Supporting Information

Well water-dispersible PCN nanosheets as light-controlled lysosome self-promoting escape type non-cationic gene carriers for tumor therapy

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Content

1. Experimental section

Materials and Instrumentation

Anhydrous solvents were purified under nitrogen *via* using standard methods. Melamine and DCFH were purchased from Energy Chemical (Shanghai, China). Lyso-Tracker Red, tris-HCl, Gold view II, loading buffer, MTT were purchased from Solarbio Science & Technology Co., Ltd (Beijing, China). Lipofectamine 2000 was obtained from Invitrogen (Life technologies, Mauricio Minotta, USA). EGFP-DNA was purchased from Clontech (Palo Alto, CA, USA). Zebrafish was obtained from Shanghai GeneBio Co., Ltd (Shanghai, China). FAM-DNA and pUC-18 DNA were purchased from Ruibiotech Co., Ltd. (Beijing, China). AFM was measured by Atomic Force Microscopy (Bruker, Germany). Particle size analysis was taken on a Brookhaven Zeta Plus Partical Size and Zeta Potential Analyzer (Midland, Canada). Photoluminescence spectra were tested via Hitachi F-4600 (Tokyo, Japan). UV-Vis absorption spectra were measured via Hitachi U-3900 Spectrophotometer (Tokyo, Japan). All the solutions were measured in 3.5 mL (10 × 10 mm), 100 and 50 μL quartz cuvettes.

DLS and zeta assay

The particle size and zeta potential of the DNA complexes were measured via dynamic light scattering. The DNA complexes were prepared by self-assembly of pUC-18 DNA (0.9 μL, 200 μg/mL) and 50 μg/mL of PCN in a buffer of Trips-HCl (pH 7.4) for 40 min. After blowing evenly with a pipette, all the samples were added

to the cuvette for measurement. The scattering angle was set at 90° and the temperature was 25 °C, as reported in the reference.[1]

Ethidium bromide displacement assay

EB (10 μg/mL) was added in the quartz cuvette filled with 5 mM Tris-HCl. Then, ctDNA (100 μM) was further added to the mixtures. The fluorescence intensity was recorded as the result of the interaction between EB and DNA. Thereafter, PCN of different concentrations were also added to the above mixtures for further measurement. Finally, the fluorescence in the system was measured and recorded. All the samples were excited at 520 nm, as reported in the reference. [1-3]

Cytotoxicity assay

The cells were seeded into 96-well plates at a concentration of 5000 cells/well, and 150 μL complete medium (90% DMEM and 10% FBS) was added to culture for 24 h. Then cells were incubated with different concentrations of PCN for 48 h. 20 μL MTT (5 mg/mL) was added to each well, let stand for 4 h, and then the medium was changed to 200 μL DMSO. The optical density (OD) at 490 nm was measured via Thermo Scientific Multiskan GO. Cell viability was calculated: cell viability = $[(OD_{samples} - OD_{DMSO})/(OD_{control} - OD_{DMSO})] \times 100\%$, as reported in the reference.[1]

***In vitro* DNA transfection**

Cells were seeded in Glass Bottom Cell Culture Dishes at a concentration of 8×10^4 cells/well and cultured in complete medium in an incubator at 37 °C for 24 h. Aqueous solutions of different concentrations of PCN and 5 μg of eGFP-DNA were added to the EP tube. DMEM was also added to EP tube to make the total volume 600

μL, and mixed for 30 min. Then, the PCN-DNA complexes were incubated with the cells for 5 h in an incubator. Then, the DMEM was replaced with 600 μL complete medium and incubated at 37 °C in an incubator with 5% CO_2 for 24 h. Finally, the medium was aspirated, and the treated cells were washed three times with 1 mL of PBS. The results of gene expression were recorded by CLSM according to the references.[1]

Cell flow experiments

Cells were seeded in 6-well plates at a density of 4×10^4 cells/well and cultured for 24h. 50 μg/mL PCN nanosheets and DNA were incubated in 1 mL DMEM for 30 min, then added to the 6-well plate and incubated in a 37 °C incubator for 24 h. Finally, the medium was removed and washed several times with PBS. The treated cells were transferred to 1 mL DMEM uniformly, and measured by flow cytometry analysis according to the references.[1]

Colocalization study of PCN-DNA and intracellular organelles

HeLa cells were seeded in Glass Bottom Cell Culture Dishes at a concentration of 2000 cells/well and cultured for 24 h. The culture medium was then removed before the cells were washed with PBS. The cells were then incubated with PCN-DNA and LysoTracker at 37 °C for 0.5 h before light irradiation (LED light, 450 mW/cm^2, 5 min). The PCN-DNA-treated group without light irradiation was used as a control. Then the medium was replaced with PBS and measured via CLSM.

Cell-uptake mechanistic studies

Pre-incubated Hela cells with 75 μM AM, 2.5 mM MβCD, 50 μM GE or 50 μM

CPZ for 1 h, and then added 1 mL DMEM containing FAM-DNA complexes to the cells. After 2 h of incubation, FAM-positive cells were compared to understand which inhibitors played a major role in reducing the cellular uptake.

1.2. Chemical Experiments

Preparation of PCN-DNA complexes: Add the corresponding concentration of DNA and PCN nanosheets to 600 uL of DEME or water to mix and assemble. Then the mixture was incubated at 37 ^{o}C for 30 minutes to prepare PCN-DNA complexes.

References

1 M.-X. Liu, L.-L. Ma, X.-Y. Liu, J.-Y. Liu, Z.-L. Lu, R. Liu, L. He, *ACS Appl. Mater. Interfaces,* 2019, **11**, 42975-42987.

2 Ding, A. X.; Tan, Z. L.; Shi, Y. D.; Song, L.; Gong, B.; Lu, Z. L. *Acs Appl. Mater. Interfaces,* 2017, **9**, 11546-11556.

3 Li, Z. F.; Chen, H. L.; Zhang, L. J.; Lu, Z. L. *Bioorg. & Med. Chem. Lett.* 2012, **22**, 2303-2307.

2. The molecular structure of PCN

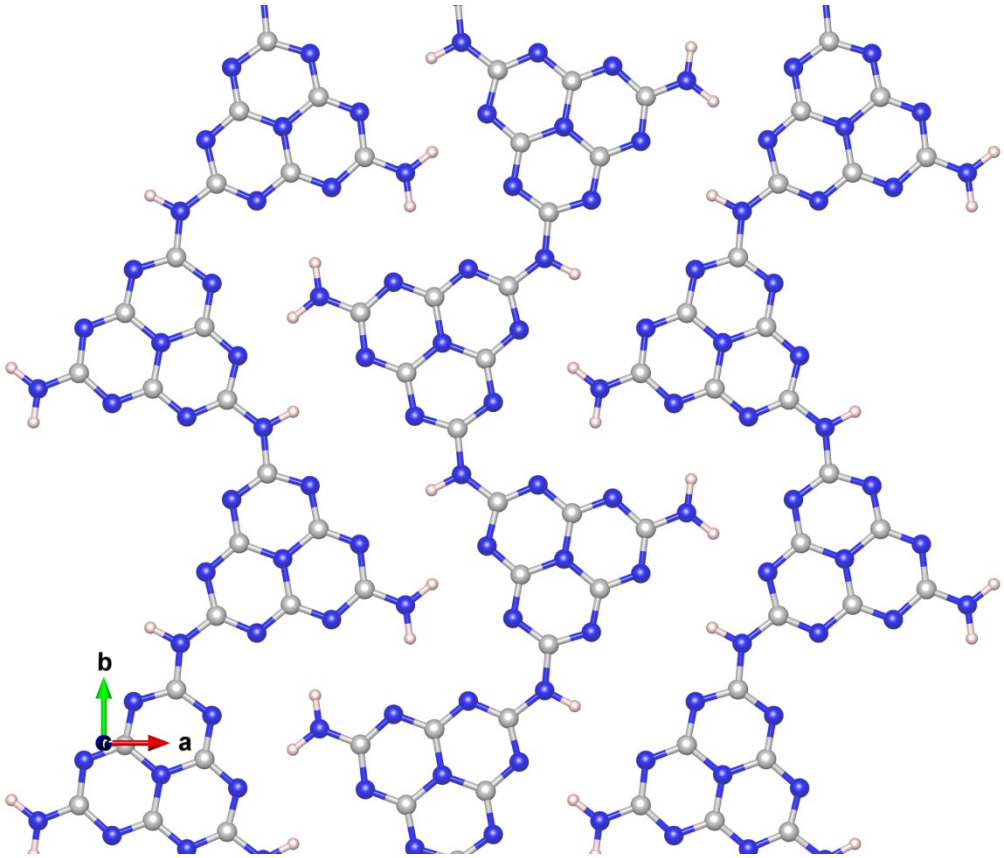

Figure S1. Schematic diagram of the structure of PCN (The gray, blue and pink-white spheres are represented by C, N and H atoms, respectively).

3. Characterization of water-dispersible PCN nanosheets

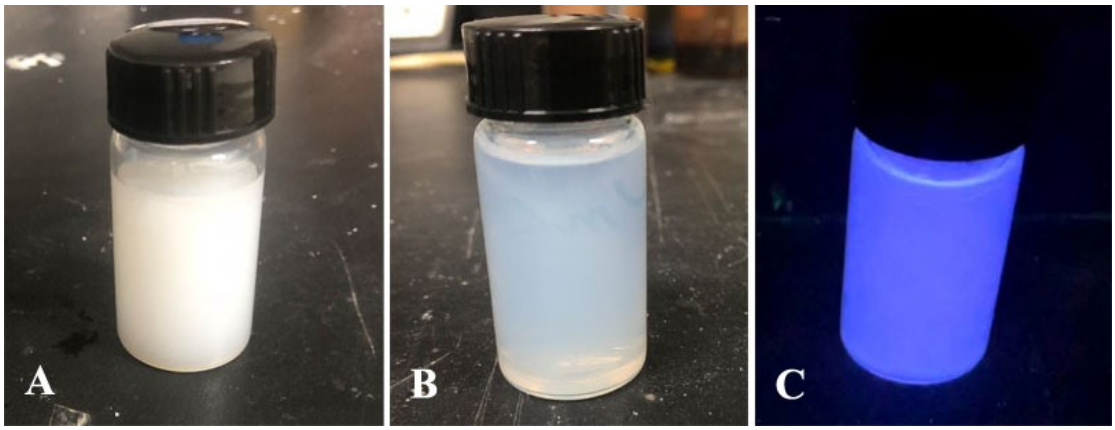

Figure S2. The photograph of (A) porous PCN, (B) water-soluble PCN and (C) the PL emission spectra of PCN under portable UV lamp.

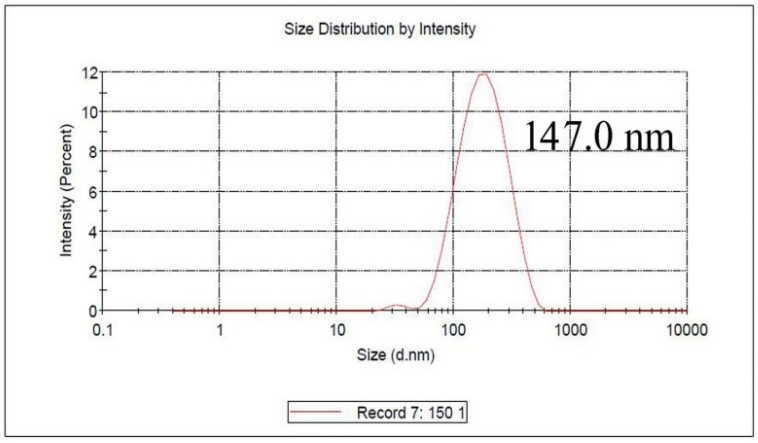

Figure S3. DLS size distribution of PCN nanosheets.

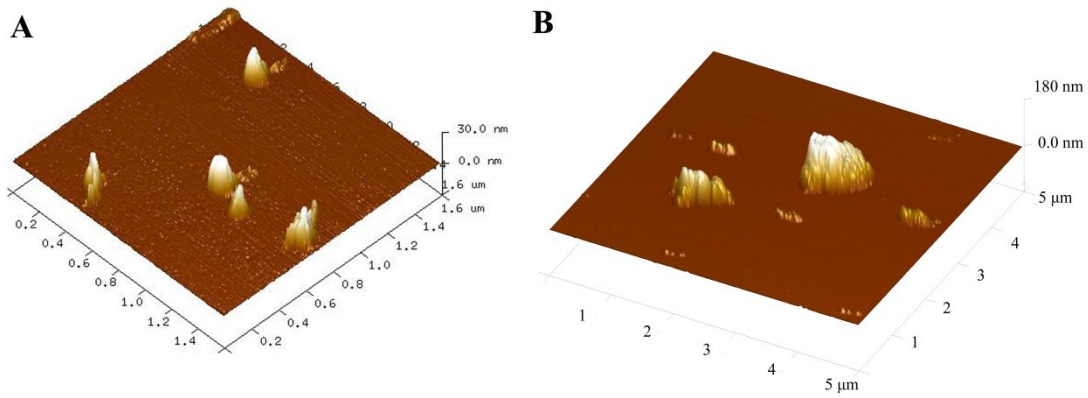

Figure S4. AFM of (A) well water-dispersible PCN nanosheets and (B) bulk PCN.

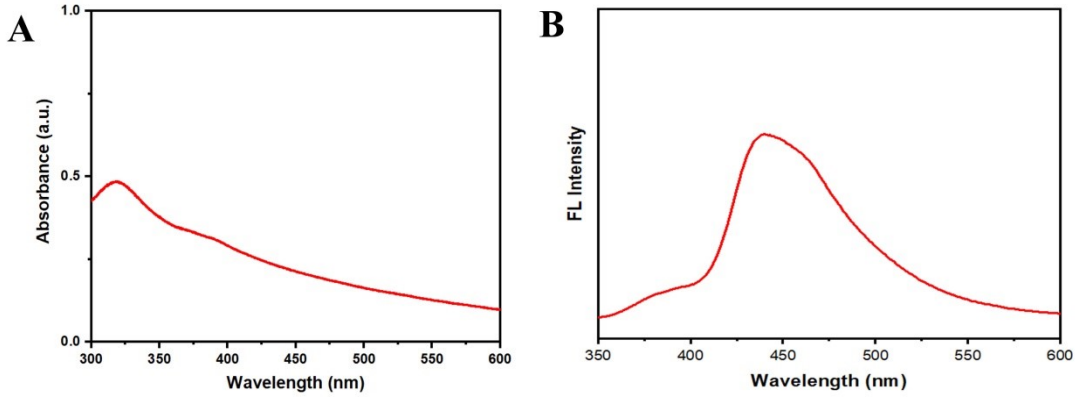

Figure S5. UV-Vis absorption and PL emission spectra of bulk PCN in water.

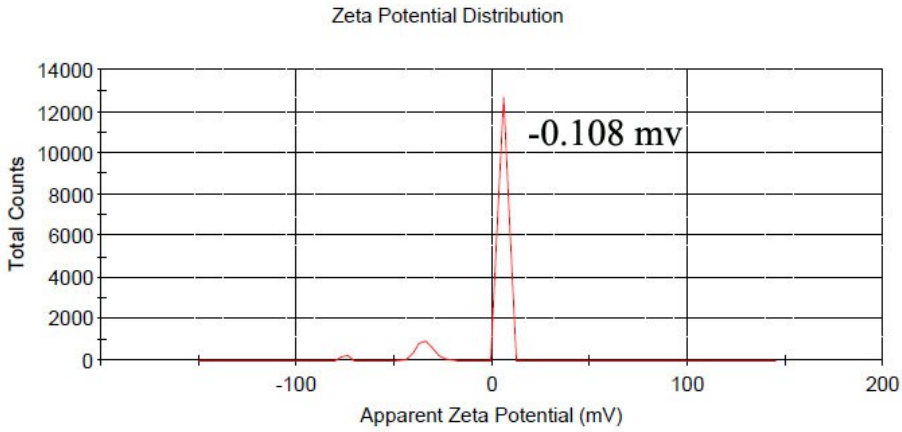

Figure S6. Zeta potentials of PCN in Trips buffer (50 mM, pH 7.4). PCN: 50 µg mL^{-1}.

4. *In vitro* experiment of water-dispersible PCN nanosheets

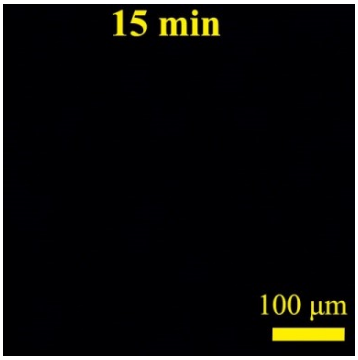

Figure S7. CLSM images of HeLa cells not treated with PCN nanosheets under LED irradiation. All the cells were incubated with DCFH-DA. LED wavelength: 401 nm, power density: 450 mW/cm^2, scale bars: 100 μm.

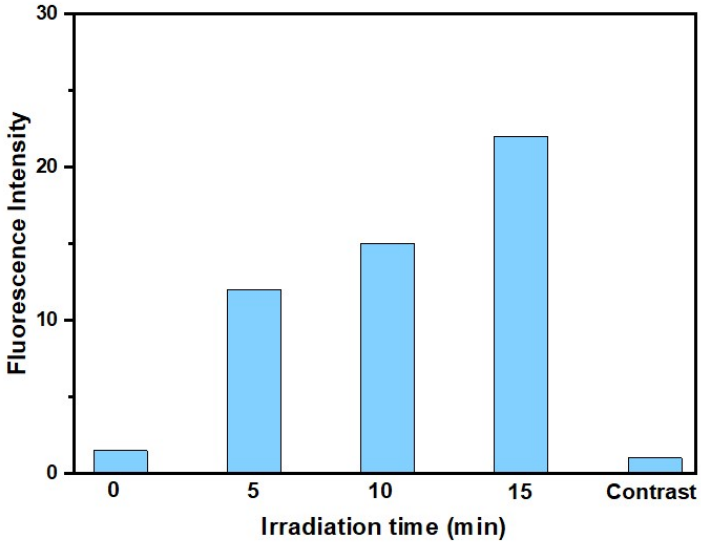

Figure S8. Analysis of DCFH fluorescence intensity in Hela cells treated with water-dispersible PCN nanosheets. LED wavelength: 401 nm, power density: 450 mW/cm^2, [PCN] = 50 μg/mL.

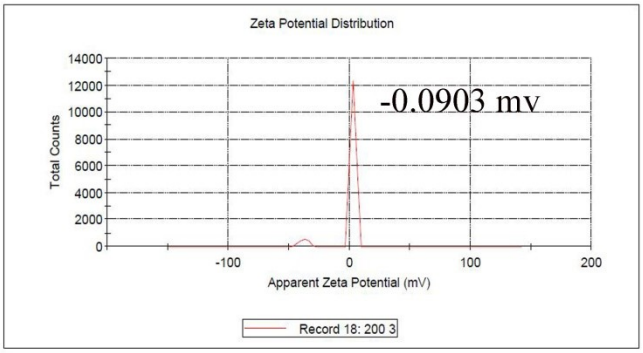

Figure S9. Zeta potentials of PCN-DNA in Trips buffer (50 mM, pH 7.4). PCN: 50 μg mL^{-1}, pUC18 DNA: 9 μg mL^{-1}.

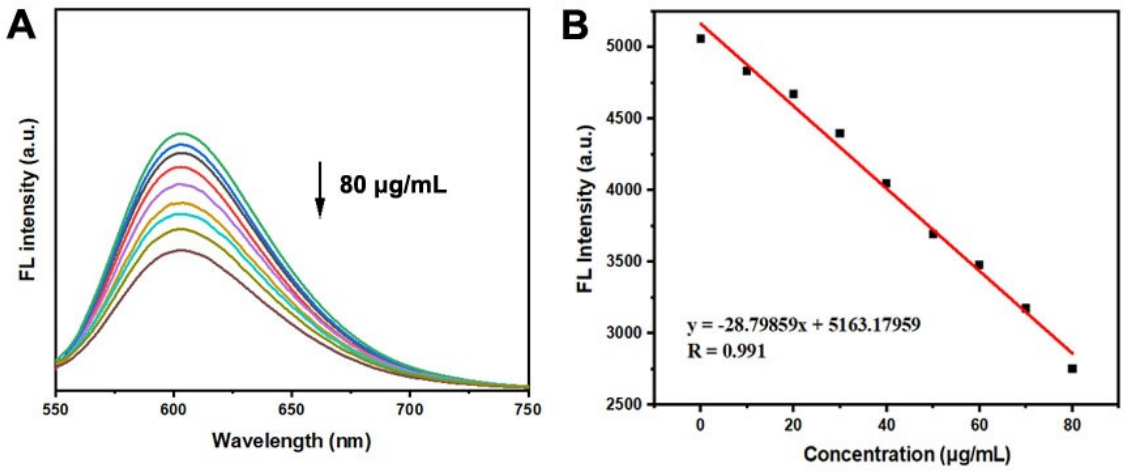

Figure S10. EB exclusion experiments of PCN nanosheets in 5 mM Tris-HCl (pH 7.4, 25 °C); [EB] = 10 μg/mL and [DNA] = 100 μM. The arrow indicates the decrease in the fluorescence intensity with increasing concentration of PCN nanosheets.

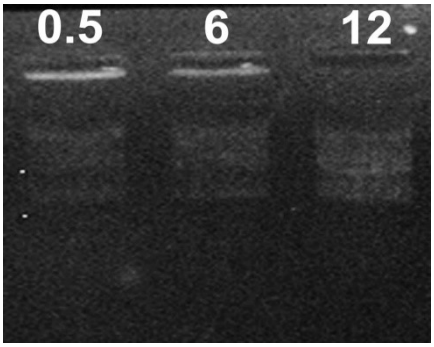

Figure S11. Aggregation of pUC-18 DNA *via* water-soluble PCN nanosheets with increasing different incubation times (h). PCN: 50 μg mL^{-1}, pUC18 DNA: 9 μg mL^{-1}.

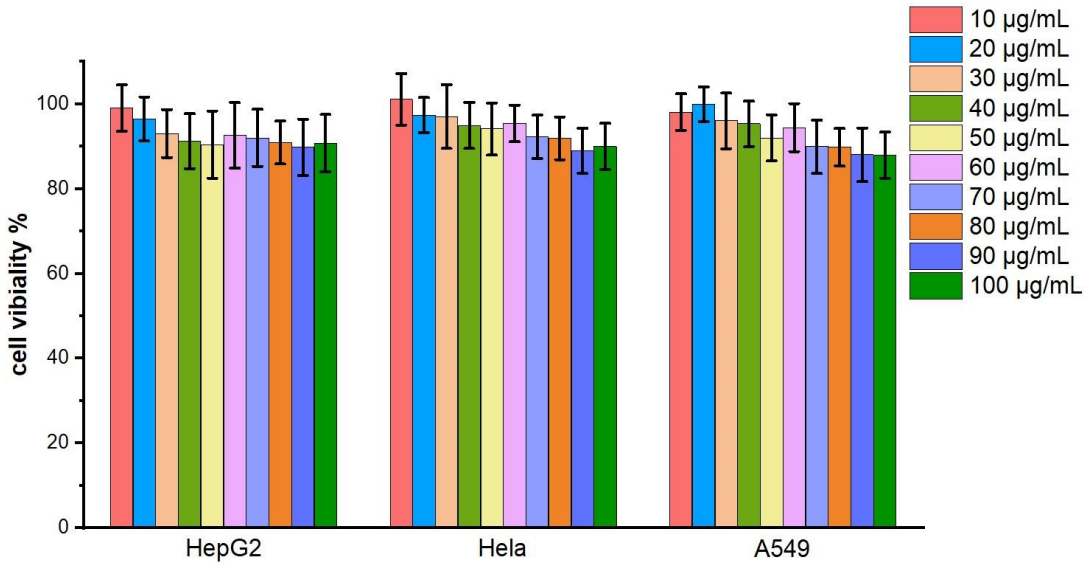

Figure S12. Tumor cytotoxicity assay of PCN nanosheets cultured at various concentrations for 24 h.

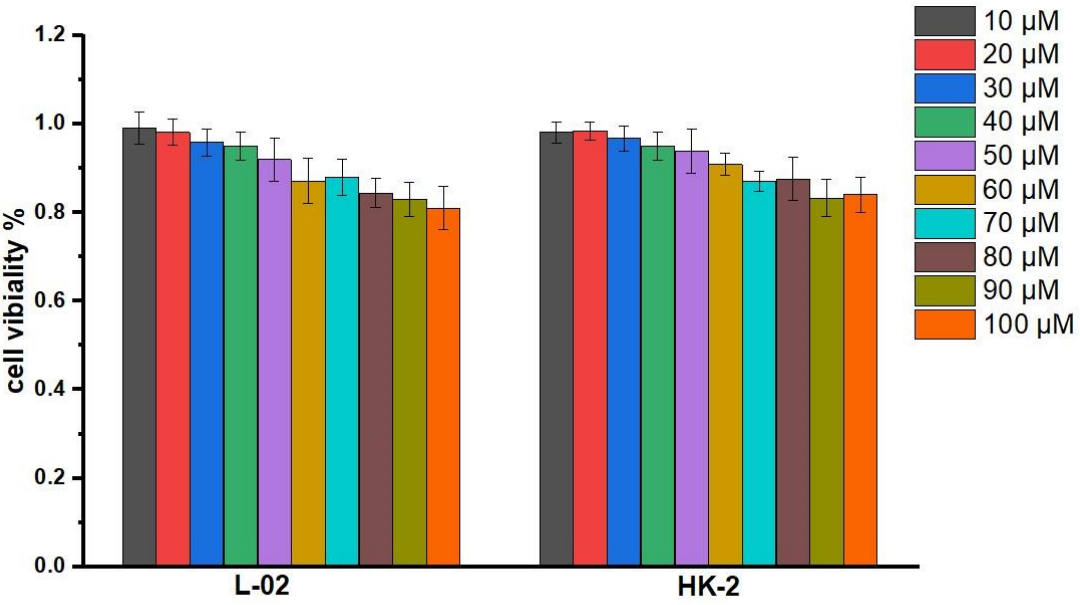

Figure S13. Normal tissue cytotoxicity assay of PCN nanosheets cultured at different concentrations for 24 h.

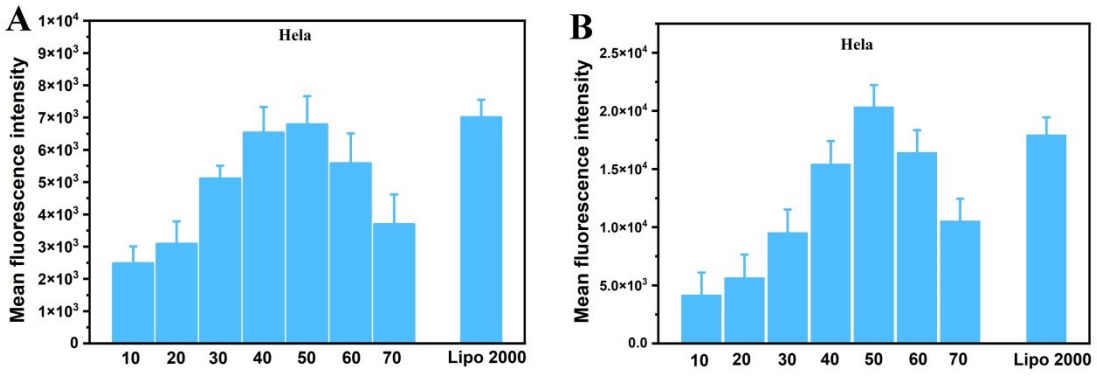

Figure S14. The expression of GFP by PCN-DNA (A) without light irradiation and (B) under light irradiation. The Lipo 2000 control group was not irradiated, PCN: 50 μg mL^{-1}, EGFP plasmid DNA: 10 μg mL^{-1}, irradiation time: 10 min, LED wavelength: 401 nm, power density: 450 mW/cm^2.

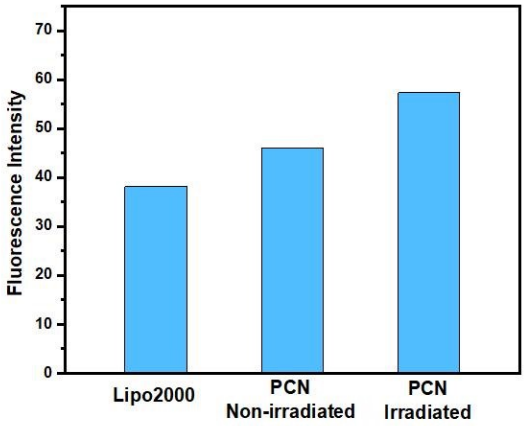

Figure S15. Fluorescence analysis of GFP expression. PCN: 50 μg mL^{-1}, EGFP plasmid DNA: 10 μg mL^{-1}, irradiation time: 10 min, LED wavelength: 401 nm, power density: 450 mW/cm^2.

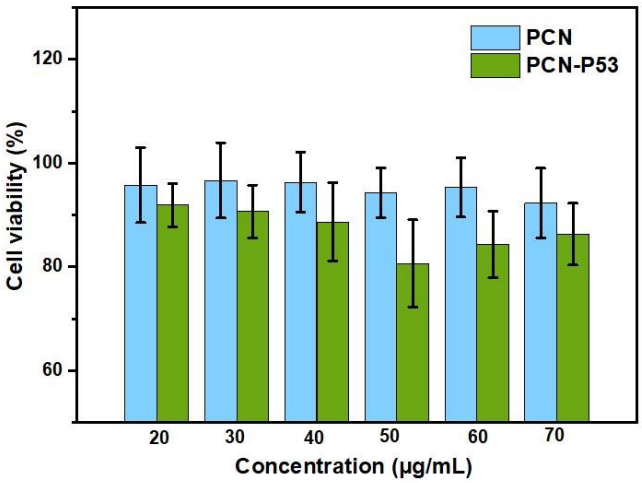

Figure S16. Viability of PCN-P53-treated HeLa cells under dark conditions, PCN-treated cells as control. [PCN] = 50 μg/mL.