Supplementary Information

Deciphering silver nanoparticle fate in liver up to biliary excretion using HepG2/C3A spheroids in scenarios mimicking different exposure pathways

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Captions for videos.

Video S1. The video presents the FIB-SEM stack of micrographs within a spheroid exposed to cit-AgNP for 7 days. The video shows the raw FIB-SEM volume and its segmentation into the different organelles: nuclei (pink), mitochondria (green), vesicles (blue), bile canaliculi (grey) and NP (black). The different segments are also shown sequentially to visualize the different cellular compartments.

Video S2. The video presents the FIB-SEM stack of micrographs within a spheroid exposed to PVP-AgNP for 7 days. The video shows the raw FIB-SEM volume and its segmentation into the different organelles: nuclei (pink), mitochondria (green), vesicles (blue), bile canaliculi (grey) and NP (black). The different segments are also shown sequentially to visualize the different cellular compartments.

Video S3. The video presents the FIB-SEM stack of micrographs within a spheroid exposed to $AgNO_3$ for 7 days. The video shows the raw FIB-SEM volume and its segmentation into the different organelles: nuclei (pink), mitochondria (green), vesicles (blue), bile canaliculi (grey) and NP (black). The different segments are also shown sequentially to visualize the different cellular compartments.

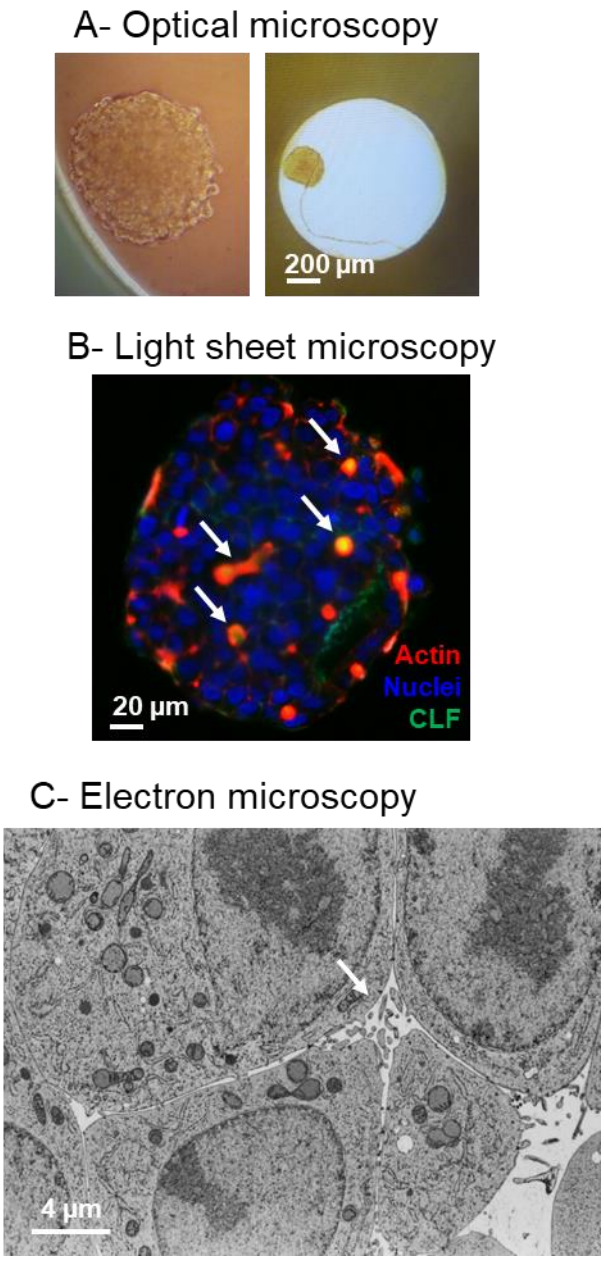

Fig. S1 Hepatocyte spheroid model characterization. (A) A mature HepG2/C3a spheroid observed by optical microscopy. (B) Single plan light sheet fluorescence microscopy of a matrix-free hepatic spheroid stained for actin (red), Hoechst (blue) and a fluorescent bile salt (CLF, green). White arrows locate active bile canaliculi regions. (C) Ultrastructure within a spheroid visualized by 3D electron microscopy (FIB-SEM). The white arrow pinpoints a bile canaliculus.

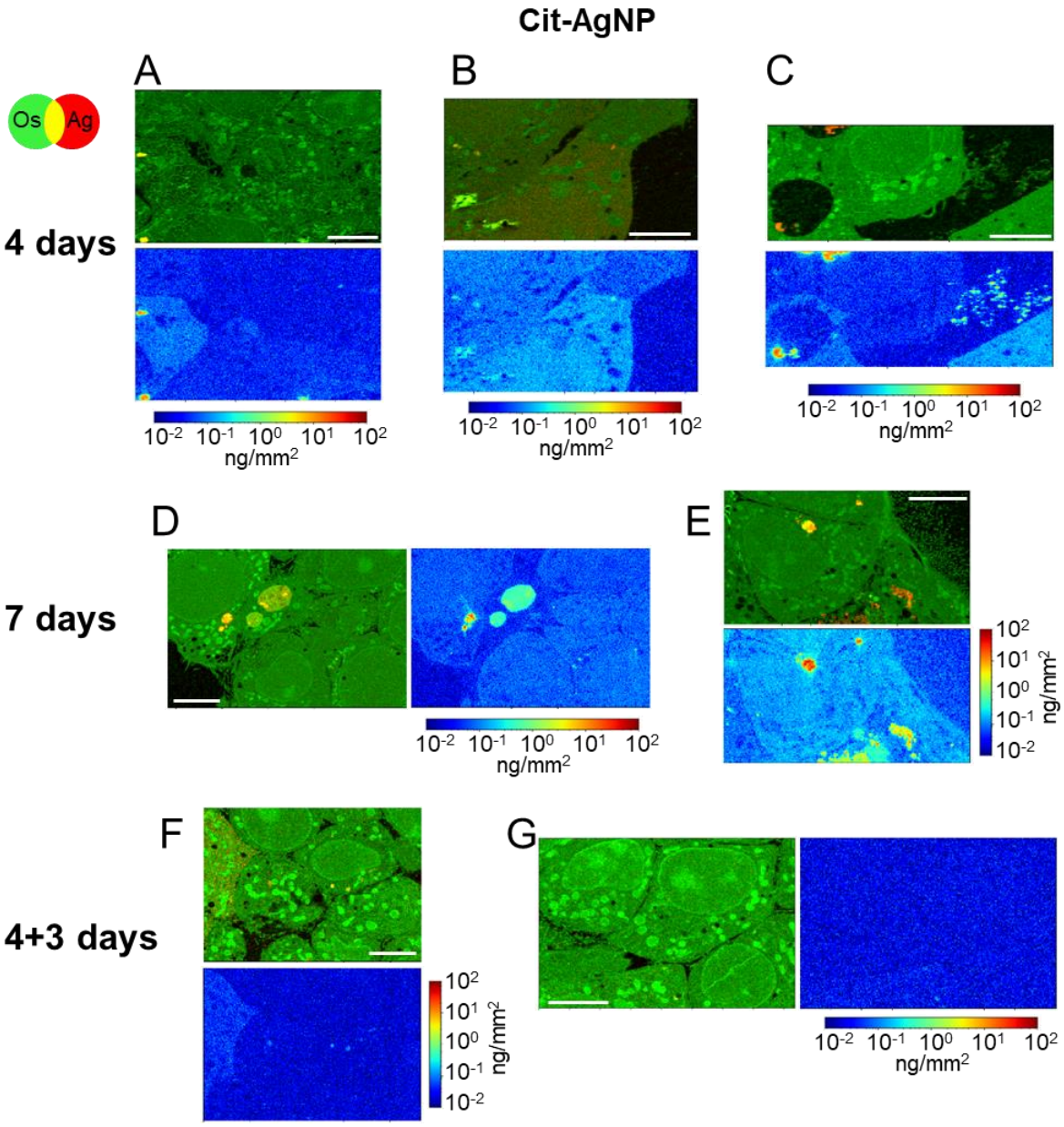

Fig. S2 False-color representation of Ag distribution in hepatocytes exposed to cit-AgNP, extracted from XRF hyperspectral images with 100 × 100 nm^2 pixel size. Cells were exposed to citrate-AgNP for 4 days (A, B, C), 7 days (D, E), or for 4 days followed by 3 days of recovery (F, G). In each figure panel, the first map represents the overlay of Os (green) and Ag (red), the second the quantitative distribution of the areal density of Ag over the same map area, in logarithmic scale. Scale bars = 5 µm.

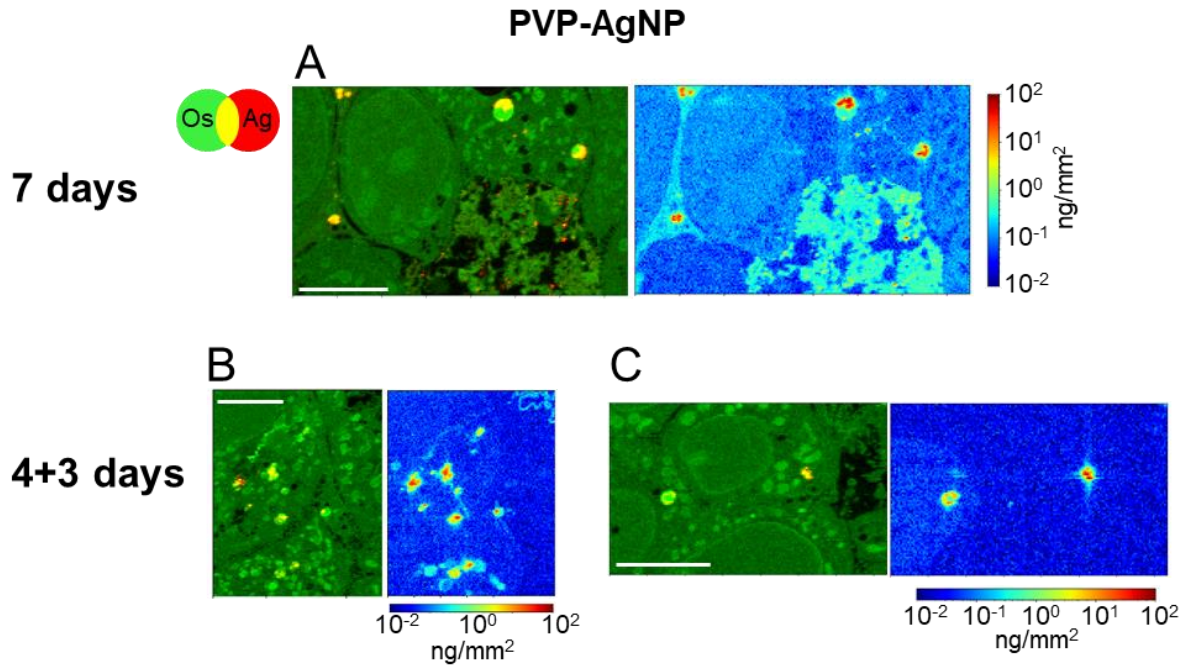

Fig. S3 False-color representation of Ag distribution in hepatocytes exposed to PVP-AgNP, extracted from XRF hyperspectral images with 100 × 100 nm^2 pixel size. Cells were exposed to PVP-AgNP for 7 days (A), or for 4 days followed by 3 days of recovery (B, C). In each figure panel, the first map represents the overlay of Os (green) and Ag (red), the second the quantitative distribution of the areal density of Ag over the same map area, in logarithmic scale. Scale bars = 5 µm.

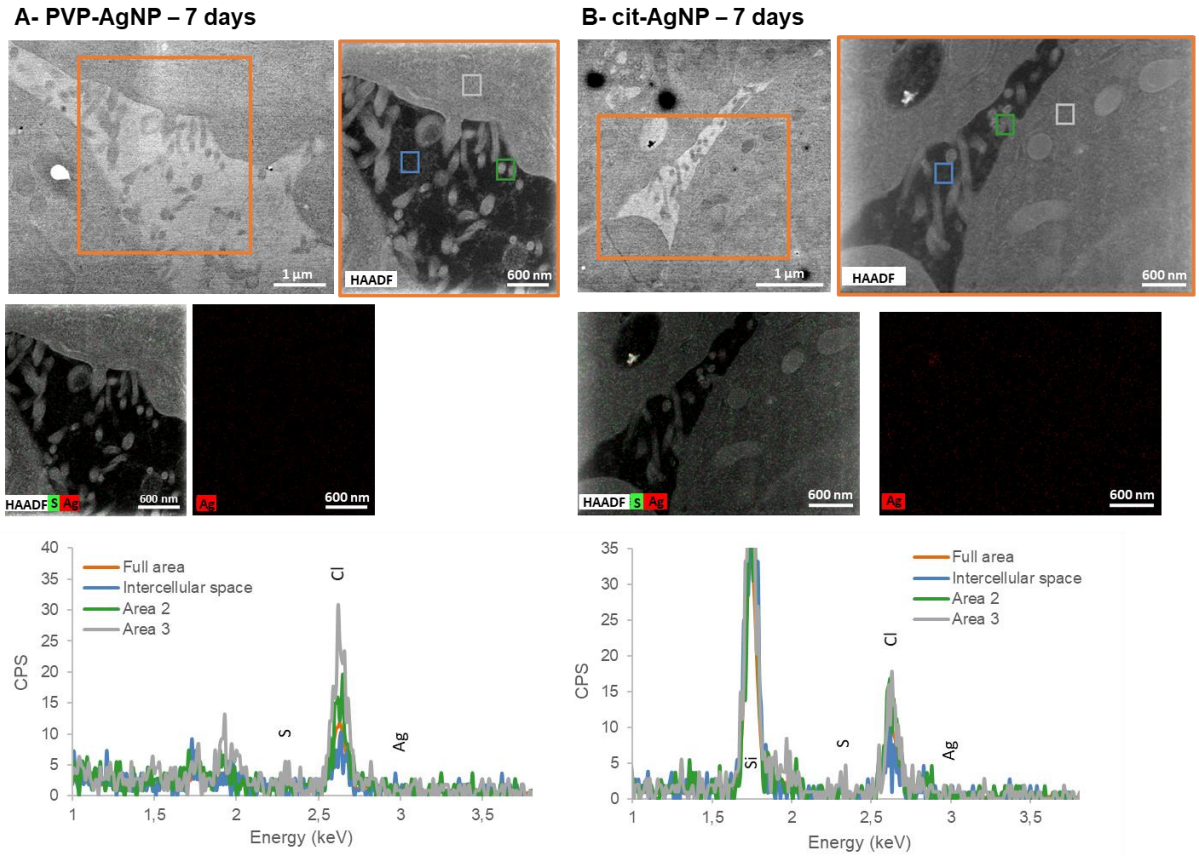

Fig. S4 Chemical analysis by STEM-EDX of spheroid sections exposed for 7 days to PVP-AgNP (A) or cit-AgNP (B). STEM micrograph field of view centered on a bile canaliculi area (upper panels). EDX maps of the region in the orange box (middle panels). EDX spectra of the different regions selected in the HAADF map (colored boxes) in the energy range 1 – 3.5 keV (lower panels). The position of Si, S, Cl and Ag peaks is highlighted.

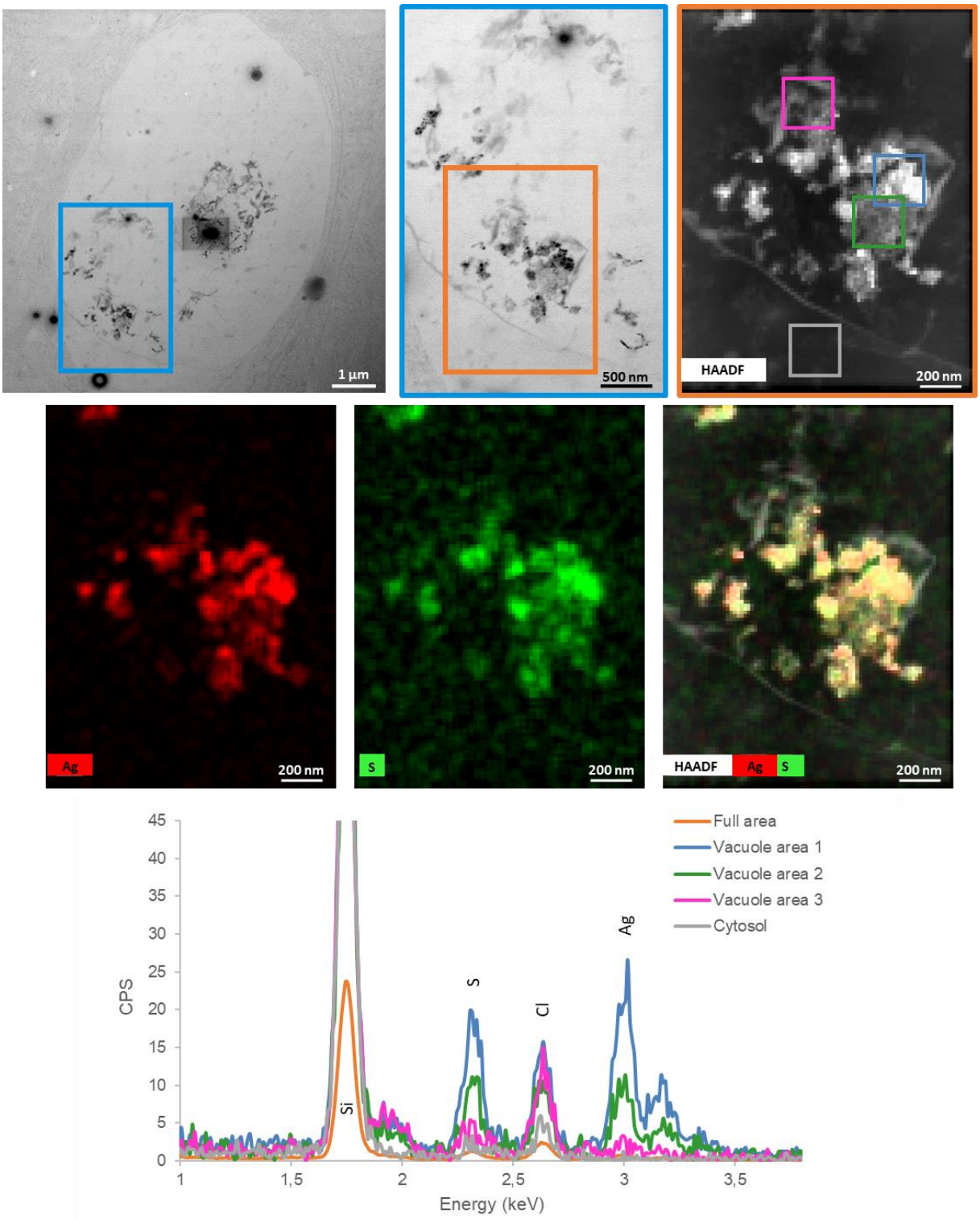

Fig. S5 Chemical analysis by STEM-EDX of a spheroid section exposed for 4 days to cit-AgNP. STEM micrograph field of view showing a vacuole containing transformed AgNP (upper panels). EDX maps of the region in the orange box (middle panels). EDX spectra of the different regions selected in the HAADF map (colored boxes) in the energy range 1 – 3.5 keV (lower panel). The position of Si, S, Cl and Ag peaks is highlighted.

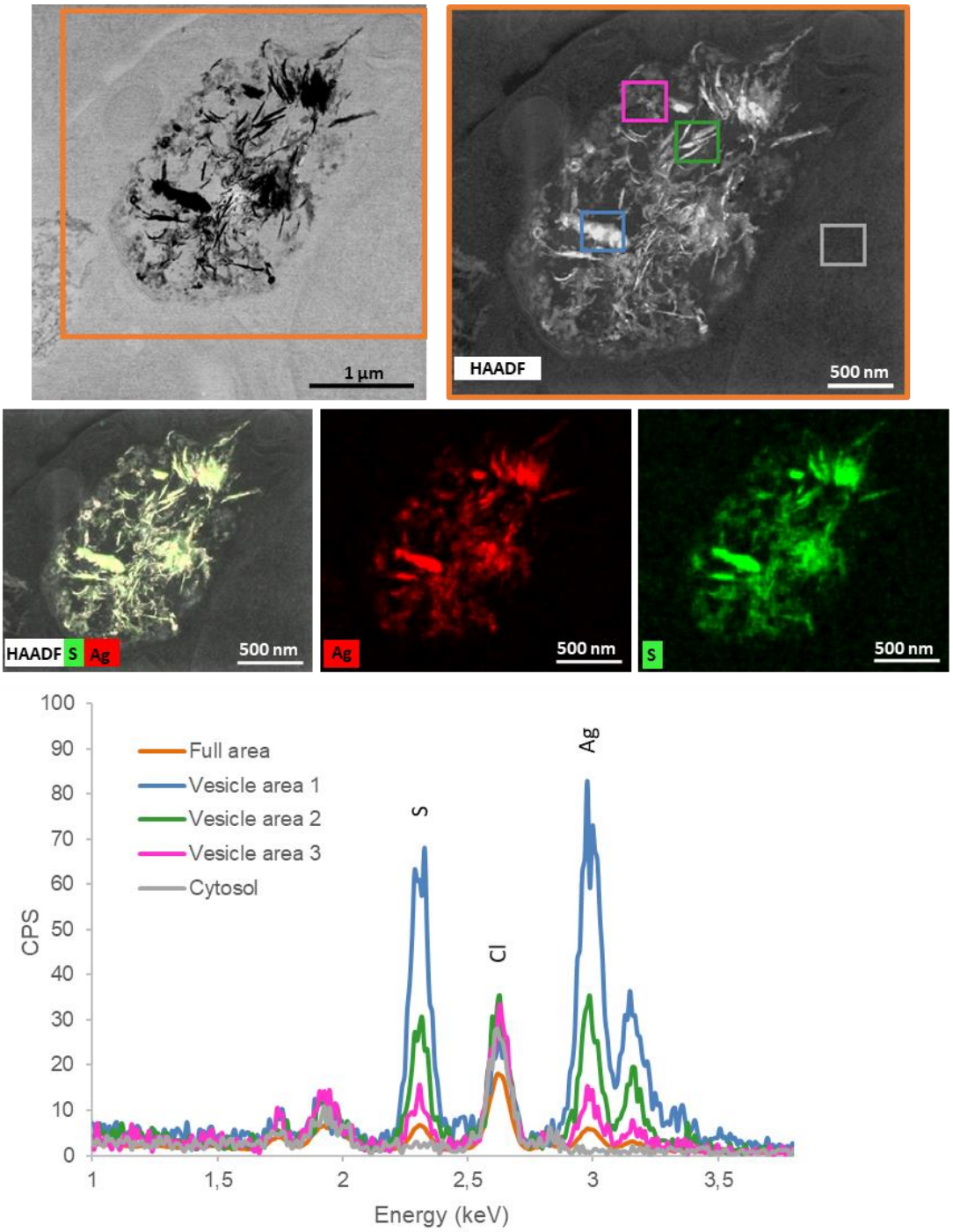

Fig. S6 Chemical analysis by STEM-EDX of a spheroid section exposed for 7 days to PVP-AgNP. STEM micrograph field of view showing a vesicle containing transformed AgNP (upper panels). EDX maps of the region in the orange box (middle panels). EDX spectra of the different regions selected in the HAADF map (colored boxes) in the energy range 1 – 3.5 keV (lower panel). The position of S, Cl and Ag peaks is highlighted.

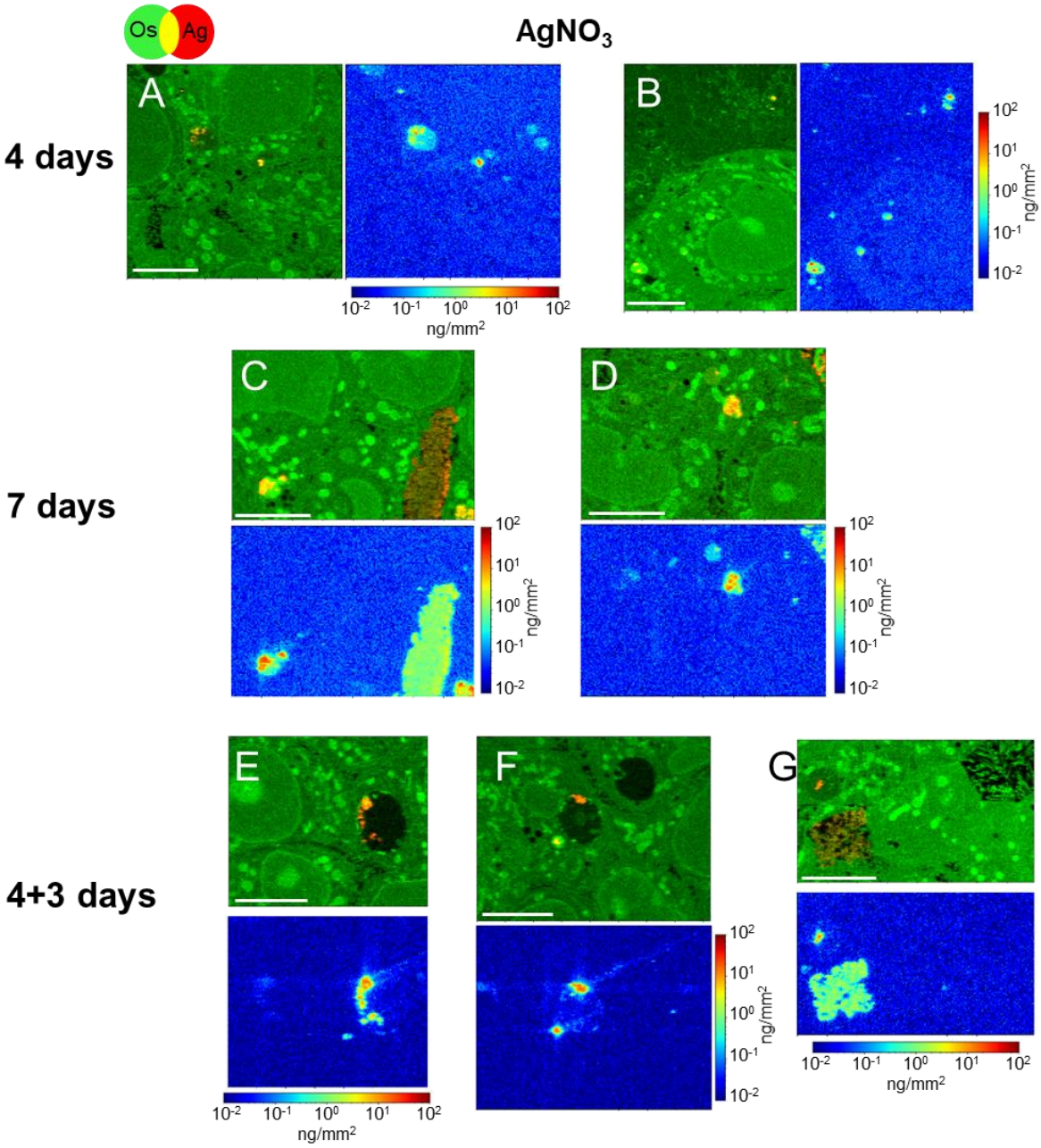

Fig. S7 False-color representation of Ag distribution in hepatocytes exposed to $AgNO_3$, extracted from XRF hyperspectral images with 100 × 100 nm^2 pixel size. Cells were exposed to a Ag salt for 4 days (A, B), 7 days (C, D), or for 4 days followed by 3 days of recovery (E, F, G). In each figure panel, the first map represents the overlay of Os (green) and Ag (red), the second the quantitative distribution of the areal density of Ag over the same map area, in logarithmic scale. Scale bars = 5 µm.

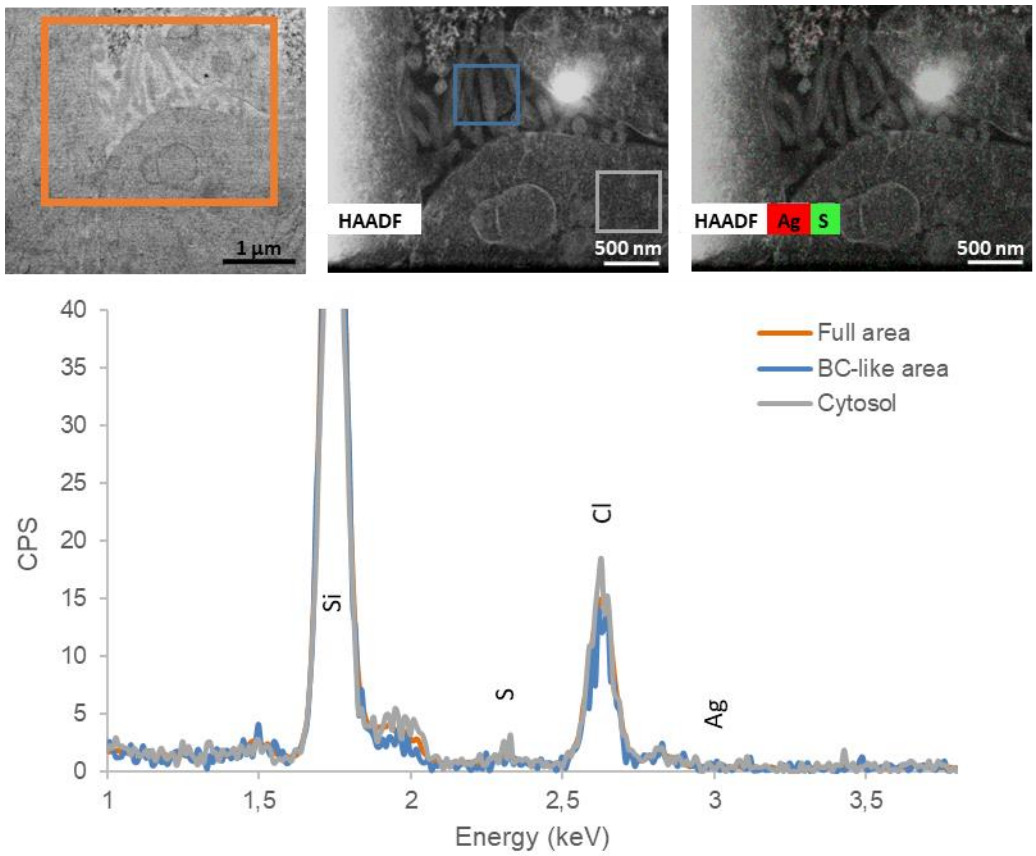

Fig. S8 Chemical analysis by STEM-EDX of spheroid sections exposed for 7 days to $AgNO_3$. STEM micrograph field of view centered on a bile canaliculi area (upper left and center panels). EDX maps of the region in the orange box (upper right panel). EDX spectra of the different regions selected in the HAADF map (colored boxes) in the energy range 1 – 3.5 keV (lower panel). The position of Si, S, Cl and Ag peaks is highlighted.

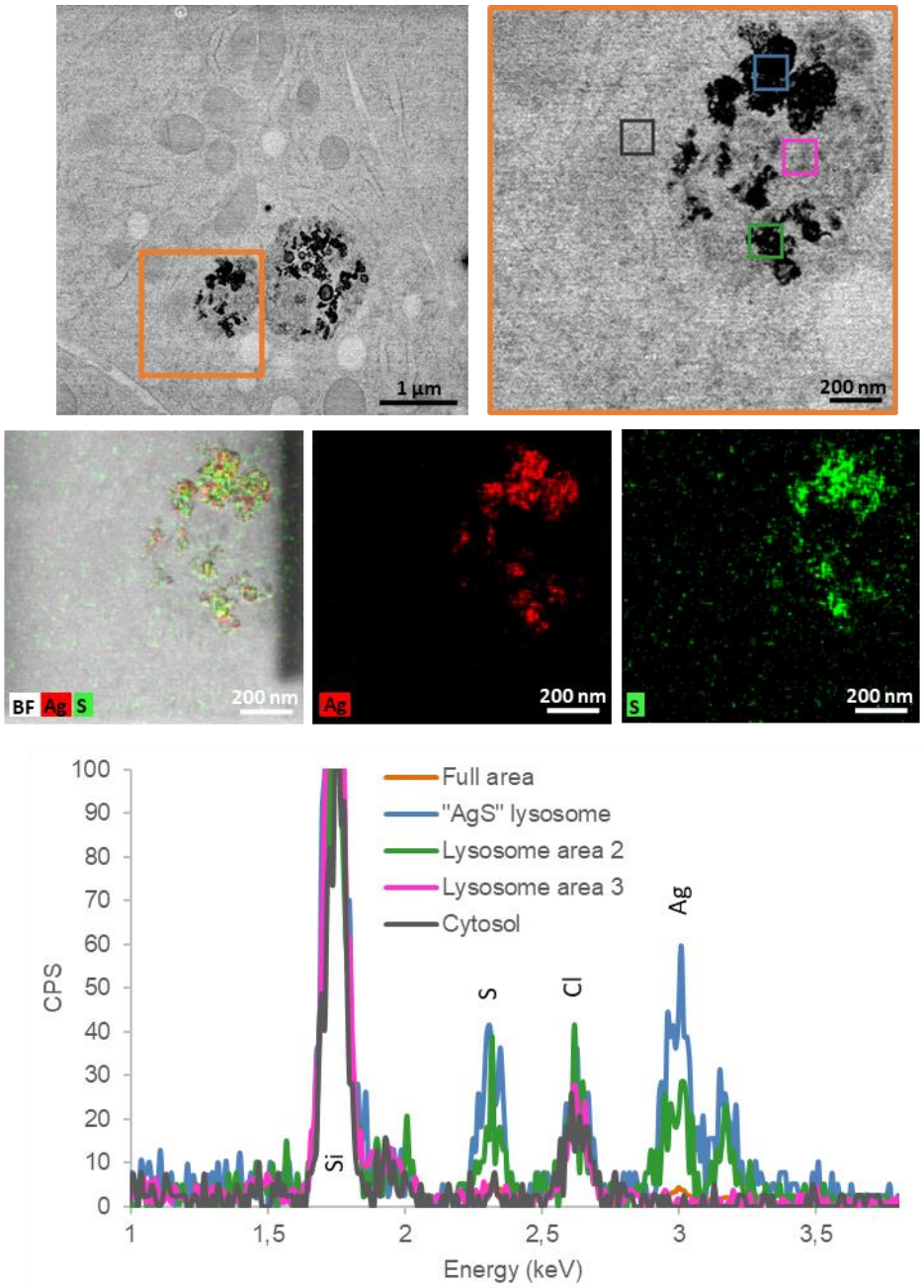

Fig. S9 Chemical analysis by STEM-EDX of a spheroid section exposed for 7 days to $AgNO_3$. STEM micrograph field of view showing vesicles containing Ag particles (upper panels). EDX maps of the region in the orange box (middle panels). EDX spectra of the different regions selected in the HAADF map (colored boxes) in the energy range 1 – 3.5 keV (lower panel). The position of Si, S, Cl and Ag peaks is highlighted.

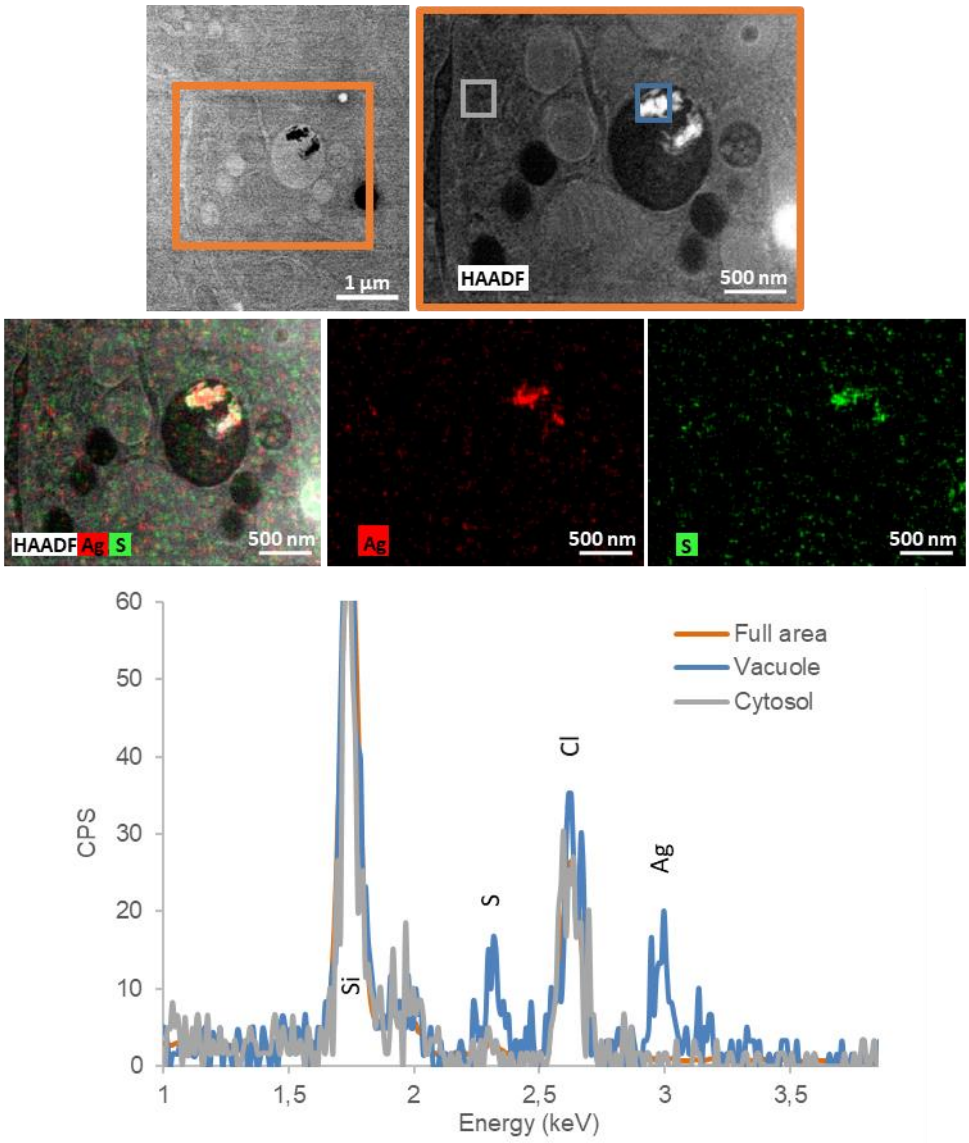

Fig. S10 Chemical analysis by STEM-EDX of a spheroid section exposed for 7 days to $AgNO_3$. STEM micrograph field of view showing a vacuole containing Ag particles (upper panels). EDX maps of the region in the orange box (middle panels). EDX spectra of the different regions selected in the HAADF map (colored boxes) in the energy range 1 – 3.5 keV (lower panel). The position of Si, S, Cl and Ag peaks is highlighted.