

Figure: S1 Lung cancer MTT assay



Figure S2. Annexin-V and PI staining in Lung cancer cells

**MTT assay**

NCI-1975 lung cancer cells were grown in 75mm culture flask. Cells were trypsanized and

were seeded onto 96 well cell culture plates at a cell density of 1 x 105 cells/well. After 24hr of incubation, fresh DMEM high glucose media with L-glutamine (2mM), 10 % Fetal bovine serum (FBS), penicillin, and streptomycin 100 U/ml. Nanoparticles used for testing in MCF-7 and in MCF10A cells, similar particles and concentration were used for NCI-1975 cancer cells. The incubation time and treatment conditions were similar to MCF-7 cells. Varying concentrations (5, 10, 15, and 20μg/ml) of Ag-NP, Cu-NP, and or different alloyed compositions of AgCu-NP were added to cells growing on 96 well plates and were incubated for 48 hours. Following incubation, MTT (Thermo Fisher Scientific V13154) assay was performed as directed by the supplier's protocol. After incubation time, slowly, media was aspirated, and 50 μL of Dimethyl sulfoxide (DMSO) was added to each well and incubated for 15 minutes to dissolve insoluble formazan crystals. The absorbance of the formazan dye was read at 540 nm using a TECAN-SPARK plate reader.

**Results**

NCI-1975 showed toxic response to AgCu-NP treatment. Our results showed that only AgCu-NP nanoparticles showed significant toxicity as shown in figure S1. The toxicity results were resembling to MCF-7 cells.

Furthermore, we tested Annexin-V and PI staining in NCI-1975 cells similar to MCF-7 and MCF-10A protocol. In this experiment, AgCu-NP 70:30 was added as used in MCF-7 and MCF-10A. Our results showed there was significant staining in pre-apoptosis and late stage of apoptosis. This data may suggest that the toxicity by agCu-NP in NCI-1975 involving apoptotic pathway. The results are shown in S2 figure. In the S2 figure we added bar graph representing the PI positive cells and apoptotic cells (early+late stage) with respect to concentration of AgCu-NP nanoparticles.