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Black Phosphorus Quantum Dots-based Novel siRNA Delivery Systems in Human Pluripotent Teratoma PA-1 cells

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Methods and materials

Chemicals and reagents

Poly(allylamine hydrochloride) (PAH, molecular weight 15,000) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromides (MTT) were purchased from Sigma Aldrich. Ultrapure water produced with a Milli-Q Integral 5 system was used in all experiments. Antibodies against LSD1 (ab17721), and H3 (ab1791) were obtained from Abcam.

BP-QDs characterizations

TEM images were obtained by a JEOL model JEM-2010 transmission electron microscope at an acceleration voltage of 200 KV. The specimens were prepared by drop-casting the sample dispersion onto a carbon coated 300 mesh copper grid (Carbon Type-B, Ted Pella, Inc.). After coating samples on the grid, uranyl acetate solution (2%, 10 μL) was dropwise added on the grid for negative staining. The UV-visible absorption spectra were obtained from a spectrophotometer (Shimadzu UV-2450). The hydrodynamic size distribution profile and the zeta potential of the BP-QDs@PAH nanocomplex were measured by a particle size analyzer system (90 Plus, Brookhaven Instruments).

Atomic force microscopy

AFM measurements were performed using a Nanoscope V multimode atomic force microscope (Veeco Instruments, USA). Samples for AFM images were diluted with deionized H_2O to yield a final concentration of 1 × 10-6 M. The sample (20 μL) was then dispersed on freshly cleaved muscovite mica, and then dried at room temperature.

Tapping mode was used to acquire the images under ambient conditions.

Raman spectra

Raman spectra were performed on a Renishaw InVia Reflex Raman system (Renishawplc, Wotton-under-Edge, UK) using a grating spectrometer with a Peltier-cooled charge coupled device (CCD) detector coupled to a confocal microscope. The spectra were then processed with Renishaw WiRE 3.2 software. Raman scattering was excited using an argon ion laser (I = 514.5 nm).

siRNA transfection

The day before transfection, PA-1 cells were seeded onto 6-well plates in EMEM medium with 10% FBS to give 30% − 50% confluence at the time of transfection. BP-QDs@PAH nanosheets dispersion (1 mg/mL, 20 μL) was mixed with LSD1 siRNA^{Cy3} (0.4mg/mL, 3.25μL) with gentle vortex and left undisturbed for 30 min. Before transfection, the culture medium was replaced with OPTI-MEM (950 μL, Invitrogen), the above mentioned BP-QDs@PAH-siRNA^{Cy3} mixture was then added to the medium and the cells were continuously cultured. Free siRNA^{Cyc3} was also used in another parallel experiment at the same dosage level. Four hours later, EMEM medium (500 μL) with 30% FBS was added to the medium. The gene expression was monitored at 48 hours post-transfection. For transfection efficiency examination, fluorescent imaging and flow cytometry assays were performed at 4 hours post-treatment.

Figures and figure legends

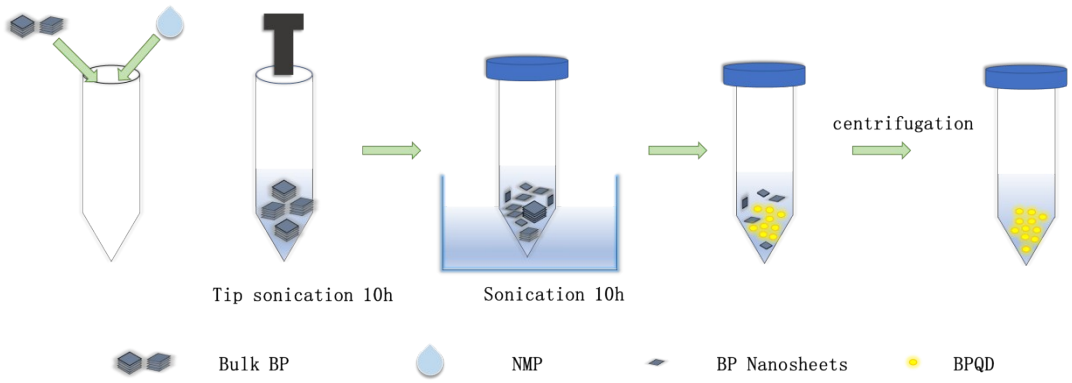

Fig. S1 Schematic illustration of Preparation of BP-QDs (see experimental section for details).

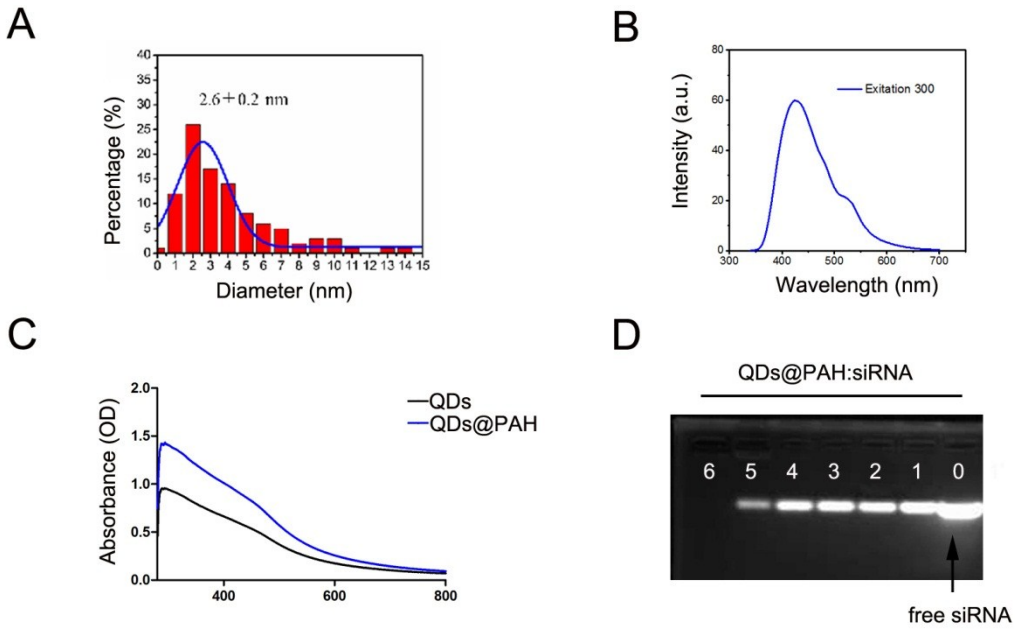

Fig. S2 Characterization of BP-QDs. (A) DLS. (B) PL of BP-QDs. (C) Absorption spectra of BP-QDs and BP-QDs@PAH. (D) The quantitative analysis of gene loading ability of BP-QDs@PAH nanocarriers. Gel retardation quantitative analysis of siRNA loading by BP-QDs@PAH, using free siRNA as reference. BP-QDs@PAH (1 mg/mL) was mixed with siRNA (0.4 mg/mL) at five different mass ratios (1 μg: 1 μg, 2 μg: 1 μg, 4μg: 1 μg, 8 μg: 1 μg, 10 μg: 1 μg and 15 μg: 1 μg).

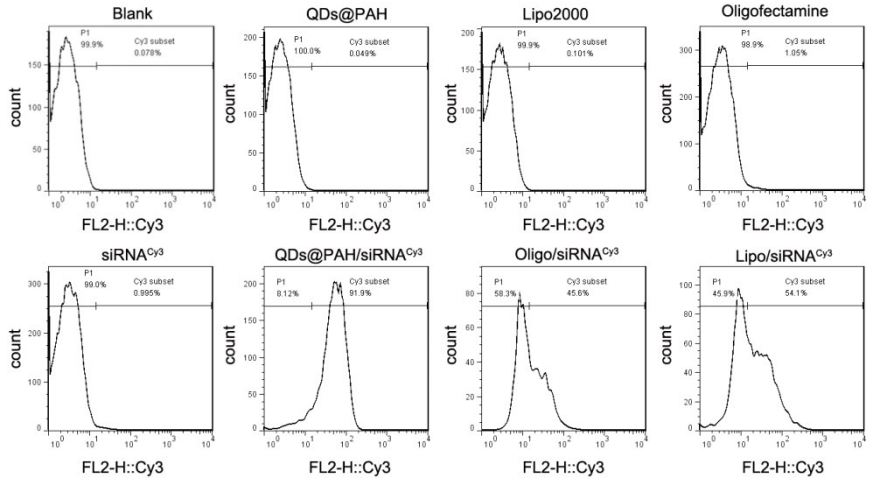

Fig. S3 Flow cytometry evaluations on the transfection efficiencies of PA-1 cells treated with PBS (as blank), BP-QDs@PAH, free LSD1-siRNACy3, BP-QDs@PAH/ LSD1-siRNACy3, Lipofectamine 2000, Lipofectamine 2000/LSD1-siRNACy3, Oligofectamine and Oligofectamine/ LSD1-siRNACy3. Representative dots plot of flow cytometry assays, where the x-axis shows the fluorescent intensities of Cyc3 and y-axis shows cell counts, respectively.

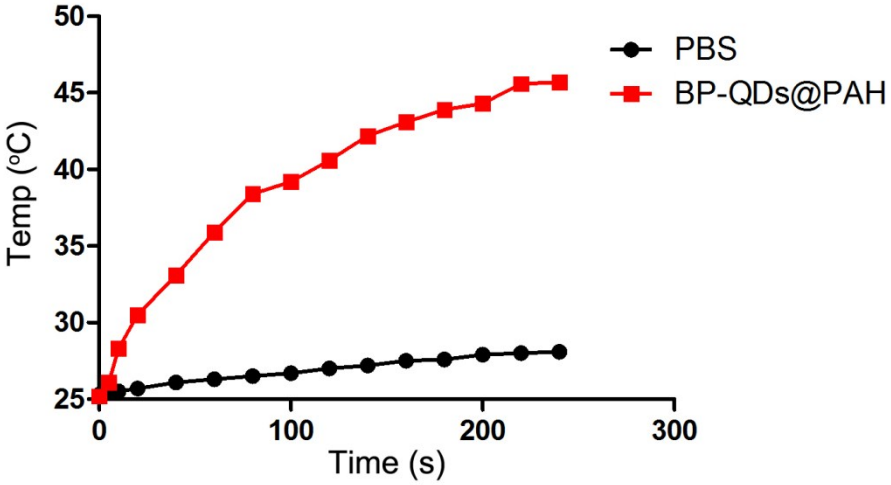

Fig. S4 Temperature dependence of BP-QDs@PAH under NIR light irradiation (808nm, 1W). BP-QDs@PAH nanocomplex was dispersed in PBS and the temperatures of the samples were measured by thermometer (T208, Digitron). Within the first 4 minutes of the NIR light exposure in vitro, a 20.5 °C increment was observed for the BP-QDs@PAH nanocomplex (from 25.2 °C to 45.7 °C). On the

other hand, only 2.8 °C increment was observed for PBS buffer solution (from 25.3°C to 28.1 °C).

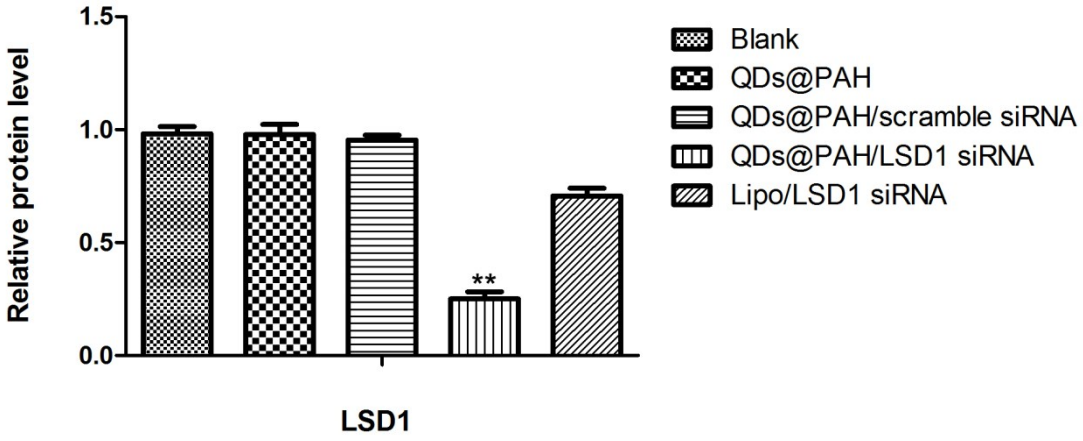

Fig. S5 Gene expression evaluations of PA-1 cells treated with different BP-QDs nanocomplexes. PA-1 cells (cultured in 6-well plate) were treated with PBS, QDs@PAH (20 μg), QDs@PAH/LSD1-siRNA (20 μg/1.3 μg), QDs@PAH/scramble-siRNA, Lipo2000/LSD1-siRNA and Oligo/LSD1-siRNA for 4 hours, and then all the cells were washed with PBS and re-incubated in fresh cell medium for additional 44 hours. The efficacy of the siRNAs was determined by Western blotting and quantified by the use of Gel-Pro Analyzer (version 4.0) software. Values are means ± SEM, n = 3, **, $P < 0.01$ vs Control and QDs@PAH.

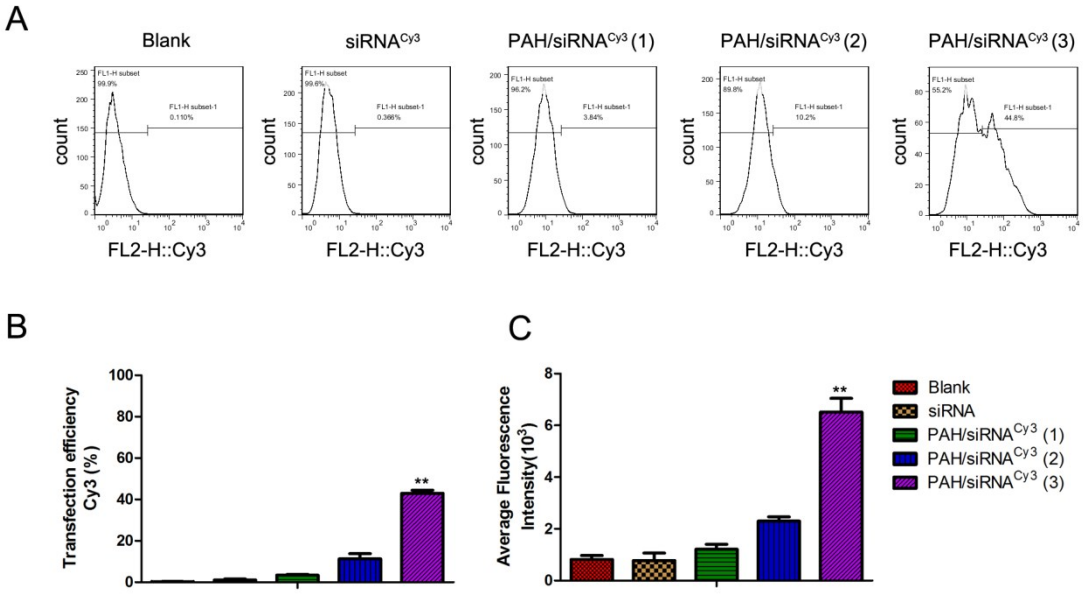

Fig. S6 Flow cytometry evaluations on the transfection efficiencies of PA-1 cells treated with PBS (as blank), free LSD1-siRNA^{Cy3} and different mass ratio between PAH/LSD1-siRNA^{Cy3}: 1μg:1.3μg (1), 2μg:1.3μg (2), 3μg:1.3μg (3). (A) Representative dots plot of flow cytometry assays, where the x-axis shows the fluorescent intensities of Cyc3 and y-axis shows cell counts, respectively. (B) and (C) Transfection efficiency and average fluorescence intensity from experiments showed in (A). Values are means ± SEM, n = 3, **, $P < 0.01$ vs Control and LSD1 siRNA^{Cyc3}.

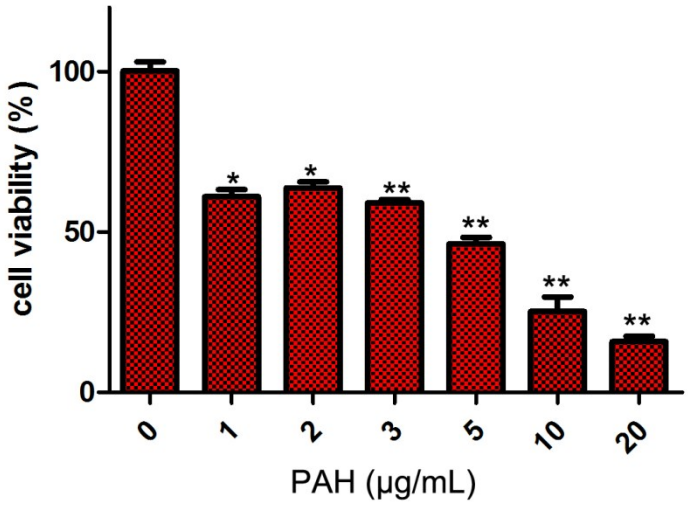

Fig. S7 Viability of cells after incubation with PAH at variation concentrations up to 20μg/mL for 24 hours. Values are means ± SEM, n = 3, *, $P < 0.05$; **, $P < 0.01$ vs Control.