

Supporting Information

Nanoparticles for co-delivery of Osimertinib and Selumetinib to Overcome Osimertinib-acquired Resistance in Non-small Cell Lung Cancer

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Equal contribution

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Methods

Determination of Intracellular ROS.

The intracellular ROS was determined with a DCFH-DA dye method. Briefly, cells were seeded into a 12-well plate at a density of 100,000 cells per well and incubated overnight. Cells were treated with DCFH-DA (20 μ M) in serum-free medium at 37 °C for 3 hours. At the end of treatment, cells were washed, collected, and analyzed by flow cytometry (BD Accuri C6 Plus).

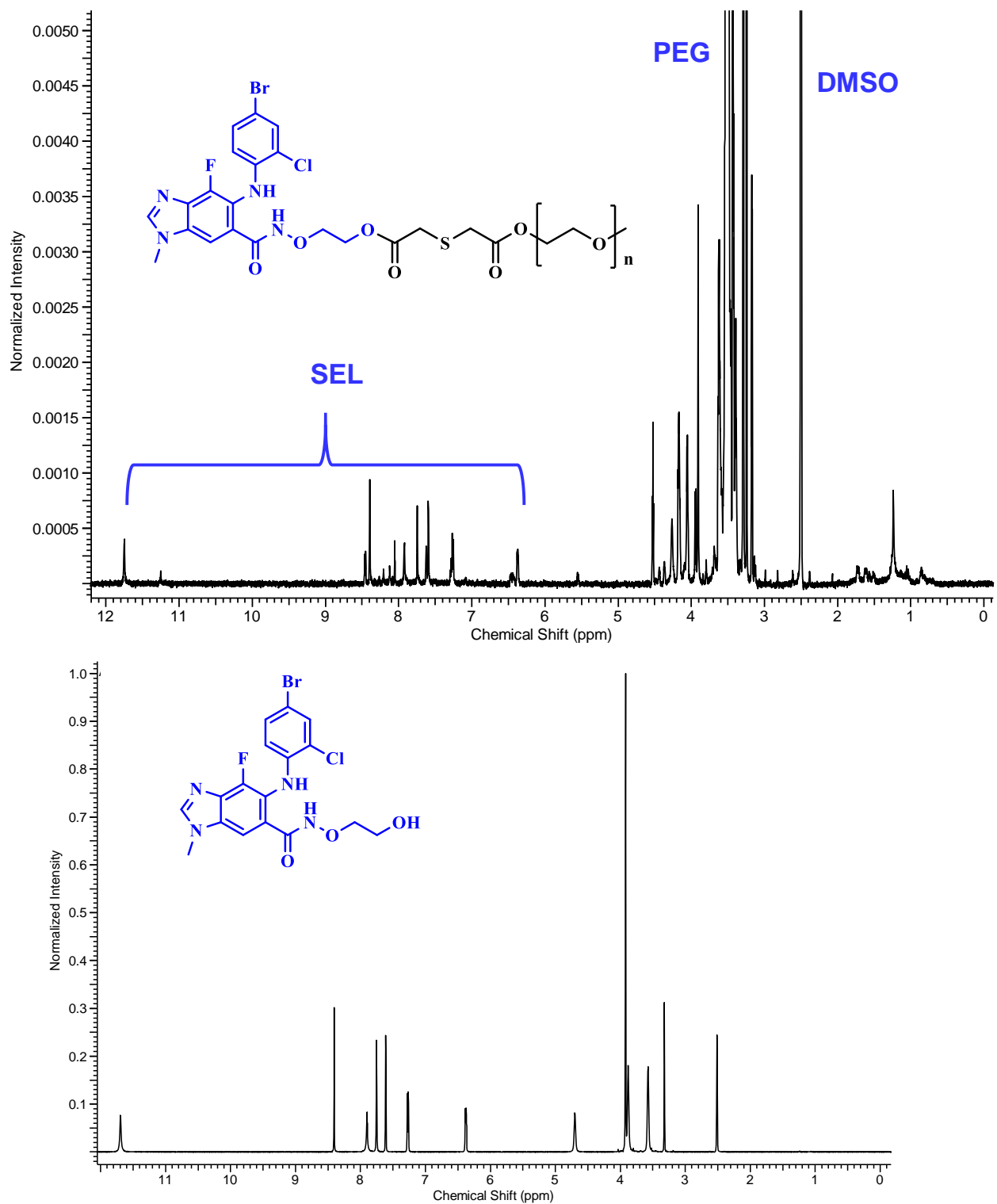


Figure S1. ¹H-NMR of PEG-SEL conjugate and SEL free drug in DMSO.

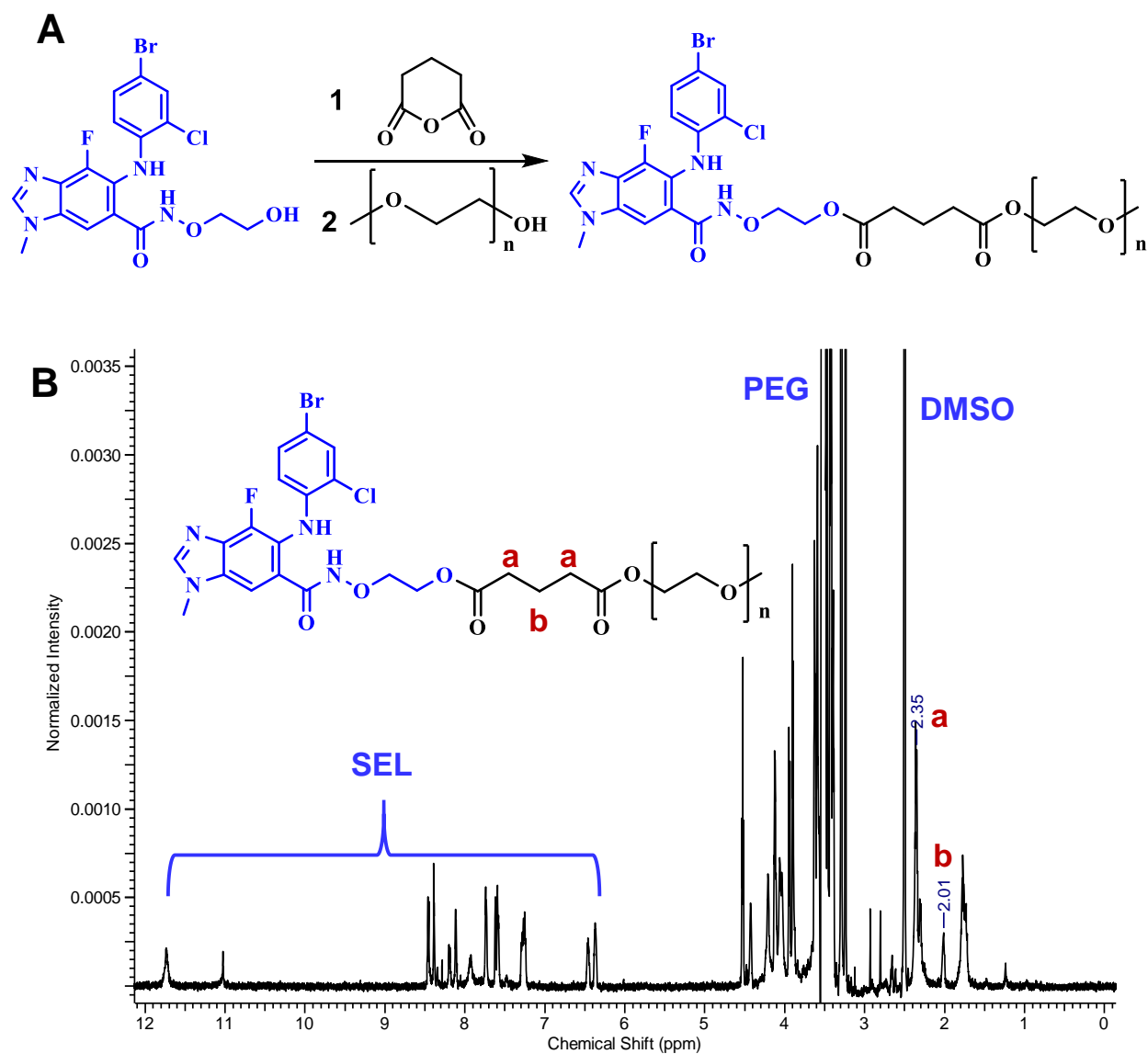


Figure S2. (A) Synthesis procedure of PEG-SEL conjugate with a NON-ROS responsive ester linker (PEG-C-SEL). 1. EDCI, HOBT, DMF; 2. DCC, DMAP, DMF. (B) $^1\text{H-NMR}$ of PEG-C-SEL in DMSO.

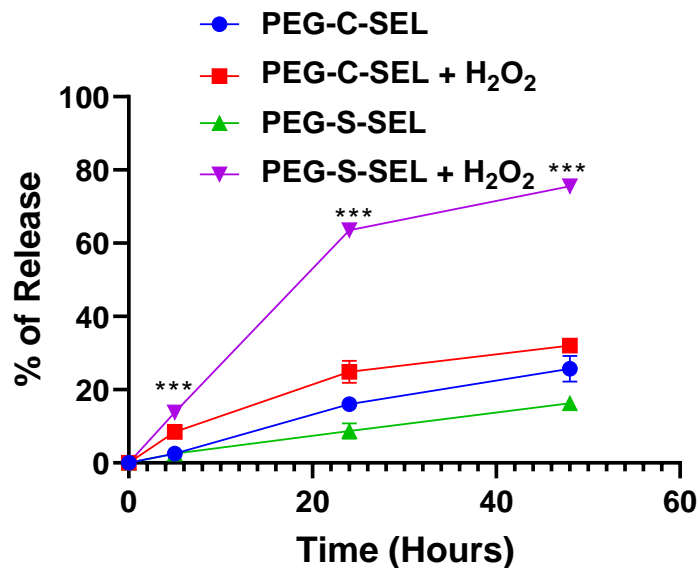


Figure S3. SEL drug release profiles of ROS responsive PEG-S-SEL conjugate and non-ROS responsive PEG-C-SEL conjugate. Results are mean \pm SD (n=3). ***, P < 0.001

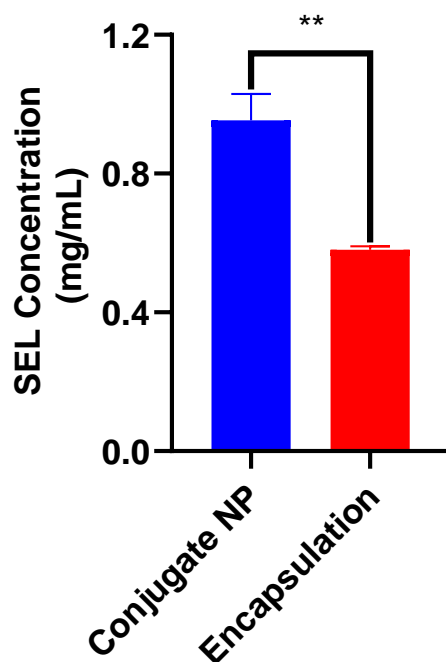


Figure S4. SEL concentration in NPs prepared with PEG-S-SEL conjugate and physical encapsulation of SEL free drug in micelle NP. Results are mean \pm SD (n=3). **, P < 0.01.

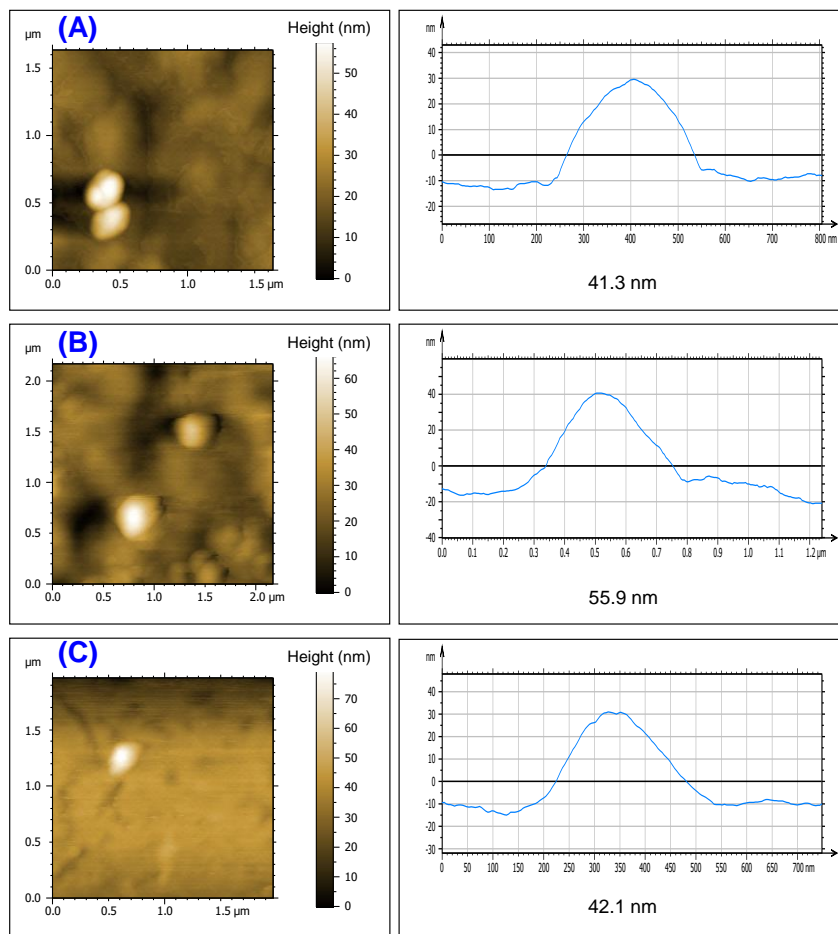


Figure S5: Atomic force microscope (AFM) images of (A) OSI-SEL NP, (B) SEL NP, (C) OSI NP

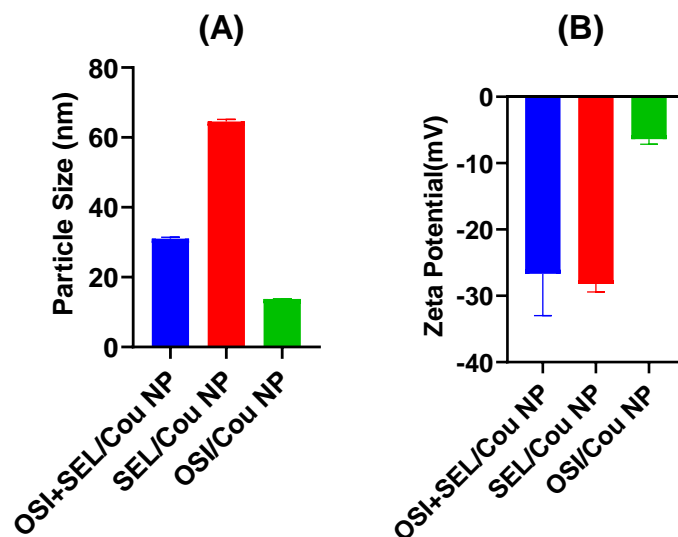


Figure S6. (A) Particle size and (B) Zeta potential of different Coumarin-6 loaded nanoparticles. Results are mean \pm SD (n=3).

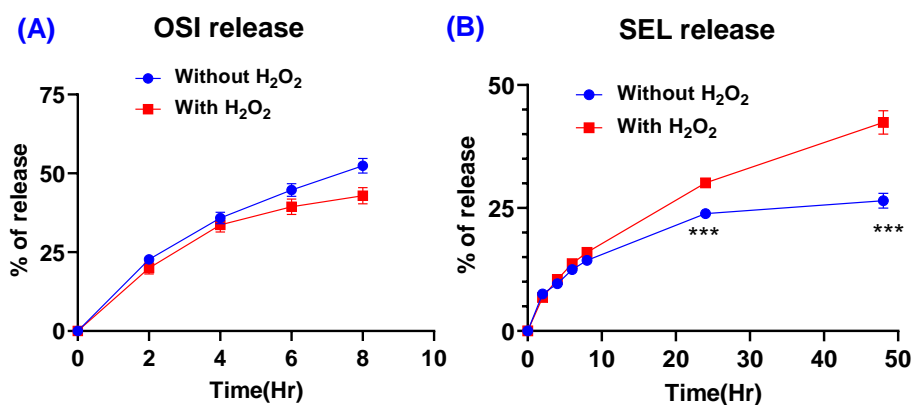


Figure S7. In vitro drug release from OSI-SEL NPs. Release of (A) OSI and (B) SEL from NPs in the presence or absence of 5 mM H₂O₂. Results are mean \pm SD (n=3). ***, P < 0.001

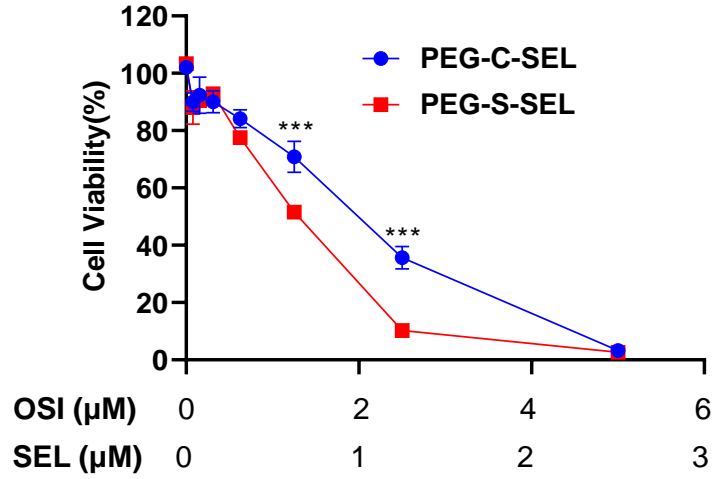


Figure S8. Anticancer activities of PEG-S-SEL and PEG-C-SEL in combination with OSI were determined with MTT assay. Results are mean \pm SD (n=4). ***, P < 0.001.

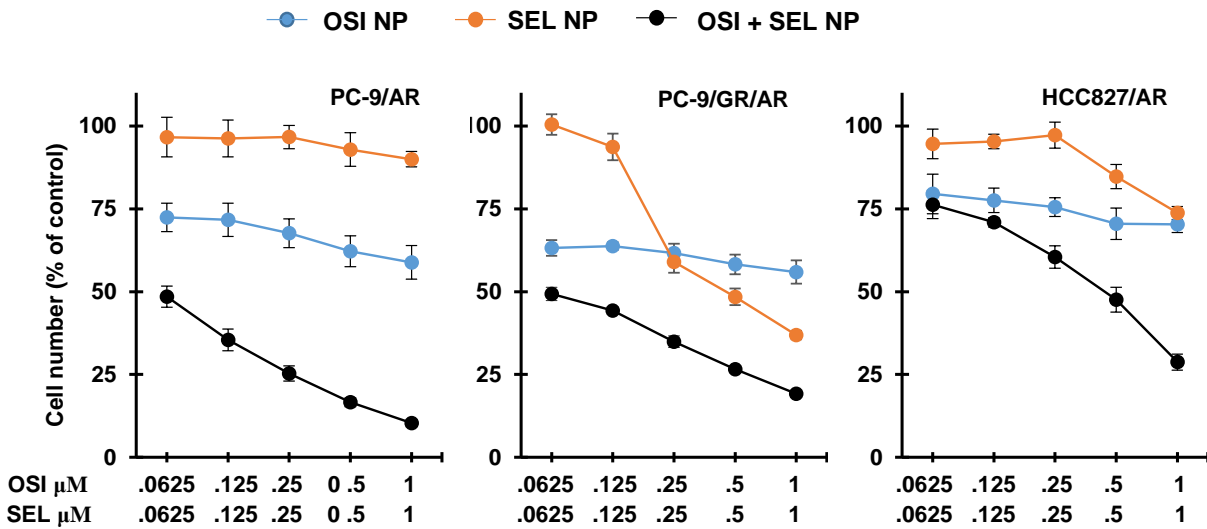


Figure S9. The indicated cell lines seeded in 96-well cell culture plates were treated with different concentrations of the tested NPs for 3 days. Cell numbers were determined with the SRB assay and expressed as the percentage of control. Results are mean \pm SD (n = 4).

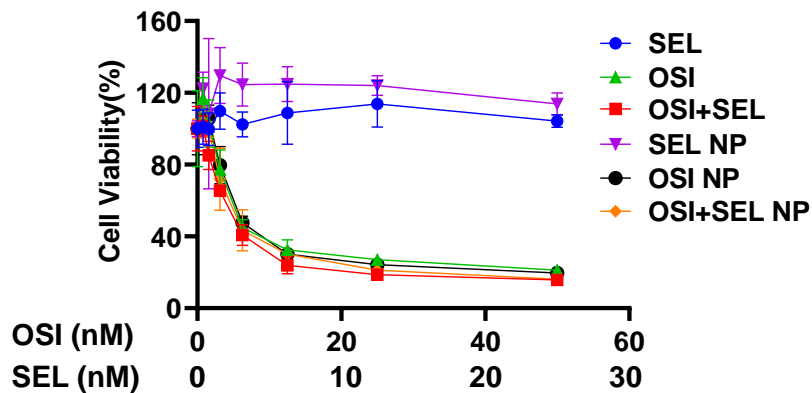


Figure S10. *In vitro* anticancer efficacy against OSI-sensitive PC9 NSCLC cells. Cell viability were determined with the MTT assay after treatment with different formulations for 48 hours. Results are mean \pm SD (n=3).

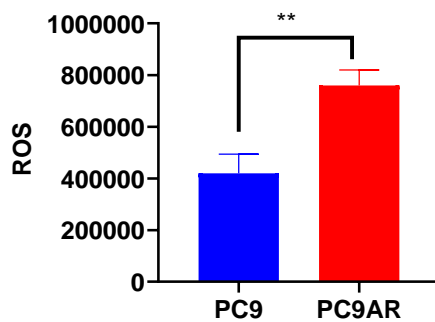


Figure S11. Intracellular ROS levels of PC9 and PC9 AR cells determined with DCFH-DA dye/flow cytometry method. Results are mean \pm SD (n=3). **, P < 0.01.