Supporting Information

Ultrasound-induced cascade amplification in a mechanoluminescent nanotransducer for enhanced sono-optogenetic deep brain stimulation

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Experimental Section/Methods

Chemicals: Soybean phosphatidylcholine (DSPC), 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) and L012 sodium salt (L012), were purchased from MedChemExpress. cholesterol, 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[amino(polyethylene glycol)-2000] (DSPE-PEG_{2000}), IR-780 iodide (IR-780), 1,3-Diphenylisobenzofuran (DPBF), salicylic acid (SA), sodium hydroxide (NaOH), 30 wt% hydrogen peroxide aqueous solution (H_2O_2), calcium chloride ($CaCl_2$), polyethylene glycol 200 (PEG_{200}) were purchased from Sigma-Aldrich.

Characterization: The morphology of nanoparticles was investigated via FEI TECNAI G2 F20 X-TWIN Transmission Electron Microscope (TEM). The hydrodynamic size and surface zeta potential of nanoparticles were measured via dynamic light scattering (DLS, Zetasizer Nano-ZS from Malvern Instruments). The UV-vis spectrum was measured via Eppendorf Biospectrometer. The sono-mechanoluminescence spectrum was measured via Fluorolog3 Fluorimeter. The focused ultrasound (FUS) generator and transducer was ordered from Image Guided Therapy. The fluorescence images and videos were recorded by Leica DMi8 fluorescence microscope. Confocal images were obtained from the Zeiss LSM 710 confocal laser scanning microscope.The powder X-ray diffraction patterns were collected by a Panalytical X'Pert powder diffractometer equipped with a Cu sealed tube (λ = 1.54184 Å) at 40 kV and 40 mA over the 2θ range of 5−40°.

The fluorescence spectrum tests of L012 at different pH and H_2O_2 concentration. We investigated the quantum yield of the L012 solution at different pH. 1 mL L012 solution (0.5 mg/mL) with pH 7.4, 8.5, and 10.0 was mixed with H_2O_2, where the final concentration of H_2O_2 was 250 μM. The fluorescence spectrum was recorded via Fluorolog3 Fluorometer. In

addition, we also investigated the quantum yield of the L012 solution with different H_2O_2 concentrations at pH 7.4. 1 mL L012 solution (0.5 mg/mL) with different H_2O_2 concentrations (50, 250, and 500 μM) at pH 7.4 was prepared at first, and the fluorescence spectrum was also recorded via Fluorolog3 Fluorometer.

Preparation of CaO_2 nanoparticles. The CaO_2 nanoparticles were prepared via $CaCl_2$—H_2O_2 reaction following the previous method.[1] In brief, 1 mL $CaCl_2$ solution (0.1 g/mL in distilled water) and 0.5 mL ammonia solution (1M) were added into 80 mL PEG_{200} with a stirring speed of 1000 rpm in the flask. After stirring for 30 min, the $CaCl_2$ solution was totally dispersed into the PEG_{200} solution, and 0.5 mL 30 wt% H_2O_2 solution was dropwise added to the mixture within 10 min. Then, the mixture was stirred for another 6 h at room temperature to obtain a colorless solution and then transfer to the sonication bath. 1 M NaOH solution was dropwise added into the mixture to adjust the pH value to 11.5. In this process, we could observe that the clear solution became slightly white. The mixture was centrifuged at speed of 15000 rpm/min, and the precipitate was washed with 0.1 M NaOH solution, distilled water, and ethanol to remove excessive PEG_{200}. The PEG_{200} coated CaO_2 nanoparticles were stored in ethanol at 4 ℃ for future use. After removing the solution, the CaO_2 nanoparticles powder was used to detect the crystal structure through X-ray diffraction analysis. 0.1 mg/mL CaO_2 nanoparticles solution was used to prepare TEM samples for morphology tests.

Preparation of drug loaded lipids nanoparticles (liposomes). To prepare the liposomes, thin film hydration strategy was used.[2,3] Briefly, 16 mg DSPC, 0.8 mg DPPC, 2.4 mg cholesterol, 0.6 mg DSPE-PEG_{2000} 1.2 mg IR780, and 0.75 mL CaO_2 ethanol solution (10 mg/mL) was dissolved in 4 mL chloroform and 1 mL methanol mixture. The solution was removed after rotary evaporation, and a thin lipid film was obtained. 3 mL L012 solution (0.5 mg/mL) was added into the lipid film, and treated in a sonication bath for 1 min at 60 ℃. The mixture

solution was extruded via a 0.45 μm filter to obtain uniform lipid suspension. Then the suspension was centrifuged at 12000 rpm/min for 20 min, the precipitate was resuspended via 2 mL distilled water. 0.5 mg/mL liposomes solution was used to do the TEM and DLS tests. In addition, the obtained liposomes solution was dried via freezing dry to obtain powder and stored with foil coverage at 4 ℃ for future use. The calcium concentration was measured via inductively coupled plasma mass spectrometry (ICP-MS). 4 mg of Lipo@IR780/CaO_2/L012 nanoparticle powder, obtained after freeze-drying, were dissolved in 4 mL of concentrated nitric acid (HNO_3) with the application of heat. Following this, the solution was diluted 2000-fold using a 2% HNO_3 solution for subsequent analysis via ICP-MS tests.

To calculate the drug loading content (DLC), the nanoparticles' powder obtained after freeze-drying, was weighed, and then dissolved in methanol at a fixed concentration. Subsequently, the characteristic absorption values of IR780 and L012 were determined using UV-Vis spectroscopy, and the concentrations of IR780 and L012 were calculated based on their respective calibration curves. The DLC was computed using the following equation:

DLC (wt%) = (Weight of drugs in the nanoparticles / Weight of nanoparticles) x 100%

The generation of $^1O_2$ under the FUS stimulation in liposomes. The IR780 loaded liposomes (Lipo@IR780), IR780/CaO_2 loaded liposomes (Lipo@IR780/CaO_2) and IR780/CaO_2/L012 laded liposomes (Lipo@IR780/CaO_2/L012) were used to evaluate the free radicals generation. 1 mL liposomes solution (1 mg/mL) was mixed with 30 μL 1 mg/mL DPBF methanol solution in a dark room. Then, the mixture was continuously irradiated with or without FUS (1.5 MHz, 1.55 MPa), and 10 μL of the solution was extracted at different time points for UV-vis spectrum tests. The characteristic absorption peak of DPBF at 420 nm was real-time detected to quantify the generation of $^1O_2$. In addition, we also measured the UV-vis spectrum of the mixture after treating 60 s under different FUS power (from 0 to 1.55 MPa), where the ultrasound power was measured via hydrophone (Onda Corporation, HGL-0200).

The generation of •OH and H_2O_2 under the FUS stimulation in liposomes. Similar to $^1O_2$ detection, 1 mL liposomes solution (1 mg/mL) was mixed with 50 μL 1 mg/mL SA methanol solution in a dark room. SA could effectively react with •OH and H_2O_2, we therefore could track the consumption of SA to quantify the generation of •OH and H_2O_2. The UV-vis characteristic absorption peak of SA is at 297 nm. After irradiation with or without FUS (1.5 MHz, 1.55 MPa), 10 μL of the liposomes mixture was extracted at different time points for UV-vis spectrum tests. Moreover, we also tested the UV-vis absorption of the liposomes mixture after treating for 60 s under different FUS power (from 0 to 1.55 MPa).

FUS triggered blue light emission from Lipo@IR780/L012 and Lipo@IR780/CaO_2/L012 liposomes. 1 mL liposomes solution (5 mg/mL) was added into a 2 mL glass vial and fixed on the top with a FUS transducer (1.5 MHz, Image Guided Therapy). The gap between the glass vial and transducer was filled with ultrasound gel, and a video camera (CS165MU1/M - Zelux® 1.6 MP Monochrome CMOS Camera, Thorlabs) was placed in front of the glass vial to record the light emission in the dark room. We first tested the light emission at a repetition frequency of 1 Hz (pulse 50 ms on 950 ms off) with different ultrasound power, from 0 to 1.55 MPa. Then, we also evaluated the light emission at different irradiation frequencies at 2 Hz (50 ms FUS on, 450 ms FUS off), 4 Hz (50 ms FUS on, 200 ms FUS off), and 5 Hz (50 ms FUS on, 150 ms FUS off) and 10 Hz (50 ms FUS on, 50 ms FUS off) at peak pressure 1.55 MPa. All the parameters were fixed to record the video, and the data were analyzed by ImageJ software. To investigate the delay time between the FUS stimulation and light emission from liposomes, we simultaneously recorded the FUS pulse LED indicator light and mechanoluminescence light, and the time gap between these two emissions is the latency.

The evaluation of FUS power transmission efficiency in tissue. In this test, we use porcine skin to mimic the normal tissue. Different depth porcine skin was placed on the top of the transducer with ultrasound gel filling. Then, the ultrasound hydrophone (Onda Corporation,

HGL-0200) was tightly placed behind the porcine skin with ultrasound gel filling. The ultrasound power was fixed, and the peak pressure behind the different depths of porcine skin was recorded via a hydrophone, thus calculating the ultrasound transmission efficiency.

The evaluation of FUS thermal effect in target brain area. A C57BL/6 mouse was anesthetized with ketamine (80 mg/kg) and dexdomitor (1 mg/kg) and was put on the top of a homoeothermic blanket (Harvard Apparatus) to prevent hypothermia. The mouse head was then depilated using hair removal lotion (Nair, Church & Dwight), and the scalp was removed using surgical scissors. The animal was then fixed on a stereotaxic frame (RWD Life Science), and a burr hole with 1-mm diameter was created on the skull with a dental drill (Marathon-III). Afterwards, a type-K thermocouple (Thermometrics) with a probe diameter of 500 μm was fixed on the stereotaxic arm and inserted into the mouse brain with a glancing angle of 30˚. The tip of the thermocouple was positioned in the motor cortex. The 1.5-MHz FUS transducer was placed on the mouse head, with the focus at the same position as the tip of the thermocouple. FUS pulse trains with 2.45 MPa, 100-ms pulse duration and 1-Hz pulse repetition rate were applied. The output of the thermocouple was connected to a real-time temperature reader (Model 210, J-KEM Scientific), which was connected to a computer for data readout.

The light emission from Lipo@IR780/L012 and Lipo@IR780/CaO_2/L012 liposomes at deep tissue. We also recorded the light emission from liposomes under the FUS irradiation at different depths of porcine skin. Similarly, the porcine skin with different depths was placed on the top of the transducer, and the liposomes solution loaded in 2 mL glass vial was placed behind the porcine skin with ultrasound gel filling. FUS (1.55 MPa, pulse 50 ms on 950 ms off) was used to treat the solution, and the light emission was recorded via CS165MU1/M - Zelux® 1.6 MP Monochrome CMOS Camera.

The sono-mechanoluminescence spectrum tests of Lipo@IR780/L012 and Lipo@IR780/CaO_2/L012 liposomes. 2 mL liposomes solution (the equivalent concentration

of L012 is 1 mg/mL) was added into a plastic cuvette and insert the fluorescence spectrometer (Fluorolog3 Fluorometer), the FUS transducer was placed on the side of the plastic cuvette, and the gap was filled with ultrasound gel. The liposomes solution was treated at peak pressure 1.55 MPa with pulse 3s on 5s off, and the sono-mechanoluminescence was recorded.ChR2 and ChiReff absorption spectra were referred from previous work,[4,5] and extracted via GetDataGraphDigitizer software.

***In vitro* sono-optogenetics tests**. We evaluated the sono-mechanoluminescence triggered firing ChR2 expressing primary neurons. Primary cortical neurons were used in our tests. Briefly, the pregnant C57BL/6 mouse (20-26 g; 8 weeks old; Jackson Laboratory) was sacrificed when the pups were 15.5 days old, and these pups' brains were used to prepare the primary cortical neurons. The 24 well plates were coated with poly-l-ornithine (0.2 mg/mL) at 37 ℃ for 2 h and washed with PBS several times to remove excessive poly-l-ornithine, then warmed at 37 °C cell incubator before use. The dissociated cortical neurons were plated into the plate with suitable cell density, and cultured in neurobasal medium with 10 % B27, glutamine, penicillin, and streptomycin. After incubating for 2 days at 37 °C under 7% CO_2, the glial inhibitor 5-fluoro-2′- deoxyuridine (0.1 mM) was added. After 4 days of incubation, 0.5 μL pAAV-hSyn-hChR2(H134R)-EYFP and 0.5 μL pAAV.Syn.NES-JRGECO1a.WPRE.SV40 were added to infect the neurons. After another 7 days of incubation, the ChR2 opsins and JRGECO1a calcium indicator were successfully expressed in the neurons for calcium imaging tests. Similar to spiking HEK cells calcium imaging tests, the vail filling with 2 mL Lipo@IR780/CaO_2/L012 liposomes solution with 1 mg/mL equivalent concentration of L012 was fixed over the cells, and the FUS irradiation (1.55MPa, pulse 100 ms on 900 ms off) was given to activate the system for light generation. The jRGECO1a red fluorescence signals were collected and recorded via Leica DMi8 fluorescence microscope. The spiking fluorescence data were analyzed by ImageJ software.

Ultrasound Heatmap determination in mice brain.

CB57BL/6 wild type mice (20-26g; 8 weeks old, Jackson Laboratory) were sacrificed via intraperitoneal injection of ketamine. The head was then removed surgically with the skin intact and stored in 1% paraformaldehyde (PFA) for 48 hours at 4°C. Upon usage, the head was removed from the PFA solution and cleansed with distilled water for 2 minutes. Increments of 1 mm from posterior to anterior measurements of the width (Medial-Lateral) and depth (Cranial-Caudal) were performed prior to dissection and heatmap measurements. A hydrophone (ONDA HGL200, Onda Corporations) was mounted to a custom-made 3-axis system and connected to a preamplifier towards an oscilloscope for measurement. During measurement, the hydrophone was placed in contact to the caudal section of the head while the commercial transducer (25mm OD @ 1.5MHz FUS, Image Guided Therapy System) was in contact with the cranial section. The placement of the transducer differs for motor cortex and VTA stimulation and was adjusted manually in accordance with the behavioral experimental procedures. For both contacts, ultrasound gel was applied to ensure maximum contact and FUS transmission (Ultrasound Transmission Gel 100, Aquasonic) To begin measuring the ultrasound heatmap, surgical scissors were used to remove sections of the skull and brain incrementally from the caudal sections. Per each removal, a reiteration of measurement in dimension was performed in conjunction with the placement of the hydrophone The hydrophone moved from anterior to posterior in increments of 1 mm and FUS pulse with peak pressure 1.55 MPa was sonicated. The measured voltage signal from the hydrophone was then converted to pressure via a calibration curve provided by the datasheet from Onda Corporations. A 10 mm by 15 mm acoustic distribution of the FUS were obtained in the sagittal plane of the mouse head with skin and skull intact. The data was then processed via linear interpolation in MATLAB for higher resolution representation of the acoustic field shown in **Figure S7** and **S10**.

Vertebrate animal subjects. Thy1-ChR2-YFP transgenic mice (20-26 g; 4 weeks old; Jackson laboratory) and C57BL/6 wild type mice (20-26 g; 8 weeks old; Jackson Laboratory) were used in our study. All procedures were designed according to the National Institute of Health Guide for the Care and Use of Laboratory Animals, approved by the Institutional Animal Care and Use Committee at the University of Texas at Austin, and were supported via the Animal Resources Center at the University of Texas at Austin.

***In vivo* Stereotaxic injection of liposomes at the motor cortex**. Thy1-ChR2-YFP transgenic mice and wild type mice were used. Before the surgery, all tools were autoclaved. The mice were anesthetized by an isoflurane anesthesia machine (Vaporizer Sales & Service Inc) with 2.5% concentration and the head was fixed in a stereotaxic frame (Kopf Stereotaxic Instruments). The head hair was shaved, and the head skin was cleaned three times with isopropanol and iodophor, respectively. Then, meloxicam (5 mg/kg) and Ethiqa (3.25 mg/kg) were subcutaneously injected before surgery, and the eyes were protected from the ophthalmic ointment. The isoflurane concentration decreased to 1.5% when the surgery was conducted. 2 μL liposomes solution (100 mg/mL dissolved in sterilized PBS) was unilaterally injected into the right motor cortex, with the coordinates relative to bregma: anteroposterior (AP) +1.0 mm, mediolateral (ML) + 0.50 mm, and dorsoventral (DV) -0.5 mm.

***In vivo* sono-optogenetic tests for motor cortex modulation**. After 24 h recovery, the mice were used to do the motor cortex behavior tests. The mice were anesthetized with 2.5% isoflurane, and the head was fixed in a stereotaxic frame, where the 37 ℃ heating pad was placed under the mouse body to maintain temperature. The eyes were protected from the ophthalmic ointment. Then, the FUS transducer was placed on the top of mice head with ultrasound gel filling to cover the whole motor cortex region, the transducer coordinates relative to bregma: anteroposterior (AP) 0.0 mm, mediolateral (ML) + 0.50 mm, and dorsoventral (DV) -0.5 mm. After that, the isoflurane concentration decreased to 0.5% to make

sure the mice were in light anesthesia before stimulation. FUS pulse (1.55 MPa, 1.5 MHz, 100 ms on 900 ms off) was given to control the limb motion, and the motions were recorded via video camera. The mice limb motion data were analyzed via DeepLabCut according to the previous method.[6]

***In vivo* Stereotaxic injection of liposomes at VTA for lever press tests**. Thy1-ChR2-YFP transgenic mice and wild type mice were used. Before the surgery, all tools were autoclaved. The mice were anesthetized by an isoflurane anesthesia machine (Vaporizer Sales & Service Inc) with 2.5% concentration and the head was fixed in a stereotaxic frame (Kopf Stereotaxic Instruments). The head hair was shaved, and the head skin was cleaned three times with isopropanol and iodophor, respectively. Then, meloxicam (5 mg/kg) and Ethiqa (3.25 mg/kg) were subcutaneously injected before surgery, and the eyes were protected from the ophthalmic ointment. The isoflurane concentration decreased to 1.5% when the surgery is conducted. 2 μL liposomes solution (100 mg/mL dissolved in sterilized PBS) was unilaterally injected into the VTA, with the coordinates relative to bregma: anteroposterior (AP) ± 3.08 mm, mediolateral (ML) + 0.40 mm, and dorsoventral (DV) -5.0 mm. After that, the head plate (Model 13, Neurotar) was mounted in the head for lever press tests.

After 24 h recovery, the mice were placed in the 3D printed holder, and the front limbs were placed on the trigger. The FUS transducer was placed on the mouse head with hydrogel filling, and the coordinates relative to bregma: anteroposterior (AP) ± 0 mm, mediolateral (ML) + 0.40 mm, and dorsoventral (DV) -5.0 mm. The FUS pulse (1.55 MPa, 1.5 MHz, 100 ms on) will be given once the mice press trigger. In the lever press behavior tests, we separated three sessions, including prestimulus (Pre), during (Dur), and poststimulus (Post) session. In the prestimulus session (day 1, 30 min), the mice were placed to obtain the level press number baseline, where the FUS generator was off, and no FUS pulse was given when the trigger was pressed, and we only recorded the level press number in 30 min. In the Dur session (day 2- day 4, 30 min each

test), the FUS generator is on, and the FUS pulse (1.55 MPa, 1.5 MHz, 100 ms on) was given when the trigger was pressed, the press number was recorded within 30 min. In Post session (day 5, 30 min), no FUS pulse was given when the trigger was pressed, and we only recorded the level press number in 30 min to study the addiction behaviors.

***In vivo* light intensity power tests in the motor cortex and VTA under the FUS stimulation**. We also determined the *in vivo* light intensity according to the previous method. The wild type mice were anesthetized by an isoflurane anesthesia machine (Vaporizer Sales & Service Inc) with 2.5% concentration and the head was fixed in a stereotaxic frame (Kopf Stereotaxic Instruments). The head hair was shaved, and the head skin was cleaned three times with isopropanol and iodophor, respectively. Then, meloxicam (5 mg/kg) and Ethiqa (3.25 mg/kg) were subcutaneously injected before surgery, and the eyes were protected from the ophthalmic ointment. Then two optical fibers (CFML12L05, Thorlabs) were inserted into the motor cortex (anteroposterior (AP) +1.0 mm, mediolateral (ML) +0.5 mm, dorsoventral (DV) −0.5 mm) or VTA (anteroposterior (AP) +3.08 mm, mediolateral (ML) +0.4 mm, dorsoventral (DV) −5.0 mm). One of the fibers was connected to a blue LED (LEDD1B-T-Cube LED Driver, Thorlabs), and another one was connected to FLIR Blackfly S BFS-U3-51S5M-C Camera. The photons density was recorded via the camera, and we recorded the photons density at different LED power densities to obtain a light density calibration curve at first.

Then, in order to test the light power density from liposomes at the motor cortex or VTA, the liposomes were firstly injected into the motor cortex (anteroposterior (AP) +1.0 mm, mediolateral (ML) +0.5 mm, dorsoventral (DV) −0.5 mm) or VTA (anteroposterior (AP) +3.08 mm, mediolateral (ML) +0.4 mm, dorsoventral (DV) −5.0 mm), and the optical fiber was implanted into the similar location. After 24 h recovery, the mice were anesthetized by an isoflurane anesthesia machine with 2.5% concentration and the head was fixed in a stereotaxic frame. The eyes were protected via coating vet ointment. The FUS transducer was placed on

the top of mice head with ultrasound gel filling, where the focal length was set as 1 mm and 5 mm for motor cortex and VTA irradiation via adjusting the transducer water balloon. After that, the FUS pulse (1.55 MPa, 1.5 MHz, 100 ms on 900 ms off) was given, and the photons from the liposomes were collected and recorded via the camera with similar parameters. The relative light power density was obtained according to the calibration curve after correction.

Histology.

(a) c-fos staining. Thy1-ChR2-YFP transgenic mice and wild type mice were first treated following the in vivo sono-optogenetics procedures. After 60-90 min, the mice were anesthetized via i.p. injection of ketamine, and perfusion was conducted with PBS and 4% paraformaldehyde. After that, the brain was extracted and stored in 4% paraformaldehyde overnight at 4 ℃ and sliced through a vibrating blade microtome (Leica VT1200). The brain slices with a depth of 60 μm were washed with 0.3% Triton-X PBS (TBS) solution, and then blocked with 5% bovine serum albumin TBS solution for 30 min at room temperature.

For motor cortex brain slices, after blocking, the solution was replaced by rabbit anti-c-Fos antibody (ab222699, Abcam)/0.3% Triton-X in PBS. The samples were incubated at 4 °C overnight and then washed with TBS solution three times. The mixture of TBS and secondary antibody goat anti-rabbit Alexa Fluor 594 (R37117, Fisher Scientific) and Hoechst 33342 (17535, ATT Bioquest) was added to incubate the slices for 1-2 hours at room temperature in a dark room. Finally, the slices were washed three times with TBS, and then mounted on the slides with mounting media (9990402, Fisher Scientific), and covered with a coverslip. The confocal images were obtained from Zeiss 710 laser scanning microscope.

For VTA brain slices, after blocking, the solution was replaced with mouse anti-c-Fos (E-8) antibody (sc-166940, Santa-Cruz)/rabbit anti-tyrosine hydroxylase antibody (AB152, Sigma-Aldrich) /0.3% Triton-X in PBS. The samples were incubated at 4 °C overnight and then washed with TBS solution three times. The mixture of TBS and secondary antibody goat anti-

rabbit Alexa Fluor 594 (R37117, Fisher Scientific) and goat anti-mouse Alexa Fluor 405 (ab175660, Abcam) was added to incubate the slices for 1-2 hours at room temperature in the dark room. Finally, the slices were washed three times with TBS, and then mounted on the slides with mounting media, and covered with a coverslip. The confocal images were obtained from Zeiss 710 laser scanning microscope.

(b) Iba1 staining and Caspase-3 staining. The mice were first treated following the in vivo sono-optogenetics procedures. After 7 days, the mice were anesthetized via i.p. injection of ketamine, and perfusion was conducted with PBS and 4% paraformaldehyde. After that, the brain was extracted and stored in 4% paraformaldehyde overnight at 4 ℃ and sliced through a vibrating blade microtome. The brain slices with a depth of 60 μm were washed with TBS solution and then blocked with 5% bovine serum albumin TBS solution for 30 min at room temperature. Then, the blocking buffer was replaced by rabbit anti-Iba1 antibody (013-27691, Wako Chem)/TBS or rabbit anti-Cleaved Caspase-3 antibody (9661, Cell Signaling Tec.)/TBS. After incubated overnight at 4 ℃ fridge, the slices were washed with TBS solution three times, and fresh TBS solution with secondary antibody Donkey anti-rabbit Alexa Fluor 594 (A32754, Invitrogen) and Hoechst 33342 (17535, ATT Bioquest) was added and incubated for 2 h at room temperature in a dark room. Finally, the slices were washed three times with TBS, and then mounted on the slides with mounting media, and covered with a coverslip. The fluorescence images were obtained from a Leica DMi8 fluorescence microscope.

(c) H&E staining. The mice were first treated following the in vivo sono-optogenetics procedures. After 7 days, the mice were anesthetized via i.p. injection of ketamine, and perfusion was conducted with PBS and 4% paraformaldehyde. After that, the brain was extracted and stored in 4% paraformaldehyde overnight at 4 ℃ and sliced through a vibrating blade microtome. The brain slices with 10 μm depths were obtained for H&E staining according to the protocol.[7] After that, the slices were mounted on the slides with mounting

media and covered with a coverslip. The images were captured with a Nikon Eclipse Ni Compound Light Microscope.

The antibody information was attached in **Table S1**.

Figures

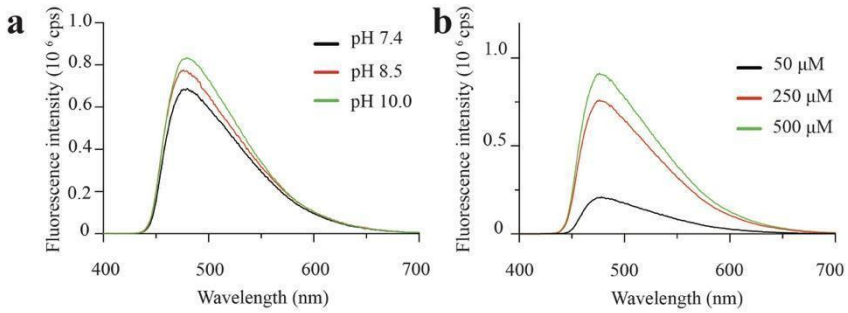

Figure S1. The fluorescence spectra of L012 (a) at different pH and (b) at different H_2O_2 concentrations.

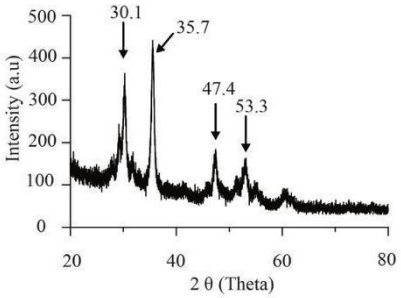

Figure S2. The XRD spectrum of CaO_2 stored at solution for 24 h after loading into the liposomes.

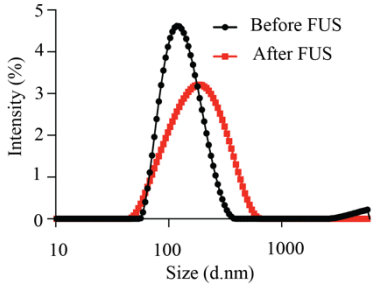

Figure S3. The DLS tests of Lipo@IR780/L012/CaO_2 before and after FUS stimulation.

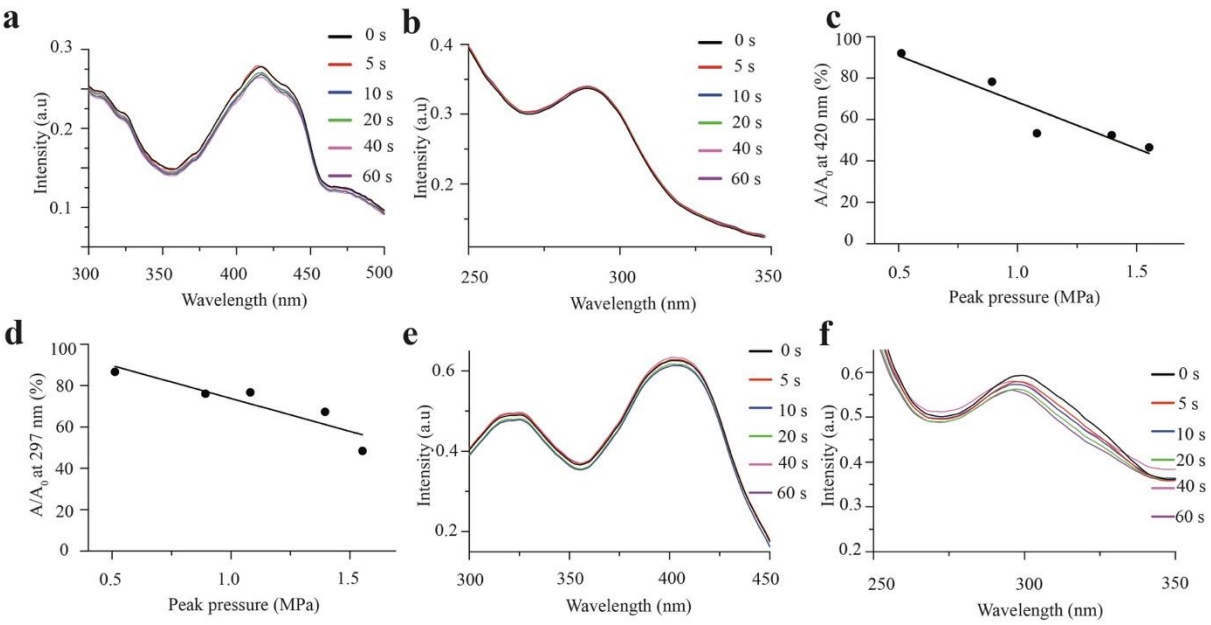

Figure S4. (a) Time-dependent UV-vis spectra of DPBF in Lipo@IR780/CaO_2 liposomes without ultrasound irradiation at different time points, indicating no generation of $^1O_2$; (b) Time-dependent UV-Vis spectra of SA in Lipo@IR780/CaO_2 liposomes without ultrasound irradiation at different time points; (c) Quantification analysis of DPBF decomposition in Lipo@IR780/CaO_2 liposomes with ultrasound irradiation (n > 3 per group) at different FUS peak pressures after 60 s stimulation; (d) Quantification analysis of SA decomposition with ultrasound irradiation (n > 3 per group) at different FUS peak pressures after 60 s stimulation; (e) Time-dependent UV-vis spectra of DPBF in Lipo@IR780/L012/CaO_2 liposomes with ultrasound irradiation at different time points; (f) Time-dependent UV-Vis spectra of SA in Lipo@IR780/L012/CaO_2 liposomes with ultrasound irradiation at different time points.

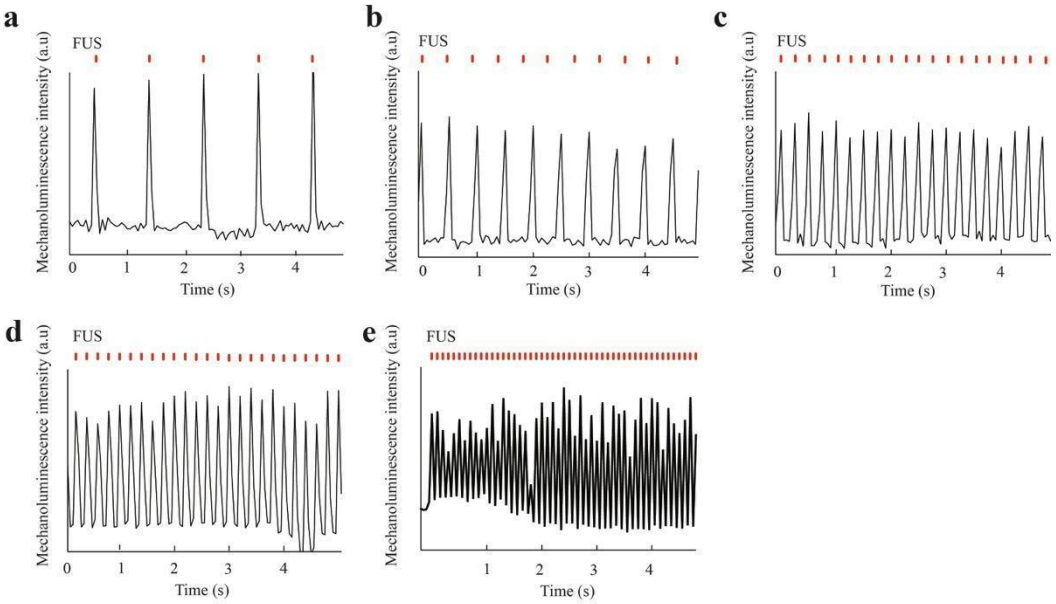

Figure S5. 470 nm blue light emission from Lipo@IR780/L012/CaO2 liposomes under different frequency FUS pulse irradiation (1.5 MHz, 1.55 MPa). (a) 1 Hz (pulse 100 ms on, 900 ms off); (b) 2 Hz (pulse 100 ms on, 400 ms off); (c) 4 Hz (pulse 100 ms on, 150 ms off); (d) 5 Hz (pulse 100 ms on, 100 ms off); (d) 10 Hz (pulse 50 ms on, 50 ms off).

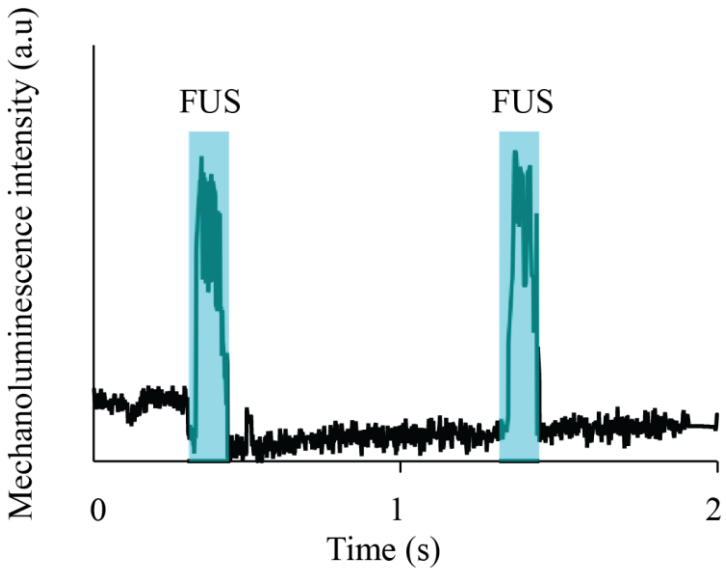

Figure S6. Blue light emission at 470 nm from Lipo@IR780/L012/CaO_2 liposomes under FUS irradiation (1.5 MHz, 1.55 MPa, 1 Hz, pulse 100 ms on, 900 ms off), presented as a zoomed-in version derived from Figure 3b.

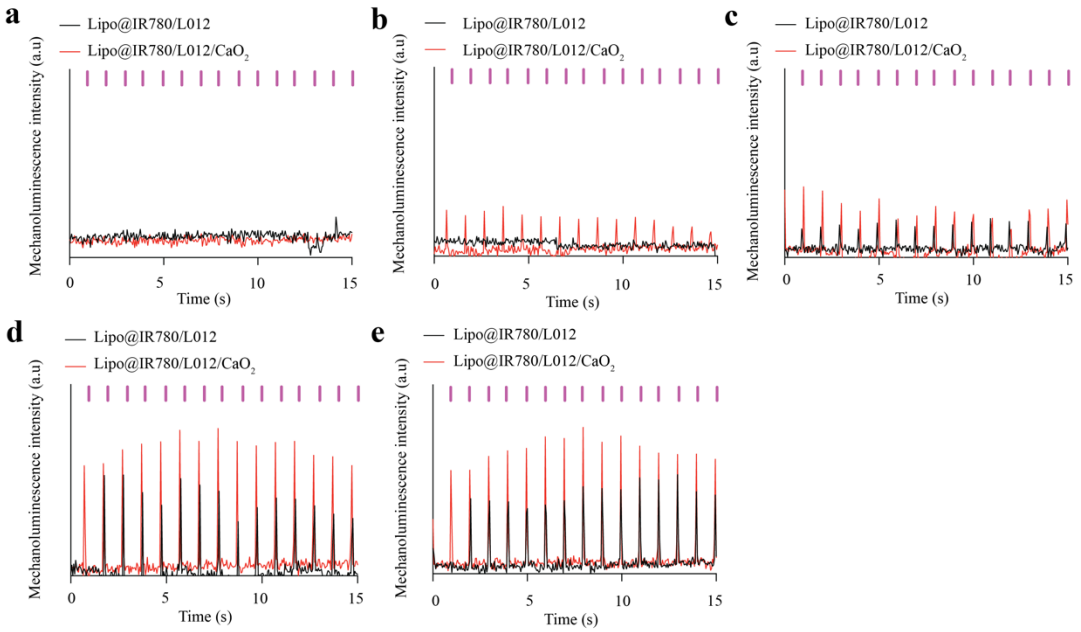

Figure S7. 470 nm blue light emission from Lipo@IR780/L012/CaO_2 and Lipo@IR780/L012 liposomes under the FUS irradiation with different peak pressure (1.5 MHz, pulse 100 ms on, 900 ms off, 1 Hz). (a) 0.51 MPa; (b) 0.89 MPa; (c) 1.08 MPa; (d) 1.40 MPa; (e) 1.55 MPa.

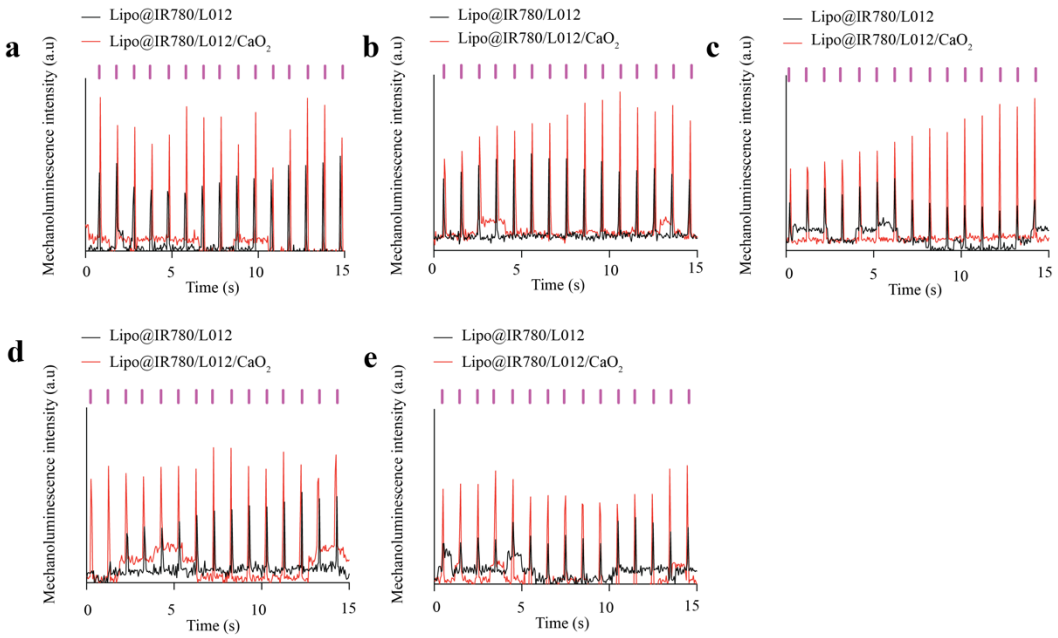

Figure S8. 470 nm blue light emission from Lipo@IR780/L012/CaO_2 and Lipo@IR780/L012 liposomes in different depth porcine skin under different pulse FUS irradiation (1.5 MHz, 1.5 MPa, pulse 100 ms on, 900 ms off). (a) there was no porcine skin; (b) the porcine skin depth was 3 mm; (c) the porcine skin depth was 5 mm; (d) the porcine skin depth was 8 mm; (e) the porcine skin depth was 10 mm.

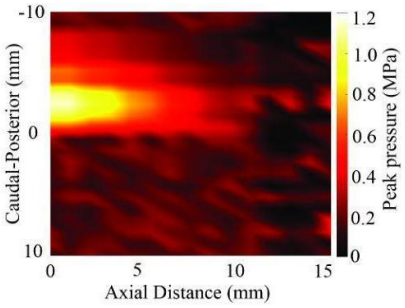

Figure S9. The heatmap of FUS peak pressure at mouse motor cortex region, where 1.55 MPa primary FUS peak pressure was used (1.5 MHz, pulse 100 ms on 900 ms off).

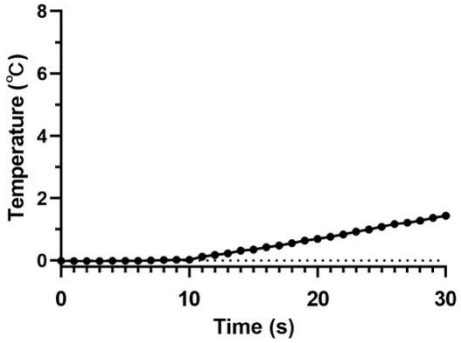

Figure S10. The temperature detection at motor cortex area through thermocouple under the FUS stimulation (1.5 MHz, 60% amplitude, 100 ms on 900 ms off).

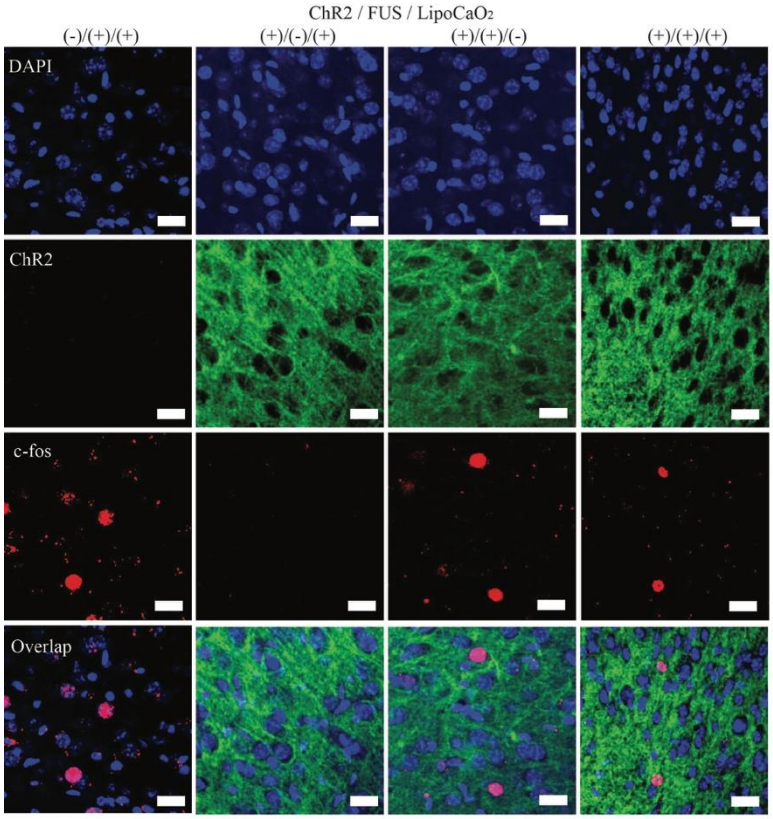

Figure S11. Confocal images of the left motor cortex region under the different experimental conditions. Scale bar: 20 μm.

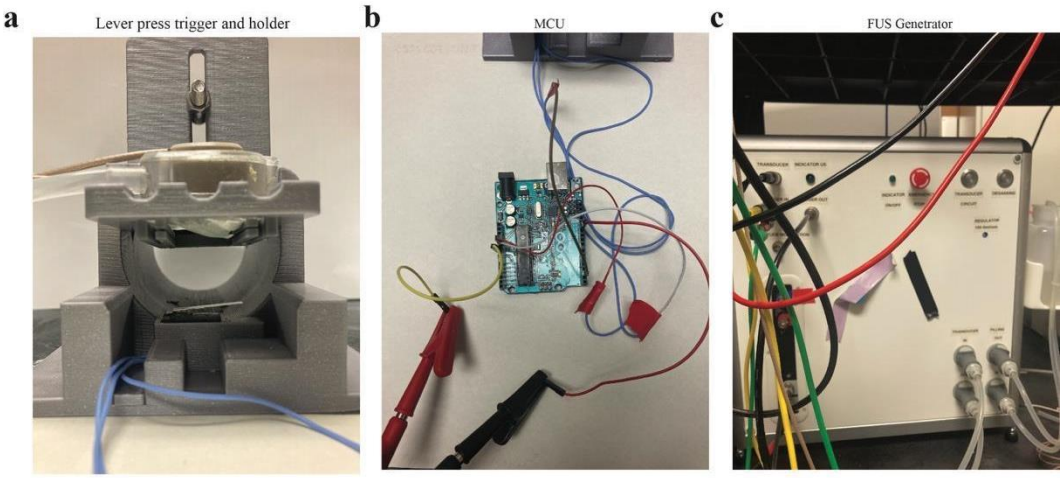

Figure S12. The photographs of lever press testing systems, including (a) trigger and holder; (b) Microcontroller Unit (MCU) system, and (c) FUS generator.

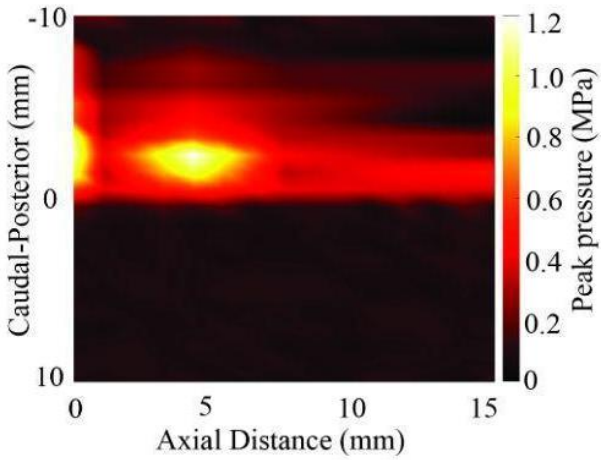

Figure S13. The heatmap of FUS peak pressure at the mouse VTA region, where 1.55 MPa primary FUS peak pressure was used (1.5 MHz, pulse 100 ms on 900 ms off).

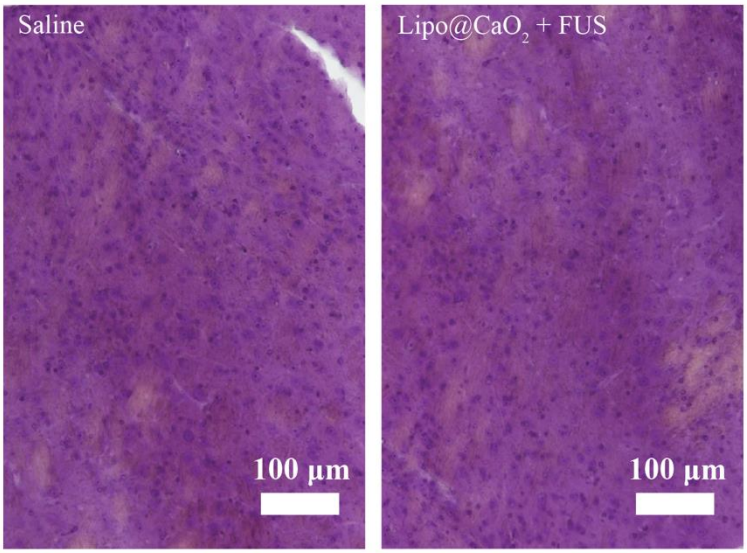

Figure S14. H&E staining of brain slices treated at different conditions after 7 days (Saline and Lipo@IR780/L012/CaO_2 liposomes with FUS stimulation).

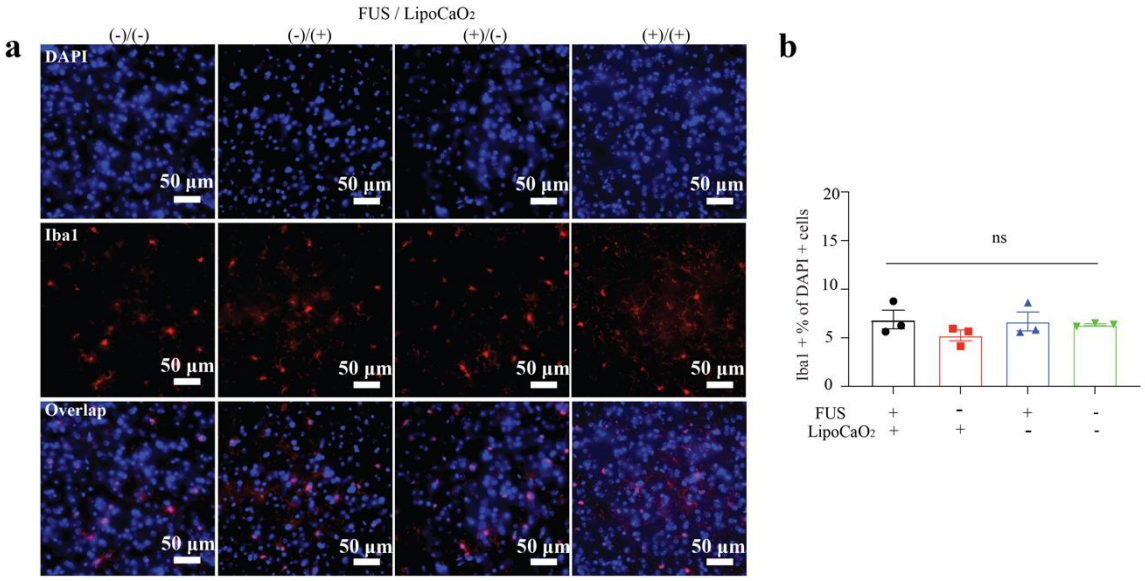

Figure S15. The biosafety evaluation through microglia activation marker Iba1 immunostaining. (a)The Iba1 fluorescence images of mouse brain after treatment at different conditions after 7 days; (b) Statistical analysis of Iba1 intensity. All plots show mean ± SEM unless otherwise mentioned. *P <0.05, **P<0.01, ***P<0.001, ****P<0.0001; ns, not significant.

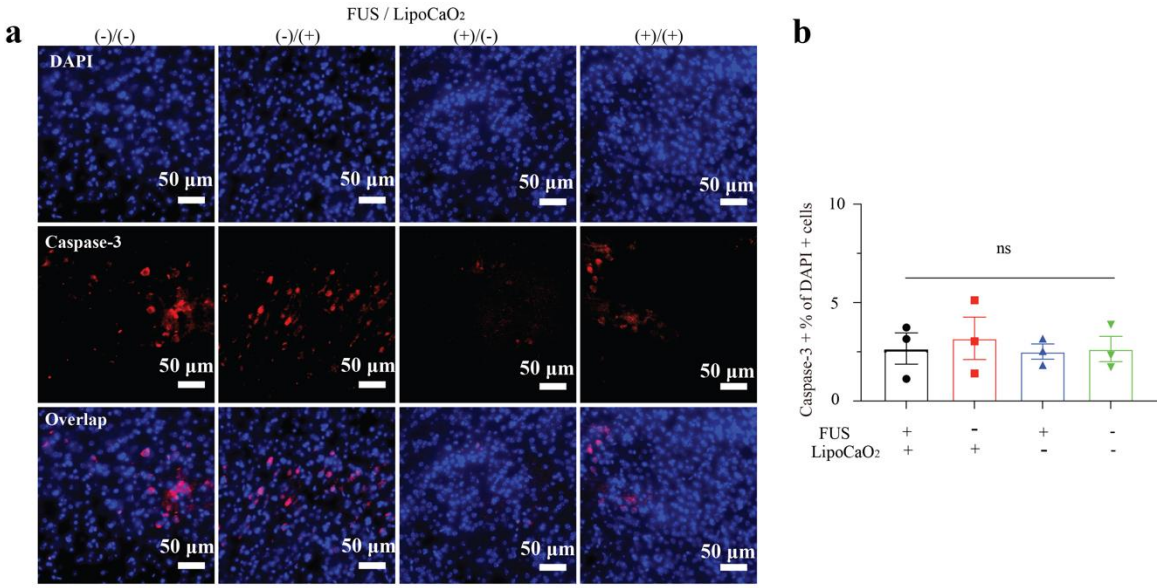

Figure S16. The biosafety evaluation through neuron apoptosis marker Caspase-3 immunostaining. (a)The Caspase-3 fluorescence images of mouse brain after treatment at different conditions after 7 days; (b) Statistical analysis of Caspase-3 intensity. All plots show mean ± SEM unless otherwise mentioned. **P* <0.05, ***P*<0.01, ****P*<0.001, *****P*<0.0001; ns, not significant.

Table S1. Antibodies used in this work.

Primary antibodies	**Secondary antibodies**
Rabbit anti-Iba1 (1:500, 013-27691, Wako Chemicals)	Donkey anti-Rabbit, Alexa Fluor 594 (1:800, A32754, Invitrogen)
Rabbit anti-Cleaved Caspase-3 (1:500, 9661, Cell Signaling Tec.)	Donkey anti-Rabbit, Alexa Fluor 594 (1:800, A32754, Invitrogen)
Anti-Tyrosine Hydroxylase Antibody (1:1000, AB152, Sigma-Aldrich)	Goat anti-Rabbit, Alexa Fluor 594 (1:500, R37117, Fisher Scientific)
Rabbit anti-c-Fos antibody (1:500, ab222699, Abcam)	Goat anti-Rabbit, Alexa Fluor 594 (1:500, R37117, Fisher Scientific)
Mouse anti-c-Fos antibody (E-8) (1:500, sc-166940, Santa-Cruz)	Goat anti-Mouse, Alexa Fluor 405 (1:500, ab175660, Abcam)
	H&E staining kit (ab245880, Abcam)
	Hoechst 33342 (1:5000, 17535, AAT Bioquest)

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