Supporting Information

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SI Methods

Animals and Tumor Model. A total of 42 male Sprague–Dawley rats and 14 male Fischer CDF (for the F98 tumor model) rats were used (~300 g; Charles River Laboratories, Inc.). Animals were anesthetized by i.p. injections of ketamine (80 mL/kg/h) and xylazine (10 mL/kg/h) for FUS experiments and with 2% isoflurane during MRI. A catheter was placed in the tail vein for i.v. administration, and the hair on the scalp was removed with clippers and depilatory cream for better ultrasound propagation.

For the experiments in the tumor model, wild-type F98 cells [passage number 6, provided by Rolf F. Barth (47) at the Department of Pathology, The Ohio State University, Columbus, OH] were cultured in Dulbecco's modified Eagle medium (1×) supplemented with 10% FBS and 0.1% Penicillin Streptomycin in a humidified incubator with 5% CO_2 at 37 °C. Following the surgical procedure as previously described (48), 4-μL cell suspension (2×10^5 cells) was injected into the caudate putamen bilaterally at 3.5 mm from the dura surface using a 10-μL gastight syringe (Hamilton) in Fischer rats. Animal behavior was monitored daily after surgery and the sutures were removed 5 d later.

Animals were killed either 1 h (healthy rats) or 24 h (tumor-bearing rats) after FUS and TB administration, under deep anesthesia with ketamine/xylazine. The brains were fixed by transcardial perfusion using 0.9% NaCl (100 mL) followed by 10% buffered formalin phosphate (250 mL). The brains were then harvested and soaked in 10% buffered formalin phosphate for at least 24 h before being sectioned into 1-mm transverse blocks for fluorescent imaging.

FUS System. A dual-transducer system for FUS transmission was designed and built in house. The system consists of two air-backed, spherically focused transducers (diameter/radius of curvature: 10/8 cm) with a resonant frequency at 274.3 kHz. The transducers were mounted in an acrylic holder at an angle of 102° with respect to each other (an angle greater than 90° was used to maximize the space between the transducers for the PCD). To break up the interference pattern in the focal region, one of the transducers was operated at a frequency of 31 Hz higher than the other. The burst length was one period of the beat frequency (32.3 ms), and the bursts were applied at a PRF of 1 or 4 Hz. A frequency difference of 31 Hz was selected so that integration of the modulation envelope over one half of a period was close to that of a 10-ms burst applied at a single frequency, a value commonly used for BBBD. The two FUS transducers were driven by two function generators (33220 A; Agilent) and two amplifiers (43 dB gain, LZY-22+; Mini-Circuits). The transducers were matched to 50 ohms, and the electrical power output was measured using a power meter (E4419 B; Agilent) and dual-directional coupler (C5948-10; Werlatone). The transducers were calibrated using scans of the focal plane acquired with a needle hydrophone (HNC-1000; Onda) and radiation force balance measurements to estimate the peak intensity at the focus. The pressure amplitude at the focus was estimated from the intensity assuming linear propagation. The pressure amplitude at the combined focus of the two transducers was estimated from the needle hydrophone after calibrating it by driving one transducer and placing a needle hydrophone at its focus. Pressure amplitudes presented here are those estimated in water considering the skull insertion loss (49).

Acoustic Emission Recording and Control. An elliptical air-backed passive cavitation detector with dimensions of 5 × 3 cm and a center frequency of 650 kHz (bandwidth: 75%) was used to record the acoustic emissions during the sonications. A digital filter (model 3944; Krohn–Hite) operating as Butterworth notch filter (stop band: 150–466 kHz) was used to suppress the fundamental frequency component in the recorded emission signals before the analysis for controller. It also amplified the signal by 20 dB. Emission signals were recorded with a high-speed digitizer (PXIe 1073; National Instruments) at a sampling rate of 5 MHz. Power spectra [PSD(f)] were then converted using fast Fourier transform and a Hanning window. The cavitation enhancement was calculated as the relative signal strength in dB for each burst:

$$S = \frac{10}{W} \log_{10}\left(\int_W \frac{PSD(f)}{PSD_{BL}(f)} df \right),$$

where $PSD_{BL}(f)$ is the power spectra obtained without microbubbles and W is the bandwidth (harmonic components, 300 Hz; broadband emission, 40 kHz) (26, 50). All time and frequency analysis, feedback controlling operations, and spectrum/controller profile display were performed in real time using in-house developed MATLAB scripts. The bursts were applied before verifying that the last one was at a safe level. If BE was detected in the closed-loop controlling, the electric input from the function generators would decrease by 1 mV.

Microbubble Administration. The microbubble contrast agent Optison (GE Healthcare) was injected either as a 4.1-s bolus or as the same bolus followed by a constant infusion. The infusion rate was 0.08 mL/min. The injections were performed with a computer-controlled syringe pump (NE-1000; New Era Pump Systems Inc.). A mechanical system was constructed that constantly rotated the syringe to keep the microbubbles mixed throughout the experiments. The syringe was rotated ±360° at a rate of ~15 rotations per minute (51).

Fluorescent Dye and Chemotherapeutic Drug. 0.08 g TB powder (MP Biomedicals) was dissolved in 2.5 mL of 0.45% NaCl and heated until boiling. This solution was then passed through a filter (MILX GV 0.22UM PVDF; Millipore Cor.) and then slowly injected i.v. at a dose of 0.1 g TB per kg of body weight (52) to visualize the BBB disruption after euthanasia. In the experiments with tumor-bearing rats, liposomal DOX chemotherapy (Doxoves, Liposomal Doxorubicin HCL, 2.0 mg/mL; FormuMax Scientific, Inc.) was administrated i.v. at a dose of 2 mg/kg before sonication following a protocol we developed previously (11). To avoid fluid overload, only a half-dose of TB was administered in these animals.

MRI. All MRI assessments were performed in a 7T Bruker Biospec animal MR-system (Biospec; Bruker). A high-resolution T2-weighted (T2w) sequence was used to access tumor volume until the tumor diameter was about 2 mm. After sonication, T1-weighted (T1w), T2w, and T2*-weighted (T2*w) images were obtained for confirming the BBBD and potential damage. T2*w and T2w images were first obtained to detect potential hemorrhage and edema that may have occurred during the sonications. Contrast-enhanced T1w imaging was then used to access BBB/BTB permeability. T1w scans were acquired before and repeated

three times after a bolus injection of 0.25 mL/kg gadopentetate dimeglumine (Gd-DTPA, molecular weight 938 Da, Magnevist; Bayer HealthCare Pharmaceuticals Inc.). All MRI parameters are shown in Table S1.

Fluorescent Imaging. Fluorescent images were acquired by the IVIS Lumina Series III imaging system (Perkin-Elmer). Data acquisition and quantification were performed in Living Image software (V4.3; Perkin–Elmer). To image TB, we used 1-s exposure time and a 640-nm/Cy 5.5 (excitation/emission) filter set. Exposure time was increased to 5 s, and the filter set was changed to 530 nm/DsRed when imaging DOX. For DOX fluorescence, the image was subtracted by 50% the intensity of an additional image that was obtained with a 470 nm/DsRed filter to reduce contributions from autofluorescence. Because DOX fluorescence is quenched when encapsulated, we assumed that our measurements were of free drug released from the liposomes over 24 h after delivery across the BBB/BTB.

Histology. Histological assessment of the sonication effects was obtained in representative examples. Following fluorescent assessments, tissue blocks were embedded in paraffin and sectioned into 5-μm serial transverse sections (perpendicular to the direction of ultrasound propagation). Every 20th section (100 μm apart) was stained with hematoxylin and eosin (H&E) for histological evaluations.

Data Analysis. Fluorescent intensities in regions of interest (ROI; circles with 4 mm in diameter) were quantified as radiant efficiency [fluorescence emission radiance per incident excitation irradiance: $(\text{photons/s/cm}^2\text{/steradian})/(\mu\text{W/cm}^2)$]. We compared fluorescent intensity in the sonicated region to similar locations in control animals that did not receive FUS. If the intensity was less than the mean value plus two SDs in the control ROI, the BBB was considered to not be disrupted. Fluorescent enhancement was quantified as the fluorescent intensity of treated location subtracted by the mean intensity of the control location without sonication. The contrast signal in MRI before and after Gd-DTPA administration was evaluated in a 3×3 voxel ROI. Contrast enhancement was calculated as the percentage enhancement relative to the contrast intensity in the same ROI before Gd-DTPA injection. Statistical comparisons between data pairs were performed using ordinary two-way ANOVA with a Tukey multicomparison test in GraphPad (PRISM 6.0). The threshold for statistical significance was $P < 0.05$. Linear regressions or segmental linear regressions were analyzed in MATLAB or GraphPad.

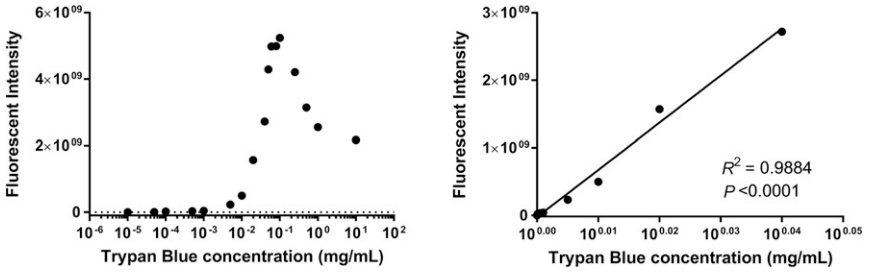

Fig. S1. Fluorescent intensities of calibration standards with different TB concentrations. TB fluorescence was found to quench at around 0.1 mg/mL The zoomed-in plot (*Right*) showed a linear relationship over the range of fluorescent intensities detected in Figs. 4 and 5. This relationship was used to estimate the delivered TB doses.

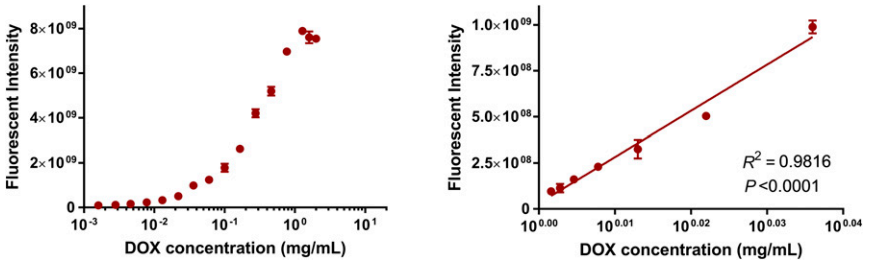

Fig. S2. Fluorescent intensities of calibration standards with different DOX concentrations. Quenching effect was observed as well: DOX fluorescence quenched at 1.28–1.6 mg/mL. The zoomed-in plot (*Right*) showed a linear relationship over the range of fluorescent intensities detected in Figs. 4 and 5. This relationship was used to estimate the delivered DOX doses.

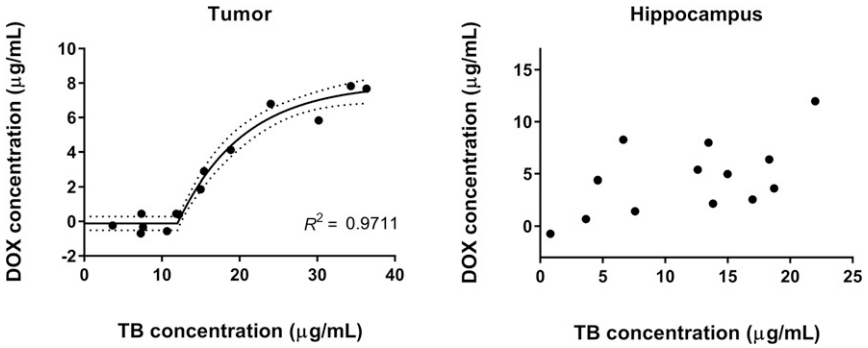

Fig. S3. TB–DOX delivery relationship in tumor and hippocampus targets. TB and DOX delivery doses were compared in tumor-bearing striatum targets (*Left*) and non–tumor-bearing hippocampus targets (*Right*).

Table S1. MRI parameters

Parameter	T2-w imaging	T1-w imaging	T2*-w imaging
Sequence	RARE-ETL: 14	RARE – ETL: 4	Gradient echo – $\alpha = 30$
Echo time, ms	52	18	15
Repetition time, ms	3,500	602	495
Field of view, mm	30 × 25.6	35 × 35	35 × 35
Matrix	300 × 252	128 × 128	128 × 128
No. of averages	6	4	2
Slice thickness, mm	1	1	1