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Supporting information for:

Induction of oxidative stress and sensitization of cancer cells to paclitaxel by gold nanoparticles with different charge densities and hydrophobicities

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General experiments

Reagents and Antibodies. Cytochalasin D (C8273), apocynin (A10809), rotenone (R8875), diphenyleneiodonium chloride (D2926), nifedipine (N7634), $DIBAC_4(3)$ (D8189), BAPTA-AM (A1076), paclitaxel (T7402), N-Acetyl-L-cysteine (A9165) and epidermal growth factor (E5036) were purchased from Sigma-Aldrich (St Louis, MO, USA). Primary antibodies against HO-1 (#5061), akt (#4691), p-akt (#4060) were purchased from Cell Signaling (Boston, MA, USA). Primary antibody against β-actin (AM1021b) was purchased from Abgent (San Diego, CA, USA). Primary antibody against $p47^{phox}$ (sc-7660) was purchased from Santa Cruz (Santa Cruz, CA, USA). Horseradish peroxidase-conjugated secondary antibodies were purchased from Biorad (Richmond, CA, USA). Western blot luminescence reagent and PVDF membranes were purchased from Millipore (MA, USA).

Cell Culture. The A549, HEK293, HepG2, MRC-5 and HeLa cell lines were cultured in DMEM (Gibco) culture medium supplemented with 10% fetal bovine serum (Gibco), 100 μg/mL of stremycin, and 100 U/mL of penicillin at 37 ℃ with 5% CO_2. Cells growing at 80% confluence were trypsinized and passaged. All the cell lines were used between passage 5 and 10.

Cellular Uptake. After incubation with GNPs for 24 hrs, cells were washed with PBS for three times. Cells were detached by trypsin−EDTA solution. After counting the numbers, cells were lysed. The concentration of GNPs was determined by ICP-MS. Endocytosis inhibitor cytochalasin D (Cyto D, 0.5 μg/mL or 1 μg/mL) was used to inhibit cellular uptake of PO **6** and HY **7**.

Cytotoxicity Assays. Cells were cultured in 96-well plates with a density of 5000 cells per well. After treatment with GNPs (6 nm, 50 μg/mL) for 24 hrs, cells were washed with PBS for 3 times. Cells were lysed and analyzed by CellTilter-Glo® luminescent cell viability assay through

luminescence determination using a microplate reader. Cell culture medium was used as negative control.

Immunoblotting. Cells were harvested and lysed by the cell extraction buffer (FNN0011, Invitrogen) supplied with proteasome inhibitor (P2714, Sigma) and phenylmethanesulfonyl fluoride (PMSF). Proteins (20 μg) were loaded onto SDS-PAGE for separation. The proteins were transferred onto a PVDF membrane, which was then blocked by nonfat dry milk (5% w/v) in TBS with 0.05% Tween-20 (TBST). After incubation with primary antibody (1:1000, 5% nonfat dry milk or BSA in TBST) at 4 ℃ overnight, the membrane was washed with TBST for three times. The PVDF membrane was then incubated with secondary antibody (1:5000, 5% nonfat dry milk or BSA in TBST) at room temperature for 1 h. Then the membrane was washed for three times with TBST. The bands emerged after incubation with a luminescent reagent. The band intensity was quantified by ImageJ.

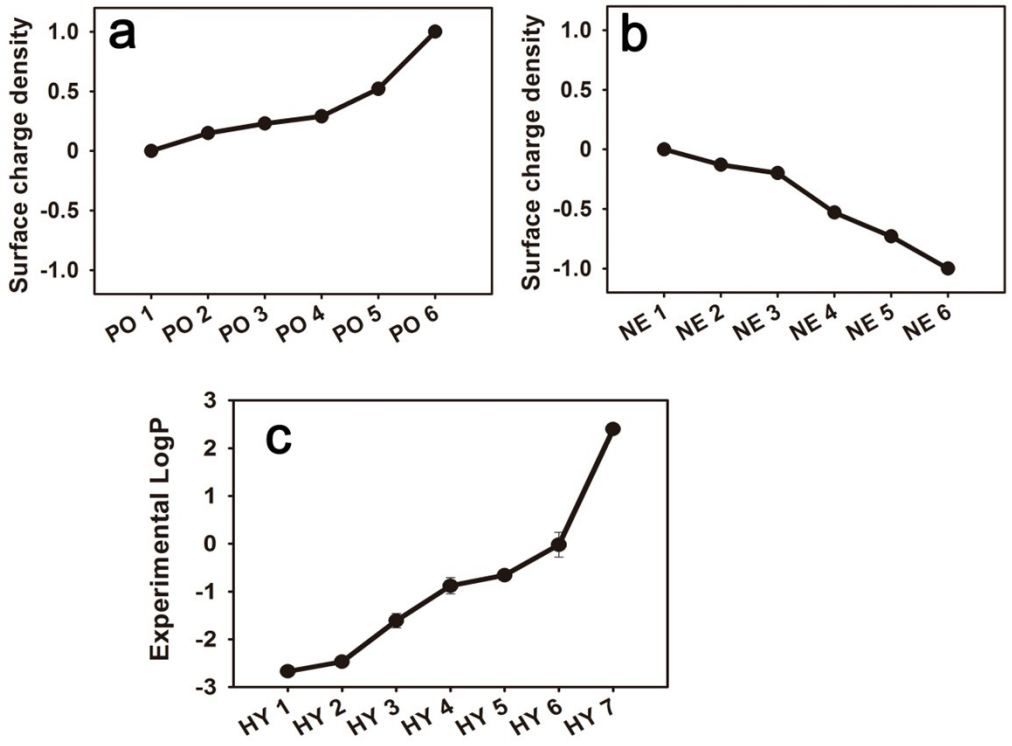

Figure S1. The surface physicochemical properties of three GNP libraries. The surface physicochemical properties include (a, b) surface charge density and (c) experimental LogP values.

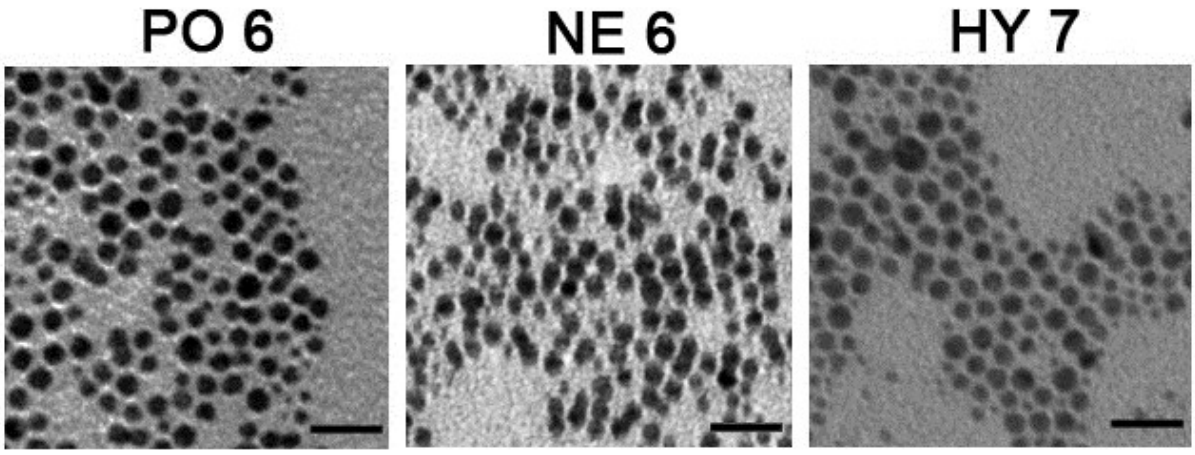

Figure S2. TEM images of selected GNPs from PO, NE, and HY libraris. The scale bar is 25 nm.

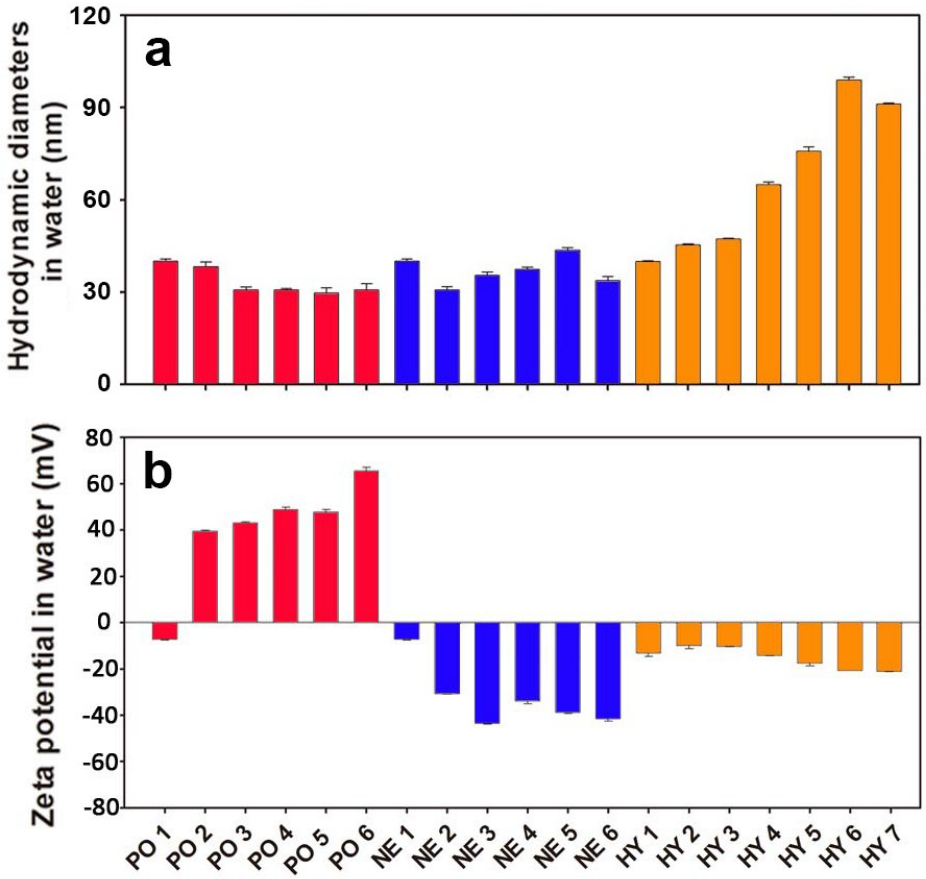

Figure S3. (a) Hydrodynamic diameters and (b) zeta potentials of GNPs from PO, NE, and HY libraries.

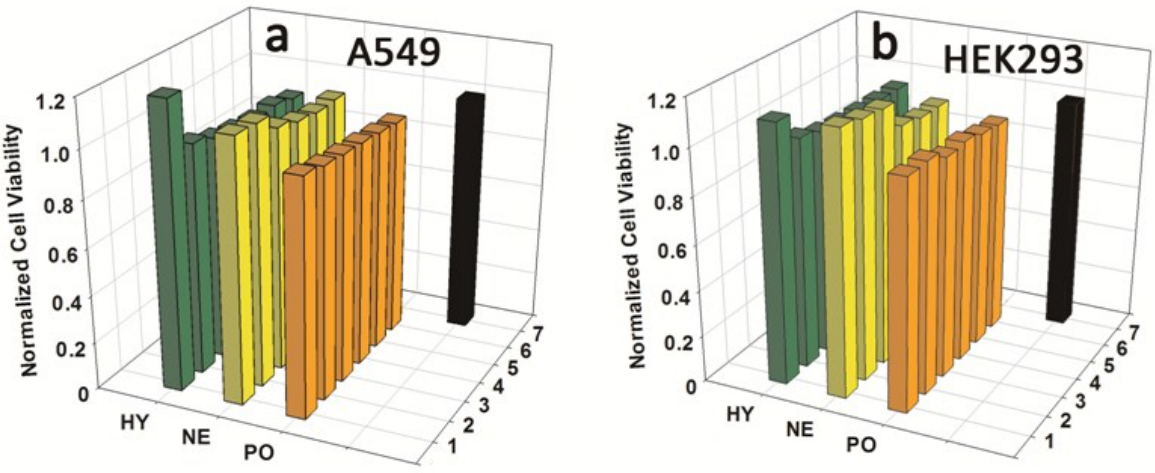

Figure S4. Cell viability of A549 and HEK293 cells treated with GNP libraries. After treatment with GNPs (6 nm, 50 μg/mL) for 24 hrs, A549 (a) and HEK293 (b) cells were lysed and analyzed by CellTilter-Glo® assay using a microplate reader. The black bar represents the cell viability of A549 and HEK293 cells in culture medium.

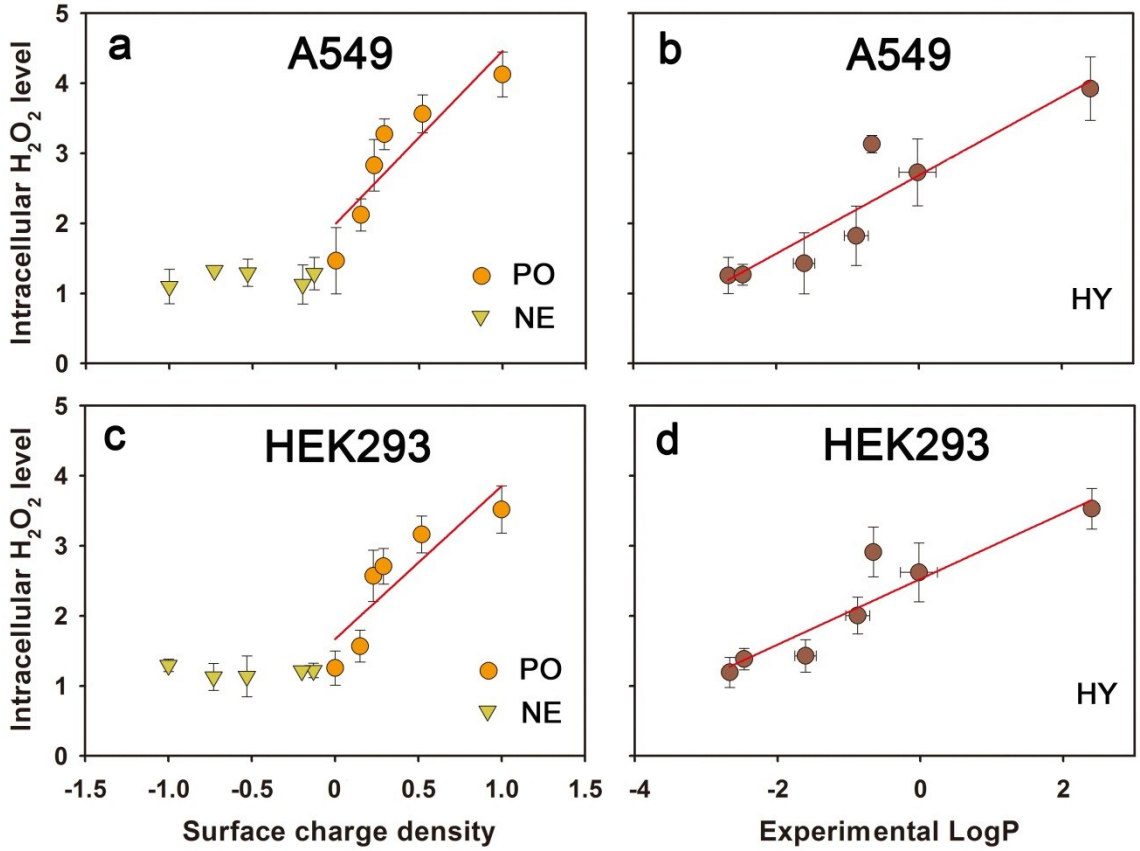

Figure S5. Intracellular H_2O_2 level in (a, b) A549 and (c, d) HEK293 cells after incubation of GNPs from different libraries. A549 and HEK293 cells were treated with GNPs (6 nm, 50 μg/mL) for 24 hrs. The intracellular H_2O_2 level was determined by ROS-Glo™ H_2O_2 Assay.

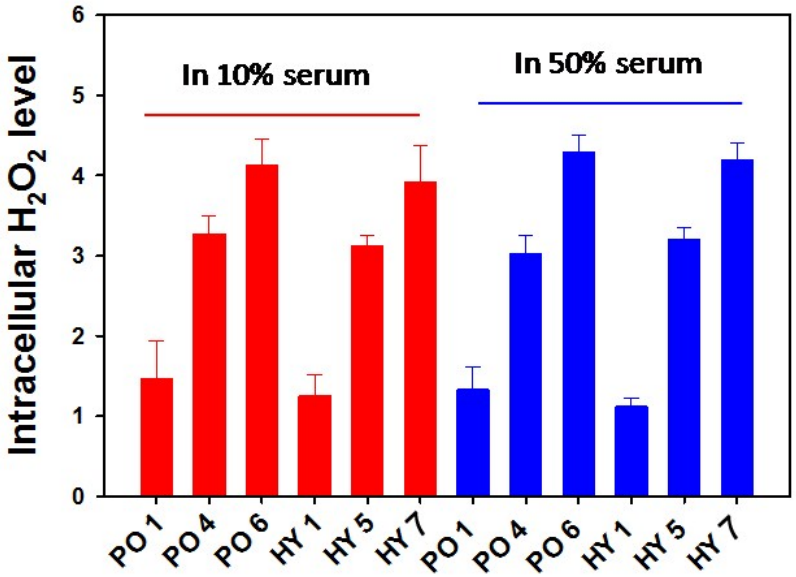

Figure S6. Comparison of intracellular H_2O_2 level in A549 cells after incubation of GNPs in medium with 10% and 50% serum. A549 cells were incubated with selected GNPs (6 nm, 50 μg/mL) for 24 hrs. The intracellular H_2O_2 level was determined by ROS-Glo™ H_2O_2 Assay.

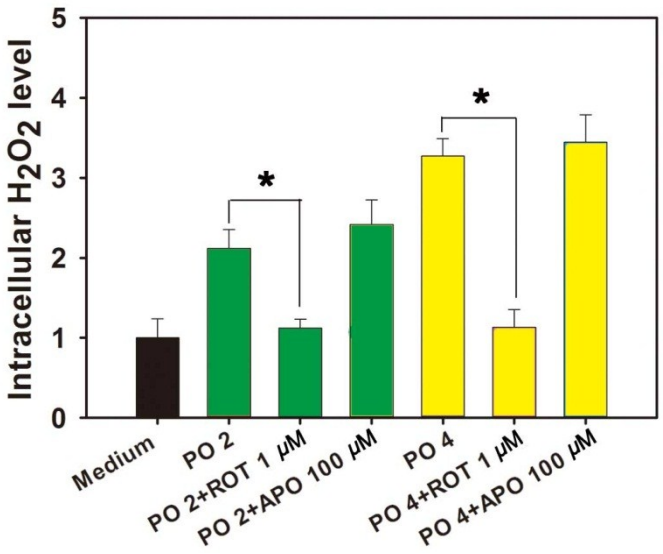

Figure S7. The effect of APO and ROT on intracellular H_2O_2 level induced by PO 2 and PO 4 GNPs. PO **2** and PO **4** induced increasing levels of H_2O_2, and H_2O_2 generation was all inhibited by ROT but not APO.

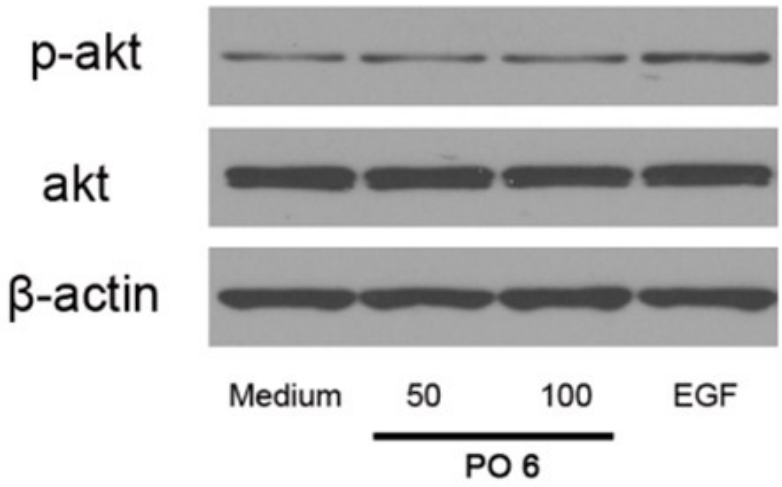

Figure S8. No P-akt activation after incubation with PO 6 GNP. A549 cells were cultured with PO **6** (6 nm, 50 μg/mL or 100 μg/mL) for 24 hrs. P-akt and total akt level was determined by Western blot; β-actin was used as loading control. Epidermal growth factor (EGF) was used as positive control.

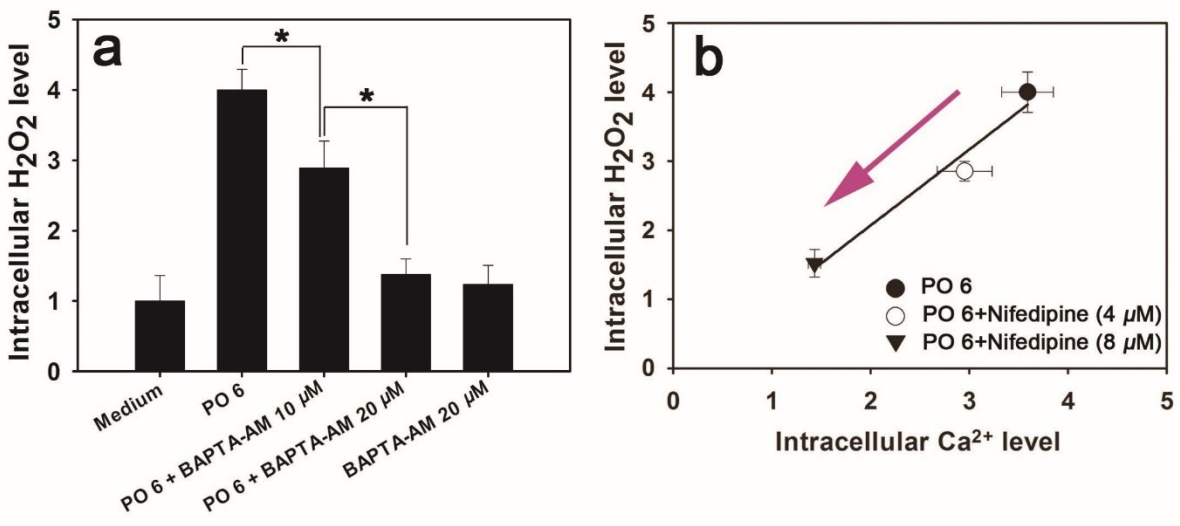

Figure S9. The effect of Ca^{2+} chelating agent and calcium channel inhibitor on intracellular H_2O_2 level induced by PO 6. (**a**) A549 cells were pretreated with a Ca^{2+} chelating agent BAPTA-AM (10 μM, 20 μM) for 30 min prior to incubation with PO **6** at a concentration of 50 μg/mL for 24 hrs. The intracellular H_2O_2 level was determined by ROS-Glo™ H_2O_2 Assay. *$P < 0.05$ *vs* value of the corresponding nanoparticle group. Error bars indicate mean ± standard deviation (n=3). (**b**) A549 cells were pretreated with a Ca^{2+}-channel inhibitor nifedipine (4 μM, 8 μM) for 30 min prior to incubation with PO **6** (50 μg/mL) for 24 hrs. Intracellular Ca^{2+} level was quantitatively determined by Fluo-3 AM. Intracellular H_2O_2 level was determined by ROS-Glo™ H_2O_2 Assay.

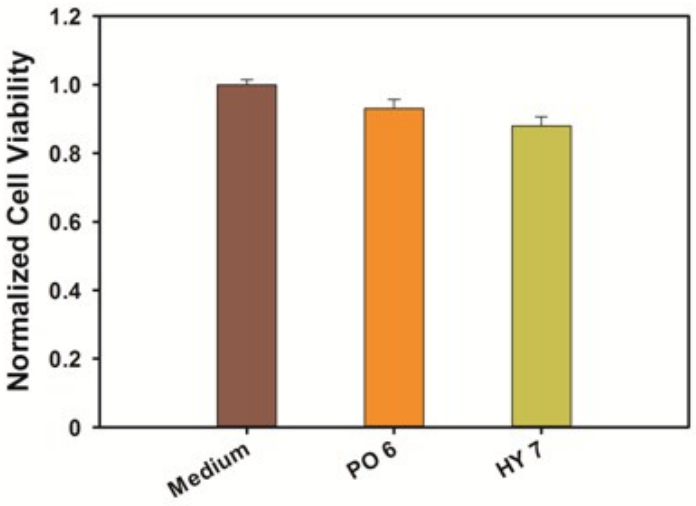

Figure S10. Cell viability of HeLa cells treated with PO 6 and HY 7 GNPs. After treatment with PO 6 or HY 7 (6 nm, 50 μg/mL) for 24 hrs, HeLa cells were lysed and analyzed by CellTilter-Glo® assay using a microplate reader. Error bars indicate mean ± standard deviation (n = 3).