Supporting Information

Enzymatically synthesised MnO_2 nanoparticles for highly efficient near-infrared photothermal therapy and dual-responsive magnetic resonance imaging

Protein expression and purification

The harvested cells were suspended in equilibration buffer (100 mM Tris-HCl, pH = 8.0; 1.5 M NaCl) supplemented with 10 mM $CaCl_2$, 1 mM $CuSO_4$, and EDTA-free protease inhibitor (Sigma) and lysed using JY92-IID (Ningbo Dongnan Instrument Co., Ltd., China) for 1 min/ml cell lysate at 60% amplitude with 10-s on/off pulses on ice. The crude extract was clarified by 20 min of incubation at 70 °C. The debris was pelleted by centrifugation at 15,000 × g at 4 °C for 30 min, and the supernatant was filtered through a 0.4-μm-pore PVDF filter. The clarified lysate was then added to a 5-ml column volume (CV) of gravity flow Strep-Tactin@XT superflow® (IBA, Germany). The column was washed 5 × with 1 CV of buffer W (100 mM Tris/HCl, pH = 8.0; 150 mM NaCl; 1 mM EDTA), the wash fractions were collected, and 10 μl of the wash fraction was saved for analytical SDS-PAGE. Then, 6 × 0.5 CV of buffer BXT (100 mM Tris/HCl, pH = 8.0; 150 mM NaCl; 1 mM EDTA; 50 mM biotin) was added to obtain a high protein concentration in one fraction, followed by the addition of 0.6 CV as elution fraction 1 (E1), then 1.6 CV (E2) and finally 0.8 CV (E3). The main protein content should be in E2. A 10-μl sample of E2 can be used for SDS-PAGE analysis. All buffers up to this point were supplemented with 50 μM $CuSO_4$ to

avoid copper leaching by Tris. This as-isolated MnxEFG was then flash frozen in liquid nitrogen and stored at −80 °C until use. The protein was quantified by the Thermo Scientific Pierce bicinchoninic acid (BCA) protein assay.

Content of protein in Bio-MnO_2 NPs

The concentration of protein in Bio-MnO_2 NPs was determined as follows: 100 μL of Bio-MnO_2 NPs (final concentration was 150 μg mL^{-1}) was reacted with 10 mM GSH for 24 h. The protein in the product after Bio-MnO_2 NP decomposition was quantified by the Thermo Scientific Pierce bicinchoninic acid (BCA) protein assay, which was determined to be 7.5 μg mL^{-1}, and thus, the content of protein in Bio-MnO_2 NPs was 5%.

Calculation of the photothermal conversion efficiency

According to a previous report[1], the photothermal conversion efficiency (η) was calculated via equation 1:

$$\eta = \frac{hS(T_{\max} - T_{surr}) - Q_s}{I(1-10^{-A})} \qquad \text{(Eq 1)}$$

where h is the heat transfer coefficient, S represents the surface area of the cell, T_{max} is the maximum steady-state temperature, T_{surr} is the ambient temperature of the surroundings, Q_s is the heat dissipated from light absorbed by the cell itself, which is measured independently to be 0.27 mW, I is the laser power (the area of the well was $3.14*(0.5)^2=0.785$ cm^2, the power density was 1.5 W/cm^2, and the laser power was 0.785*1.5*1000=1177.5 mW), and A is the absorbance of Bio-MnO_2 NPs at an

excitation wavelength of 808 nm, which was calculated to be 0.303 (Figure 3d).

We define θ as the following:

$$\theta = \frac{T - T_{surr}}{T_{\max} - T_{surr}} \qquad \text{(Eq 2)}$$

where ($T - T_{surr}$) represents the temperature increase compared to the surroundings, and ($T_{max} - T_{surr}$) represents the temperature change at the maximum steady-state temperature.

$$\tau_s = -\frac{t}{\ln \theta} \qquad \text{(Eq 3)}$$

τ_s is the sample system time constant, which can be determined to be 157 s by the linear curve fitting of the temperature cooling time according to Figure 3f.

$$hS = \frac{m_s C_s}{\tau_s} \qquad \text{(Eq 4)}$$

where m_s (0.2 g) and C_s (4.2 J g^{-1}) are the mass and heat capacity of the solvent, respectively. Putting this value into equation (4), the final photothermal conversion efficiency (η) of Bio-MnO_2 NPs can be calculated to be 44%.

The photothermal conversion efficiency (η) of Chem-MnO_2 was calculated according to the same method. The mean absorbance at 808 nm and τ_s of Chem-MnO_2 was determined to be 0.3724 and 257 s, respectively. The final photothermal conversion efficiency (η) of Chem-MnO_2 was calculated to be 27.9%.

References

[1] Roper, D. K.; Ahn, W.; Hoepfner, M. Microscale Heat Transfer Transduced

by Surface Plasmon Resonant Gold Nanoparticles. *J. Phys. Chem. C Nanomater. Interfaces* **2007**, *111*, 3636–3641.

Table S1. Surface elemental composition of Bio-MnO_2 NPs analysed by dot-scan EDS.

Element	Family	Atomic Fraction (%)	Mass Fraction (%)
C	K	80.51	66.93
N	K	2.34	2.17
O	K	7.37	8.11
Na	K	0.04	0.06
P	K	1.03	1.91
K	K	0.96	2.24
Mn	K	5.74	18.58

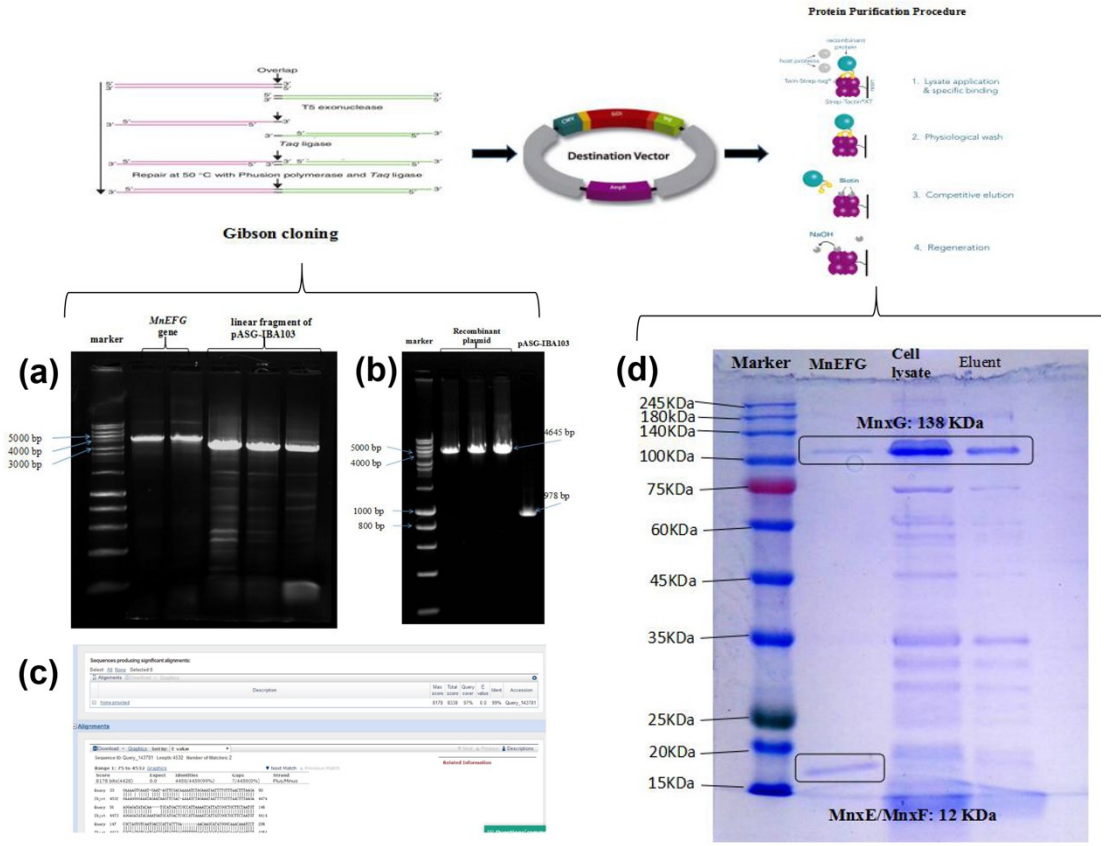

Figure S1 (a) PCR amplification of *mnxEFG* gene fragments with homologous sequences from the vector pASG-IBA103 and linearization of pASG-IBA103 by PCR. (b) PCR amplification of the recombinant plasmid and the acceptor plasmid pASG-IBA103 (control) using sequencing primers. (c) BLAST results between the insert fragment and the *mnxEFG* gene. (d) SDS-PAGE results of purified protein.

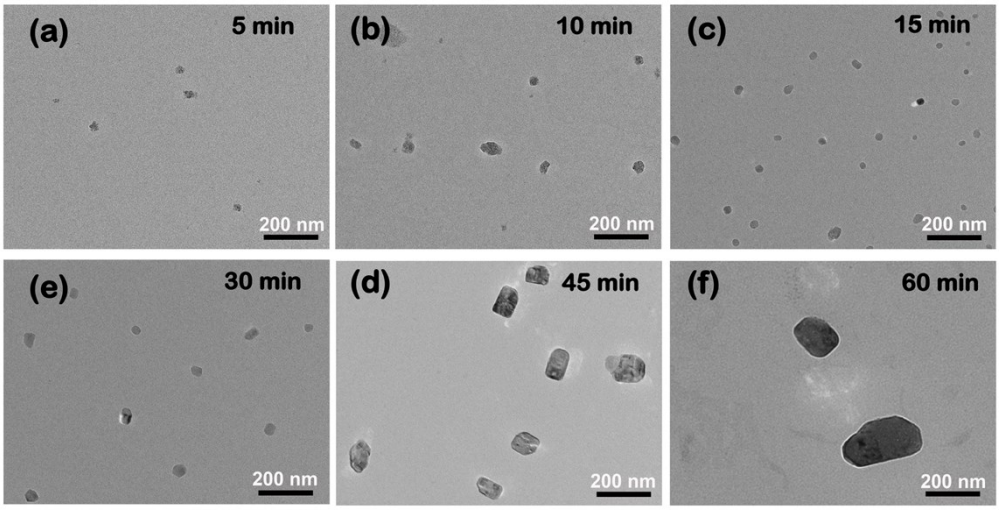

Figure S2 TEM images of Bio-MnO_2 NPs formed by the biomineralization of Mnx at 30 ℃ for different time periods.

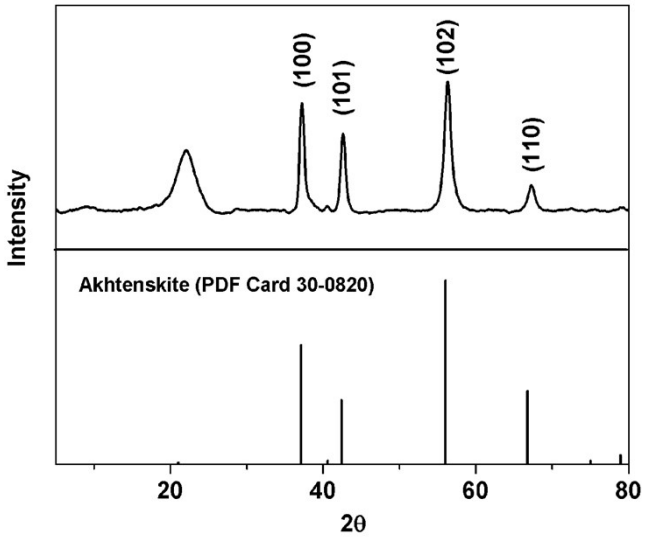

Figure S3 XRD profile of Chem-MnO_2.

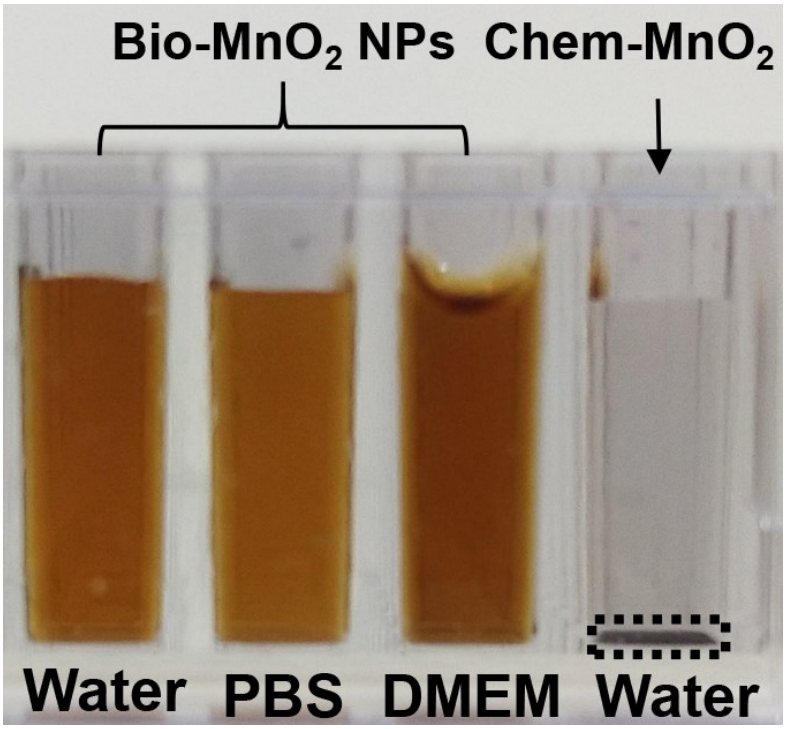

Figure S4 Photos of Bio-MnO_2 NPs dispersed in deionized water, PBS and DMEM for 9 days and Chem-MnO_2 dispersed in deionized water for 1 h.

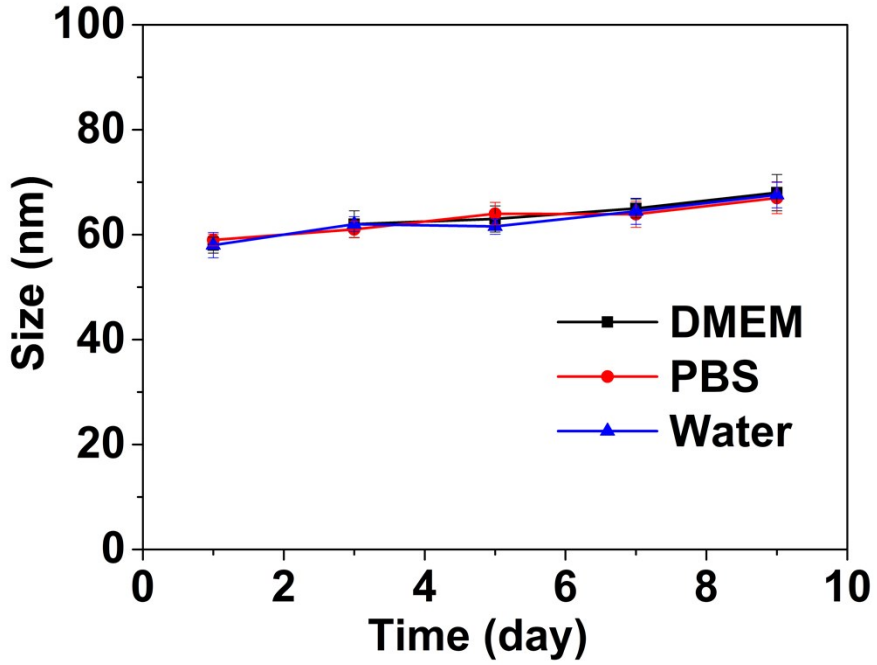

Figure S5 Hydrodynamic diameter of Bio-MnO_2 NPs as a function of incubation time.

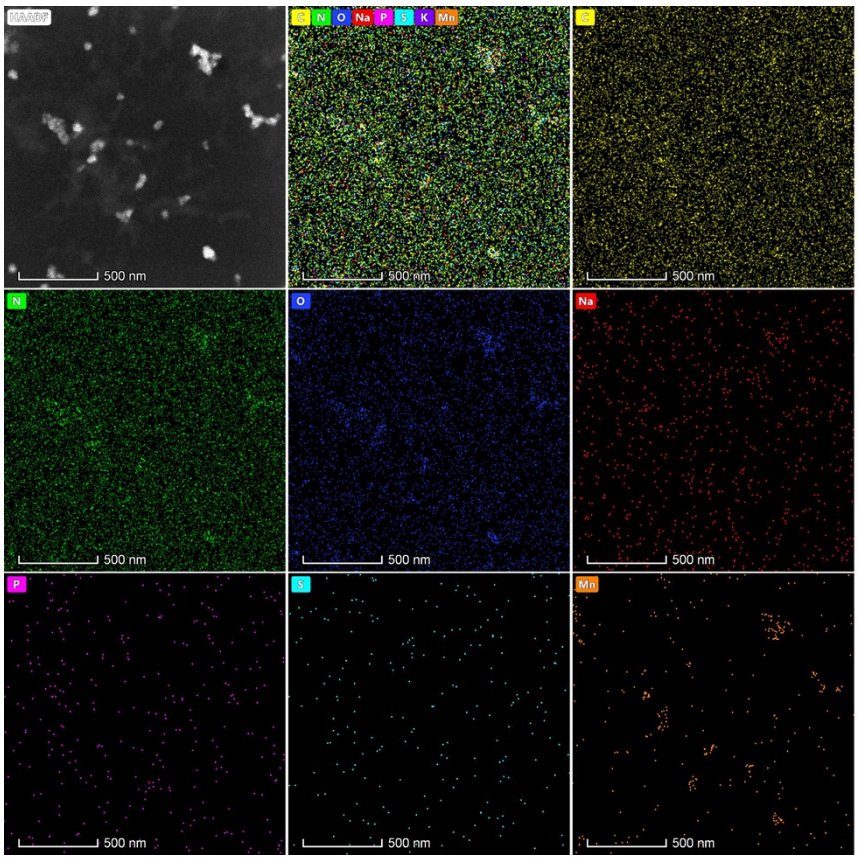

Figure S6 EDS maps of Bio-MnO_2 NPs (30 min).

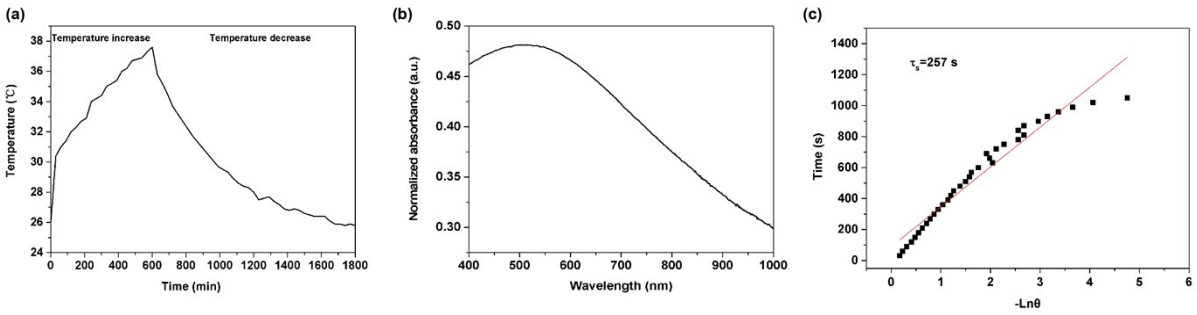

Figure S7 (a) Photothermal profile of Chem-MnO_2 (150 μg mL^{-1}) upon exposure to an 808-nm laser at 1.5 W cm^{-2} for 10 min, after which the laser was shut off. (b) UV-vis-NIR spectra of Chem-MnO_2. (c) Time constant for heat transfer from the system (τs =257 s) obtained by applying the linear time data from the cooling stage (1200 s)

versus the negative natural logarithm of the driving force temperature.

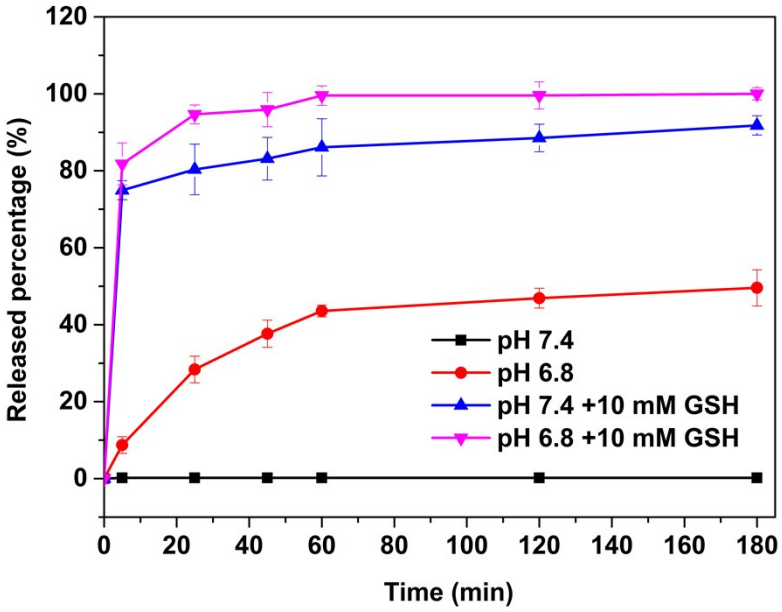

Figure S8 Percentage of Mn^{2+} released from Bio-MnO_2 NPs (150 μg mL^{-1}) as a function of incubation duration.

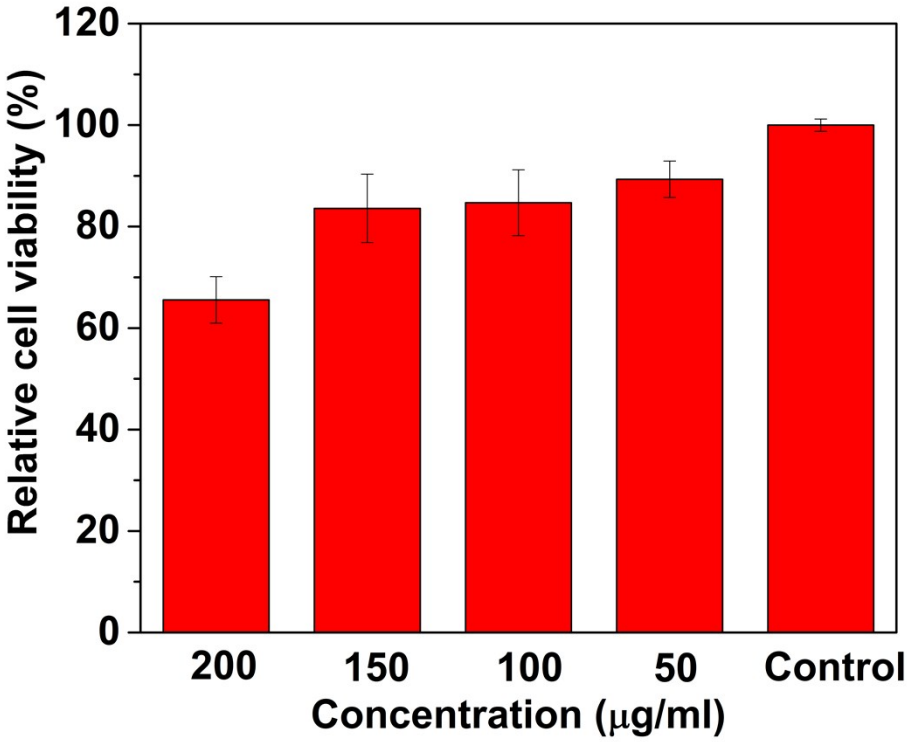

Figure S9 Relative viability of 4T1 cells after incubation with Chem-MnO_2 NPs for 24 h at different concentrations.

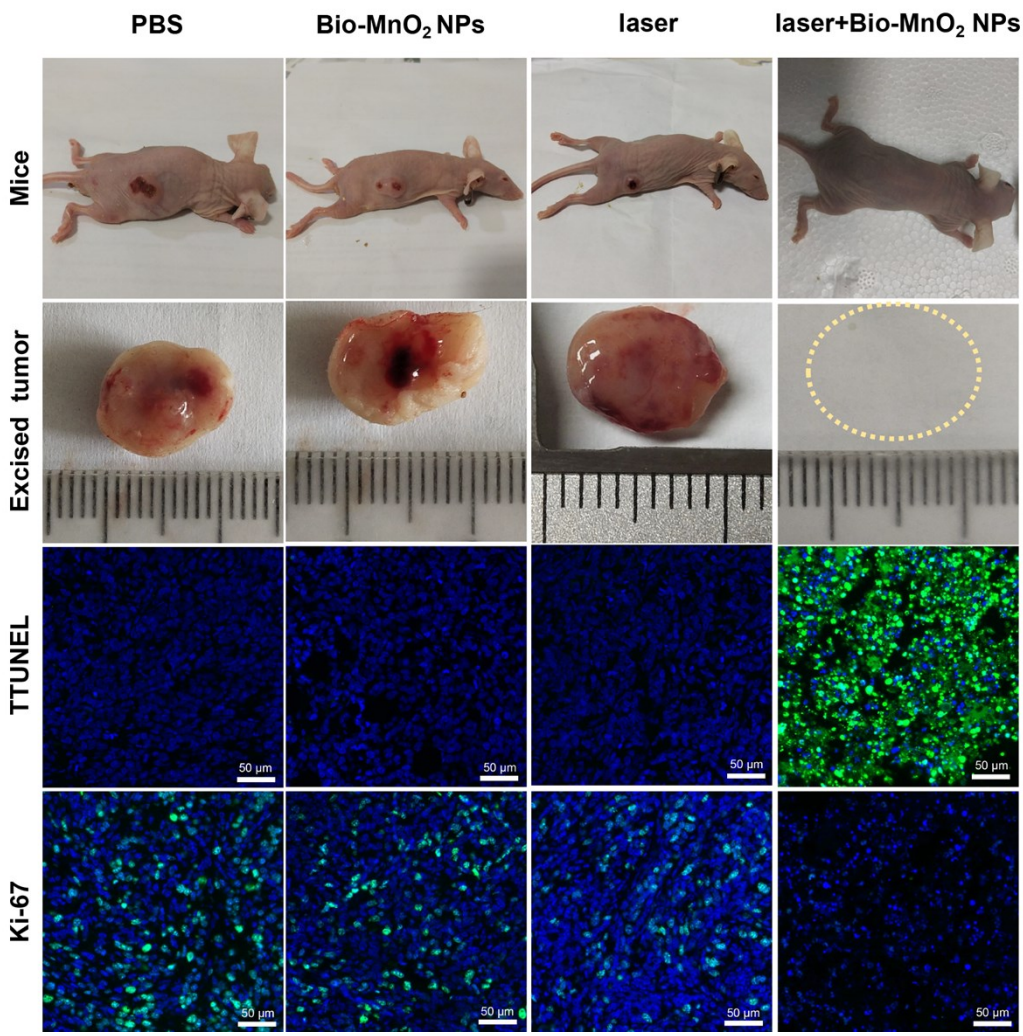

Figure S10 Photographs of representative mice after different treatments (16 days after the first treatment), excised 4T1 tumours from tumour-bearing mice and the results of TUNEL and antigen Ki-67 immunofluorescence staining of tumour tissues.