**Supplemental Information**

# Inhibition of NADPH Oxidase-ROS Signal using Hyaluronic Acid Nanoparticles for Overcoming Radioresistance in Cancer Therapy

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## Materials and methods

### Internalization of HANP/GKT831 in tumor cells

The MCF7 and Breast VII PDX derived tumor cells were cultured in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum and 1% penicillin−streptomycin at 37°C in a $CO_2$ incubator. Cells were seeded onto an eight-well chamber slide ($1\times 10^5$cells/well) and cultured for 24 h at 37°C. The culture medium was then replaced with the medium containing NIR 830 dye labeled HANP/GKT831 and incubated for 4 h at 37 °C. After washing with phosphate-buffered saline (PBS) (pH 7.4), cells were fixed in cold ethanol for 15 min. Following nuclear staining with 4′,6-diamidino-2-phenylindole (DAPI) for 10 min, the slide was examined and imaged under an inverted fluorescence microscope with a NIR filter set (Ex/Em: 780/800 nm).

### Drug delivery in tumor cells

MCF7 cells were incubated with 6 mL of culture medium containing 1 mg of GKT831 equivalent concentration of HANP/GKT831 or free GKT831 overnight at 37 °C. Cells were then washed with PBS and collected by trypsinization. Harvested cell pellets ($1\times 10^6$ cells for each group) were mixed with acetonitrile containing 0.01% trifluoroacetic acid (v/v) to precipitate cellular proteins and extract the intracellular GKT831. The mixtures were then centrifuged at 3,000 rpm for 3 min and the supernatant (20 μL) was injected into HPLC with a reverse-phase C18 column. The amount of GKT831 released from HANP/GKT831 in cells was analyzed and calculated using a standard GKT831 curve ($y = 2156.65x-1.05$, $R^2 = 0.9999$).

### Detection of apoptotic cell death using flow cytometry analysis

The Breast VII PDX tumor cells were seeded onto six-well plates and cultured overnight at 37 °C. The culture medium was then replaced with fresh medium containing free GKT831 (5 μM), HANP (0.1 μM), and HANP/GKT831 (containing 5 μM of GKT831 and 0.1 μM of HANP) and incubated

at 37°C for 48 h. After washing the harvested cells in 1× Binding Buffer, cells were labeled with 5 μL of Annexin V/7-AAD apoptosis detection agents (BD Bioscience Catalog Number 556454 and 559925) for 15 min at room temperature. Cells were examined using a BD FACS A3 Cell Analyzer (BD Biosciences, Franklin Lakes, NJ).

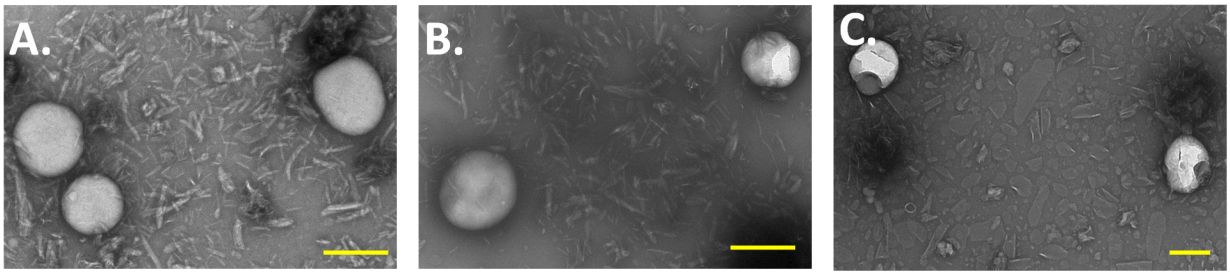


**Figure S1.** TEM images of HANP and HANP/GKT831. **A**. A TEM image of freshly made HANPs. **B**. A TEM image of HANPs after one-month storage at 4°C. C. A TEM image of HANP/GKT831 after one month storage at 4°C. Scale bars equal 200 nm.

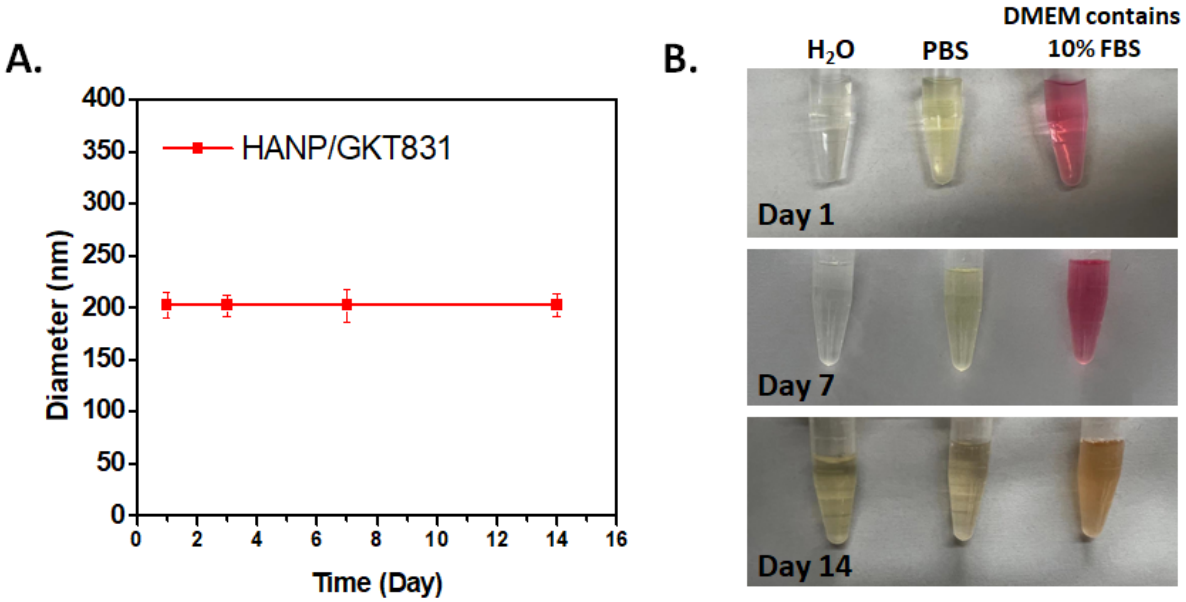


**Figure S2.** Stability of HANP/GKT831. **A.** hydrodynamic sizes of HANP/GKT831 after storing in $H_2O$ for 14 days. No significant changes in the nanoparticle size were noticed. **B.** Stability of HANP/GKT831 in solutions, including $H_2O$, PBS, and cell cultural DMEM medium containing 10% FBS. No precipitant was observed.

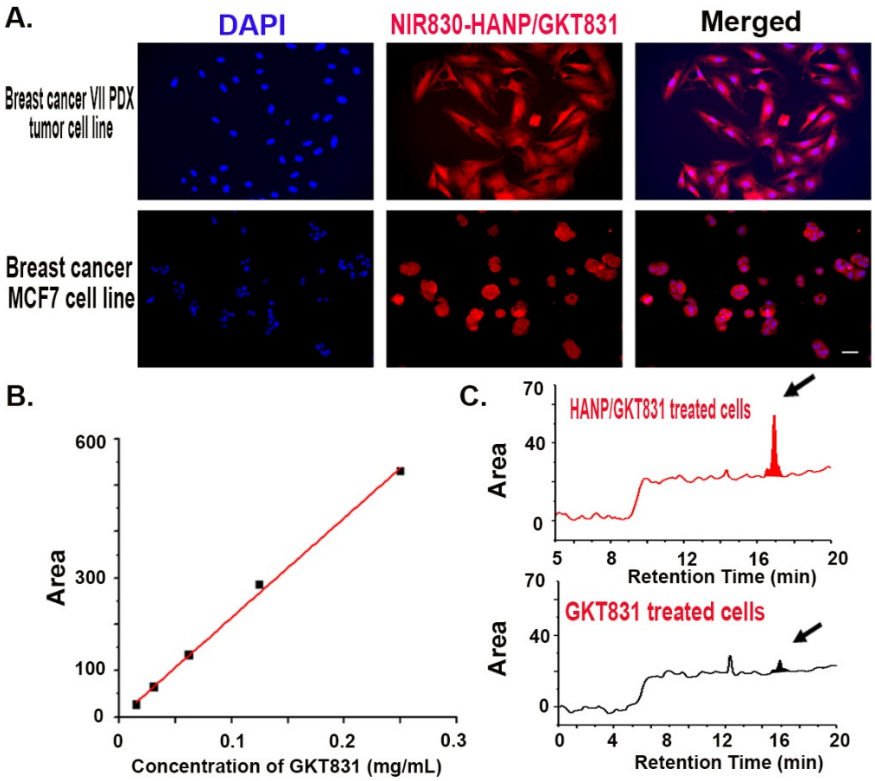


**Figure S3. Internalization of HANP/GKT831 in breast cancer cells. A.** Following 4 hr incubation, NIR830 dye labeled HANPs/GKT831 were detected in breast cancer cells. NIR 830 signal: red. Scale bar: 50 μm. **B.** A standard curve of GKT831 determined by HPLC. **C.** Quantification of GKT831 in breast cancer cells using HPLC. *Upper*: 18 μg of GKT831 was detected and calculated in HANP/GKT831 incubated MCF7 cells ($1\times 10^6$ cells). *Lower:* 5 μg of GKT831 was detected and calculated in free GKT831 incubated MCF7 cells ($1\times 10^6$ cells). Retention time of GKT831 is 16.93 min.

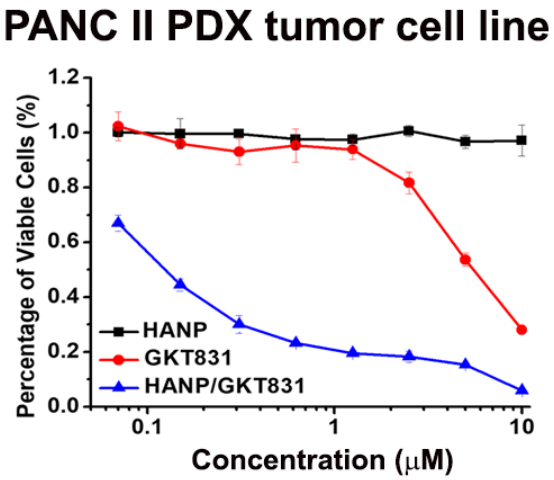


**Figure S4.** Cell proliferation assay determined using SRB assay. $IC_{50}$ of HANP/GKT831 on the pancreatic II PDX tumor derived cell line is 0.38 μM, and $IC_{50}$ of free GKT831 is 3.5 μM.

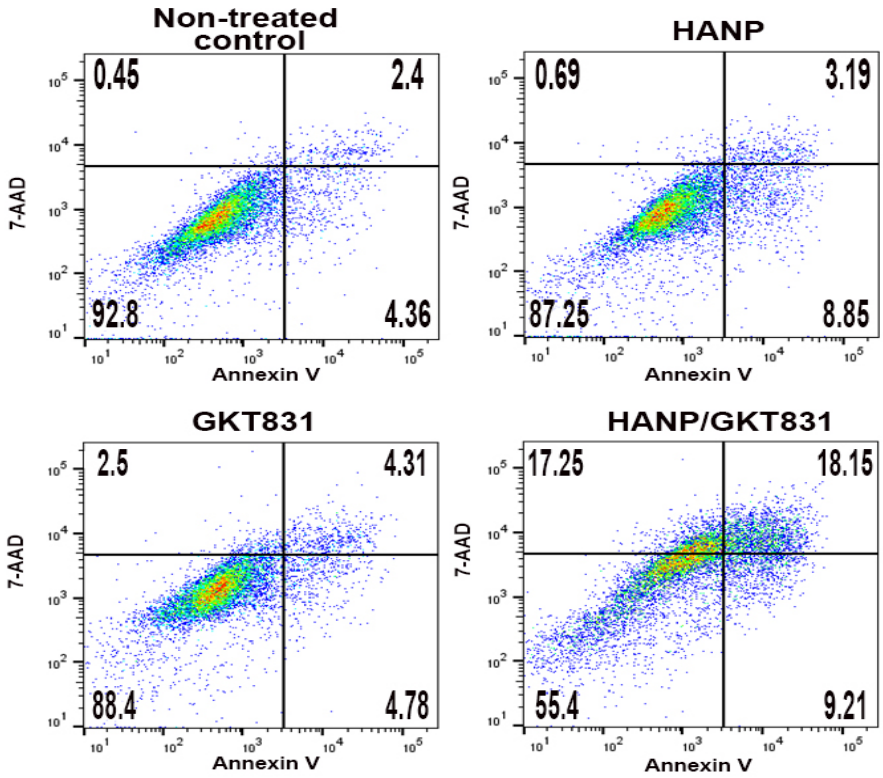


**Figure S5.** HANP/GKT831 induced apoptosis in the Breast VII PDX tumor derived cell line determined by Annexin V/7AAD assay. Representative results of flow cytometry analysis of treated tumor cells. The percentages of the apoptotic cells are shown in the lower right (early apoptotic cells: Annexin-V+) and upper right (late apoptotic cells: Annexin-V+/7-AAD+) of the quadrant. The percentages of the total apoptotic cells from the mean of two independent experiments are the following: HANP/GKT831 treated cells: 27.4%, HANP treated cells: 12.0%, GKT831-treated cells: 9.1%, and No-treatment control: 6.8%.

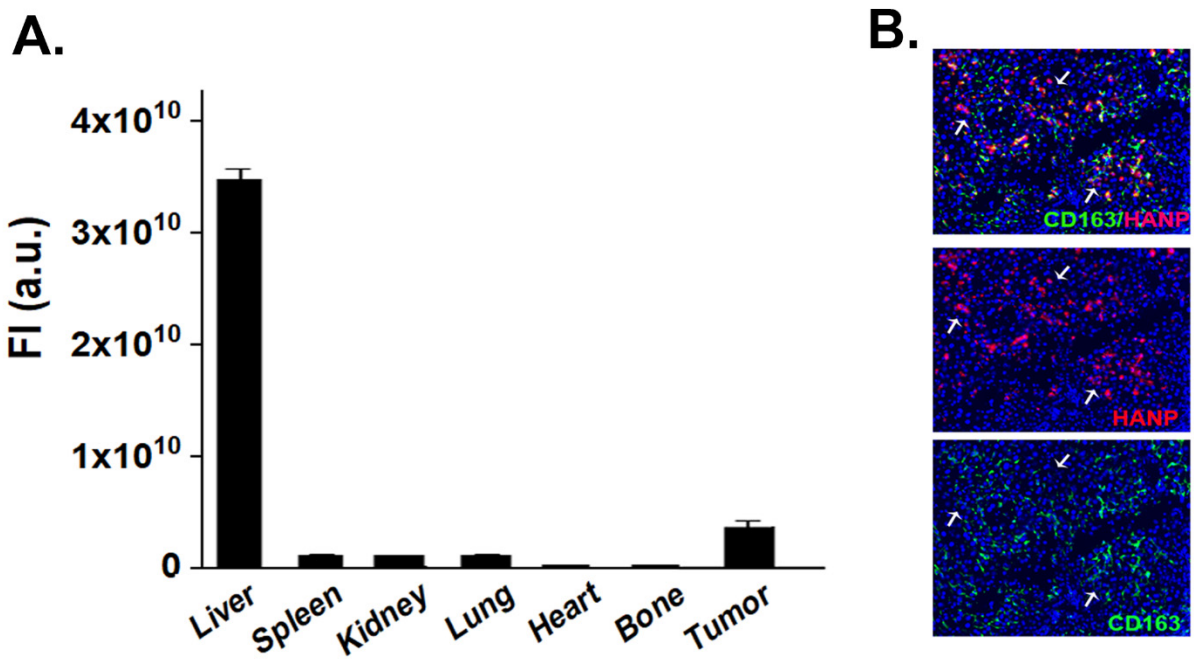


**Figure S6. Evaluation of biodistribution of HANP/GKT831 following i.v. delivery.** Nude mice bearing the orthotopic Breast VII PDX tumor received i.v. delivery of NIR830 dye labeled HANP for 24 hrs. Biodistribution of NIR 830-HANP in the tumor bearing mice was determined by *ex vivo* optical imaging of the tumor and normal organs after sacrificing mice. **A.** Quantification of NIR fluorescence signal in tumor and normal organs. **B.** Detection of localization of NIR830-HANP in the liver. A high level of NIR signal (red, NIR 830-HANP) positive cells are not co-localized with CD163+ macrophages in liver.