Supporting Information

Biosynthetic nanobubbles for targeted gene delivery by focused ultrasound

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Cytotoxicity and Hemolysis Assay.

Cell viability 293T and 4T1 was measured at 48 h after the gene transfection using Cell Counting Kit-8 (CCK-8) according to the manufacture's protocol (Dojindo, Japan). Relative cell viability (RCV) was assessed by CCK-8 assay and then determined in a 96-well plate reader (BioTek Synergy 4) at the 450 nm wavelength with equation: RCV(%) = At－Anc/ Apc－Anc×100%.

The effect of BNBs on the hemolytic behavior of RBCs was also investigated. In brief, fresh blood sample (1.0 mL) was stabilized by ethylene diaminetetraacetic acid (EDTA), and then mixed with PBS (2.0 mL, 4 °C). After centrifugation (2500 rpm•min-1, 15 min) to discard the serum, the obtained red blood cells (RBCs) were further washed five times, followed by dispersing in PBS (10 mL). To study the hemolysis of the CBNBs, RBCs dispersions (1mL) were incubated with the CBNBs at various concentrations (OD = 0.5, 1.0, 2.0, 3.0) at room temperature for 4 h. After centrifugation (10,000 rpm, 10 min), the supernatant was collected and analyzed with a UV-Vis-NIR spectrometer at 541 nm. The hemolysis percent of the RBCs after incubation was determined based on the following formula: hemolysis percentage (%) = (ODsample–ODPBS)/(ODwater – ODPBS) × 100%, where ODsample, ODPBS, and ODwater are the absorbance of the CBNBs and the deionized water (positive control) and PBS (negative controls), respectively.

Serum biochemistry analysis

To check the *in vivo* biocompatibility of the BNBs, Healthy Balb/c mice were intravenously injected with different concentrations of BNBs (OD500 = 1.0, 2.0, 3.0). At days 15 post-injection, fresh blood samples (1.0 mL) were obtained by removing the eyeballs of the mice for serum biochemistry study.

Statistical analysis

The data were analyzed using SPSS 21.0. All results are summarized as the mean ± standard deviation. ANOVA was used to evaluate statistical significances between different groups. Multiple comparisons were performed through Bonferoni analysis. $P< 0.05$ were considered statistically significant.

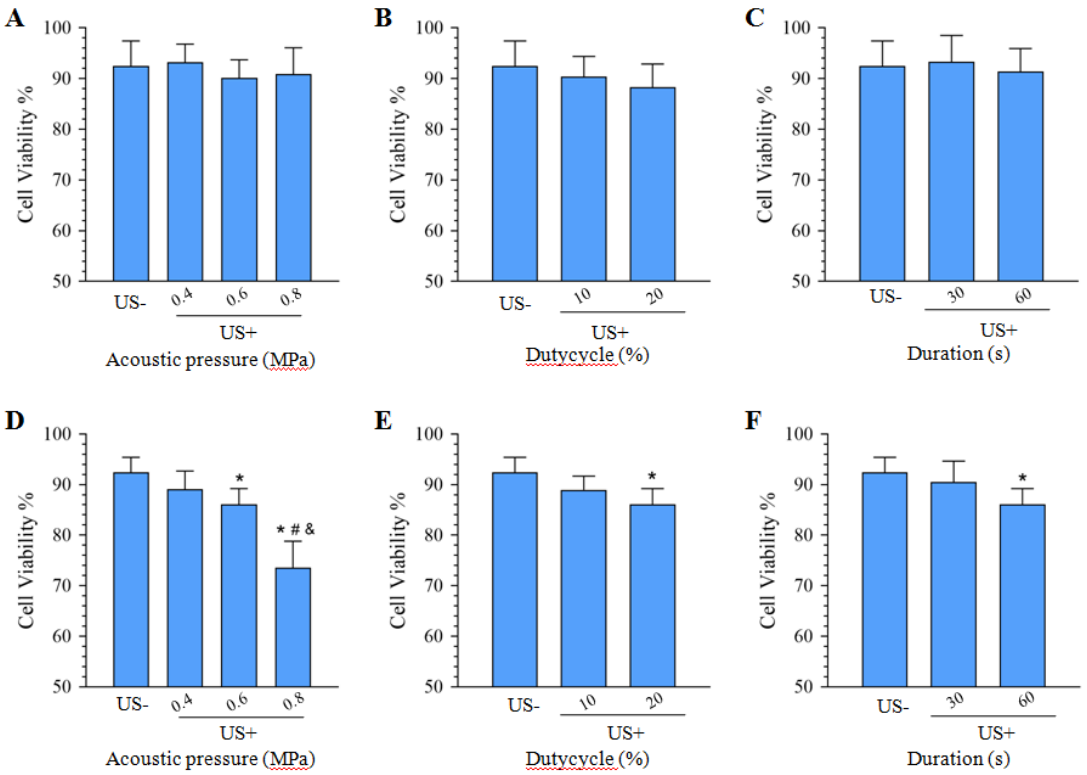

Fig. S1 Cell viability under different conditions for 293T cells: (A-C) Effect of different conditions on cell viability without CBNBs; (D-E) Effect of different conditions on cell viability with CBNBs. Dutycycle/duration was set as 20%/60 s for A and D; acoustic pressure/duration were set as 0.6 MPa/60 s for B and E; acoustic pressure/dutycycle were set as 0.6 MPa/20% for C and F.*: $P < 0.05$ vs. US-. #: $P < 0.05$ vs. 0.4 Mpa, &: $P < 0.05$ vs. 0.6 MPa.

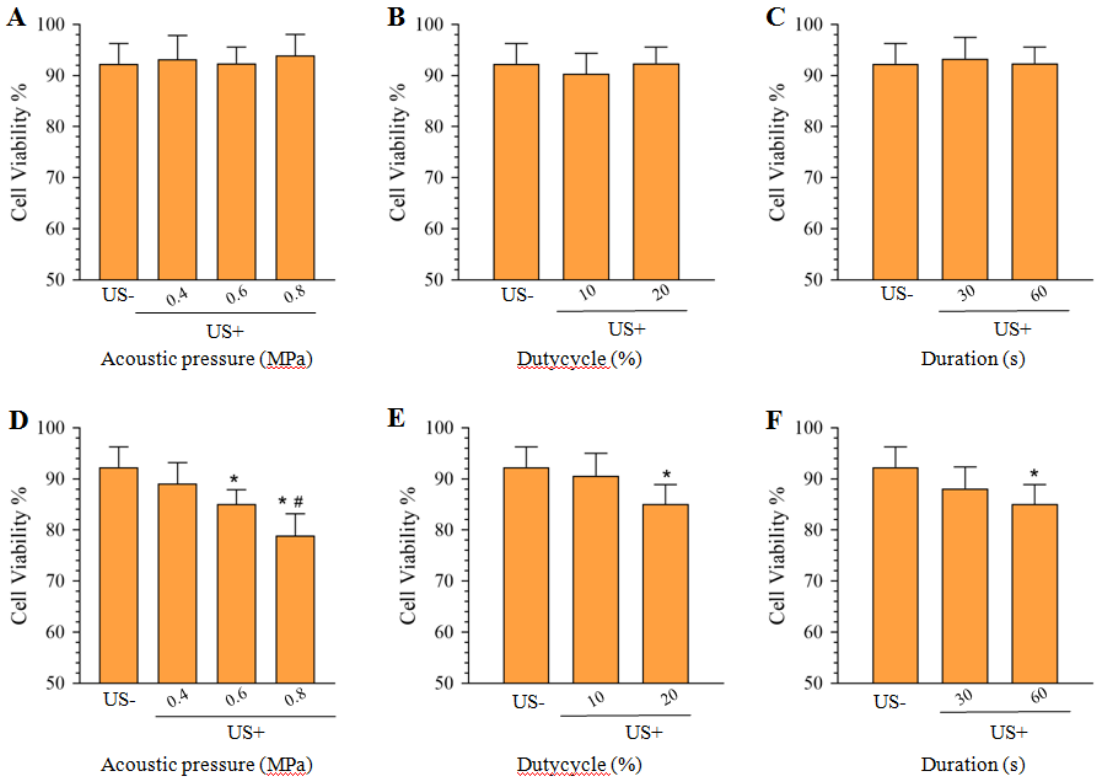

Fig. S2 Cell viability under different conditions for 4T1 cells: (A-C) Effect of different conditions on cell viability without CBNBs; (D-E) Effect of different conditions on cell viability with CBNBs. Dutycycle/duration was set as 20%/60 s for A and D; acoustic pressure/duration were set as 0.6 MPa/60 s for B and E; acoustic pressure/dutycycle were set as 0.6 MPa/20% for C and F.*: $P < 0.05$ vs. US-. #: $P < 0.05$ vs. 0.4 MPa.

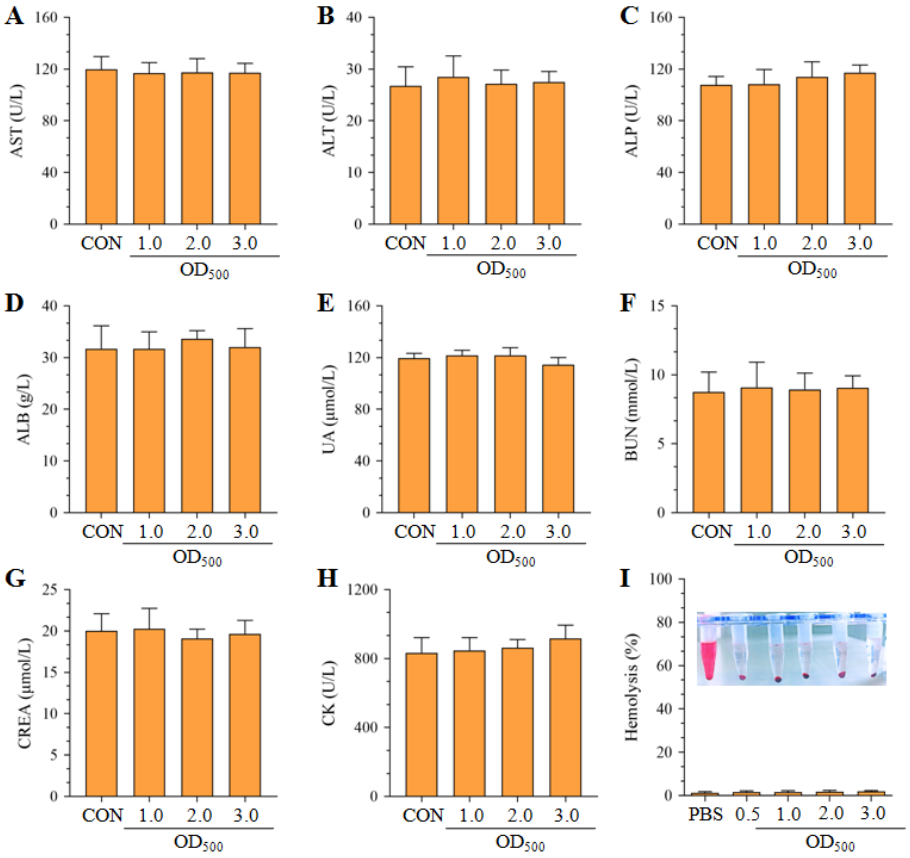

Fig. S3 Serum biochemistry analysis of the mice at different concentration: (A) AST: aspartate aminotransferase; (B) ALT: alanine aminotransferase; (C) ALP: alkaline phosphatase; (D) ALB: albumin; (E) UA: uric acid; (F) BUN: blood urea nitrogen; (G) CREA: creatinine; (H) CK: creatine kinase; (I) Hemolysis percentage of RBCs after treatment with different concentrations of BNBs, distilled water and PBS was applied as positive and negative controls respectively. CON: Healthy mice without any treatment.

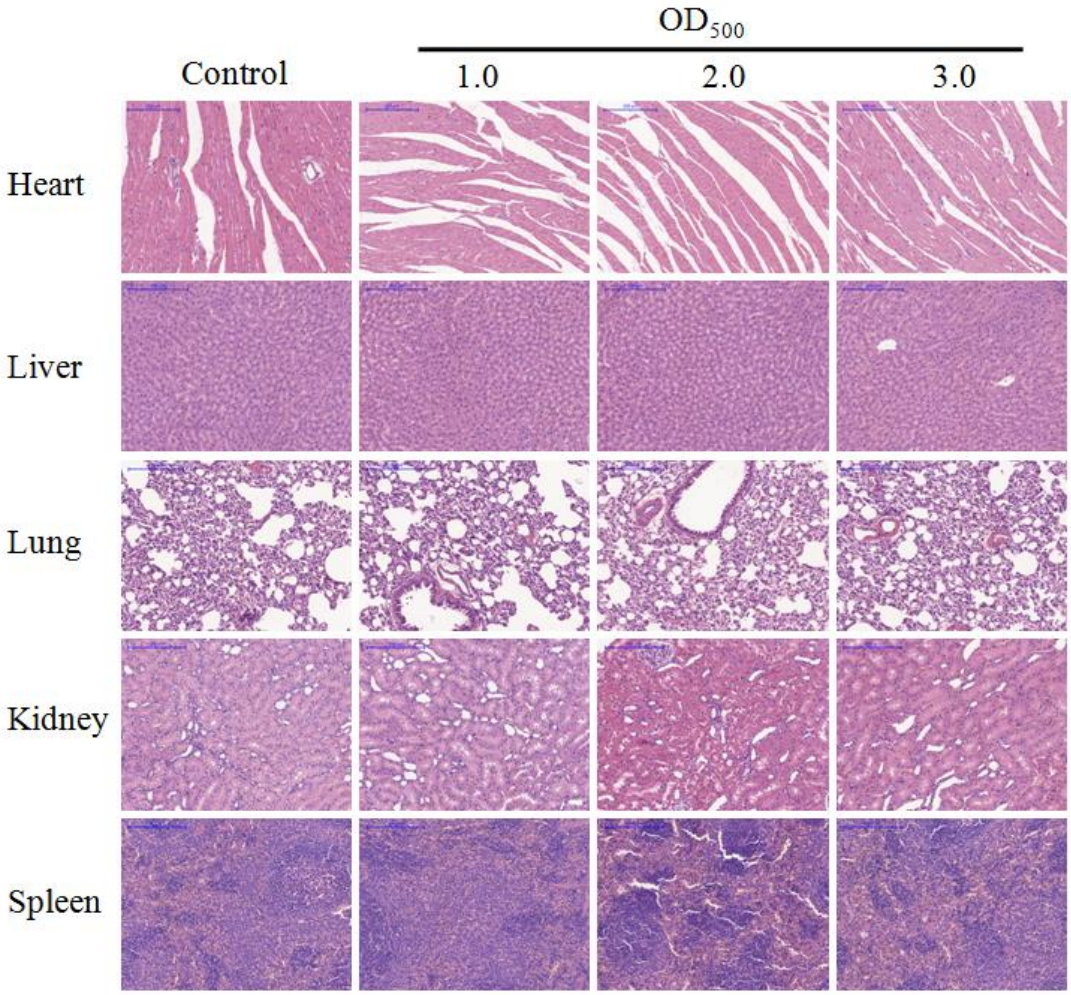

Fig. S4 Representative H&E images of major organs from the mice at the end of the experiments: no obvious damage or inflammation was observed in the BNBs-treated group and control group