Electronic Supplementary Material (ESI) for Nanoscale Advances.

Supplementary Material

Iron oxide nanoparticles trigger endoplasmic reticulum damage in steatotic hepatic cells

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Table S1. List of chemical probes used in the study.

Reagent	Manufacturer	Catalogue No
PBS	Gibco	10010015
Minimum Essential Medium Eagle	BioConcept	1-31S01-I
Glutamine	Serana Europe	RGL-001-100ML
Triton-X100	PanReac AppliChem	A4975,0100
Micro BCA Protein Assay Kit	Thermo Fisher Scientific	23235
RIPA buffer	Millipore	20188
Protease Inhibitor Cocktail	Sigma Aldrich	P8340-1ML
Phosphatase Inhibitor Cocktail 3	Sigma Aldrich	P0044-1ML
Paraformaldehyde	VWR	100503-917
BSA	Sigma Aldrich	2153
Blotting-Grade Blocker	Bio-Rad	170-6404
Clarity Max ECL Western Blotting Substrate	Bio-Rad	1705062
Ethanol	Sigma Aldrich	1.07017
Fetal Bovine Serum	Gibco	10270-106
Penicilin/Streptomycin	Biosera	XC-A4122/100
Oil Red O	Sigma Aldrich	O0625
Hematoxylin	DiaPath	C0302
Oleic acid	Sigma Aldrich	O1383
Palmitic acid	Sigma Aldrich	P0500
Iron oxide nanoparticles fluidMAG-CMX (200 nm)	Chemicell GmbH	4106-5
NE-PER™ Nuclear and Cytoplasmic Extraction Reagents	Thermo Fisher Scientific	78833
Thapsigargin	Sigma Aldrich	T9033
Camptothecin	Sigma Aldrich	C9911

Table S2. List of fluorescent probes used in the study.

Probe/Kit	Manufacturer	Catalogue No
Hoechst 33342	Thermo Fisher Scientific	62249
alamarBlue™ Cell Viability Reagent	Thermo Fisher Scientific	DAL1025
ROS/Superoxide Detection Assay Kit	Abcam	ab139476
BODIPY™ 581/591 C11 (Lipid Peroxidation Sensor)	Thermo Fisher	D3861

	Scientific	
Acridine Orange	Thermo Fisher Scientific	A3568
LipidSpot™ 610 Lipid Droplet Stains	Biotium	#70069
LipidSpot™ 488 Lipid Droplet Stains	Biotium	#70065
LysoTracker™ Green DND-26	Thermo Fisher Scientific	L7526
LysoTracker™ Red DND-99	Thermo Fisher Scientific	L7528
MitoTracker™ Red CMXRos	Thermo Fisher Scientific	M7512
ER-Tracker™ Red	Thermo Fisher Scientific	E34250
Dead Cell Apoptosis Kit (Annexin V Alexa Fluor 488 and Propidium Iodide)	Thermo Fisher Scientific	V13245
thioflavin T	Abcam	ab120751
JC-1	Thermo Fisher Scientific	T3168

Table S3. List of antibodies used in the study.

Antibody	**Clone/catalogue No**	**Dilution**		**Manufacturer**
		WB	IF	
Anti-NF-kB p65 antibody	ab16502	1:2000	N.A.	Abcam
Anti-HDAC2	2540	1:1000	N.A.	Cell Signaling Technology
Anti-β-actin	10D10	1:1000	N.A.	Thermo Fisher Scientific
Anti-BiP	C50B12	1:1000	N.A.	Cell Signaling Technology
Anti-CHOP	L63F7	1:1000	N.A.	Cell Signaling Technology
Anti- mouse-HRP	1858413	1:10 000	N.A.	Pierce Biotechnology
Anti-rabbit- HRP	1858415	1:10 000	N.A.	Pierce Biotechnology
Anti-LAMP1	D4O1S/15665	N.A.	1:100	Cell Signaling Technology
Anti-Cathepsin B	D1C7Y/31718	N.A.	1:100	Cell Signaling Technology
AlexaFluor 488 goat anti-rabbit antibody IgG	A-11008	N.A.	1:1000	Thermo Fisher Scientific

AlexaFluor 568 goat anti-rabbit IgG	A-11011	N.A.	1:1000	Thermo Fisher Scientific

N.A. – not applicable; WB – western blot; IF – immunofluorescence.

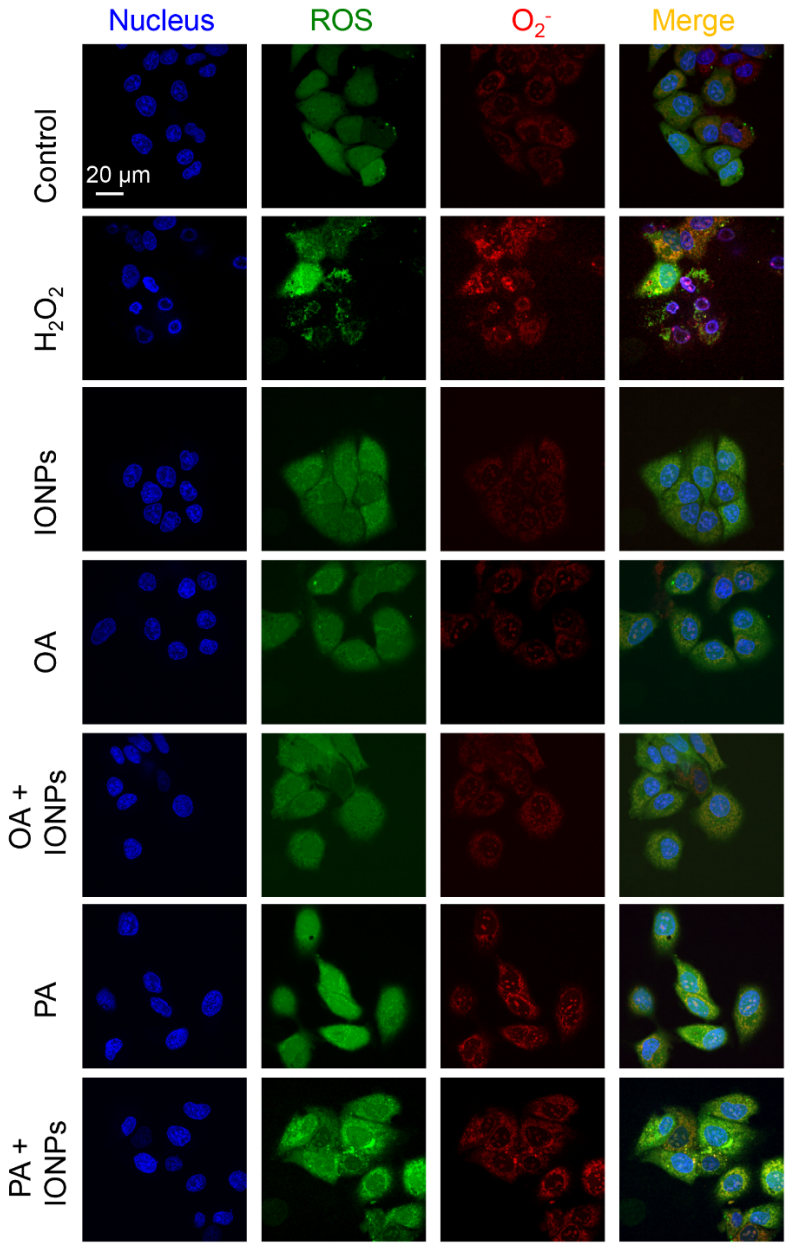


Figure S1. Analysis of ROS induction in hepatic cell lines upon co-treatment with IONPs and fatty acids. Alexander cells were co-treated with IONPs (50 µg/ml) and either OA or PA (20 µM) in accordance with scheme Figure 1b. Treated cells were stained with ROS/Superoxide Detection Assay Kit (Abcam, Cambridge, United Kingdom) and imaged by confocal microscopy. Representative images out of three independent experiments are shown. Positive control 100 µM H_2O_2 for 30 min was used.

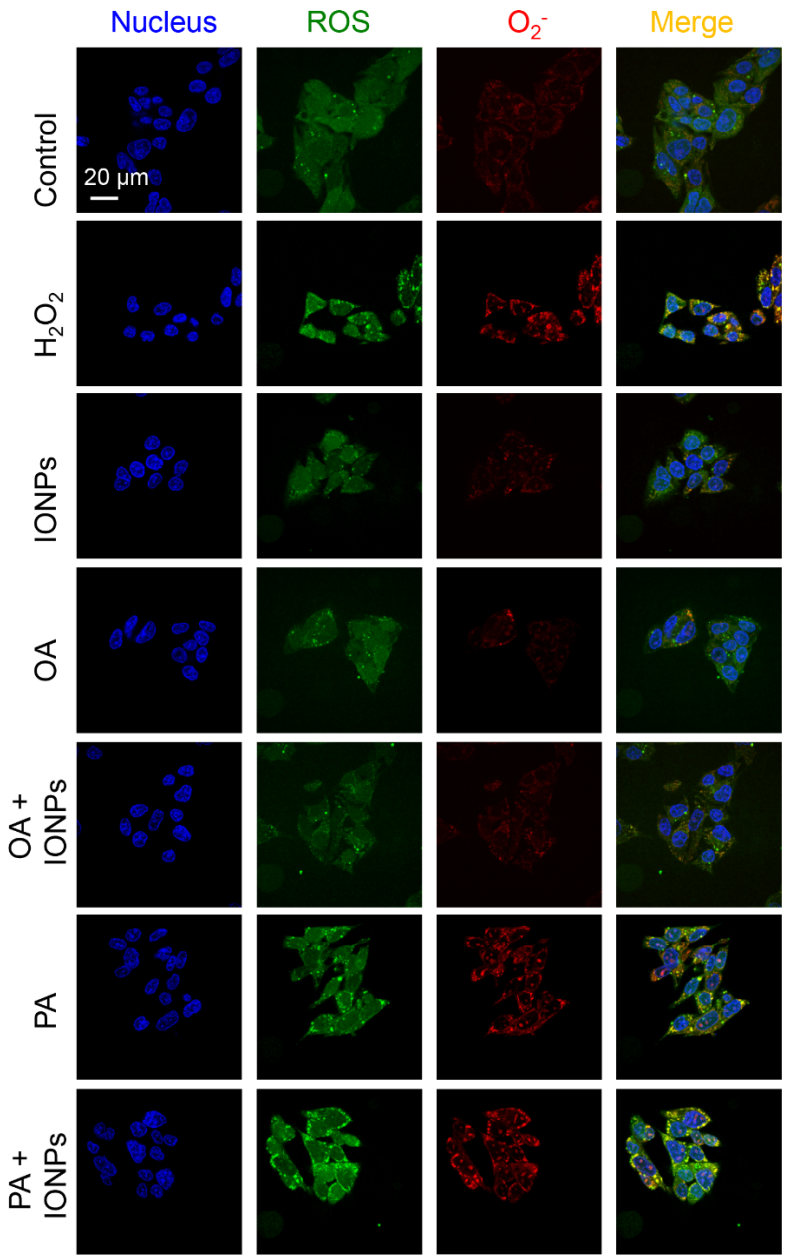


Figure S2. Analysis of ROS induction in hepatic cell lines upon co-treatment with IONPs and fatty acids. HepG2 cells were co-treated with IONPs (50 µg/ml) and either OA or PA (200 µM) in accordance with scheme Figure 1b. Treated cells were stained with ROS/Superoxide Detection Assay Kit (Abcam, Cambridge, United Kingdom) and imaged by confocal microscopy. Representative images out of three independent experiments are shown. Positive control 100 µM H_2O_2 for 30 min was used.

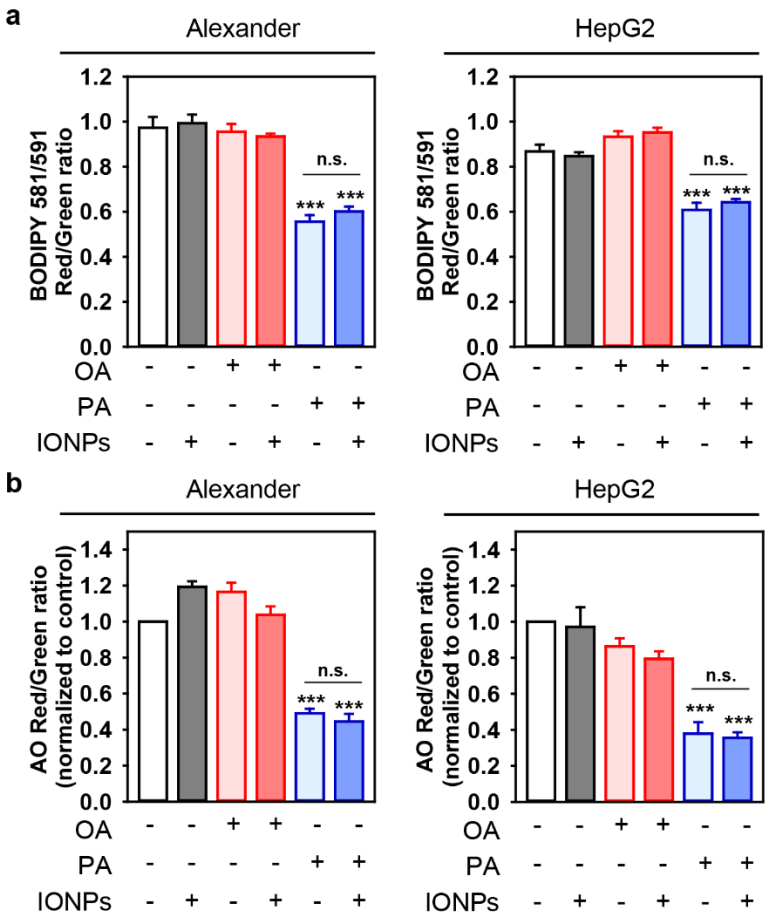


Figure S3. Analysis of lipid peroxidation (a) and lysosomal integrity (b) in hepatic cell lines upon co-treatment with IONPs and fatty acids. Alexander or HepG2 cells were co-treated with IONPs (50 µg/ml) and either OA or PA (20 µM for Alexander and 200 µM for HepG2 cells) in accordance with scheme Figure 1b. (a) Detection of lipid peroxidation using BODIPY™ 581/591 C11 lipid peroxidation sensor (ThermoFisher Scientific). Quantification of the lipid peroxidation ratio (red/green) was measured using a Tecan microplate reader SpectraFluor Plus (Tecan, Männedorf, Switzerland). Data are expressed as means ± SEM (n = 3). (***) $P < 0.001$ denotes significant differences. (b) Lysosomal integrity as measured by acridine orange (AO) red/green ratio fluorescence decrease. Cells were treated in accordance with scheme Figure 1b. stained with AO and then red and green AO fluorescence intensities were measured using a fluorescent microplate

reader. The data are expressed as mean ± SEM, n = 3 each. (***) $P < 0.001$ denotes significant differences.

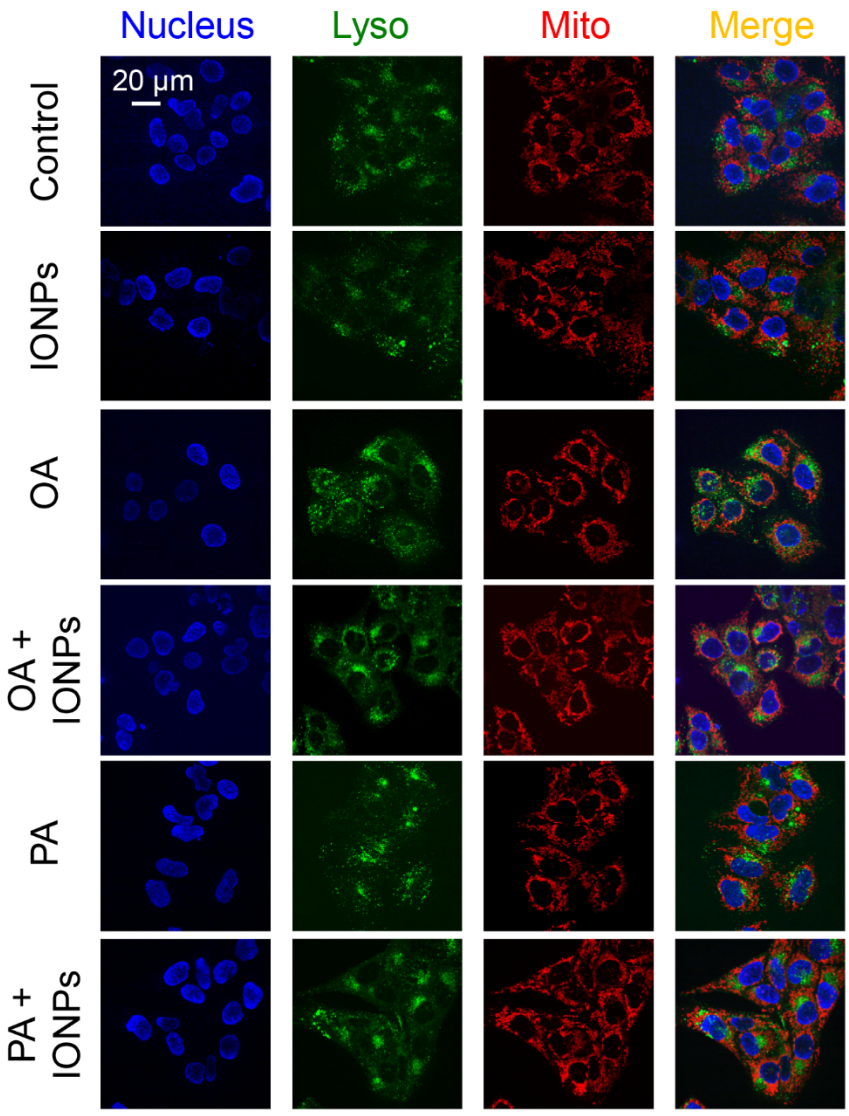


Figure S4. Effect of co-treatment with IONPs and fatty acids on morphology of lysosomes and mitochondria in Alexander cells. (a) Alexander cells were co-treated with IONPs (50 µg/ml) and either OA or PA (20 µM) in accordance with scheme Figure 1b. Cells were labeled with LysoTracker™ Green DND-26 (green) and MitoTracker™ Red CMXRos (red). Nuclei were

stained with hoechst 33342 nuclear stain (blue). Labeled cells were then imaged using spinning disk confocal microscopy.

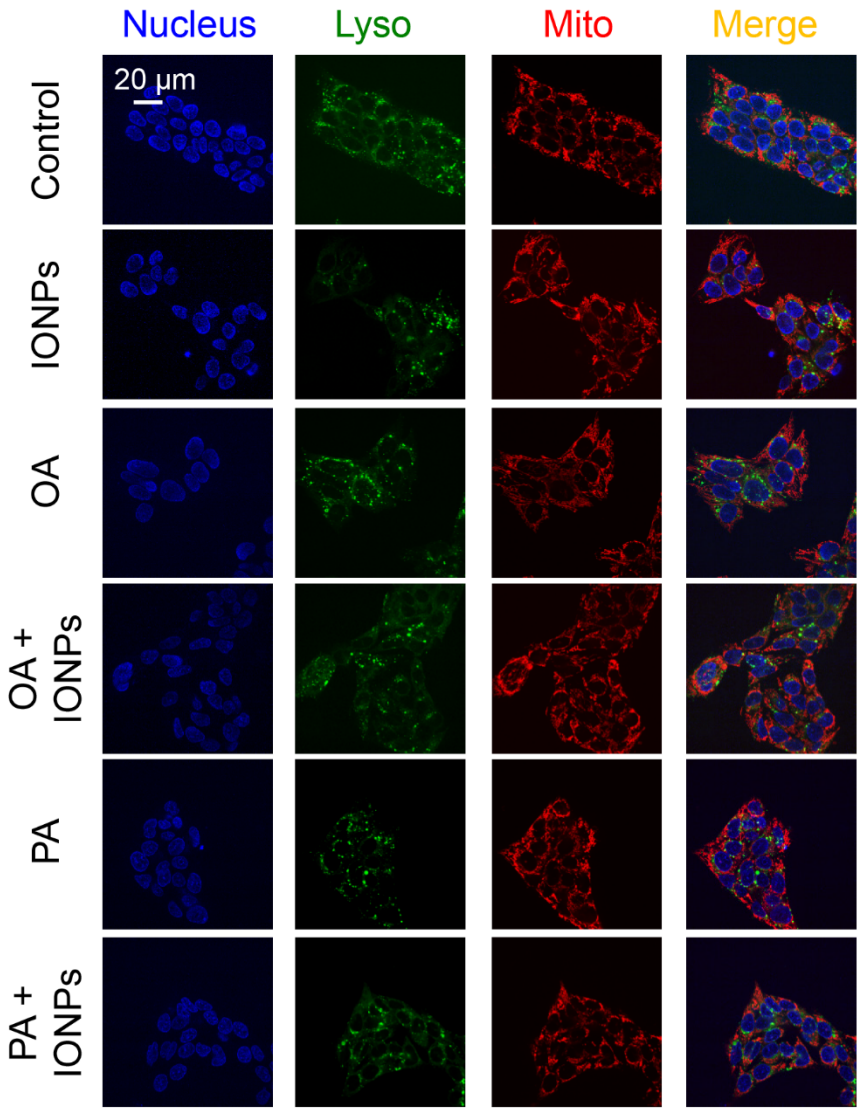


Figure S5. Effect of co-treatment with IONPs and fatty acids on morphology of lysosomes and mitochondria in HepG2 cells. (a) HepG2 cells were co-treated with IONPs (50 µg/ml) and either OA or PA (200 µM) in accordance with scheme Figure 1b. Cells were labeled with LysoTracker™ Green DND-26 (green) and MitoTracker™ Red CMXRos (red). Nuclei were stained with hoechst

33342 nuclear stain (blue). Labeled cells were then imaged using spinning disk confocal microscopy.

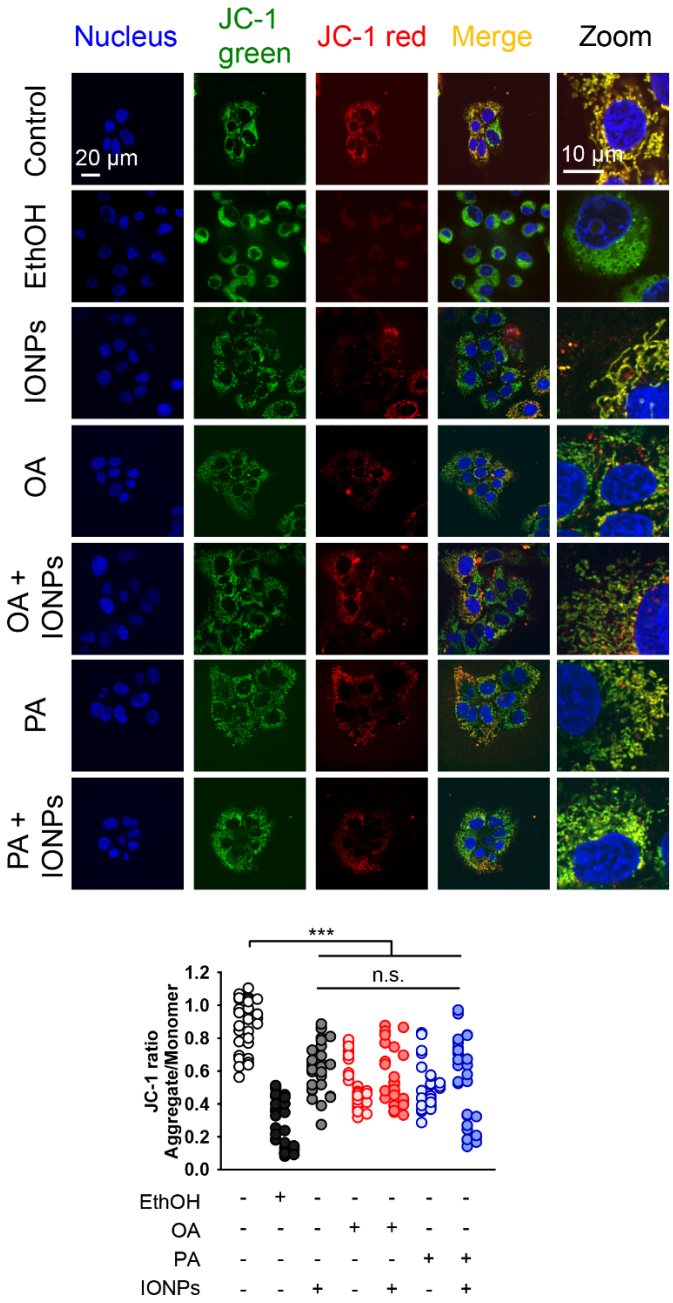
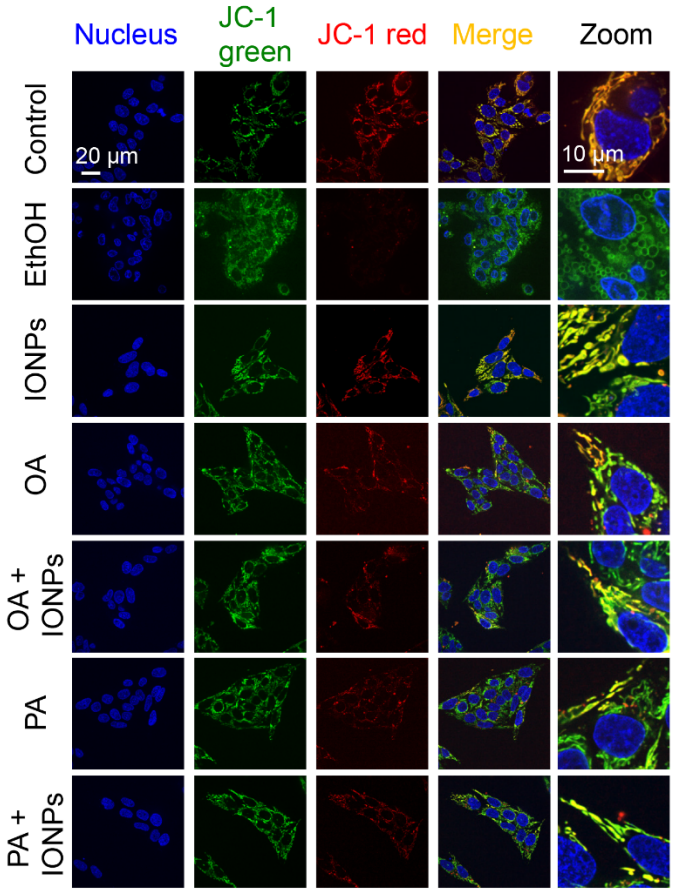


Figure S6. Assessment of mitochondrial membrane depolarization upon co-treatment with IONPs and fatty acids on morphology of lysosomes and mitochondria in Alexander cells. Alexander cells were co-treated with IONPs (50 µg/ml) and either OA or PA (20 µM) in accordance with scheme

Figure 1b. Cells were stained with JC-1 (1 µM) and then imaged using spinning disk confocal microscopy. Nuclei were stained with hoechst 33342 nuclear stain (blue). Positive control – 20 % ethanol for 30 min. Confocal images were quantified using ImageJ software. Red/green JC-1 ratio was measured. Quantifications are presented as means of n = 30 cells. (***) $P < 0.001$ denotes significant differences.



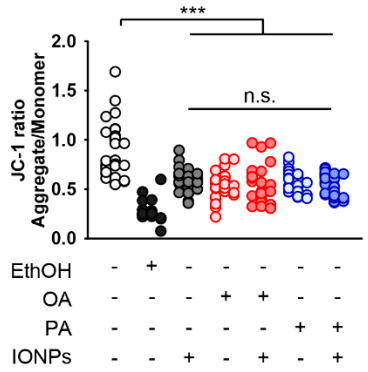


Figure S7. Assessment of mitochondrial membrane depolarization upon co-treatment with IONPs and fatty acids on morphology of lysosomes and mitochondria in HepG2 cells. HepG2 cells were co-treated with IONPs (50 µg/ml) and either OA or PA (200 µM) in accordance with scheme Figure 1b. Cells were stained with JC-1 (1 µM) and then imaged using spinning disk confocal microscopy. Nuclei were stained with hoechst 33342 nuclear stain (blue). Positive control – 20 % ethanol for 30 min. Confocal images were quantified using ImageJ software. Red/green JC-1 ratio was measured. Quantifications are presented as means of n = 30 cells. (***) $P < 0.001$ denotes significant differences.

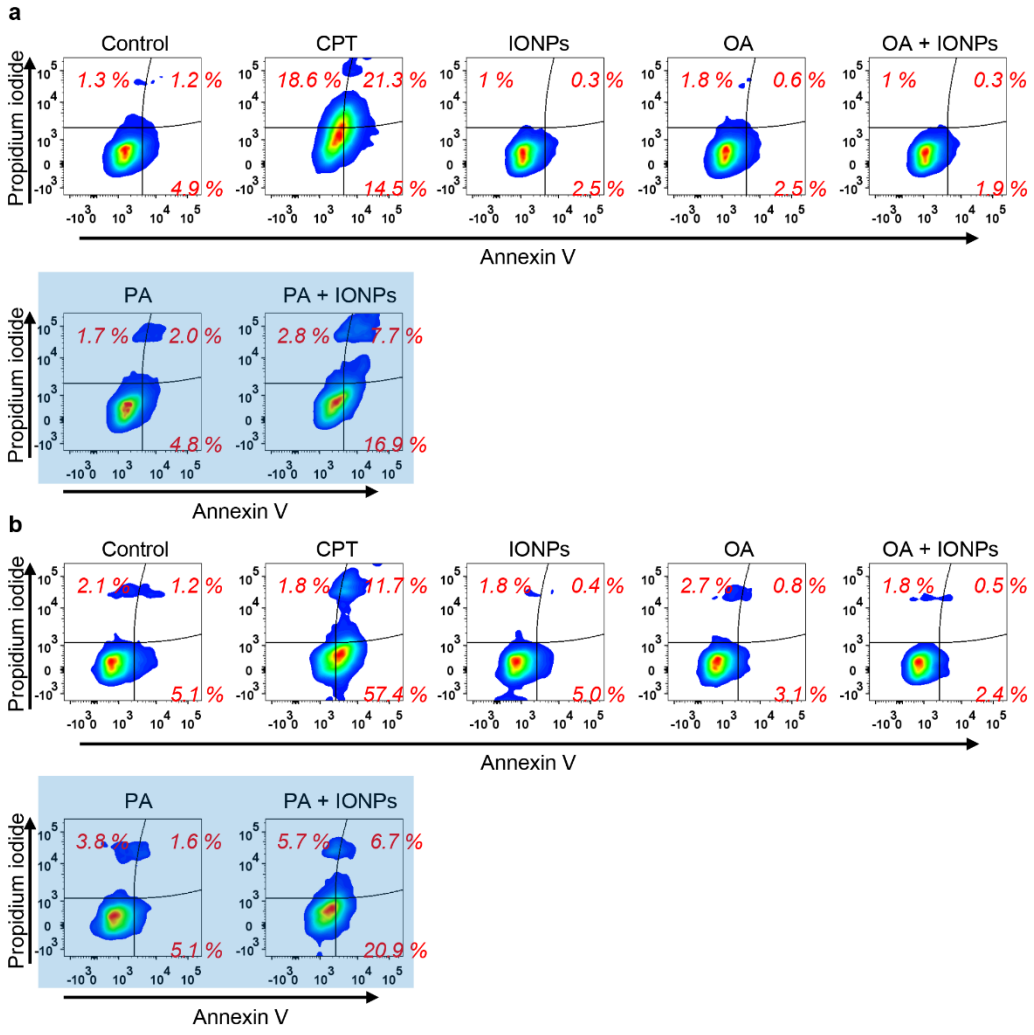


Figure S8. Alexander (a) or HepG2 (b) cells were co-treated with IONPs (50 µg/ml) and either OA or PA (20 µM for Alexander and 200 µM for HepG2 cells) in accordance with scheme Figure 1b. After treatment cells were labelled with annexin V, and propidium iodide (PI). Labelled cells were analyzed by flow cytometry. As a positive control, cells were treated with camptothecin (CPT) 30 µM for 24 h. Representative flow charts out of three independent experiments are shown.

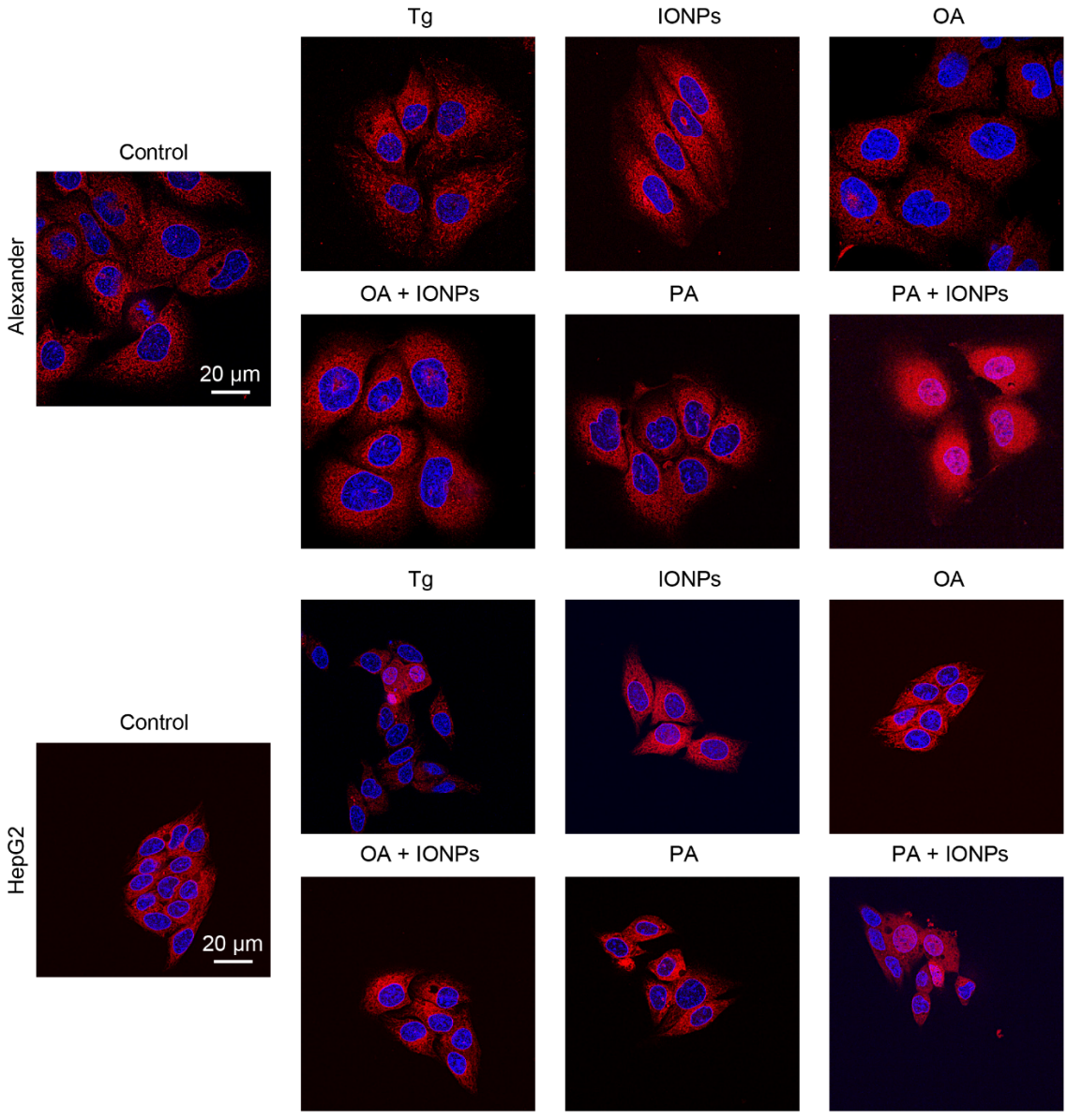


Figure S9. Cells were stained with ER-Tracker Red and visualized by confocal microscopy. Nuclei were stained with hoechst 33342 nuclear stain (blue). Positive control – treatment with s

thapsigargin (Tg) 1 µM for 12 h. Stained cells were imaged using spinning disk confocal microscopy IXplore SpinSR (Olympus, Tokyo, Japan). Images were deconvolved using CellSens software (Olympus, Japan).

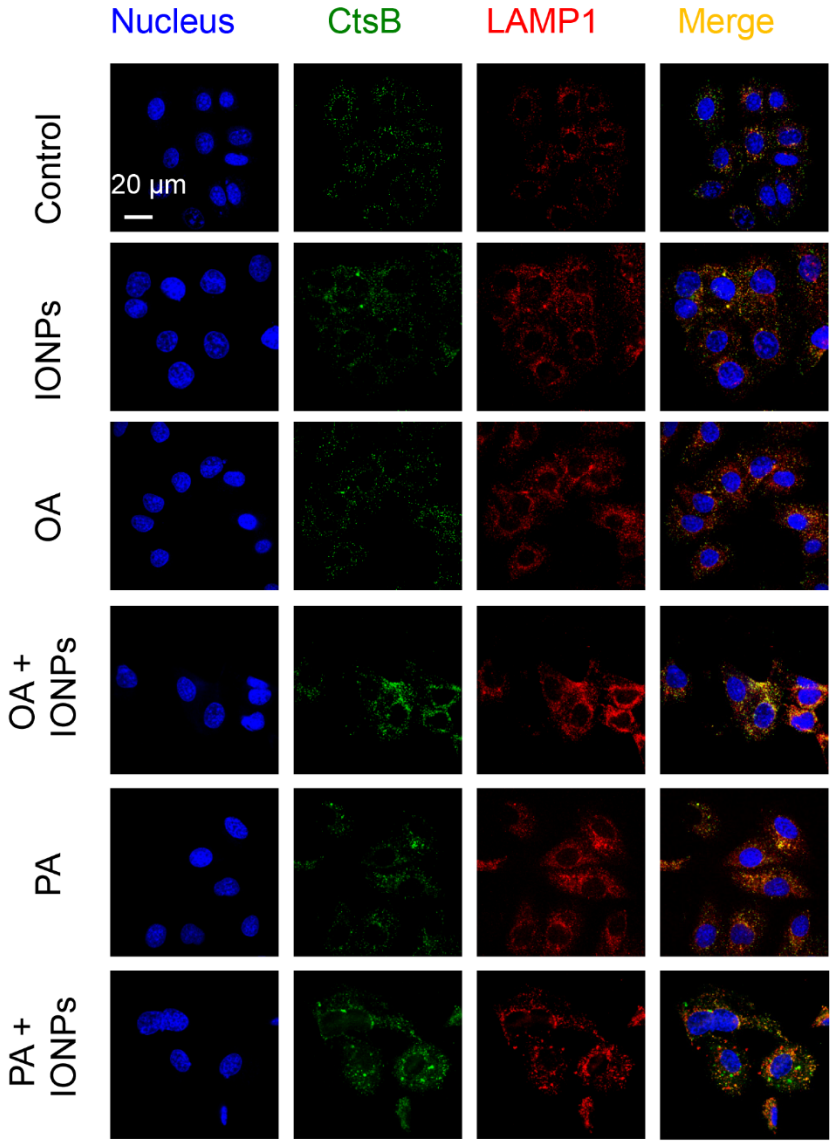


Figure S10. Alexander cells were co-treated with IONPs (50 µg/ml) and either OA or PA (20 µM) in accordance with scheme Figure 1b. After cells were fixed, permeabilized and immunostained with anti-CtsB (green) and anti-LAMP1 (red) antibodies. Nuclei were stained with hoechst 33342

nuclear stain (blue). Stained cells were imaged using spinning disk confocal microscopy IXplore SpinSR (Olympus, Tokyo, Japan).

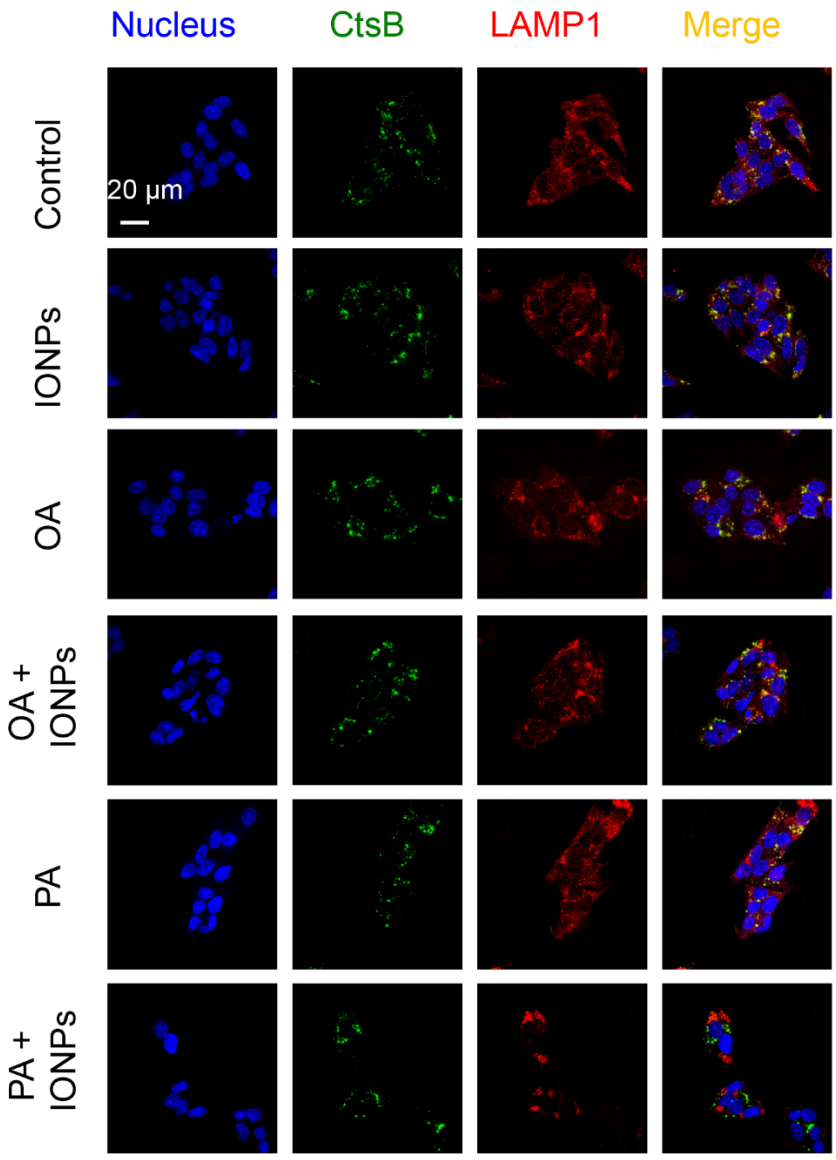


Figure S11. HepG2 cells were co-treated with IONPs (50 µg/ml) and either OA or PA (200 µM) in accordance with scheme Figure 1b. After cells were fixed, permeabilized and immunostained with anti-CtsB (green) and anti-LAMP1 (red) antibodies. Nuclei were stained with hoechst 33342 nuclear stain (blue). Stained cells were imaged using spinning disk confocal microscopy IXplore SpinSR (Olympus, Tokyo, Japan).

Uncropped immunoblot scans

Figure 6B

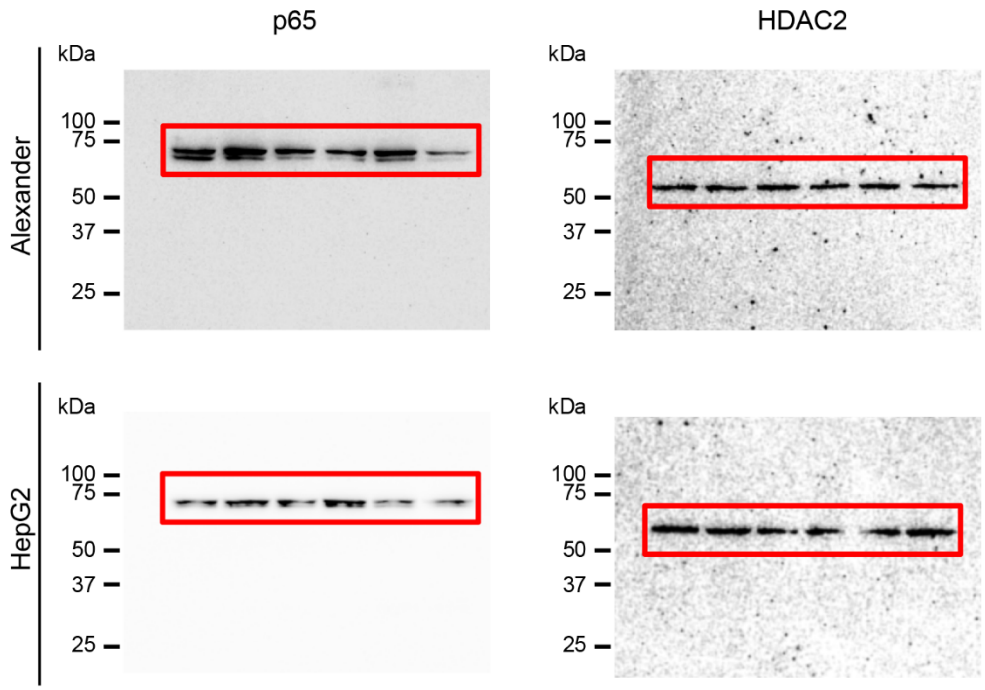


Figure 6C

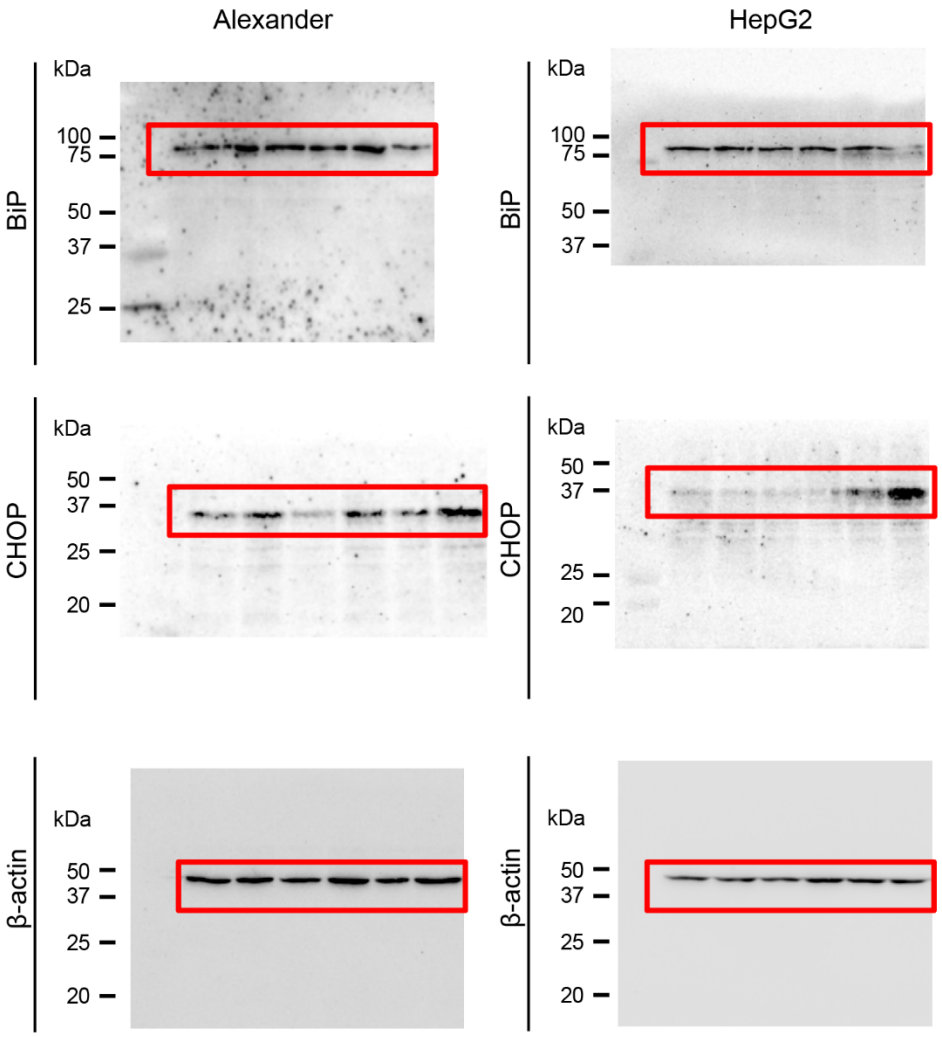


Figure 6D