Supporting Information

NaCl nanoparticles as a cancer therapeutic

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Abbreviation list

NPs	Nanoparticles
SCNPs	Sodium Chloride Nanoparticles
PSCNPs	Phospholipid-coated Sodium Chloride Nanoparticles
RB-PSCNPs	Rhodamine B-labeled PSCNPs
TEM	Transmission Electron Microscopy
DLS	Dynamic Light Scattering
SEM	Scanning Electron Microscopy
EDS	Energy Dispersive Spectroscopy
XRD	X-ray Diffraction
FT-IR	Fourier-transform Infrared
DMEM	Dulbecco's Modified Eagle's Medium
RPMI 1640	Roswell Park Memorial Institute Medium 1640
SBFI-AM	Sodiumbinding Benzofuran Isophthalate Acetoxymethyl Ester
MQAE	N-(Ethoxycarbonylmethyl)-6-Methoxy-Quinolinium Bromide
PBFI-AM	Potassium-Binding Benzofuran Isophthalate Acetoxymethyl Ester
OCR	Oxygen Consumption Rate
MSR	Mitochondrial Respiration Rate
ROS	Reactive Oxygen Species
PBS	Phosphate-buffered Saline
EthD-III	Ethidium homodimer III
$[Na^+]_{int}$	Intracellular sodium concentration
$[K^+]_{int}$	Intracellular potassium concentration
TDLNs	Tissue Draining Lymph Nodes
DMAPs	Damage Associated Molecular Patterns
CRT	Calreticulin
ATP	Adenosine Triphosphate
HMGB-1	High-Mobility Group Box 1 protein
APCs	Antigen-Presenting Cells
DCs	Dendritic Cells
ICD	Immunogenic Cell Death
F/T	Freeze and Thaw

Materials and Methods

Synthesis of sodium chloride nanoparticles (SCNPs). In a typical synthesis, 20 mg of sodium oleate (TCI, 97%, Lot No.: W76EGFQ), 1 mL of oleylamine (70%, Sigma-Aldrich, Lot No.: STBF9554V) and 50 mg 1,2-tetradecanediol (90%, Sigma-Aldrich) were dissolved in a mixed solution containing 10 mL hexane (99.9%, Fisher) and 10 mL ethanol (99.9%, Fisher). 1,2-tetradecanediol serves as a reductant in the reaction. Into the mixture, 15 mg of molybdenum (V) chloride (95%, Sigma-Aldrich, Lot No.: MKBQ9967V) was added and the solution was magnetically stirred for 24 h at 60 ^{o}C. The raw products were collected by centrifugation at 12000 RPM for 10 min. The particles were redispersed in hexanes with brief sonication and the centrifugation/hexane washing process was repeated 3 times to remove unreacted precursors.

Phospholipid-coated sodium chloride nanoparticles (PSCNPs). The above-synthesized SCNPs (10 mL) in hexane were sonicated for 30 s and mixed with 80 µL phospholipid solution (1 mg/mL) DSPE-PEG (2000) Amine (1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-N-[amino(polyethylene glycol)-2000] (ammonium salt), Avanti, Lot No.: 180PEPEPEG2NH2-64). For rhodamine B labeled PSCNPs (RB-PSCNPs), Liss Rhod PE (1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine-N-(lissamine rhodamine B sulfonyl, ammonium salt, Avanti, Lot No.: F160LRPE-33) solution in chloroform (40 µL, 1 mg/mL) was added into 10 mL SCNPs as well. The mixture was allowed to sonicate for 30 s. The solvent was removed under reduced pressure at 40 °C using a Buchi R II Rotavapor. 10 mL PBS/water was then added to the flask and the mixture was sonicated for 30 s. Fresh-made PSCNPs were used for characterizations *in vitro* and *in vivo* studies unless specified otherwise. All the particle doses were calculated based on NaCl concentration unless specified otherwise.

Characterizations of NPs. X-ray diffraction (XRD) analysis was carried out on a Bruker D8-Advance using dried samples placed on a cut glass slide with Cu Kα1 radiation (λ = 1.5406 Å). Scanning electron microscopy (SEM) and energy dispersive X-ray spectra EDS elemental mapping images were acquired on a FEI Teneo field emission SEM equipped with an Oxford EDS system. Transmission electron microscopy (TEM) was carried out on an FEI Tecnai20 transmission electron microscope operating at an accelerating voltage of 200 kV. High resolution TEM analysis was performed on a Hitachi transmission electron microscope H9500 operating at a 300 kV accelerating voltage. Particle size and zeta potential measurements were carried out on a Malvern Zetasizer Nano ZS system. Fourier-transform infrared (FT-IR) spectra were recorded on a Nicolet iS10 FT-IR spectrometer.

Stability and release experiments. PSCNPs were dispersed in 100 µl ammonium acetate buffers (pH=5 or pH=7) and added into a Slide-A-Lyzer™ MINI Dialysis Device (MWCO = 2K, Cat# 69550, Thermofisher, US). Place the unite into a 5 mL Eppendorf tube containing 4.5 mL ammonium acetate buffer. Keep the tube on the shaker (20 rpm) at room temperature. At different time points (0, 10 min, 0.5, 1, 2, 4, 6, 12, 24 h), take 400 µL of PSCNPs solution from the Eppendorf tube to test the free ions concentration. A Na^+ electrode (HORIBA LAQUAtwin Na-11) was used to measure free Na^+ ions, while MQAE (N-(ethoxycarbonylmethyl)-6-methoxy-quinolinium bromide, Setareh Biotech, Lot No.: 50610) were used to measure the free Cl^- ions in the solution. All measurements were performed following the manufacture's protocol and repeated in sextuplicate.

Cell culture. 4T1 (murine mammary carcinoma), HT29 (human colorectal adenocarcinoma), A549 (human lung carcinoma), SGC7901 (human gastric adenocarcinoma), PC-3 (human prostate adenocarcinoma) and UPPL-1541 cells (murine bladder carcinoma) were grown in RPMI-1640 (Corning, 10-040-CV). U87MG (human glioblastoma) and RAW264.7 cells (murine macrophage) were grown in DMEM (Corning, 10-013-CV). B16-F10 cells (murine melanoma) were grown in high glucose DMEM (ATCC® 30-2002™). SCC VII cells (murine head and neck squamous carcinoma) were grown in Corning® DMEM (Dulbecco's Modified Eagle's Medium)/Hams F-12 50/50 Mix (Corning, 10-090-CV). All the cell culture medium were supplemented with 10% fetal bovine serum (FBS) and 100 units/mL of penicillin and 100 units/mL streptomycin (MediaTech, USA). Human primary prostate epithelial cells (HPrECs, ATCC, PCS440010) were maintained in serum free conditions with prostate epithelial cell growth kit (ATCC PCS440040). The mouse spermatogonial cell line (C18-4) was established from germ cells isolated from the testes of 6-day-old Balb/c mice,[1] and the cells were cultured in DMEM (Corning, 10-013-CV), containing 5% FBS, and 100 U/ml streptomycin and penicillin. All cells were maintained in a humidified, 5% carbon dioxide atmosphere at 37 °C.

MTT assay to study cytotoxicity. Cells were seeded into 96-well plates at the density of 1×10^4 cells per well and incubated overnight. Then the cells were treated with PSCNPs dispersed in PBS, PSCNPs pre-aged in PBS (1, 3, 8 and 24 h), or NaCl salt at a dose range of 13.2-320 μg·NaCl/mL for 6 or 24 h. MTT assays (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide, Sigma) were performed following the manufacture's protocol. The absorbance at 570 nm was measured by a microplate reader (Synergy Mx, BioTeK).

Live/dead assay to assess time-dependent cytotoxicity. The time-dependent cytotoxicity of PSCNPs was evaluated using a live/dead viability/cytotoxicity kit (Biotum, Cat No.: 30002). After incubating with PSCNPs at a dose of 52.5, 105 or 160.0 μg/mL (NaCl concentration, the same below), PC-3 cells were washed with PBS twice and stained with 2 μM Calcein AM and 3 μM of PI for live and dead cells detection, respectively. All the cells were co-stained with 10 μM Hoechst 33342 (Life Technologies) for nucleus observation. Quantitative time-lapse fluorescence microscopy was conducted and sequential images were automatically acquired on an Arrayscan™ VTI HCS reader using the HCS StudioTM 2.0 Target Activation BioApplication module (Thermo Scientific, MA) at 0, 2, 4, 6, and 12 h after treatment with PSCNPs. PBS and 10 μM $CdCl_2$ (termed as Cd) were analyzed as a negative and a positive control, respectively. For all measurements, 49 fields per well and approximately 5000 cells were analyzed using a 40× objective (NA 0.5), a Hamamatsu ORCA-ER digital camera in combination with a 0.63× coupler, and Carl Zeiss microscope optics in an auto focus and high resolution mode. Channel two (Ch2) used a BGRFR 485-20 filter for Calcein AM dye (live cell) imaging. Channel three (Ch3) used a BGRFR 549-15 filter for ethidium homodimer-III (dead cell) imaging. High-content multichannel analysis (HCA) was analyzed using HCS Studio 2.0 Target Activation BioApplication (Thermo Scientific, MA).

Intracellular concentrations of PSCNPs, Na^+, Cl^-, K^+ fluorescence staining, image acquisition and high-content analysis. Microscope studies were carried out on a Cellomics® ArrayScan® VTI HCS Reader with a live cell chamber and the HCS Studio™ 2.0 Cell Analysis Software (Thermo Scientific). For all measurements, 49 fields per well and approximately 5000 cells were analyzed using a 40× objective (NA 0.5), a Hamamatsu ORCA-ER digital camera in combination with a 0.63× coupler, and Carl Zeiss microscope optics in auto focus and high resolution mode with three channels. Image smoothing was applied to reduce object fragmentation prior to object detection. Channel one (Ch1) used the BGRFR 386-23 filter for Hoechst 33342 staining that was

used for auto-focus, object identification, and segmentation. Ch2 used a BGRFR 485-20 filter for SBFI-AM (sodiumbinding benzofuran isophthalate acetoxymethyl ester, Setareh Biotech, Lot No.: 50609), PBFI-AM (potassium-binding benzofuran isophthalate acetoxymethyl ester, Setareh Biotech, Lot No.: 5027), and MQAE imaging. Ch3 used a BGRFR 549-15 filter for RB-PSCNPs imaging. High-content multichannel analysis (HCA) was analyzed using HCS Studio 2.0 Target Activation BioApplication (Thermo Scientific, MA). Single-cell based HCA provided multiple parameters to characterize the nucleus, the number of cells, and total or average intensity of each cell. Total intensity was defined as all pixels within a cell. Average intensity was defined as all the pixels within a cell divided by the total area of the cell. Specifically, for PSCNP cellular uptake, PC-3 cells were incubated with RB-PSCNPs for 0, 2, 4 and 6 h. Then, LysoTracker® Green DND-26 (molecular probes) and Hoechst 33342 dyes were co-stained for 10 min. Fluorescent images were obtained every 10 min. For intracellular Na^+, Cl^- and K^+ characterization, PC-3 cells were treated with RB-PSCNPs for 0, 2, 4 or 6 h. The cells were then incubated with 10 μM SBFI-AM in 0.04% Pluronic F-127 (Sigma, Lot No.: SLBB4267V), 10 mM MQAE, or 10 μM PBFI-AM in 0.04% Pluronic F-127, respectively for Na^+, Cl^- and K^+ staining. The final fluorescence signal was measured by Ch2.

Mitochondrial electric potential ($\Delta\Psi_m$). The change of mitochondrial membrane potential was measured by a JC-1 mitochondrial membrane potential detection kit (Biotium, Cat No.: 30001). The JC-1 working solution was prepared by adding 10 μL of the concentrated dye to 1 mL of FBS-free RPMI medium. PSCNPs (52.5, 105 or 160 μg/mL), PBS, and NaCl (160.0 μg/mL in PBS) were incubated with cells for 6 h. The medium was removed and replaced with the JC-1 working solution and the incubation took another 15 min. The stained cells were analyzed on an Array Scan VTI reader by analyzing Ch2 (green, JC-1 monomeric dye), and Ch3 (red, JC-1 aggregated dye) signals. The red/green ratio was analyzed by HCS Studio 2.0 Target Activation BioApplication software (Thermo Scientific, MA).

Oxygen consumption rates (OCR). PC-3 cells (20,000/well) were seeded in Seahorse XFe 24 assay plates and cultured in 250 μL of RPMI1640 medium overnight. Cells were washed and incubated with Seahorse base medium supplemented with 2 mg/mL of glucose, 1 mM of glutamine, and 1 mM of sodium pyruvate (pH 7.4) for 1 h. After 3 consecutive measurements of basal metabolic rates, PSCNPs (52.5, 105 or 160 μg/mL) or PBS was mixed with the cells. The metabolic rates were measured every 30 min up to 6 h. For each measurement, the cells were sequentially treated with 2 μM of oligomycin, 3 μM of FCCP (Carbonyl cyanide 4-(trifluoromethoxy) phenylhydrazone), and 3 μM antimycin/3 μM rotenone and analyzed 3 times for each stage. Respiration rate in support of ATP production was calculated as OCR differences before/after the oligomycin treatment.

ATP level. Luminescent ATP detection assay kit (Abcam, ab113849) was used to determine cellular ATP contents following the manufacturer's protocol. PC-3 cells were grown in a 96-well plate at the density of 1×10^4 cells per well, and were incubated with various concentrations of PSCNPs (52.5, 105 or 160 μg/mL) for 6 h. 50 μL of Lysis buffer was added into each well and incubated for 5 min under shaking on an orbital shaker at 700 RPM. Then, 50 μL of the reconstituted substrate solution was added into each well and the mixture was shaken for 15 min in dark. The luminescence intensity of each well was measured on a microplate reader (Synergy Mx, BioTeK) and normalized to that in control cells.

ROS generation and lipid peroxidation. PC-3 cells were subcultured in a 96-well plate at the density of 1×10^4 cells per well, then were incubated with PSCNPs at a concentration of 52.5, 105, or 160 μg/mL for 6 h. The treated cells were incubated with 10 μM of DCFH-DA (2′,7′-dichlorofluorescin diacetate, Sigma) and the 529-nm fluorescence intensity was measured on a microplate reader (Synergy Mx, BioTeK). Cells were incubated with PSCNPs at a concentration of 52.5, 105, or 160 μg/mL for 6 h for lipid peroxidation analysis. The treated cells were incubated with 10 μM of lipid peroxidation sensor (Life technologies) for 30 min in complete growth medium at 37 °C. The cells were washed once with PBS and then the fluorescence intensity of the reduced state (red, ex/em: 530/590 nm) and oxidized state (green, ex/em: 488/560 nm) were analyzed. The data were represented as red/green fluorescence intensity ratios.

DNA damage and caspase-3 activation. γ-H2AX and caspase-3 double immunostainings were preformed to confirm DNA damage and the activation of caspase-3 apoptotic pathway. PC-3 cells were seeded in a 96-well plate at a density of 1×10^4 cells per well and cultured overnight. Cells were then incubated with PSCNPs at a dose of 52.5, 105 or 160 μg/mL for 24 h. The treated PC-3 cells were fixed with 4% paraformaldehyde for 30 min at room temperature, followed by 3 repeated washes with PBS. After fixation, the cells were permeabilized by 0.1% Triton X-100 in PBS, incubated with a mouse anti-phospho-Histone-H2AX antibody (Ser139, γ-H2AX, Millipore, Massachusetts), and a mouse anti-cleaved-caspase-3 antibody (Cell Signaling, #9664) in a PBS/BSA/0.5% Tween 20 solution at 4 °C overnight. After washing twice with PBS/BSA, the cells were incubated with goat anti-rabbit Dylight 650, mouse anti-rabbit Dylight 488 (Thermo Scientific, MA), and Hoechst 33342 in a PBS/BSA solution for 90 min at room temperature. Flow cytometry (Beckman Coulter, CytoFLEX S) was conducted for signal quantification.

Cytochrome *c* release induced by PSCNPs. PC-3 cells were seeded in 2-well chamber slide (Nunc™ Lab-Tek™ II Chamber Slide™ System, ThermoFisher) at the density of 4×10^5 cells per well for overnight growth. Then the cells were incubated with PSCNPs at a concentration of 26.3, 52.5 or 160 μg/mL for 6 h. Cytochrome *c* were analyzed by ApoTrack™ Cytochrome c Apoptosis ICC Antibody Kit (ab110417) following manufacture's protocol. Confocal images were taken at 100× magnification on a Zeiss LSM 710 Confocal Microscope and analyzed by ImageJ to compare the fluorescence intensity.

Western blot analysis. Antibodies used were phospho-JNK1/2 (Thr183/Tyr185) (Cell Signaling; 4668), JNK1/2 (Cell Signaling; 9252), phospho-ERK1/2 (Cell Signaling; 4370), ERK1/2 (Cell Signaling; 4695), phospho-p38 MAPK (Cell Signaling; 4511), p38 MAPK (Cell Signaling; 8690), cleaved caspase-3 (Cell Signaling, 9661), α-Tubulin (Abcam, 7291), NLRP3 (D4D8T) Rabbit mAb (Cell Signaling, 15101).

PC-3 cells were incubated with PSCNPs at a concentration of 160 μg/mL for 6 h. The cells were then analyzed for cell stress, in particular the impact on the JNK/p38 MAPK pathways. PBS, NaCl solution (160 μg/mL), and PSCNPs pre-aged in PBS (160 μg/mL) were used as negative controls. For NLRP3 inflamasome studies, PC-3 cells were incubated with PSCNPs at a concentration of 52.5 or 160 μg/mL for 6 h. PBS, NaCl solution (160 μg/mL), and PSCNPs pre-aged in PBS (160 μg/mL) were used as negative controls. Cell lysates were prepared by homogenizing cells in a RIPA buffer supplemented with 1× proteinase inhibitor cocktail (Amresco). Protein concentration was determined using a bicinchoninic acid (BCA) protein assay kit (Thermo Fisher Scientific). Protein lysates were loaded onto 10% SDS-PAGE and were transferred to PVDF membrane. Nonspecific binding to the membrane was blocked by incubation with 5% nonfat milk at room

temperature for 1 h. The membranes were incubated for 16 hours at 4 °C with primary antibodies at the dilutions specified by the manufacturers. After secondary antibody incubation for 1 h at room temperature, membranes were treated with ECL reagents (Thermo Fisher Scientific) and exposed to X-ray films (Santa Cruz). All the imaging results were analyzed by ImageJ.

Cell morphology changes and cell expansion. The PC-3 cell morphology changes were monitored by taking bright-field images every 20 minutes between 2 and 6 h of incubation with PSCNPs (160 μg/mL) on a Cellomics® ArrayScan® VTI HCS Reader. A time-lapse video was generated using the bright-field images to show the morphology changes. For cell volume changes, PSCNPs of different concentrations (52.5, 105, and160 μg/mL) were incubated with PC-3 cells and individual cell volume (n = 5000, measured in pixels) at different time points were analyzed by the HCS Studio™ 2.0 Cell Analysis Software (Thermo Scientific). The 98% quantile of PBS treated cells (37500 pixels) was used as a benchmark.

TEM images of cells. PC-3 cells were incubated with PSCNPs (160 μg/mL) for 0, 2, 4, or 6 h. Cell cultures were briefly rinsed with 0.1 M Cacodylate-HCl buffer with 5% sucrose (w/v, pH 7.25). The buffer was immediately poured out of the culture dish and replaced with a fixative containing 2.5% glutaraldehyde in 0.1 M Cacodylate-HCl buffer (pH 7.25). Cells were fixed for 1 h at room temperature. The fixative was removed from the culture dish and the cells were rinsed briefly with buffer and then post-fixed in buffered 2% (v/v) osmium tetroxide for 1 h at 4 °C. A rubber policeman was used to detach cells from the culture dish. The samples were pipetted into Eppendorf snap-cap microcentrifuge tubes and centrifuged for 10 min to concentrate cells into a sample pellet before each of the following changes: Samples were rinsed three times in distilled water for 10 min each; dehydrated in a graded ethanol series for 10 min at each step: 25%, 50%, 75%, 95%, 100% and 100% followed by two changes of 10 min each in 100% acetone; infiltrated in acetone and Spurrs resin (Electron Microscopy Sciences) for 1 h or overnight: 75% acetone and 25% Spurrs, 50% acetone and 50% Spurrs, 75% acetone and 25% Spurrs, 100% Spurrs, 100% Spurrs. Samples were embedded in fresh 100% Spurrs resin and polymerized at 60 °C for 24 h in the Eppendorf tubes. The samples were removed from the tubes and mounted on plexiglass cylinders (Ted Pella) with Loctite super glue. The pelleted cell region was trimmed with a razor blade and sectioned using a Reichert-Jung Ultracut S ultramicrotome. Sixty nanometer thick sections were picked up on slot grids and allowed to dry down onto Formvar-coated aluminum racks. Grids were post-stained with uranyl acetate and lead citrate and viewed with a JEOL JEM 1011 transmission electron microscope operating at 80kV with an AMT mid-mount digital camera with 3000x3000 resolution.

Plasma membrane potential. The plasma membrane potential change was measured with $DiBAC_4(3)$ (bis-(1,3-dibutylbarbituric acid) trimethine oxonol, Invitrogen, Lot No.: 14D1001). After the addition of PSCNPs at different concentrations (52.5, 105 or 160 μg/mL) and time points (30-150 min), PC-3 cells were incubated with 5 μM $DiBAC_4(3)$ for 30 min at 37°C. The green fluorescence from $DiBAC_4(3)$ was measured by a Cellomics® ArrayScan® VTI HCS Reader and analyzed using the HCS Studio™ 2.0 Cell Analysis Software.

Apoptosis/necrosis cell death. Apoptosis/necrosis was assessed through Annexin V/EthD-III staining by Apoptotic, Necrotic, and Healthy Cells Quantification Kit (Biotium, Cat No.:30018). PC-3 cells (5×10^4) were seeded on a tissue culture dish (Corning, 35 mm×10 mm) and were grown overnight. PSCNPs (160 μg/mL) were added to the dish. A working dye solution was made according to the manufacture's protocol. Briefly, into a 100 μL diluted binding buffer, 5 μL

Hoechst 33342, 5 μL of FITC-Annexin V, and 5 μL ethidium homodimer-III (EthD-III) was added. After incubating with PSCNPs for 0, 2, 4, or 6 h, the cells were washed with PBS, and incubated with the dye working solution for 15 min. Fluorescence images were acquired on a fluorescence microscope using the DAPI channel for Hoechst 33342, the FITC channel for AnnexinV-FITC, and the TRITC channel for EthD-III.

Cathepsin B release and caspase-1 activation. The Magic Red Cathepsin B kit and the FAM-FLICA® Caspase-1 Assay kit were purchased from ImmunoChemistry Technologies, LLC (Bloomington, MN). PC-3 cells were seeded in a 8-well chamber slide (Nunc™ Lab-Tek™ II Chamber Slide™ System, ThermoFisher) at the density of 5×10^4 cells per well and were cultured overnight. Then the cells were incubated with PSCNPs (160 μg/mL) or NaCl salt (160 μg/mL) for 6 h. Nigericin (20 μM) was used as a positive control (24 h incubation). The materials treated cells were stained with either Magic Red or FAM-FLICA® Caspase-1 at 37 °C following the manufacture's protocols. The cells were then fixed in a 4% paraformaldehyde PBS solution and mounted with VECTASHIELD anti-fade mounting medium containing DAPI (H-1200) (Vector Laboratories, US). Confocal images were taken at 100× magnification on a Zeiss LSM 710 Confocal Microscope.

Caspases-1 and caspases-3/7 activation measured by flow cytometry. For caspase-1 analysis, PC-3 cells were seeded at a density of 1×10^6 cells per well in a 6-well plate overnight and then incubated with PSCNPs (160 μg/mL) for 1 or 6 h. The FAM-FLICA® Caspase-1 kit was used for cellular staining following the manufacturer's protocol. All the cells were collected and analyzed on a Beckman Coulter CytoFLEX system using the FITC channel. The results were analyzed with FlowJo v10 for caspase-1 activation. For caspase-1 and caspase-3/7 side-by-side comparison study, PC-3 cells were seeded at a density of 1×10^6 cells per well in a 6-well plate and were cultured overnight. The cells were incubated with 160 μg/mL PSCNPs for 6 h, or at 52.5 μg/mL for 24 h. H_2O_2 (0.5 mM, 24 h incubation) and Nigericin (20 μM, 24 incubation) were used as caspase-3/7 and caspase-1 positive controls, respectively. The FAM-FLICA® Caspase-1 and FLICA 660 Caspase-3/7 Assay Kits (ImmunoChemistry Technologies, LLC) were used for cell staining. All the cells were collected and analyzed on a Beckman Coulter CytoFLEX system, using 488-nm excitation for caspase-1 measurement and 633-nm excitation for caspse-3/7 measurement. All the data were analyzed with FlowJo v10.

IL-1β secretion. PC-3 cells at a density of 1×10^4 cells per well were seeded in a 96-well plate one day before the experiment. The cells were incubated with PSCNPs (105 or 160 μg/mL) for 6 h. NaCl salt (160 μg/mL) and Nigericin (20 μM) with 24 h incubation were studied as negative and positive control, respectively. The supernatants were collected and the IL-1β contents were quantified using R&D Systems Human IL-1beta DuoSet ELISA (Minneapolis, MN).

LDH assays. (a) LDH release study: PC-3 cells were plated overnight at a density of 1×10^4 cells per well in a 96-well plate. The cells were incubated with PSCNPs in a dose of 13.2, 26.25, 52.5, 105, 160, 220 or 320 μg/mL for 6 h. PBS and NaCl salt at the same dose were used as control materials. Supernatants were collected and the LDH contents were analyzed by LDH Assay Kit–WST (CT01-05, Dojindo, Japan). The results were normalized to PBS treated control cells. **(b) Necrosis inhibition study**: PC-3 cells were plated overnight at a density of 1×10^4 cells per well in a 96-well plate. These cells were pre-treated with necrosis inhibitor glycine (5 mM) or capsase-1 inhibitor Ac-YVAD-cmk (30 μg/mL) for 1 h, and then incubated with PSCNPs (160 or 320 μg/mL) for 6 h. Cell without glycine or Ac-YVAD-cmk treatment were studied as controls. Supernatants

were collected and the LDH contents were analyzed by LDH Assay Kit–WST (CT01-05, Dojindo, Japan). The results were normalized to lysing control cells.

Intracellular sodium contents. A panel of cell lines, including 4T1, HT29, A549, SGC7901, PC-3, U-87 MG, B16-F10, RAW264.7, HPrECs and C18-4 cells, were cultured in 75 cm^2 Corning cell culture flasks in a humidified, 5% carbon dioxide atmosphere at 37 °C. Cells were collected when they reached 85% confluency and the cell numbers were counted using a hemocytometer. After centrifugation (1200 rpm, 5 min), the cell pellets were washed with 5 mL Na^+-free HEPES buffer three times. The final cell pellets were suspended in D.I. water and homogenized by probe sonication. The intracellular sodium concentration $[Na^+]_{int}$ was measured using a Na^+ electrode (HORIBA LAQUAtwin Na-11). The results were normalized to cell numbers to obtain intracellular sodium content ($[Na^+]_{int}$) for each cell line.

***In vivo* therapy study.** Animal studied were performed according to a protocol approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Georgia. All the animals were purchased from Charles River and maintained under pathogen-free conditions. PC-3 tumor model was generated by subcutaneously injecting 2×10^6 cells in 50 μL PBS into the right flank of 5-6 week old male athymic nude mice. U-87 MG tumor model was generated in female athymic nude mice following the same method as PC-3 model. UPPL-1541 tumor model was generated by subcutaneously injecting 1×10^6 cells in 50 μL PBS into the right flank of 5-6 week old female C57BL6 mice. B16F10 tumor model was generated by subcutaneously injecting 2×10^5 cells in 50 μL PBS into the right flank of 5-6 week old female C57BL/6 mice. SCC VII tumor model was generated by subcutaneously injecting 2×10^5 cells in 50 μL PBS into the right flank of 5-6 week old female C3H/HeN mice.

For therapy studies, PC-3 tumor bearing mice were randomly divided into 2 groups (n = 5 for each group). When the average tumor volume was about 100 mm^3, PSCNPs (9 mg/mL, 50 μL) were intratumorally injected on day 0, 2 and 4. For control, saline at the same volume was injected. For both PSCNPs and saline, the injection was performed at five sites of the tumor to ensure good coverage. The tumor size and body weight were inspected every two days. The tumor was measured in two dimensions with a caliper, and the tumor volume was estimated as $(\text{length})\times(\text{width})^2/2$. U-87 MG tumor model followed the same therapy method as PC-3 tumor model. B16F10, SCC VII tumor models were treated with PSCNPs when the average tumor volume was about 40 mm^3, while UPPL-1541 tumor model was treated at 100 mm^3. PSCNPs (27 mg/mL, 50 μL) were intratumorally injected on day 0, while saline at the same volume was injected as control group. The tumor size and body weight measurements were the same as PC-3 model. At the end of the PC-3 tumor therapy experiment, autopsies were performed. The tumor were dissected for morphological and histological examination. In particular, these tissues were sectioned into 4 μm slices for H&E, TUNEL staining (*in situ* Apoptosis Detection Kit, ab206386, Abcam, US) and caspase-1 staining. The caspase-1 IHC staining kit was purchased from Abcam, US. The kit includes anti-caspase-1 antibody (ab1872), goat anti-rabbit IgG H&L (HRP) (ab6721), rabbit specific HRP/DAB (ABC) detection IHC kit (ab64261), and methyl green pyronin (RNA DNA Stain) (ab150676). All the staining followed the manufacturing protocols.

CRT expression on cell surface in Flow cytometry assessment. B16F10 or SCC VII cells are seeded into the 6 wells plate at 1×10^6 per well. After overnight incubation, use PSCNPs (160 μg/mL) to treat cells for 2 h. PBS treated cells were used as a control. All the cells were collected with cell lifters, and an Alexa Fluor® 647-conjugated anti-CRT antibody (ab196159, 1/500,

Abcam) for 30 min at 4 °C. The cells are incubated in 500 µL PBS containing 50 µg/mL propidium iodide before washing and assessment in a flow cytometer. The data were expressed in histogram compared to the PBS treated control cells.

ATP and HMGB-1 release. Cells were seeded into 96-well plates at the density of 1×10^4 cells per well and incubated overnight. Then the cells were treated with PSCNPs dispersed in PBS at a dose range of 13.2-320 µg/mL for 1, 2, 4 h and 24 h. Cell supernatant was collected after 1-4 h incubation and tested in ATP 1step Luminescence Assay System, 100 mL ATP Assay Kit (PerkinElmer, US) following the manufacture's protocol. A 10-fold serial dilution series of ATP in culture medium (1 µM to 1 pM) were created to build up a standard curve and calculate the absolute amount of ATP in the supernatant. The luminescence was measured by a microplate reader (Synergy Mx, BioTeK). Cell supernatant was collected after 24 h incubation and tested in an ELISA kit (IBL International GmbH), according to the manufacturer's instructions. NaCl salt (160 µg/mL) and PBS were used as controls.

***In vivo* vaccination approach to induce immune response.** Animal studies were performed according to a protocol approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Georgia. B16F10 cells were exposed to PBS, 320 µg/mL NaCl NPs for 6 h, as well as F/T method to induce ICD. The dying 2×10^5 B16F10 cells were injected into the right flank of 5-6 week old female C57BL6 mice (Charles River) (n=5). 6 days after the injection, the animals received SC injection of viable B16F10 cells 2×10^5 in the contralateral (left) flank. Similar as B16F10 cells, SCC VII cells were exposed to PBS and 320 µg/mL NaCl NPs for 6 h to induce IDC biomarkers release. The dying 2×10^5 SCC cells were injected twice into the right flank of 5-6 week old female C3H/HeN mice (Charles River) (n=5), 6 days apart. 12 days after the injection, the animals received SC injection of viable SCC cells 2×10^5 in the contralateral (left) flank. Tumor size was measured by a digital caliper every 2-3 days. The tumor volume was calculated according to the formula $(\text{length})\times(\text{width})^2/2$. Animals were sacrificed on Day 22 for B16F10 tumor model and on Day 24 for SCC VII tumor model.

***In situ* vaccination and cancer therapy in SCC VII bilateral tumor model.** Cell were mixed with Corning Matrigel Matrix (#356234) for tumor inoculation. SCC VII bilateral tumor model was created by SC injecting 1×10^6 SCC cells into the right flank as the primary tumor and 0.5×10^6 SCC cells in the left flank as the secondary tumor of 5-6 week old female C3H/HeN mice (Charles River) (n=5). 12 days after the injection, the animals received one time NaCl NPs treatment. Each mouse in NPs group was injected 1.35 mg NaCl NPs in 50 µL saline. Saline treated group (50 µL) was used as a negative control. The tumor volume was calculated according to the formula $(\text{length})\times(\text{width})^2/2$. On Day 12, primary and secondary tumors, spleen, blood and TDLNs were collected after euthanizing the animal to conduct flow cytometry study.

Flow cytometry analysis. The tumor pieces obtained for single-cell analysis were cut into smaller pieces with scissors and digested in DMEM with 0.5 mg/mL collagenase type I (Worthington Biochemical Corporation) at 37 °C for 1 h. The digested tissues were gently meshed though a 70 µM cell strainer, twice. Red blood cells were lysed by Ack lysing buffer (Gibco) according to the manufacturer's instructions. The single-cell suspensions were washed twice and resuspended in staining buffer. Following cell counting and aliquoting, staining was performed by using various combinations of fluorophore-conjugated antibodies for 20 min at 4 °C. The following anti-mouse antibodies were purchased from BD Biosciences: CD45-APC-Cy7 (#557659), CD4-BV605 (#563151), CD8α-FITC (#553030), CD11c-V450 (#560521), CD86-BV605 (#563055), CD80-

PerCP-Cy5.5 (#560526). Foxp3-PE-Cy7 (#60-5773), live/dead cell assay Ghost Red 710 (#13-0871) were purchased from TONBO biosciences. IFN-γ-APC (#505810), CD3-PE (#100206), CD45R/B220-FITC (#103206) and CD19-PE (#152408) were purchased from BioLegend. Multi-parameter staining was used to identify the following populations of interest: (a) $CD8^+$ T cells ($CD45^+CD3^+CD8^+$), (b) $CD8^+IFN\gamma^+$ T cells ($CD45^+CD3^+CD8^+IFN\gamma^+$) (c) Tregs ($CD45^+CD3^+CD4^+Foxp3^+$), (d) $CD80^+CD86^+$ DCs ($CD45^+CD11c^+CD80^+CD86^+$) (e) $CCR7^+$ DCs ($CD45^+CD11c^+CD80^+CD86^+CCR7^+$). For intracellular Foxp3 and IFN-γ staining, cells were further fixed and permeabilized using Fixation/Permeabilization Solution Kit (BD, 554714). After washing, cells were used for flow cytometry analysis (CytoFLEX, Backman Coulter). The data were processed by FlowJo 10.0. Doublets were excluded based on forward and side scatter. Dead cells were excluded beased on negative signal of Ghost Red 710 staining.

Statistical analysis. For *in vitro* study, all measurements were performed in sextuplicate unless specified otherwise. Data obtained from high-content BioApplication Studio 2.0 were exported and further analyzed using a JMP statistical analysis package (SAS Institute, North Carolina). Half-maximum inhibitory concentration (IC_{50}) was determined by Doseresp using Origin 9. The median lethal concentrations (LC_{50}) were calculated with a curve-fitting program using GraphPad Prism 5 (San Diego, California). For *in vivo* study, all measurements were performed three times unless specified otherwise. One tailed Student's *t* test was used for comparison among groups, with *P* values of 0.05 or less representing statistical significance.

Discussion on Computational Simulation

Model and Methodology

The simulation box described in **Figure S13** contained 18,000 lipid molecules that formed a spherical cell. Moreover, 289,000 water beads were included to mimic the aqueous environment. Periodic boundary conditions were applied in three directions of the simulation box. The mass, length, and time scales were all normalized in the simulations, with the unit of length taken to be σ, the unit of mass to be that of the lipid beads, and the unit of energy to be ε. All other quantities are expressed in terms of these basic units. We used a Velocity-Verlet algorithm to perform time integration, and Langevin thermostat to control the system temperature T. The integration time step is

$$\Delta t = 0.01\tau \qquad (1)$$

(where τ is 15 ns).

All simulations were performed using a LAMMPS package.[2] The radius of the cell was 50 σ. The cell as shown contained enough lipids on membrane to mimic the mechanical rupture occurring in a real cell. Similar approximation was used to study the mechanical deformation of red blood cell by Yuan et al.[3]

Each lipid molecule in the computational model was represented by one head bead followed by two tail beads.[4] The following potentials were used in the simulation to describe interactions between lipid beads:

The size of a lipid was fixed via a Weeks-Chandler-Andersen potential

$$U_{WCA} = 4\epsilon\left[\left(\frac{b}{r_{ij}}\right)^{12} - \left(\frac{b}{r_{ij}}\right)^{6} + \frac{1}{4}\right], r_{ij} < r_c = \sqrt[6]{2}b \quad (2)$$

where ϵ is the depth of the potential well, b is the finite distance at which the inter-particle potential is zero, and r_{ij} is the distance between the particles. In order to ensure the cylindrical lipid shape, b was set as $b_{head,head} = b_{head,tail} = 0.95\sigma$ and $b_{tail,tail} = \sigma$. The three beads in a single lipid were linked by two FENE bonds:

$$U_{FENE} = \sum_{bonds} -\frac{1}{2}k_{fene}R_{max}^2 \ln\left(1 - \frac{{r_{ij}}^2}{R_{max}^2}\right) \quad (3)$$

with the stiffness $k_{fene} = 30\,\varepsilon/\sigma^2$ and the divergence length $R_{max} = 1.5\sigma$. Lipids were straightened by a harmonic spring

$$U_{stretching} = \sum_{bonds} k_{stretch}(r_{ij} - r_0)^2 \quad (4)$$

with the bending stiffness $k_{stretch} = 10\,\varepsilon/\sigma^2$ and the equilibrium length $r_0 = 4\sigma$ between the head bead and the second tail bead. The hydrophobic effect was compensated by an attractive interaction between the tail beads as

$$U_{cos} = \begin{cases} -\epsilon, & r_{ij} < r_c \\ -\epsilon cos^2[\pi(r_{ij} - r_c)/2w], & r_c \le r_{ij} \le r_c + w \\ 0, & r_{ij} > r_c + w \end{cases} \quad (5)$$

which describes an attractive potential with depth ε that smoothly tapers to zero for $r > r_c$. In our case, the decay range w was set as 1.6σ. The interaction between solvent and lipid heads in cell membrane was described by the Lennard-Jones potential function

$$U_{LJ} = 4\epsilon\left[\left(\frac{b}{r_{ij}}\right)^{12} - \left(\frac{b}{r_{ij}}\right)^{6}\right], r_{ij} < r_c = 2.5\sigma \quad (6)$$

(where b is set as $b_{water,head} = \sigma$).

Relationship between the ion concentration gradient and water flux

We assumed that the sodium and chloride concentrations were spatially uniform and the cell membrane was semipermeable to water, meaning that water particles can freely pass through the membrane. In our simulation, this process was represented by adding the water beads to the cytoplasm step-by-step. Mathematically, the osmotic pressure Π under certain sodium and chloride concentrations can be estimated using the Van't Hoff equation [5]:

$$\Pi = cRT \quad (7)$$

(where c is the osmolarity, R is the gas constant, and T is the absolute temperature). To simplify the problem, we don't consider the hydrostatic pressure. The net chemical osmotic pressure difference $\Pi_{in} - \Pi_{out}$ drives the water flux across the semipermeable membrane. Therefore, the volume of water passing through a unit area of membrane per unit time can be modeled as proportional to the chemical osmotic pressure difference

$$J_0 = qV_w(c_{in} - c_{out}) = \frac{qV_w}{RT}(\Pi_{in} - \Pi_{out}) \quad (8)$$

(where q represents the permeability rate for cells as 10^{-5}~10^{-4} m/s; V_w represents the molar volume of water, 18.016 mL [5]). Supposing that cells are symmetrical spheres, the total volume of water injected to the interior of a cell can be estimated as

$$\boldsymbol{V = J_0 D^2 \int_0^{2\pi} d\phi \int_0^{\pi} sin\theta d\theta = \pi D^2 J_0 = \pi D^2 \alpha (c_{in} - c_{out}) RT} \quad (9)$$

(where D is the cell diameter). Therefore, the concentration difference across the plasma membrane can be expressed as

$$\Delta \boldsymbol{c} = (\boldsymbol{c_{in} - c_{out}}) = \frac{\boldsymbol{V}}{\boldsymbol{\pi D^2 \alpha RT}} \quad (10)$$

The notion of membrane tension with regard to membrane rupture is widely used in cell biological literature. Based on the Law of Laplace, the membrane tension γ is directly proportional to the pressure in a cell and the radius of a cell. It can be calculated by

$$\gamma = \frac{p \cdot D}{4} \quad (11)$$

(p is the pressure on the membrane).

The latter can be calculated by

$$p = \frac{\sigma_{xx} + \sigma_{yy} + \sigma_{zz}}{3} \quad (12)$$

(σ_{xx}, σ_{yy} and σ_{zz} is the stress).

For different size of cells, **Figure S14** shows the critical concentration gradients (Δc) upon which the plasma membrane begins to rupture (red square shadow). By curve fitting these data points, we've obtained an interesting curve that has allowed us to predict the critical concentration for 25 μm cells.

Supporting Figures and Tables

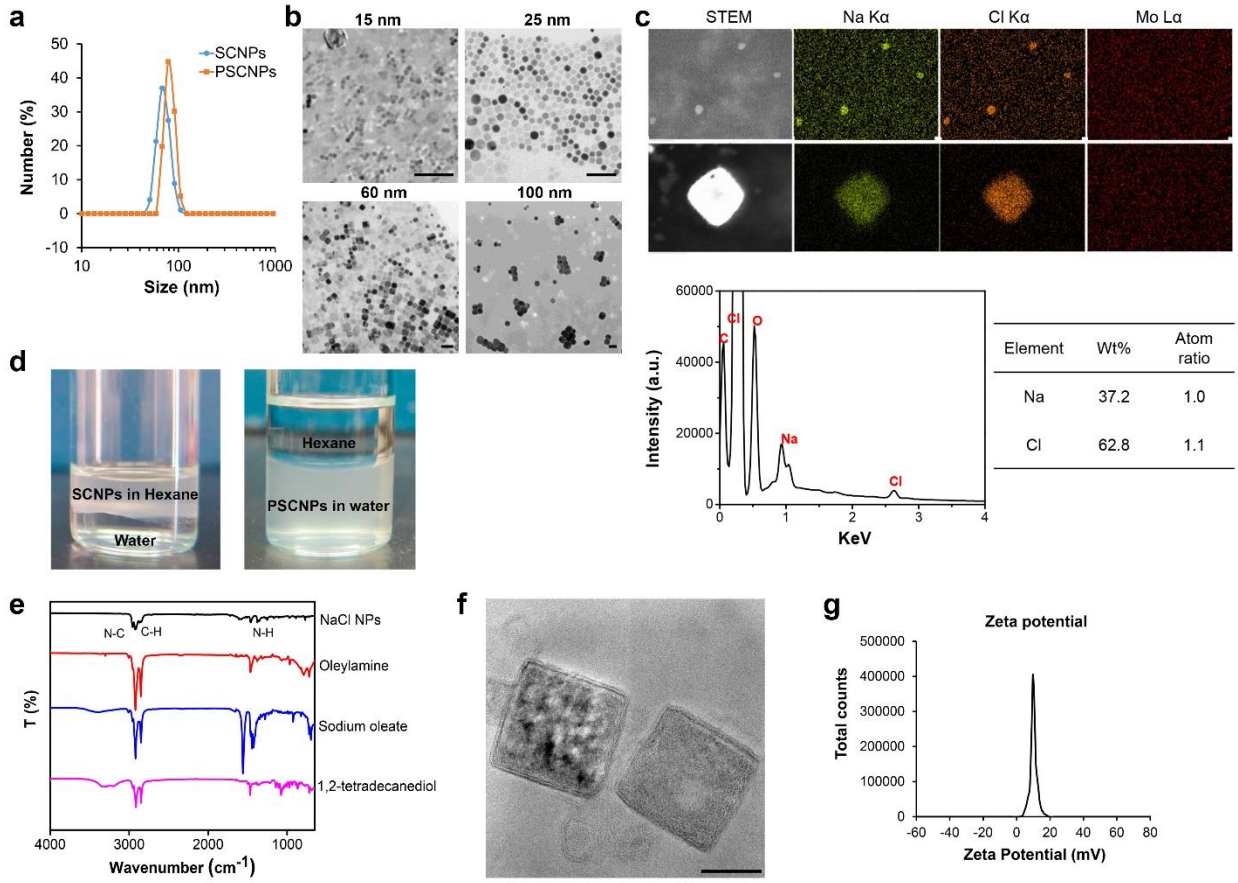

Figure S1. a, Dynamic light scattering (DLS) analysis of SCNPs and PSCNPs. The hydrodynamic size of SCNPs in hexane was 84.6 ± 9.8 nm. After phospholipid coating, the hydrodynamic size was increased to 98.0 ± 13.1 nm in water. **b**, Representative TEM images of SCNPs with different sizes. Scale bars, 100 nm. **c**, STEM images of NaCl NPs as well as EDS elemental mapping. EDS analysis spectrum confirmed that the Na to Cl atom molar ratio was ~1:1. **d**, Uncoated SCNPs dispersed in hexane and phospholipid-coated PSCNPs dispersed in water. **e**, FT-IR spectra of as-synthesized SCNPs, oleylamine, sodium oleate, and 1,2-tetradecanediol. **f**, High-resolution TEM image of PSCNPs. The lipid coating layer can be visualized as a dim rim around the SCNP core. Scale bar, 50 nm. **g**, Zeta potential of PSCNPs in D.I. water, which was +9.7 mV.

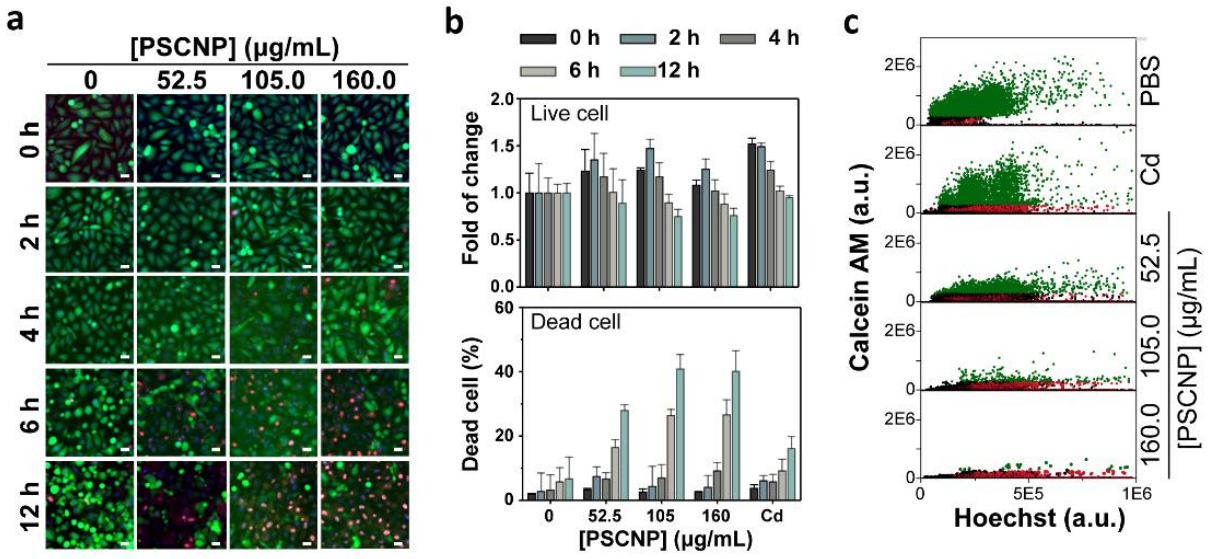

Figure S2. Kinetic cytotoxicity study using Live/Dead (Calcein AM/PI) assay. PSCNPs were incubated with PC-3 cells at a dose range of 0-160 μg/mL from 0 and 12 h. **a**, Representative live/dead assay images at different time points. Live cells were stained with Calcien AM (green), the dead cells were stained with PI (red), and the nuclei were stained with Hoechst 33342 (blue). Scale bars, 50 μm. **b**, Changes in the number of live cells and the percentages of dead cells, based on the results in **a**. For each time point, 5000 cells were analyzed. Cadmium (Cd, 10 μM) was studied as a positive control. **C**, Multi-parametric analysis, based on the 12 h imaging results from **a**. We used the Compartmental Analysis BioApplication software (Thermo Scientific Cellomics V4) to quantify the total fluorescence activity in individual cells (n = 5000) and derived the geometric means for each condition. Green, live cells; red, dead cells; blue, nucleus.

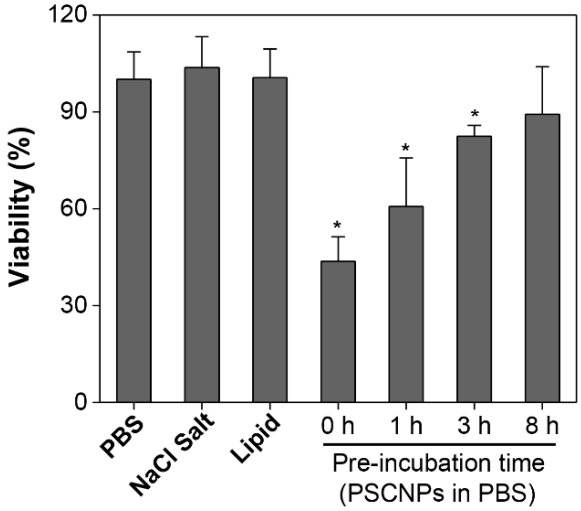

Figure S3. Cell viability, analyzed by MTT assay. PSCNPs (160 μg/mL) were pre-aged in PBS for 1, 3 or 8 h before incubating with PC-3 cells. Standard MTT assays were conducted at 24 h of cell incubation. PBS, NaCl salt (160 μg/mL), and the surface coating material, DSPE-PEG2000 amine (phospholipid), were studied for comparison. The results are expressed as mean ± S.E.M. (* $p < 0.05$ compared to PBS treated control cells).

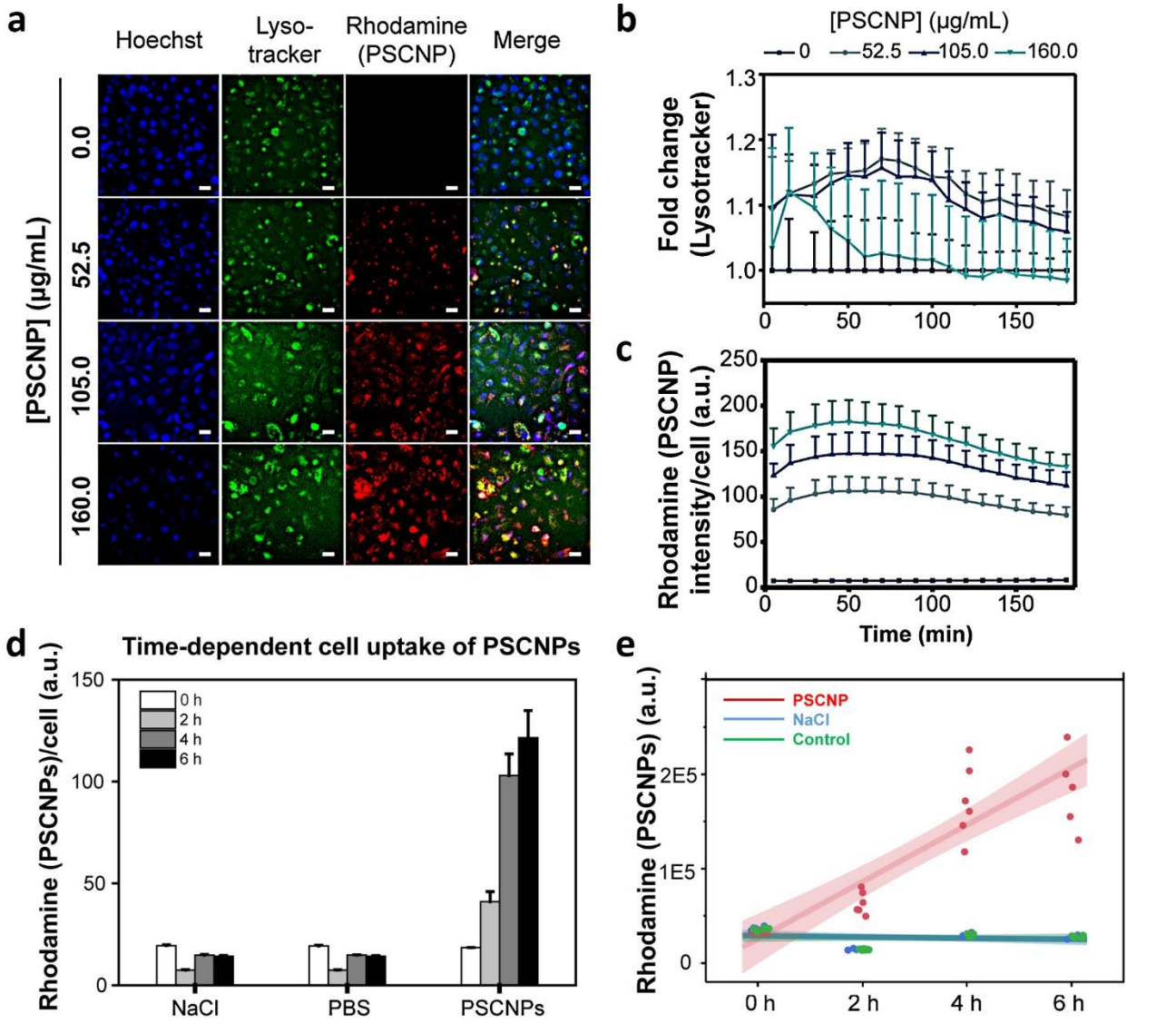

Figure S4. PSCNP cell uptake and intracellular degradation. **a**, Representative live cell images. PC-3 cells were incubated with RB-PSCNPs (0-160 μg/mL). Images were acquired every 10 min for 5000 cells between 0 to 180 min, as well as at 4 and 6 h. Only representative 2 h imaging results were shown. Cell endosomes/lysosomes were labeled with LysoTracker. Red, Rhodamine B (RB-PSCNPs); green, LysoTracker; blue, Hoechst 33342 (nucleus). Scale bars, 50 μm. Good overlap was observed between RB-PSCNPs and LysoTracker signals. **b**, Time-dependent LysoTracker intensity changes based on imaging results from **a** from 0 to 180 min. **c**, Time-dependent Rhodamine intensity changes based on imaging results from **a** from 0 to 180 min. **d**, Histogram comparing intracellular Rhodamine B signals between RB-PSCNPs (160 μg/mL), NaCl (160 μg /mL), and PBS treated cells at 0, 2, 4 and 6 h. The results are based on imaging results from **a** and represented as Rhodamine B intensity per cell (n = 5000 cells). **e**, Scatter plot comparing intracellular Rhodamine B signals between RB-PSCNPs (160 μg/mL), NaCl salt (160 μg/mL), and PBS treated cells. The results are based on imaging results from **a** and represented as Rhodamine B intensity per well (n = 6 wells). Shadow areas represent the 95% confidence intervals of the fit.

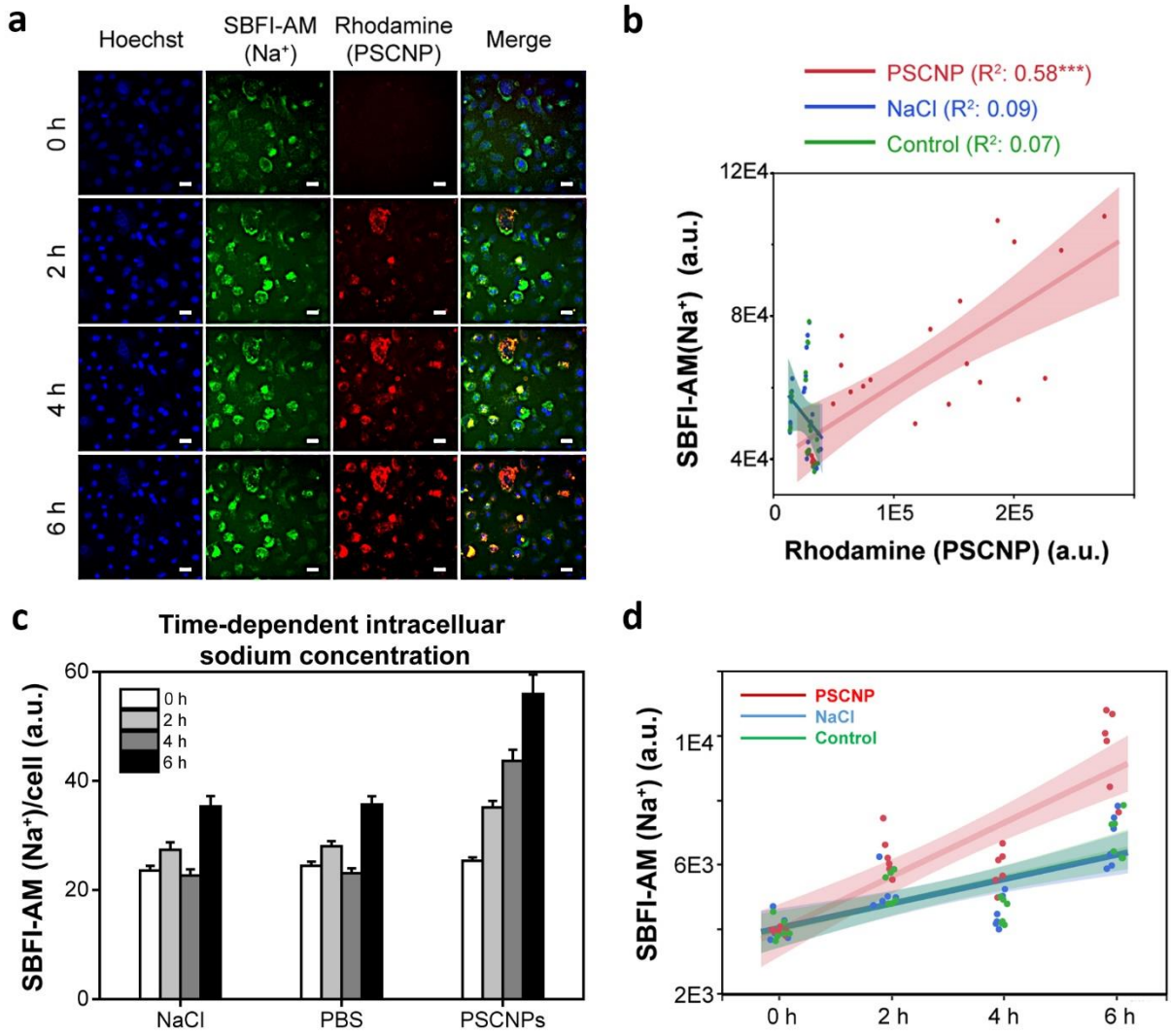

Figure S5. PSCNP cell uptake and intracellular sodium change. **a**, Representative live cell images taken to examine intracellular Na^+ level changes at 0, 2, 4 and 6 h. PC-3 cells were incubated with 160 μg/mL RB-PSCNPs. Red, Rhodamine B (RB-PSCNPs); green, SBFI-AM (Na^+); blue, Hoechst 33342 (nucleus). Scale bars, 50 μm. **b**, Spearman's correlation, based on the fluorescence intensity results in **a**. Shadow areas represent the 95% confidence intervals of the fit. Good correlation was found between Rhodamine B (RB-PSCNPs) and SBFI-AM (intracellular Na^+ concentration). R^2 = 0.58, $p<0.001$. **c&d**, Intracellular Cl^- level changes, based on imaging results from **a**. **c**, Histogram comparing intracellular Na^+ concentrations at different time points. The results are represented as SBFI-AM intensity per cell (n = 5000 cells). PBS and NaCl salt (160 μg/mL) treated cells were studied as controls. **h**, Scatter plot comparing intracellular Na^+ concentration changes. The results are represented as SBFI-AM intensity per well (n = 6 wells).

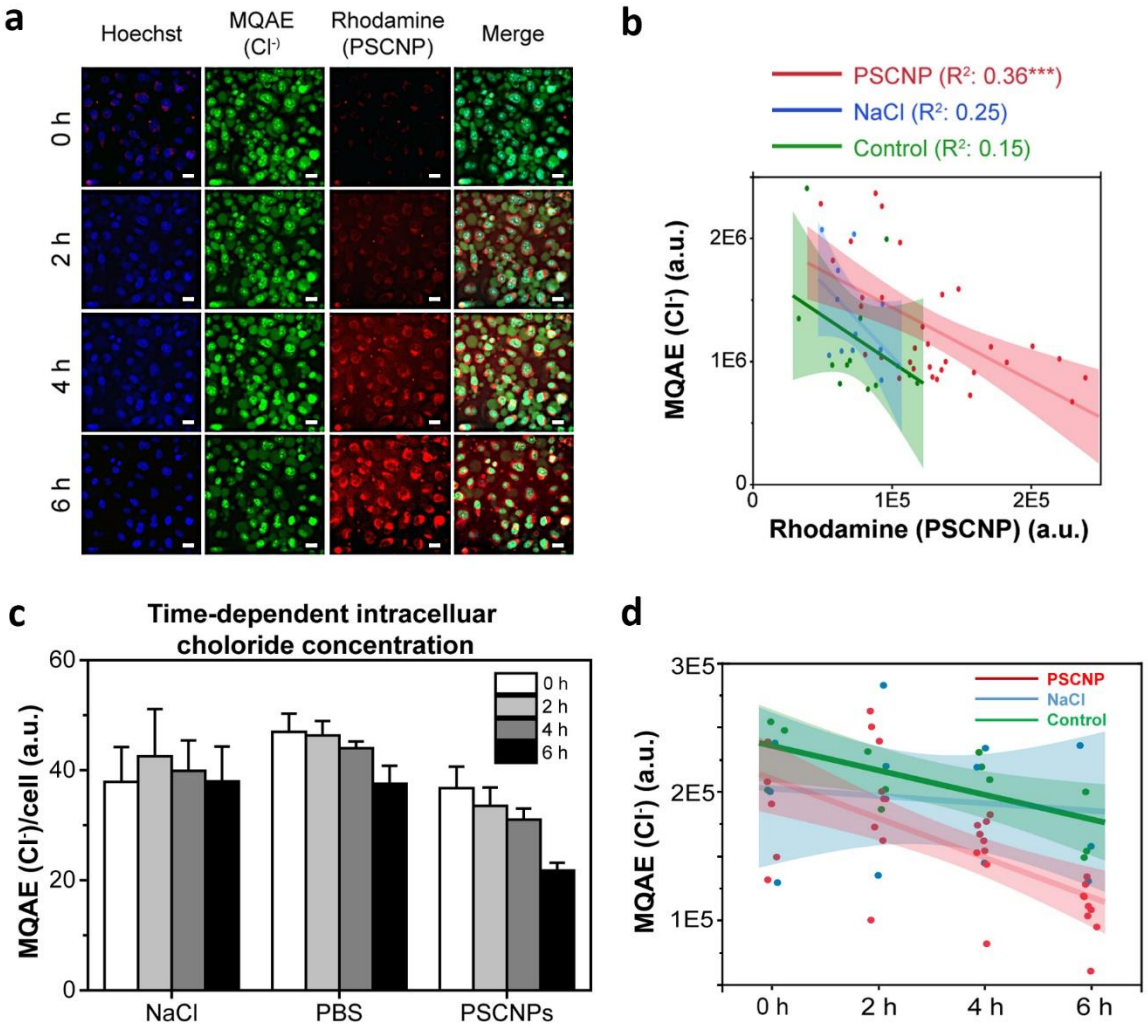

Figure S6. PSCNP cell uptake and intracellular chlorine change. **a**, Representative live cell images taken to examine intracellular Cl^- level changes at 0, 2, 4 and 6 h. PC-3 cells were incubated with 160 μg/mL RB-PSCNPs. Red, Rhodamine B (RB-PSCNPs); green, MQAE (Cl^-); blue, Hoechst 33342 (nucleus). Scale bars, 50 μm. **b**, Spearman's correlation, based on the fluorescence intensity changes in **a**. Shadow areas represent the 95% confidence intervals of the fit. Good correlation was found between Rhodamine B (RB-PSCNPs) MQAE (intracellular Cl^-). $R^2 = 0.36$, $p<0.001$. **c&d**, Intracellular Cl^- level changes, based on imaging results from **a**. Notably, the MQAE signal is reversely correlated with Cl^- concentrations because of fluorescent signal quenches by high Cl^- concentration.[6] **c**, Histogram comparing intracellular Cl^- concentrations at different time points. The results are represented as MQAE intensity per cell (n = 5000 cells). PBS and NaCl salt (160 μg/mL) treated cells were studied as controls. **d**, Scatter plot comparing intracellular Cl^- concentration changes. The results are represented as MQAE intensity per well (n = 6 wells).

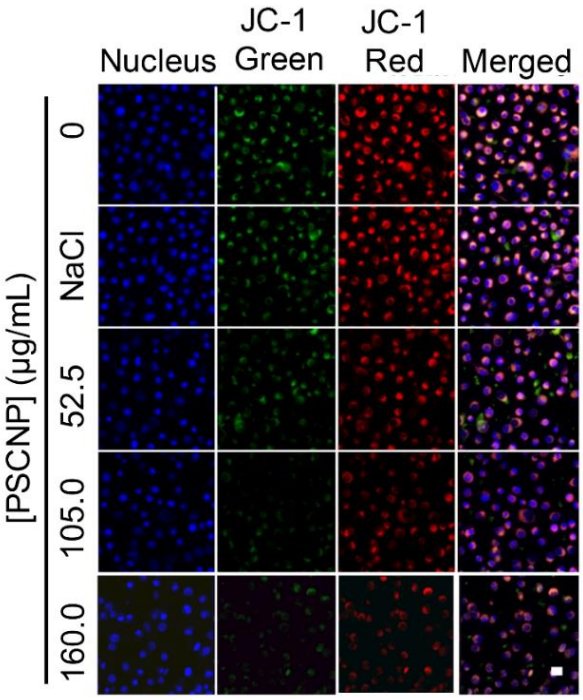

Figure S7. Representative JC-1 staining results. PC-3 cells were incubated with PSCNPs at 52.5, 105 or 160 μg/mL for 6 h before being stained with JC-1 dye. Blue, Hoechst 33342; Green, JC-1 monomer; Red, JC-1 aggregate. Scale bar, 50 μm.

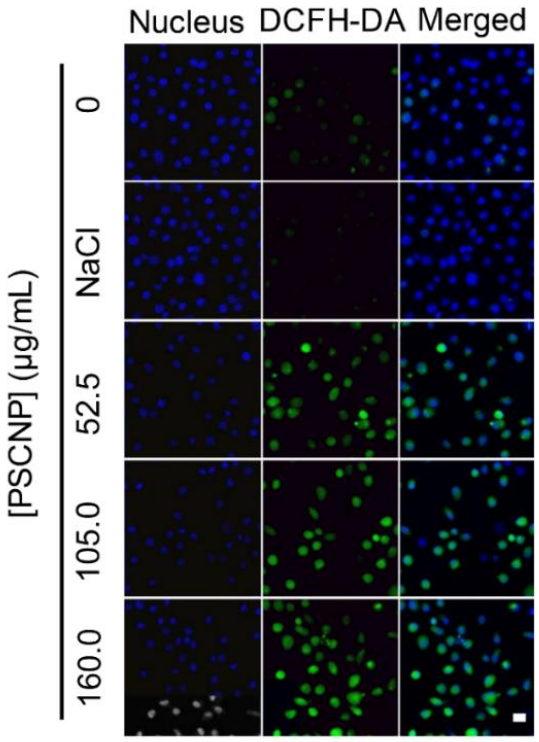

Figure S8. Representative DCFH-DA staining results. PC-3 cells were incubated with PSCNPs of different concentrations (52.5, 105 or 160 μg/mL) for 6 h, followed by staining with 10 μM DCFH-DA. Blue, Hoechst 33342; Green, DCFH-DA. Scale bar, 50 μm.

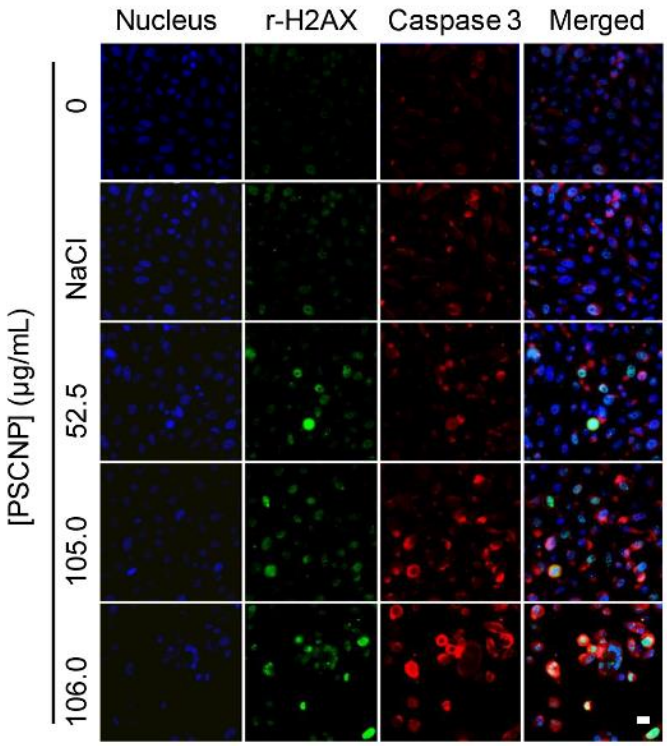

Figure S9. Representative γH2AX and caspase-3 double staining images. PC-3 cells were incubated with PSCNPs (52.5, 105 or 160 μg/mL) for 24 h, followed by staining with both anti-γH2AX and anti-caspase-3 antibodies. Green, γH2AX; Red, Caspase-3; Blue, Hoechst 33342 (nucleus). Scale bars, 50 μm.

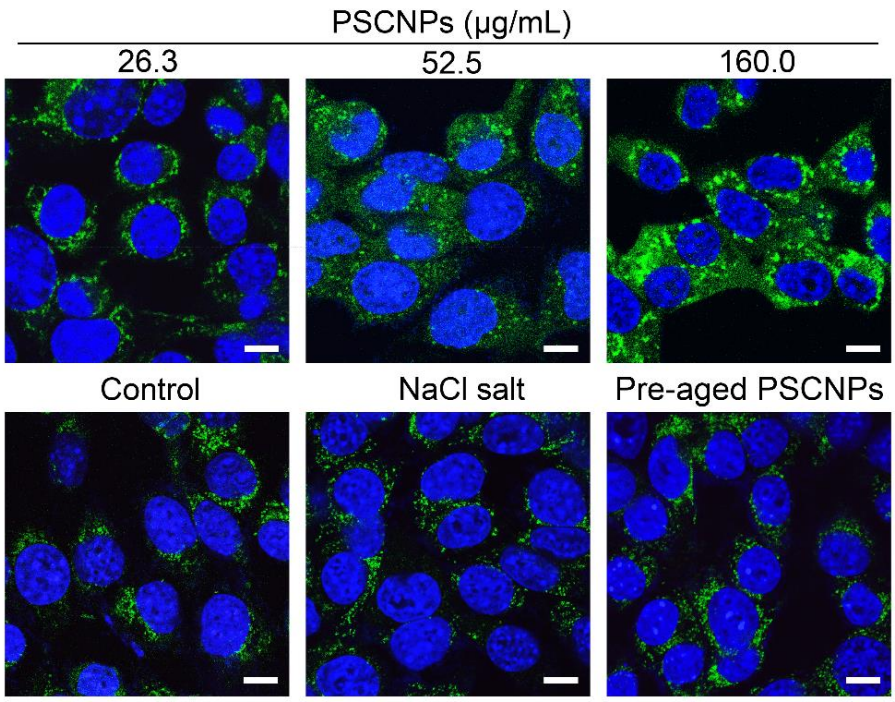

Figure S10. PSCNPs led to cytochrome *c* release into the cytosol. The PC-3 cells were incubated with PSCNPs at 26.3, 52.5 or 160 μg/mL for 6 h. PBS, NaCl salt (160 μg/mL), and PSCNPs pre-aged in PBS (160 μg/mL) were studied as negative controls. The cells were observed on a Zeiss LSM 710 Confocal Microscope at 100× magnification. Scale bars, 10 μm.

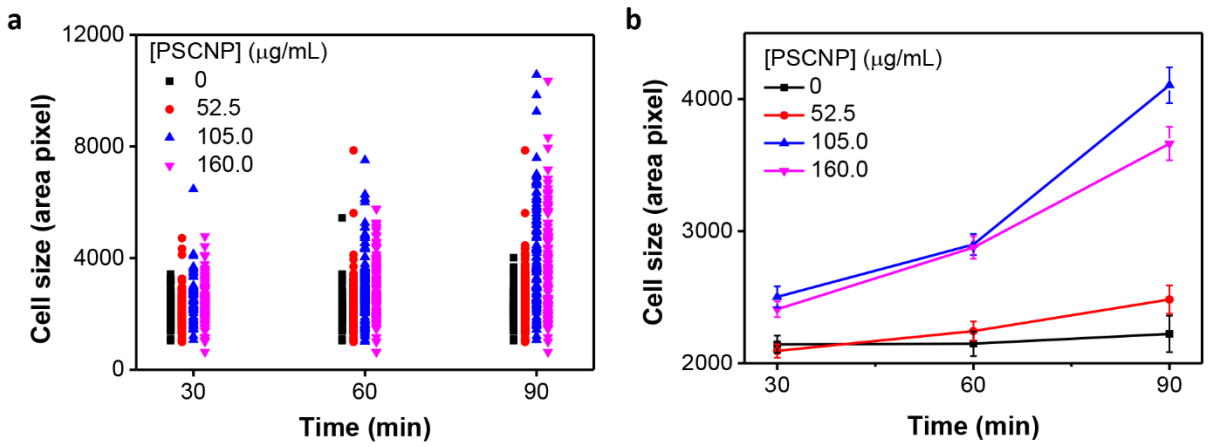

Figure S11. Cell volume changes, based on the imaging results from **Figure 3a**. PC-3 cells were incubated with PSCNPs at varied concentrations (52.5-160 μg/mL). Cell areas (in pixels) at 30, 60, and 90 min were analyzed and compared (n = 5000 cells). **a**, Results shown in a scatter plot. **b**, Average cell sizes at different time points.

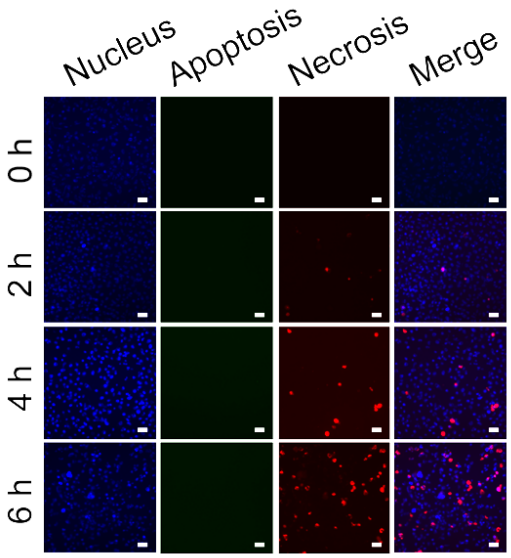

Figure S12. Representative apoptosis/necrosis double staining images. Green, apoptotic cells, stained with FITC-labeled Annexin V; red, necrotic cells, stained with EthD-III; blue, nucleus, stained with Hoechst 33342. Scale bars, 50 μm.

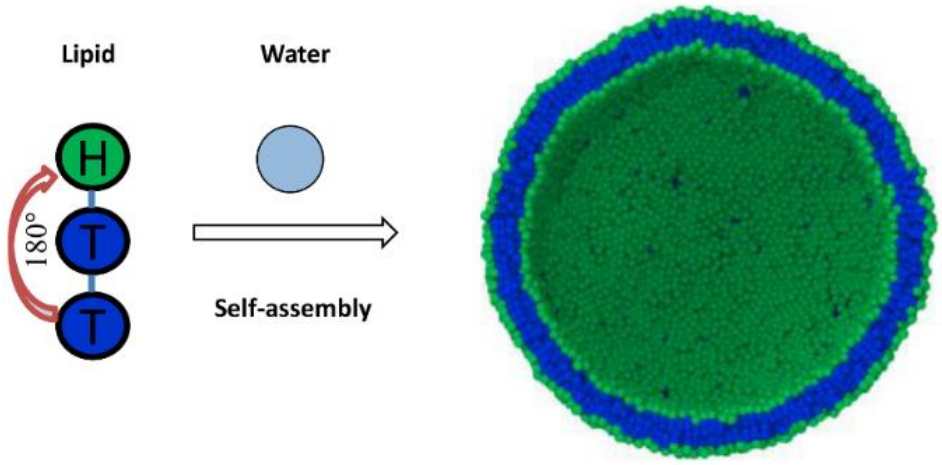

Figure S13. Schematic representation of the computational model for ion concentration induced cell cytolysis**.**

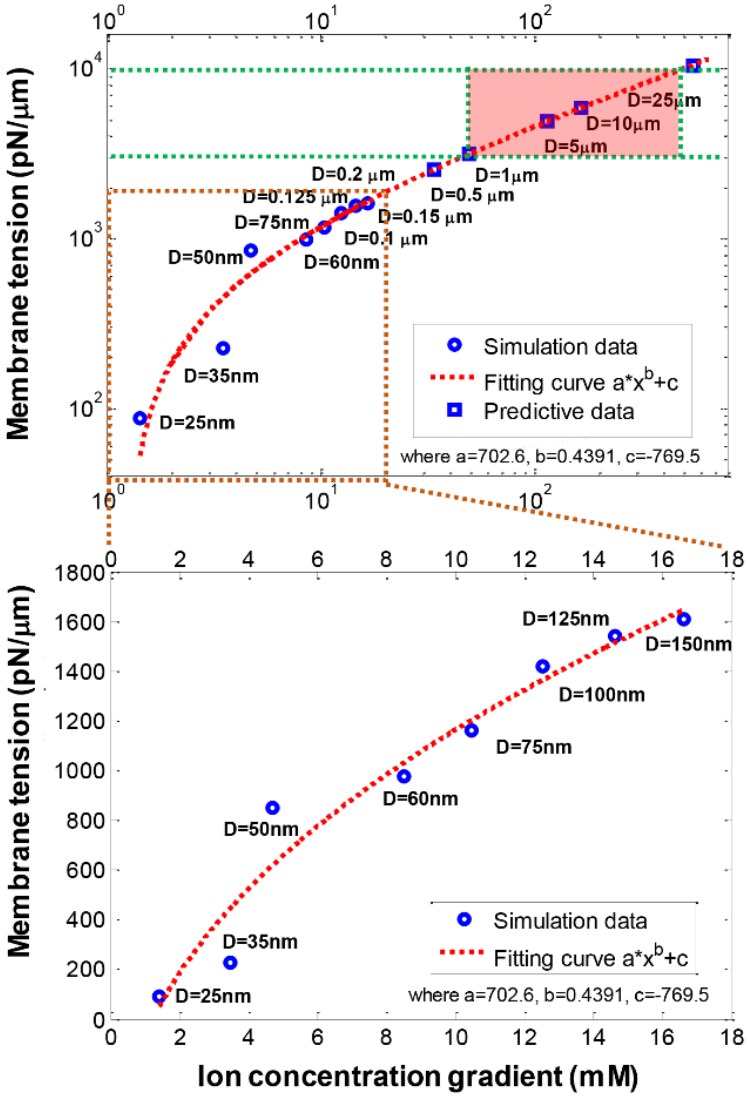

Figure S14. Relationship between the membrane tension and ion concentration gradient across the membrane. For different sizes of a cell, the critical concentration gradients (Δc) upon which the plasma membrane begins to rupture (red square shadow) change. By curve fitting these data points, we obtained a curve that allowed us to predict the critical concentration for 25 μm cells.

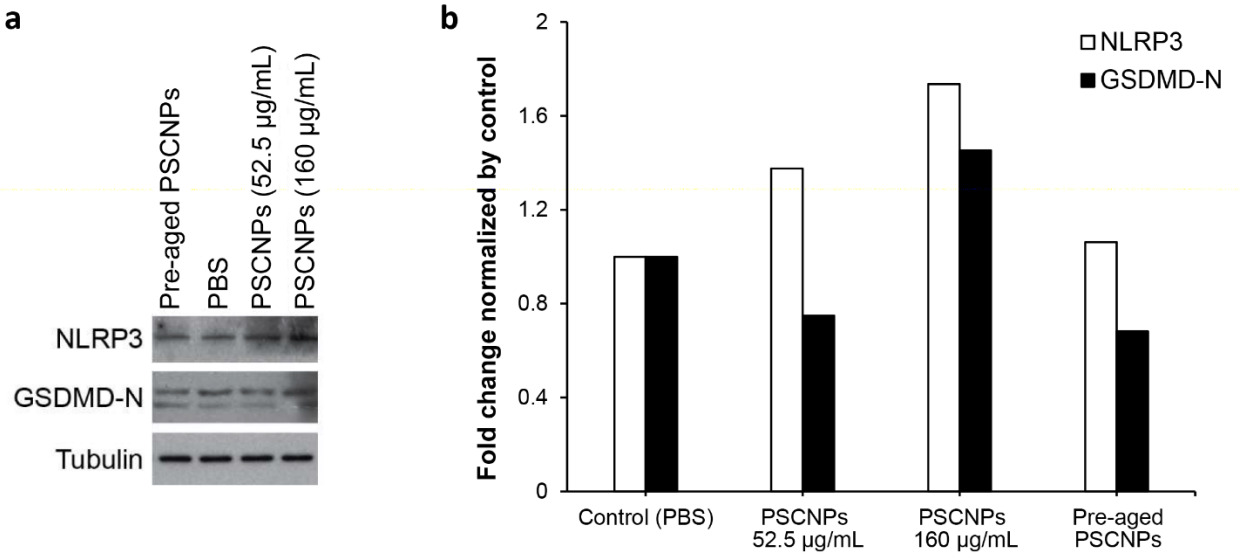

Figure S15. a, Western blotting to analyze the impact of PSCNPs on NLRP3 inflammasome activation and GSDMD-N terminal release. PC-3 cells were treated with 52.5 and 160 µg/mL PSCNPs for 6 h. PBS and PSCNPs pre-aged in PBS (160 µg/mL) were studied as negative controls. **b**, NLRP3 and GSDMD-N levels relative to the PBS controls, based on ImageJ analysis of the Western blotting results from **a**.

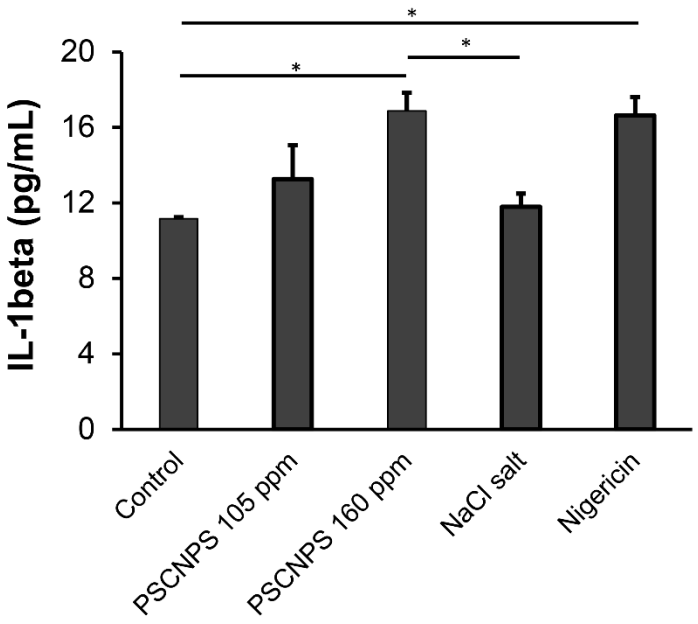

Figure S16. IL-1β release, analyzed by ELISA. PC-3 were incubated with PSCNPs (105 and 160 µg/mL) for 6 h (* $p < 0.05$ compared to PBS treated control cells).

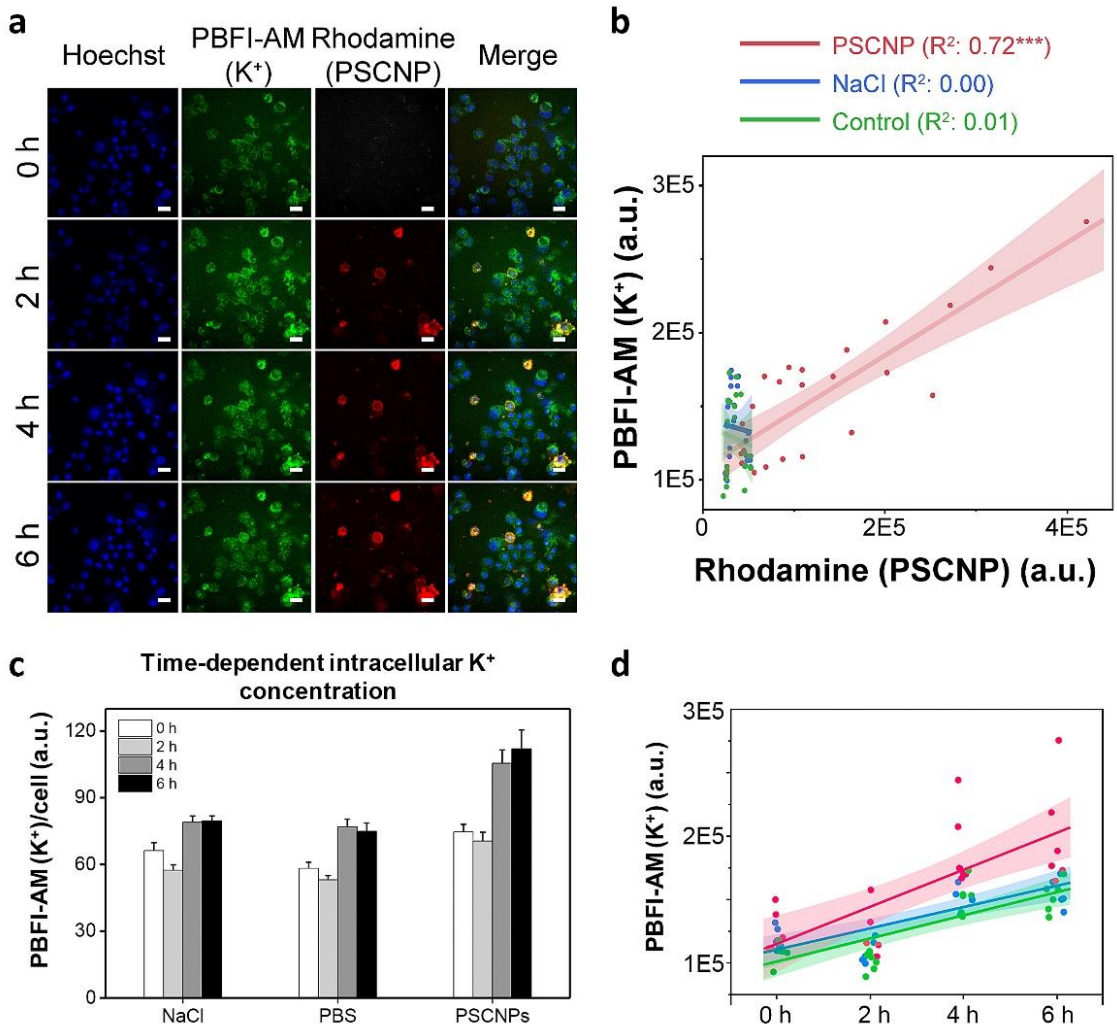

Figure S17. a, Fluorescent microscopy to examine intracellular K^+ levels at 0, 2, 4 and 6 h of PSCNP incubation. Red, Rhodamine B (RB-PSCNPs); green, PBFI-AM (K^+); blue, Hoechst 33342 (nucleus); scale bars, 50 μm. **b**, Spearman's correlation, based on the fluorescence intensity changes in **a**. **c** and **d**, Intracellular K^+ level. **c**, Histogram comparing intracellular K^+ concentrations at different time points. The results are represented as PBFI-AM intensity per cell (n = 5000 cells). PBS and NaCl salt (160 μg/mL) treated cells were studied as controls. **d**, Scatter plot comparing intracellular K^+ concentration changes. The results are represented as PBFI-AM intensity per well (n = 6 wells). Shadow areas represent the 95% confidence intervals of the fit.

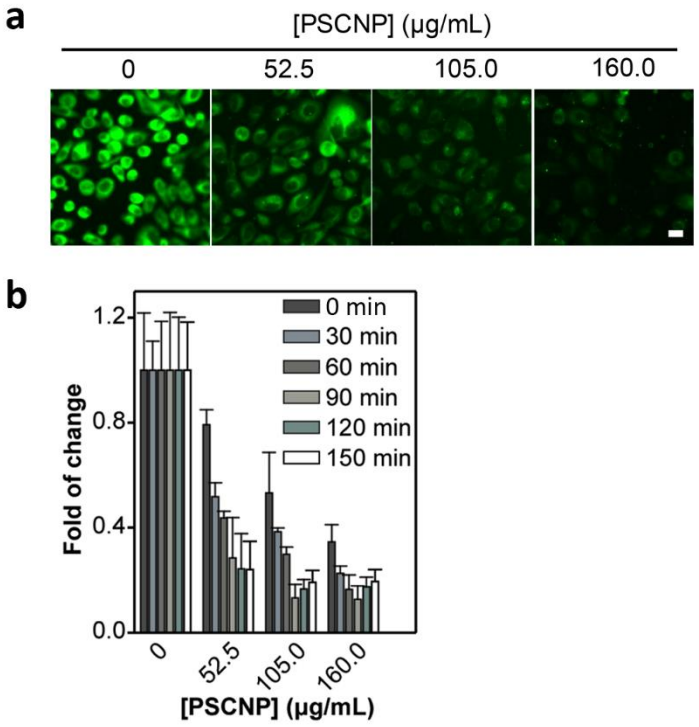

Figure S18. a, Representative DiBAC$_4$(3) staining results. PC-3 cells were incubated with PSCNPs of different concentrations (52.5, 105 or 160 μg/mL) for 30-150 min before the staining. A dose-dependent decrease of DiBAC$_4$(3) fluorescence intensity was observed, indicating PSCNP-induced membrane hyperpolarization. Scale bar, 50 μm. **b**, Plasma membrane potential changes. The fluoresce intensity was analyzed by ImageJ based on the imaging results from **a** and was normalized to PBS treated control cells. (mean ± s.d., n = 5000 cells).

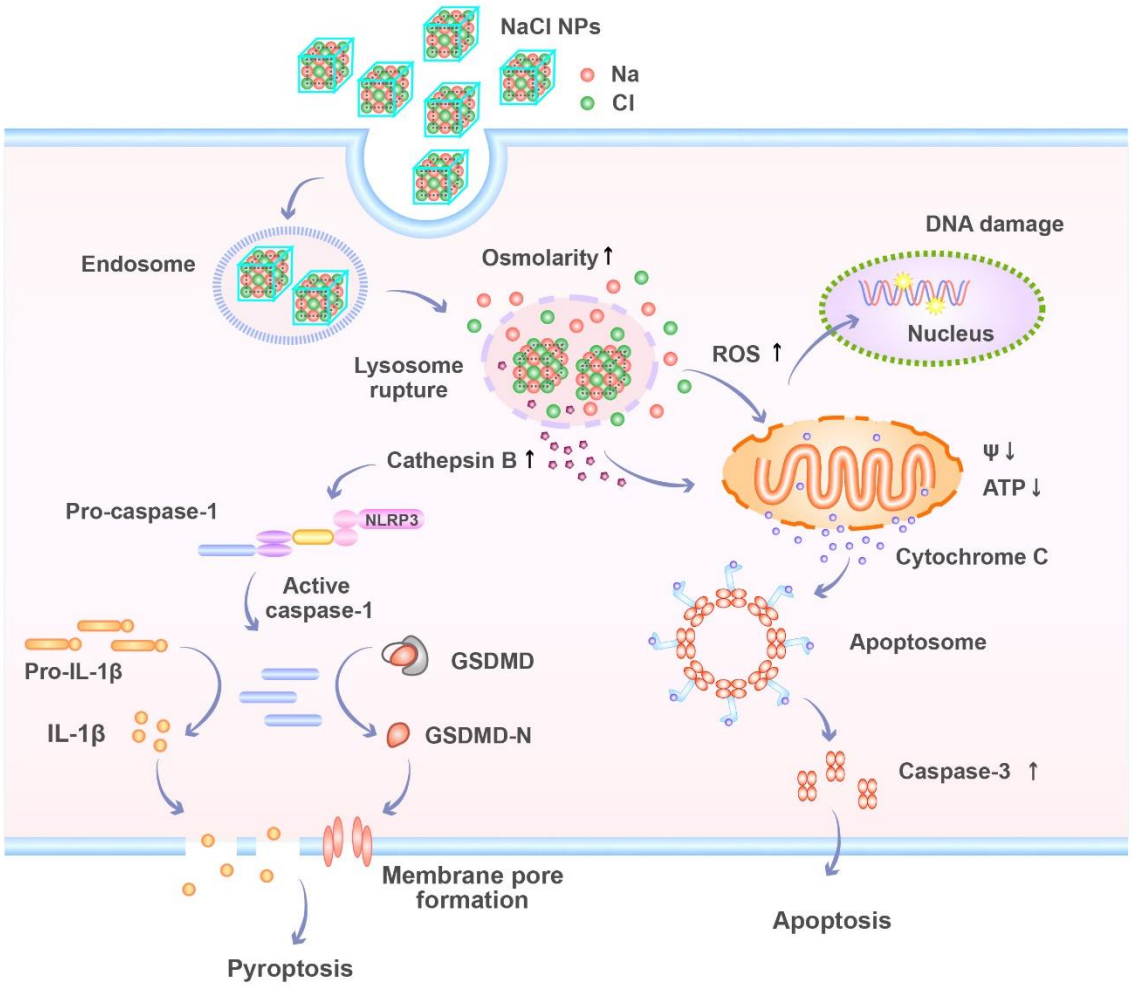

Figure S19. Proposed mechanisms for SCNP induced cell death.

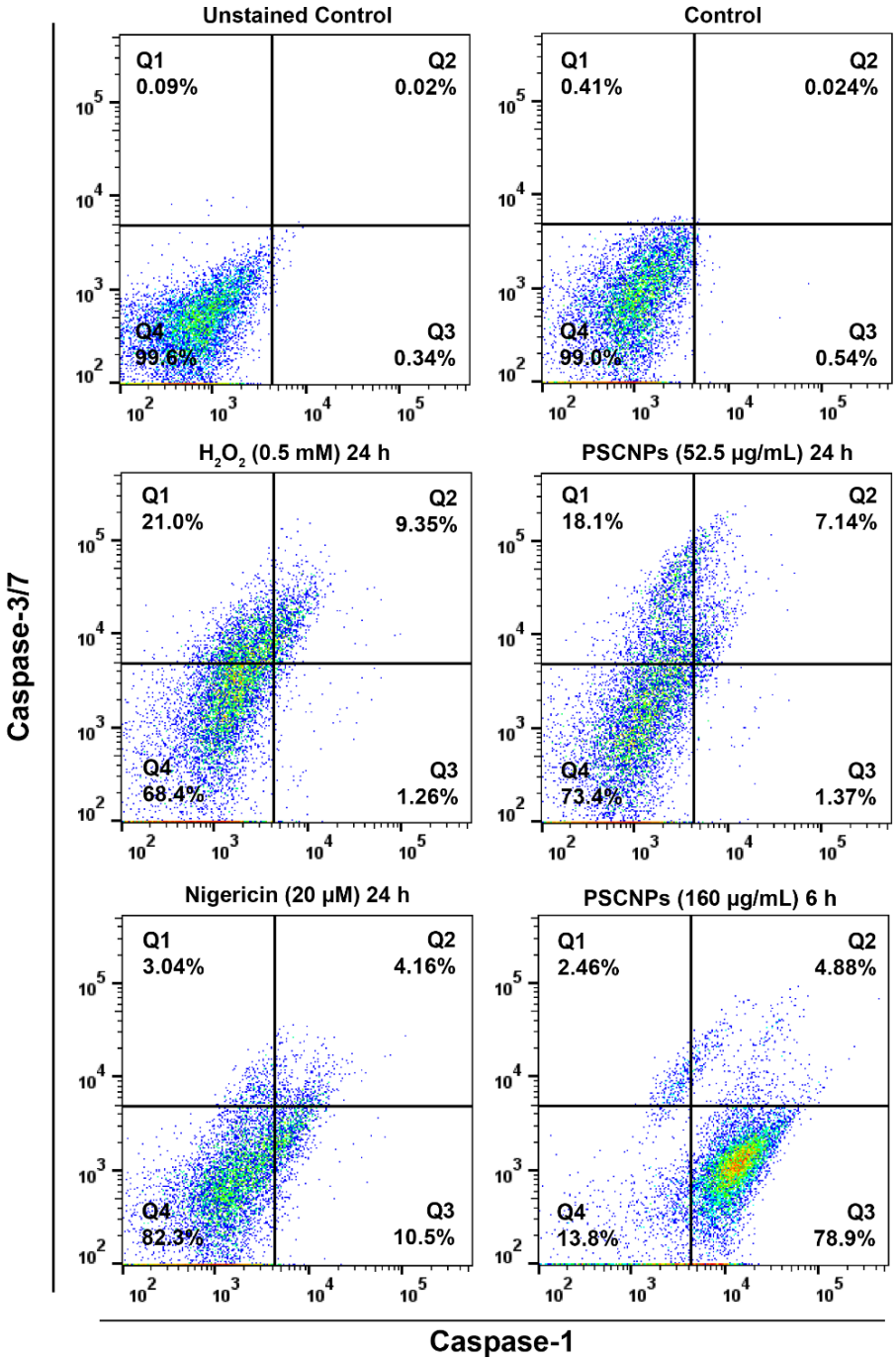

Figure S20. Flow cytometry to analyze caspase-3/7 activation (apoptosis) and caspase-1 activation (pyroptosis). PSCNPs at relatively low doses, such as 52.5 μg/mL, mainly caused apoptotic cell death at late time point (e.g. 24 h) through caspase-3/7 activation. In contrast, high concentrations of PSCNPs, such as 160 μg/mL, mainly induced rapid pyroptotic cell death (e.g. 6 h) through caspase-1 activation.

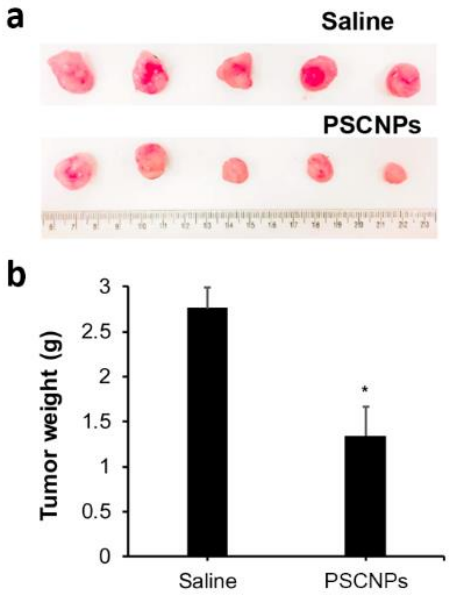

Figure S21. a, Photo of the dissected PC-3 tumors after 16 days of PSCNP treatment. Animals were *i.t.* injected with either saline or PSCNPs with the same NaCl dose (9 mg/mL, 50 µL). **b**, Tumor weight (* $p < 0.05$ compared to saline group).

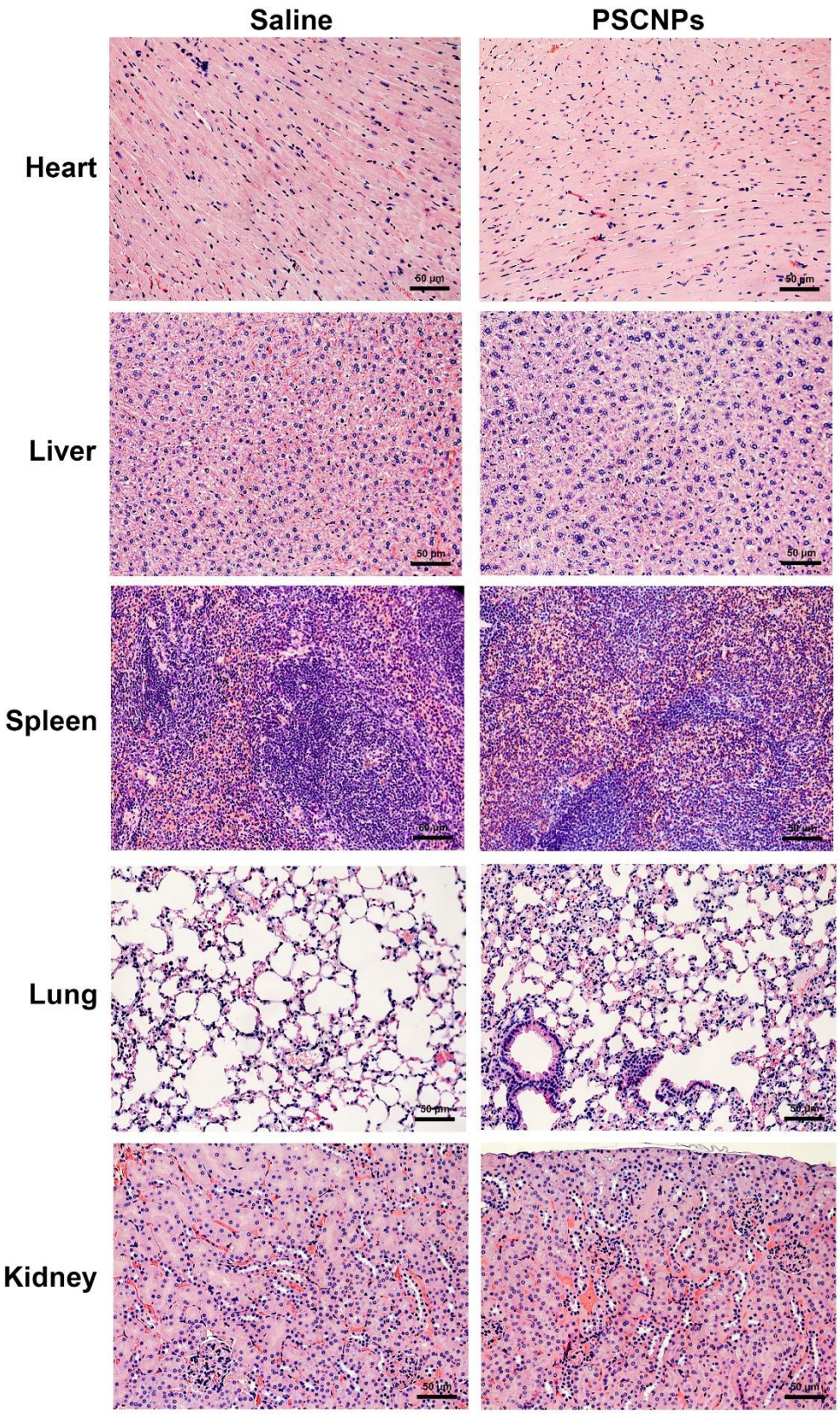

Figure S22. H&E staining for major organs for PC-3 tumor bearing mice. No significant pathological changes were detected (Scale bars, 50 μm).

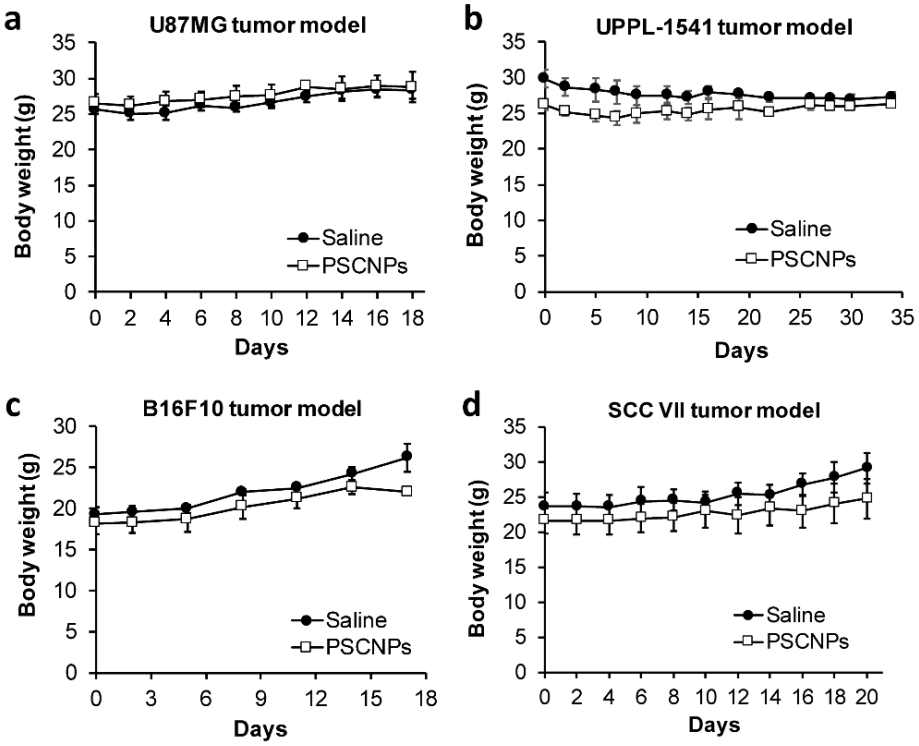

Figure S23. *In vivo* tumor therapy study outcomes. Animal body weight changes of **a**, U87MG, **b**, UPPL-1541, **c**, B16F10, and **d**, SCC VII tumor models. For all the tumor models, animals were injected with either saline or PSCNPs.

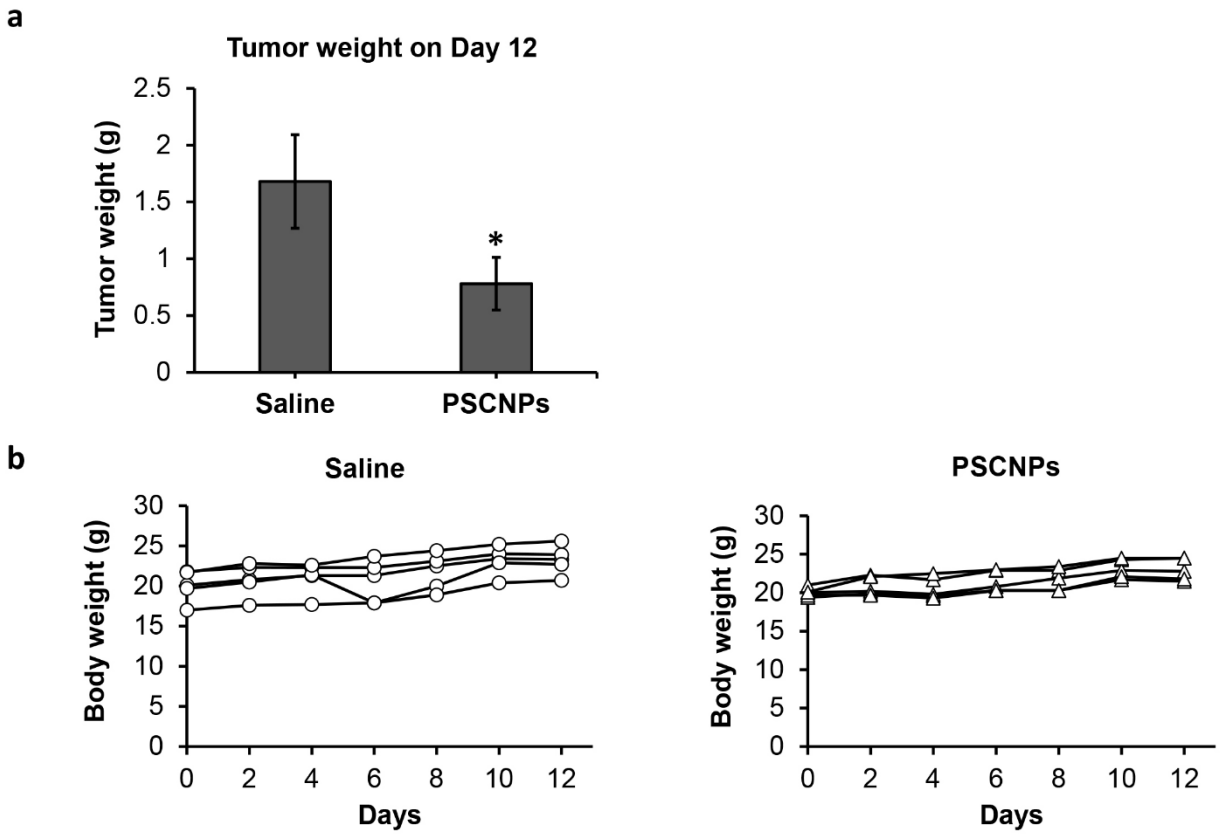

Figure S24. Tumor therapy results from SCC VII bilateral tumor models. **a**, Summary of the secondary tumor weight on Day 12 (* $p < 0.05$ compared to saline group). **b**, Animal body weight changes of saline and PSCNPs groups.

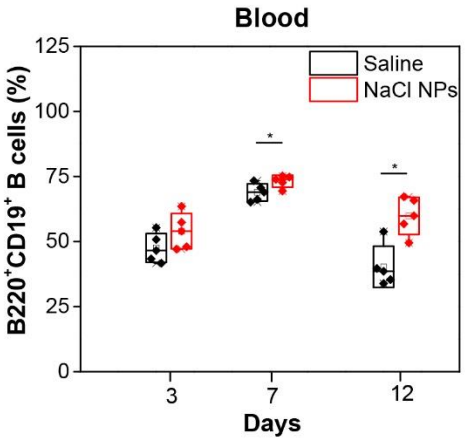

Figure S25. Flow cytometry analysis of B cells ($B220^{+}CD19^{+}$) in the blood on Day 3, 7 and 12 after PSCNP injection. The study was performed in SCC VII bilateral tumor models. (* $p < 0.05$)

Table S1. Time-dependent increase of intracellular ion concentrations upon incubation with PSCNPs (160 µg/mL).

Time (h)	$\Delta[Na^+]_{int}$ (mM)*	$\Delta[K^+]_{int}$ (mM)*	Δc (mM)
0	0	0	0.0
1	0.6	19.4	20.0
2	3.8	35.9	39.7
4	13.4	41.8	55.2
6	8.5	59.3	68.8

* The concentrations were estimated by fluorescence intensity changes in **Figure S5c** and **S17c**. Linear response for SBFI-AM [7] and PBFI-AM [8] was assumed.
** The cells were incubated in an isotonic solution [9]. Hence, Δc is equal to 0 at 0 h.
*** It is assumed that the extracellular ion concentrations remained unchanged [9].

Movie S1-S4. Time-lapse imaging to minitor cell morphology and volume changes. PC-3 cells were incubated with PSCNPs (Rhodamine B-labeled, red) of different concentrations (52.5, 105, and 160 μg/mL) or PBS. Cell nuclei were stained by Hoechst 33342 (blue) and the plasma membrane by $DiBAC_4(3)$ (green). Time-lapse movies were taken after 2 hours of incubation. The fluorescence imaging results were superimposed onto bright-field images that delineated the cell boundaries (purple). Incubation with PSCNPs led to effective plasma membrane hyperpolarization, manifesting as a decrease of $DiBAC_4(3)$ fluorescence. Meanwhile, cell swell and burst were observed, especially for a high concentration at 160 μg/mL.

Movie S1_PBS: cells were incubated with PBS.

Movie_S2_PSCNPs_52.5: cells were incubated with PSCNPs at 52.5 μg /mL.

Movie_S3_PSCNPs_105: cells were incubated with PSCNPs at 105 μg/mL.

Movie_S4_PSCNPs_160: cells were incubated with PSCNPs at 160 μg/mL.

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