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## Internalization and toxicological mechanisms of uncoated and PVP-coated cerium oxide nanoparticles in the freshwater alga *Chlamydomonas reinhardtii*

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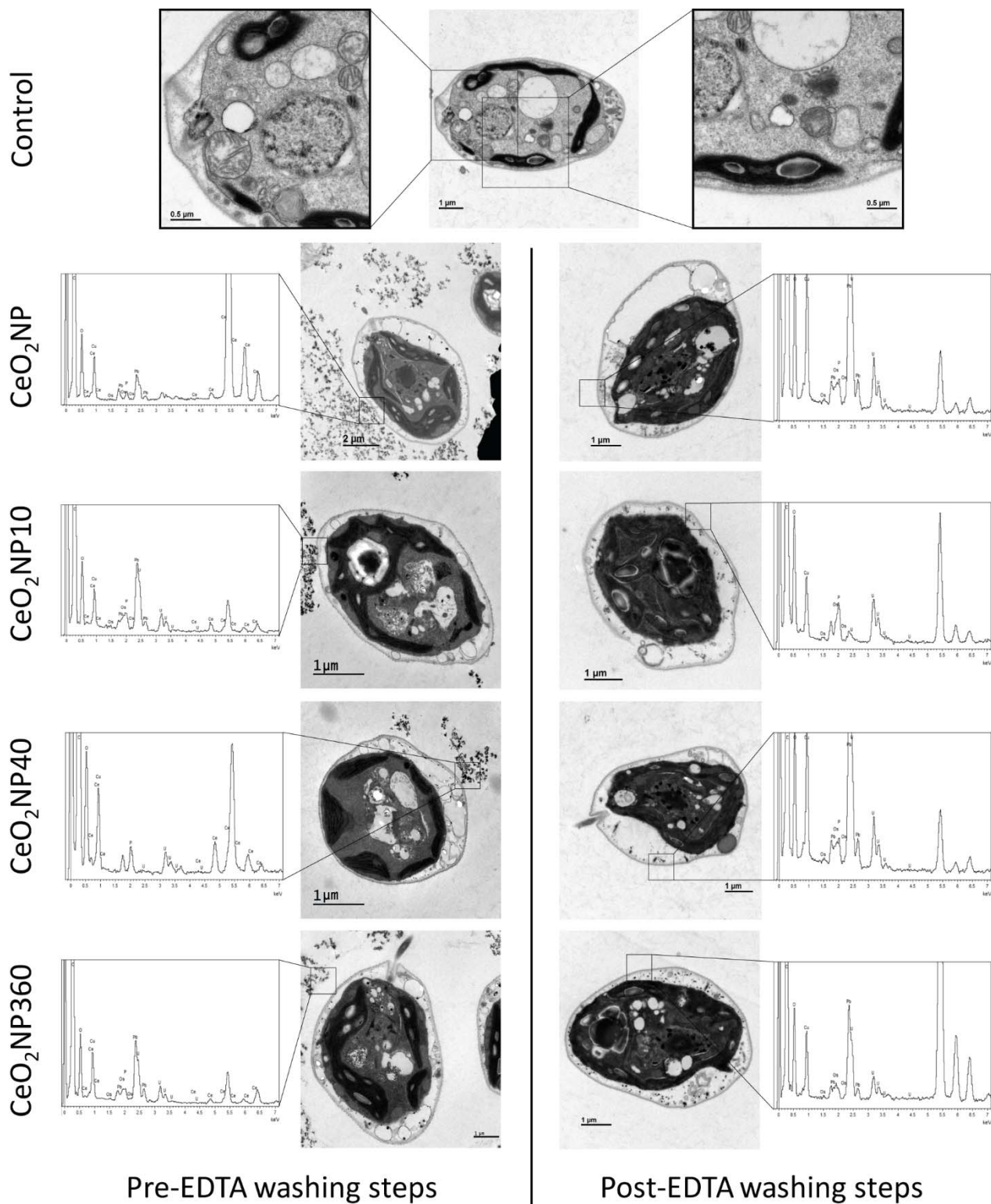
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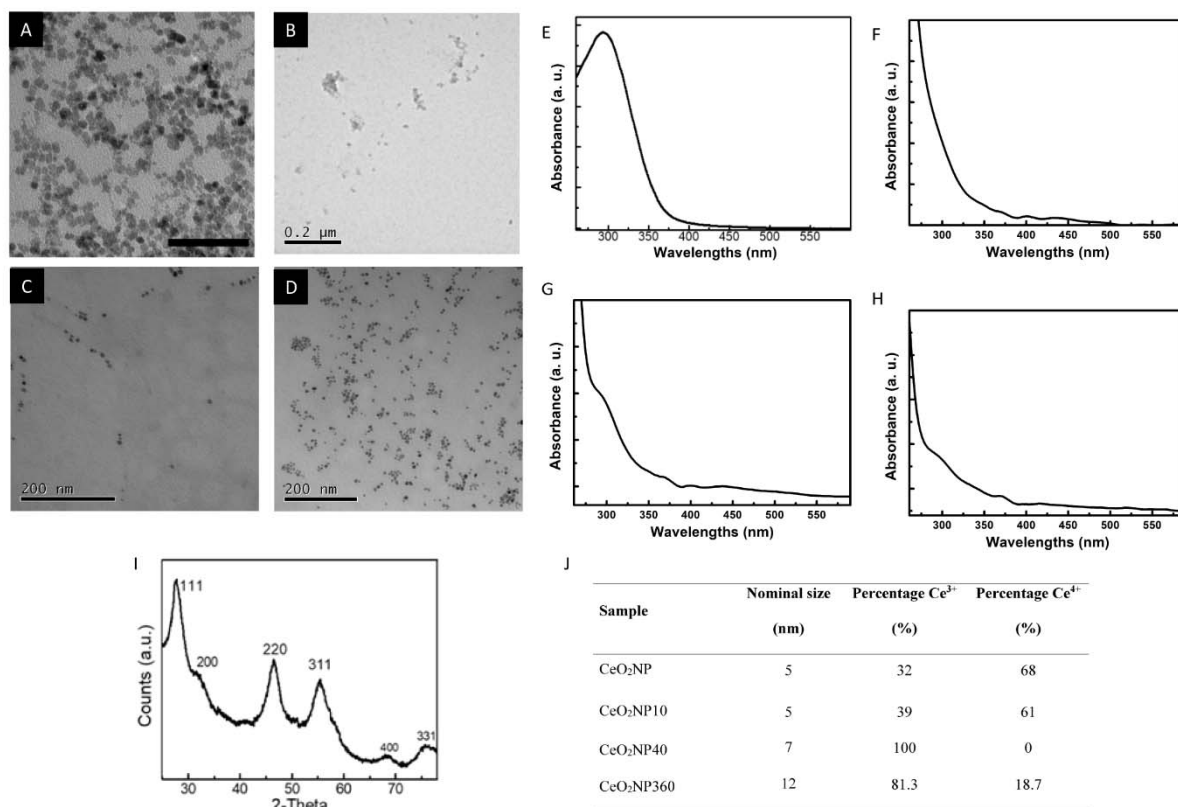
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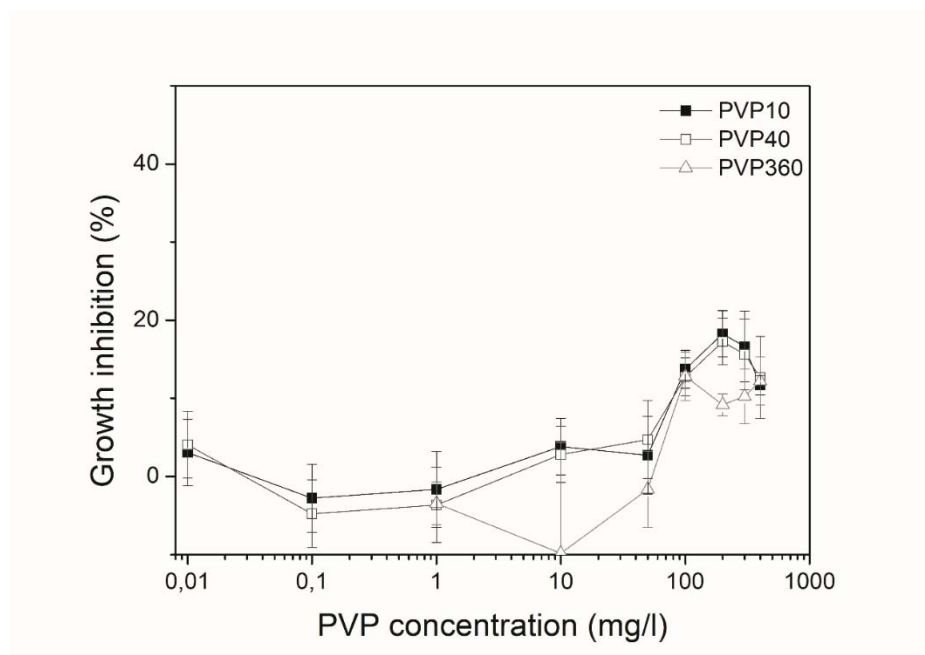
## Electronic Supplementary Information (ESI)



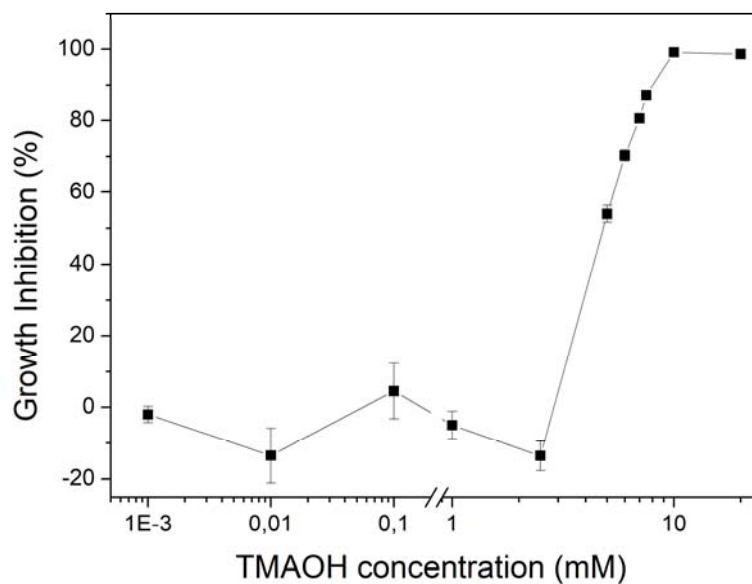
**Figure S1.** TEM images and EDX spectra of *C. reinhardtii* cells before (left column) and after (right column) the application of EDTA washing steps.



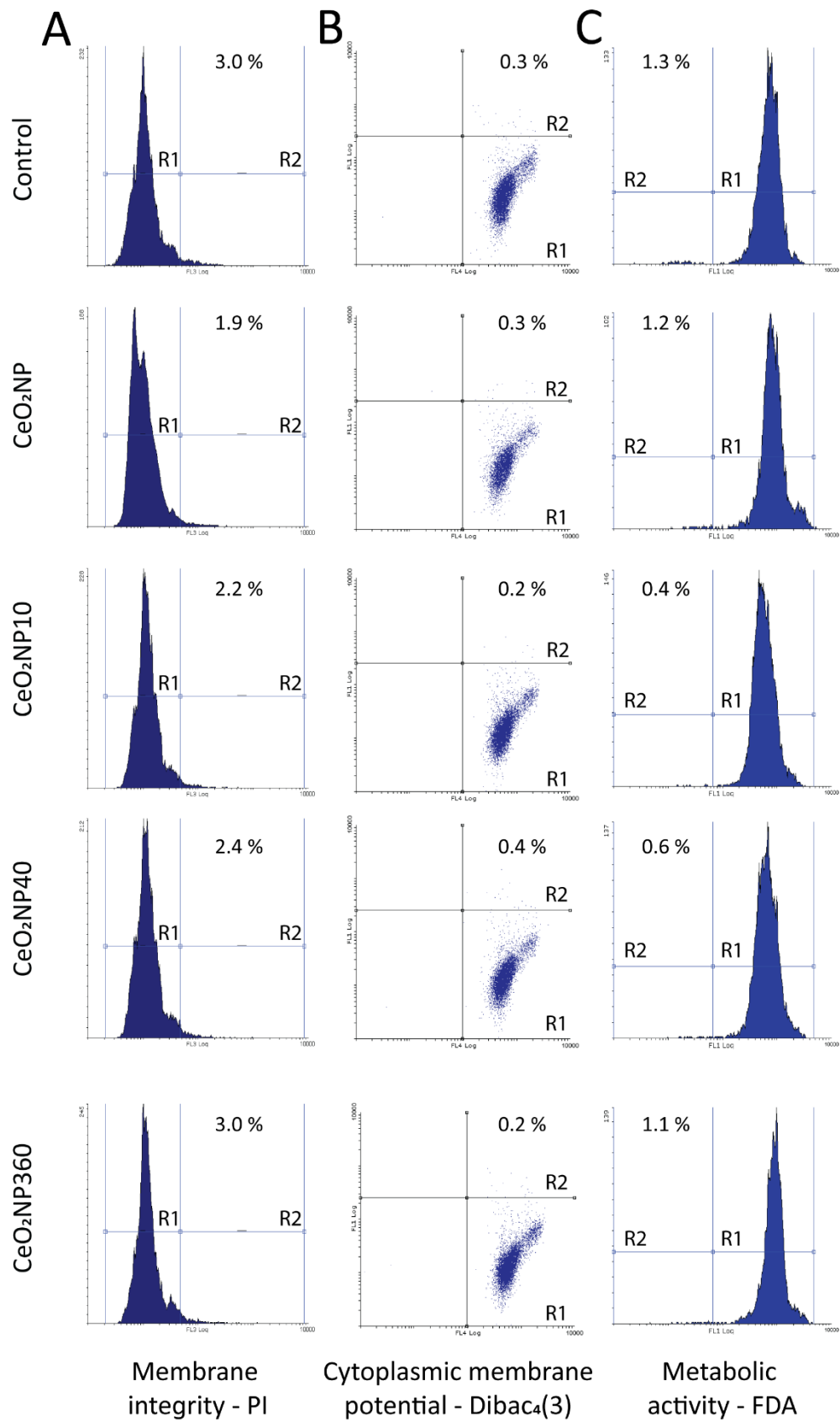
**Figure S2.** Characterization of the non-coated and coated CeO<sub>2</sub>NPs used in this work. TEM image of CeO<sub>2</sub>NP (A – scale bar: 50 nm), CeO<sub>2</sub>NP10 (B), CeO<sub>2</sub>NP40 (C) and CeO<sub>2</sub>NP360 (D). UV-vis absorbance spectrum of CeO<sub>2</sub>NP, CeO<sub>2</sub>NP10, CeO<sub>2</sub>NP40 and CeO<sub>2</sub>NP360 is shown in E, F, G and H, respectively. XRD spectra of the CeO<sub>2</sub>NP showing the characteristics peaks of CeO<sub>2</sub> crystals is shown in I (there was no good XRD data for PVP-coated nanoparticles due to the external presence of PVP as capping agent). Section J shows the nominal size and percentage of surface Ce<sup>3+</sup>/Ce<sup>4+</sup> of CeO<sub>2</sub>NPs calculated by X-Ray photoelectron spectroscopy. TEM and UV-Vis spectra of PVP-coated CeO<sub>2</sub>NPs are reproduced from Briffa *et al* (2017) with permission from the Royal Society of Chemistry. Additional characterization of these NPs can be also found in the cited reference.



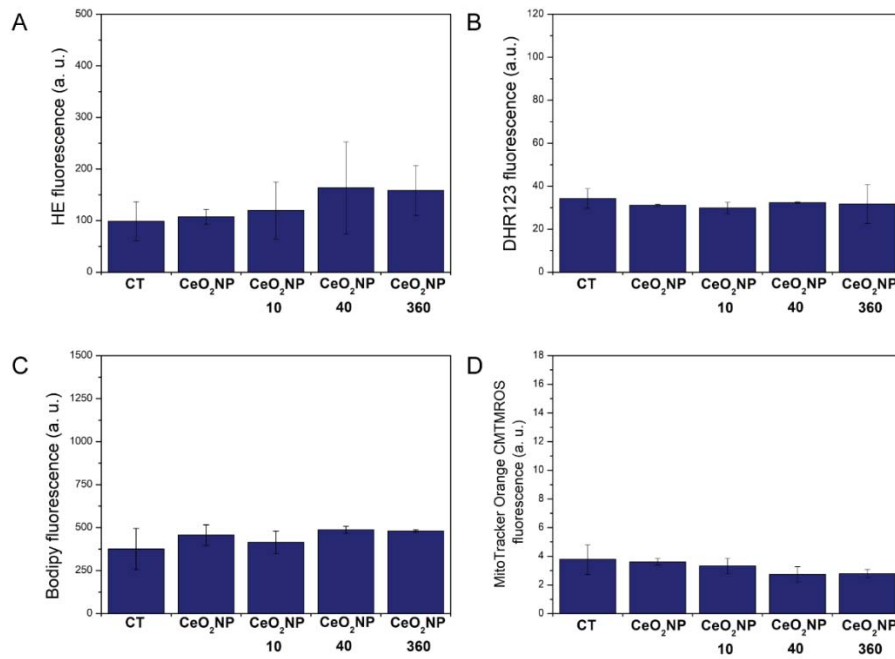
**Figure S3.** The biological effect of 72 h exposure to the three PVP used to synthesize the different CeO<sub>2</sub>NPs on the growth of *C. reinhardtii*. Data are expressed as percentages of the value of untreated cells (mean ± standard deviation).



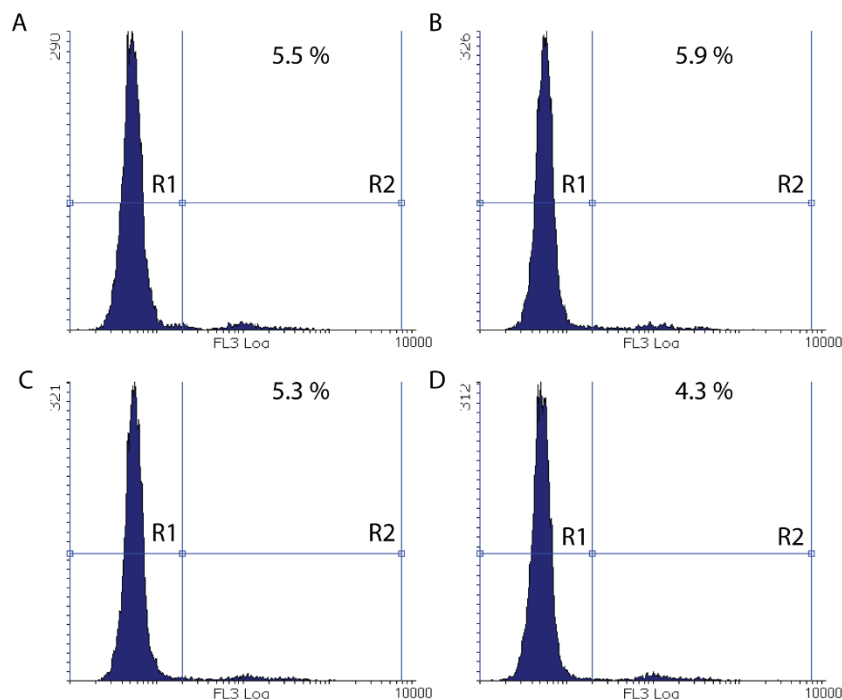
**Figure S4.** The biological effect of 72 h exposure to TMAOH used to stabilize the uncoated CeO<sub>2</sub>NPs on the growth of *C. reinhardtii*. Data are expressed as percentages of the value of untreated cells (mean ± standard deviation).



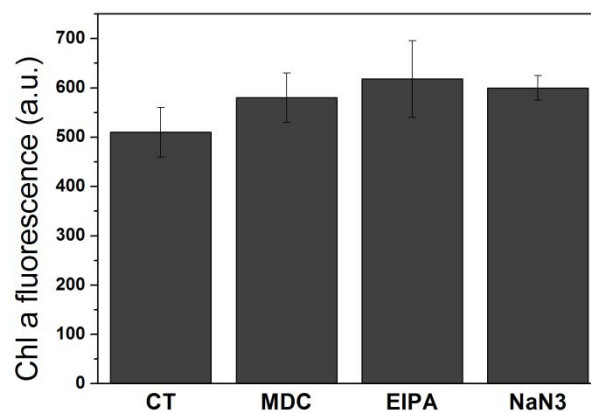
**Figure S5.** The biological effect of 0.1 mg/L of CeO<sub>2</sub>NP, CeO<sub>2</sub>NP10, CeO<sub>2</sub>NP40 and CeO<sub>2</sub>NP360 on cell membrane integrity (A), cytoplasmic membrane potential (B) and metabolic activity (C) of *C. reinhardtii*.



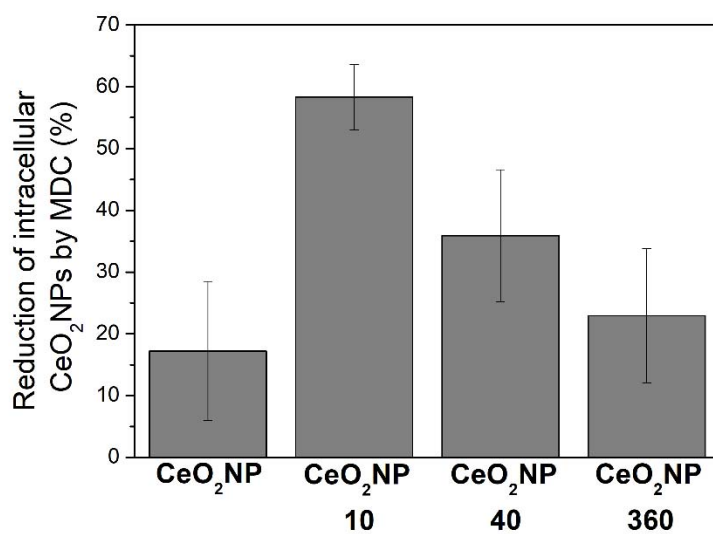
**Figure S6.** Effect of 0.1 mg/L of CeO<sub>2</sub>NPs on intracellular superoxide anion and hydrogen peroxide levels of *C. reinhardtii* by FCM using the fluorochrome HE (A) and DHR123 (B), respectively. Alterations derived of oxidative stress in mitochondria and intracellular lipid peroxidation are also shown in (C) and (D), respectively.



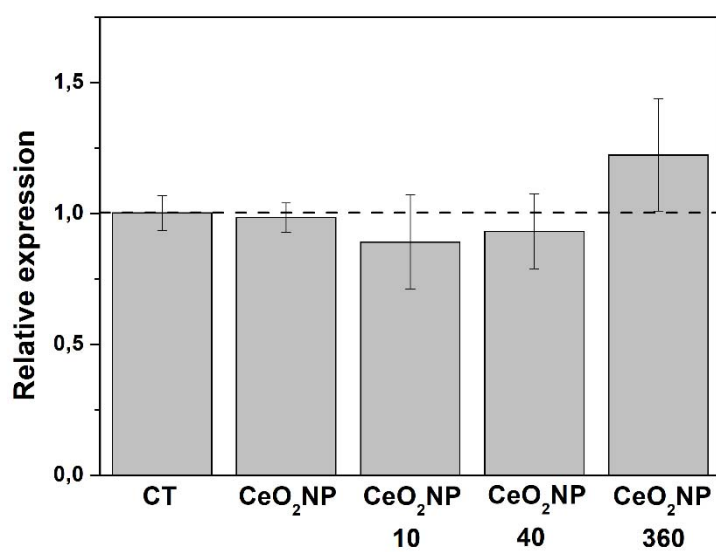
**Figure S7.** The effect of TMAOH (compound used to stabilize uncoated CeO<sub>2</sub>NPs) at 0.1 mM (B), 1 mM (C) and 2.5 mM (D) on the cell membrane integrity of *C. reinhardtii*. A: control cells without TMAOH.



**Figure S8.** The effect of the different endocytic inhibitors towards chlorophyll a fluorescence of *C. reinhardtii*. CT: control. MDC: monodansylcadaverine. EIPA: 5-(N-ethyl-N-isopropyl)-amiloride. NaN<sub>3</sub>: Sodium azide.



**Figure S9.** The level of reduction of intracellular CeO<sub>2</sub>NPs after the treatment with the MDC inhibitor. Data are expressed as percentage of reduction in comparison with CNPs samples without inhibitor (mean ± standard deviation).



**Figure S10.** Effect of CeO<sub>2</sub>NPs on expression of *CHC1* gene after 4 h of exposure. Data are represented as relative expression of the genes with respect to the unexposed control. Control values were set to 1 for easy comparison.



**Table S1:** Fluorochromes used to analyze several physiological parameters of *C. reinhardtii* by flow cytometry.

<b>Fluorochrome</b>	<b>Acronym</b>	<b>Applications</b>	<b>Stock concentration (mg mL<sup>-1</sup>)</b>	<b>Final concentration (µg mL<sup>-1</sup>)</b>	<b>Incubation time (min)</b>
Dihydrorhodamine123	<b>DHR 123</b>	Intracellular levels of hydrogen peroxide	2	10	40
Hydroethidine	<b>HE</b>	Intracellular levels of superoxide anion	3.154	5	30
Propidium iodide	<b>IP</b>	Membrane integrity	1	5	10
Fluorescein Diacetate	<b>FDA</b>	Unspecific esterase activity	5	2.5	15
bis-(1,3-dibutylbarbituric acid) trimethine oxonol	<b>DiBAC<sub>4</sub>(3)</b>	Cytoplasmic membrane potential	0.5	2.5	10
5-Butyl-4,4-Difluoro-4-Bora-3a,4a-Diaza-s-Indacene-3-Nonanoic Acid	<b>BODIPY- C4-C9</b>	Lipid peroxidation	101.08	0.01	10
MitoTracker® Orange CM-H2TMRos	<b>Mitotracker</b>	Mitochondrial ROS homeostasis	0.05	2.5	60

## References

Briffa, S. M., Lynch, I., Trouillet, V., Bruns, M., Hapiuk, D., Liu, J., ... & Valsami-Jones, E. (2017). Development of scalable and versatile nanomaterial libraries for nanosafety studies: polyvinylpyrrolidone (PVP) capped metal oxide nanoparticles. *RSC Advances*, 7(7), 3894-3906.