Electronic Supplementary Material (ESI) for Biomaterials Science

Supporting Information for

Uptake of poly(2-hydroxypropylmethacrylamide)-coated gold nanoparticles in microvascular endothelial cells and transport across the blood-brain barrier

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Cell viability and cytotoxicity assay

The assays were performed as explained in the main text.

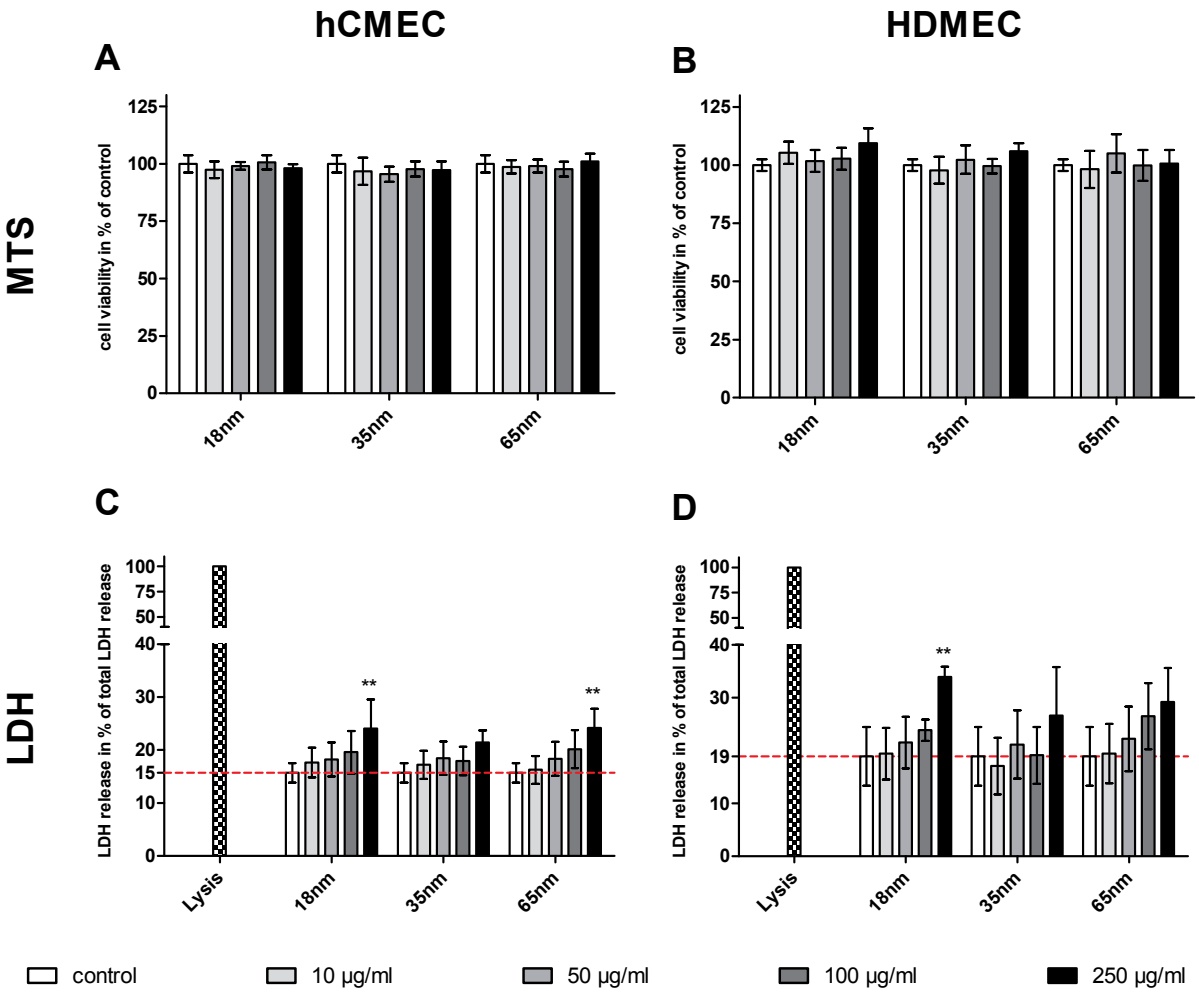

Figure S1. Cell viability (A, B) and cell toxicity (C, D) of HDMEC and hCMEC/D3 after treatment with various gold nanoparticles.

hCMEC (A, C) and HDMEC (B, D) were treated with different concentrations of different-sized poly(2-hydroxypropylmethacrylamide)-coated gold nanoparticles. After 24 hours the cell viability (A, B) and cytotoxicity (C, D) were detected by MTS assay and LDH release assay, respectively. Each result represents the mean value in % ± standard deviation of triplicates of three independent experiments ($n \geq 3$). The controls (MTS: untreated control; LDH: lysed cells) were set to 100%. **: $p < 0.01$ (Two-way ANOVA with Bonferroni post test).

Cellular uptake of gold nanoparticles into endothelial cells

As shown in Figure S2 the internalized gold nanoparticles could be detected by optical microscopy after 4 hours of treatment. The particles (black dots) were mostly located in the perinuclear region of hCMEC/D3 cells while the AuNPs in HDMEC samples were mostly detected in the periphery or even on top of the cells. Since optical microscopy is limited by the resolution, no single

particles can be visualized, thus preventing image analysis to quantify the amount of internalized gold nanoparticles. The resolution of optical microscope also explains why none of the 18nm poly(2-hydroxypropylmethacrylamide) AuNPs (Hydroxy@Au_{18}) could be detected within the cells.

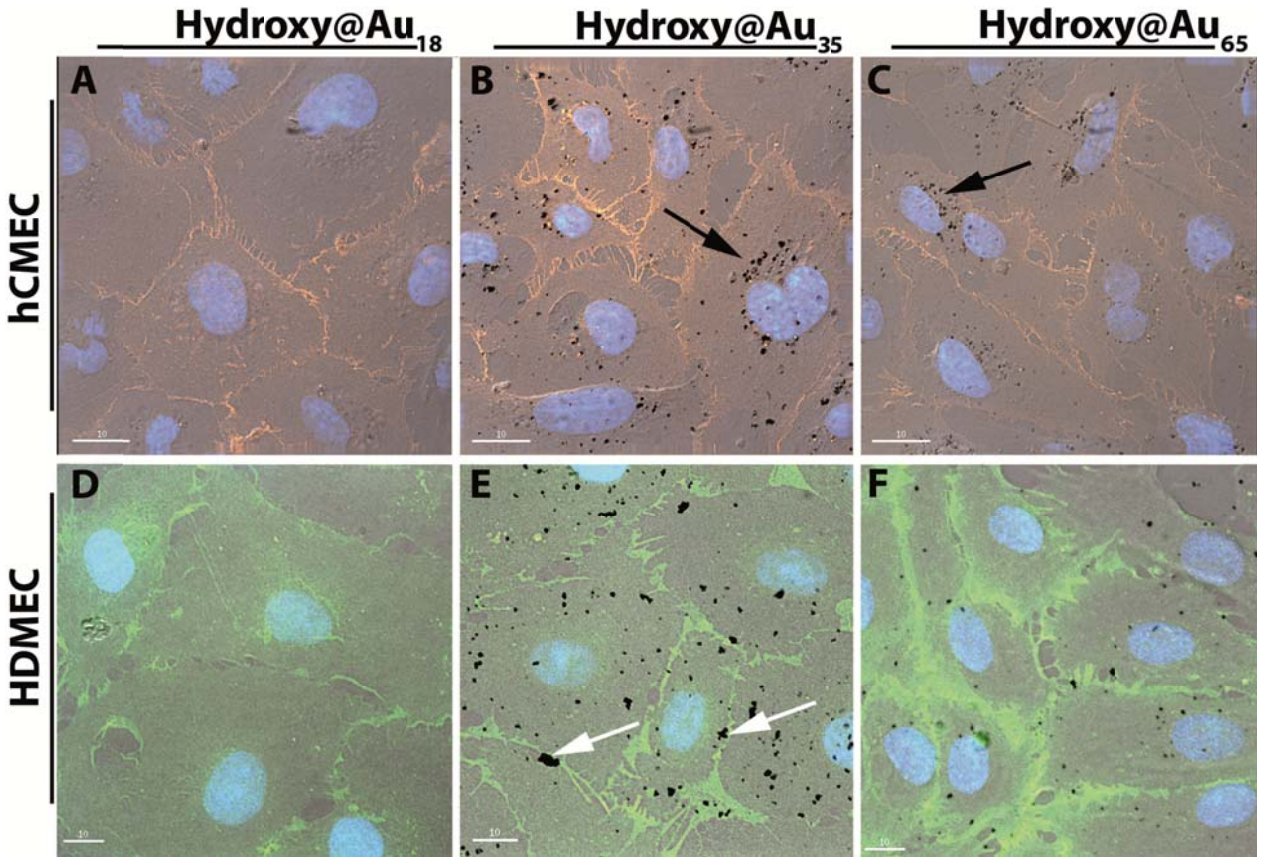

Figure S2. Optical microscopy of internalized gold nanoparticles in hCMEC/D3 and HDMEC after 4 hours of treatment.

hCMEC/D3 (**A**-**C**) and HDMEC (**D**-**F**) were incubated with 100 µg/ml gold nanoparticles for 4 hours, fixed with 3.7% paraformaldehyde and cell membranes were stained by immunostaining of the membrane protein, CD31 (red/green). Cell nuclei were stained by Hoechst dye (blue). The 35 nm- and 65 nm-sized gold nanoparticles could be detected within both cell types but also on the top of the cells (white arrows). Light/fluorescence microscopy (60x). Scale bar: 10 µm.

To confirm the uptake of poly(2-hydroxypropylmethacrylamide)-coated AuNPs transmission electron microscopy was performed. After treatment for 4h HDMEC and hCMEC/D3 internalized all sizes of gold nanoparticles. The nanoparticles are located within vesicles, but could not be found in the nucleus or freely distributed in the cytoplasm (Figure S3).

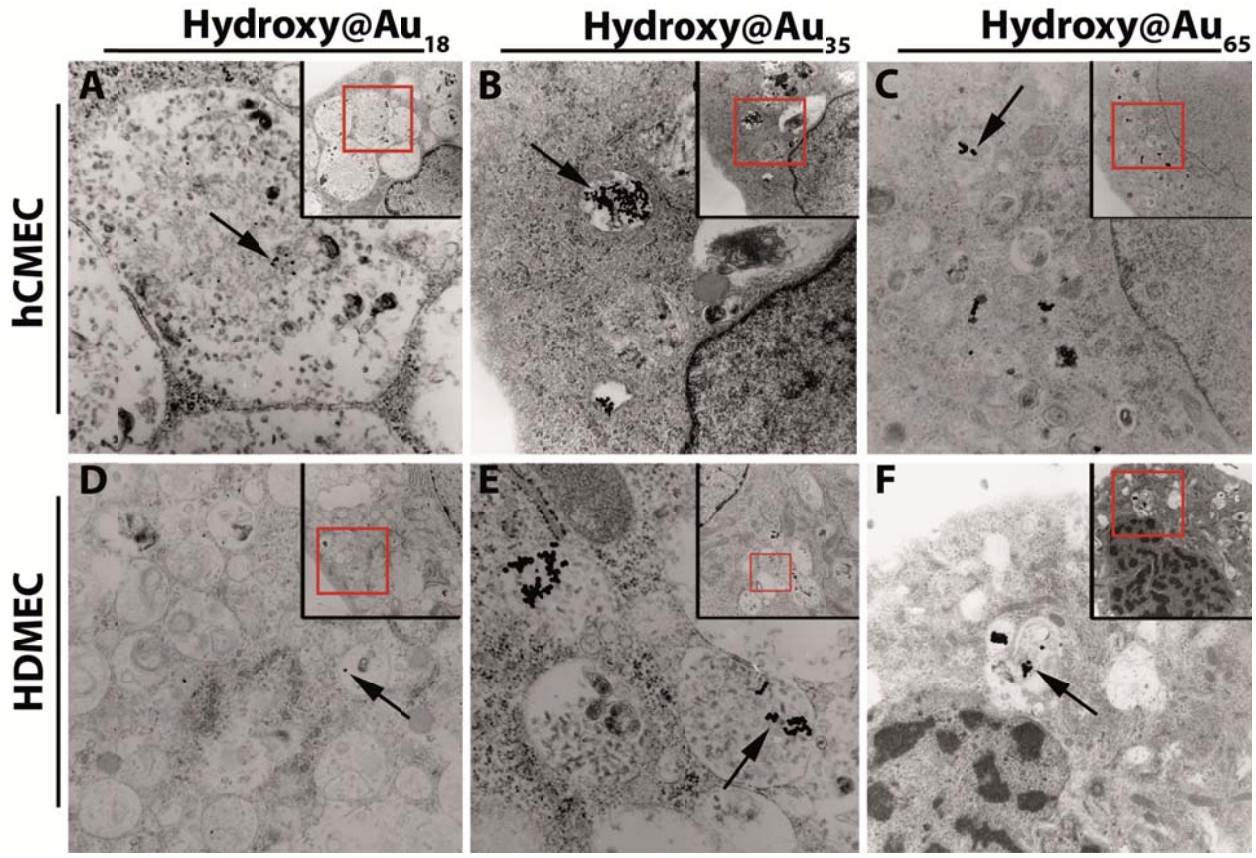

Figure S3. TEM images of hCMEC/D3 and HDMEC after treatment with different-sized poly(2-hydroxypropylmethacrylamide)-coated gold nanoparticles for 4 hours.

hCMEC/D3 (**A**-**C**) and HDMEC (**D**-**E**) were treated with 10 µg/ml gold nanoparticles, fixed with glutaraldehyde and prepared for TEM microscopy. Arrows indicate gold nanoparticles within the cells. Images in the corners with the red selection box represent the overview of the magnified main image. All gold nanoparticles are located within vesicles. Images 3500x; small images 2100x (**A**-**C** and **F**); 8900x (**E**).

Uptake properties of gold nanoparticles with different neutral-charged coatings.

We demonstrated that the functionalization of AuNPs by a neutral-charged coating with hydroxypropylamine can influence the uptake behavior in different endothelial cell types and may act as a first targeting to brain endothelium without the conjugation of a specific targeting peptide. In contrast to these observations, a different neutral surface modification such as PEG and

glucosamine did not show these cell type-specific effects (Figure S4).

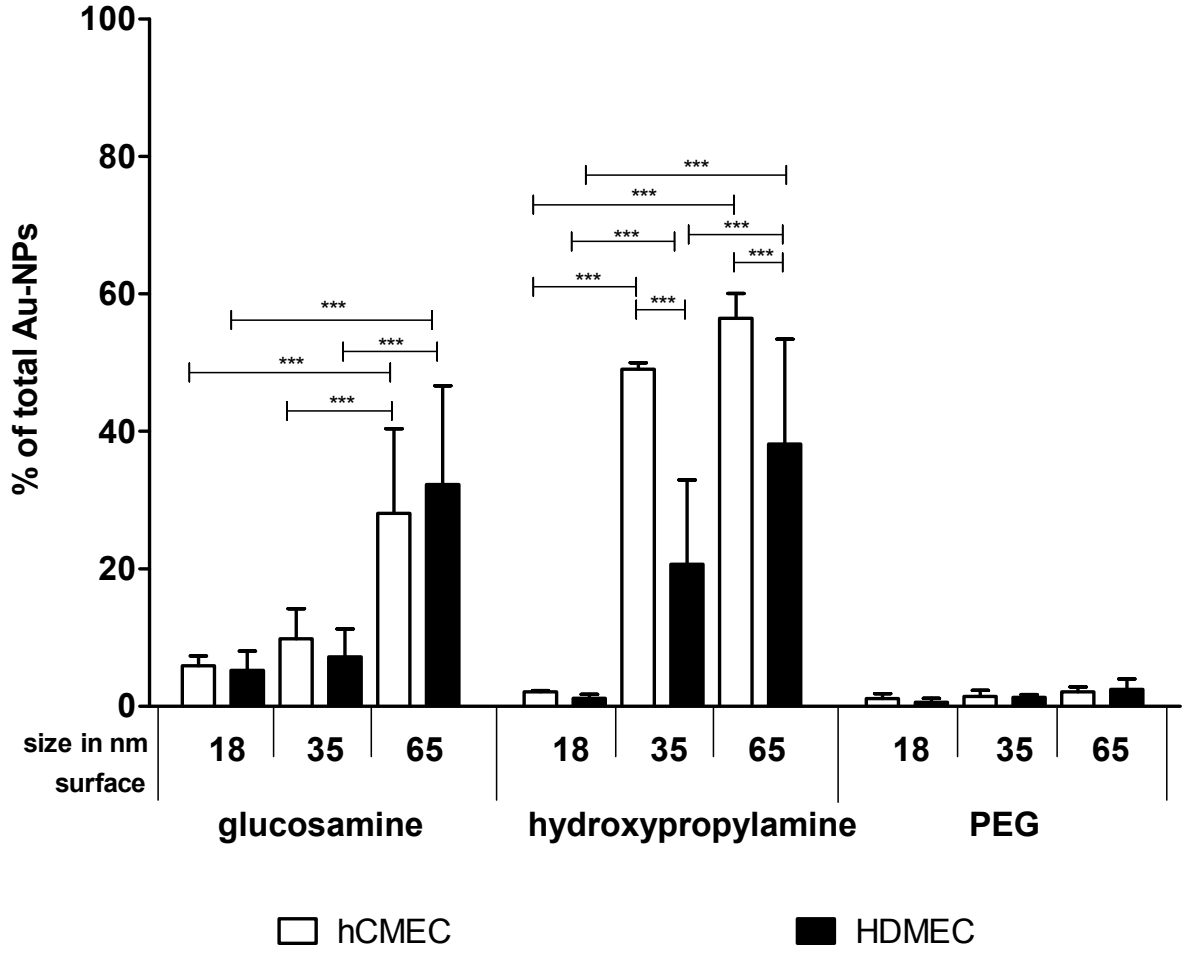

Figure S4. Quantification of internalized neutral-charged gold nanoparticles in HDMEC and hCMEC/D3 by ICP-AES. hCMEC/D3 and HDMEC were treated with 10 µg/ml gold nanoparticles for 24 hours. After the incubation cells were extensively washed and collected in a sufficient volume of PBS. After treatment with nitric acid, the total amount of gold in the different wells were measured by ICP-AES (mean ± SD; n = 3-6). Differences in the uptake behavior into HDMEC and hCMEC/D3 were determined by spectroscopy. The initial applied gold was set to 100%. ***: $p < 0.001$ (Two-way ANOVA with Bonferroni post test).

Evaluation of cell viability of primary porcine brain endothelial cells (PBEC) after AuNP treatment

To exclude the decrease of cell viability after AuNP treatment, PBECs were incubated with different amounts of AuNPs. The results depicted in Figure S5 demonstrates that the cell viability was unaltered even after the treatment with 500 µg/ml poly(2-hydroxypropylmethacrylamide)-coated AuNPs which differ in size.

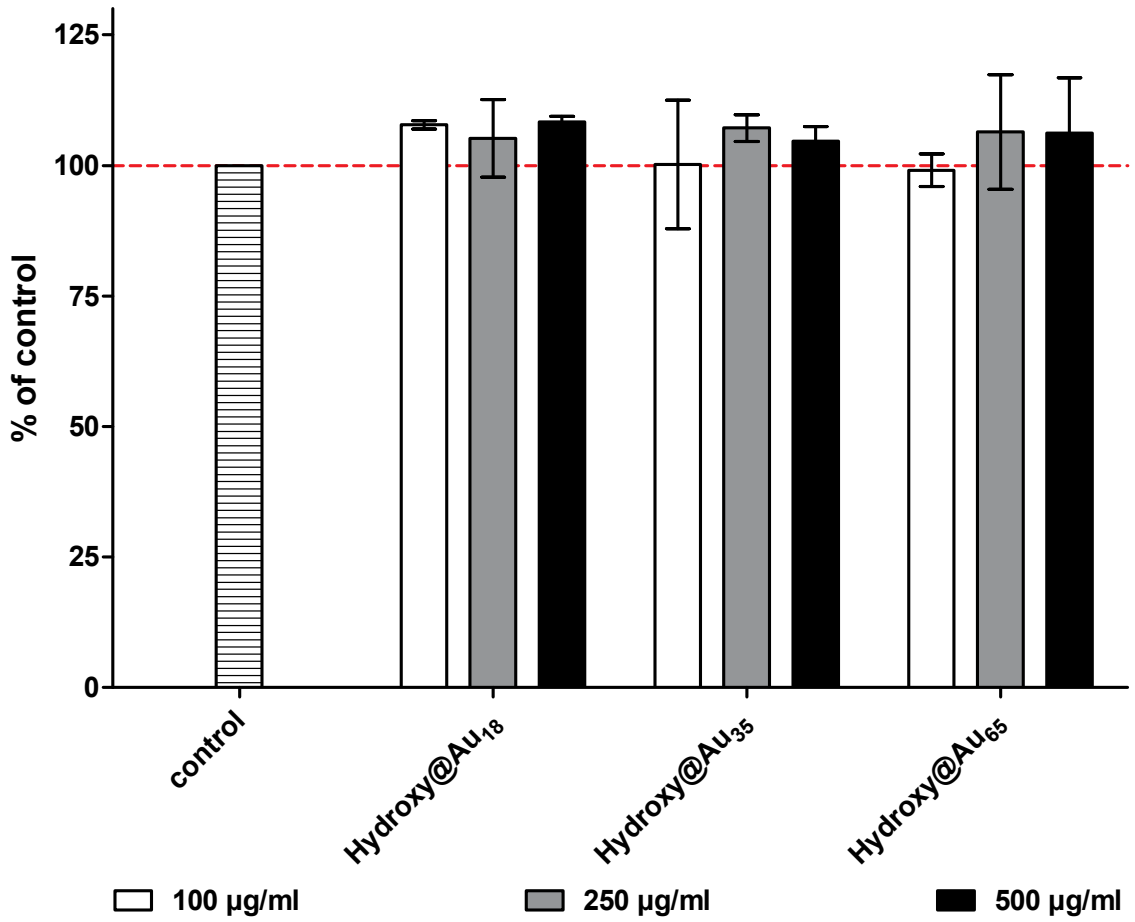

Figure S5. MTS data of PBEC after exposure to various concentrations of poly(2-hydroxypropylmethacrylamide)-coated gold nanoparticles.

Cells were incubated with different concentrations of poly(2-hydroxypropylmethacrylamide)-coated AuNPs for 24 hours. The cell viability was measured by using the MTS assay. Untreated cells were used as control and set to 100% cell viability (two donors, mean ± SD).

Uptake mechanism and intracellular localization of internalized Hydroxy@Au

Internalized poly(2-hydroxypropylmethacrylamide)-coated AuNPs were shown located within vesicles (see Figure S3). After treatment of hCMEC/D3 and HDMEC with 35nm-sized AuNPs and staining of flotillin-1 it was shown that the vesicles in which the particles are located were positive for flotillins (Figure S6). On the basis of previously published data the localization within flotillin-positive vesicles suggests an uptake mechanism which is clathrin- and caveolin-independent (Kasper et al., 2012).

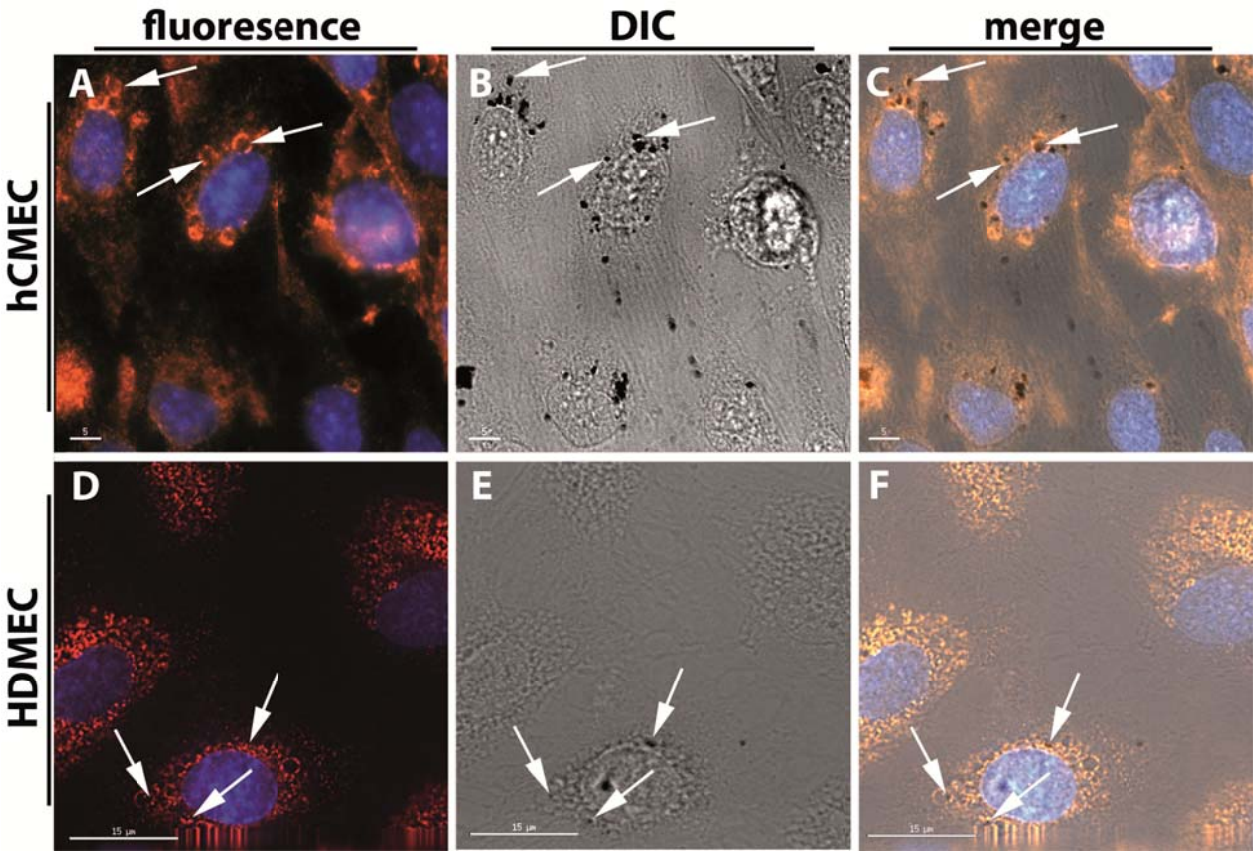

Figure S6. Co-localization of Hydroxy@AuNP$_{35}$ with flotillin-1 in hCMEC/D3 and HDMEC.

hCMEC/D3 were treated with 100µg/ml poly(2-hydroxypropylmethacrylamide)-coated gold nanoparticles (Hydroxy@AuNP$_{35}$) for 24 hours. Cells were washed and then fixed with methanol/ethanol and stained with anti-flotillin-1 (**A**, **D**). Differential interference contrast (DIC) images are presented in images **B** and **E**, while merged images are shown in **C** and **F**. Gold nanoparticles can be detected in vesicles surrounded by flotillin-1, mostly located in the perinuclear region. Nuclei are stained in blue (Hoechst dye). Delta Vision, 100x. Scale bar 5 µm or 15 µm as indicated.