Supporting information

Intracellular trafficking of fluorescent nanodiamonds and regulation of their cellular toxicity.

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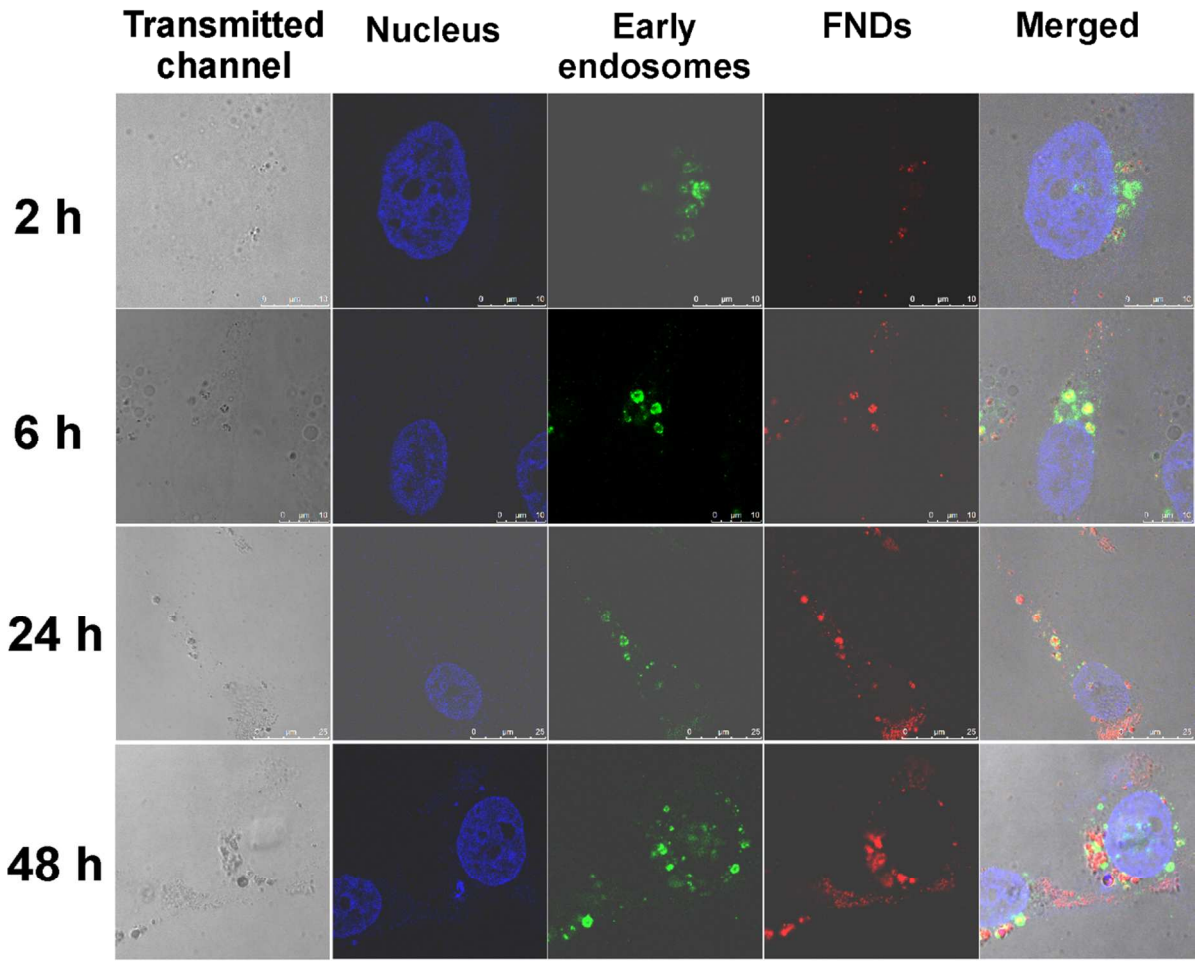

Figure S1. Temporal localization of FNDs in cells by early endosomes (EEA1). After 2h incubation FNDs (red) are seen within early endosomes (green), and in cytoplasm (blue-nuclei). Some early endosomes do not contain any FNDs. After 6 h, FNDs were seen localized in early endosomes forming large aggregates. Another FND population was observed to be not early endosome bound, forming smaller aggregates and localized more dispersed in cells. After 24 h, three distinct populations of FNDs were observed: FNDs co-localized with early endosomes, FNDs aggregated but not bound to early endosomes and a dispersed population of FNDs, which were mainly localized in proximity to plasma membrane. After 48 h, a small but significant population of FNDs is localized in early endosomes. Larger population of FNDs were not bound to early endosomes but remained clustered and dispersed in proximity of plasma membrane.

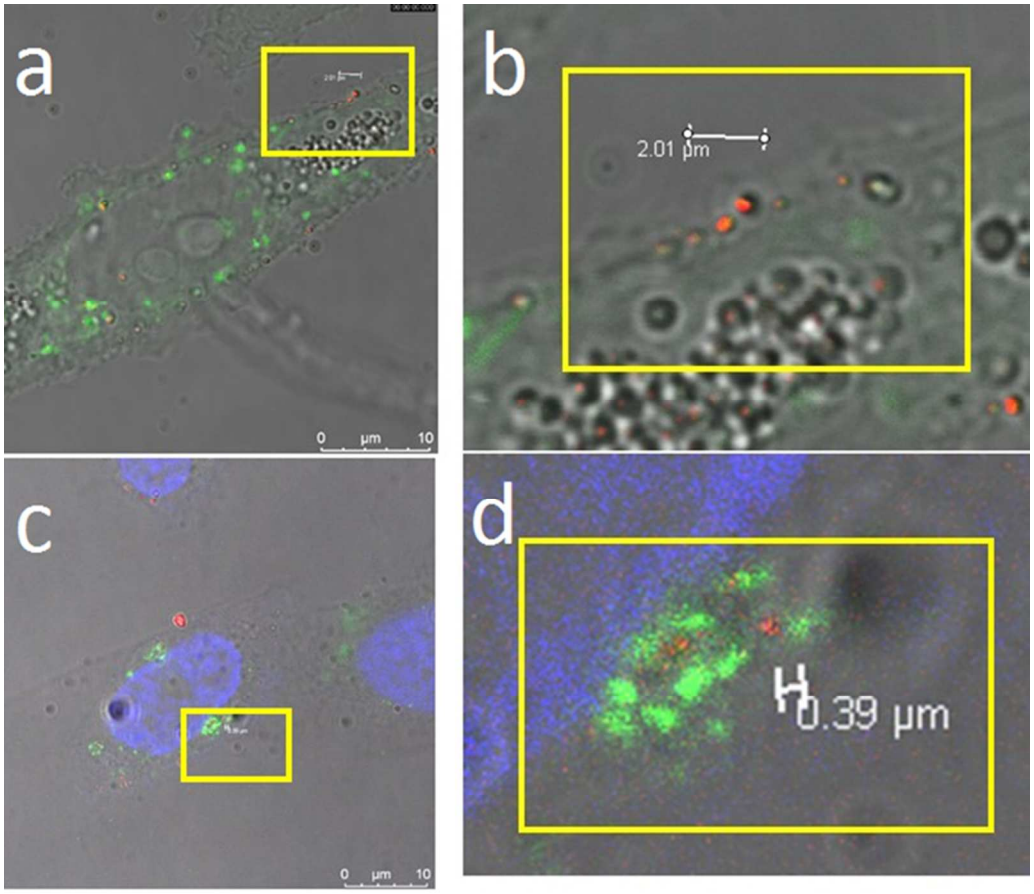

Figure S2. FND were observed to be internalized in form of smaller aggregates in at 0-2h. a) FND (red) can be seen localized near plasma membrane and lysosomes (green). b) The size of FND next to plasma membrane is approximately 400 nm. c) 1-2 µm (approximately) aggregated FNDs (red) can be seen next to plasma membrane. d) Inset image of FNDs localized in EEA1 (green). The approximately size of FNDs co-localized within EEA1 is roughly 300 nm.

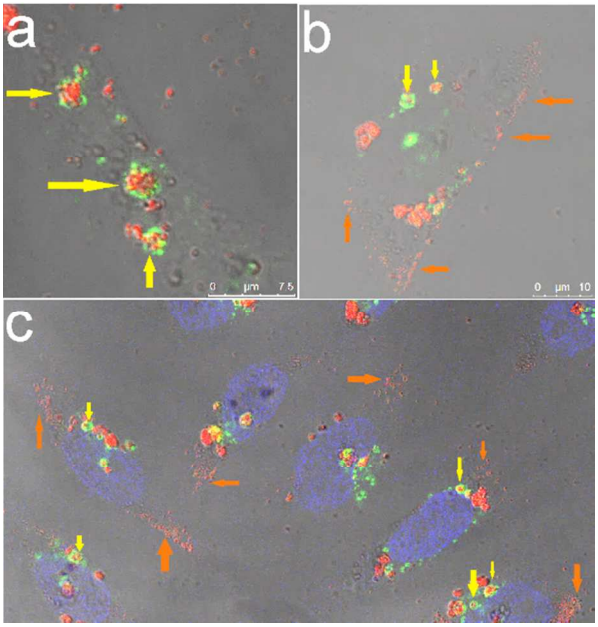

Figure S3. Early endosomal localization and presence of distinct FND populations after 24h internalization. a) FNDs localized within large early endosomes (yellow arrow). b-c) Presence of more dispersed and comparatively less aggregated FNDs (orange arrow) in proximity of plasma membrane.

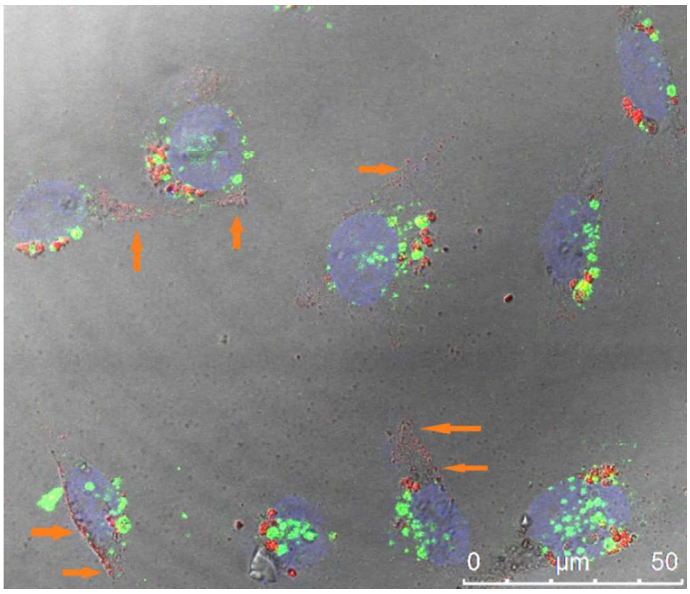

Figure S4. Early endosomal localization and presence of distinct FND populations after 48h internalization. Lesser population of FNDs were localized in early endosomes. However, there

was presence of other aggregated FND population but mainly outside early endosomes. Non-aggregate and more disperse FNDs can be seen in proximity of plasma membrane (arrow).

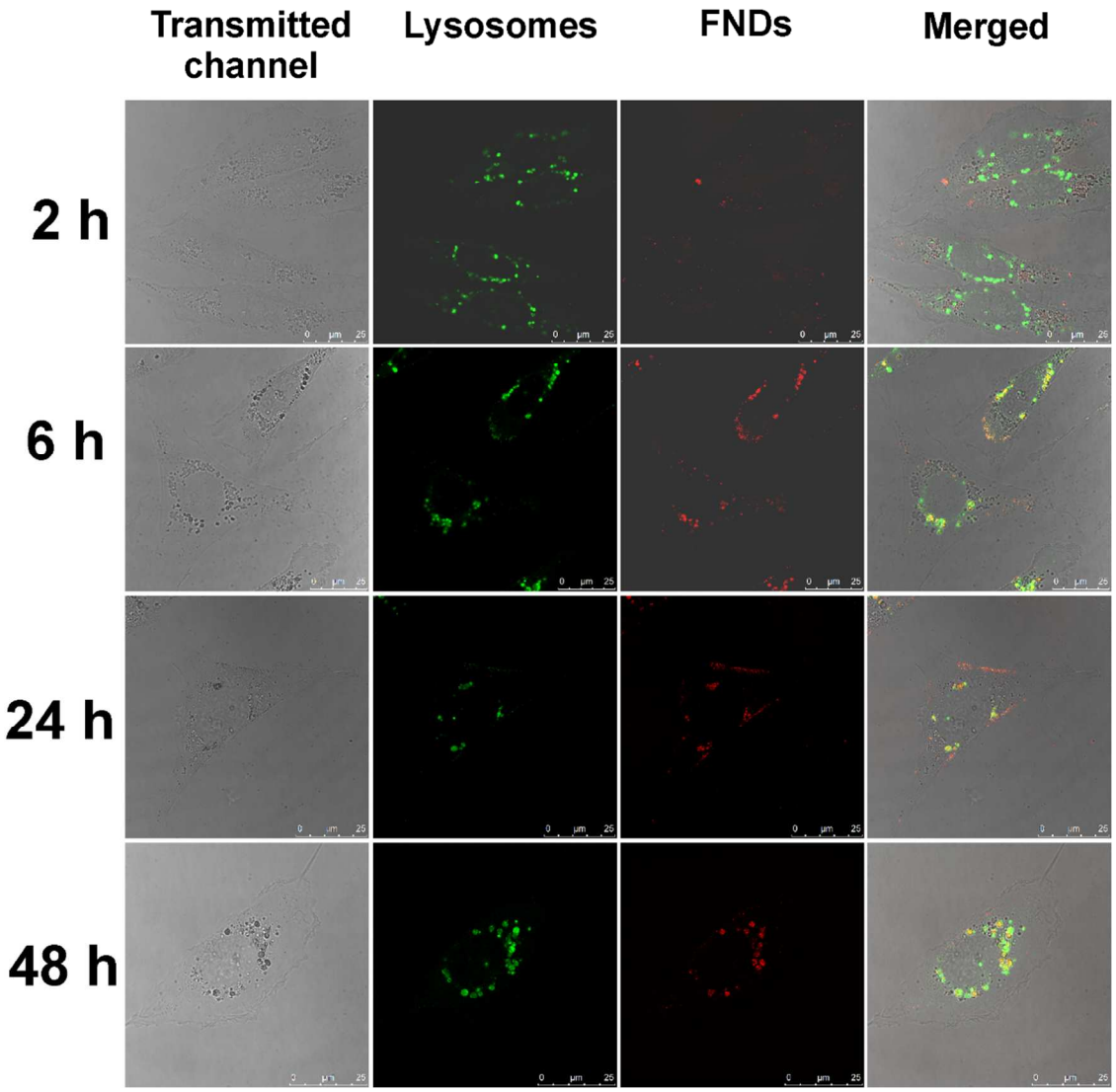

Figure S5. Live cell microscopy of FND localization with lysosomes (2-48h). After 2h internalization, FNDs (red) can be seen mainly outside lysosomes (green). After 6 h, a population of FNDs co-localized with lysosomes. Another population was aggregated but non-confined to lysosomes and some lysosomes were seen without any FNDs. After 24 h, one population of FNDs was aggregated and co-localized with lysosomes, and another distinct, dispersed FND population was observed mainly at the edges of plasma membrane. After 48 h, progressive co-localization of FNDs with lysosomes was observed. A distinct and dispersed population of FNDs was seen localized in proximity of plasma membrane.

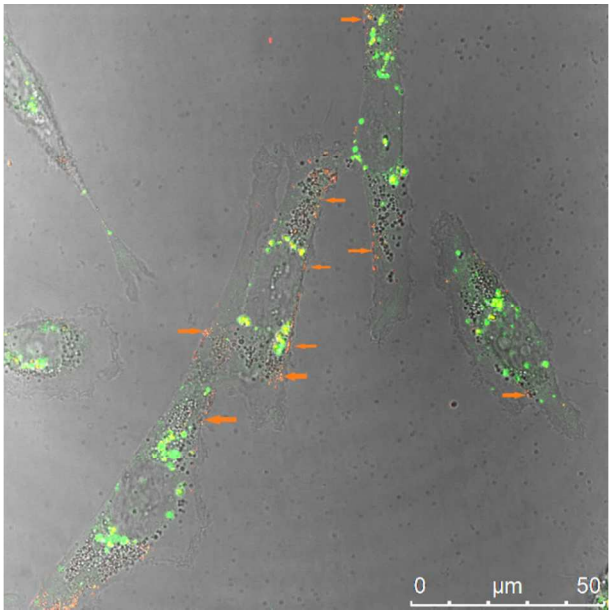

Figure S6. Distinct populations of FNDs were observed at 24h internalization with lysosomal localization as reference. The main observations were FND co-localized with lysosomes, Lysosomes without any FNDs and localization of FNDs on the edges of cell (arrow).

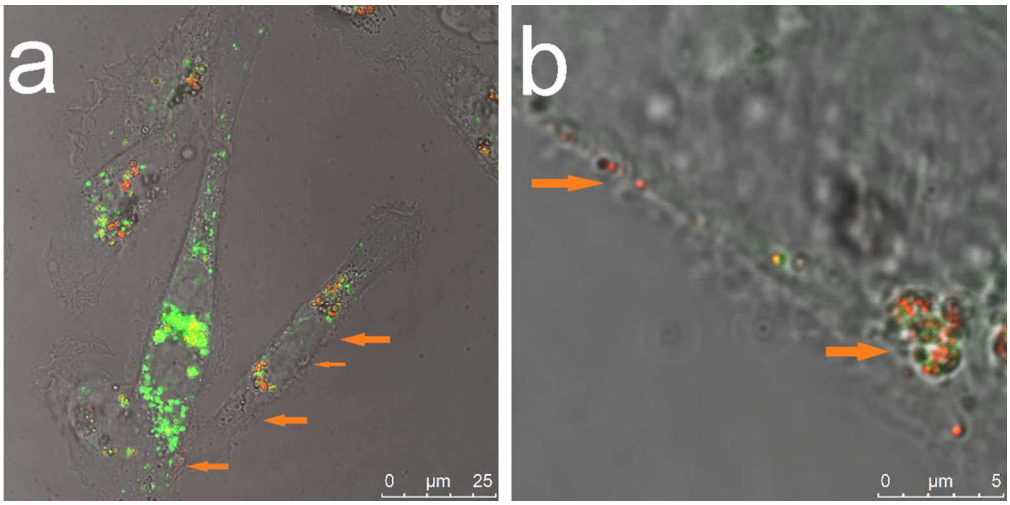

Figure S7. a) Densely packed lysosomes with FNDs. The main observation were FND co-localized with lysosomes, Lysosomes without any FNDs and b) localization of FNDs on the edges of cell (arrow).

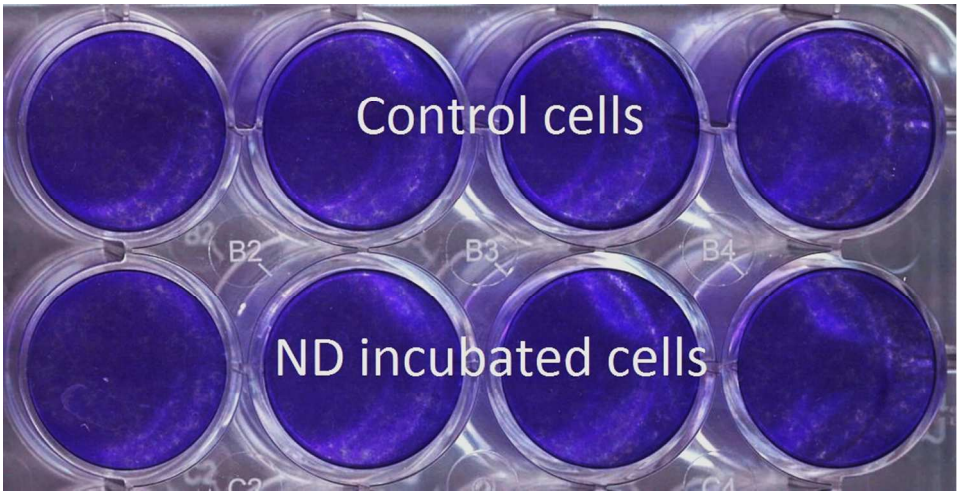

Figure S8. Comparison of 120h colony growth of control cells (no FNDs) with FNDs incubated cells. The results were demonstrated by crystal violet staining for colony growth analysis.

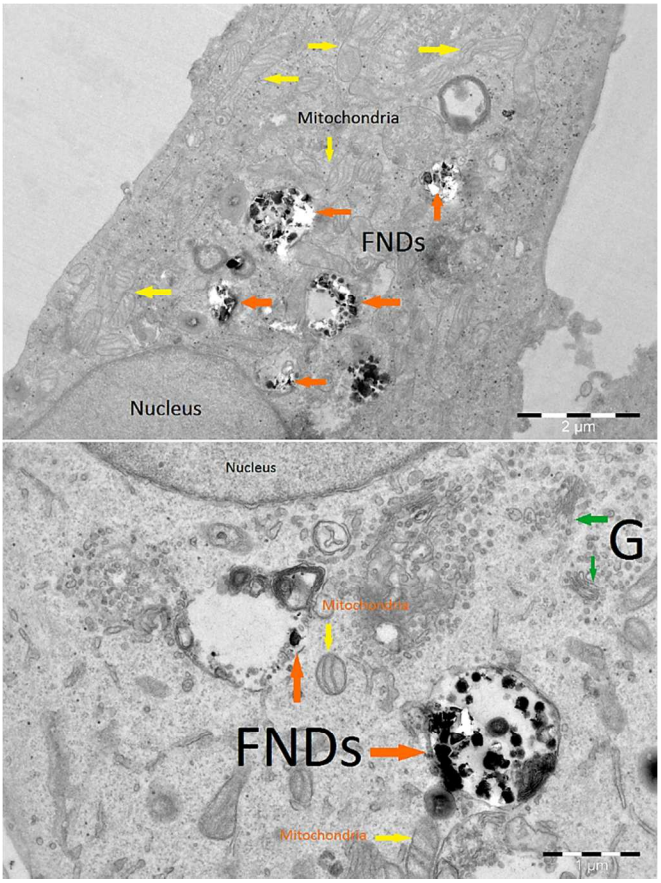

Figure S9. Intracellular localization of FNDs in vesicular space. FNDs (orange arrow) were not observed interacting with nucleus, mitochondria (yellow arrow), Golgi (green arrow).

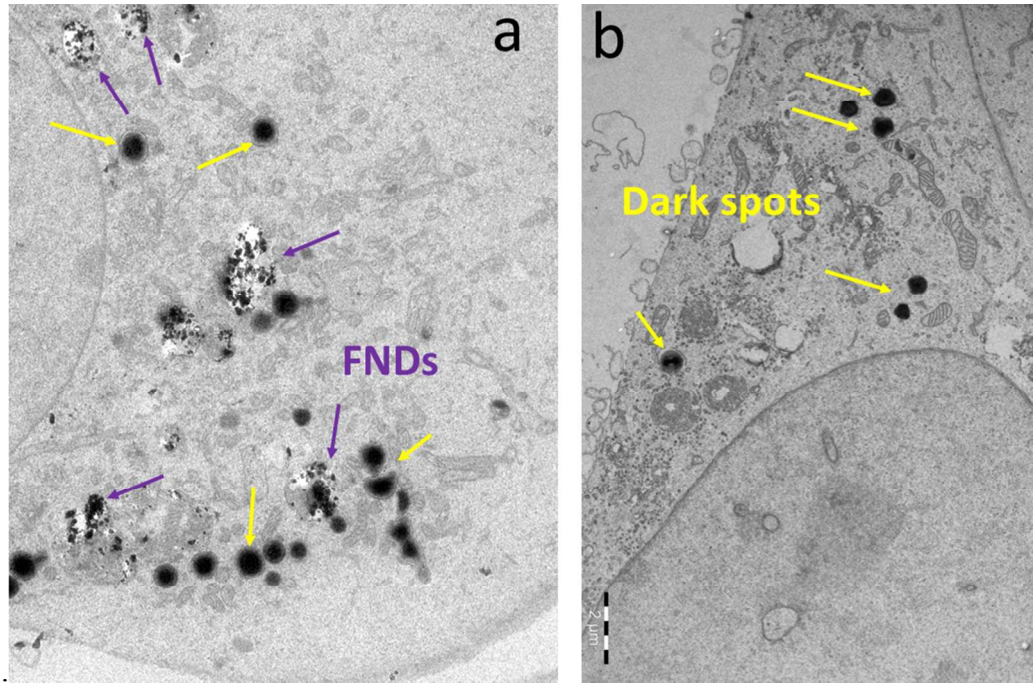

Figure S10. The dark spots present in images have been seen with both FND containing cells and control cells. The darks spots (yellow arrow) are mostly spherical and easily distinguishable from FNDs (purple).