Supporting Information

Size and ligand effects of gold nanoclusters in alteration of organellar state and translocation of transcription factors in human primary astrocytes

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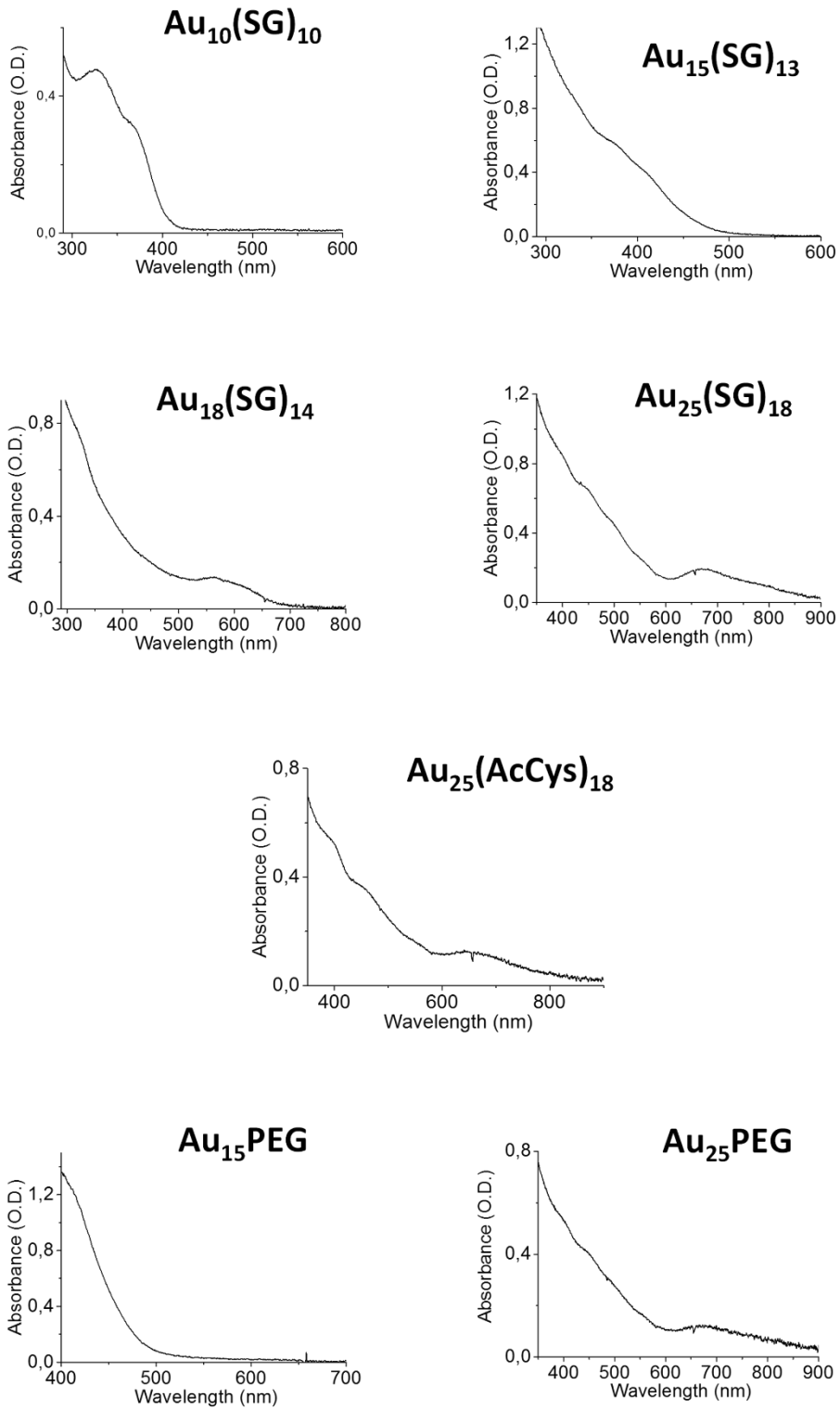

Fig. S1 UV-vis absorption spectra of AuNCs ($Au_{10}SG_{10}$, $Au_{15}SG_{13}$, $Au_{18}SG_{14}$ and $Au_{25}SG_{18}$, $Au_{25}AcCys_{18}$, $Au_{25}PEG$, $Au_{15}PEG$) in water.

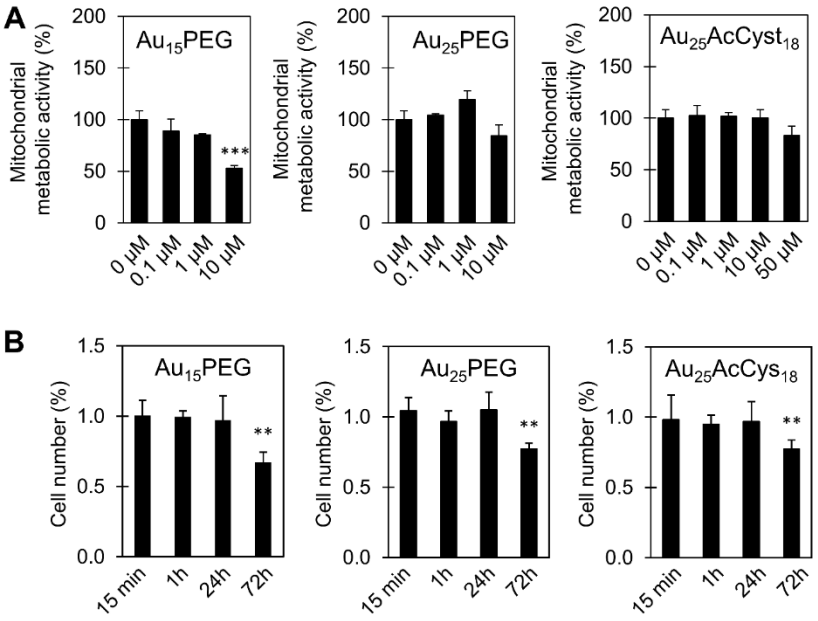

Fig. S2 **(A)** Human astrocytes were treated for 24 hours with $Au_{15}PEG$, $Au_{25}PEG$, or $Au_{25}AcCyst_{18}$ at the final concentrations indicated. Mitochondrial metabolic activity of human primary astrocytes was measured using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, as described in Methods. **(B)** Primary human astrocytes were treated with 10 µM with $Au_{15}PEG$, $Au_{25}PEG$, or $Au_{25}AcCyst_{18}$ for 15 min, 1 h, 24h and 72h. Cell viability was assessed by cell counting, using Hoechst 33342 nuclear stain. Cell number was quantified and normalised to control±SD. A p value of less than 0.05 was identified as statistically significant and indicated as: **p<0.01, ***p<0.001.

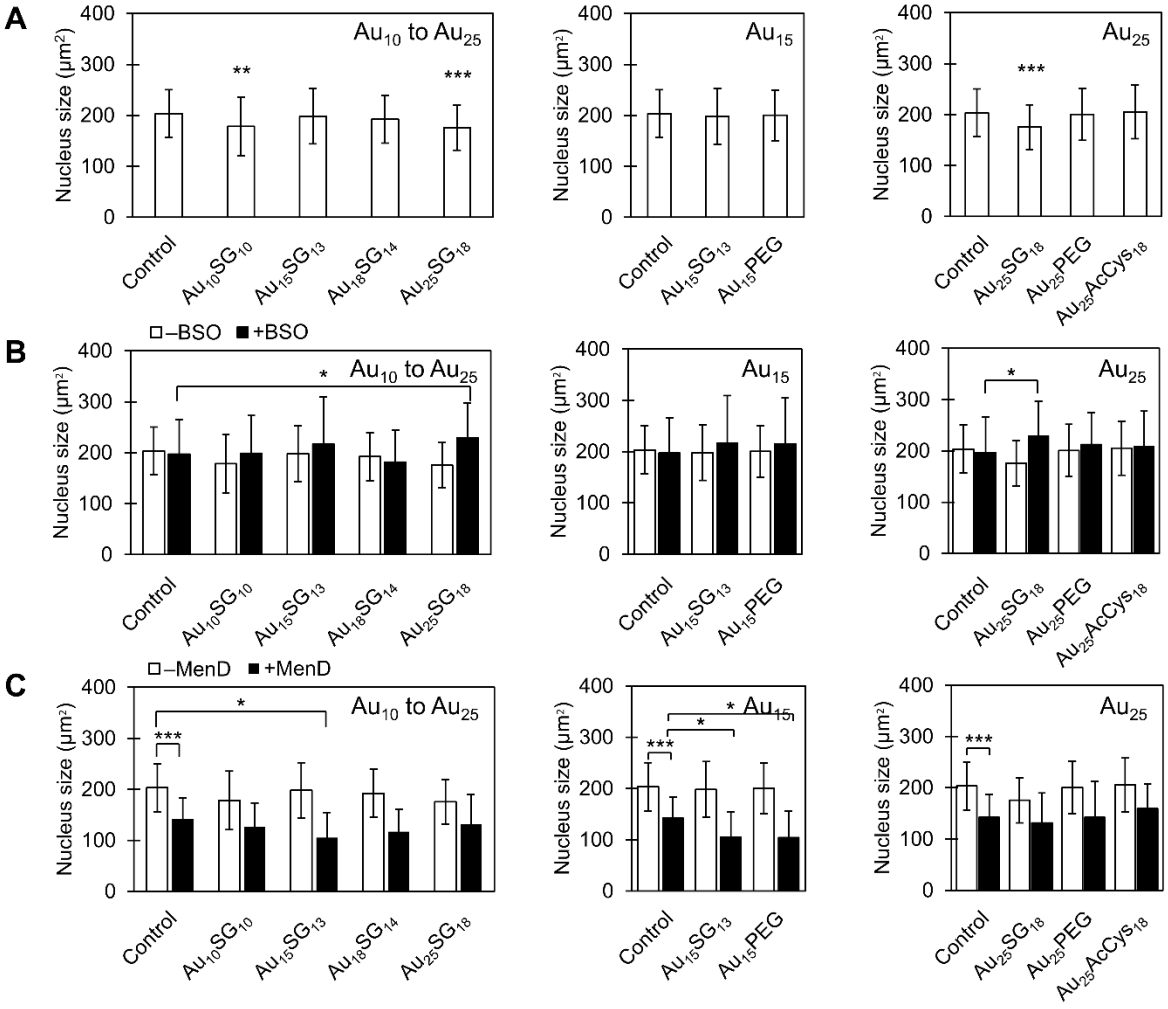

Fig. S3 AuNCs cause minor changes in nuclear area size. Human astrocytes were treated with 10 μM AuNC for 24 hours in serum-deprived media in the **(A)** absence or presence of **(B)** 100 μM L-buthionine-sulfoximine (BSO) or **(C)** 25 μM menadione (MenD). BSO or MenD was used as inducer of oxidative stress. Following treatment, cells were fixed and processed for immunocytochemistry. Nuclei were stained with Hoechst 33342. Nuclear area (μm2) was quantified using ImageJ, and normalized to vehicle control± SD. A minimum of 65 cells in (A), 40 cells in (B), and 14 cells in (C) were analyzed per condition. Over 1000 cells were analyzed in total. A p value of less than 0.05 was identified as statistically significant and indicated in figures as follows: *p<0.05, **p<0.01, ***p<0.001.

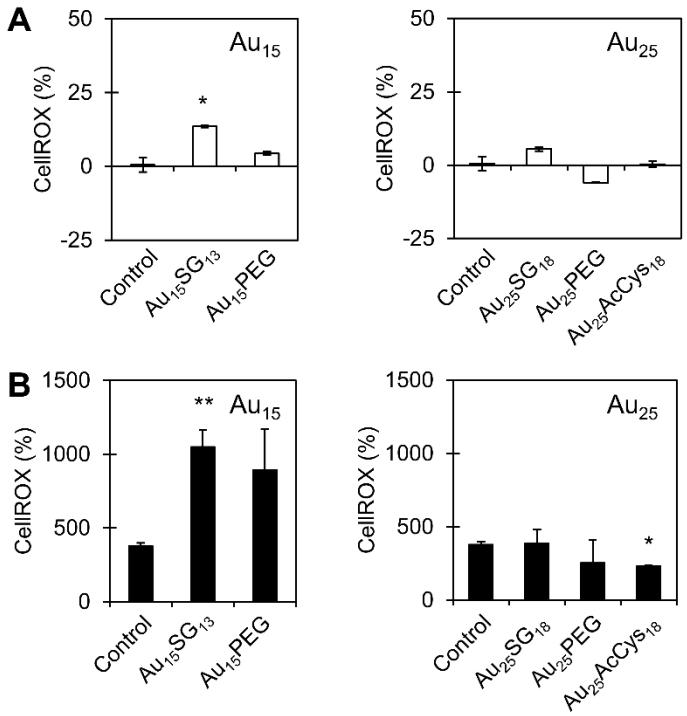

Fig. S4 Au_{15}NCs and Au_{25}NCs impact intracellular redox status. Primary human astrocytes were treated with 10 µM AuNC in the **(A)** absence or **(B)** presence of ROS-inducer, menadione (MenD, 25 µM) for 24 hours. **(A-B)** Percentage change in mean CellROX fluorescence intensity per cell relative to control ± standard error of the mean. A p value of less than 0.05 was identified as statistically significant as follows: * $p<0.05$, ** $p<0.01$.

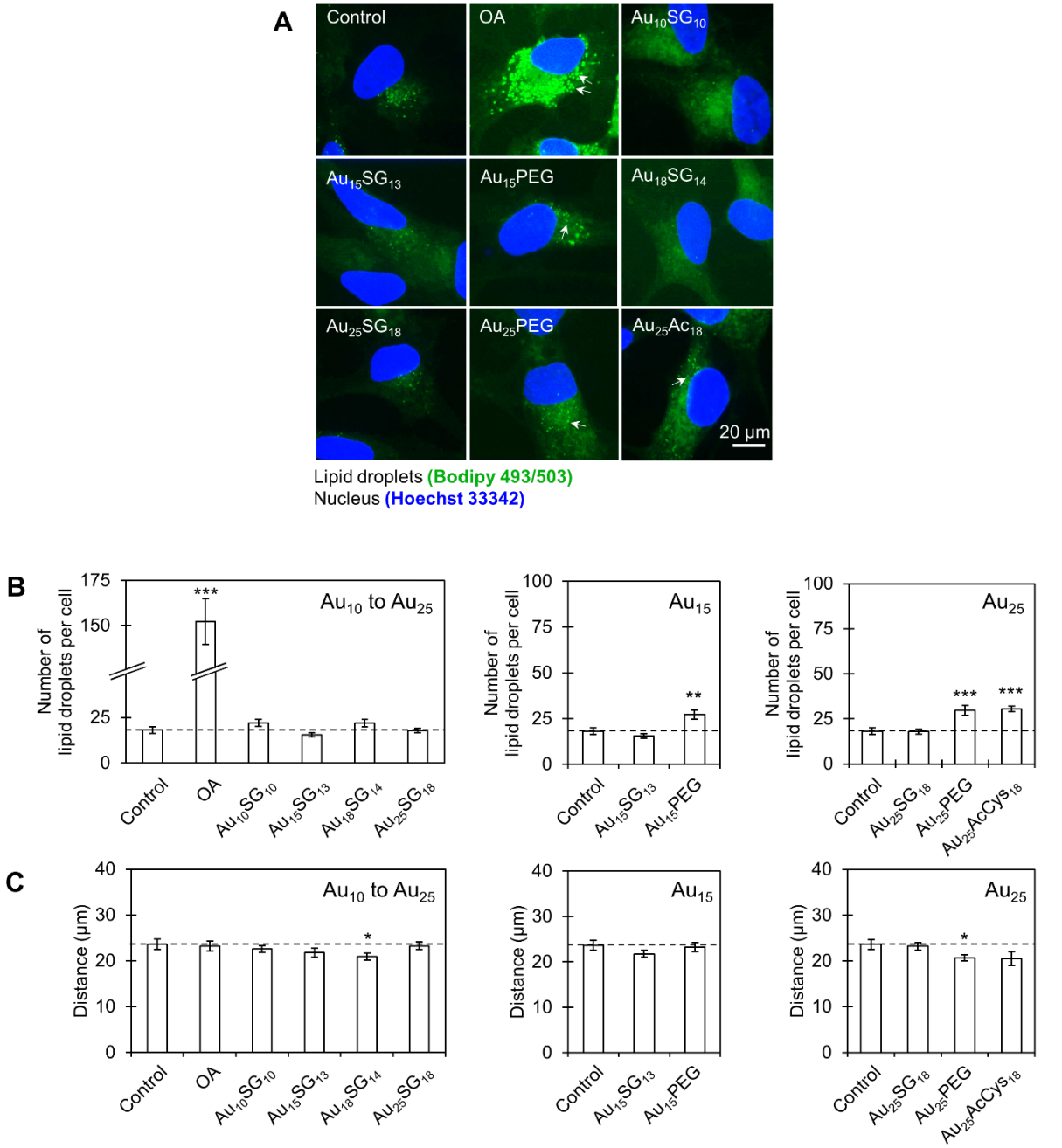

Fig. S5 Reorganization of lipid droplets caused by AuNCs. **(A)** Human astrocytes were treated with 10 µM AuNC for 24 hours. Oleic acid (300 µM) was used as positive control. Following treatment, neutral lipid droplets were labelled using Bodipy 493/503. Lipid droplet average **(B)** number, and **(C)** distance from center of nucleus were quantified and normalized to control ± standard error of the mean. A minimum of 80 cells were analyzed per condition. Over 800 cells were analyzed in total. A p value was identified as statistically significant as follows: *p<0.05, **p<0.01, ***p<0.001. Oleic acid (OA) treatment, served as a positive control showing over 152±12.81 increase in lipid droplet number. A mean distance of lipid droplets from the center of the nucleus was unchanged with smaller AuNCs (Au_{10}, Au_{15}), but the treatments with Au_{25}PEG or $Au_{25}AcCys_{18}$ caused a small increase of lipid droplets in the perinuclear region. Au_{25}PEG and $Au_{25}AcCys_{18}$ also increased lipid droplet number to 30±2.79 ($p<0.001$) or 31±1.59 ($p<0.001$) relative to the vehicle-treated control, respectively.

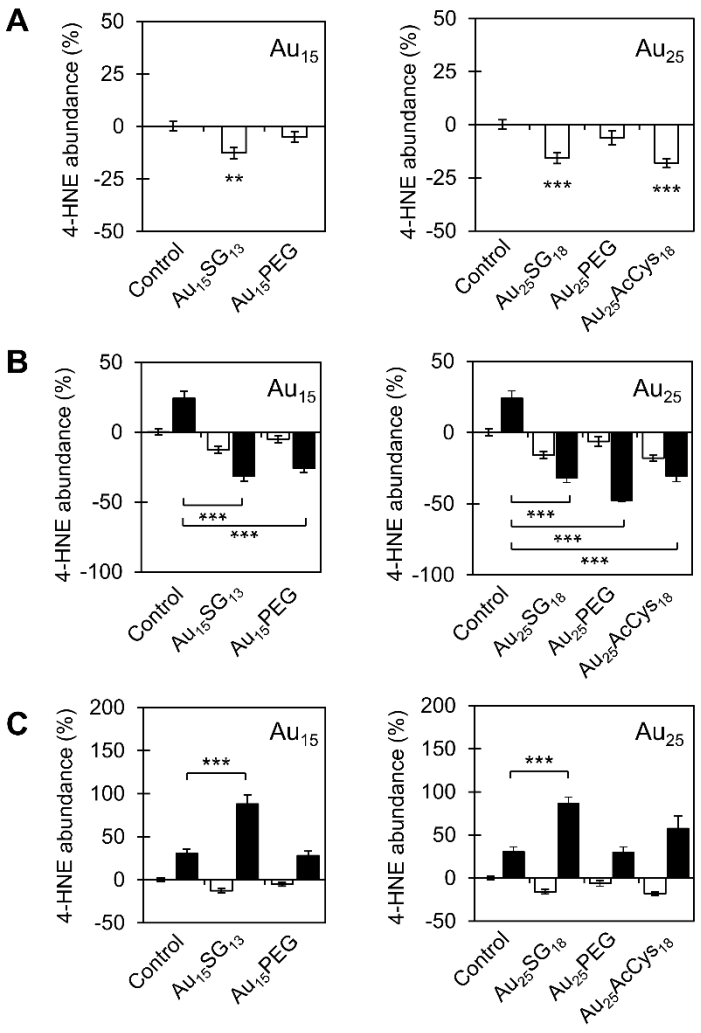

Fig. S6 Effects of Au_{15}NCs or Au_{25}NCs on 4-hydroxynonenal (4-HNE) abundance in human primary astrocytes. **(A-C)** Human astrocytes were treated with 10 µM AuNC for 24 hours **(A)** in the absence or presence of **(B)** 100 µM L-buthionine-sulfoximine (BSO) or **(C)** 25 µM menadione (MenD). Abundance of 4-HNE was determined by immunocytochemistry. Nuclei were stained with Hoechst 33342. F-actin was labeled using Alexa Fluor phalloidin 488. Mean fluorescence intensities per unit area (μm^2) are normalized to control ± SEM. A minimum of 68 cells in **(A)**, 51 cells in **(B)**, and 14 cells in **(C)** were analyzed per condition. Over 1600 cells were analyzed in total. A p value of less than 0.05 was identified as statistically significant as follows: ** p<0.01, *** p<0.001.

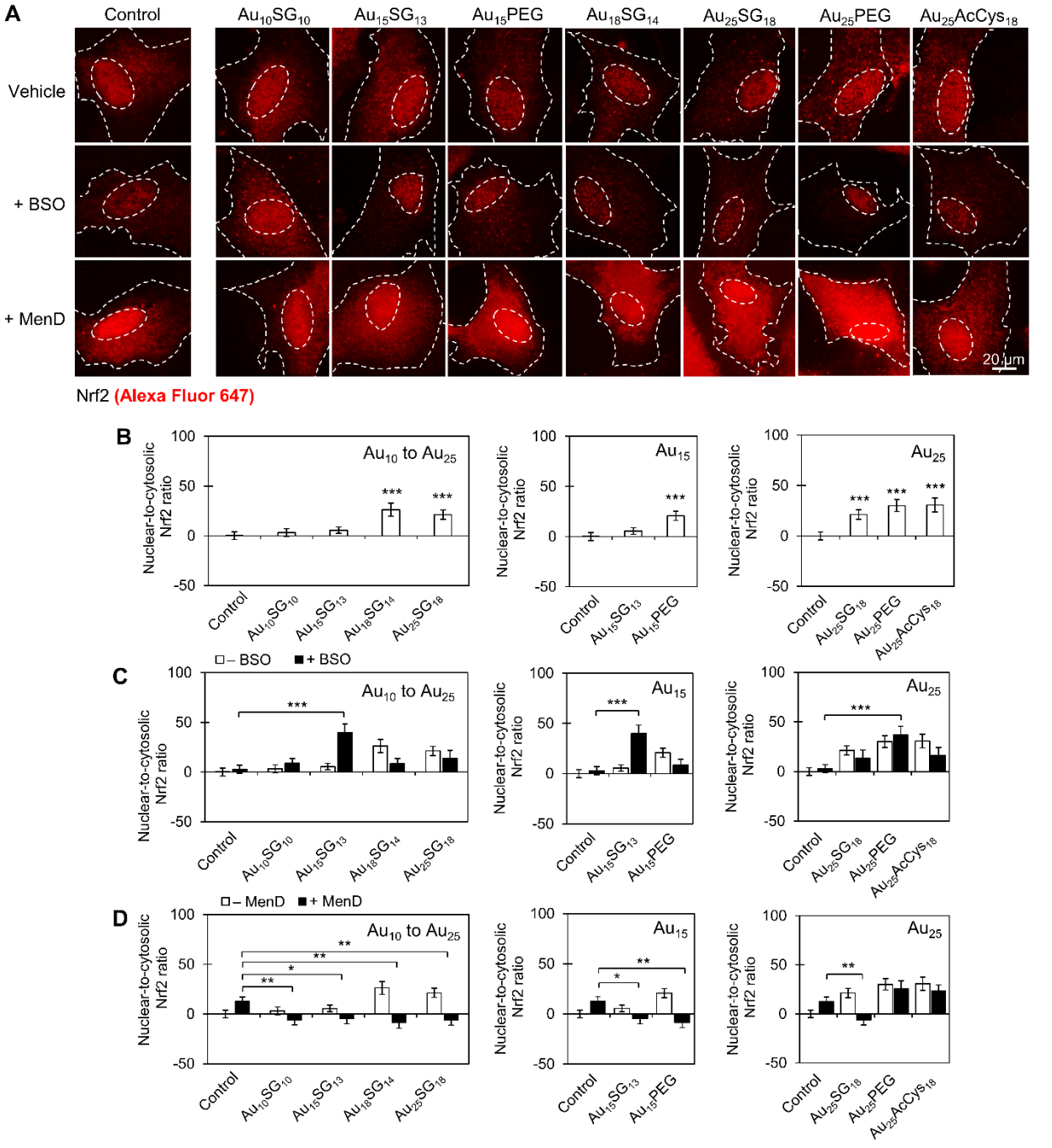

Fig. S7 AuNC induces redistribution of Nrf2. **(A)** Primary human astrocytes were treated with 10 μM AuNC for 1 hour in the **(B)** absence or presence of **(C)** 100 μM L-buthionine-sulfoximine (BSO) or **(D)** 25 μM menad ione (MenD).Nrf2 cellular distribution was assessed by antibody labelling. Nuclei and cells are outlined. Shown are percentage change of nuclear-to-cytoplasmic ratio of mean fluorescence intensities per unit area (μm^2) ± SEM. A minimum of 84 cells in **(B)**, 32 cells in **(C)**, and 54 cells in **(D)** were analyzed per condition. Over 1800 cells were analyzed in total. A p value was identified as statistically significant as follows: *p<0.05, **p<0.01, ***p<0.001. Under oxidative stress due to the reduction of glutathione by BSO, all AuNCs, regardless of size or ligand composition, caused a greater increase of 8.31±5.19% to 39.91±8.53% (p<0.001) in nuclear-to-cytosolic Nrf2 ratio compared with BSO treatment alone (2.82±4.06%). However, when cells were treated with menadione, AuNCs failed to increase nuclear-to-cytosolic Nrf2 ratio, except for Au_{25}PEG and $Au_{25}AcCys_{18}$.

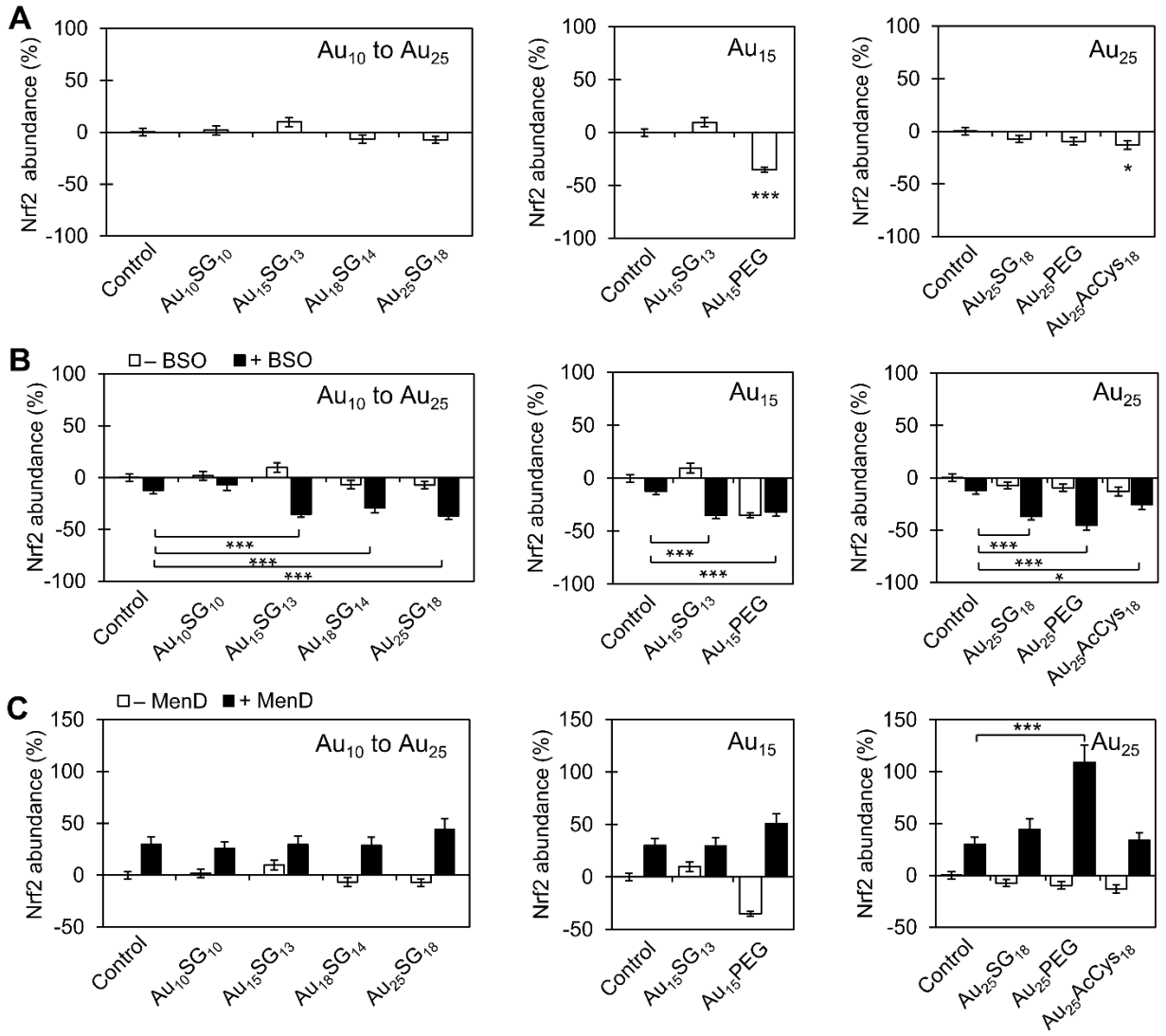

Fig. S8 AuNCs change total Nrf2 abundance. Human astrocytes were treated with 10 µM AuNC for 1 hour in serum-deprived media in the **(A)** absence or **(B)** presence of 100 µM L-buthionine-sulfoximine (BSO) or **(C)** 25 µM menadione (MenD). BSO or MenD was used as inducer of oxidative stress. Following treatment, cells were fixed and processed for immunocytochemistry. Nuclear factor erythroid 2-related factor 2 (Nrf2) cellular abundance was assessed by antibody labelling. Nuclei and cells are outlined in Supplementary Figure 2. Shown are percentage change of total Nrf2 mean fluorescence intensities per unit area (μm^2) ± SEM. A minimum of 84 cells in **(A)**, 32 cells in **(B)**, and 54 cells in **(C)** were analyzed per condition. Over 1800 cells were analyzed in total. A p value was identified as statistically significant as follows: *p<0.05, **p<0.01, ***p<0.001.