Electronic Supplementary Information (ESI) for:

Manganese-Phenolic Networks Coated Black Phosphorus Nanosheets for Theranostics Combining Magnetic Resonance/Photoacoustic Dual-Modal Imaging and Photothermal Therapy

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Experimental Section

Materials

Bulk black phosphorus (BP) was purchased from Nanjing Xianfeng Nanomaterials Technology Co., Ltd. Tannic acid and manganese chloride tetrahydrate ($MnCl_2$•$4H_2O$, 99.99%) were purchased from Sinopharm Chemical Reagent Co. Ltd. 3-(N-morpholino)-propanesulfonic acid (MOPS) was purchased from Nanjing Exploration Biotechnology Co., Ltd.

Apparatus

Transmission electron microscopy (TEM) images and energy-dispersive X-ray (EDX) element mapping were obtained on a Tecnai G2 F20 at an accelerating voltage of 200 kV. Atomic force microscopy (AFM) analyses were performed on a Bruker Multimode 8 atomic force microscope. X-ray photoelectron spectroscopy (XPS) spectra were collected by a Thermo escalab 250Xi XPS spectrometer. Fourier transform infrared (FTIR) spectra were obtained by a Thermo fisher Nicolet 6700 spectrometer. Hydrodynamic diameter and zeta potential were measured by a Zetasizer Nano ZS instrument (Malvern, England).

Synthesis of BPNSs.

First, the block BP crystals were sufficiently ground into BP powders using a mortar. Then 40 mg BP powders were dispersed in 30 mL ultrapure water. The mixture was placed in an ultrasonic cleaner for ultrasonic exfoliating for 6 h (In this process, the ultrasonic cleaner needs to change the water several times to prevent the temperature from exceeding 40 °C). After the sonication, the unexfoliated massive BP crystals were first removed by centrifugation (3000 rpm, 10 min). Then the BPNSs were obtained by further centrifugation (30000 rpm, 10 min). Finally, the resulting BPNSs were dispersed in 10 mL ultrapure water and stored at 4 °C for further use.

Synthesis of BPNSs@TA-Mn.

2 mL of the above BPNSs solution was dispersed in 5 mL of MOPS buffer solution (10 mM, pH=8) containing 2.4 mM TA. After stirring for 30 min, 5 mL of MOPS buffer solution (10 mM, pH=8) containing 2.4 mM $MnCl_2$•$4H_2O$ was added, and the mixture was stirred for another 30 min. The resulting product was collected by centrifugation (10000 rpm, 10 min). The product was washed with ultrapure water for three times (collected by centrifugation, 20000 rpm for 20 min) and finally dispersed in 5 mL ultrapure water. The resulting BPNSs@TA-Mn was stored at 4 °C for further use.

***In vitro* photothermal effect measurement.**

To investigate the *in vitro* photothermal effect, BPNSs@TA-Mn with different concentrations were added into a quartz cuvette (total volume of 1 mL) and irradiated by a 808 nm laser (KS3-11312-110, Beijing Kaplan Optoelectronics Technology Co., Ltd.) at 1 W cm^{-2} for 10 min. The temperature changes of the solutions were recorded by a digital thermometer with a thermocouple micrprobe (φ = 0.5 mm) submerged in the solutions. IR thermographs of the solutions were collected by a FLIR Ax5 infrared camera. To investigate the tissue penetration efficiency of 808 nm laser (1 W cm^{-2}), thermal imaging camera was used to record the temperature changes of BPNSs@TA-Mn solution with the cover of pork tissues with different thicknesses (2-20 mm). The obtained temperature changes were normalized to the maximum temperature increase which measured in the absence of tissue cover.

Measurement of photothermal conversion efficiency.

The photothermal conversion efficiency (η) was calculated according to the following equation[1].

$$\eta = \frac{hA\Delta T_{max} - Q_S}{I(1-10^{-A_{808}})} \quad (1)$$

Where ΔT_{max} is the temperature change at the maximum steady-state temperature. Q_S is the heat dissipation from the light absorbance of the solvent, which is measured independently to be 25.1 mW

using pure water. I is the laser power (1 W cm^{-2}), A_{808} is the absorbance of BPNSs@TA-Mn, BPNSs, or gold nanorods (GNRs) at 808 nm. hA is determined by the following equation:

$$\tau_s = \frac{m_D C_D}{hA} \quad (2)$$

Where τ_s is sample system time constant that obtained from the slope of the plot of cooling time vs -Ln($\Delta T/\Delta T_{max}$), m_D and C_D are the mass (1 g for H_2O) and heat capacity (4.2 J g^{-1} for H_2O) of the used solvent. GNRs were synthesized according to the previous method.[2]

***In vitro* MRI studies.**

To study the *in vitro* MRI phantom and relaxivity performance, BPNSs@TA-Mn and Gd-DTPA (as a control sample) aqueous solutions with different metal ion concentrations were prepared. T_1-weighted phantom images were collected on a 0.5 T NMI20-Analyst NMR system (Niumag Corporation, Shanghai, China) using a 2D multi-slice spin-echo (MSE) sequence with the following parameters: TR/TE = 100/2 ms, 512 × 512 matrices, slices = 1, thickness = 1 mm, NS = 4. The T_1 times were measured by an inversion recovery (IR) sequence. The r_1 values were calculated from the slope of the plot of $1/T_1$ against metal concentrations.

***In vivo* MRI.**

Animal experiments were executed in accordance with the Guide for the Care and Use of Laboratory Animals (Ministry of Science and Technology of China, 2006) and were approved by Institutional Animal Care and Use Committee of Fuzhou University. Tumor-bearing mice were prepared by subcutaneously injecting a suspension of 2×10^6 HeLa cells in 100 μL PBS into the back of the hind leg of BALB/c nude mice (weight ~29 g). *In vivo* MRI was performed on a Siemens Prisma 3.0-T MR scanner (Erlangen, Germany). T_1-weighted multislice spinecho images were collected before and after intratumor injection of BPNSs@TA-Mn (100 μL, 70 μg mL^{-1}) using the following parameters: TR/TE =

800/12 ms, 256 × 256 matrices, FOV = 50 × 50, thickness = 1 mm.

***In vitro* photothermal therapy.**

First, different concentrations of BPNSs@TA-Mn solutions were incubated with HeLa cells at 37 °C for 4 h. After that, the cell viability was evaluated by standard MTT assay. To collect fluorescence images, the cells were co-stained with Calcein AM (stains live cells) and propidium iodide (stains dead cells), and then were imaged by a Nikon Eclipse Ti-S inverted microscope.

***In vivo* photothermal therapy.**

HeLa tumor-bearing BALB/c nude mice were divided into four groups (five mice per group): In BPNSs@TA-Mn + laser group, the mice were intratumorally injected with BPNSs@TA-Mn (100 μL, 70 μg mL^{-1}) and then were irradiated with 808 nm laser (1 W cm^{-2}) for 10 min; In BPNSs@TA-Mn group, the mice were injected with BPNSs@TA-Mn (100 μL, 70 μg mL^{-1}) only; In PBS + laser group, the mice were intratumorally injected with 100 μL PBS and then were irradiated with 808 nm laser (1 W cm^{-2}) for 10 min; In blank group, the mice received no treatment. The tumor volume was calculated according to the equation: tumor volume = (length × $width^2$)/2. Relative tumor volume was calculated as V/V_0, where V is the tumor volume after the treatments, while V_0 is the initial tumor volume before the treatments.

Histology.

Sixteen days after the treatments, the mice in four groups were sacrificed. The tumor, heart, liver, spleen, lung, and kidney were excised to prepare histological sections and then stained with hematoxylin and eosin (H&E) following the standard protocol for analysis.

[1] Y. Liu, K. Ai, J. Liu, M. Deng, Y. He, L. Lu, *Adv. Mater.* 2013, 25, 1353.

[2] T. Guo, Y. Lin, Z. Li, S. Chen, G. Huang, H. Lin, J. Wang, G. Liu, H. H. Yang, *Nanoscale*, 2017, 9, 56.

Supporting Figures

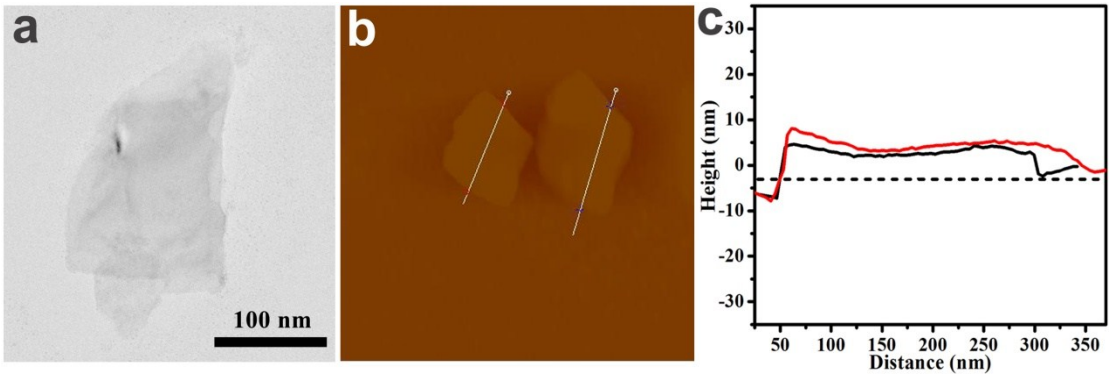

Figure S1. (a) TEM image, (b) AFM image and (c) the corresponding height profiles of BPNSs.

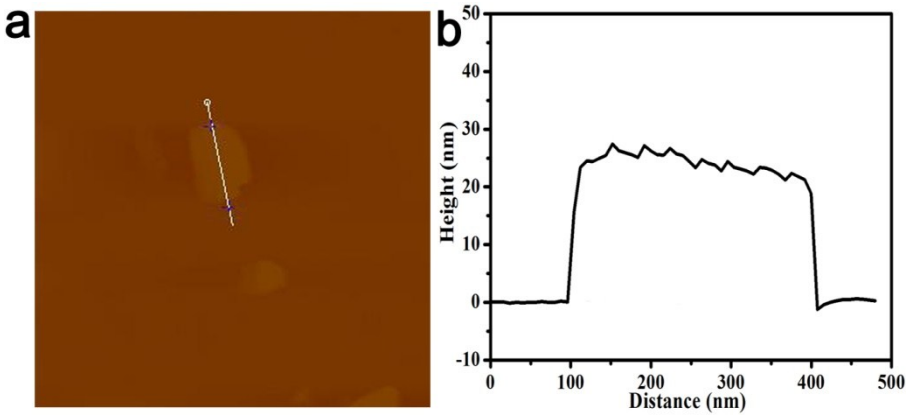

Figure S2. (a) AFM image and (b) the corresponding height profile of BPNSs@TA-Mn.

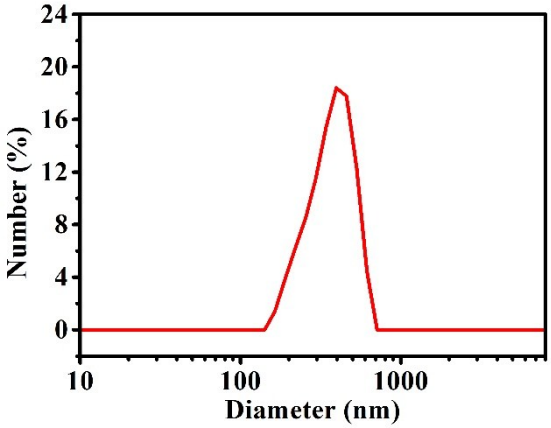

Figure S3. Dynamic light scattering (DLS) measured hydrodynamic diameter distribution of BPNSs@TA-Mn in water.

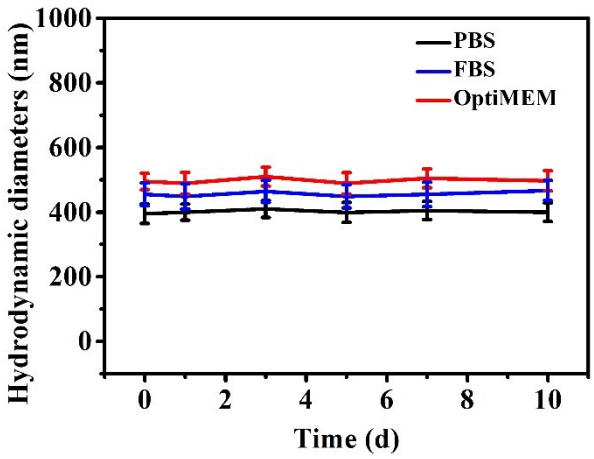

Figure S4. Hydrodynamic diameter changes of BPNSs@TA-Mn in different media.

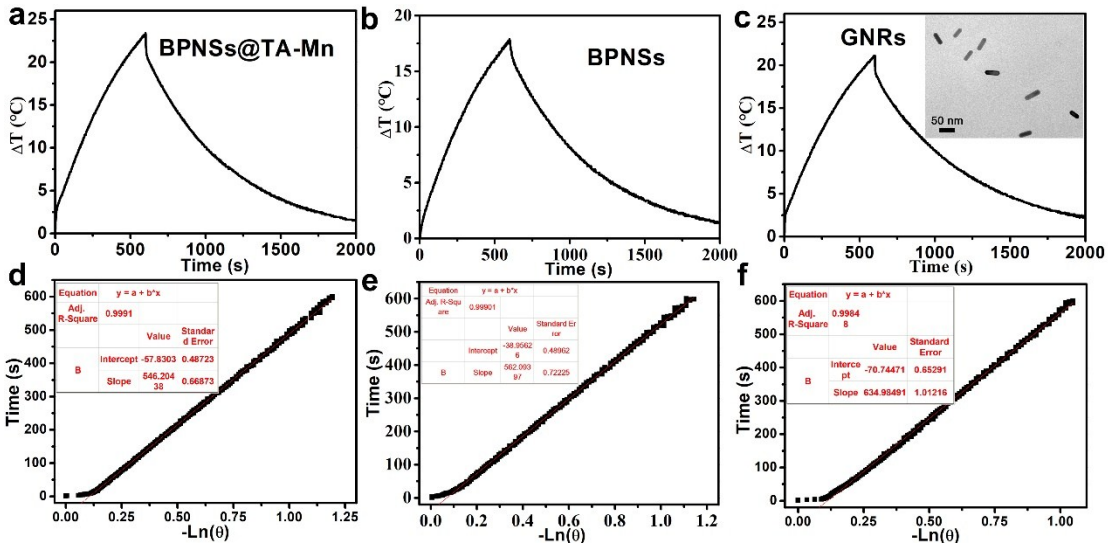

Figure S5. The temperature change curves of (a) BPNSs@TA-Mn, (b) BPNSs, and (c) GNRs (inset: TEM image of GNRs) aqueous solutions under 808 nm laser irradiation for 10 min, respectively. The linear fitting of laser irradiation times versus -Ln(ΔT/ΔTmax) obtained from the cooling period for (d) BPNSs@TA-Mn, (e) BPNSs, and (f) GNRs, respectively.

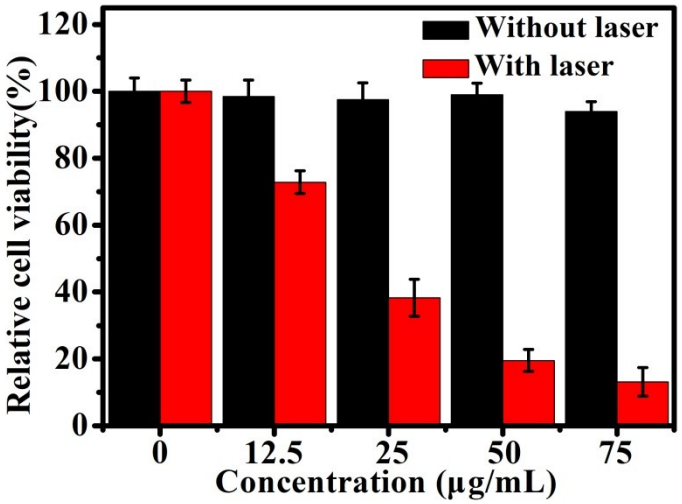

Figure S6. Cell viability of HeLa treated with different concentrations of BPNSs@TA-Mn with or without laser irradiation.

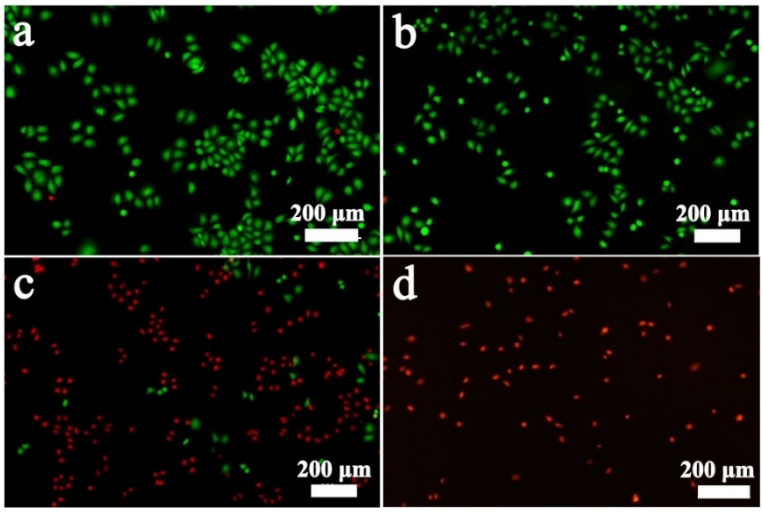

Figure S7. Confocal fluorescence images of PI/Calcein-AM co-stained HeLa cells with different treatments: (a) 10 min of laser irradiation only; (b) incubated with BPNSs@TA-Mn only; incubated with BPNSs@TA-Mn and then treated with (c) 5 min, and (d) 10 min of laser irradiation (1 W cm^{-2}), respectively.

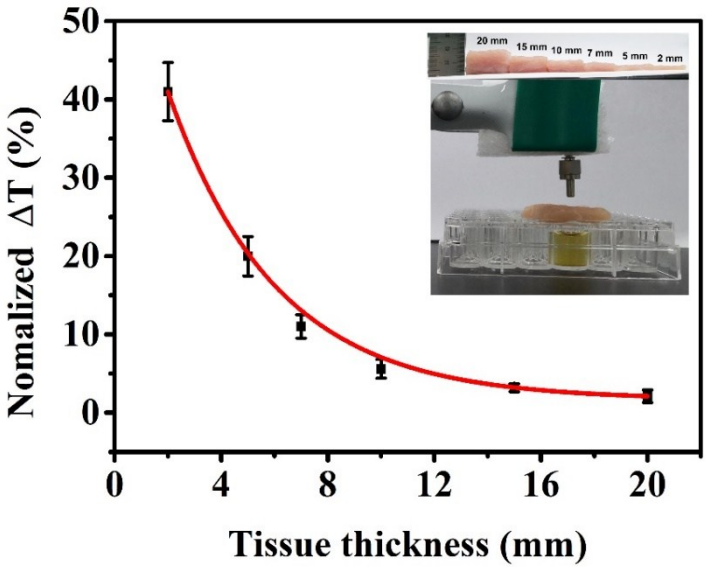

Figure S8. The normalized temperature changes of BPNSs@TA-Mn aqueous solution under 808 nm laser irradiation with the cover of different thicknesses pork tissues. Inset: photographs of the pork tissues with different thicknesses and the experimental setup.

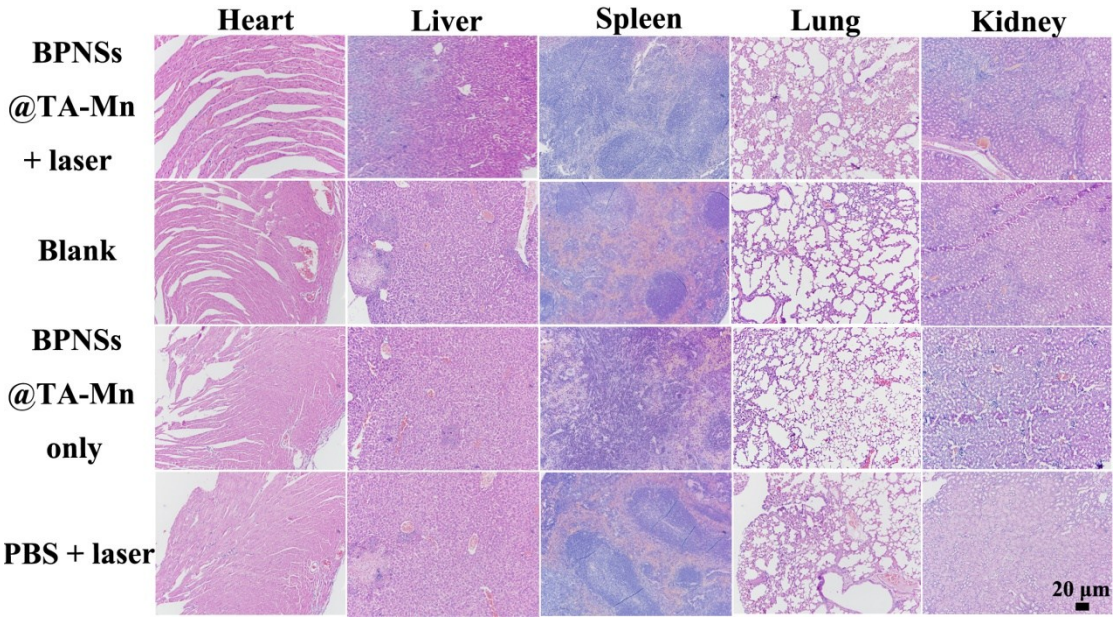

Figure S9. H&E stained images of major organs of mice collected at 16 days after different treatments.

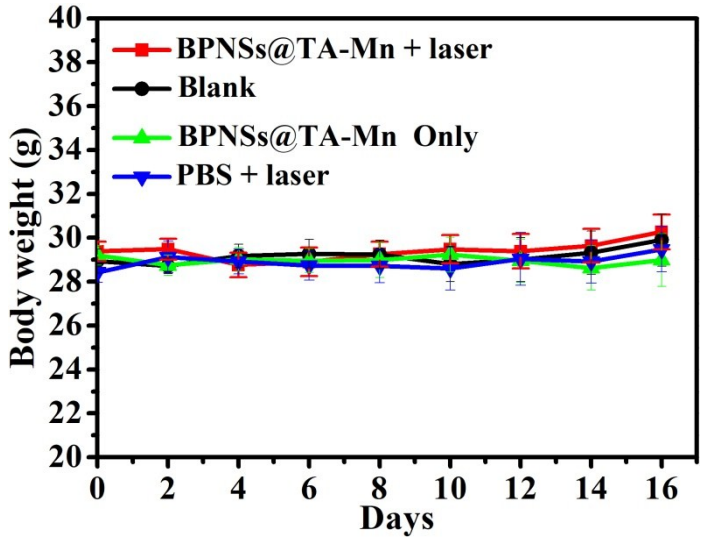

Figure S10. Body weight changes of the HeLa tumor-bearing nude mice after different treatments.