Electronic Supplementary Material (ESI) for Nanoscale.

Supplemental Information

Gold nanourchins and celastrol reorganize the nucleo- and cytoskeleton of glioblastoma cells

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Supplemental experimental section

AuNS synthesis

CTAB covered spherical AuNS (d=13.5 nm) were prepared by adding an aqueous ice-cold $NaBH_4$ solution (0.500 mL, 0.01 M) to a mixture of aqueous $HAuCl_4*3H_2O$ (0.125 mL, 0.01 M) and an aqueous solution of CTAB (4.375 mL, 0.075 M).[1] The seed growth was allowed to proceed for 2 h. After this time, an aliquot of the seed solution (0.1 mL) was added to a solution obtained by adding aqueous CTAB (1.6 mL, 0.10 M), aqueous $HAuCl_4*3H_2O$ (2 mL, 0.01 M), an aqueous AA solution (0.6 mL, 0.10 M) to 90 mL water. Immediately upon addition of the seed solution, the mixture was mixed gently for 10 s. It was then kept undisturbed for at least 3 h. The solutions were kept at 27 °C (in a water bath) throughout the entire procedure to prevent the crystallization of CTAB. At the end of the reaction, the mixture was centrifuged in an Eppendorf centrifuge model 5403 (6,000 rpm, 60 min). The residue was redispersed in water (5 mL).

AuNR synthesis

Gold seeds were prepared by adding an aqueous ice-cold $NaBH_4$ solution (0.6 mL, 0.01 M) to a solution obtained by adding aqueous $HAuCl_4*3H_2O$ (0.250 mL, 0.01 M) to an aqueous solution of CTAB (7.5 mL, 0.10 M). The seed growth was allowed to proceed for 2 h. After this time, an aliquot of the seed solution was added to a solution obtained by mixing, first, aqueous CTAB (95 mL, 0.10 M), aqueous $HAuCl_4*3H_2O$ (4 mL, 0.01 M), and aqueous $AgNO_3$ (0.6 mL, 0.01 M) and, last, an aqueous AA solution (0.64 mL, 0.10 M). Immediately upon addition of the seed solution, the mixture was mixed gently for 10 s. It was then kept undisturbed for at least 3 h. The solutions were kept at 27 °C (in a water bath) throughout the entire procedure to prevent the crystallization of CTAB. At the end of the reaction, the mixture was centrifuged by an Eppendorf centrifuge model 5403 (9,000 rpm, 90 min). The residue (AuNR) was redispersed in water and HS-PEG-OMe (12 μL, 25 mM) was added to this suspension (0.3 mL). The mixture was kept overnight. It was subjected to centrifugation (14,000 rpm 20 min). The residue was redispersed in water (0.5 mL). The concentration of atomic gold in the rod AuNP suspension was determined by inductively coupled plasmon atomic emission spectroscopy (ICP-AES).

Supplemental Figures and Tables

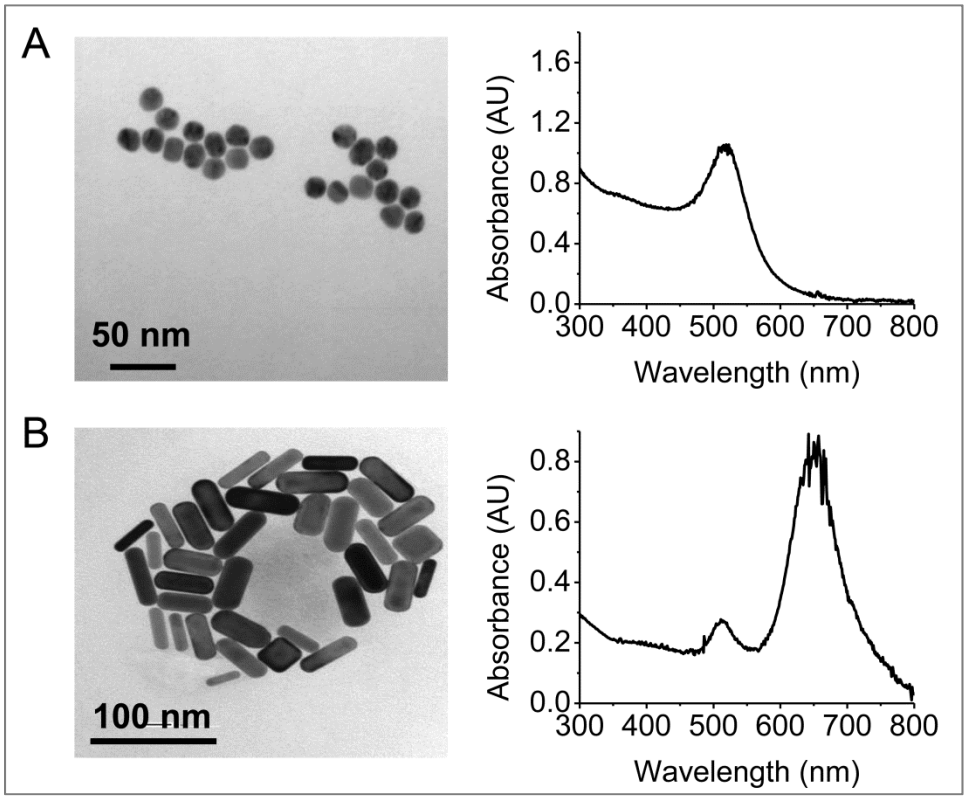

Figure S1. Characterization of PEGylated (A) Gold nanospheres (AuNS) and (B) gold nanorods (AuNR). Left panels: electron micrographs of gold nanoparticles; scale bar is 50 nm for gold nanospheres, 100 nm for nanorods. Right panels: UV absorbance spectra for different AuNPs.

	AuNS-PEG	**AuNR-PEG**
Size	13.50 nm, diameter	L=50.10 nm × W=15.80 nm
Au atoms/NP	1.02×10^5	1.99×10^5
Absorption maximum (λ_{max})	524 nm	514 nm and 675 nm
Surface per NP	572.56 nm^2	2878.95 nm^2
Volume per NP	1288.25 nm^3	9822.95 nm^3
Surface-to-volume ratio	0.44 nm^{-1}	0.29
IC_{50} (Au molarity)	100 μM	180 μM
IC_{50} in U251N cells (NP/mL)	5.89×10^{11}	5.45×10^{11}
Surface area at IC_{50} (per mL)	337.20 mm^2	1569.00 mm^2
ζ-potential	-23 mV	-23 mV

Table S1. Properties of PEGylated AuNPs. Size, absorption peaks, ζ-potential, surface, volume, surface/volume ratio and IC_{50} for U251N glioblastoma cells are listed for PEGylated gold nanospheres (AuNS-PEG) and gold nanorods (AuNR-PEG).

Short name	Name	M_w	Functional group
PEG	α-Methoxy-ω-mercapto poly(ethylene glycol)	5 kDa	Thiol group for attachment to Au surface
SB	Poly(3-dimethyl(methacryloyloxyethyl) ammonium propanesulfonate) or PDMAPS	M_n = 17.3 kDa, M_w/M_n = 1.5	Thiol group and poly(sulfobetaine)
CTAB	Cetyl trimethyl ammonium bromide	364.5 g/mol	Quartenary amine and alkyl chain
Celastrol	10-Hydroxy-2,4a,6a,9,12b,14a-hexamethyl-11-oxo,2,3,4,4a,5,6,6a,11,12b,13,14,14a,14b-tetradecahydro-picene-2-carboxylic acid	450.6 g/mol	Carboxyl, hydroxyl

Table S2. Chemical properties of compounds used to functionalize AuNP surfaces, and formulae of CTAB and poly(sulfobetaine), referred to as SB, surface molecules.

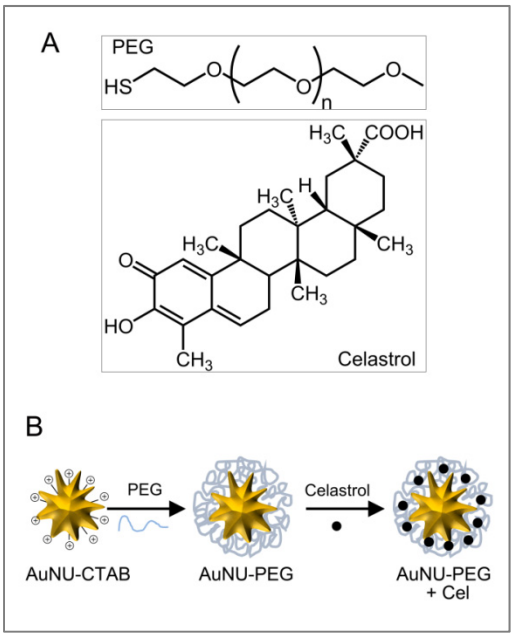

Figure S2. Functionalization of AuNU surfaces. (A) PEG, thiolated poly(ethylene glycol) and celastrol. (B) Combination of PEGylated gold nanourchins (AuNU) with celastrol. CTAB-coated AuNUs were PEGylated with 5 kDa thiolated PEG-methoxy. Celastrol (Cel) was then combined with the PEGylated AuNUs.

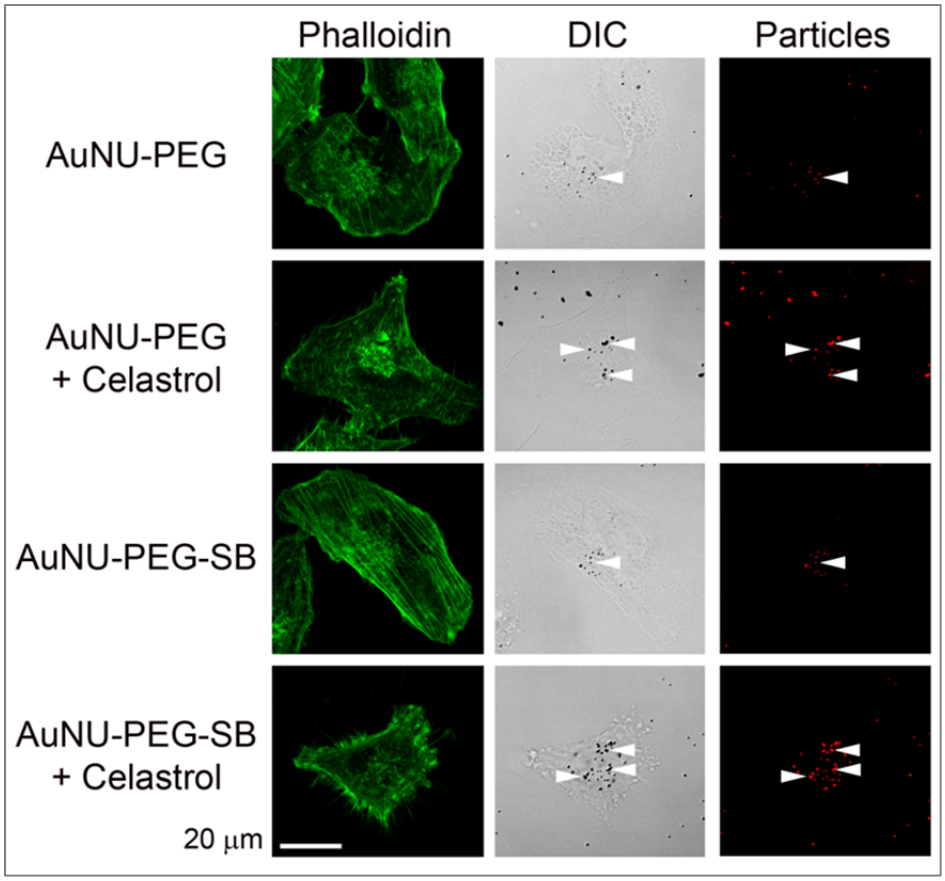

Figure S3. Celastrol increases the AuNU content in U251N cells. Cells were incubated for 24 h with AuNU-PEG or AuNU-PEG/SB in the absence or presence of celastrol. Following treatment, cells were fixed, labeled with Alexa Fluor® 488 Phalloidin (165 nM, 20 min) and imaged with a Zeiss LSM780 confocal microscope. Differential interference contrast (DIC) was used to detect AuNUs. The gradient map function of Adobe Photoshop was applied to visualize particles (right column, red particles). Arrows point to AuNUs in U251N cells.

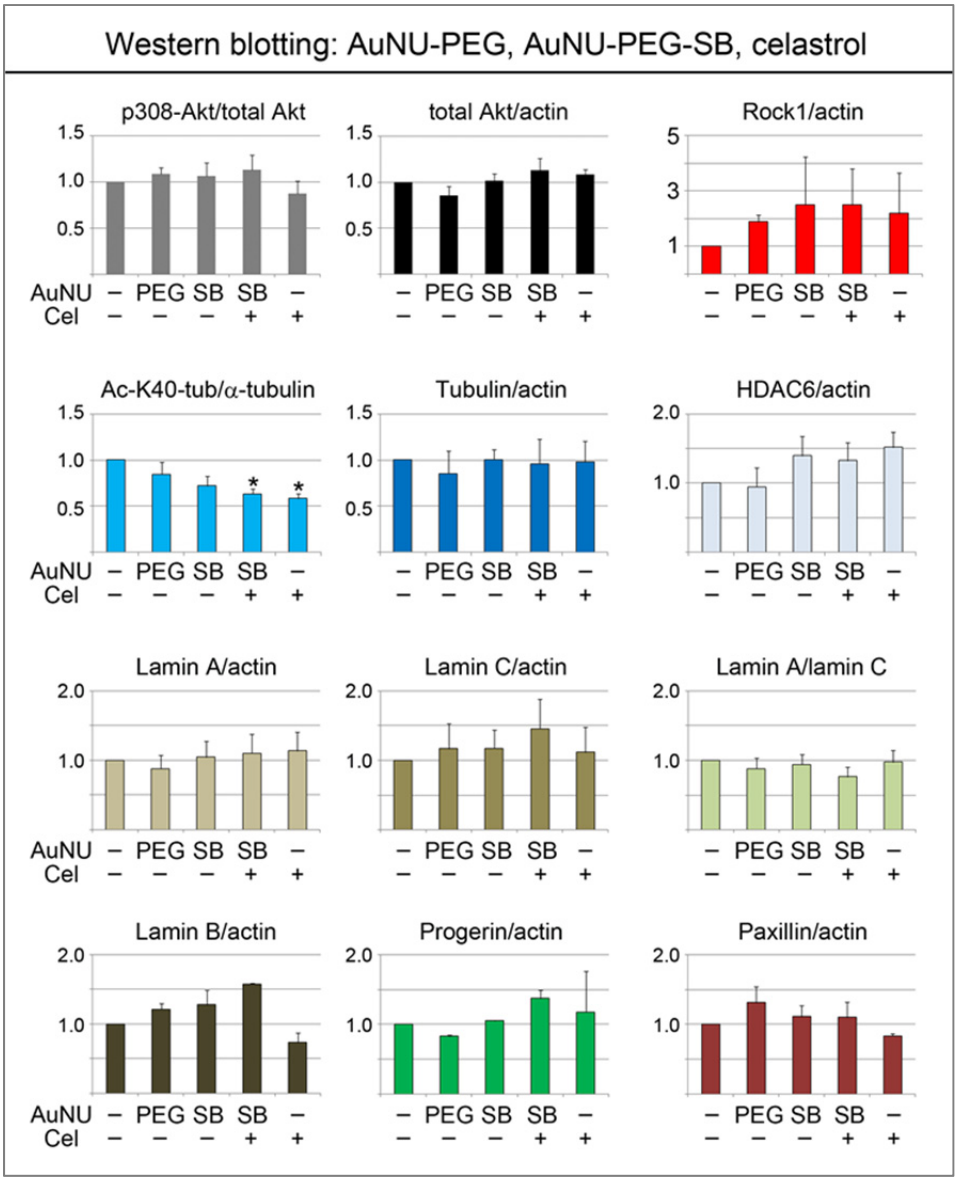

Figure S4. Effects of AuNU-PEG, AuNU-PEG-SB and celastrol on cell signaling, and components of the cytoskeleton or nuclear lamina. Western blotting was carried out as for Fig. 6. U251N cells were treated with AuNU-PEG (PEG) or AuNU-PEG-SB (SB). Celastrol was present as indicated. Results were normalized to vehicle-treated controls and depicted as means + SEM for two to four independent experiments. Significant differences were identified by One-way ANOVA with Bonferroni posthoc analysis; vehicle controls served as reference; *, $p<0.05$.

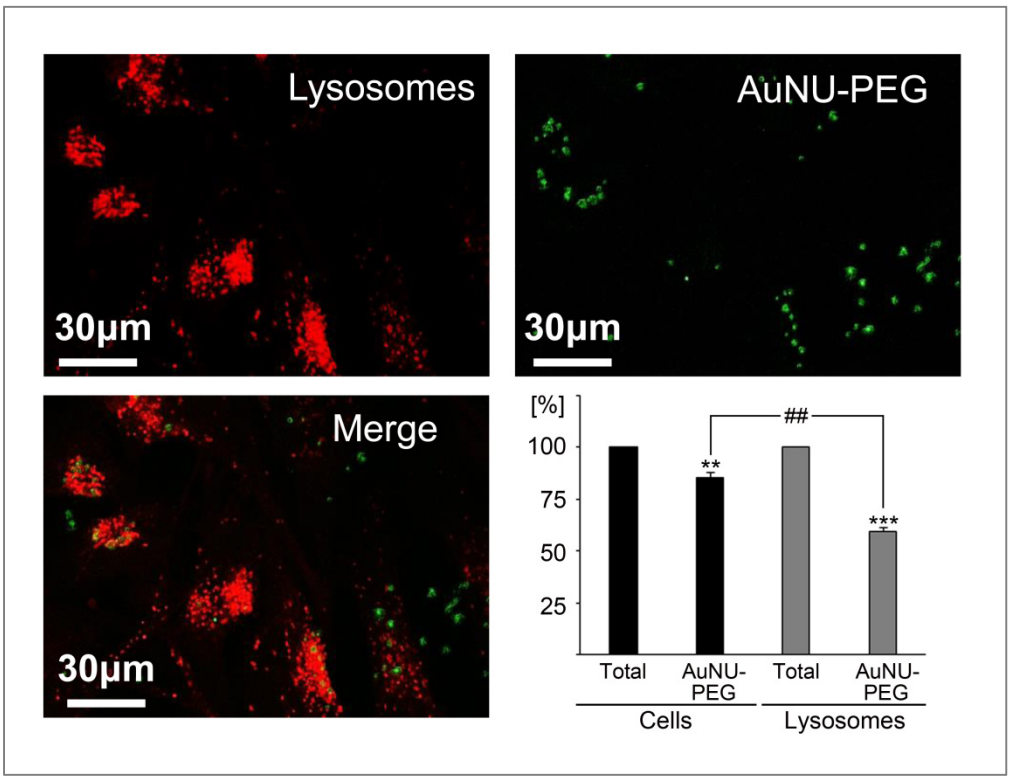

Figure S5. Two-photon luminescence (TPL) imaging of internalized gold nanoparticles in U251N glioblastoma cells. U251N cells were treated with AuNUs (10^{10} NPs/mL, 48 h at 37 °C) and stained with LysoTracker Red DND-99. TPL AuNU-PEG images were acquired for live cells with a Leica TCS SP-8 microscope ($\lambda_{ex.}$ 680 nm, $\lambda_{em.}$ 450-550 nm). Quantification for three independent experiments shows that AuNU-PEG particles were primarily in the cytosol, where ~59% co-localized with lysosomes. For comparison, ~52% of AuNU-PEG-SB particles associated with lysosomes (Fig. S6). Results are shown as means + SEM. Student's t-test identified significant differences between the total number of cells and cells containing AuNPs (black bars). In addition, the percentage of AuNU-PEG particles co-localizing with lysosomes was determined (gray bars); **, $p<0.01$; ***, $p<0.001$. The relationship between AuNU-PEG cellular uptake and lysosomal association was also compared; ##, $p<0.01$.

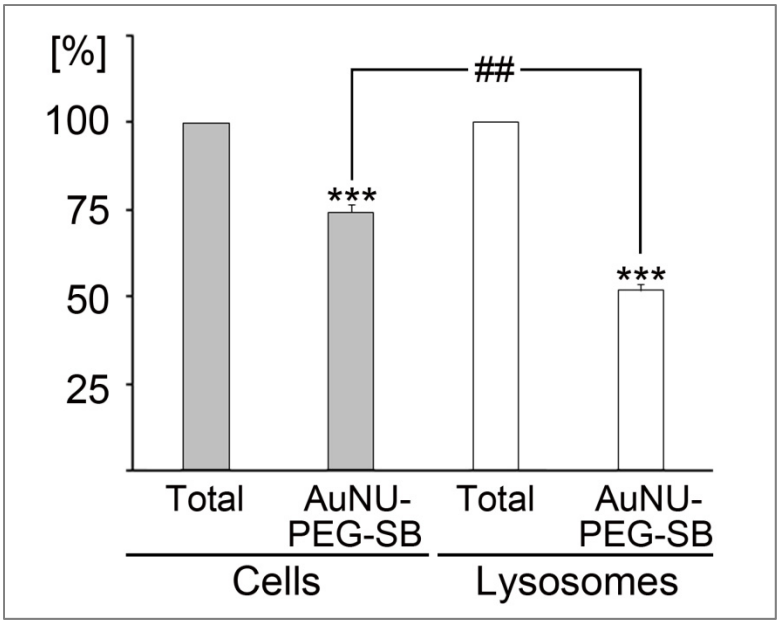

Figure S6. Two-photon luminescence (TPL) imaging of internalized gold nanoparticles in U251N glioblastoma cells. U251N cells were incubated with AuNU-PEG-SB (10^{10} NPs/mL, 48 h at 37 °C) and stained with LysoTracker Red DND-99. TPL AuNU-PEG images were acquired for live cells with a Leica TCS SP-8 microscope ($\lambda_{ex.}$ 680 nm, $\lambda_{em.}$ 450-550 nm); quantification was performed for three independent experiments. AuNU-PEG-SB particles were predominantly in the cytosol; ~52% of the particles co-localized with lysosomes. Results are shown as means + SEM. Student's t-test identified significant differences. The total number of cells was compared to cells containing AuNPs (grey bars), and lysosomes were evaluated for the presence of AuNU-PEG-SB particles (white bars); ***, $p<0.001$. There was also a significant difference between AuNU cellular uptake and lysosomal association; ##, $p<0.01$.

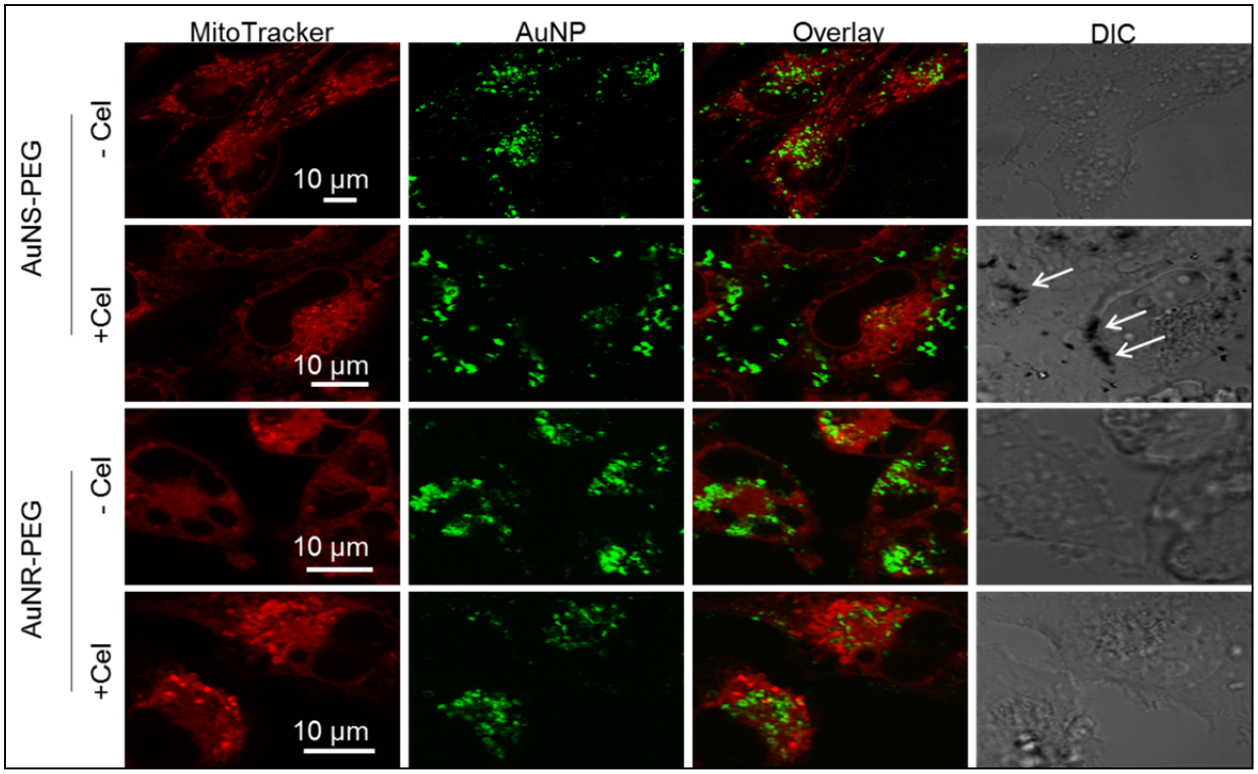

Figure S7. AuNP shape and celastrol determine nanoparticle uptake, intracellular localization and aggregation. U251N cells were treated with PEGylated gold nanospheres (AuNS) or nanorods (AuNR) in the absence or presence of celastrol (± Cel). The Au concentration was 10 μM Au. After 12 h treatment, cells were labelled with MitoTracker Deep Red and imaged live by confocal microscopy. Note that AuNS tend to aggregate upon co-treatment with celastrol (arrows in DIC image). This correlated with minimal uptake by U251N cell. By contrast, aggregates were not visible for AuNS, AuNR or AuNR+Cel. Internalized particles did not accumulate in mitochondria.

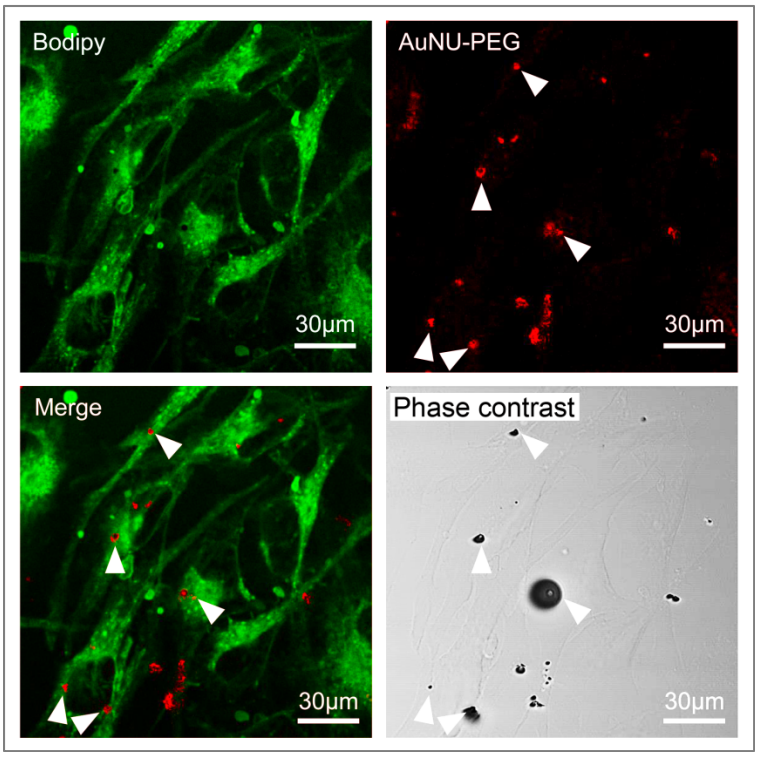

Figure S8. A portion of internalized AuNUs locate in the vicinity of lipid droplets of glioblastoma cells. U251N cells treated with AuNU-PEG (red) were fixed and stained with Bodipy (green; λ_{ex} 488 nm/λ_{em} 503 nm). AuNPs were excited at 720 nm at 80 MHz and 4.7% laser intensity. Phase contrast shows microbubble formation as absorbed light is converted into heat.

Supplemental reference

1. J. Wang, Y. F. Li, C. Z. Huang and T. Wu, *Anal. Chim. Acta*, 2008, **626**, 37-43