Supporting Information

Imaging Inwards and Outwards Trafficking of Gold Nanoparticles in Whole Animals

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This file includes additional information for the following sections:

Long term toxicity effects of AuNP in *Hydra*

Additional TEM images on AuNP uptake, trafficking and exocytosis.

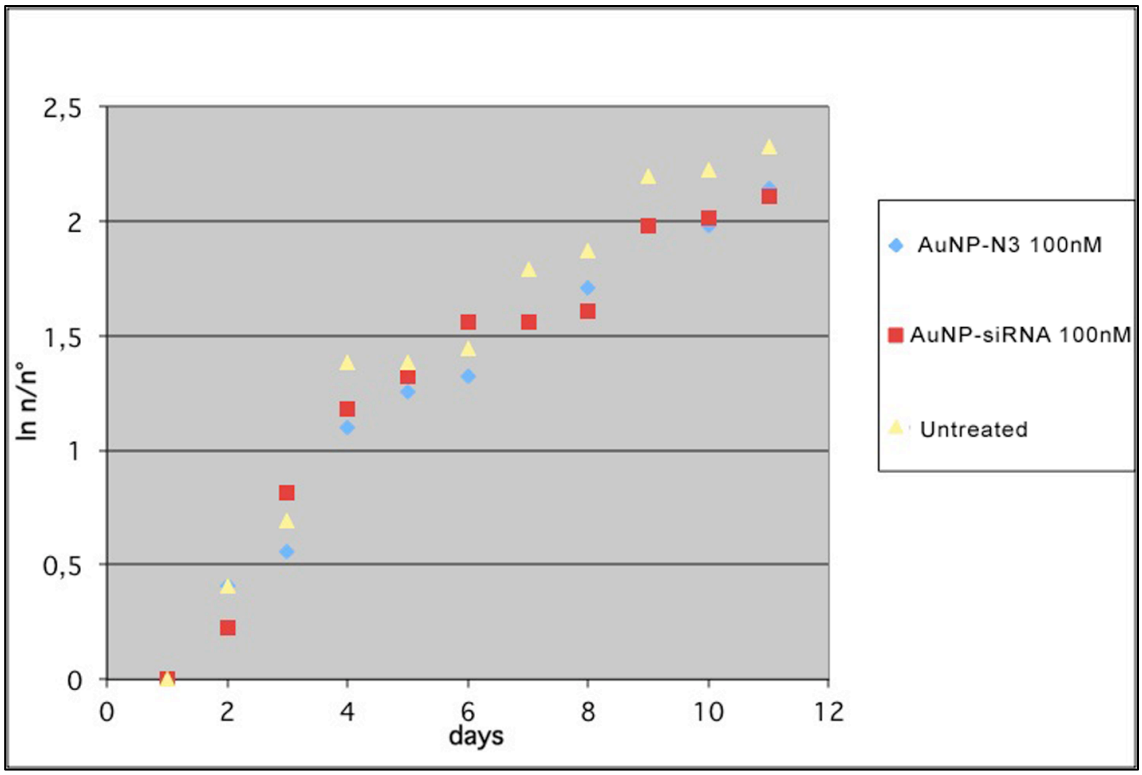

Figure S1. Long-term toxicity effects of AuNP treatment on *Hydra.*

As Hydra reproduce by budding, the population growth rate is an indirect measure of the *Hydra* tissue growth rate and cell viability, and it is routinely used as toxicity parameter. A group of founder animals (n°) either untreated or incubated for 24 h with 100 nM AuNP-N_3 or AuNP-siRNA were monitored over two weeks, and the total number of individuals (n) used to calculate the growth rate constant (k) over the duration of the experiment (t), was defined by the equation ln(n/n°) = kt. Control animals at the same developmental stage were not treated. Both experimental and control *Hydra* were fed once daily and monitored for bud formation and detachment. Not significant differences were observed between growth parameters of treated or untreated animals, showing the absence of toxic effects of these NP on *Hydra* reproductive capabilities.

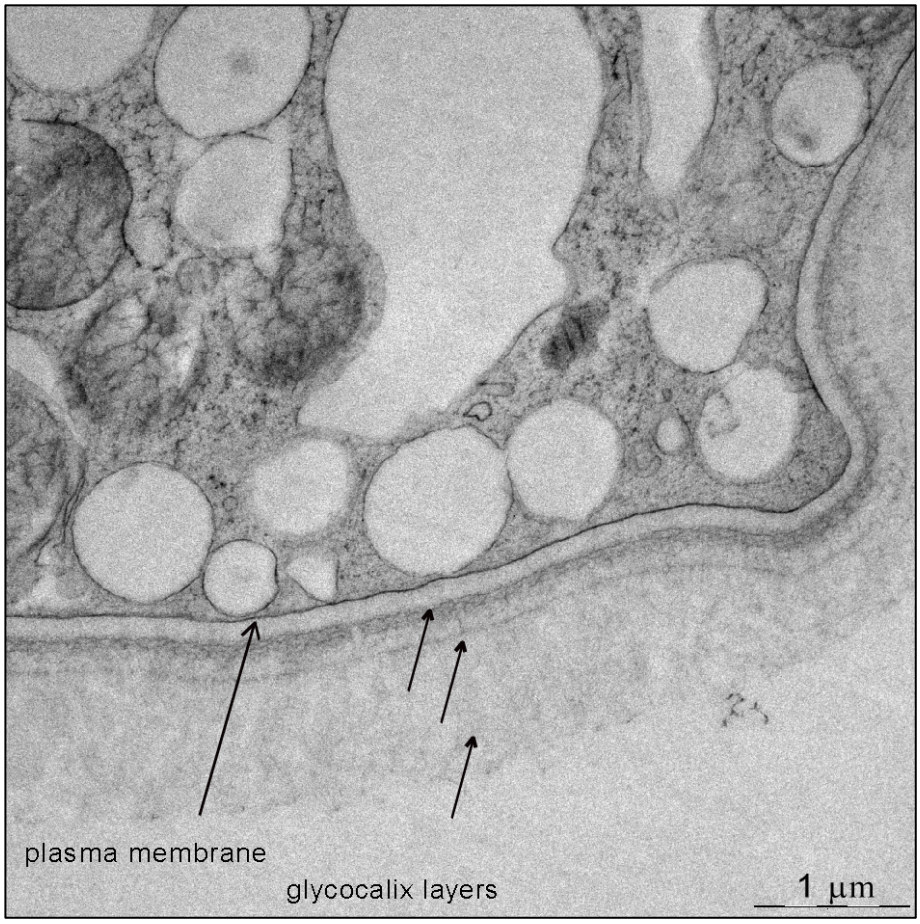

Figure S2. Electron micrograph of the apical portion of an ectodermal cell

Image of a portion of *Hydra* ectodermal cell analysed by high-pressure freezing TEM. The electron micrograph shows the multi-layered structure of *Hydra* glycocalix coating the plasma membrane, as reported by A. Bottger recent work. [32] Arrows indicate identifiable layers with diverse electron densities, not visible by other TEM techniques. Scale bar: 1μM.

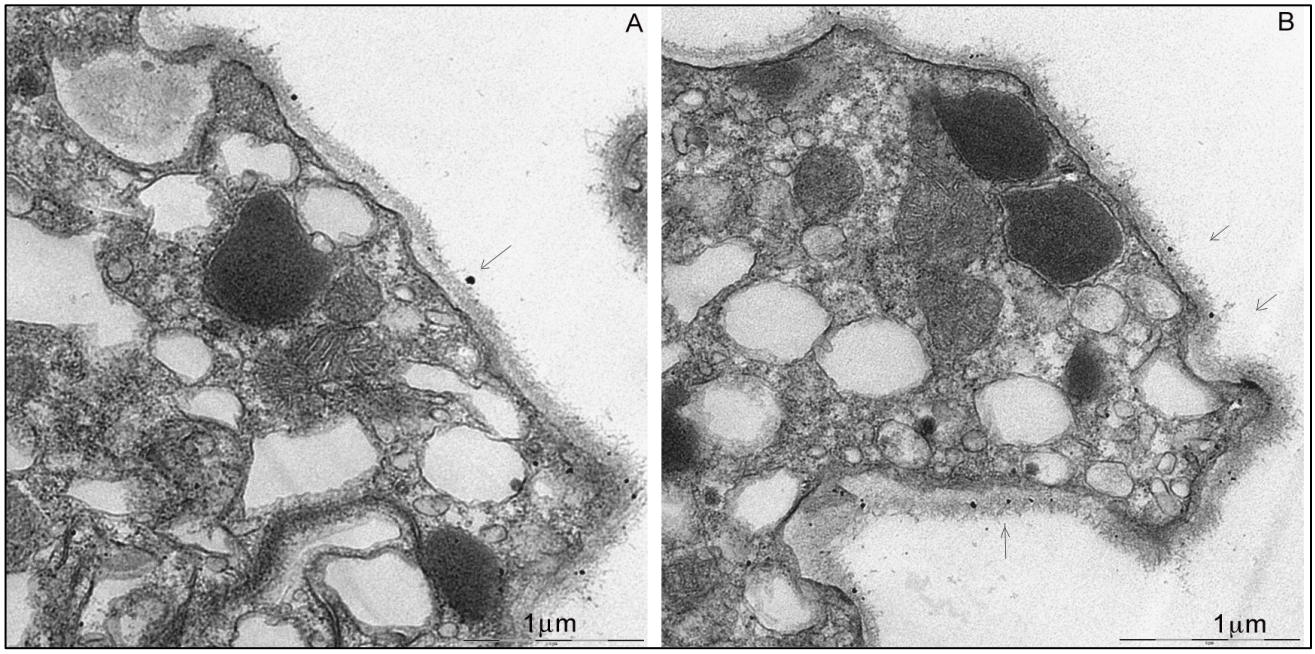

Figure S3. Early interactions of AuNP-N_3 with cell membranes

TEM images of *Hydra* ectodermal cell prepared from polyps treated with AuNP-N_3 for 5 min. NPs are attached, as single unit or small clusters, to the glycocalix layer (arrowed in A) or embedded in it (arrows in B). Scale bars: 1 μM in A and B.

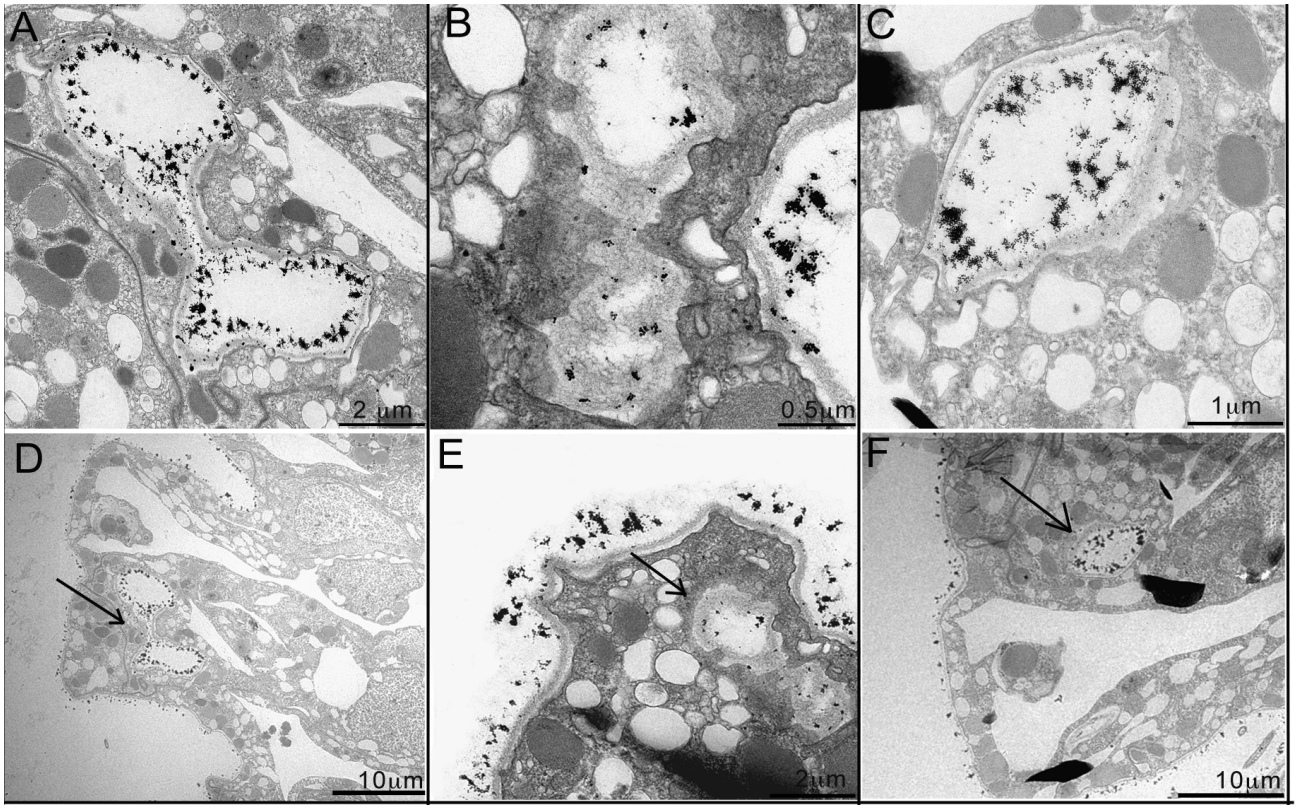

Figure S4. Massive nanoparticle uptake through endocytosis

Polyps were incubated 30min with 70nM AuNP-N_3, then washed and processed for cross sectioning and TEM analysis. In A, B and C, large endocytic vacuoles derived from membrane invagination are shown, while their location relative to the whole tissue sections are shown below each image, in D, E, and F, obtained from three different animals. In A and B the fusions/divisions of intracellular vesicles reveal active dynamics taking place during the first 30 minutes. In all cases the vacuoles are located in the apical part of the ectodermal cells, facing the animal outer layer, suggesting that they originate from recent membrane invaginations. The inner lining by glycocalix-like structures further support this hypothesis. AuNP-N_3 are also found escaping from such structures, showing the capability of AuNP-N_3 to freely traverse such membranes and to reach the cytoplasm. Scale bars: 2μM in A, E; 0.5μM in B; 1μM in C; 10μM in D, F.

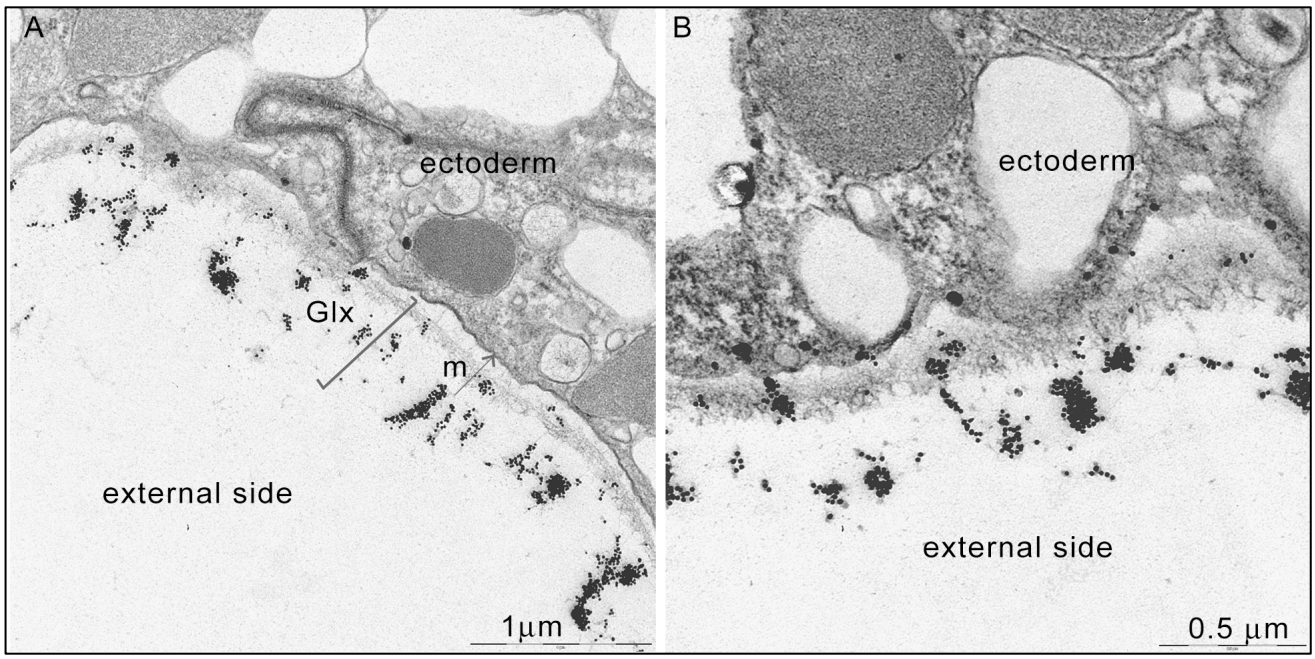

Figure S5. AuNP-N_3 directly cross the cell membrane

AuNP-N_3 are shown well monodisperse, embedded into the glycocalix (Glx), which appears only partially preserved after processing the sample for TEM. Single NPs are located by at various glycocalix depths, and crossing the cell membrane (m). In A and B two tissue regions are shown. AuNP-N_3 in same case are not monodisperse, and appear as small aggregates. Scale bars: 1μM in A; 0.5μM in B.

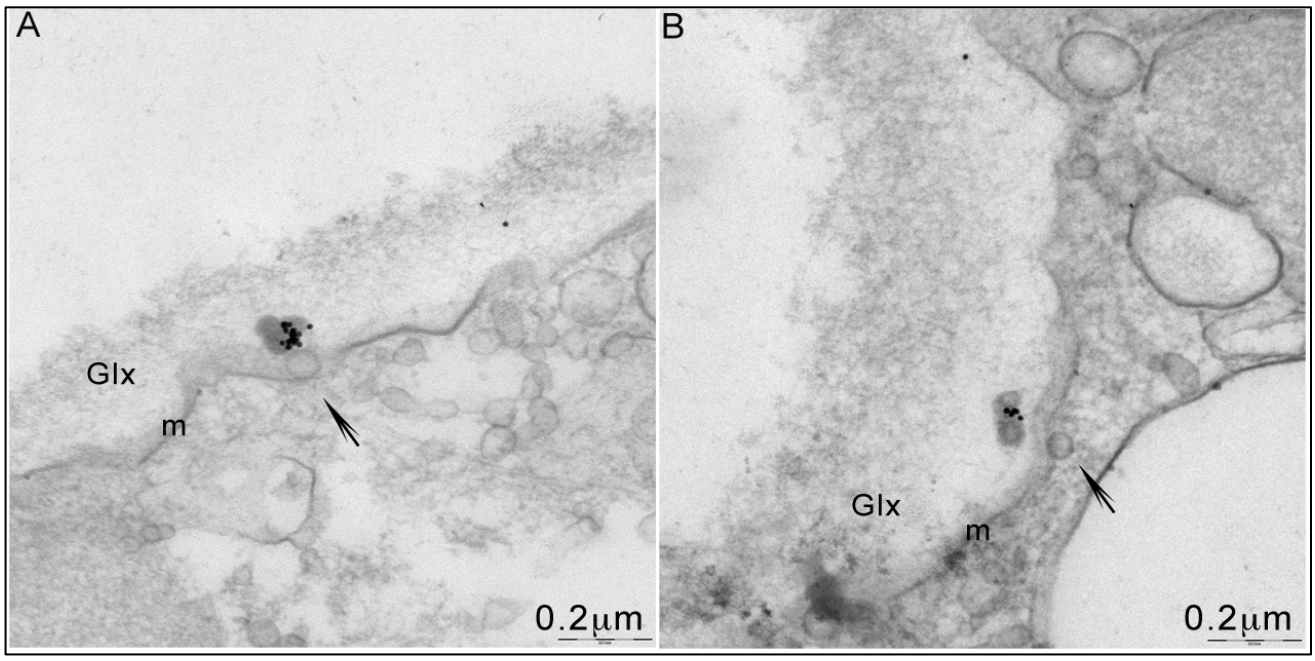

Figure S6. Nanovesicle-mediated exocytosis of AuNP-N_3

A) Nanovesicles underlying the cell membrane release their content outside upon fusion with the phospholipid layer. Gold nanoparticles may be shuttled in and out the membrane through binding to these nanovesicles. The membrane appears interrupted on different regions to enable cell release, and empty vesicles (arrowed) are clearly detected on the cytosolic region below the external electron dense material. B) Another example of an empty nanovesicle in close proximity of external one decorated by gold nanoparticle. Scale bar 0.2μM in A and B.

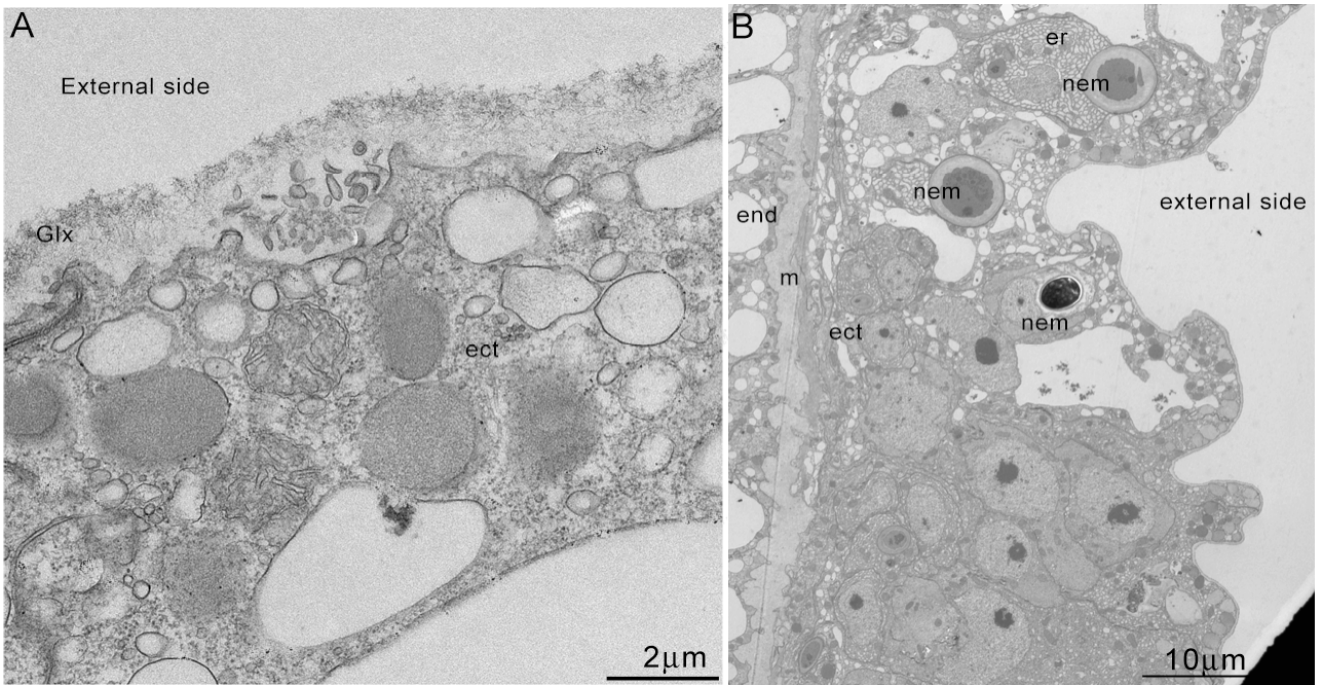

Figure S7. TEM images of ectodermal tissue prepared from an untreated animal.

A) Lysosome fusion with the cell membrane causes the external release of its content. B) TEM image of a different tissue region, showing the bilayer structure of the *Hydra* tissue. A nest of more than 8 nematoblasts (stem cell committed to nematocyte differentiation) is clearly recognizable in the lower part of the image, interspersed within epithelial cells. The nematocyst appears in different developmental stages. An extended endoplasmic reticulum is evident in differentiated nematocytes (nem), the specialized cells characterizing the whole Cnidaria phylum, identifiable also by the presence of a dark electrondense spherical nematocyst. Glx, Glycocalix; m, mesoglea. ect, ectoderm; end, endoderm.