# Supporting Information

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### SI Materials and Methods

**Preparation of Gd@$C_{82}(OH)_{22}$.** Gadolinium metallofullerenes (Gd@$C_{82}$) were synthesized by arc-burning composite rods consisting of $Gd_2O_3$ and graphite in a He atmosphere. The Gd@$C_{82}$ was separated by a two-step high-performance liquid chromatography (HPLC; LC908-C60, Japan Analytical Industry Co.) technique with COSMOSIL 5PBB guard columns and Buckyprep guard columns (Nacalai Tesque). The purity of the final Gd@$C_{82}$ product was greater than 99.5%. Gd@$C_{82}$(OH)x was synthesized using an alkaline reaction. Briefly, Gd@$C_{82}$ toluene solution was first mixed with a 50% NaOH aqueous solution. A few drops of 40% tetrabutylammonium hydroxide (TBAH) were added to the mixture as catalyst. After vigorous stirring at room temperature, a brown sludge precipitate appeared and the toluene solution became colorless. Remaining TBAH and NaOH were removed with water and methanol. The brown precipitate was then dissolved in deionized water with continuous stirring for 24 h until the solution became reddish brown. Finally, the solution was purified by a Sephadex G-25 column chromatography ($5 \times 50$ $cm^2$) with distilled water as an eluent. To obtain a final Gd@$C_{82}$(OH)x product with a narrow range of attached hydroxyl groups, the fraction (eluate) was collected in several fractions. The molecular weight was determined by elemental analysis, MALDI-TOF–MS, and x-ray photoemission spectroscopy. The number of attached hydroxyl groups for Gd@$C_{82}$(OH)x was determined to be $22 \pm 2$. The dry Gd@$C_{82}(OH)_{22}$ powder was weighed and dissolved in 0.9% sterile saline solution.

**Cell Lines and Establishment of Cisplatin-Resistant Cells.** PC-3-luc cells, a human prostate cancer cell line transfected with luciferase gene, were the parent cell line for the selection of drug-resistant variants. The PC-3-luc cells were maintained in RPMI medium 1640 supplemented with 10% FBS (HyClone) and 100 U/mL of penicillin and streptomycin each (Invitrogen). To establish cisplatin-resistant cells, PC-3 luc cells were first cultured in RPMI medium with 0.5 μg/mL cisplatin. The surviving cells were then maintained in medium containing 1 μg/mL cisplatin for 4 weeks until the growth of the cells was stable enough to form solid colonies. Individual colonies were harvested as cisplatin-resistant PC-3-luc cells. The cisplatin-resistant variants were cultured with 1 μg/mL cisplatin to maintain cellular resistance to cisplatin. All cell lines were grown as monolayer cultures at 37 °C.

**Animals.** The animals used were 8– to 10-week-old male athymic nude mice (Harlan). The mice were kept in a pathogen-free (SPF) animal facility under a controlled environment ($22 \pm 1$ °C, $60 \pm 10$% humidity, and a 12-h light/dark cycle) with free access to food and water. All animal experiments were performed in compliance with the local ethics committee guidelines.

**Cytotoxicity Assay.** Cytotoxicity was determined by the viability of cells with MTT assay. Cells were seeded in 96-well plates at $3 \times 10^3$ cells with 100 μL medium per well. After 6 h incubation, the test compounds dissolved in 50 μL medium were added to each well, and the cells were incubated for 3 days. Next, 10 μL MTT solutions (5 mg/mL in PBS) were added to each well and incubated at 37 °C in 5% $CO_2$ for 2 h. The medium in the wells was then aspirated gently, and 150 μL DMSO was added to each well to dissolve the formazan crystals. After shaking for 10 min, the absorbance of each well at 490 nm was measured using a multimode plate reader (Infinite M200, Tecan Group). Results were expressed as the mean percentage of cell viability relative to untreated cells. To measure the adjuvant effect of metallofullerene nanoparticles, 0.2 μM cytochalasin D (MW:507.62) and 0.01μM bafilomycin A1 (MW:622.83, Sigma-Aldrich) as intracellular endocytotic vesicle trafficking inhibitors were used to pretreat cells for 30 min before nanoparticles and/or cisplatin treatment for cytotoxic measurement. The intracellular platinum amount was measured with inductively coupled plasma mass spectroscopy (ICP-MS) technique (Elemental ×7 ICP-MS, Thermo Fisher Scientific).

**Confocal Microscopy for Measurement of Viability Using LIVE/DEAD Viability/Cytotoxicity Kit-Labeled Cells.** The cytotoxicity measurement of Gd@$C_{82}(OH)_{22}$ nanoparticles was compared to cisplatin in CP-s and CP-r PC-3 cells exposed to nanoparticles and/or cisplatin for 24 h. Briefly, $\approx 2.0 \times 10^4$ cells were plated onto 30-mm glass microscopy coverslip and cultured at 37 °C overnight until the monolayer was confluent. The cells were washed with PBS (pH 7.4). The cell monolayer was then incubated with serum-free medium containing different concentrations Gd@$C_{82}(OH)_{22}$ nanoparticles and/or cisplatin for 24 h. The cytotoxity was measured using a LIVE/DEAD Viability/Cytotoxicity Kit (Invitrogen, Rockville, MD) for staining with calcein AM (live cells fluoresce green) and ethidium homodimer (dead cells fluoresce red) and viewed by confocal microscopy (Ultraview ERS, Perkin-Elmer).

**Measurement of Transferrin-Mediated Endocytosis.** Cisplatin-sensitive and cisplatin-resistant PC-3-luc cells were labeled with 10 μg/mL Texas Red–transferrin for 20 min at 37 °C. Free Texas Red–transferrin was removed by washing three times with PBS. Cells labeled with Texas Red–transferrin were incubated in RPMI medium 1640 at 37 °C for 30 min, and labeled cells were suspended by detachment with trypsin/EDTA without calcium. Suspended cells were collected collected by centrifugation. Cell pellets were resuspended in PBS and fixed with 70% ethanol in PBS. In each individual experiment, 20 nmol/mL $[Gd@C_{82}(OH)_{22}]_n$ nanoparticles and 1 μg/mL cisplatin were used to measure the endocytosis of Texas Red–transferrin. Cisplatin-sensitive and cisplatin-resistant cells were counted, and the same numbers of cells labeled with Texas Red–transferrin were measured by spectrophotometer.

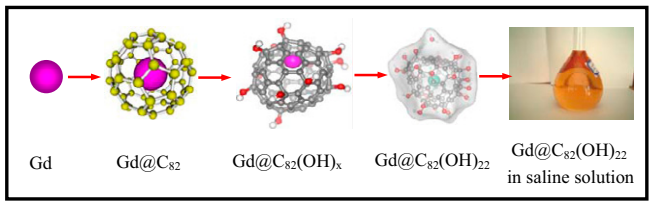



**Fig. S1.** Formation and structures of $Gd@C_{82}$, $Gd@C_{82}(OH)x$, $Gd@C_{82}(OH)_{22}$, and $Gd@C_{82}(OH)_{22}$ in saline solution.

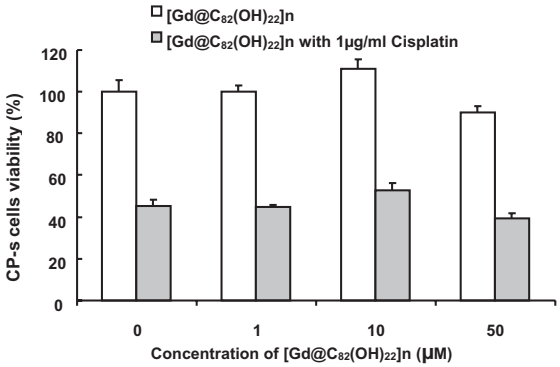



**Fig. S2.** Effects of $[Gd@C_{82}(OH)_{22}]_n$ nanoparticles on cisplatin cytotoxicity (1 μg/mL). CP-s cells ($3 \times 10^3$) were plated in 96-well plates with 100 μL medium per well. After 6 h, cisplatin (in 50 μl medium) was added, and the cells were incubated at 37°C for 3 days. Next,10 μL of MTT solution (Kumamoto) was added per well and incubated for ~2 h. Cell viability was measured using a spectrophotometer at the wavelength suggested in the kit's protocol. The results shown are the mean of three different experiments. A significant difference was assumed to exist when $P < 0.05$.

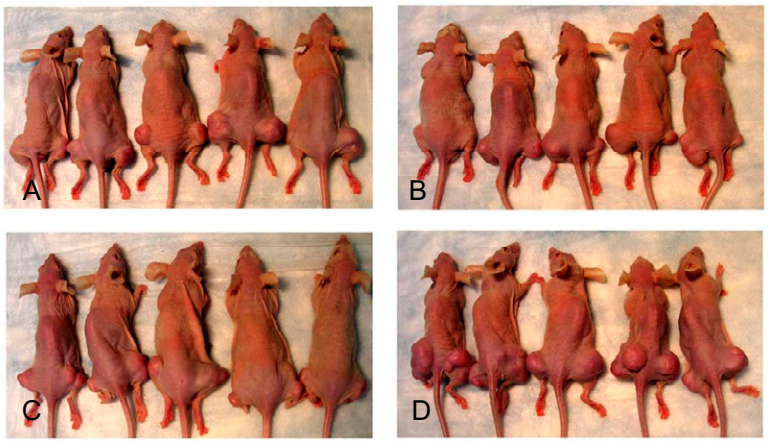



**Fig. S3.** Morphological observation of tumor after 4 weeks treatment. Animals treated with the following: (*A*) $Gd@C_{82}(OH)_{22}]_n$ nanoparticles (NP); (*B*) cisplatin; (*C*) cisplatin/nanoparticles (cisplatin + NP); and (*D*) saline as control. (*Right*) Drug-sensitive tumor. (*Left*) Drug-resistant tumor. The experiment is representative of three individual experiments.

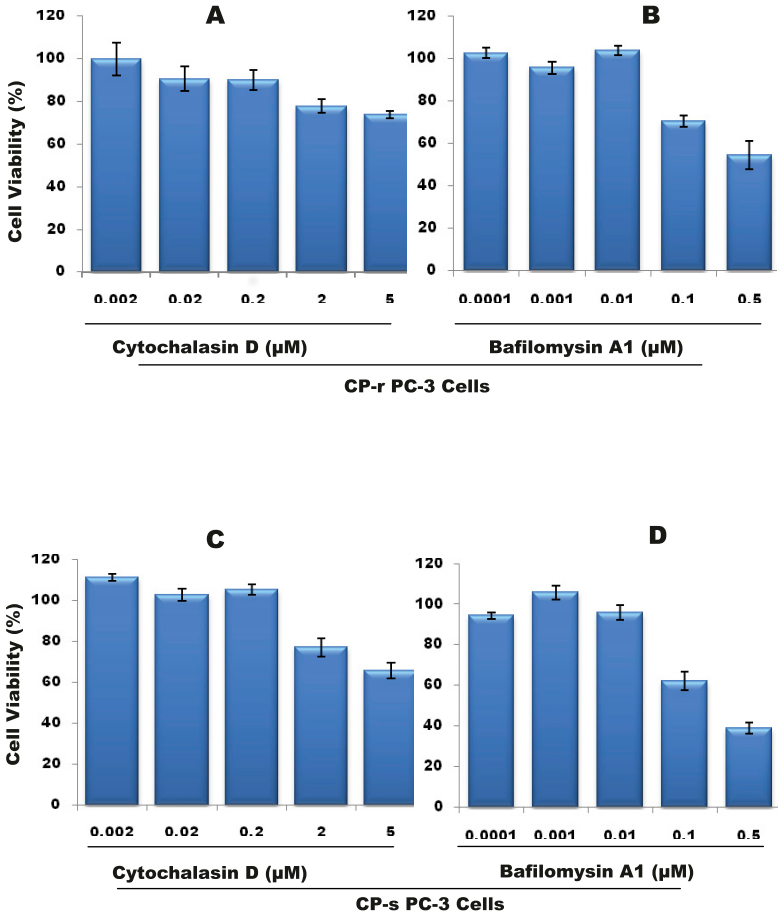



**Fig. S4.** Optimized concentration of Cytochalasin D and Bafilomysin A1 without significant toxicity on CP-s and CP-r PC-3 Cells. CP-r PC-3 cells treated with different concentrations of Cytochalasin D (*A*) and Balifomysin A1 (*B*), and CP-s PC-3 cells treated with different concentration of Cytochalasin D (*C*) and Balifomysin A1 (*D*). Briefly, cells were seeded in the 96-well plastic disk by 5,000 cells/well. After 24 h culture, cells were incubated with cytochalasin D or bafilomysin A1 at 37 °C for 30 min. Cytochalasin D (MW: 507.62, 2,000 μM stock solution) was used to treat cells at 0.002, 0.02, 0.2,2, or 5 μM . Bafilomycin A1 (MW:622.83, 160 μM stock concentration), at 0.0001, 0.001, 0.01, and 0.1, 0.5 μM, was used to inhibit endocytosis. After washing with PBS three times, the cells were incubated with cisplatin, nanoparticles, or cisplatin plus nanoparticles at 37 °C for 48 h. The viability was examined using the MTT method. Briefly, 10 μL of MTT solution was added to each well. Cell viability was measured using a spectrometer at 490 nm. Cytochalasin D and Bafilomycin A1 prohibited cell proliferation with increasing concentration. For further experimental treatment, 0.2 μM cytochalasin D and 0.01 μM bafilomycin A1 were selected based on their effects on cell viability assay. The result shown is the mean of five individual experiments.

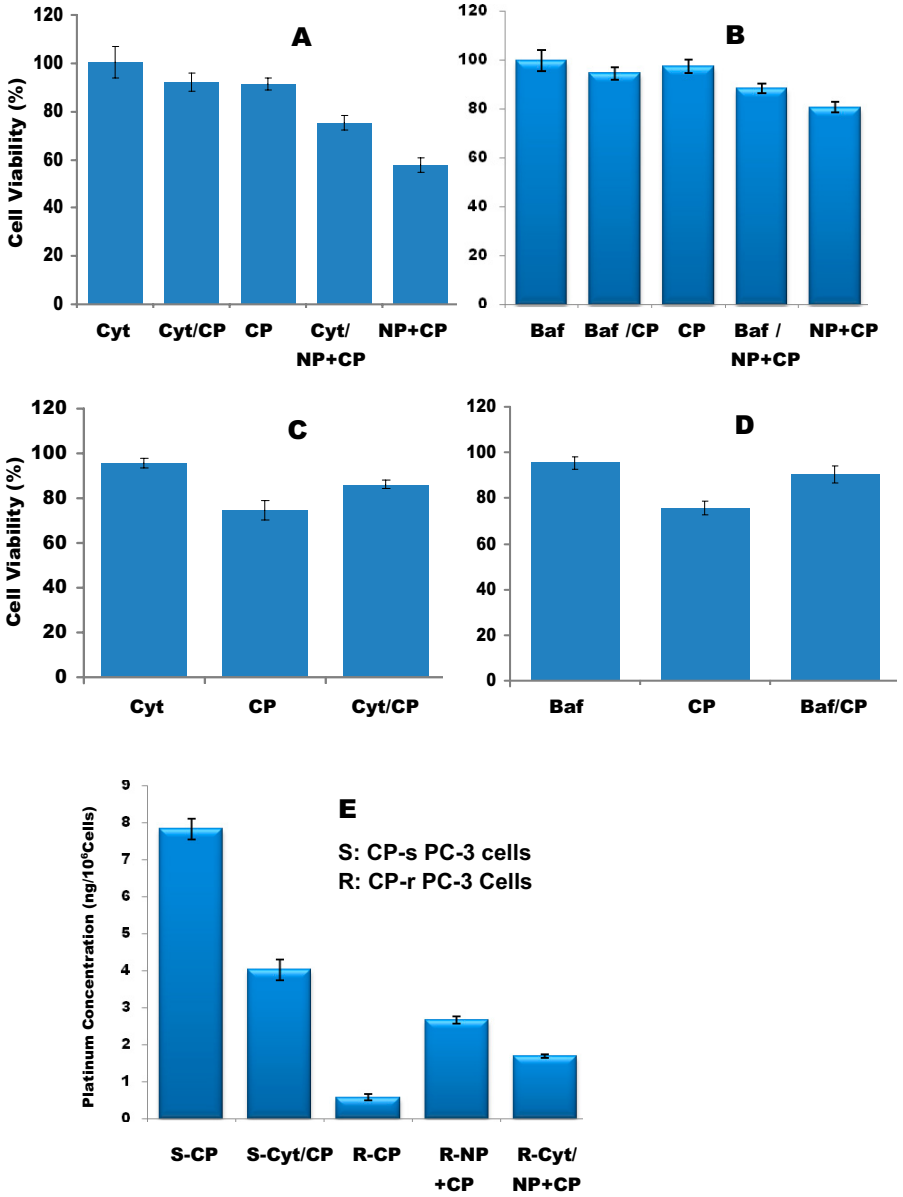



**Fig. S5.** CPs and CP-r PC-3 cell viability was affected by increased intracellular cisplatin accumulation due to Cytochalasin D or Bafilomysin A1 inhibiting endocytosis. Viability of CP-r PC-3 cells was measured following exposure to cytochalasin D (*A*) and Bafilomysin A1 (*B*). Viability of CP-s PC-3 cells was measured after exposure to cytochalasin D (*C*) and Bafilomysin A1 (*D*). Intracellular platinum concentration was measured by MALDI-TOF–MS as described in *SI Materials and Methods* (*E*). Baf, Bafilomycin A1; CP, cisplatin; Cyt, Cytochalasin D; NP, nanoparticles.

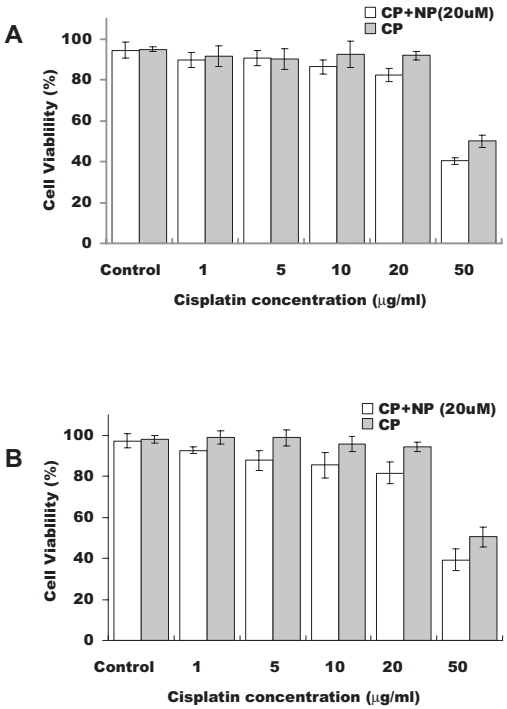



**Fig. S6.** KB-CP20 (*A*) and BEL 7404 CP-20 (*B*) CP-r cells were treated with nanoparticles and cisplatin for measurement of cytotoxicity. Optical absorbance of cells suspended with CCK-8 was measured using a spectrophotometer at 520 nm. Columns indicate mean; bars indicate SD. Comparisons of groups were evaluated by one-way ANOVA. When twice the SD was higher than the mean, a nonvariable test was used to evaluate the difference. A significant difference was assumed to exist when $P < 0.05$. Data presented represent the mean of at least three experiments. CP x, x μg/mL cisplatin; NP20, 20 μmol/mL nanoparticles.

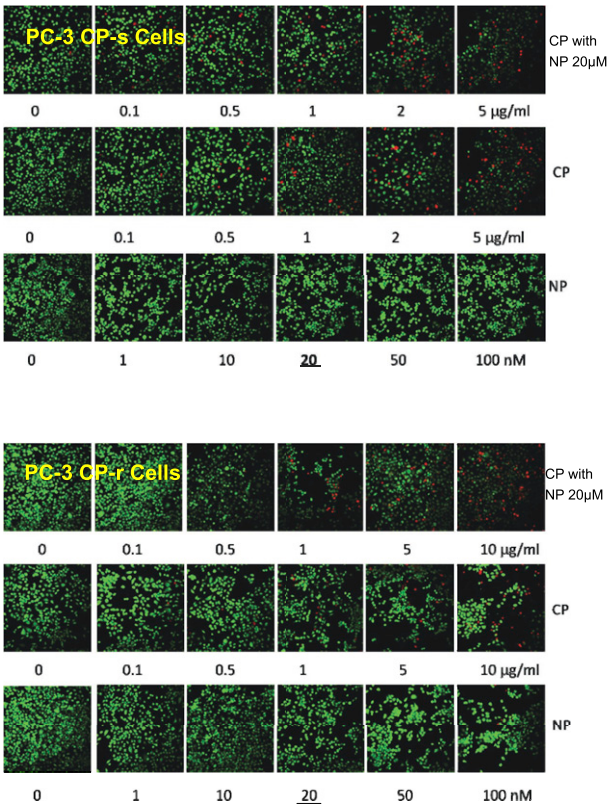



**Fig. S7.** Cytotoxicity of $Gd@C_{82}(OH)_{22}$ nanoparticles was compared with cisplatin in PC-3 and PC-3 CP-r cells that were exposed to nanoparticles and/or cisplatin for 24 h. Cytotoxity was measured using a LIVE/DEAD Viability/Cytotoxicity Kit for staining with calcein AM (live cells fluoresce green) and ethidium homodimer (dead cells fluoresce red) by confocal microscopy. Results are representative of three individual experiments.

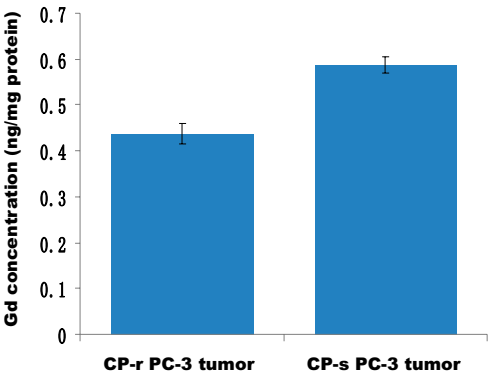
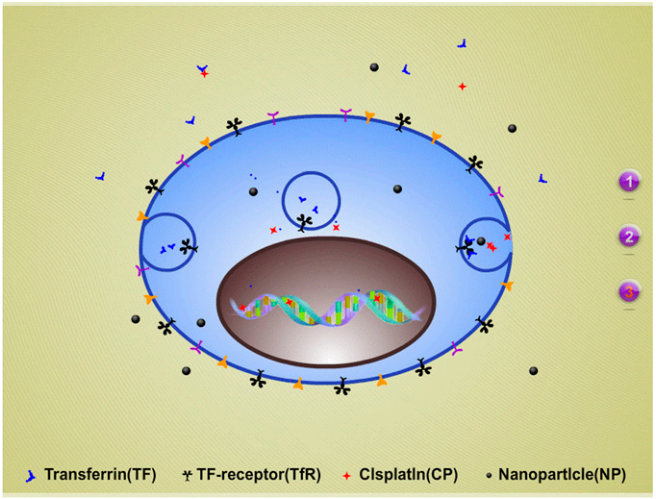



**Fig. S8.** Biodistribution study of nanoparticles in tumors was measured by inductively coupled plasma mass spectroscopy (ICP-MS) instrument. For intratumor localization of the nanoparticles, $2 \times 10^6$ CP-s and CP-r PC-3 cells (in 200 μL saline solution) per mouse were injected intraperitoneally (i.p.) into 5-week-old Balb/C mice (five mice per group). Each mouse was administered i.p. with a dose of 20 μmol $[Gd@C_{82}(OH)_{22}]_n$ in 2 weeks. The weight of the mice was measured every 24 h. Elemental ×7 ICP-MS (Thermo Fisher Scientific) was used to quantitatively measure of the amount of cisplatin (Fig. S5*E*) and gadolium in the isolated tumor tissue. Results represent the mean of three experiments.




**Movie S1.** A movie is created to make the cartoon in Fig. 5 easily understandable. This dynamic cartoon shows that cisplatin resistant cancer cells can be sensitized by increasing cisplatin intracellular concentration through nanoparticle-mediated endocytosis. This movie is divided into three dynamic cartoons. The first cartoon explains the normal endocytosis mechanism, which includes the ligands binding with receptor on plasma membrane, penetration and ingestion into cytoplasm, intracellular vesicles transportation, releasing the payload, and recycling back to the plasma membrane. The second cartoon shows the cisplatin entry into cells by receptor-mediated endocytosis. Cisplatin is trapped in endocytosis vesicles and is released during endocytotic vesicle trafficking. The defective endocytosis in CP-r cells leads to less effective cisplatin intracellular accumulation. The third cartoon defines nanoparticle-activated endocytosis, by which more cisplatin is transported and released into the cytoplasm of CP-r cells. The active cisplatin can then effectively bind with nucleic acids and sensitize CP-r cells by inhibiting cell proliferation. The movie indicates that cisplatin-resistant cancer cells can be sensitized by increasing cisplatin intracellular concentration through nanoparticle-mediated endocytosis.

Movie S1