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Enhancing Doxorubicin Anticancer Activity with a Novel Polymeric Platform Photoreleasing Nitric Oxide

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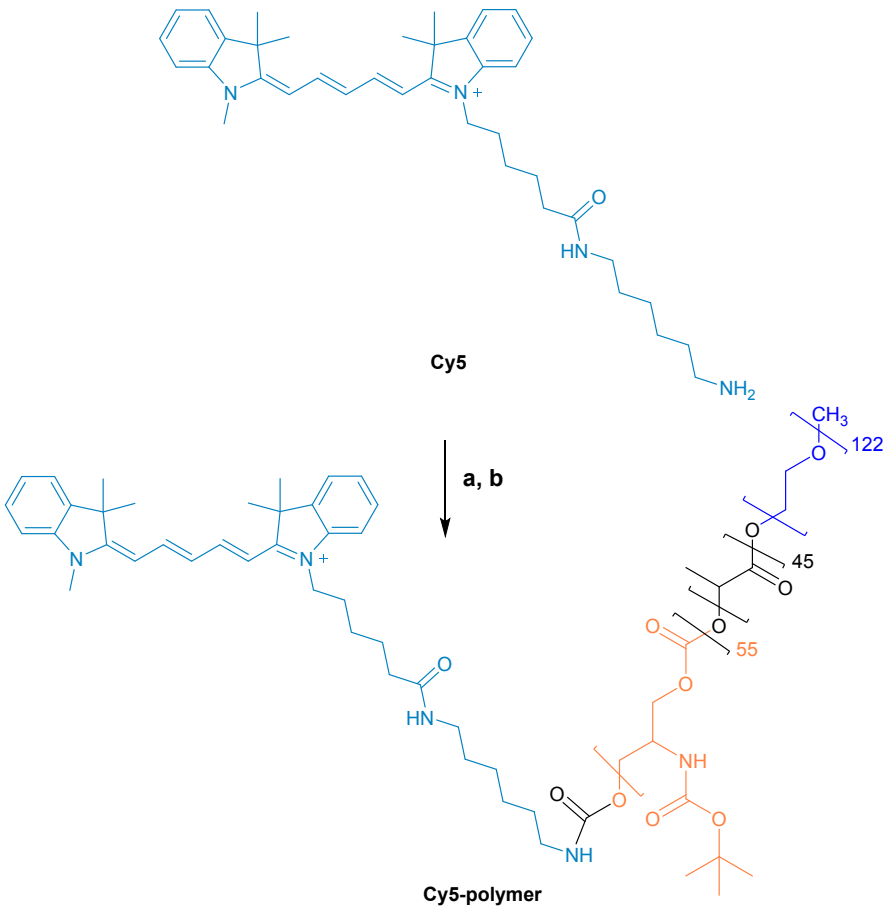
TABLE OF CONTENTS



Scheme S1. Synthesis of Cy5-polymer. Reagents and conditions: a) DSC, TEA, CH_3CN, 0 °C; b) protected polymer, TEA, CH_3CN, 0 °C.

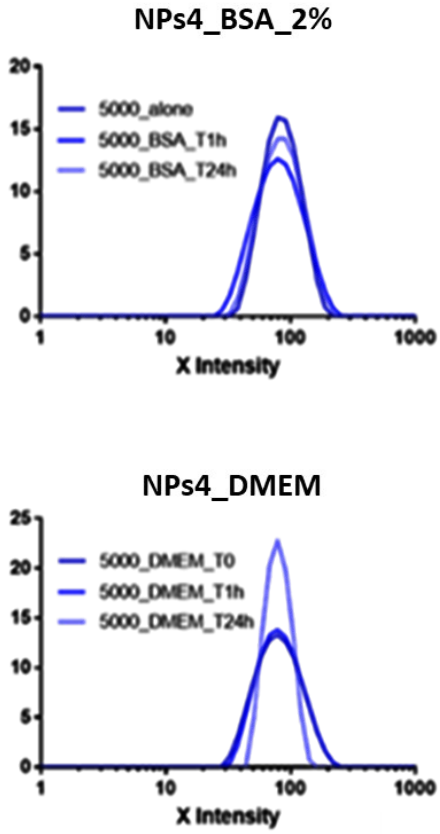


Figure S1. Stability of the NP4 in BSA and DMEM at different times.

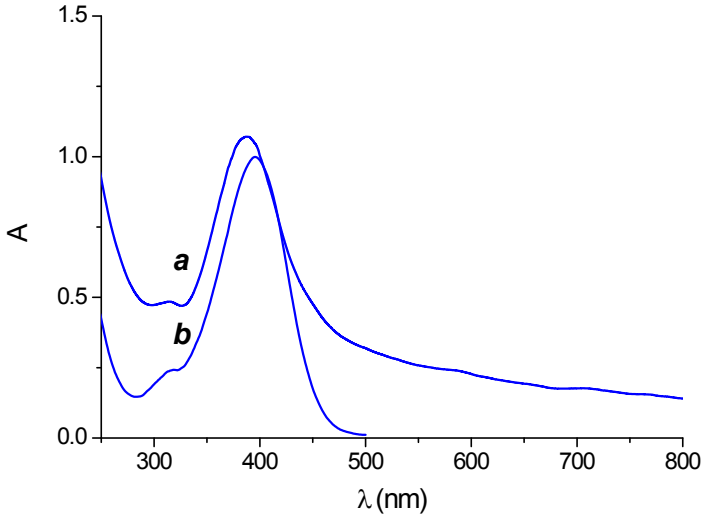


Figure S2. Absorption spectrum of NP2 (**a**) and, for comparison the free NOPD (**b**) in water.

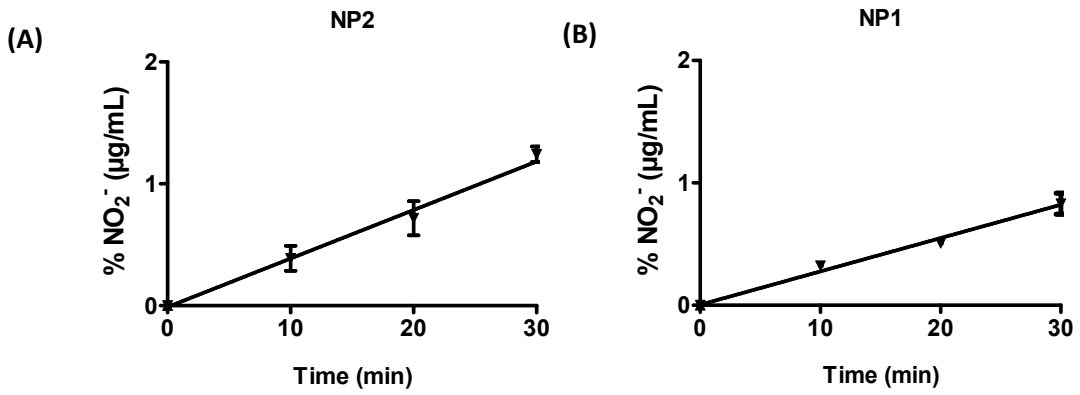


Figure S3. NO photorelease by Griess assay from NP2 (A) and NP1 (B) at the concentration of 0.2 mg/mL.

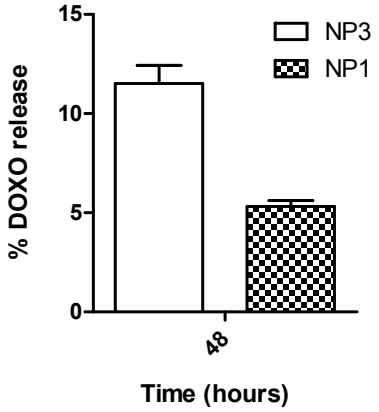


Figure S4. DOXO release profile observed after 48 h from NP1 (0.81 mg/mL) and NP3 (0.73 mg/mL) at pH 5.0 lysosome mimicking.

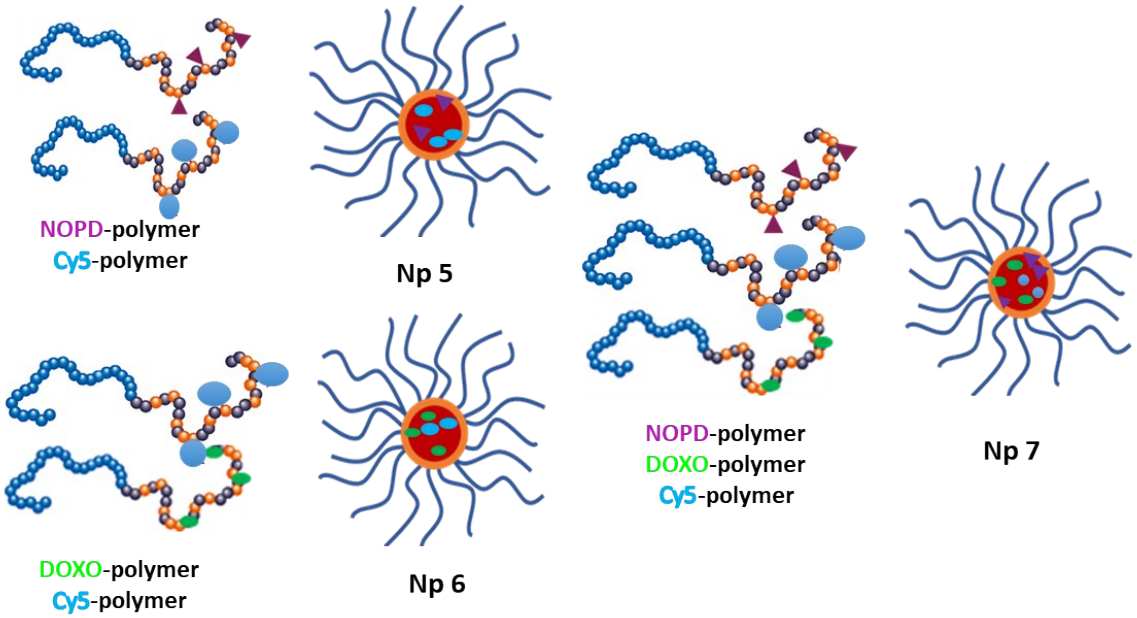


Figure S5. Schematic illustration of NPs 5-7.

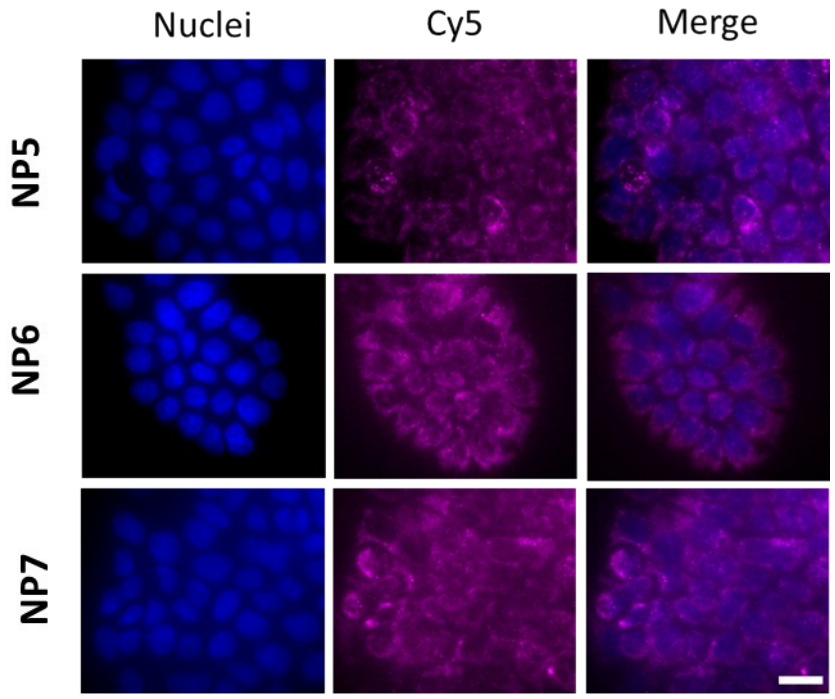


Figure S6. Micrographs of nanoparticle internalization in A431 cells. Cells were incubated with nanoparticle solutions (NPs 5-7) at the concentration of 6.5 x 10 µg/mL for 120 minutes. Scale bar = 30 µm. Images are representative from three independent repeats.

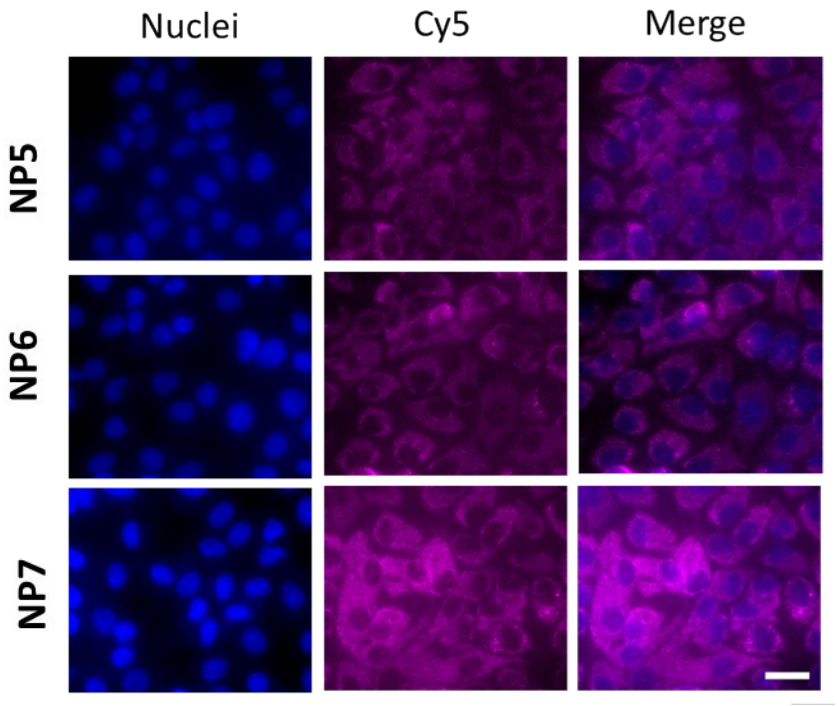


Figure S7. Micrographs of nanoparticle internalization in A549 cells. Cells were incubated with nanoparticle solutions (NPs 5-7) at the concentration of 6.5 x 10 µg/mL for 120 minutes. Scale bar = 30 µm. Images are representative from three independent repeats.

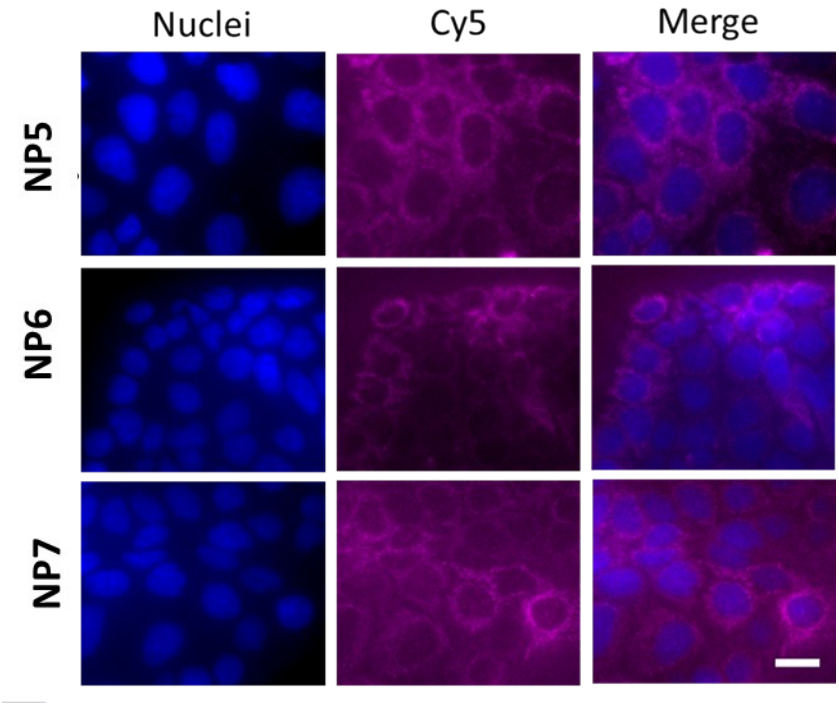


Figure S8. Micrographs of nanoparticle internalization in Caco-2 cells. Cells were incubated with nanoparticle solutions (NPs 5-7) at the concentration of 6.5 x 10 µg/mL for 120 minutes. Scale bar = 30 µm. Images are representative from three independent repeats.

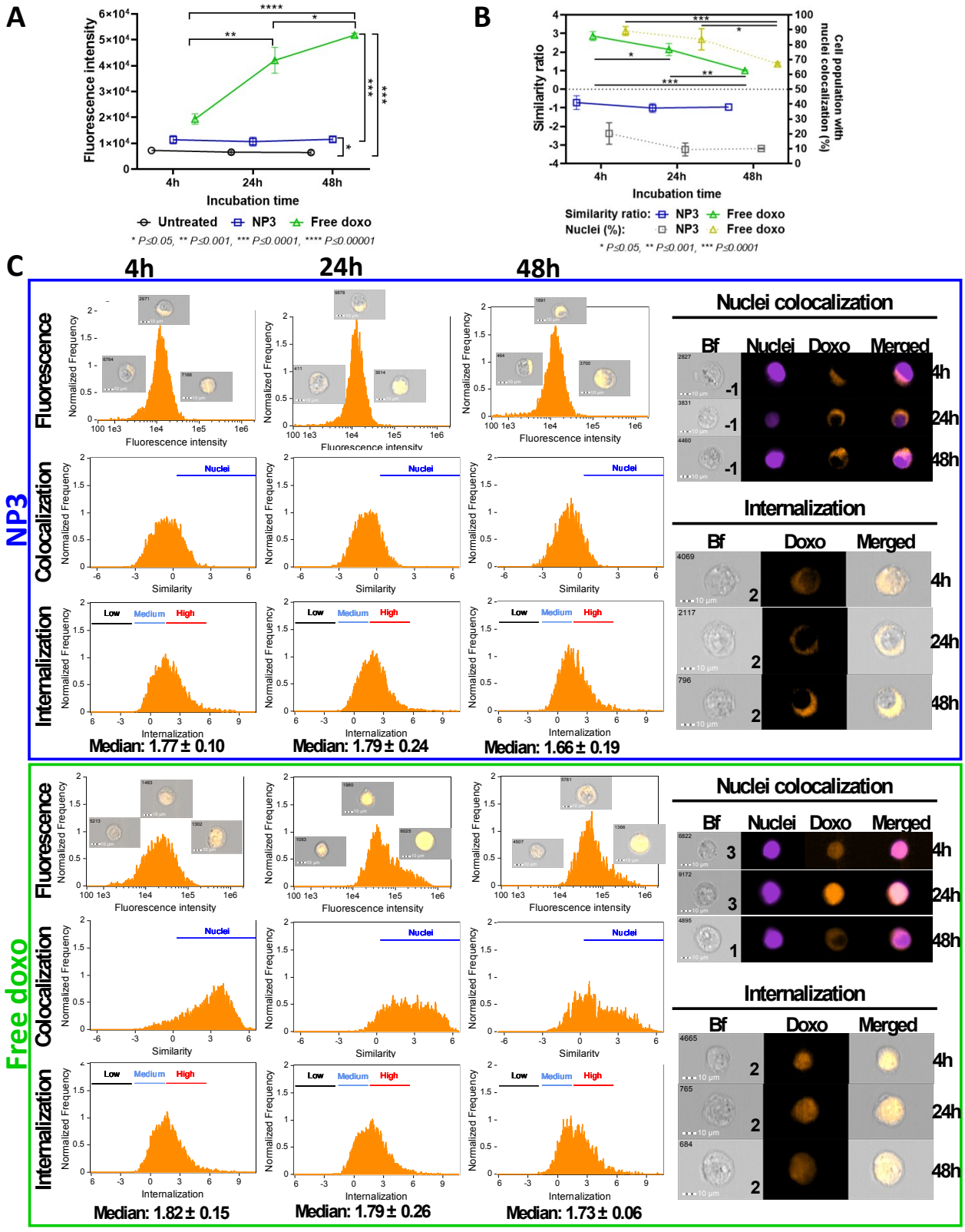


Figure S9. Median doxorubicin fluorescence intensity of NP3 and free drug at increasing time points (A). Ratio of doxorucin nuclei colocalization with Hoechst nuclei dye and percentage of cells with positive nuclei colocalization (B). Caco-2 cells were incubated with NP3 and free drug at 1 µM doxorubicin for 4/24/48h (n=3). Representative images of nuclei colocalization and internalization and histograms of doxorubicin fluorescence intensity, colocalization and internalization for 4, 24 and 48h incubation times. Data was acquired in a ImageStream Flow Cytometer and analysed using IDEAS software (v. 6.2, Amnis).

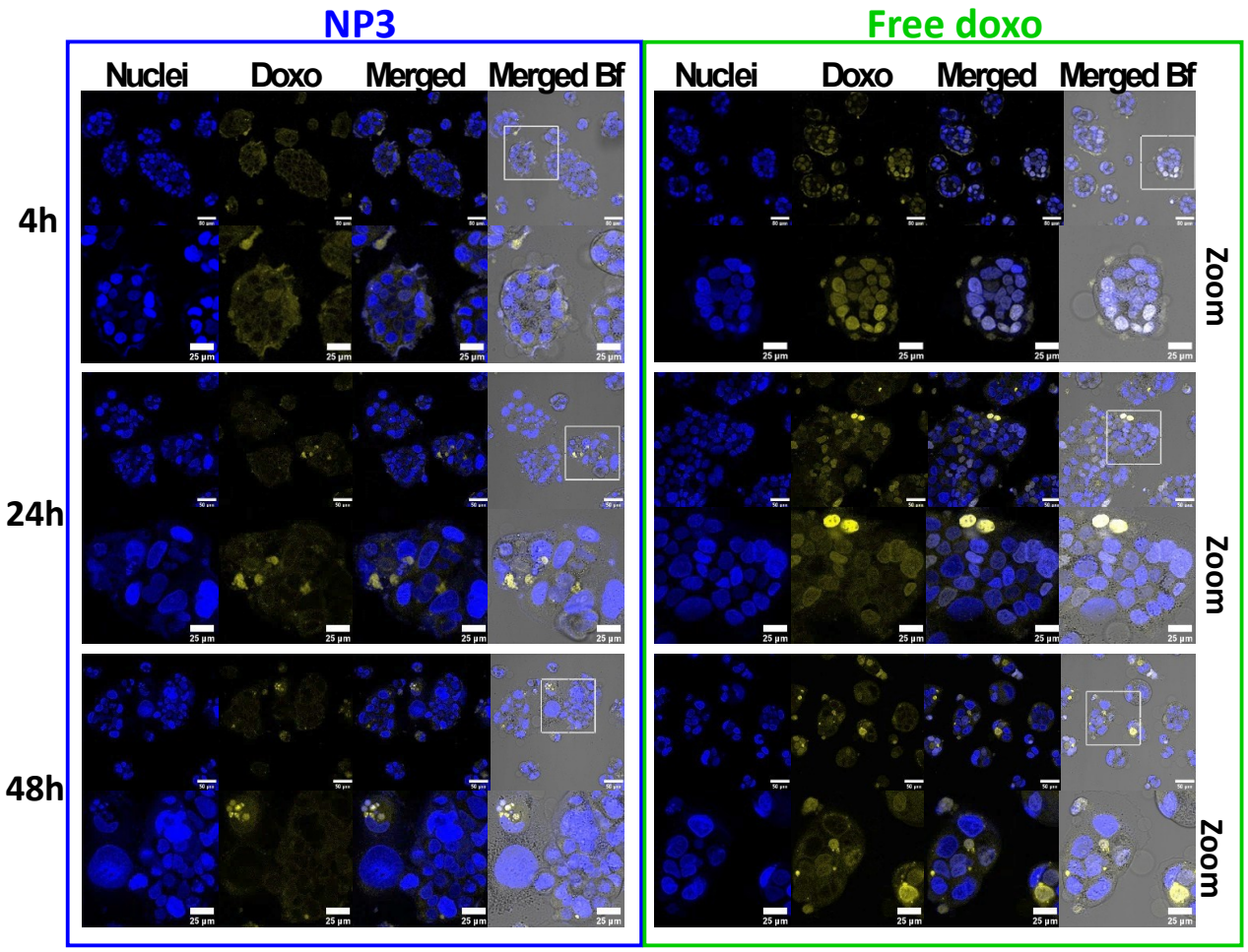


Figure S10. Confocal images of NP3 and free drug uptake at 1 µM doxorubicin following 4/24/48 h incubation. Bar represents 50 µM and 25 µM (zoomed images).

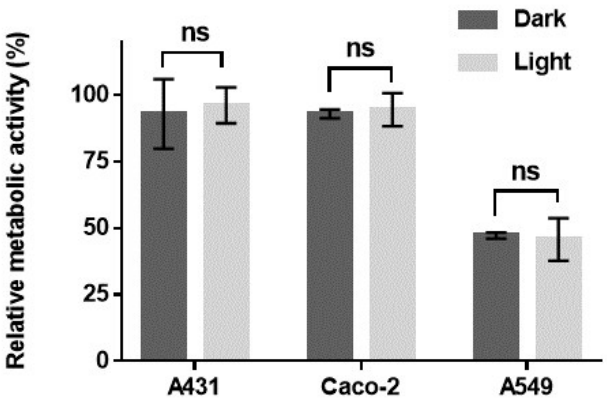


Figure S11. NP3 effect potency. Cells were exposed to NP3 for 48 hours either in the dark or under irradiation (λ = 400 nm, 7 mW cm^{-2}, for 10 min). Metabolic activity was assessed using the PrestoBlue assay. Data represents the mean ± S.D. (n=3).