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Supplementary Materials for

Intraoperative visualization of the tumor microenvironment and quantification of extracellular vesicles by label-free nonlinear imaging

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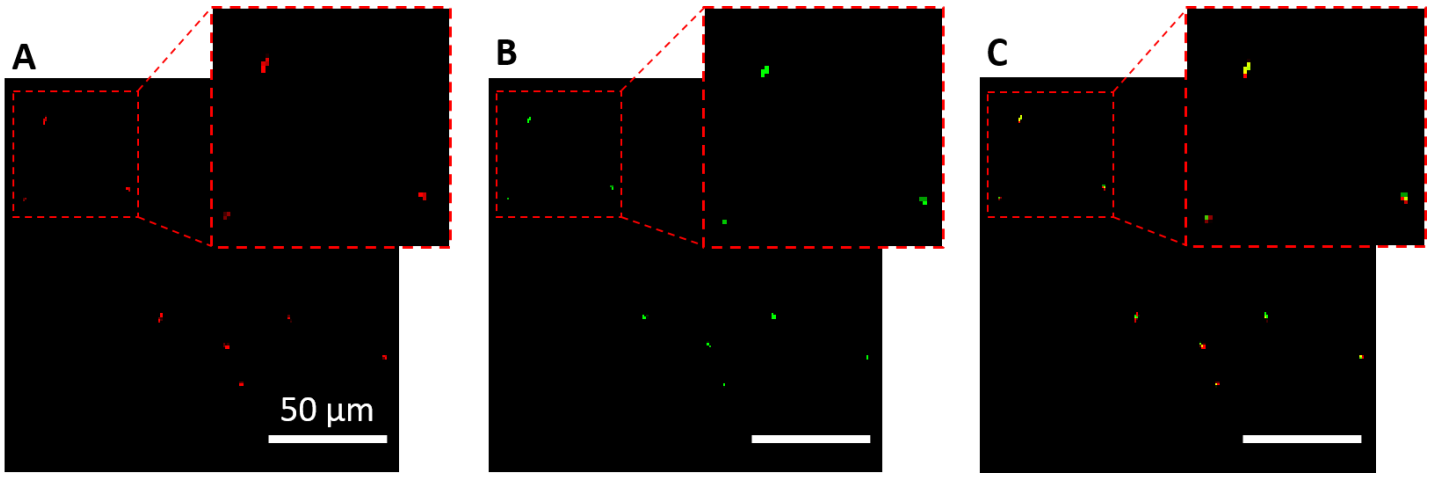

Fig. S1. Validation of THG imaging of EVs by immunohistochemical-based detection. Images of EVs (isolated from MCF-10A cell line) are acquired by (**A**) label-free THG and (**B**) labeled two-photon fluorescence using CD40L antibody. (**C**) Composite image of the two imaging modalities shows the overlapping of EVs. Red channel represents the label-free THG image, while green channel represents the labeled two-photon fluorescence image. The inserted zoomed-in images (red dashed squares) show the spatial overlapping of EVs visualized by the two imaging modalities, and the EVs are yellow due to the intensity addition of the red and green channels. Scale bar is the same for all images.

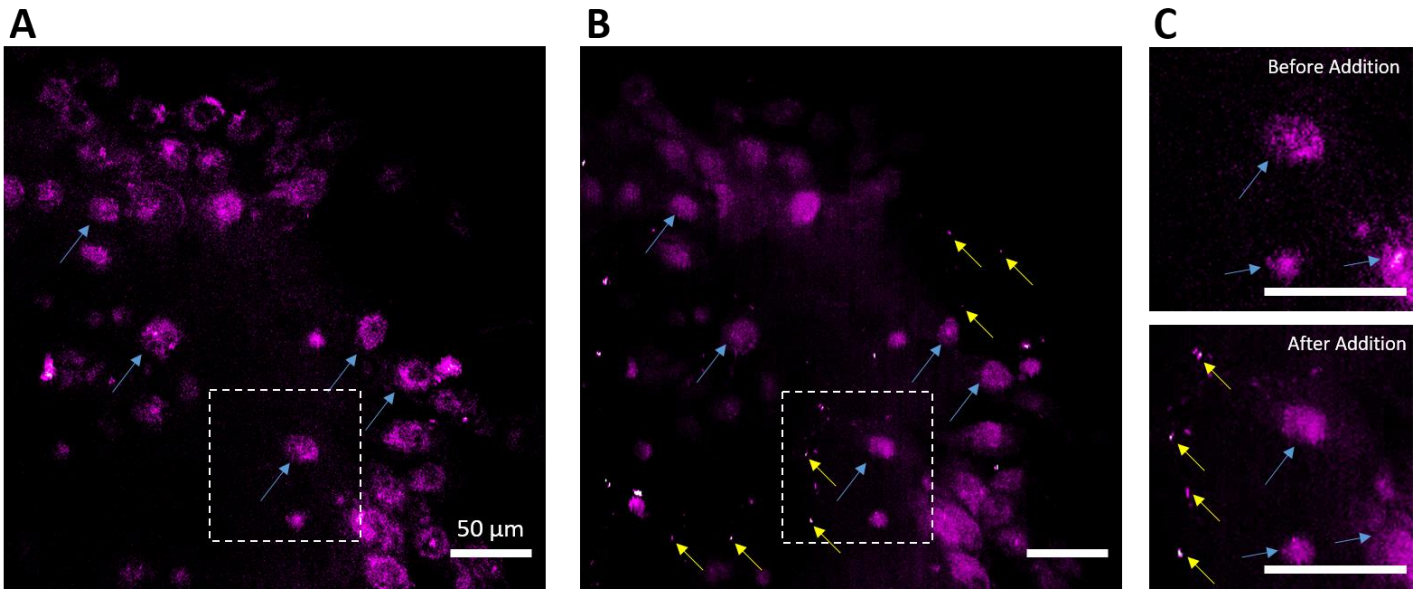

Fig. S2. Increase of EV density in cell culture by adding isolated EVs. THG Images of MCF-10A cells (blue arrows) in culture are acquired (**A**) before and (**B**) after mixing with isolated MDA-231-drived EVs (yellow arrows), with a region of interest (white dashed square). (**C**) Two zoomed-in images of the boxed areas highlight the drastic changes of EV numbers (Before: 36.3 ± 18.4 nL^{-1}, After: 205.7 ± 60.2 nL^{-1}) because of the addition of EVs. Scale bar is the same for all images.