Supporting information:

Carbon nanocages-based nanozyme as an endogenous H_2O_2-activated oxygenerator for real-time bimodal imaging and enhanced phototherapy on esophageal cancer

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Methods

Live-dead cell staining evaluation

To visually verify the therapy effects, live-dead cell staining was evaluated by Calcein-AM/PI double dyeing kit. The KYSE-30 cells were seeded in 12-well plates overnight. Then the cells were incubated with PBS, OCNCs (200 μg/mL), IR820 (8 μg/mL), IR820@OCNCs (equivalent OCNC and IR820 concentrations), and BMIOC (equivalent OCNC and IR820 concentrations) with or without irradiation on the condition of 1% O_2. The laser-irradiated groups were exposed to 0.8 W/cm^2 of 808-nm irradiation for 5 min. After that, the cells were washed with PBS and stained with calcein-AM and PI. Finally, the stained cells were imaged by the fluorescence microscope with 488 nm and 543 nm excitation, respectively.

Intracellular ROS detection

The ROS generation of KYSE-30 cells was investigated via incubation with DCFH-DA using the Reactive Oxygen Species Assay Kit (Beyotime Institute of Biotechnology, Shanghai, China). The cells were cultured with PBS, OCNCs (200 μg/mL), IR820 (8 μg/mL), IR820@OCNCs (equivalent OCNC and IR820 concentrations), and BMIOC (equivalent OCNC and IR820 concentrations) for 24 h, with or without irradiation. The laser-irradiated groups were exposed to 0.8 W/cm^2 of 808-nm irradiation for 5 min. Subsequently, the cells were incubated with DCFH-DA solution (20 μM) for 20 min. The fluorescence intensity of DCFH-DA inside the cells, which was in parallel with the amount of intracellular ROS generated, was measured by flow cytometry.

Pharmacokinetics of the BMIOC nanosystem

The pharmacokinetics of prepared BMIOC nanosystem was detected by measuring Mn content in blood. In brief, blood samples were collected at 5 min, 15 min, 1 h, 4 h, 8 h, 18 h of tumor-bearing mice after intravenously injected with BMIOC. And then, the Mn content in blood samples was determined by an inductively coupled plasma mass spectrometry (ICP-MS, Agilent 7500a). Take the initial injection dose (ID) as a control, the percentage of drug content in blood versus time was analyzed and used to calculate the blood circulation half-lives. Blood circulation data curve was shown in Fig. S9. The blood circulation half-lives were calculated using the following formula: K=(Inco-Inc)/t, $t_{1/2}$=0.693/k.

Western blot analysis

The cells and tumor tissues were lysed with cell lysis buffer (Beyotime Institute of Biotechnology, Shanghai, China) assisted with 0.5 mM phenylmethanesulfonyl fluoride. After centrifugation (12,000 rpm, 15 min, 4°C), protein concentrations were evaluated by a Bicinchoninic Acid Protein Assay Kit (Beyotime Institute of Biotechnology, Shanghai, China). Sample lysates were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene difluoride (PVDF) membranes (0.22; Millipore, Billerica, MA, USA). Membranes were blocked for 2 h with 5% nonfat milk in Tris-buffered saline at room temperature and then incubated with HIF-1α, Caspase 3 antibody (1 : 1,000 dilution; Cell Signaling Technology, Boston, MA, USA) at 4°C overnight, followed by HRP-conjugated secondary antibodies (1 : 5,000; Proteintech, Chicago, IL, USA) for 1 h. Protein bands were determined using an enhanced chemiluminescence detection kit (Cell Signaling Technology, Boston, MA, USA) and photographed by a GE Amersham Imager 600 imaging system (GE Life Sciences, Chicago, IL, USA).

Figures

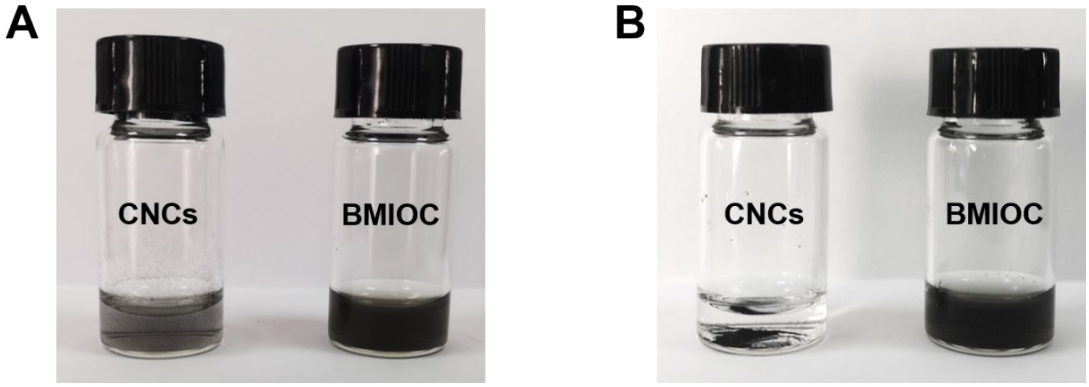

Fig. S1. (A) Digital photographs of CNCs and BMIOC in PBS (B) Digital photographs of CNCs and BMIOC after dispersed in PBS for 7 days.

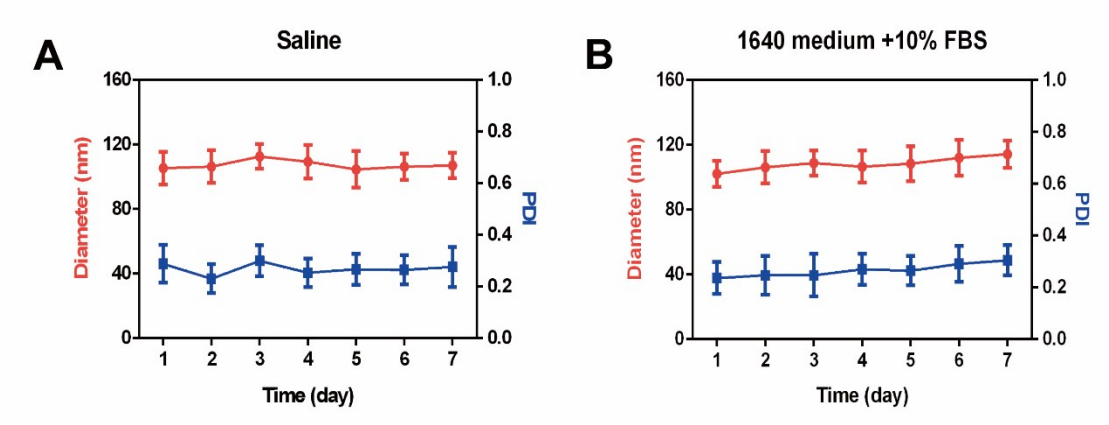

Fig. S2. The size distribution and the polydispersity index (PDI) of BMIOC dispersed in (A) saline and (B) 1640 medium +10% FBS for 7 days (n=3).

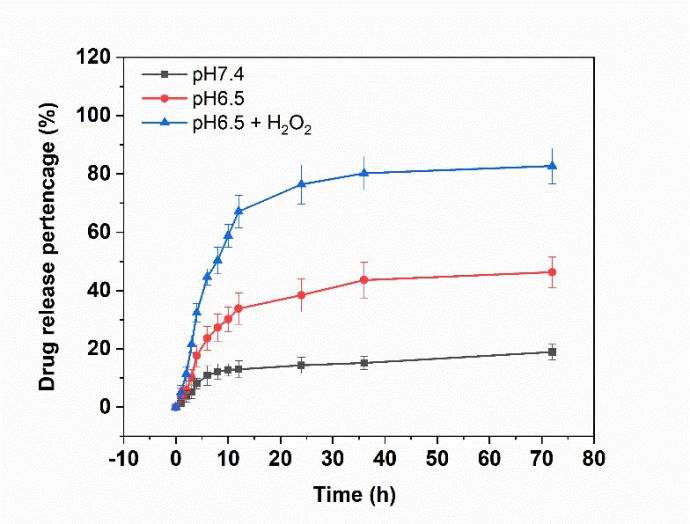

Fig. S3. In vitro release profiles of the loaded IR820 from BMIOC.

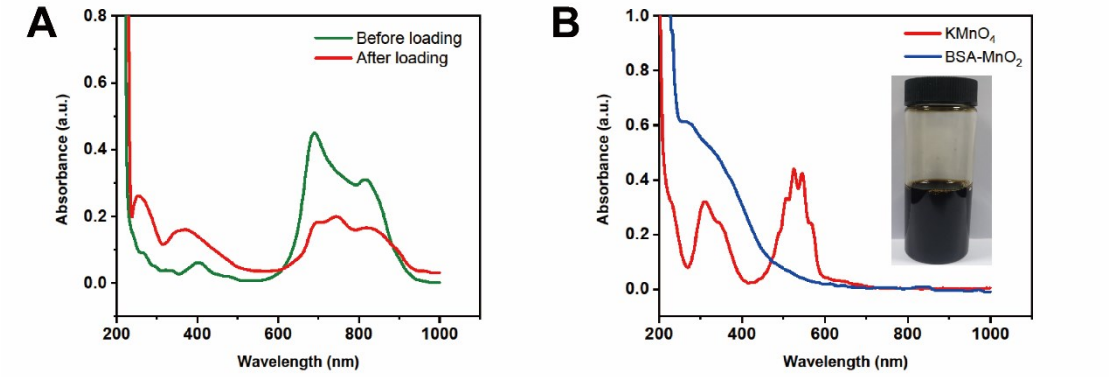

Fig. S4. (A) UV-vis absorption spectra of the solution before and after loading of IR820. (B) UV−vis absorption spectra of $KMnO_4$ and BSA-MnO_2. Inset: the

corresponding photograph of BSA-MnO_2 solution.

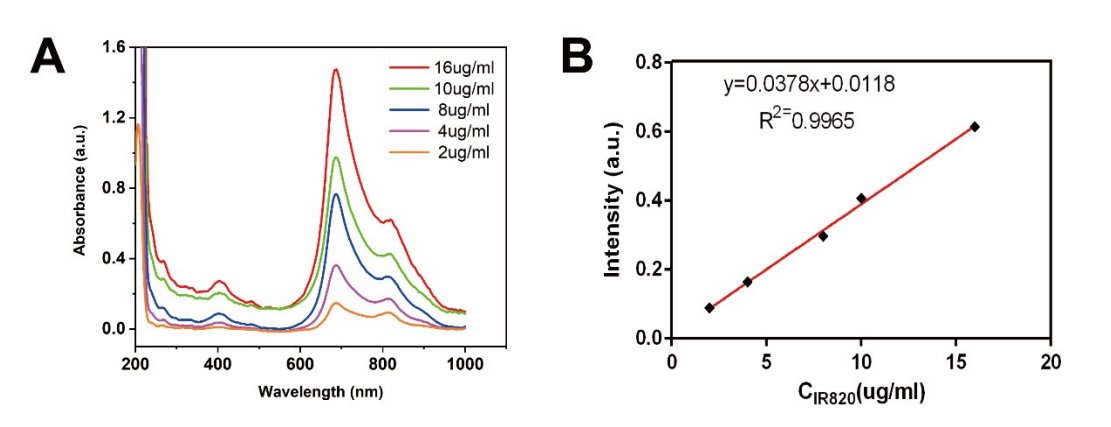

Fig. S5. (A) UV-vis absorption spectra of IR820 under different concentrations. (B) The standard curve between absorption intensity and concentrations of IR820.

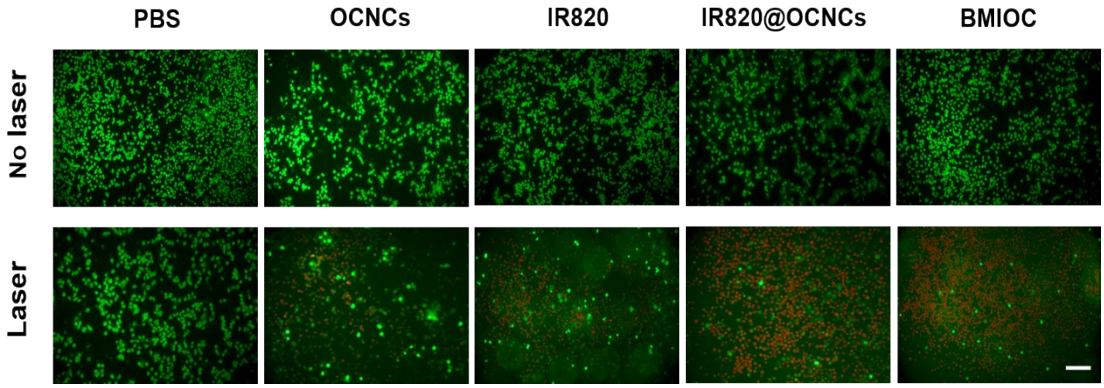

Figure S6. Live/dead cell staining of KYSE-30 cells treated with various nanoparticles in the absence/presence of 808 nm laser irradiation using double-staining with PI and Calcein-M. The green fluorescence marked live cells, while the red fluorescence indicates dead cells. Scale bar: 200 μm.

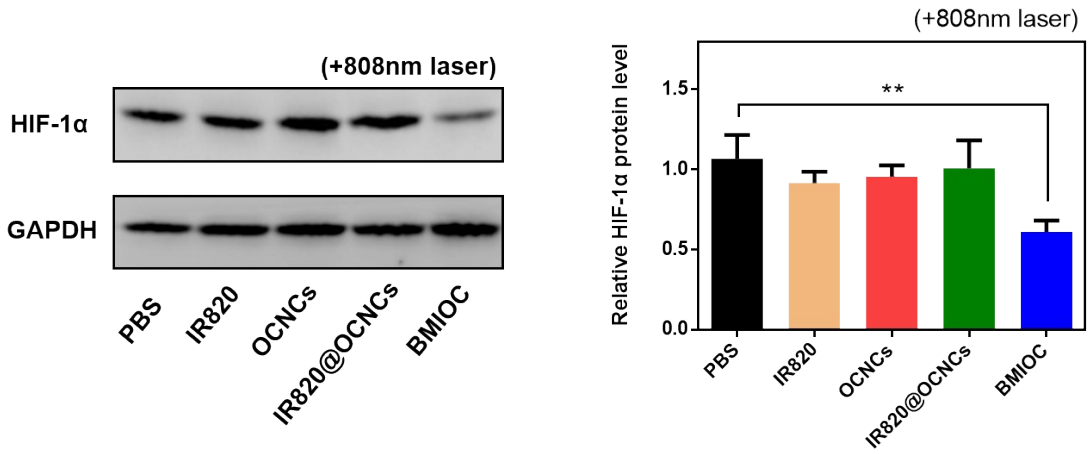

Fig. S7. Representative protein bands of HIF-1α by western blot and quantitative analysis of the relative protein levels of KYSE-30 cells incubated with different nanomaterials under 808 nm laser (n=3, **P < 0.01).

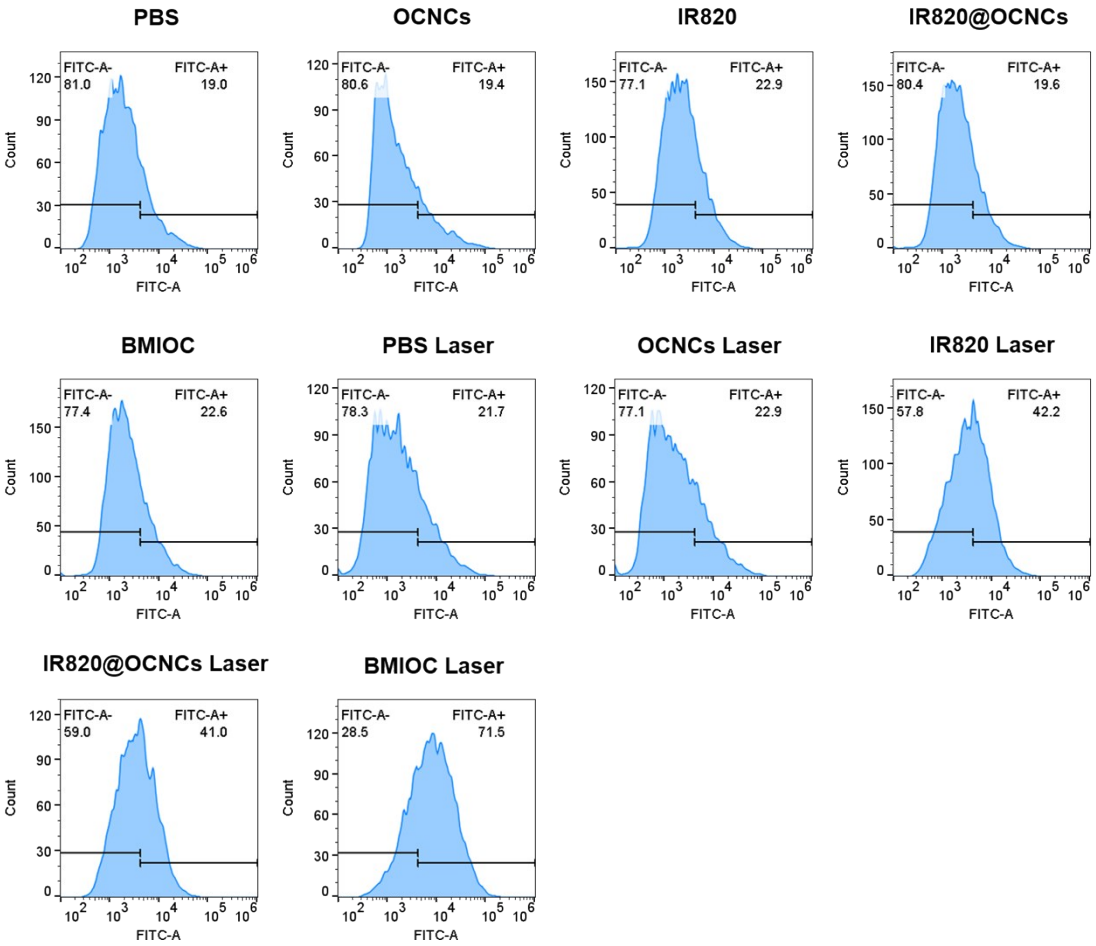

Fig. S8. Flow cytometry analysis on the intracellular ROS levels of KYSE-30 cells treated with various nanoparticles in the absence/presence of 808 nm laser irradiation by DCFH-DA staining.

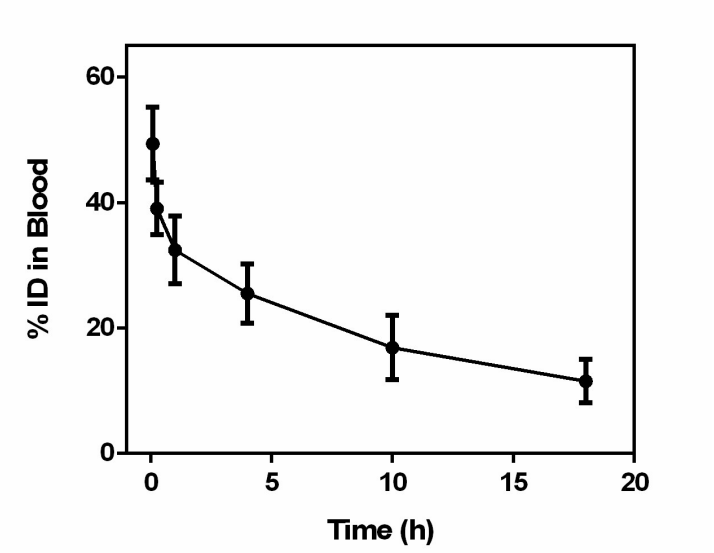

Fig. S9. Pharmacokinetics of the BMIOC nanosystem after intravenous injection (n=3).

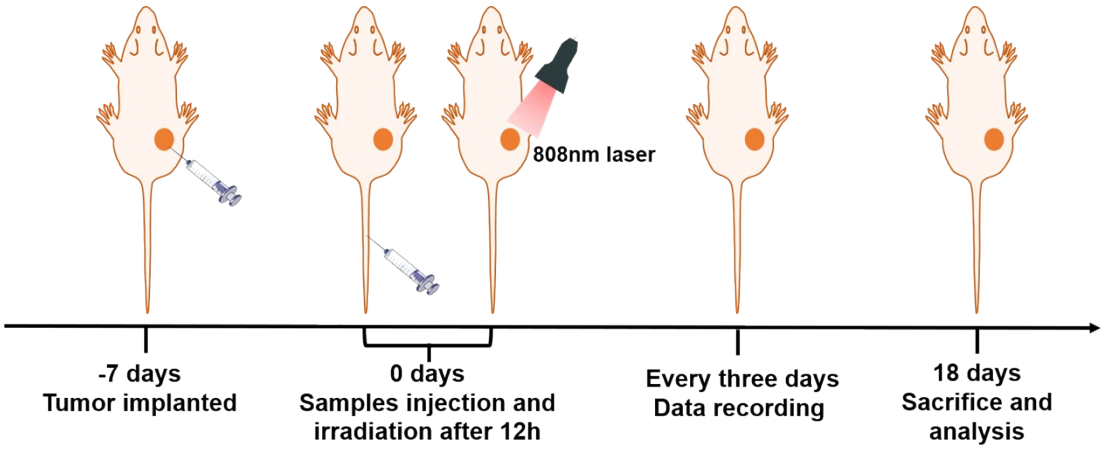

Fig. S10. Schematic illustration for the therapeutic model and procedure.

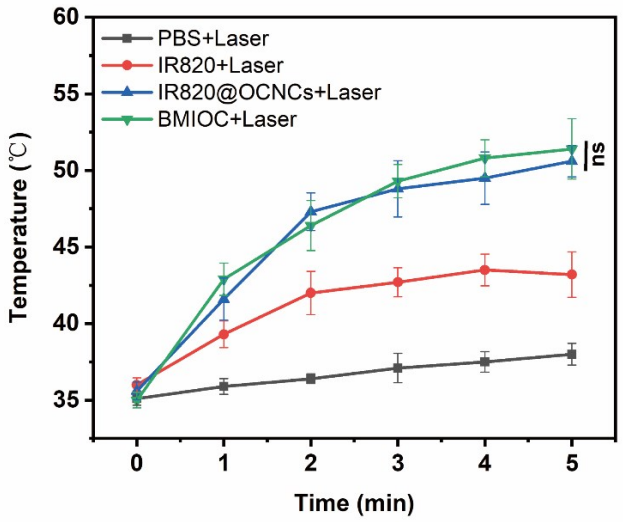

Fig. S11. Temperature changes of the tumor sites from mice treated with various samples in the presence of laser irradiation (n=5, ns indicates $P > 0.05$).

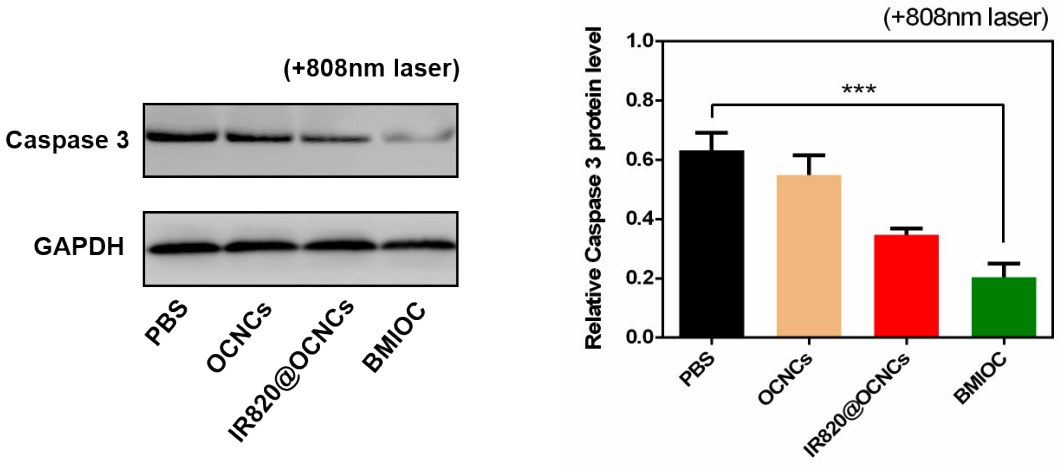

Fig. S12. Representative protein bands of Caspase 3 by western blot and quantitative analysis of the relative protein levels in tumors of mice treated with different nanomaterials under 808 nm laser (n=3, **$P < 0.01$).

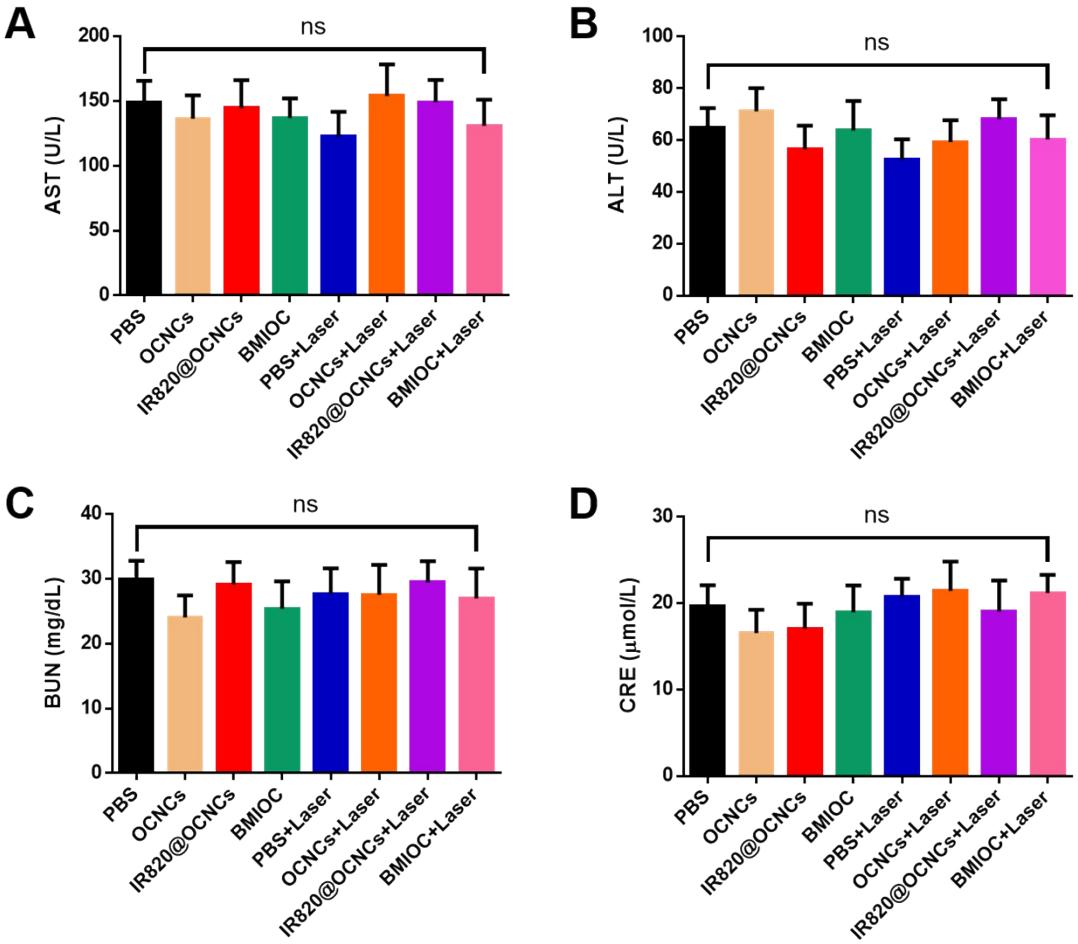

Fig. S13. The level of (A) AST, (B) ALT, (C) BUN, and (D) CRE of serum biochemistry parameters (n=5, ns indicates $P > 0.05$).