

## Supporting Information

### **NIR-II light responsive hydrogel based on 2D engineered tungsten nitride nanosheets for multimode chemo/photothermal therapy**

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## **MATERIALS AND METHODS**

### **Materials.**

Ammonium tungstate hydrate was purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Doxorubicin hydrochloride (DOX) and Agarose were obtained from Aladdin Reagents (Shanghai, China). *N*-methyl-2-pyrrolidone (NMP) was purchased from Heowns Biochem. (Tianjin, China). Calcein-AM, propidium iodide (PI) and CCK-8 cell proliferation assay kits were provided by Beyotime Inst. Biotech. (Haimen, China). 4T1 cells, HeLa cells and L929 cells were obtained from Xiangya Hospital of Central South University (Changsha, China). Fetal bovine serum (FBS) and DMEM cell culture medium were obtained from Hyclone (Beijing, China). BALB/c mice were provided by Hunan Silaike Experimental Animal Co. Ltd. (Changsha, China). Other reagents were of analytical purity and used without further purification.

### **Preparation of WN nanosheets.**

The WN nanosheets (WNNS) were prepared using a simple liquid exfoliation. Firstly, the bulk WN was prepared according to previously reported methods<sup>[1]</sup>. Briefly, ammonium tungstate hydrate (300 mg) was dissolved in HCl solution (10 mL, 1 M) to form tungstic acid solution ( $H_2WO_4$ ) under stirring. The  $H_2WO_4$  solution was put in an oven at 80 °C for 24 h and recovered the precipitation. The precipitation was heated at 600 °C for 2 h under an  $NH_3$  flow. After cool down, the prepared WN powder was collected. Then, WN powder was immersed in 45 mL of saturated sodium hydroxide NMP solution and the mixture solution was sonicated in ice bath for 10 h. The resulting

suspension was centrifuged at 4000 rpm for 5 min to remove the residual unexfoliated particles and the supernatant was collected for further use. The WN nanosheets were collected by centrifugation at 13000 rpm for 5 min and washed for three times with water to remove the residual NMP.

### **Characterization of WN nanosheets.**

The morphology of WN nanosheets was observed by TEM (JEOL, JEM-2100F, Japan). The zeta potential and size distribution were determined by Malvern Zetasizer Nano-ZS (ZEN-3600, Malvern Instruments, UK). Raman scattering was performed on a Raman microspectrometer (Jobin Yvon LabRam-010, France). XPS spectrum was performed on an X-ray photoelectron spectroscopy (Thermo Fisher, ESCALAB 250, USA). The UV-vis absorbance spectrum was recorded on UV-vis absorbance spectrometer (Shimadzu, UV-2450, Japan).

### **One-pot fabrication of WNNS@hydrogel.**

WNNS aqueous solution (0.1 mg mL<sup>-1</sup>) was added into agarose and the mixture solution was stirred at 60 °C, then different concentrations of DOX (0.2, 0.5, 0.8 mg mL<sup>-1</sup>) was loaded into the above mixture solution under stirring for 5 min, followed by rapid cooling down to room temperature to form WNNS@hydrogel. The final agarose content is 1%.

### **Photothermal effect and photostability of WNNS@hydrogel.**

To explore the photothermal effect, WN nanosheets aqueous solutions (100, 200, 250, 500  $\mu\text{g mL}^{-1}$ , 1 mL) and WNNS@hydrogel (ca. 0.5 mL) were exposed to a fiber-coupled continuous semiconductor diode laser (1064 nm, Viasho Technology, Beijing, China) at the power of  $2.0 \text{ W cm}^{-2}$ . A thermal infrared imaging camera (Flir C2, USA) was used to monitor the temperature change.

To investigate the photothermal stability, WNNS (50  $\mu\text{g mL}^{-1}$ ) and WNNS@hydrogel were irradiated under 1064 nm laser irradiation at the power of  $1.0 \text{ W cm}^{-2}$  for 5 min, then naturally cooled to room temperature. It was performed for 5 cycles and the temperature was recorded using an infrared thermal camera.

#### **Calculation of photothermal conversion efficiency.**

WNNS@hydrogel (ca. 1 g) was irradiated under 1064 nm NIR light ( $1.0 \text{ W cm}^{-1}$ ) for 5 min, and then transferred to the dark. The temperature was recorded with an infrared thermal camera. The photothermal conversion efficiency ( $\eta$ ) of laser at 1064 nm can be determined using the following equations [2].

$$\eta = \frac{hS(T_{\max} - T_{\text{surr}})}{I(1 - 10^{-A_{1064}})} \quad (1)$$

$$\theta = \frac{T - T_{\text{surr}}}{T_{\text{Max}} - T_{\text{surr}}} \quad (2)$$

$$\tau = \frac{t}{-\ln\theta} \quad (3)$$

$$hS = \frac{mC_p}{\tau} \quad (4)$$

where,  $h$  is the heat transfer coefficient,  $S$  is the surface area of the sample vial,  $T_{\text{surr}}$  is the room temperature, and  $T_{\text{max}}$  is the maximum temperature of the system during light irradiation.  $I$  represents NIR light power ( $1.0 \text{ W cm}^{-2}$ ).  $A_{1064}$  is the absorbance of WNNS@hydrogel ( $A_{1064} = 0.353$ ).  $\tau$  is the sample system time constant, and the value of  $\tau$  is 252.8 s according to Fig. S2.  $m$  is the mass of samples.  $C_p$  is specific heat capacity of water ( $C_p = 4.2 \text{ J mL}^{-1}$ ). Finally, the light photothermal conversion efficiency of WNNS@hydrogel was calculated to be 29.5%.

### **NIR-triggered DOX release from WNNS@hydrogel**

The fetal bovine serum in phosphate-buffered saline (PBS, pH 7.4, 0.5 mL) was added with 0.5 mL of DOX-loaded WNNS@hydrogel ( $200 \mu\text{g mL}^{-1}$  WNNS,  $500 \mu\text{g mL}^{-1}$  DOX, 1% LA). For the NIR triggering group, the samples were irradiated under 1064 nm light within an interval of 10 min. At the desired time point, 0.3 mL of solution was taken out and centrifuged at 13000 rpm. The absorption of DOX in the supernatant was measured using UV-vis absorbance spectrometer, then the suspensions was recovered and added to the sample for next measurement. The DOX concentration was quantified by the standard curve.

### **Cell culture.**

4T1 cells, L929 cells and HeLa cells were cultured in DMEM medium with 10% fetal bovine serum (FBS), 1% streptomycin/penicillin. Cells were kept at 37 °C with 5% CO<sub>2</sub>.

### **In vitro cytotoxicity assay.**

The cytotoxicity of WNNS to cells was determined by CCK-8 cell viability assay kit. 4T1, L929 and HeLa Cells were seeded into the 96-well plates at the density of  $5 \times 10^4$  cells per milliliter and cultured overnight, the medium was replaced with fresh medium containing different concentrations of WNNS (10, 20, 50, 100 and  $200 \mu\text{g mL}^{-1}$ ). After 12 h of incubation, the medium was replaced with CCK-8 reagent and incubated for 1 h. The absorbance at 450 nm ( $\text{OD}_{450\text{nm}}$ ) was measured on a microplate reader (Bio-Tek, ELx800, USA).

#### **In vitro WNNS@hydrogel photothermal therapy.**

4T1 cells were seeded into a 96-well plate at density of  $5 \times 10^4$  cells per well. After 12 h of incubation, the culture medium was replaced by fresh medium. Then 0.1 g of WNNS@hydrogel ( $200 \mu\text{g mL}^{-1}$  WNNS,  $200 \mu\text{g mL}^{-1}$  DOX, 1% LA ) were placed onto the surface of wells and incubation for another 2 h. The cells were irradiated under 1064 nm laser at  $1.0 \text{ W cm}^{-2}$  for 5 min. After 1 h of incubation, the cells were washed twice with PBS, the viability was determined by a CCK-8 cell cytotoxicity assay. Meanwhile, to further verify the therapy effect of WNNS@hydrogel, cells were seeded into a 24-well plate and incubated for 12 h, the culture medium was replaced by fresh medium, and WNNS@hydrogel were put into the wells. The cells were exposed to a 1064 nm laser at  $1.0 \text{ W cm}^{-2}$  for different time. The cells were further incubated for 1 h, the culture medium was removed and washed with PBS. The medium containing propidium iodide (PI) and calcein-AM was added to differentiate the live and dead cells. Finally, the cells were washed twice with PBS and fluorescence images were taken using an inverted fluorescence microscope.

### **In vivo antitumour efficiency.**

The 4T1 cells were subcutaneously injected into the Male BALB/c mice. When the tumour volume reached above 100 mm<sup>3</sup>, the mice were divided into four groups: (1) PBS, (2) DOX, (3) WNNS@hydrogel, (4) WNNS@Hydrogel + NIR light. The mice were received an intratumoural injection of 150 μL DOX or WNNS@Hydrogel. After 1 h, the mice were irradiated with the 1064 nm laser (1.0 W cm<sup>-2</sup>) for 5 min, and the local temperature of tumour were recorded by infrared thermal camera. The tumour volumes were measured every other day. The body weight was recorded on the 4<sup>th</sup>, 8<sup>th</sup>, and 16<sup>th</sup> day, respectively. Sixteen days later, the mice were sacrificed, the tumours and main organs (heart, liver, spleen, lung and kidney) were examined by H&E staining. Male BALB/c mice (5 weeks old) were obtained from Silaike Experimental Animal Co., Ltd. (Changsha, China). All the animal experiments were performed according to the regulation approved by the Xiangya Laboratory Animal Center of Central South University, Changsha, China.

## Supplemental figures

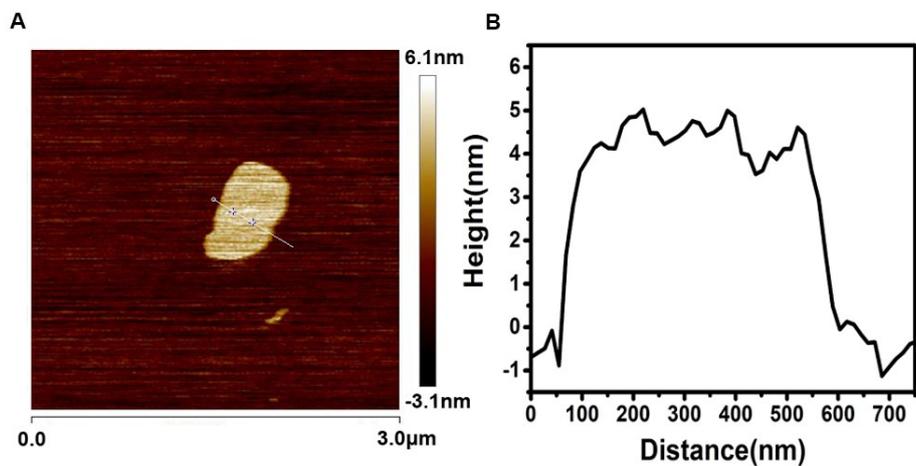


Fig. S1. A) AFM image of WNNS and B) the height profile along the white line.

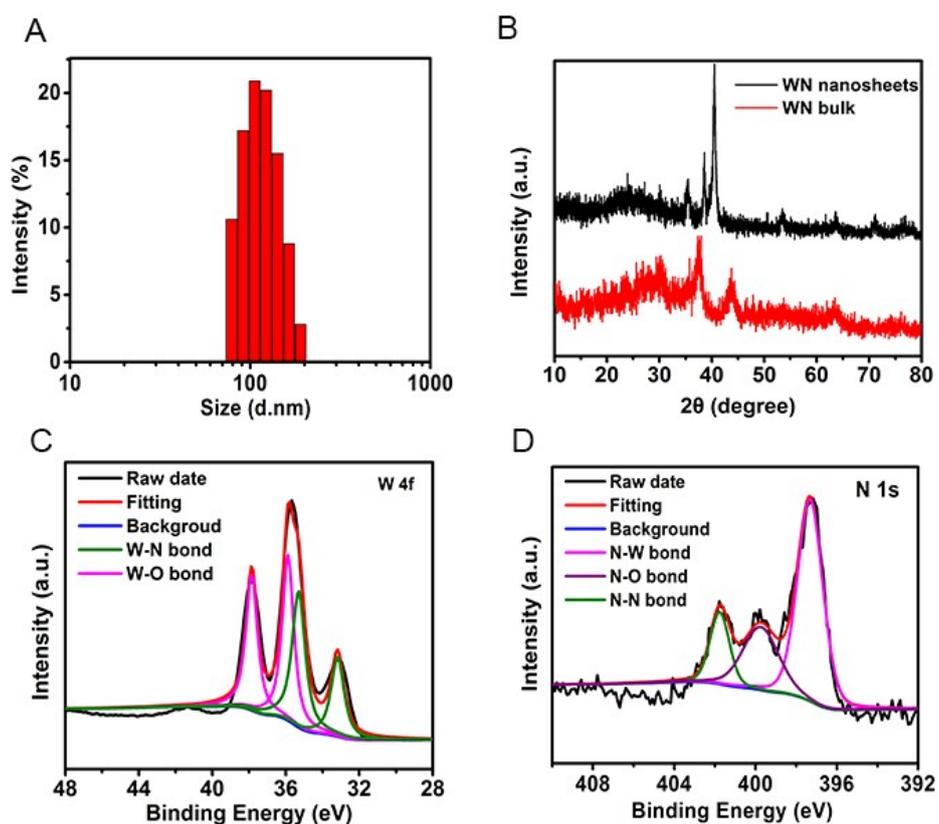


Fig. S2. (A) Size distribution of WNNS in water was measured from dynamic light scattering. (B) XRD of WNNS and bulk WN. (C, D) XPS spectra of W and N of WNNS.

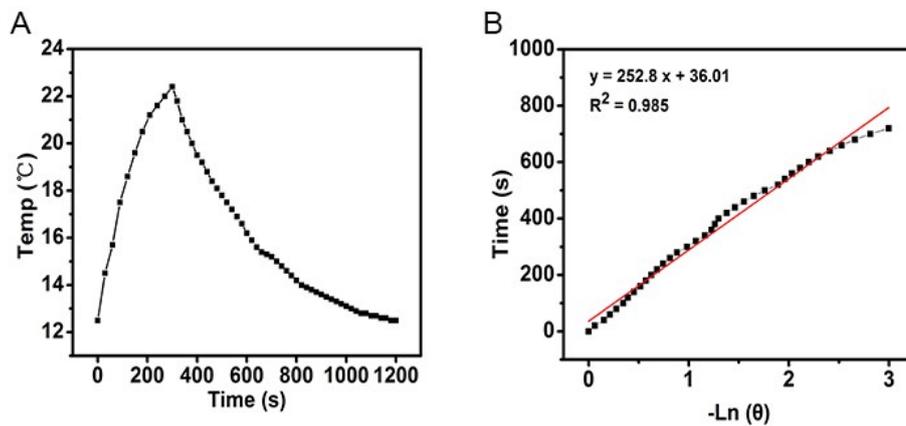


Fig. S3. (A) Temperature variations of WNNS@hydrogel under irradiation with 1064 nm laser at the intensity of  $1.0 \text{ W cm}^{-2}$  for 5 min, and then natural cool. (B) The linear regression of the cooling process referred to calculate the time constant for heat transfer system.

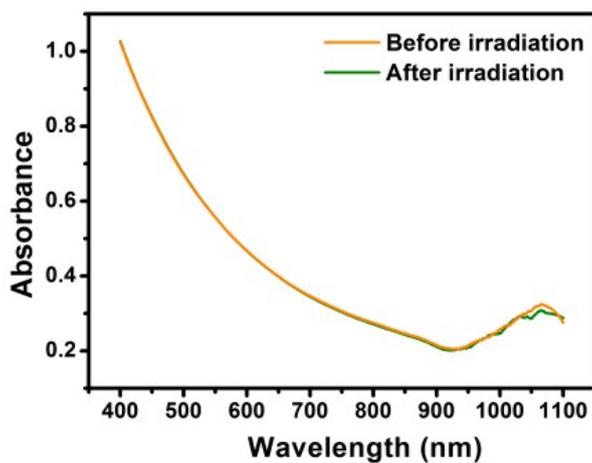


Fig. S4. UV-vis absorption spectra of WNNS before and after 1064 nm laser irradiation ( $1.0 \text{ W cm}^{-2}$ ).

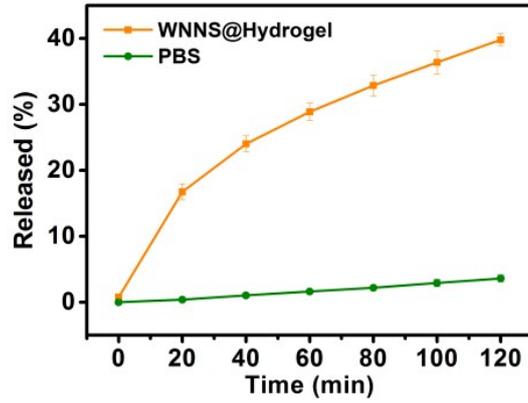


Fig. S5. DOX release from WNNS@hydrogel in the presence of PBS under NIR-II irradiation.

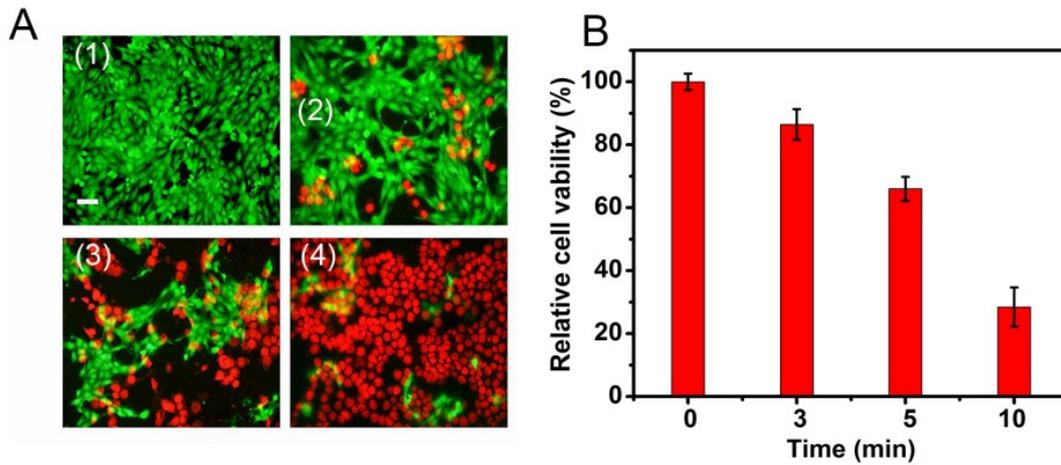


Fig. S6. (A) Calcein-AM/PI staining assay to study the photothermal-killing efficiency of 4T1 cells under exposure to laser for 0, 3, 5 and 10 min. (B) Relative cell viabilities of 4T1 cells under different laser irradiation time.

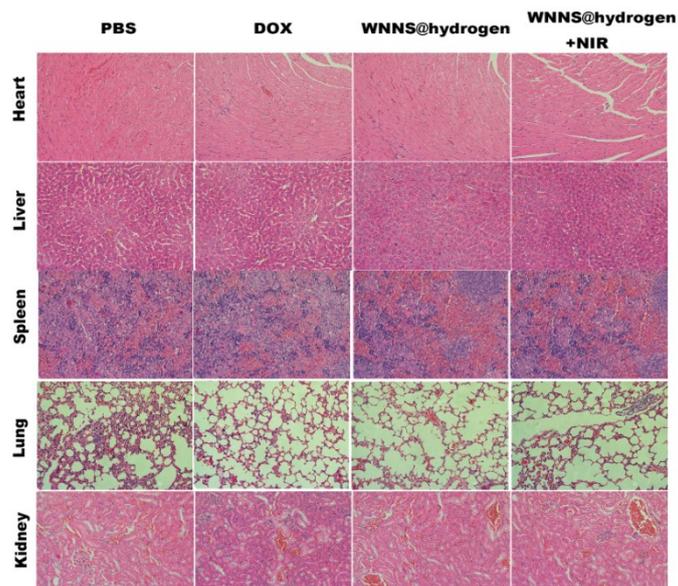


Fig. S7. H&E stained images of major organs collected from the treated mice.

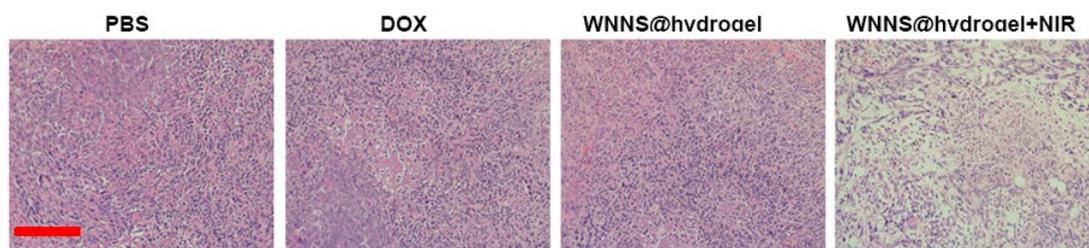


Fig. S8. H&E stained images of representative tumour obtained from different groups of the treated mice. (Scale bar  $\mu = 50$  nm)

## Reference

- [1] Y. Wang, T. Nie, Y. Li, X. Wang, L. Zheng, A. Chen, X. Gong and H. Yang. *Angew. Chem. Int. Ed.*, 2017, 56, 7430-7434.
- [2] C. Sun, L. Wen, J. Zeng, Y. Wang, Q. Sun, L. Deng, C. Zhao and Z. Li. *Biomaterials*, 2016, 91, 81-89.