Supplemental Material

Materials and methods

Cell cultures: Human ovarian cancer cells A2780 and CP70 were cultured in DMEM and RPMI 1640 medium respectively. Human bronchial epithelial cells (BECs) and airway smooth muscle (ASM) cells were enzymatically isolated using previously described techniques[1, 2] from 3rd generation or higher bronchi excised from lung samples incidental to patient surgery. BECs and ASM cells were maintained (up to 3 passages) in serum-free bronchial epithelial growth medium (BEGM) [3] or phenol red-free DMEM/F12, respectively. Phenotypes were verified by expression of cytokeratins and CD31 (BECs) vs. smooth muscle myosin or actin (ASM). Experiments were performed in cells at ~80% confluence.

Cellular Imaging: Previously published imaging protocols are summarized here [2]. Cells were plated onto Labtek 8-well glass coverslip-bottomed chambers. Cells were loaded for 45 min with either 25 μM RH414 (cell-permeant membrane potential-sensitive fluorescent dye), or 5 μM fura-2/AM (ratiometric Ca^{2+} indicator). Cells were visualized using an Universal Imaging MetaFluor system (Nikon microscope, 40X/1.3NA oil immersion lens, 12-bit Roper Scientific CCD camera) with images obtained at ~2 Hz and fluorescence levels measured for 10-15 cells per field. RH414 calibration was performed in situ for each cell type using a range of KCl concentrations in 2mM Ca^{2+} Hanks balanced salt solution (HBSS), while fura-2 was calibrated as described previously [2,4]. In a subset of CP70 cells cells, temporal relationships between changes in membrane potential vs. $[Ca^{2+}]_I$ was determined using co-loading with RH414 and fluo-3 (non-

ratiometric Ca^{2+} indicator) and simultaneously visualization using appropriate filters.

^{3}H-Thymidine incorporation assay for cellular proliferation*:* Cells ($2x10^4$) in 24-well culture plates were treated with $^{+}$AuNPs for 30 min, rinsed with phosphate buffered saline (PBS) and exposed to cell-appropriate fresh media. Thymidine incorporation assay was performed as previously published [5]. In brief, after 24h, 1 μCi/ml $^{[3H]}$thymidine was added, and 4h later, cells washed with ice-cold PBS, fixed with 100% cold methanol, washed again, and lysed with 250 μl 0.1N NaOH. $^{[3H]}$thymidine incorporation was measured in scintillation solution.

Cellular apoptosis assay: Cells seeded in 60 mm dishes at 3 x 10^5 and at ~80% confluence were treated with 0 (vehicle), 0.4 or 0.8 μM $^{+}$AuNPs for 30 min. They were then rinsed with PBS, replenished with fresh media and maintained in culture overnight. Annexin FITC-propidium iodide staining flow cytometry was performed per manufacturer's protocol (BioVision, CA).

Cellular viability assay: Cells in 96-well plates at ~80% confluence (10,000–20,000 cells) were exposed for 30 min to $^{+}$AuNPs (0 (vehicle), 0.4, 0.8, or 1.2 μM), washed, and then maintained for 24h. Subsequently, 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) was added for 30 min, and absorbance at 490 nm measured.

Nanoparticle Fabrication: Gold nanoparticles were synthesized according to reported procedures [6]. Dynamic light scattering DLS and zeta potential

measurements were carried out using a Nano series Zetasizer (Malvern Instruments).

Quantification of Gold Uptake (Instrumental Neutron Activation Analysis; INAA)*:* Samples were analyzed by INAA as previously described [7]. Cells were grown in 60 mm dishes. Once 80% confluent, the cells were incubated with 0.4 μM $^{+}$AuNPs for 30 min and rinsed in PBS five times. After being trypsinized for 10 minutes, the cells were collected into eppendorf tubes and pelleted (10 min, 1400 rpm, 4°C). They were subsequently transferred and weighed into pre-cleaned, high-density polyethylene irradiation vials and lyophilized to constant dry weight. Samples were then reconstituted with 100 μL sample solution, loaded in polyethylene transfer "rabbits" and irradiated for 90 s in a thermal flux density of ~5×10^{13} ncm^{-2} s^{-1}. Samples were allowed to decay for 24-48 h and counted in real-time on a high-purity germanium detector for 3600 s at a sample-to-detector distance of ~5 cm. Gold mass was quantified by measuring the 411.8 keV gamma ray from β^- decay of ^{198}Au ($t_{1/2}$=2.7 days), and calibrated using certified gold standard solutions as described previously.

TEM Imaging: Cells grown on Aclar film in 24 well plates were exposed to 0.4 μM $^{+}$AuNPs for fixed durations (5 min, 2h, 6h), thoroughly rinsed with PBS, and fixed with Trumps fixative for microtome sectioning and TEM microscopy [8].

References

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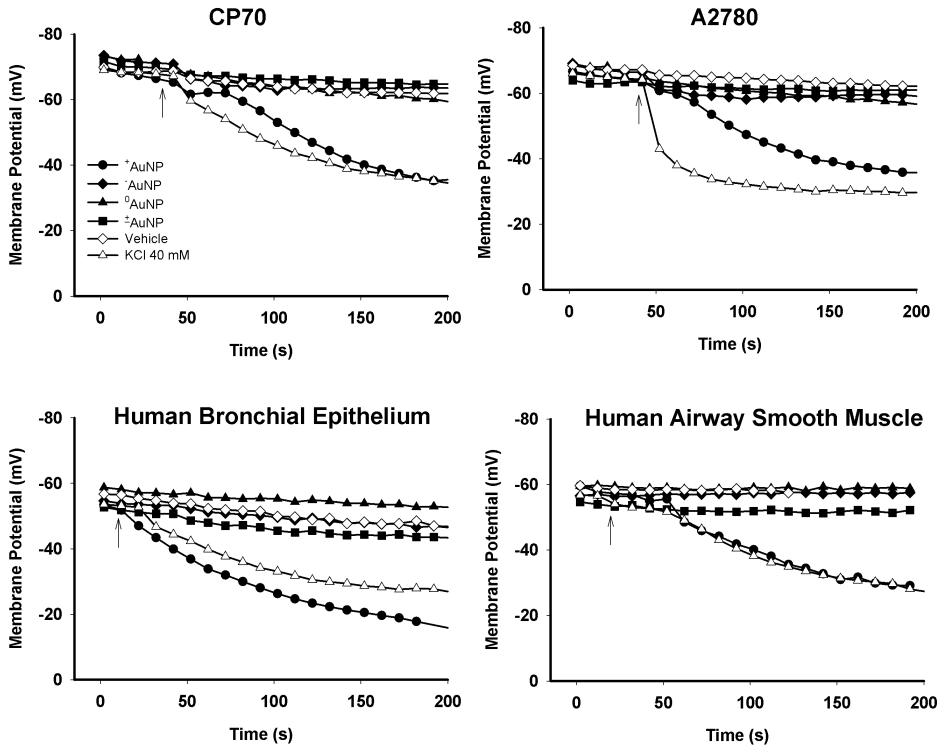

Figure S1: Nanoparticle effects on cellular membrane potentials. Four cell types (two ovarian cancer cell lines (CP70, A2780), human bronchial epithelial cells and human airway smooth muscle cells) were loaded with the cell-permeant fluorescent membrane potential indicator RH414 and imaged using real-time fluorescence microscopy. Following measurement of basal membrane potential (ranging from -75 to -55, depending on cell type), cells were exposed to AuNPs (1.2 μM, black arrow indicates time of addition) of different surface charges. Membrane depolarization was observed in less than 1 min following exposure to AuNPs with positive charge, reaching a maximum level of depolarization in

approximately 3 min. The extent of depolarization was greater in the ovarian cancer cells and in epithelium, compared to airway smooth muscle cells. In comparison to positively charged AuNPs, those with other charges had negligible effects on membrane potential in any cell type. In control experiments, each of these cell types were exposed to 40 mM KCl (black arrow also indicates time of KCl application), which produced fairly immediate depolarization across cell types (see Figure 1 of main text for summary).

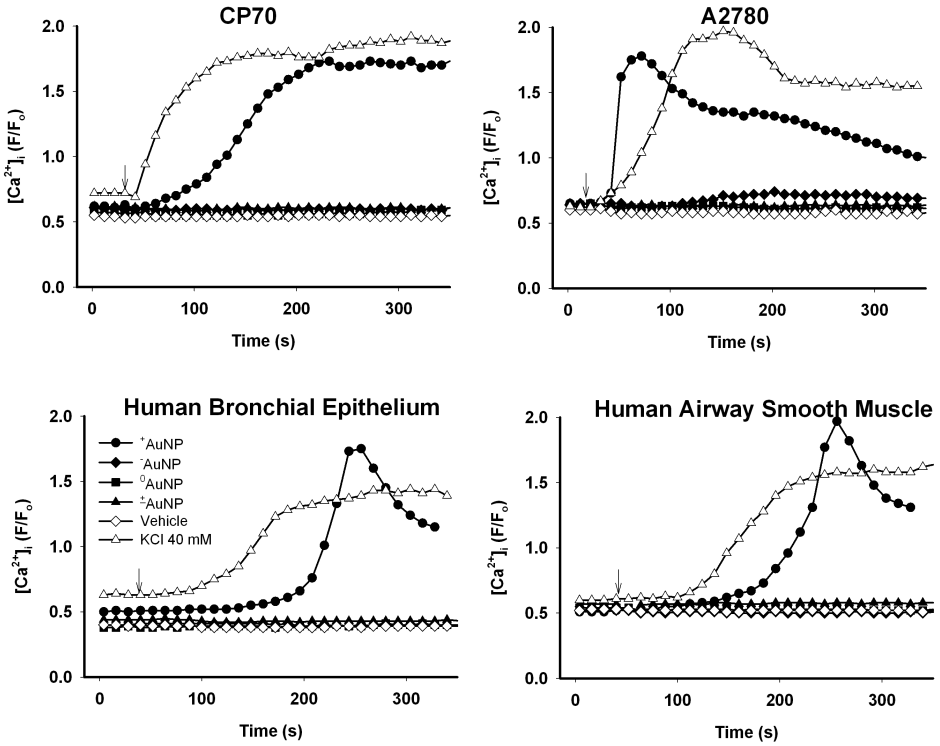

Figure S2: Positively charged AuNPs rapidly increase $[Ca^{2+}]_i$ levels. In ovarian cancer cells (CP70, A2780), addition of positively charged AuNPs (1.2 μM; black arrow indicates time of addition) produced immediate increases in $[Ca^{2+}]_i$ (measured using the ratiometric fluorescent Ca^{2+} indicator fura-2) that were sustained at 5 min in CP70 cells, while levels had reduced to a lower level (still above baseline) in A2780 cells. In human bronchial epithelial cells as well as airway smooth muscle cells, changes in $[Ca^{2+}]_i$ did not occur for 1-2 min following AuNP exposure. In all cell types, compared to positively charged AuNPs, particles with other types of charges had negligible effects on $[Ca^{2+}]_i$ levels even after 5 min. In control experiments, each of these cell types were exposed to 40

mM KCl (black arrow also indicates time of KCl application), which produced $[Ca^{2+}]_i$ elevations across cell types albeit with different time delays and profiles (see Figure 3 of main text for summary).

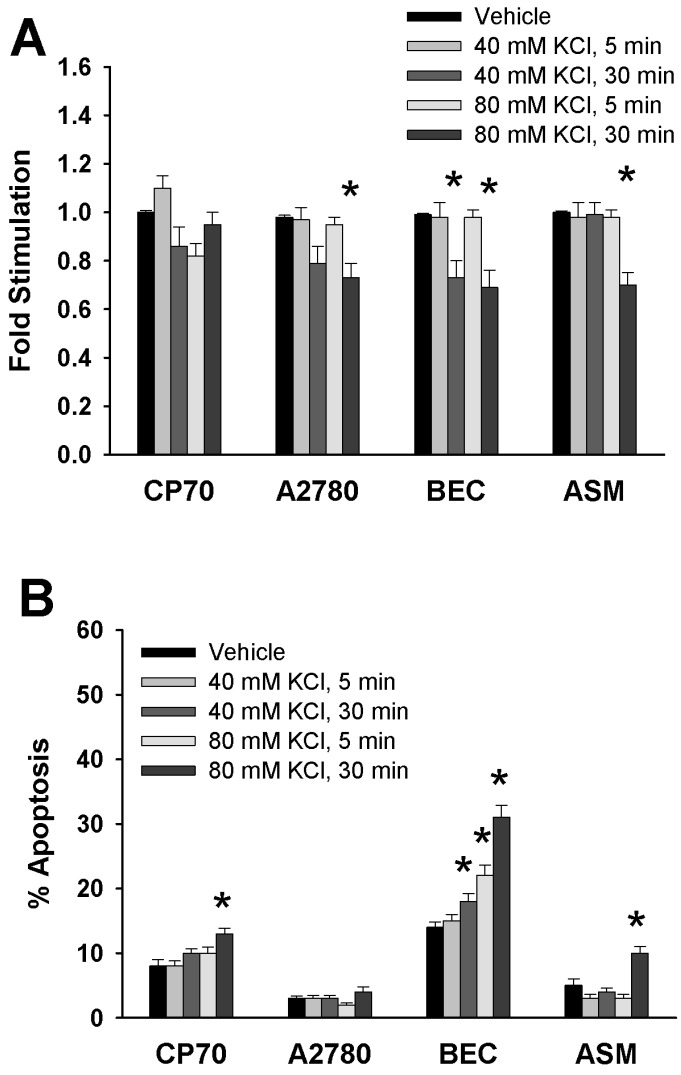

Figure S3: Effect of KCl on cellular proliferation and apoptosis. CP70, A2780, BEC or ASM cells were exposed to 40 mM or 80 mM KCl to induce membrane depolarization and $[Ca^{2+}]_i$ elevation (with exposures of 5 min or 30 min, mimicking evaluation of $^{+}$AuNP effects). Cellular proliferation was slightly decreased in some cell types after 30 min KCl exposure. However, compared to AuNPs (see Figure 4 of main text), the decrease in proliferation was substantially

smaller, especially in BECs. Similarly, KCl by itself did induce some degree of apoptosis in BECs and ASM, but again not to the substantial extent induced by $^{+}$AuNPs (Figure 4). Values are means $\pm$ SE. * indicates significant KCl effect ($p<0.05$).