Supporting Information

Filling Core-Shell Microneedles with Pressurized Oxygen-embedded Particles to Improve Photodynamic Therapy

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1. Experimental Section

Materials: Sucrose, corn syrup (20% in water), methylene blue (MB), rose bengal (RB), curcumin (CUR), IR-780, acrylamide, polyvinylpyrrolidone (PVP, Mw 360kDa), and D-Luciferin sodium salt were supplied by Aladdin. Double sided adhesive tape (VHB 4959) was obtained from 3M. Reactive oxygen species assay kit (S0033S) and enhanced BCA protein assay kit (P0010) were provided by Beyotime. Hypoxyprobe assay kit (P1-100Kit) was purchased from Hypoxyprobe, Inc. AnaeroPack (C1) was purchased from Mitsubishi Gas Chemical Company. Anti-VEGF Receptor 1, anti-HIF-1 alpha, and anti-beta Actin were provided by Abcam. Microneedle positive molds were fabricated by BMF Precision Tech Inc. Cell Counting Kit-8 (CCK-8, CK04) was obtained from Dojindo Molecular Technology. Optical cutting temperature compound (O.C.T) was purchased from Sakura Finetek. Polydimethylsiloxane (PDMS) Sylgard 184 was purchased from Dowsil.

Method: Ultraviolet-visible light absorption spectrum was measured using a UV-vis spectrophotometer (UV-2550, Shimadzu). The morphology of MN patches was observed using a digital microscope and a scanning electron microscope (SEM, S4800, Hitachi). Cell fluorescence and drug fluorescence distribution in the MN patches were observed using an inverted fluorescence microscope (LSM780, Zeiss). Oxygen content was detected using a portable dissolved oxygen meter (JPF-605B, Rex). The compression mechanical properties of MNs were analyzed using an electronic universal testing machine (Criterion® C41.103Y, MTS). Tissue samples were sectioned

(thickness 12 μm) using a cryostat (CM1950, Leica). The absorbance of the cell sample was detected using a microplate reader (MODEL550, Bio Rad).

Fabrication of Particles: The production of POPs involved the use of a high-temperature high-pressure reaction vessel. Initially, 21 g of sucrose, 9 g of corn syrup, and 5 mL of deionized water were mixed in a glass container. The mixture was then stirred and heated to 137°C, resulting in a molten fluid. Subsequently, this molten substance was poured into the inner chamber of the vessel and pressurized with oxygen. Upon sealing the container, the reaction vessel was stirred at 750 rpm for 5 minutes and then allowed to cool and solidify at room temperature for 12 hours. The obtained pressurized oxygen solid was subsequently crushed and sieved using meshes of varying sizes in a dry environment, starting from 70-mesh, then 40-mesh, and finally 20-mesh. These particles were stored in Ziplock bags at 4°C. To prepare photosensitizer-loaded POPs, 0.5 g of MB, RB, CUR, or IR-780 was added to the initial mixture and processed using the same fabrication protocol. MB particles were created by incorporating 0.5 g of MB into the initial mixture without oxygen infusion and underwent the same sieving process.

Measurement of the Oxygen Loading Capacity of Particles: Initially, a 25 mL three-necked flask was added with 5 mL of deoxygenated deionized water and hermetically sealed using a rubber stopper. The oxygen concentration was continuously monitored using a portable dissolved oxygen meter probe immersed in the solution. Subsequently, 0.5 g of POPs were introduced into the sealed flask, and the oxygen concentration was recorded upon reaching equilibrium.

Cell Culture and Animals: The mouse melanoma cells, B16F10 and the luciferase-labeled B16F10 (B16F10-luc), were acquired from the China Type Culture Collection. These cells were cultured in DMEM medium supplemented with 10% (v/v) fetal bovine serum and 1% (v/v) penicillin/streptomycin, within a 37°C constant-temperature incubator with 5% carbon dioxide. Four-week-old male BALB/c nude mice were obtained from the Zhejiang Academy of Medical Sciences. All animal experiments strictly adhered to the “Principles of Laboratory Animal Care” (NIH publication no. 86-23, revised 1985) and were approved by the Laboratory Animal Welfare and Research Committee at Zhejiang University.

Cell Scratch Migration Assay: For the cell scratch migration assay, B16F10 cells were initially seeded at a density of 10^5 cells per well in a 24-well plate. After 24 hours of incubation, perpendicular scratches were created on the well plate’s surface using a 100 μL pipette tip. Each

experimental group received particles, and the hypoxic group's well plate was subsequently placed in an anaerobic gas-generating bag for 24 hours to maintain an oxygen content of less than 1%. Cell migration was then observed under a microscope.

Intracellular ROS detection: For the intracellular detection of ROS, B16F10 cells were initially seeded into a 24-well plate at a density of 10^5 cells per well. After cell adhesion, particles were added to each experimental group. The well plate of the hypoxic group was subsequently transferred to an anaerobic gas-generating bag and cultured for 6 hours with an oxygen content maintained at less than 1%. Subsequently, the treated cells were exposed to light irradiation (0.4 W/cm^2, 5 minutes), washed with PBS, and treated with 200 μL of diluted ROS probe DCFH-DA (at a 1:1000 ratio) per well. After incubation at 37°C for 20 minutes, the plate was observed under a fluorescence microscope.

Cell Viability Assay: For the cell viability assay, B16F10 cells were initially seeded at a density of 5000 cells per well in a 24-well plate. After 12 hours incubation, a MB particle or a MB POP (3.7 mg) was added into each well of the plate. The plate of the hypoxic group was subsequently transferred to an anaerobic gas-generating bag and cultured for 6 hours with an oxygen content maintained at less than 1%. Subsequently, the treated cells were exposed to light irradiation (0.4 W/cm^2, 5 minutes), The treated cells underwent an additional 12-hour incubation, and the cell survival rate was assessed using the CCK-8 method. This involved adding 50 μL of CCK-8 solution to each well and continuing incubation for 4 hours. Subsequently, the absorbance value at a wavelength of 450 nm was measured using a microplate reader.

Immunoblot Analysis: B16F10 cells were initially seeded into 24-well plates at a density of 10^5 cells per well. Following cell adhesion, the hypoxic group plate was incubated in a hypoxic environment (oxygen concentrations of less than 1%) for 6 hours in a cell incubator, while the normoxic group plate was cultured under normal oxygen conditions in the cell incubator for 6 hours. Subsequently, a POP or MB POP (3.7 mg) was added to each well of the plate of the hypoxic group and normoxic group. These plates were then incubated under their former oxygen incubation condition for 12 hours. After that, B16F10 cells were lysed, and their proteins were extracted using RIPA buffer. The protein concentration was quantified using a BCA kit. After centrifugation (4°C, 10,000 rpm, 10 min), the supernatants were collected, separated by electrophoresis in sodium dodecyl sulfate polyacrylamide (SDS-PAGE) gels, and then transferred to polyvinylidene fluoride

(PVDF) membranes. These PVDF membranes were blocked in 5% skim milk buffer for 1 hour to reduce nonspecific protein binding. Subsequently, the membranes were incubated with the primary antibody HIF-1α overnight at 4°C, followed by incubation with the fluorescence conjugated secondary antibody for 1 hour. Finally, the membrane was rinsed using an enhanced chemiluminescence detection kit and visualized through chemiluminescence development.

Fabrication of MNs: The PDMS MN mold is fabricated by molding the customized 3D printed resin MN obtained from BMF Precision Tech Inc. The MN specifications are as follows: 25 MNs are arranged in a 5×5 array over 1 cm^2, with a spacing of 2000 μm; each MN has a height of 1400 μm and a base diameter of 700 μm. The process of creating the PDMS negative mold involves stirring the PDMS prepolymer and curing agent at a 10:1 ratio to achieve a uniform mixture. The mixture is then poured onto the 3D printed resin MNs, allowed to sit for 30 minutes to remove air bubbles, and heated in an 80°C oven for 1 hour to solidify. The preparation process for the MNs involves three steps. Firstly, 1 mL of a 10% w/v PVP solution was poured into the PDMS molds and dried in a desiccator for 6 hours to form the shell layer. Subsequently, particles with diameters between 224 μm and 355 μm were filled into the cavities of the MN shell in a dry environment. Finally, an adhesive tape was applied to the base of the particle-filled MN shell, which was then used to entirely remove the MN patch from the PDMS mold. These MN patches were stored in a room-temperature desiccator for future use.

Mechanical Test: The mechanical properties of the MN patch were assessed using an electronic universal testing machine to record the force displacement curve. The MN patch was placed on the stainless-steel plate. Initially, the distance between the needle tip and the upper stainless-steel plate was set to 0.1 mm, and the compression speed was maintained at 1 mm/min. The instrument continuously monitored compression force and displacement until the displacement exceeded 1.5 mm after MN tip contacted with the upper pressure plate.

Skin penetration and payload diffusion study: Skin penetration test was performed on an isolated SD rat skin. After MN application for 30 minutes, skin samples were sectioned into 12 μm-thick slices using a freezing microtome for H&E staining analysis. The payload diffusion study was performed on a porcine skin. After MB POPs MNs or MB MNs application for 20 minutes, the skin was cut at the insertion site for observation under a digital camera.

Animal studies: A 50 μL suspension of B16F10-LUC cells (containing approximately 5×10^6 cells) was subcutaneously injected into the right lateral thigh of male BALB/c nude mice to establish the subcutaneous tumor model. Subsequently, the mice were randomly divided into 5 groups, each comprising 4 mice: Control group, with no treatment; MB (i.v.) group, where mice were injected with MB solution via the tail vein followed by light irradiation; POPs MN group, where MNs loaded with POPs were utilized for treatment; MB MN group, where MNs loaded with MB particles were applied to the tumor site followed by light irradiation; MB POPs MN group, where MNs loaded with MB POPs were applied to the tumor site followed by light irradiation. The dosage of MB in the experiment was 450 μg/kg. When the tumor volume reached approximately 100 mm^3, the mice received administration (MN application for 5 min or injection of MB solution) and light irradiation (5 minutes, 0.4W/cm^2) every 3 days for a total of 3 times. The bioluminescence imaging was performed by intraperitoneally injecting 200 μL (15 mg/mL) D-luciferin sodium salt solution every 3 days. Ten minutes after injection, the fluorescence distribution was observed using an intravital imager, and the tumor volume and body weight of the mice were recorded simultaneously. Throughout the experiment, mice with a tumor volume exceeding 1500 mm^3 were euthanized. Before euthanasia, all mice were injected with 100 μL (15 mg/mL) of the hypoxia probe Hypoxyprobe-1 via the tail vein. After 90 minutes, the tumors and various organs were collected for subsequent immunochemical analysis, including immunofluorescence staining for HIF-1α, VEGF, and hypoxic immunofluorescence.

Statistics: All experimental results were presented as mean ± standard deviation (SD). Statistical comparisons between groups were performed using one-way analysis of variance (ANOVA). Significance was defined as $p < 0.05$. The symbols *, **, and *** represent increasing levels of significance at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. The symbol “n.s.” indicates a lack of significant difference.

2. Supplementary Figures

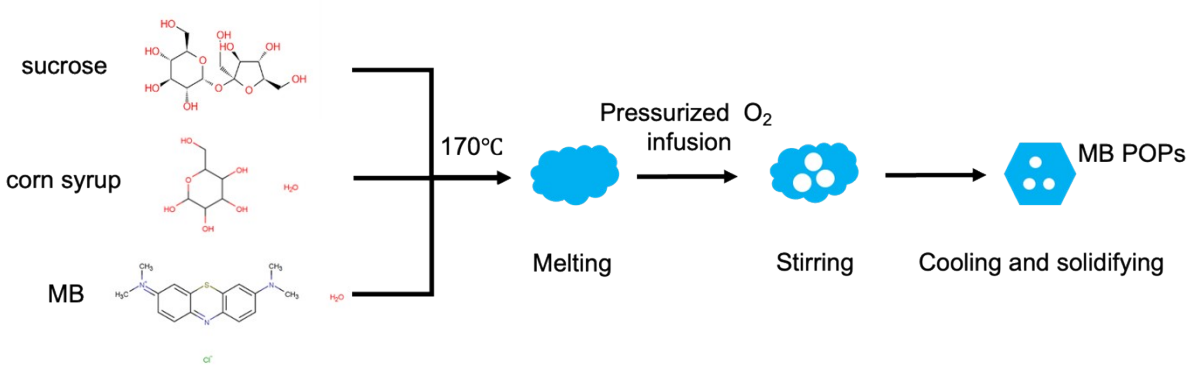

Fig. S1. Fabrication of MB POPs.

Fig. S2. Photograph of POPs individually loaded with different photosensitizers: MB, RB, CUR, and IR780. Scale bar: 1 cm.

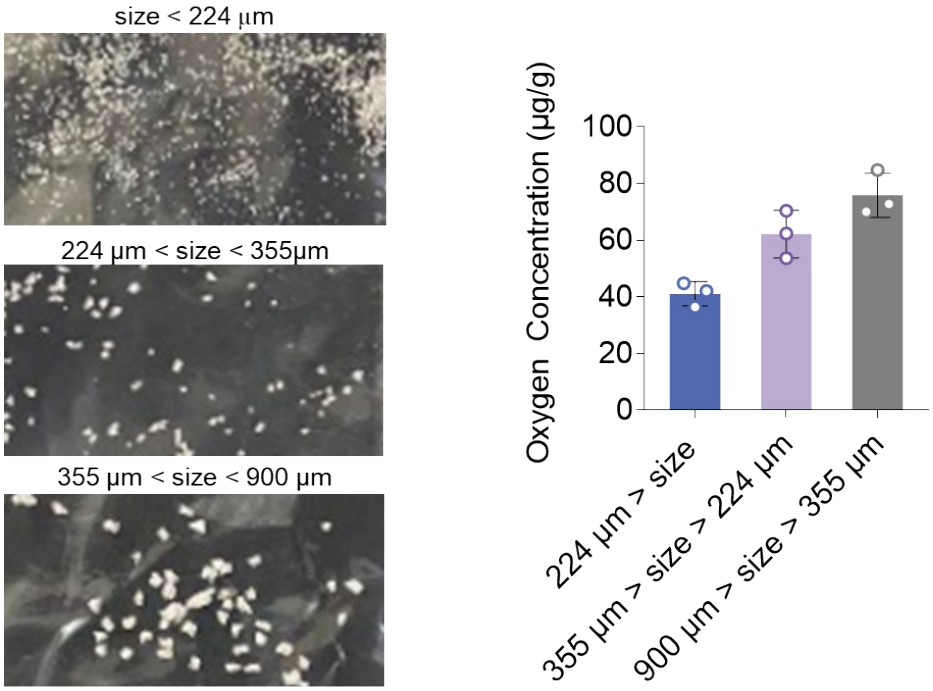

Fig. S3. Pressurized oxygen-embedded particles sorted using various sieves, alongside their respective oxygen content.

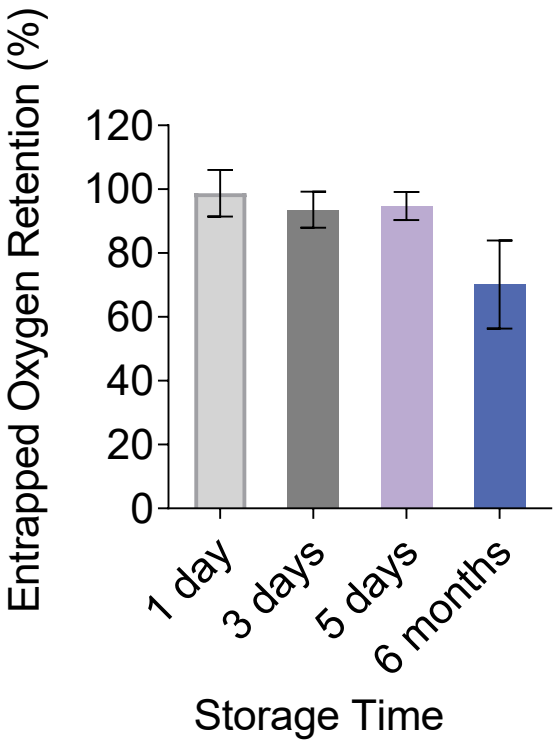

Fig. S4. Retention of entrapped oxygen in particles after storage for different durations.

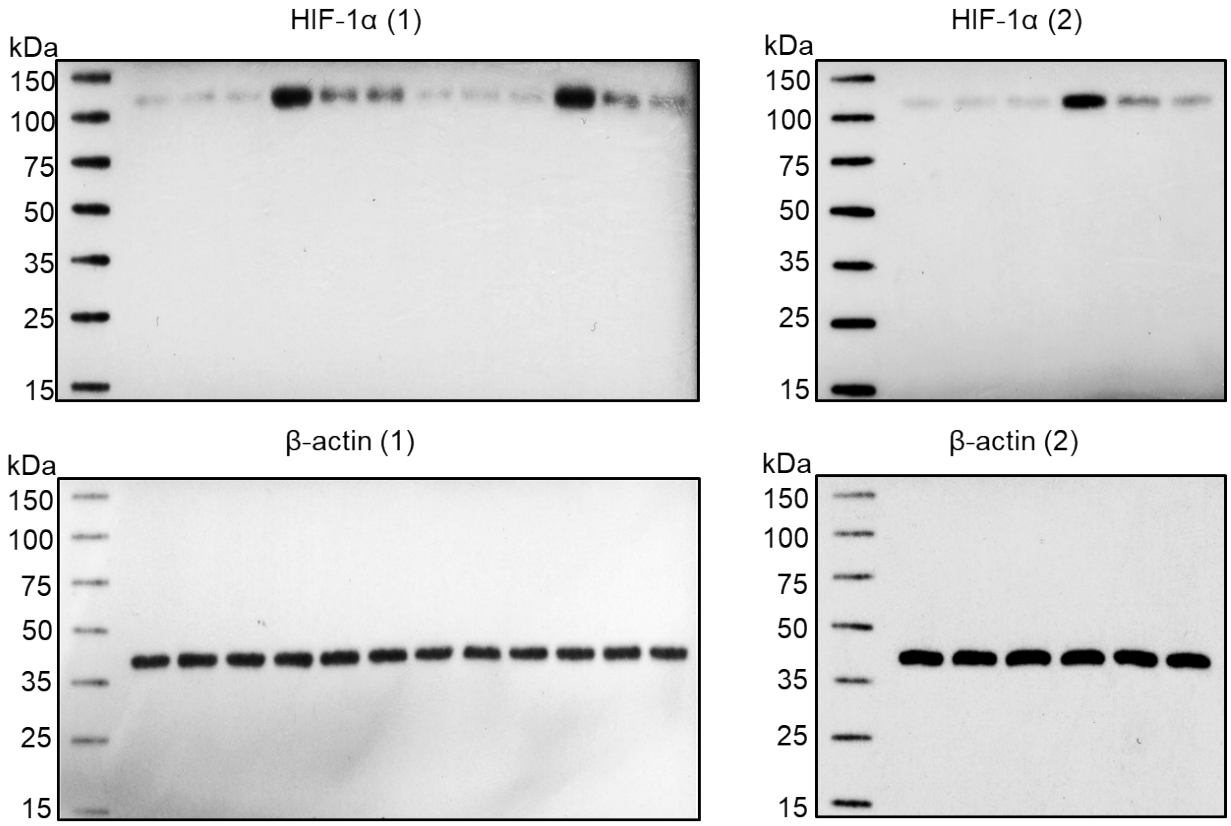

Fig.S5. Uncropped original images of WB.

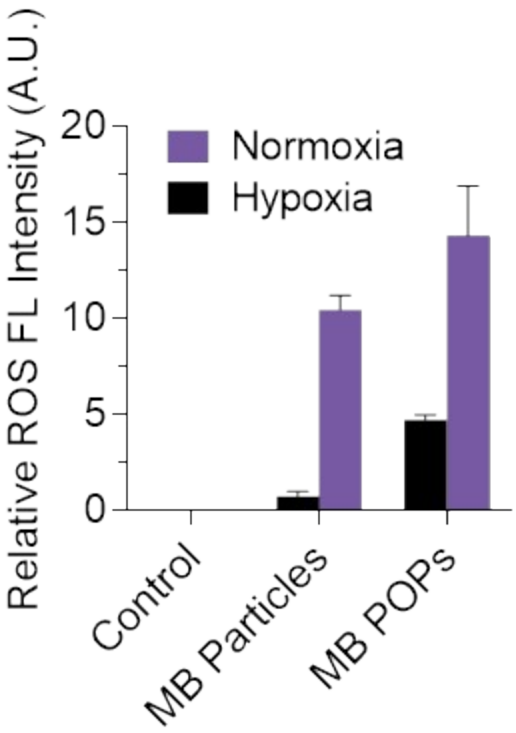

Fig.S6. Quantification analysis of ROS levels in B16F10 cells after different treatments.

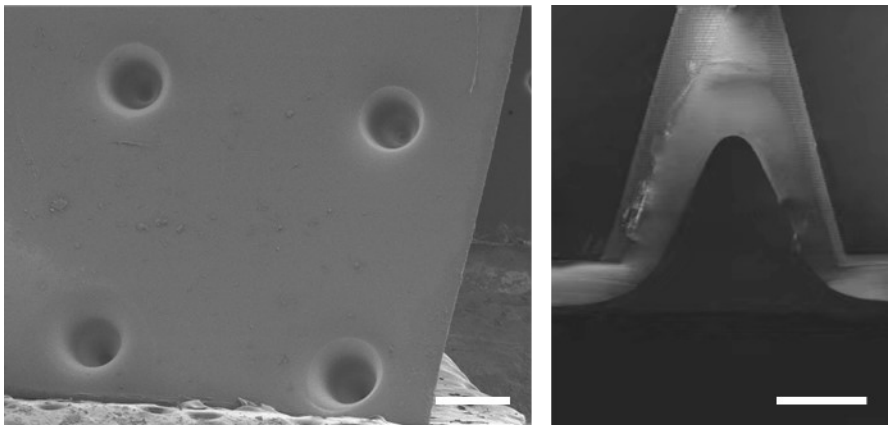

Fig.S7. SEM images of the backside and vertical slice side of an MN shell. Scale bar: 500 μm.

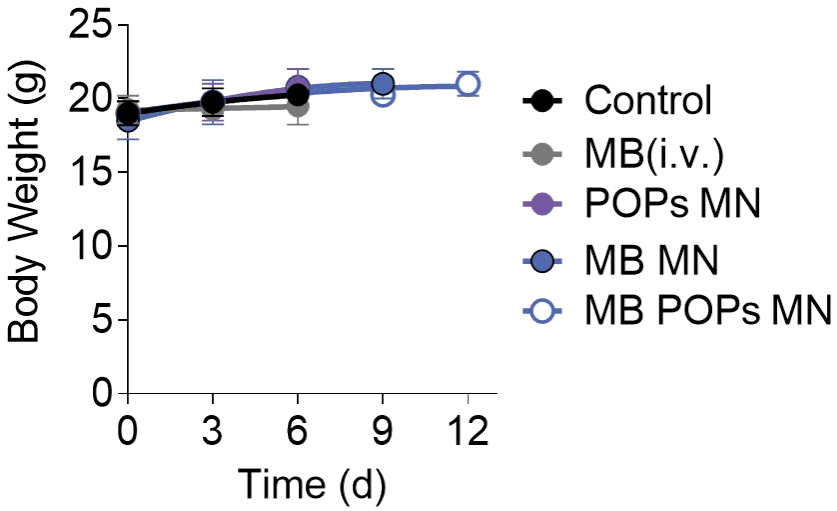

Fig.S8. Body weight profiles of B16F10-luc-bearing mice following different treatments.

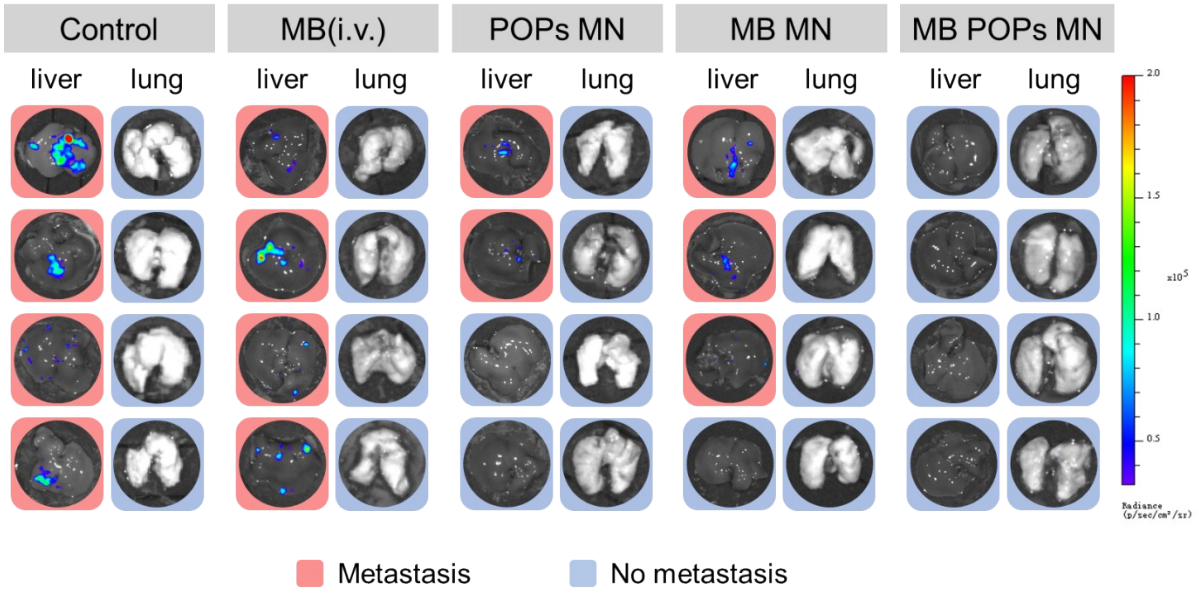

Fig.S9. Bioluminescence imaging photographs of the isolated lungs and livers after mice euthanasia.