Supplementary Results:

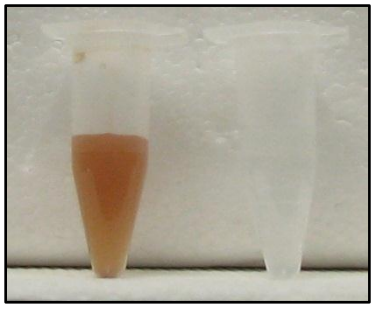
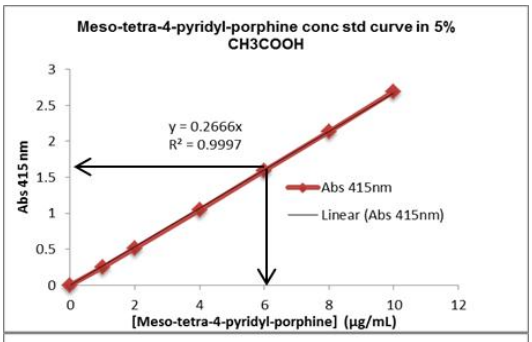
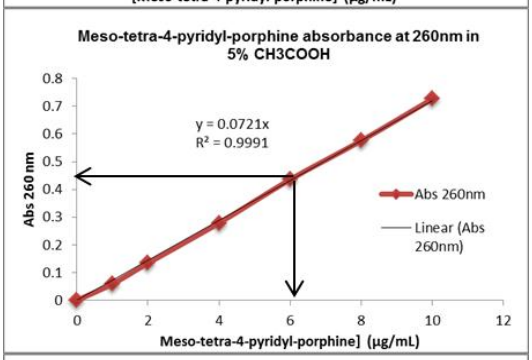


Figure S1: Photograph of resultant PDN suspension obtained upon sonication of MTP in the presence of DNA followed by centrifugation (left). In the absence of DNA, the identical procedure did not produce any nanoparticle as insoluble porphyrin aggregates were removed by centrifugation resulting a clear phosphate buffer (right).

Table S1: Absorbance of porphyrin and DNA at 415 and 260 nm

	Abs-415 nm	Abs-260 nm	Concentration (µg/mL)
Meso-tetra-4-pyridyl-porphine	1.629 (pH-4.5) 0.551 (pH-7.25)	0.668	6.5
ss-d$(GT)_{20}$	------	0.222	6.94





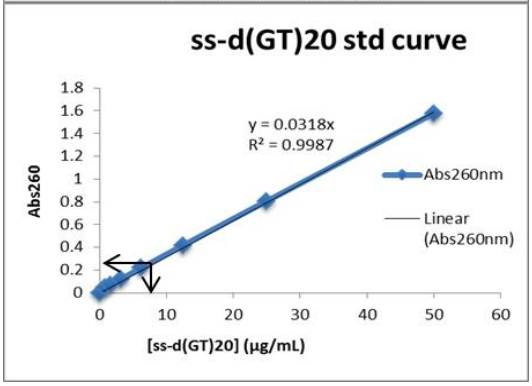


Figure S2: Standard curves used to quantify the concentrations of the DNA and porphyrin components in the PDN suspension following acidification. Meso-tetra-4-pyridyl-porphine absorbance curve at 415 nm (top); meso-tetra-4-pyridyl-porphine absorbance curve at 260 nm (middle); ss-d$(GT)_{20}$ absorbance curve (bottom). The ratio of porphyrin and DNA in PDN (~19:1) was determined from the concentrations resulting from nanoparticle dissolution using the molecular weights for each component. Experiment was performed in three independent sets.

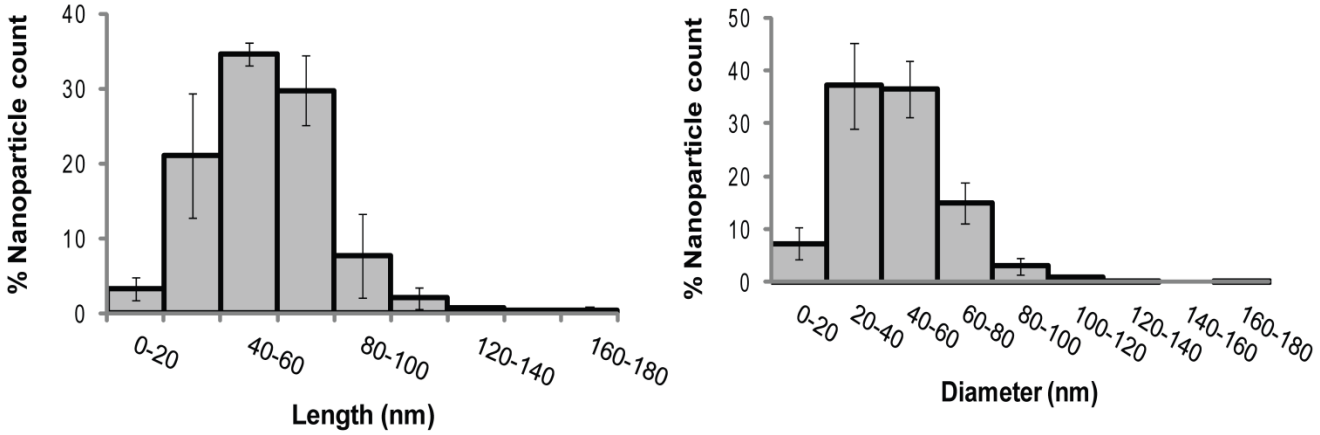


Figure S3: Representative graphs of major (left) and minor diameter (right) analysis from TEM images. Length as well as diameter of the dehydrated particles is primarily distributed in 20 – 100 nm with the majority around 60 nm. Experiment was performed in three independent sets.

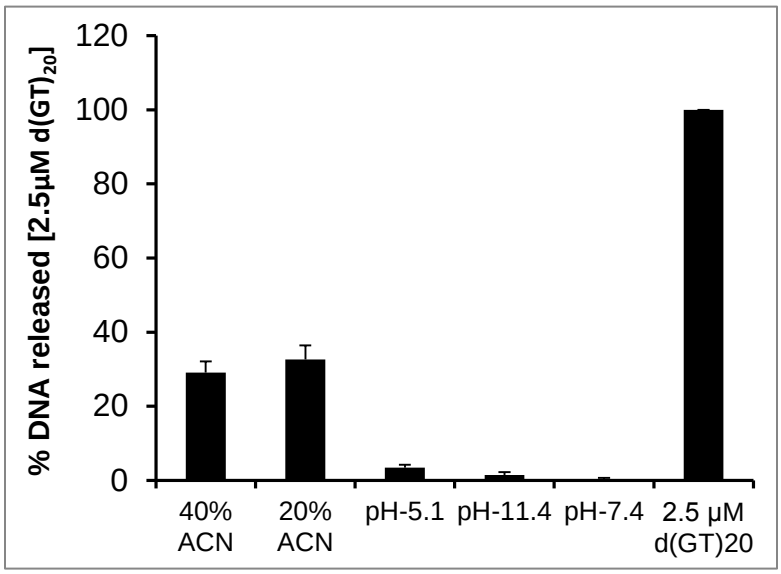


Figure S4: Quantitative analysis of DNA release in different pH and polarity by native gel electrophoresis. Results demonstrated DNA dissociation is primarily hydrophobicity dependent. 2.5 µM $d(GT)_{20}$ (not sonicated) was used as a control to confirm that small piece of DNA like 40 mer remains unfragmented upon sonication. The concentration of DNA solution used here as positive control is almost double (2.5 µM) than the total DNA concentration of the PDN suspension (1.275 µM); thus the actual percentage of DNA release is approximately double with respect to the presented graphs. The quantification was done with Image Quant 5.2 software. Experiment was done in three independent sets.

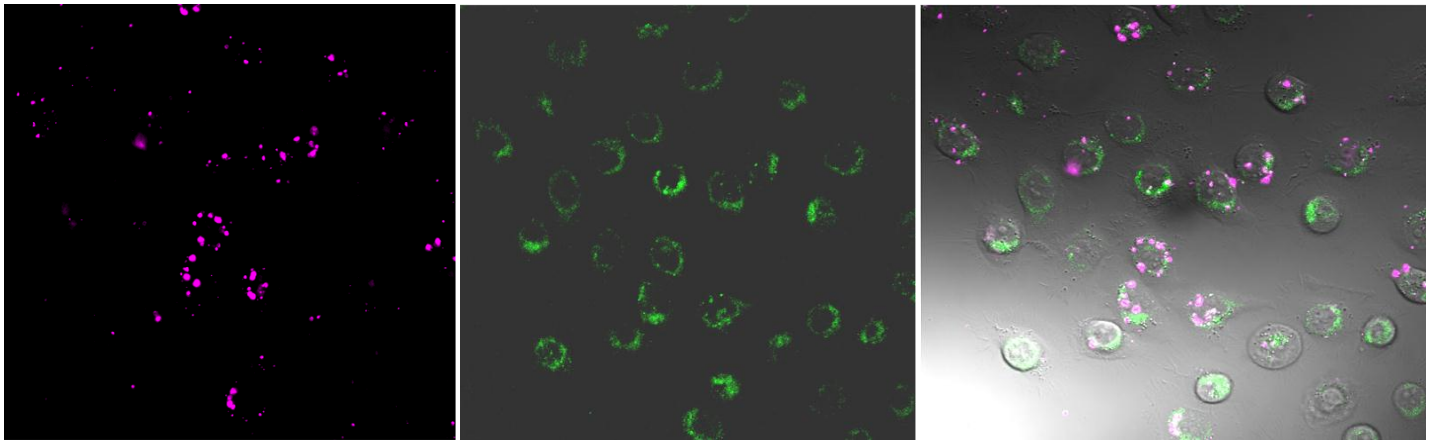


Figure S5: Confocal microscopy images evaluating PDN dissociation and DNA release. PDN were prepared with fluorescently-labeled (6-FAM) DNA: (left) porphyrin fluorescence; (middle) DNA fluorescence; (right) overlay of porphyrin and DNA fluorescence. Porphyrin fluorescence remains localized with a punctate pattern while DNA fluorescence gets distributing around the periphery indicating DNA dissociation in the hydrophobic cell membrane. Experiment was performed in three independent sets.

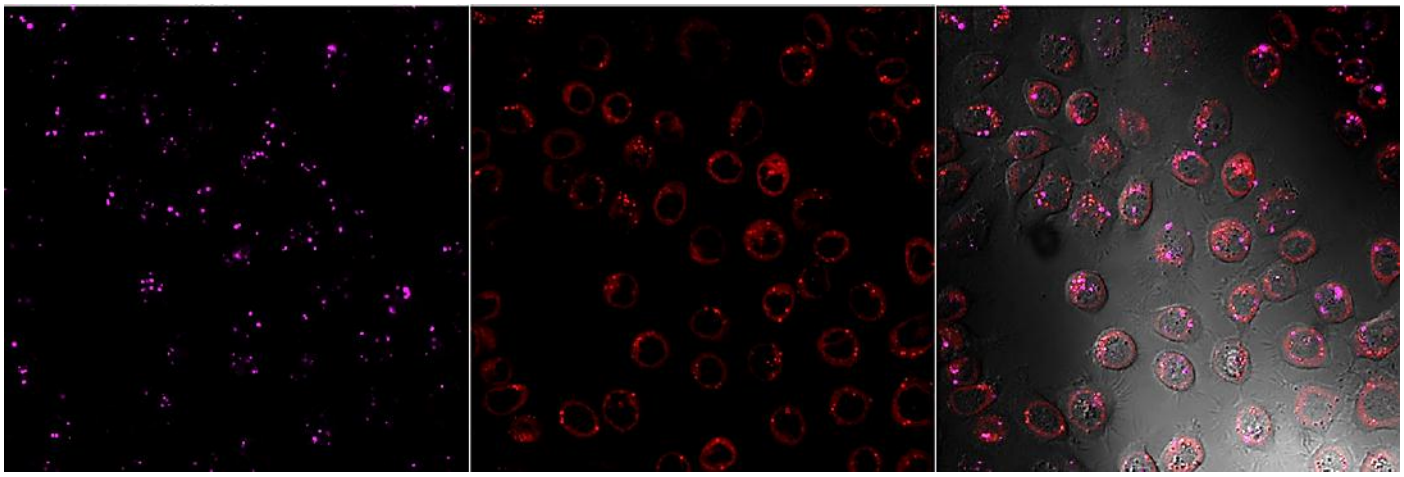


Figure S6: Confocal microscopy images demonstrating the membrane association of PDN around the periphery. Cells were treated with membrane localizing fluorescent dye C-11 Bidipy (red, middle) and PDN (magenta, left). Overlay image (right) demonstrates significant colocalization of PDN with membrane localizing dye around the periphery with a circular pattern. Experiment was performed in three independent sets.

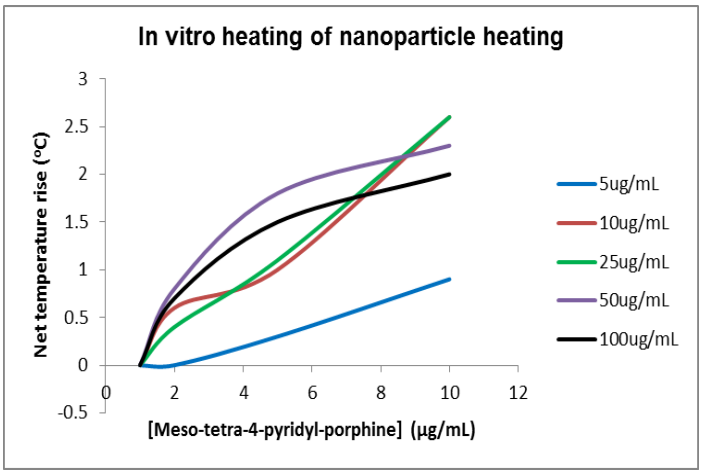


Figure S7: Representative plots from *in vitro* heating experiments of PDN upon irradiation with 125mW blue laser (420 nm). Concentration- and time-dependent heating of nanoparticle showed measurable amount heat. However the observed heating might not be sufficient alone to explain the light-dependent cytotoxic and anti-tumor activity; but it can enhance the malignant cell killing activity of PDN.

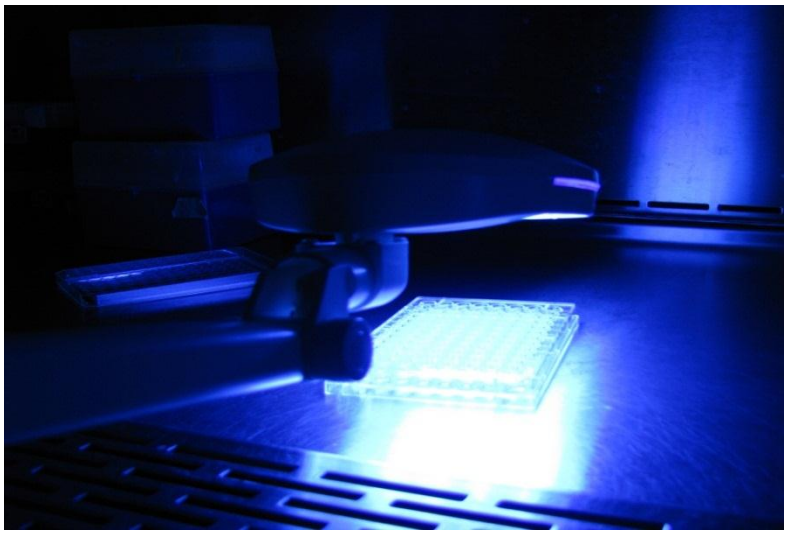


Figure S8: Photograph demonstrating the blue light irradiation set-up for cell culture experiments. All wells are equally receiving blue beam from the 420 nm light source in the biosafety cabinet under aseptic condition.

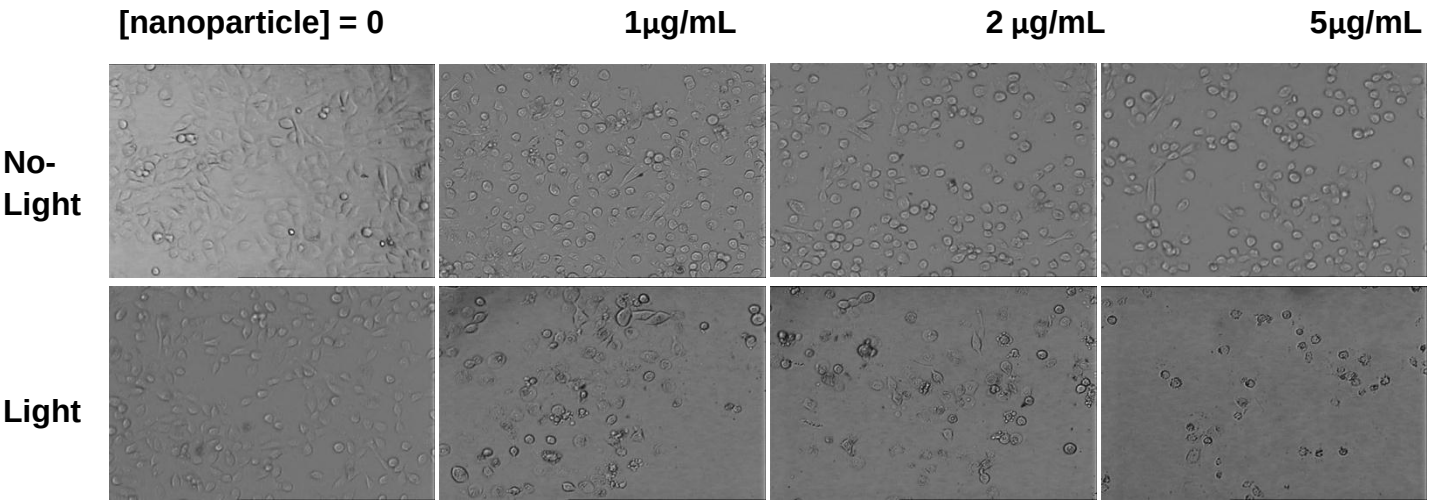


Figure S9: **Representative Phase-contrast microscopy images evaluating cytotoxicity of PDN,** visually demonstrating the cell density is reduced upon treatment with PDN in a light-dependent manner. Experiment was performed in three independent sets.

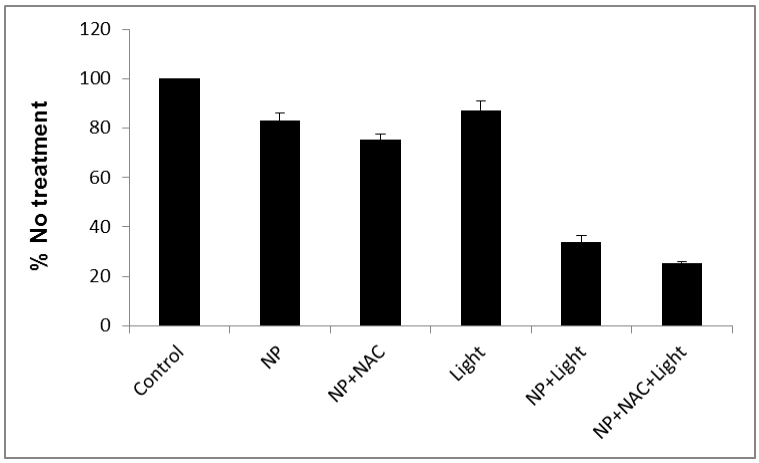


Figure S10: Graphs representing the NAC rescue from PDN mediated cytotoxicity. Cell viability data were graphed after treating cells with nanoparticle and blue light in presence or absence of the radical scavenger N-acetyl-cysteine (NAC). Data did not showed any significant amount of rescue by the NAC indicating diffusible radicals are not the major contributor to cytotoxicity. Experiment was performed in three independent replicates.

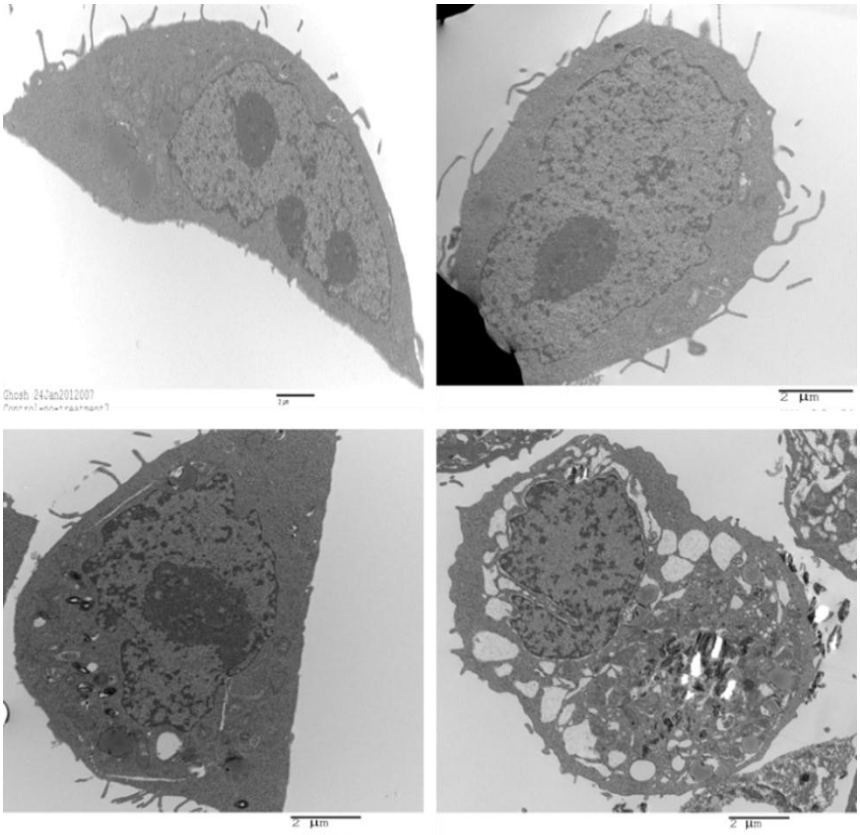


Figure S11: Representative TEM images demonstrating the overall effect of PDN & light treatment on cellular physiology. PDN treatment in combination with 420 nm light caused severe membrane damage and vacuole formation. Light-only treatment (**top-right**) did not resulted any significant physiological damage with similar effect as no-treatment (**top-left**). PDN-only (**bottom-left**) cell showed moderate vacuole formation but no significant membrane damage. PDN & Light (**bottom-right**) Experiment was performed in three independent replicates.

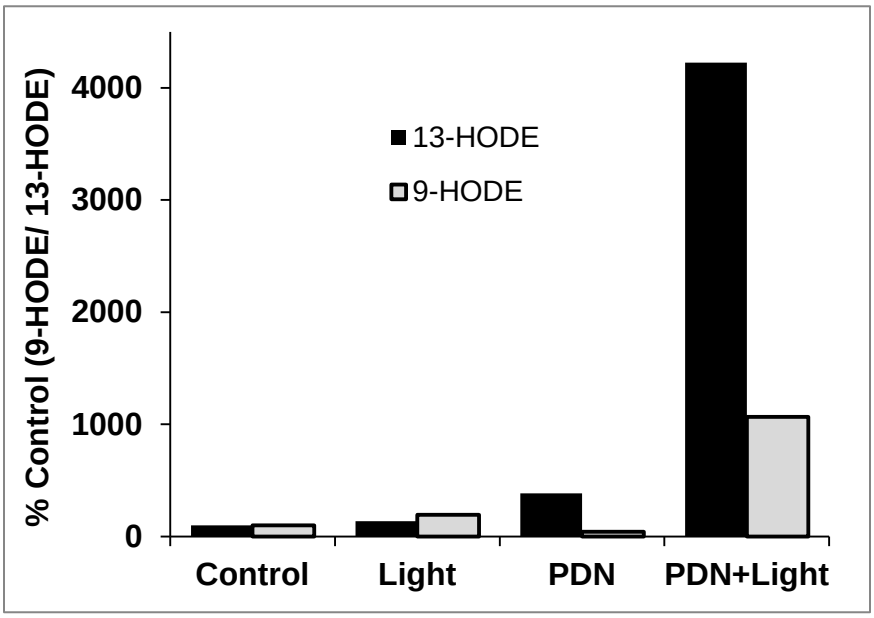


Figure S12: Quantification of membrane lipid peroxidation products 9-HODE and 13-HODE from plasma membrane fraction of bladder cancer cells treated with PDN/light, light-only, PDN-only, and no treatment. PDN treatment in combination with blue light generated significant membrane lipid peroxidation products 9-HODE and 13-HODE, oxidised product of linoleic acid. Experiment was performed in three independent sets and samples were processed independently for mass spectrometry. Corresponding replicates were pulled to run together to get a better signal to noise ratio and an instrument generated average result instead of digitally averaged result.

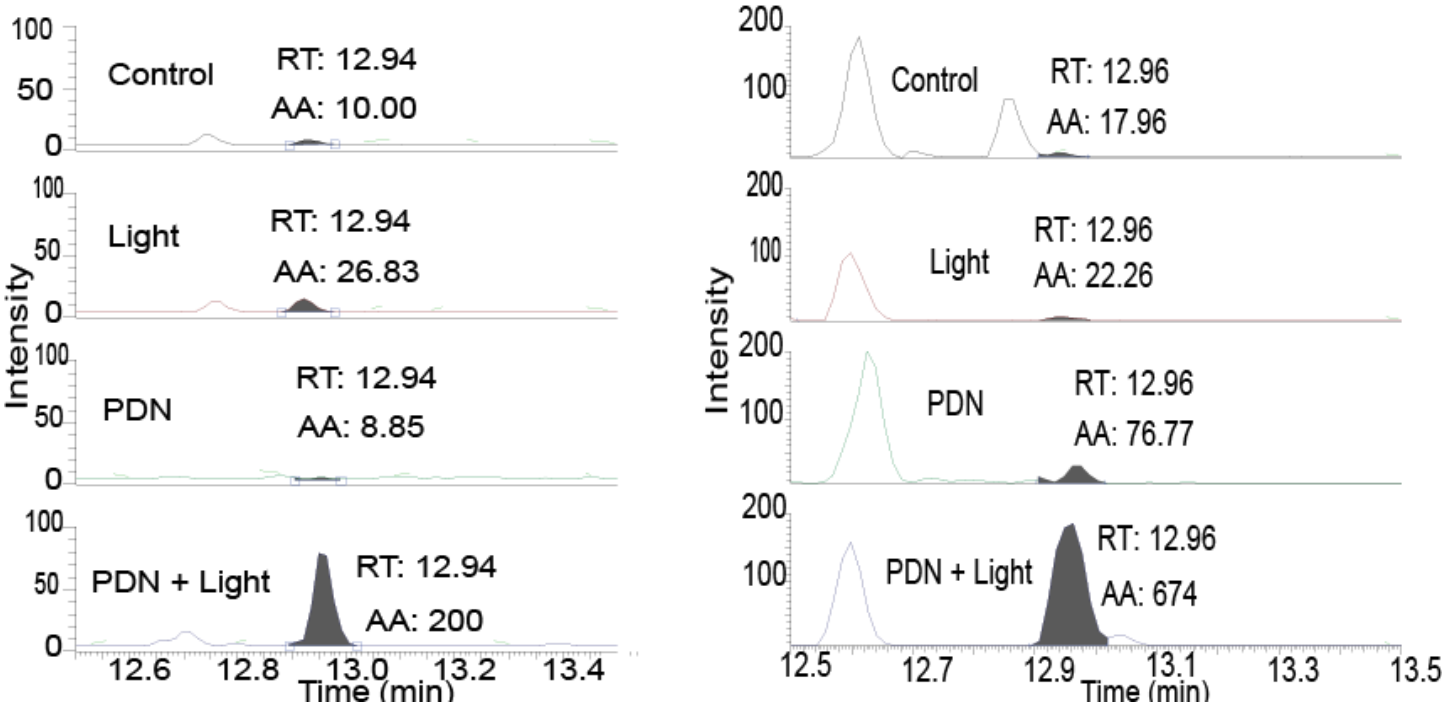


Figure S13: Representative Chromatograms from membrane lipid mass-spec indicating elevated level of 9-HODE (left) and 13-HODE (right), the peroxidation product of linoleic acid upon PDN/light treatment. Experiment was performed in three independent sets and samples were processed independently for mass spectrometry then replicates were pulled together to run together to get a better signal to noise ratio and an instrument generated average result instead of digitally averaged result.

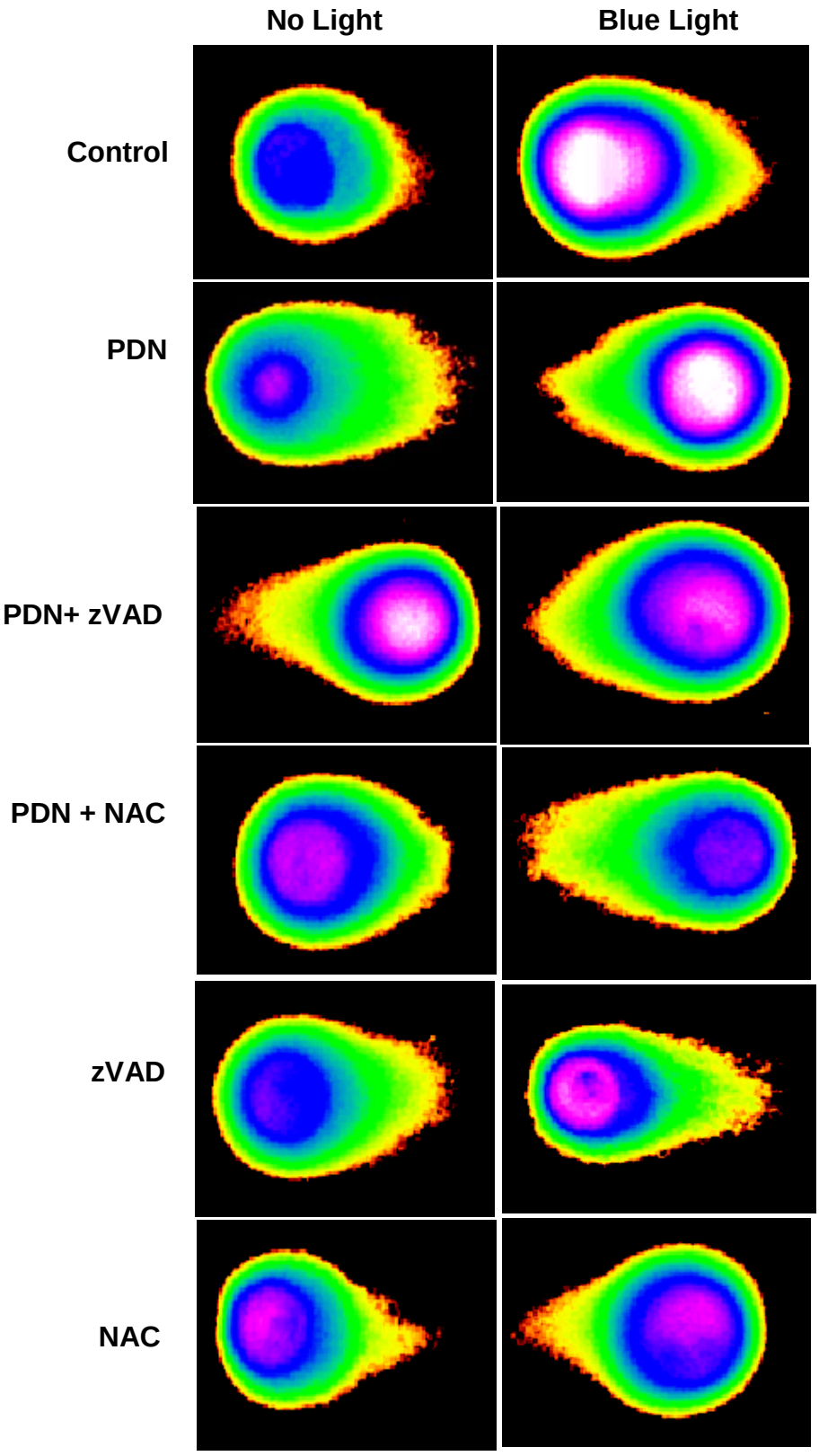
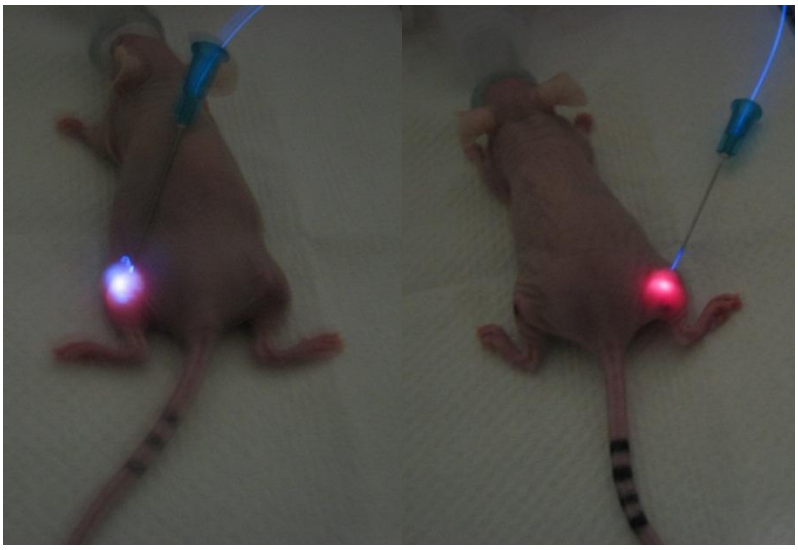
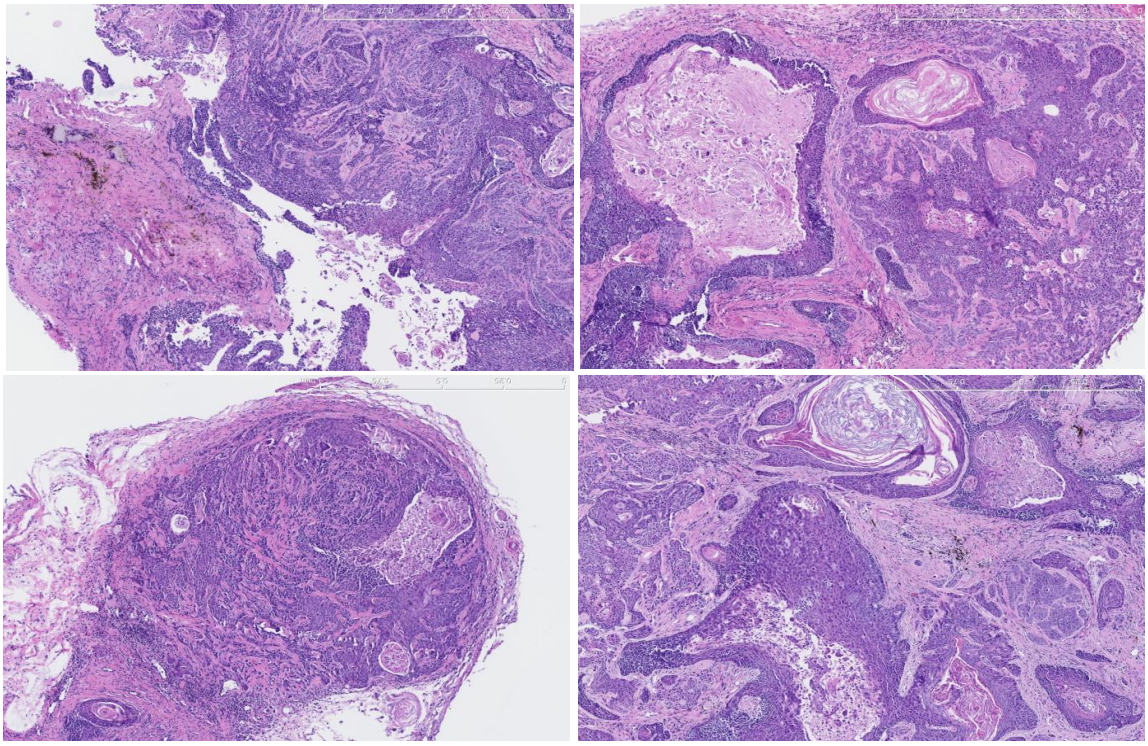


Figure S14: Representative images from single cell gel electrophoresis (Comet) assay showing no significant difference in tail moment caused either by PDN or PDN & light treatment. These results indicate that either PDN alone or in combination with light does not induce significant DNA damage. No further reduction in DNA fragmentation by NAC (a free-radical scavenger) or z-VAD (an inhibitor of caspase-mediated apoptosis) confirmed that diffusible free radicals were not a significant source of DNA damage and that apoptosis was not a predominant form of cell death. Experiment was performed in three independent sets.



Supplementary Figure S15: Photograph of representative animals from the *in vivo* experiment demonstrating the laser irradiation set up. **(Left)** - Saline-treated tumor receiving laser irradiation. **(Right)** – PDN-treated tumor receiving laser irradiation. As conditions are otherwise identical between the irradiated tumors the observed color difference reflects the absorbance of laser light by the PDN.



Supplementary Figure S16: Bright field image of histopathological analysis of in vivo study from the same regions as shown in Fig. 6C with lower magnification (50x) showing the overall effect. PDN with laser **(top-left)** demonstrated a significant tumorpathic effect and reduced mallignanent growth including immunological changes. PDN only **(bottom-left)** tumor showed significant growth with little immune response. Laser only **(top-right)** like the no treatment **(bottom-right)** tumor showed significant tumorgenesis including no significant immune response.

Supplementary Materials and Methods:

Preparation of Porphyrin:DNA Nanoparticles: Meso-tetra-4-pyridyl porphine (MTP; ~0.25 mg - Frontier Scientific) was suspended in 5 mL of 20mM Phosphate Buffer (pH-7.4) containing 5 nmol ss-d$(GT)_{20}$ and sonicated in a bath sonicator for 1.5hrs with temperature maintained at 5-7^{o}C. A second addition of MTP (~0.25 mg) was followed by an additional 1 h sonication, followed by addition of 5 nmol DNA and further sonication for 1 hour. The mixture was then centrifuged at 4000 x g to pellet large aggregates and insoluble materials. The resulting transparent, brownish-yellow aqueous suspension of PDN (**Supplementary Fig. 1**) was filtered through membrane filter tubes (Amicon Ultra, 100 KDa MWCO) and used for subsequent experiments.

Quantification of DNA and Porphyrin Ratio in PDN: A PDNA suspension was prepared in 20 mM phosphate buffer (pH 7.25) and absorbance was measured at 415 nm. Concentrated HCl was added to the suspension lowering the pH to ~3.0. Absorbance of the resulting solution was measured at 260 nm and 415 nm. The porphyrin concentration was determined from the absorbance at 415 nm using a pre-established standard curve (**Supplementary Fig. 2**). A HCl solution of porphyrin of similar concentration was prepared and its absorbance at 415 nm and 260nm was confirmed. The 260 nm absorbance of the pure porphyrin solution was subtracted from the 260 nm absorbance for the dissociated nanoparticle to determine the absorbance contributed by ss-d$(GT)_{20}$ only. The concentration of d$(GT)_{20}$ was then determined from the pre-established standard curve. The ratio of porphyrin to DNA in PDN (~19:1) was determined using the porphyrin and DNA concentrations calculated from the standard curves together with the known molecular weights. Experiment was performed in three independent sets.

Porphyrin:DNA Nanoparticle Molecular Model: The model for the PDN nanoparticle was derived from coordinates of a complex of telomeric DNA and porphyrin (pdb 2HRI). Conversion to meso-tetra-4-pyridyl-porphine (MTP) was performed by deleting the methyl groups from 5,10,15,20-tetrakis(N-methyl-4-pyridyl)porphyrin structure. Coordinates of the porphyrin were then replicated to five molecules and spaced suitably to depict all as stacked on one another. DNA strands were constructed using original coordinates from the pdb file and deleting six nucleotides from the 3' end. The coordinates of the DNA strand were then replicated to form a second DNA strand. The two DNA strands were then oriented so that hydrophobic bases of DNA remain proximal to the hydrophobic pyridyl ring of porphyrin. All modifications to the original pdb coordinates were performed using PyMOL.

Transmission Electron Microscopy: TEM images were acquired under ambient conditions using a FEI Thcnai-Spirit TEM. A drop of PDN nanoparticle suspension (5μg/mL) was placed on a carbon-supported copper grid and incubated for 30min at ambient condition then excess solution was absorbed very slowly through the edge of the grid using blotting paper followed by negative staining with a mixture containing 5%phosphomolybdate and 0.1% trehalos. The grid was then dried and investigated under TEM with different magnifications. TEM images were analyzed and particle sizes were determined using Matlab software.

Dynamic Light Scattering: DLS was performed under ambient conditions using a Malvern Zetasizer nano series ZEN-1600 in particle size measurement mode. Each sample was read for 60 sec using a 442.0 Kcps count rate. Data were analyzed using Malvern software. Experiments were performed in three independent sets and averaged to determine final size distribution.

Spectroscopic Characterization of PD Nanoparticles: UV-Vis spectroscopy was performed under ambient conditions by placing 1 mL PDN suspension (5μg/mL) in a quartz cuvette of path length 1cm using DU800 UV-Vis spectrophotometer (Beckman Coulter). The region 230 – 800 nm was scanned at 120nm/min. Data were analyzed using Beckman software. The experiment was performed in three

independent sets and representative graphs are displayed. Fluorescence spectra were acquired under ambient conditions using a Perkin-Elmer-F1000 fluorometer. The nanoparticle suspension (~5μg/mL) was placed in a quartz cuvette with excitation at 420nm and emission scans performed over the range 550 -900nm. The experiment was performed in three independent sets. Raman spectra of PDN (~250μg/mL) were recorded using a Deltanu Advantage-532 Raman spectrometer. The region from 3400 - 200 cm^{-1} was scanned under ambient conditions following excitation with a 532nm laser. Spectra were averaged over 10 acquisitions. The experiment was performed in three independent sets and representative data were graphed.

pH and hydrophobicity dependent melting of PDN: PDN were prepared in 20 mM phosphate buffer to a concentration of 250 μg/mL. PDN suspension was then diluted to 2.5 μg/mL in 20mM phosphate buffer mixture containing either 0%, 20% or 40% acetonitrile. Desired pH was obtained by adding concentrated HCl or NaOH to negligible volume. The pH of all mixtures was further confirmed using a pH meter after addition of all components and incubated at room temperature for overnight followed by filtration through membrane filter tubes (Amicon Ultra; 100KDa MWCO). 25μL of each filtrate was then loaded in 15% native polyacrylamide gel and run it in TBE buffer (pH-7.4) at 50 mA for 90 min. The gel was then analysed by incubating in Syber Gold solution for 45 min followed by scanning with Typhoon FLA 9500. The pH and hydrophobicity dependent DNA dissociation was quantified by investigating absorbance of the filtrates at 260 nm. Experiment was performed in three independent sets.

PDN dissociation and DNA release in the cellular environment: DNA dissociation from the PDN in cellular environment was investigated using fluorescently (6-FAM) labeled DNA by confocal microscopy. PDN was prepared with fluorescently-labeled green dye 6-FAM. Free DNA was removed by filtering through Amicon ultra (100 KDa MWCO) centrifugal filter tube. Human bladder cancer cells HTB-9 (ATCC 5637) were seeded in each chamber of two sterile chamber slides in 200 μL complete media (RPMI + 10%FBS + 1% Penicillin & Streptomycin). After 24 hours, media was removed and

cells were treated with complete media containing PDN (final concentration 0 or 2 μg/mL) followed by overnight incubation at 37^{o}C in a CO_2 incubator. Cells were then washed three times with sterile PBS and imaged using a Zeiss Axiovert LSM-510 microscope. PDN were excited using the 633 nm laser and emission was collected with the 650nm long-pass filter set. DNA labeling green dye (6-FAM) was excited using the 488 nm argon laser and emission collected with 505 – 530 nm bandpass filter set. DIC images of cells were collected in a separate channel for overlay.

Cellular internalization of PDN by Endocytosis: Cellular uptake of PDN by endocytosis was followed by colocalization with FITC labeled dextran using confocal microscopy. Human bladder cancer cells HTB-9 (ATCC 5637) were seeded in sterile chamber slides in 200 μL complete media (RPMI + 10%FBS + 1% Penicillin & Streptomycin). After 24 hours, media was removed and cells were treated with media containing PDN (final concentration 2 μg/mL) and FITC-dextran complex (final concentration 1mg/mL) followed by incubation overnight at 37^{o}C in a CO_2 incubator. Cells were then washed three times and maintained in sterile PBS followed by imaging using a Zeiss Axiovert LSM-510 microscope. PDN were excited using the 633 nm laser with emission collected with the 650nm long-pass filter set. Dextran labeling FITC was excited using the 488 nm argon laser with emission collected with 505 – 530 nm bandpass filter set. DIC images of cells were collected in a separate channel for overlay.

In vitro **Heating of Nanoparticle Suspension**: PDN suspensions were irradiated at 420 nm using a pulsed laser with constant power of 158mW. Sample heating was evaluated for a range of nanoparticle concentrations (5 – 100μg/mL) at four (1,2,5,10 min) different time points.

Light-Dependent Cytotoxicity of PDN: The cytotoxicity of PDN upon blue light irradiation was determined using a MTS assay. Human bladder cancer cells, HTB-9 (ATCC 5637) were seeded in each of two 96 well plates at a cell density of 5000 cells/well in 200 μL complete media (RPMI + 10%FBS + 1% Penicillin & Streptomycin). After 24 hours, media was removed and cells were washed twice with 500 μL sterile PBS. Cells were then treated with complete media containing nanoparticle at

final concentrations of 0, 1, 2, 5 µg/mL followed by overnight incubation at 37^{o}C in a CO_2 incubator. Cells were then washed three times thoroughly with sterile PBS and maintained in PBS. One of the two plates was exposed to 27 W, 420nm blue light (Trophy Skin Blue MD) for 10min inside the biological safety cabinet. PBS was replaced by complete media followed by incubation at 37^{o}C in a CO_2 incubator for 24 hours. Cell viability was assessed using the CellTiter 96 AQ_{ueous} One Solution Cell Proliferation Assay reagent (Promega) following the manufacturer's instructions. All experiments were done in three independent sets. Each set of data (net absorbance) were expressed as a percentage, considering the no treatment group as 100%.

N-acetyl cysteine rescue in cell viability: N-acetyl cysteine (NAC) was used as a free radical scavenger to evaluate the role of diffusible free radical in PDN-mediated cytotoxicity. Tissue culture, PDN and/or light treatment including cell viability assay were performed following the same protocol as mentioned for the light dependent cytotoxicity of PDN. A final concentration of 2.5 mM NAC was included as required. All experiments were done in triplicate. Each set of data (net absorbance) were expressed as a percentage, considering the no treatment group as 100%.

Evaluation of apoptosis induced by PDN upon blue light irradiation: Apoptosis induced by the nanoparticle upon blue light irradiation was evaluated using a caspase activity assay. Tissue culture and PDN treatment including light treatment were performed following the same protocol as mentioned for the light dependent cytotoxicity of PDN. Caspase activity was then assessed using the Caspase Glo 3/7 assay (Promega) following the manufacturer's instructions. Luminescence was measured using GENios (TECAN) microplate reader. All experiments were done in three independent sets. Each set of data (net luminescence) were expressed as a percentage, considering the no treatment group as 100%.

Estimation of DNA Damage by Comet Analysis: PDN-mediated DNA damage upon blue light irradiation was estimated by analyzing comet tail moments in a single cell gel electrophoresis assay. Human bladder cancer cells HTB-9 (ATCC 5637) were seeded in two six-well plates replicating each

other at a cell density of 100,000 cells/well in 2 mL complete media (RPMI + 10%FBS + 1% Penicillin & Streptomycin) and incubated at 37^{o}C in a CO_2 incubator. After 24 hours, media was removed and cells were washed twice with 500 µL sterile PBS. Cells were then treated with complete media containing PDN at final concentration 0 or 2 µg/mL in combination with N-acetyl cysteine (NAC; 0 or 2.5 mM) or z-VAD (0 or 0.01mM) and incubated overnight in a CO_2 incubator. Next morning cells were washed three times with sterile PBS to remove excess nanoparticles and maintained in PBS with the same level of NAC or z-VAD. One of the two plates was then exposed to the 420nm blue light (Trophy Skin Blue MD) for 10min inside the biological safety cabinet. PBS was then replaced by fresh complete media containing the same level of NAC or z-VAD and incubated at 37^{o}C overnight. Cells were washed with sterile PBS and trypsinized followed by addition of fresh complete media such that the final cell count was 1x10^{5} cell/mL. 50µL of the cell suspension was then mixed with low melting agarose and 50 µL of the resulting mixture were spread on the comet slide (TREVIGEN). The slides maintained at ambient temperature for 30 min then incubated at 4^{o}C in pre-chilled lysis solution (TREVIGEN) for 1 hour. Slides were washed with water and placed in neutral electrophoresis buffer (TREVIGEN) followed by electrophoresis at 24V for 1 hour at 4^{o}C. Slides were further washed with water and placed in DNA-precipitating solution in the dark at ambient temperature. Then washed with water and incubated for 30 min in 70% EtOH at room temperature in the dark followed by oven-drying at 50^{o}C for 1 hour. Stained with Syber Green and visualized using a Zeiss Axioplan-2 microscope using excitation at 488 nm and emission with a 520 nm filter. Images were captured and analyzed with HCSA version-2.3.6.1 (Loats Associates Inc.) software.

Light-Dependent Membrane Damage by PDN (Calcein-AM): Localized membrane damaging activity of PDN upon blue light irradiation was evaluated using confocal microscopy with a cytoplasm localizing fluorescent dye. Human bladder cancer cells HTB-9 (ATCC 5637) were seeded in each of two sterile chamber slides in 200 µL complete media (RPMI + 10%FBS + 1% Penicillin & Streptomycin). After 24 hours, media was removed and cells were treated with complete media

containing PDN (final concentration 0 or 2 µg/mL) followed by overnight incubation at 37^{o}C in a CO_2 incubator. Cells were then washed three times with sterile PBS followed by addition of Calcein-AM (Invitrogen) at final concentration 2.5µM and incubation for 15min at 37^{o}C. One of the two chamber slides was then exposed to 420nm blue light (Trophy Skin Blue MD) for 10min inside the biosafety cabinet under aseptic condition. Excess dye was washed with sterile PBS and cells were imaged using a Zeiss Axiovert LSM-510 microscope. PDN were excited using the 633 nm laser and emission was collected with the 650nm long-pass filter set. Calcein-AM dye was excited using the 488 nm argon laser and emission was collected with 505 – 530 nm bandpass filter set. DIC images of cells were collected in a separate channel for overlay. All experiments were done in three independent sets.

Detection of ROS generated by PDN upon light irradiation using C11 Bodipy: PDN suspension was prepared in 20 mM phosphate buffer (pH-7.4) as described above to a final concentration of 250 µg/mL. Acetonitrile buffer mixture was prepared by adding appropriate amount of 100 mM sodium phosphate and water to acetonitrile. PDN suspension was added to the mixture to final concentration 5 µg/mL followed by addition of the dye C11 Bodipy (final concentration 20 µM) in a 96 well black flat bottom plate (Coster). Mixtures were prepared in triplicates in two plates. One of the plate was then exposed to the blue light (420 nm) for 2 min. Plates were immediately scanned using Tecan-Safire-II microplate reader in fluorescence mode with 480 nm excitation and 500 – 800 nm emission. Slit opening was set to 10nm and reading were averaged for 5 actuations.

Light-Dependent Membrane Lipid Peroxidation by PDN (C11-Bodipy): Membrane lipid peroxidation upon blue light irradiation of cancer cells treated with PDN was evaluated using confocal microscopy detecting a membrane localizable fluorescent dye (C11-Bodipy). Tissue culture, PDN and/or light treatment including C11-Bodipy (final conc 0.5µM) was performed following the same protocol as mentioned in the membrane damage detection by Calcin-AM. Excess dye was removed using sterile PBS and cells were imaged with Zeiss axiovert LSM-510 microscope. PDN excitation

used a 633 nm laser with emission collected using a 650nm longpass filter set. The green fluorescence of the oxidized dye was excited with 488 nm argon laser with emission collected using the 505 – 530 nm bandpass filter set. Red fluorescence of the non-oxidized dye was excited with a 543 nm He-Ne laser and emission was collected using 565 – 615 bandpass filter. DIC images of cells were collected in a separate channel for overlay. All experiments were done in three independent sets and representative images are displayed in figures.

Detection of PDN mediated membrane damage by TEM: The nanoparticle mediated membrane damage upon blue light irradiation was detected using Transmission Election Microscopy (TEM). Tissue culture as well as PDN and/or light treatment were performed using same protocol as mentioned in the light dependent cytotoxicity of PDN. Cells were fixed with 2.5% glutaraldehyde applied followed by incubation for 30 min in room temperature after which cells were washed three times with PBS, treated with 2% osmium tetroxide for 30min and washed again three times with PBS. Cells were then incubated with 25% ethanol solution for 10min followed by incubation in 50% ethanol solution for another 10 min to dehydrate. Cells were then scraped with rubber policeman into microfuge tubes and spun for 10 min. Cells were then slowly dehydrated to 100% ethanol by incubating in 75%, 85%, or 95% ethanol solution for 10min followed by centrifugation. Cells were then incubated in 100% propylene oxide for 10 min. Cell pellets were then infiltrated with Spurr's resin using the following dilutions: 1:1 for 1 hour; 1:2 for 2 hours; pure resin for 1 hour followed by embedding in Beem capsules and cured overnight at 60 ^{o}C. 80 nm sections were then cut using a ultra-microtome followed by staining with uranyl acetate and lead citrate and viewed with Tecnai Spirit BioTwin TEM at 80 kEv.

PDN mediated Light-Dependent Membrane Lipid Peroxidation by mass-spec: The PDN mediated peroxidation of membrane lipid upon blue light stimulation was estimated by mass-spectrometry. Tissue culture as well as PDN and/or light treatment were performed using same protocol as mentioned in the light dependent cytotoxicity of PDN. The cell suspension was then

transferred to a glass vial and 100 µL methanol-cocktail was added. All glass vials were immediately filled with argon and froze at -20^{o}C. Experiments were performed in triplicate. All corrosponding replicate from each group was then combined and lipid extraction was performed followed by running samples into mass-spec.

PDN-Mediated Light-Dependent Antitumor Activity *in vivo*: All animal experiments were performed under protocols approved by the animal care and use committee of Wake Forest University Baptist Medical Center. Tumor xenografts were generated by sub-cutaneous injection of $1.5x10^{6}$ human bladder cancer cells suspended in 200 µL of 1:1 PBS/Matrigel (BD bioscience) in both flanks of 10 female nude mice. Mice were used for experimental procedures 3 weeks following inoculation with tumor cells, after tumor size had reached approximately 75 mm^{3}. Each of the 10 mice was injected with 100 µL of 250 µg/mL nanoparticle suspension in one flank and 100 µL saline in the other flank. Approximately 12 hours later, tumors on both flanks of 6 mice were irradiated with a blue beam of 420nm from Ti:Sapphire laser (Coherent Inc., Santa Clara, CA) for 3 min. The beam was transmitted directly into the tumor using a multimode optical fiber (SFS105/125Y, Thorlabs Inc., Newton, NJ) with a numerical aperture of 0.22 and core diameter of 105 microns. The output power at the final end of the fiber was 100 mW. The remaining four mice were treated identically but were not exposed to the laser beam. The laser irradiation was performed every 5th day for a total of five doses. Tumor sizes were measured using calipers and photographs of tumors were taken once per week. Tumor volumes were calculated using the formula $xy^{2}\pi/6$ (where x and y are the long and short diameters of the tumor, respectively). The tumors were analyzed as four independent groups: i) no treatment; ii) light-only; iii) PDN-only; iv) PDN + light. Relative tumor volumes (V/V_{o}) were graphed vs time (where V is the present tumor volume and V_{o} was the tumor volume when treatment started). Repeated measures mixed models were fit to compare tumor volumes between groups. In these models, animals were treated as random effects and group (four-levels) and time (days) were treated as fixed-effects. The group-by-time interaction was examined to determine whether the rate of

change in tumor volume differed over time among the four groups. All statistical analyses were performed using SAS 9.1.

Histopathological Analysis of Tumor Tissue : At the conclusion of the study animals were sacrificed and tumors were excised carefully and placed in 4% paraformaldehyde solution overnight at ambient temperature. The following day, tumors were embedded in molten paraffin and thin sections were prepared from different layers of tumors and placed on glass slides followed by H&E staining.