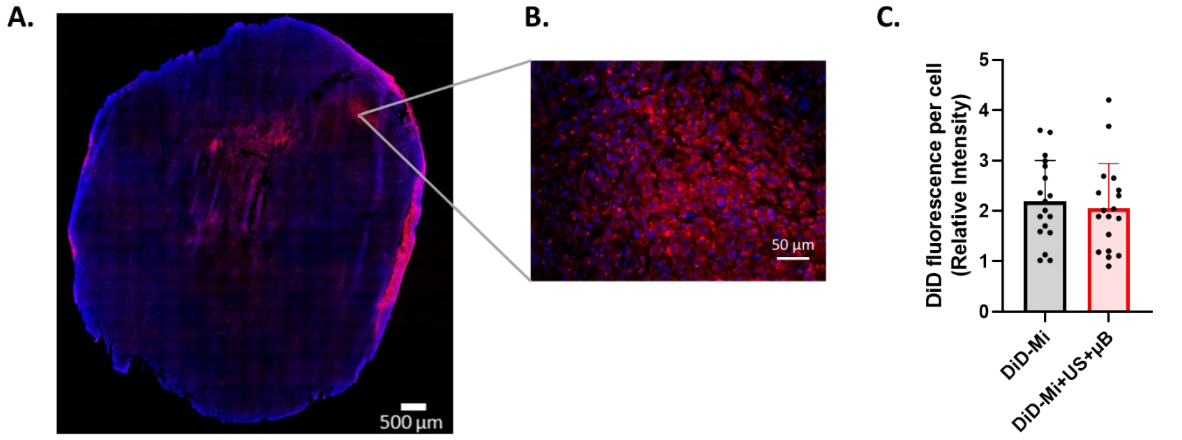


Figure S8. Assessing drug delivery through the DiD release within the tumor microenvironment. A. Representative images from the enestic tumor one week after the injection of DiD@pDA-PEG/DFO[^{89}Zr] micelles (150 µL, 10 mg/mL, 113 µCi). Bi-photonic scan of whole tumor sections at **A.** low and **B.** high magnification with signal from cell nucleus (blue) and micelles (red). **C.** The quantification of DiD release was conducted within two groups: DiD-Mi alone and DiD-Mi with ultrasound (US) and microbubbles (µB) one tumor slices one week after injection. Fluorescence intensity was measured across six slices obtained from three different tumors derived from three mice. Each slice was divided into 3 distinct areas measuring 0.323 x 0.323 µm². The fluorescence of DiD was normalized by the number of cells within each slide, providing quantification for the twelve distinguishable areas. Quantification was performed using ImageJ software, and the number of cells per tile was determined through DAPI labeling.

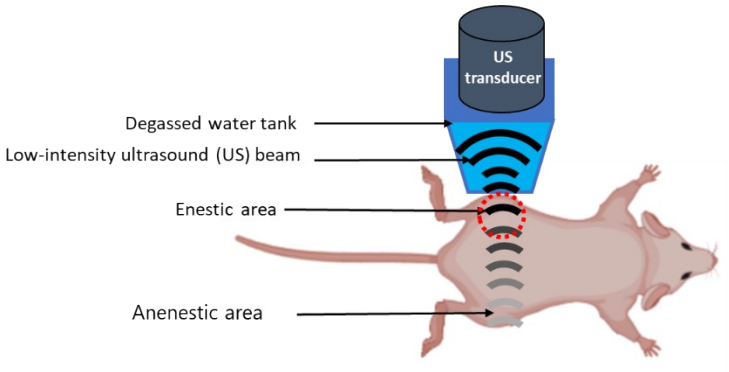


Figure S9. Experimental setup for sonoporation procedure.

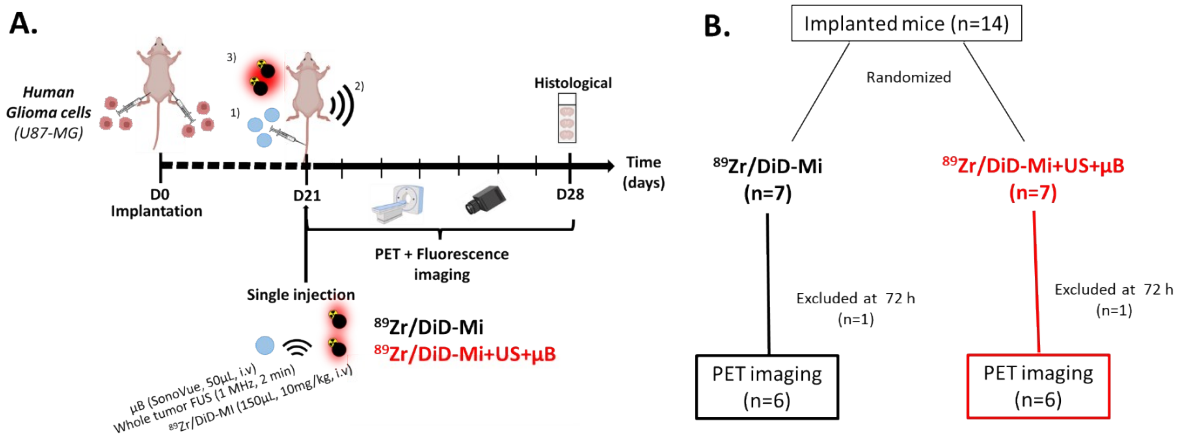


Figure S10. Study design for the longitudinal PET evaluation of DiD@pDA-PEG/DFO[^{89}Zr] micelle delivery with and without US-induce sonoporation in mice bearing subcutaneous U87-MG tumors. A. Timeline for the experiments. Human glioma cells (U87-MG, 5.0×10^6) were subcutaneously implanted in both flanks of nude mice. Tumor were allowed to growth for 3 weeks. Then, µB (Sonovue, 50 µL) were intravenous injected followed (n = 7) or not (n = 7) by US exposure. Four minutes after US ended, DiD@pDA-PEG/DFO[^{89}Zr] micelles (150 µL, 10 mg mL^{-1}, 113 µCi) were intravenously injected into the tail vein. Finally, PET (n = 14) and optical (n = 2) acquisitions were performed at different time points after DiD@pDA-PEG/DFO[^{89}Zr] micelle injection. One mouse per group were sacrificed 72 h post injection for histological analysis. B. Number of animals used for the experiment. In total 14 mice were implanted and randomized into 2 groups with (n = 7) or without (n = 7) US exposure.