Electronic Supplementary Material (ESI) for Nanoscale.
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Supplementary Information

GRPr Mediated Photothermal and Thermodynamic Dual-therapy for Prostate Cancer with Synergistic Anti-apoptosis Mechanism

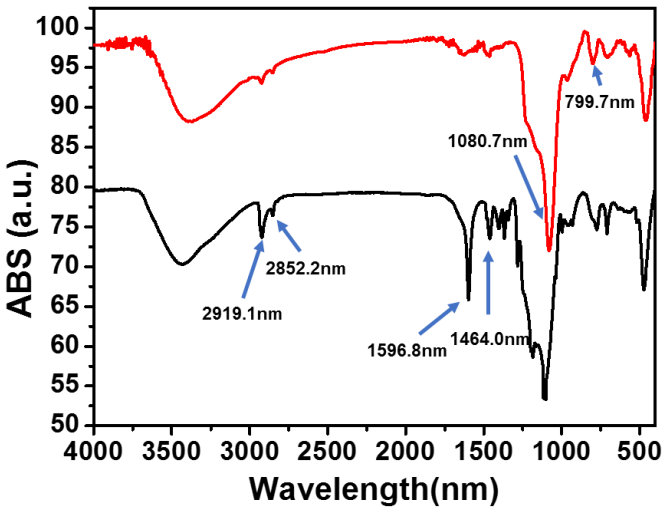

Figure. S1 FTIR spectrum of MS-GNR(red) and MS-GNR-A (black)

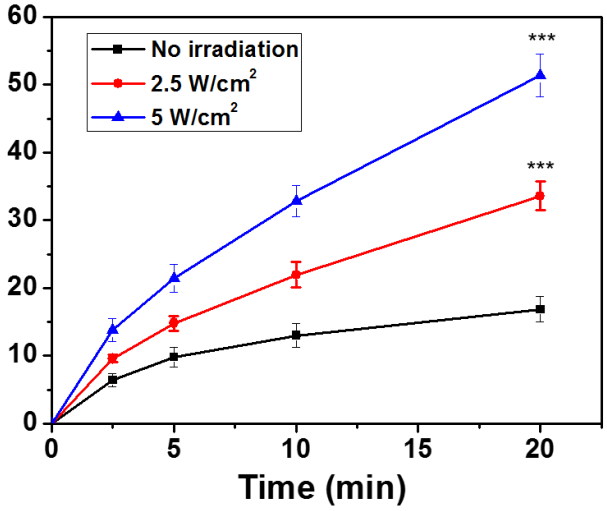

Figure. S2 Profile of AIPH release from MS-GNR under different irradiation conditions.

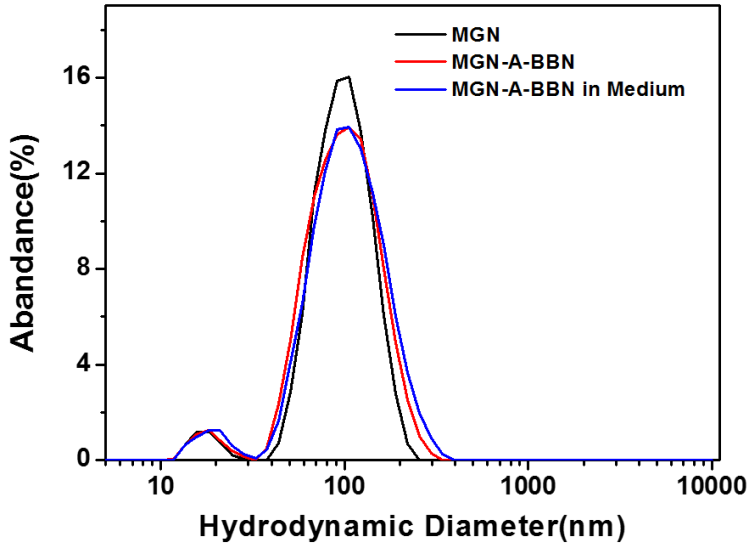

Figure. S3 Hydrodynamic diameter of MGN, MGN-A-BBN and MGN-A-BBN in serum-containing medium after 2 weeks.

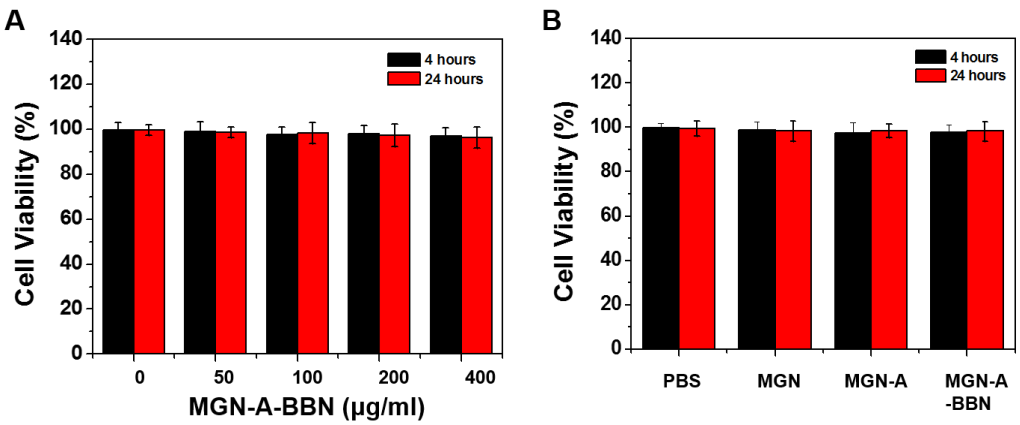

Figure S4. A Viability of PC-3 cell after treatments with MGN-A-BBN at different concentrations. **B** Viability of PC-3 cells after treatments with different nanoparticles.

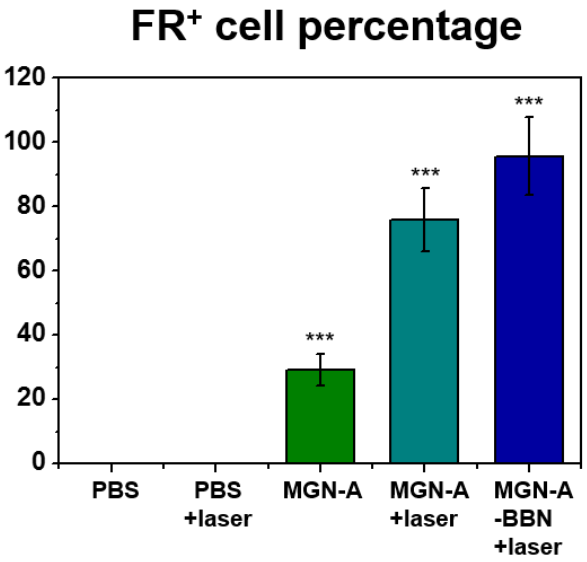

Figure S5. Percentages of cells with free radical signals.

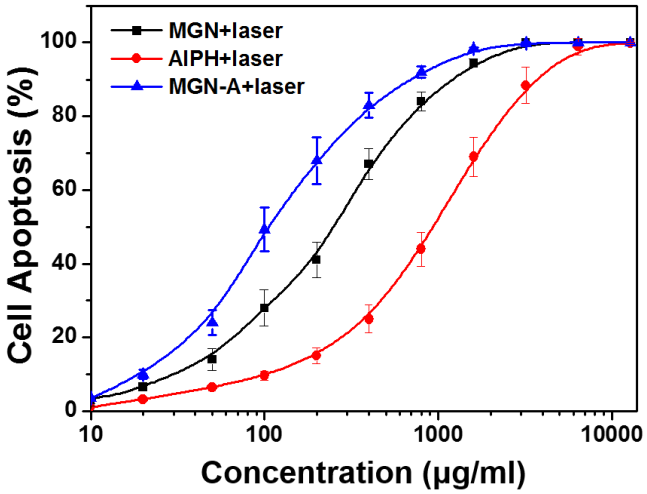

Figure S6. IC50 assays for MGN+laser, AIPH+laser and MGN-A+laser.

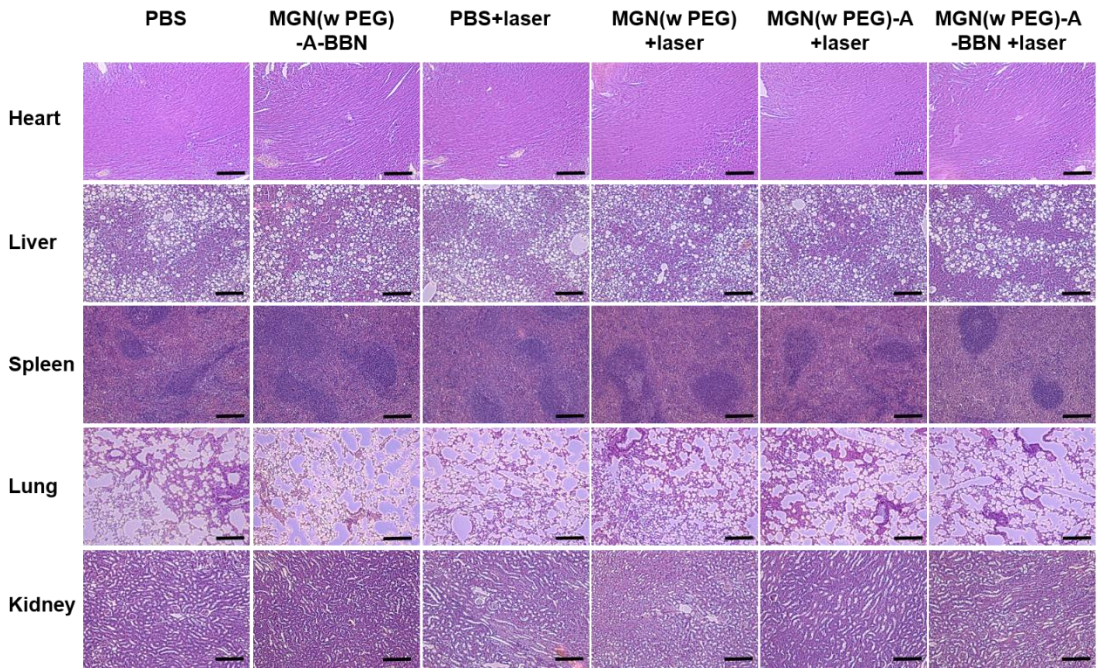

Figure. S7 H&E stained images acquired from the major organs after intravenous injection with control (PBS) and different nanomedicines for 30 days feeding (Scale bar = 100 μm).

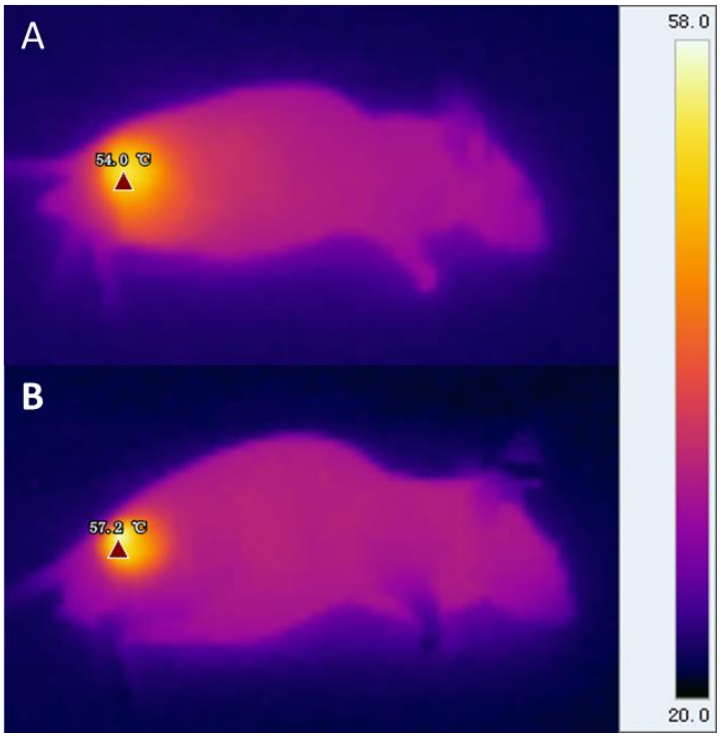

Figure. S8 The thermal images of MGN-A **(A)** and MGN-A-BBN **(B)** at 5 min 1 W/cm^2 808 nm laser irradiation.

Quantitative RT-PCR procedure

The cell samples were homogenized and total RNA was extracted using RNAiso Plus (Takara, Shiga, Japan). First strand cDNA was synthesized by a reverse-transcription reaction with 500 ng total RNA using the PrimeScript RT reagent Kit Perfect Real Time (Takara) driven by the primer pairs in a 20 μl reaction system according to the manufacturer's instructions. Quantitative RT-PCR was performed in 96-well plates using SYBR Premix Ex Taq (Takara) equipped with a Mastercycler ep realplex (Eppendorf, Hamburger, Germany). The forward primer sequence of GRPr mRNA is GCTCTCGGCAGACAGATACAA, and the reverse primer sequence of GRPr mRNA is GGTCTGGTTGGTGCTTTCCT. The forward and reverse primer sequence of GAPDH are GGAGCGAGATCCCTCCAAAAT and GGCTGTTGTCATACTTCTCATGG. The RNA expression Ct values were automatically calculated on the commercial software with the Mastercycler ep realplex normalized to GAPDH. Data were expressed relative to a calibrator using the 2-(DDCt) ± s formula.

Light to heat conversion efficiency calculation

The photothermal conversion efficiency was determined according to the previous literatures.[1, 2] Briefly, the value of the photothermal efficiency η was calculated by the equation **(1)**:

$$\eta = \frac{hS\,(T_{max}-T_{surr})-Q_{Dis}}{I(1-10^{A808})} \quad \textbf{(1)}$$

Where *h* is the heat transfer coefficient, and *S* is the surface area of the container. The maximum temperature T_{max} of the sample solution was determined to be 64.0 °C and the Temperature of the surrounding environment T_{surr} was determined to be 25.0 °C. Q_{Dis} represents the heat dissipated from the light absorbed by the solvent and the container. It was determined to be 41.0 mW using a crystal cuvette and 0.5ml Milli-Q water. *I* indicates the laser power, which is 1W in this experiment. A_{808} represents the absorbance of the sample solution in this test, which is determined to be 1.468 on a microplate reader.

Equation **(2)** was employed to determine the value of *hS*.

$$hS = \frac{\Sigma miCi}{\tau_s} \quad \textbf{(2)}$$

Where $\Sigma miCi$ is the product sum of sample's mass and the specific heat coefficients. In our system, the mass is equal to 0.5g, and the specific heat coefficient is approximately equal to 4.2 J/g. °C. The time constant τ_s can be derived by the fitting curve of the equation **(3)**,

$$t = -\tau_s \ln(\theta) \quad \textbf{(3)}$$

$$\text{Where } \theta = \frac{T-Tsurr}{Tmax-Tsurr} \quad \textbf{(4)}$$

The τ_s was determined to be 230.79s by the cooling loop of the sample with the concentration of 200μg/ml.

Take all together, the photothermal conversion efficient was calculated to be 32.5%.

Combination index calculation:

Combination index (CI) is calculated by Equation (**5**):

$$CI = \frac{C_{A,x}}{IC_{x,A}} + \frac{C_{B,x}}{IC_{x,B}} \quad \textbf{(5)}^{3}$$

In this equation, the concentrations required to produce the given effect (IC50 in our case) are determined for drug A ($IC_{x,A}$) and drug B ($IC_{x,B}$). The concentrations of A and B contained in combination that provide the same effect are denoted as ($C_{A,x}$, $C_{B,x}$). A denotes MGN+laser, and B denotes AIPH +laser. $IC_{x,A}$ was measured to be 249.88 μg/ml, $IC_{x,B}$ was measured to be 921.52 μg/ml. $C_{A,x}$ was measured to be 100.55 μg/ml, and $C_{B,x}$ was measured to be 4.71 μg/ml. Therefore, *CI* was equal to 0.408.

IC50 determination for the drug A, B and the combination.

The PC-3 cells seeded on the 6-well glass slide were incubated with different concentration of MGN or AIPH. Then they were washed with PBS and treated with 5min 1 W/cm^2 808 laser for 5 minutes, following by a standard CCK-8 assay for an evaluation of cell viability. The cell apoptosis percentages were drawn with agents' concentrations and the concentration of drug that conducts 50% apoptosis of cells was picked as the IC50 value.

The IC50 determination procedure was the same for the combination. The AIPH percentage in the combination was measured according to the absorbance method described in the main text.

Reference

1. C. Dai, Y. Chen, X. Jing, L. Xiang, D. Yang, H. Lin, Z. Liu, X. Han and R. Wu, *ACS Nano*, 2017, **11**, 12696-12712.
2. C. Bi, J. Chen, Y. Chen, Y. Song, A. Li, S. Li, Z. Mao, C. Gao, D. Wang, H. Möhwald and H. Xia, *Chemistry of Materials*, 2018, **30**, 2709-2718.
3. Z. Liang, A. Jessie, W. Guillaume, *Frontiers in Bioscience,* 2010, **2**, 241-249.