Electronic Supplementary Material (ESI) for Nanoscale.

Immunogold Labeling Reveals Subcellular Localisation of Silica Nanoparticles in a Human Blood-Brain Barrier Model

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Supplementary Figures

Table 1. Size and zeta-potential of 50 nm SiO_2-NPs in water.

SiO_2 NPs nominal size	Temperature (^oC)	Hydrodynamic diameter in de-ionised water (nm)[a]	PDI in de-ionised water[b]	Zeta Potential in de-ionised water (mV)
50 nm	37	52 ± 2	0.17	-22 ± 1

[a] Z-average hydrodynamic diameter extracted by cumulant analysis of the data. [b] Polydispersity index (PDI) from cumulant fitting.

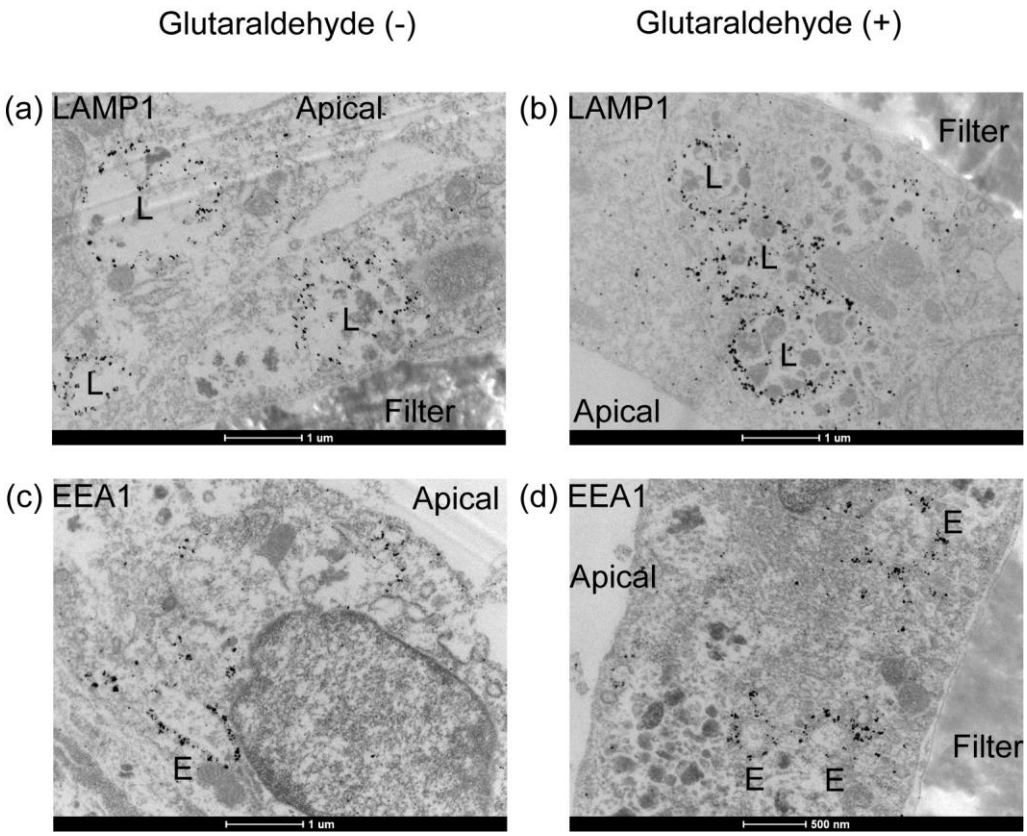

Figure 1. Morphological comparison on preservation of subcellular structures with or without fixation of glutaradehyde after a pre-embedding immunogold labeling procedure. In (a) and (c), nanogold-Fab' conjugates were applied to counterstain the subcellular compartments pre-stained with LAMP 1 or EEA 1 antibody respectively, samples were not post-fixed with glutaradehyde; In (b) and (d), the LAMP 1 and EEA 1 antigens were detected with counterstaining of immunogold probes in the BBB, followed by post-treatment of glutaradehyde for one hour. Briefly, without post-fixation of glutaradehyde (a, c), the apical plasma membranes appeared structurally disorganised and morphologically permeabilised. Membrane integrity of endosomal or lysosomal compartments seemed compromised, and their enclosed contents were somewhat lost. These structural artifacts were overcome after glutaradehyde was introduced according to (b, d), where the apical cell membranes were observed intact and the border of membrane-enclosed organelles was well-preserved. L, lysosomes; E, endosomes.

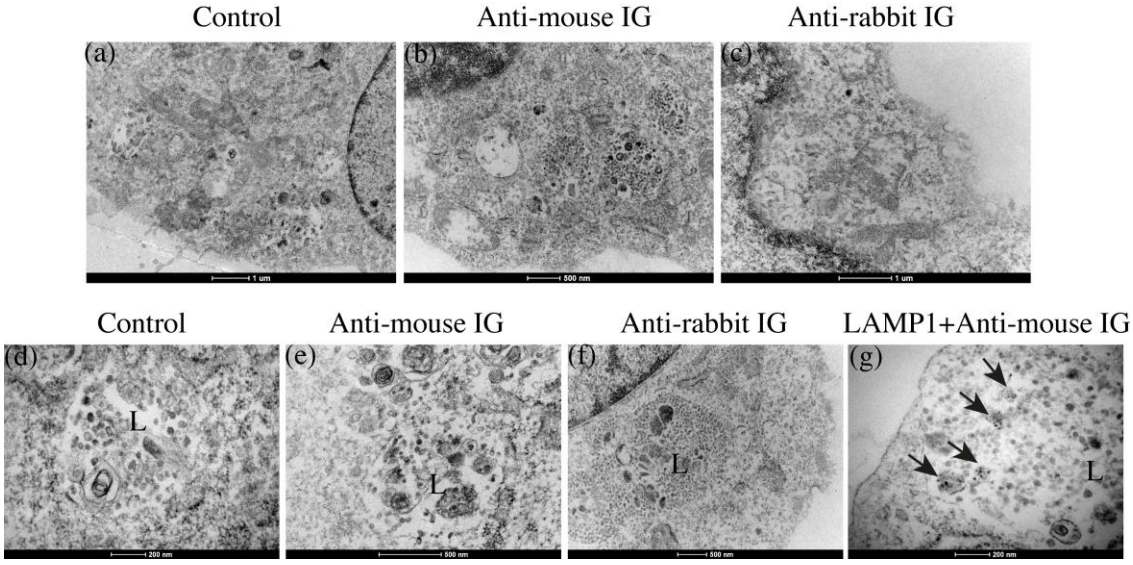

Figure 2. The immunolabeling controls for 1.4 nm secondary nanogold-antibody conjugates. The BBB cells were stained with secondardy anti-mouse (b, e) or anti-rabbit (c, f) immunogold (IG) probes, without pre-treatment of a primary antibody. The untreated BBB cells were included as negative controls (a, d). Overview of staining was provided in a, b&c respectively. In g, the sample was labeled with LAMP 1 antibody and secondary anti-mouse IG probe, whereby a lysosome was immunolocalised by IG particles (without silver enhancement, as indicated by arrows), in comparison with d, e&f that lysosomes (L) were observed with no presence of IG particles.

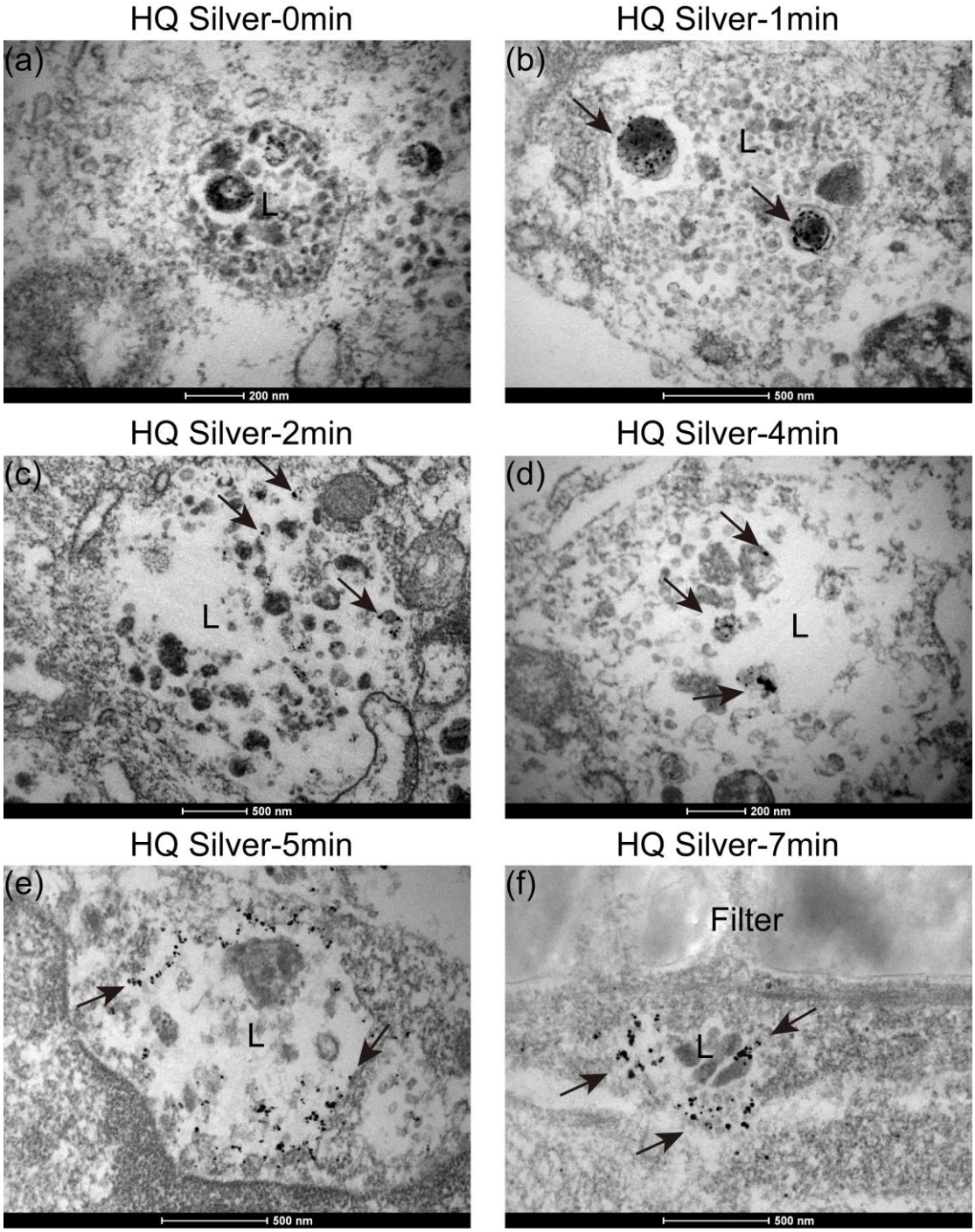

Figure 3. Time-dependent size amplification of colloidal nanogold particles during the silver enhancement. The BBB cell monolayers were stained with LAMP1 primary antibody and subsequently stained with secondary nanogold-conjugated Fab' antibody. All samples were processed using HQ SILVER™ Enhancement kit for silver enhancement at various treatment times, i.e., 0, 1 min, 2 min, 4 min, 5 min, 7 min as shown above. In results, the nanogold particles were seen effectively enlarged inside the BBB cells, and 5-7 min was the optimal treatment time for the silver

enhancement. The locations of silver-gold colloidal grains were indicated by the black arrows.

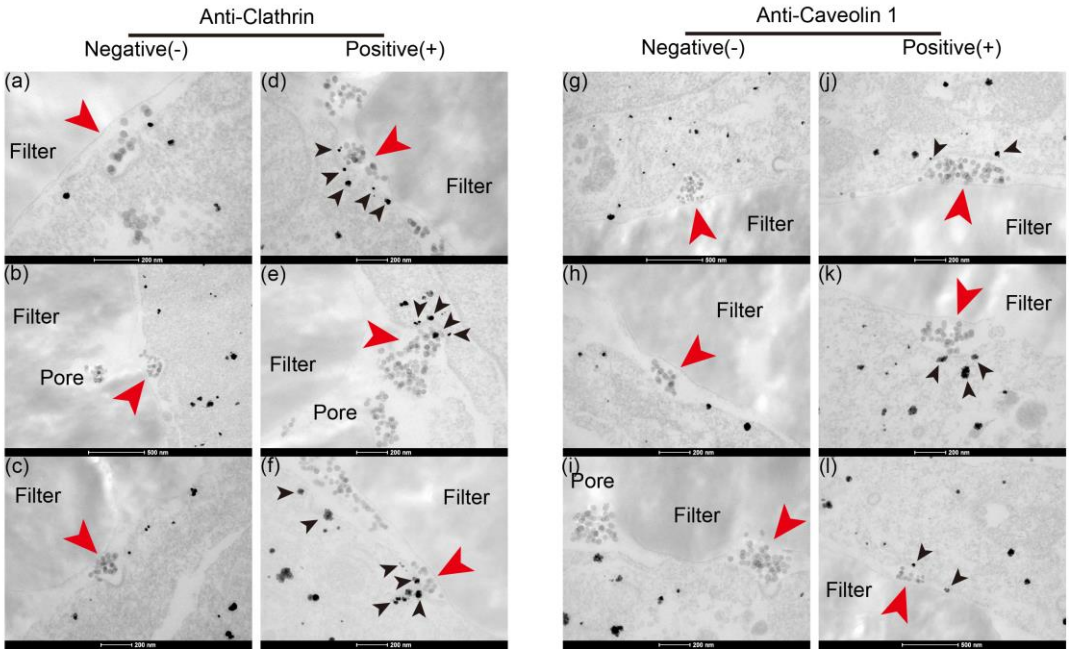

Figure 4. Comparison of positive and negative immunogold labeling results from detection of clathrin- and caveolin-regulated basal membrane activities in the BBB. Each positive or negative labeling result was demonstrated with three images acquired from three different regions at the basal membrane of the BBB. For the clathrin immunolabeling, the BBB monolayers were exposed to 50 nm SiO_2-NPs for 24 hours, and the basal membranes invaginated and associated SiO_2-NPs after their translocation. Such interactions were examined via immunogold labels, with two staining outcomes: positive (d, e, f) and negative (a, b, c) staining of clathrin-regulated vesicular endocytosis; For the caveolin 1 labeling, the barriers were exposed to 50 nm SiO_2-NPs for 12 hours. The basal invaginations were also observed to associate with (j, k, l) or without (g, h, i) the labels of immunogold particles, indicating their positive or negative immunoreactivity with caveolin-regulated events.