

—Supporting Information—

## **Uptake of Polystyrene Nanospheres by Wheat and Arabidopsis Roots in Agar, Hydroponics, and Soil**

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Number of pages: 14

Number of tables: 3

Number of figures: 10

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**Table S1.** Confocal microscope settings used for imaging of plant roots.

<b>Microscope parameter</b>	<b>Setting</b>
Confocal microscope	TCS SP8 Leica Microsystems
Laser	White light laser (WLL)
Excitation wavelength (spheres)	488 nm
Emission wavelength (spheres)	500–550 nm
Excitation wavelength (PI)	561 nm
Emission wavelength (PI)	597–776 nm
Laser intensity (spheres)	3%
Laser intensity (PI)	30–60%
z-stack increments (wheat)	150–200 $\mu\text{m}$
z-stacks increments (Arabidopsis)	40–60 $\mu\text{m}$
Magnification (wheat)	10x
Magnification (Arabidopsis)	40x

PI: propidium iodide

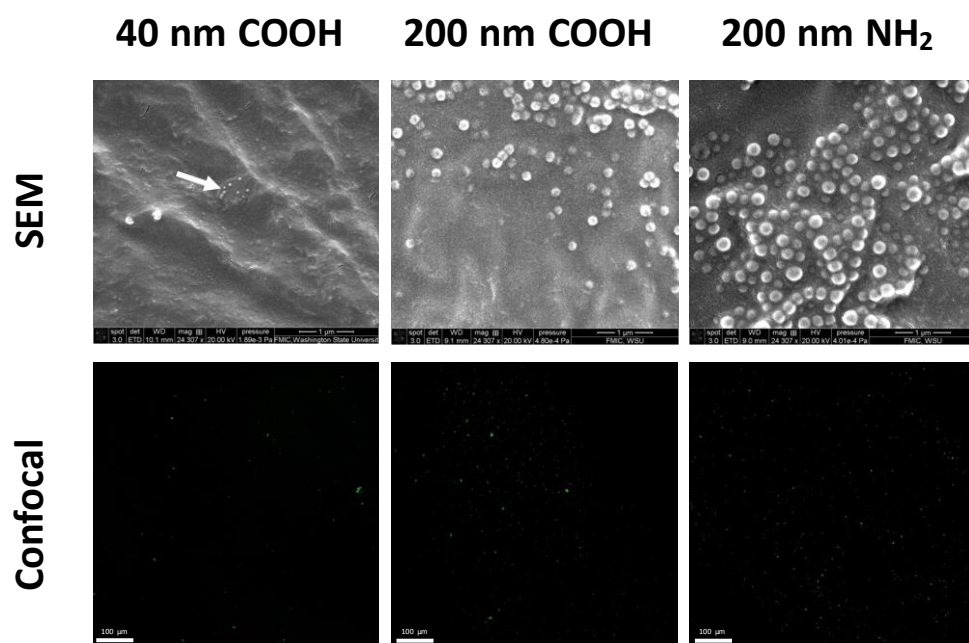
**Table S2.** Root and shoot dry biomass of Arabidopsis and wheat grown in agar and hydroponics.

Treatments	Arabidopsis (Agar)			Arabidopsis (Hydroponics)			Wheat (Agar)			Wheat (Hydroponics)		
	Root dry	Shoot dry	Root:Shoot	Root dry	Shoot dry	Root:Shoot	Root dry	Shoot dry	Root:Shoot	Root dry	Shoot dry	Root:Shoot
	biomass	biomass	ratio	biomass	biomass	ratio	biomass	biomass	ratio	biomass	biomass	ratio
	(mg)	(mg)	(-)	(mg)	(mg)	(-)	(mg)	(mg)	(-)	(mg)	(mg)	(-)
Control	7.3 ± 1.2	22.5 ± 2.6	0.32	0.72 ± 0.28	2.9 ± 0.4	0.25	19.3 ± 4.9	46.8 ± 18.7	0.41	28.5 ± 3.0	63.3 ± 15.3	0.45
40 nm – COOH	4.6 ± 1.2	18.3 ± 4.5	0.25	0.30 ± 0.10	1.7 ± 0.6	0.18	14.7 ± 0.3	38.9 ± 5.9	0.38	16.5 ± 1.1	53.3 ± 15.3	0.31
200 nm – COOH	4.5 ± 1.9	17.2 ± 3.8	0.26	0.74 ± 0.16	4.3 ± 1.3	0.17	19.0 ± 6.5	27.0 ± 6.9	0.7	23.1 ± 2.2	66.7 ± 5.8	0.35
200 nm – NH <sub>2</sub>	4.3 ± 1.5	19.9 ± 1.0	0.21	0.35 ± 0.05	1.8 ± 0.3	0.19	12.3 ± 5.2	33.3 ± 5.8	0.37	23.9 ± 2.7	65.0 ± 8.7	0.37

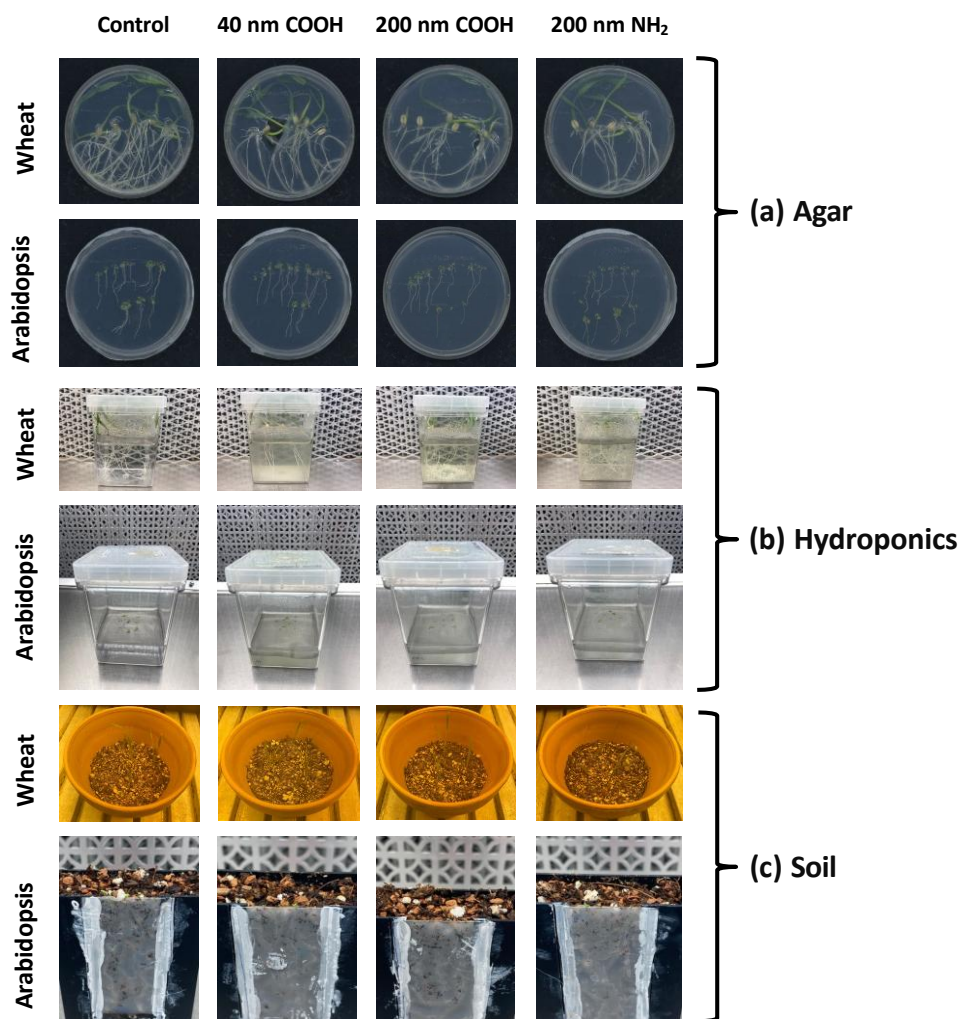
† Root and Shoot biomass are expressed as mean ± standard deviation

**Table S3.** TukeyHSD test  $p$ -values based on ROS accumulations in *Arabidopsis*. Test was made based on the stained areas of DAB (3,3-Diaminobenzidine) for hydrogen peroxide and NBT (Nitrioblue Tetrazolium) for superoxide.

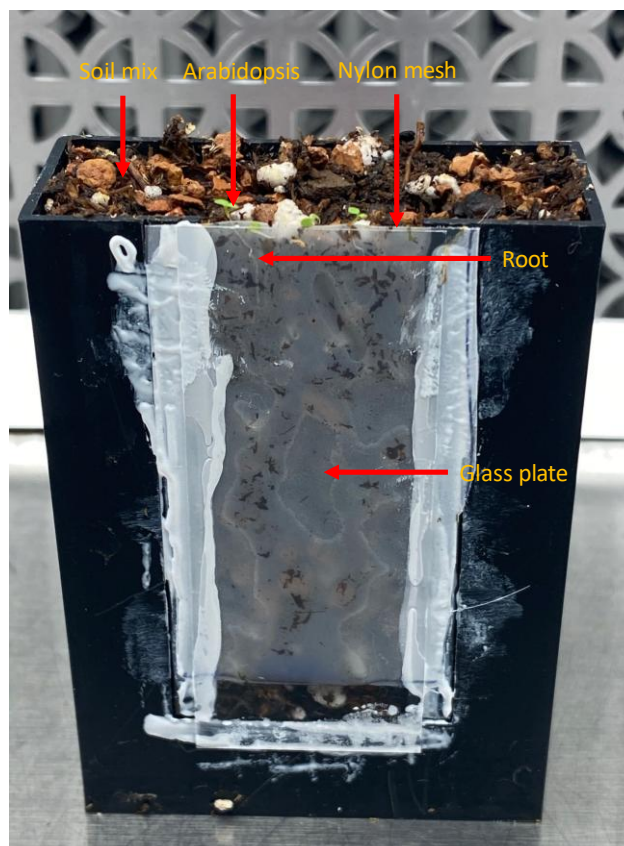
<b>Treatments</b>	<b>AreaDAB</b>	<b>AreaNBT</b>
Control*40 nm COOH	0.0114	0.3248
Control*200 nm COOH	0.023	0.7429
Control*200 nm NH <sub>2</sub>	0.0009	0.0262
40 nm COOH*200 nm COOH	0.9506	0.8454
40 nm COOH*200 nm NH <sub>2</sub>	0.2339	0.3271
200 nm COOH*200 nm NH <sub>2</sub>	0.114	0.1095



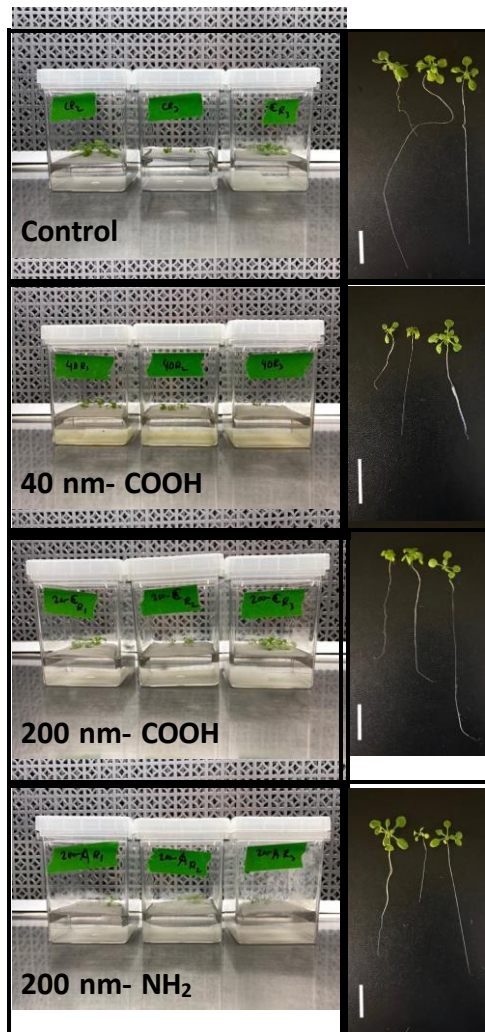
**Figure S1.** Scanning Electron Microscopy images of 40 and 200 nm polystyrene nanospheres in deionized water (top row), and confocal images of nanospheres in agar medium with half- strength Murashige and Skoog (MS) solution (bottom row).



**Figure S2.** Wheat and *Arabidopsis thaliana* grown in (a) agar (Petri dishes), (b) hydroponics (Magenta boxes), and (c) soil (clay pots and micro-ROCS).

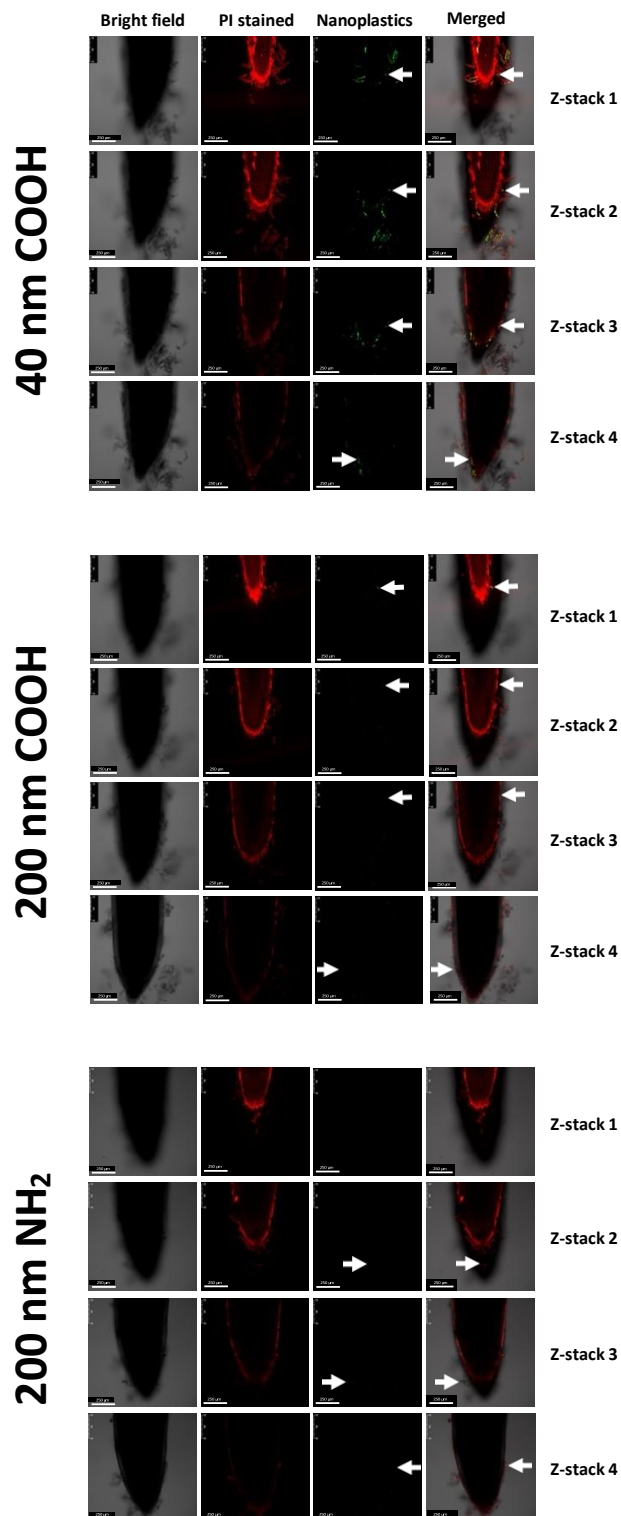


**Figure S3.** *Arabidopsis thaliana* grown in micro-ROC system filled with a greenhouse soil. The front of the systems consists of a glass slide and a Nylon mesh (38  $\mu\text{m}$  in contact with the soil). Plant roots grow in the space between the glass slide and the Nylon mesh. The chamber size is 7 cm  $\times$  5 cm  $\times$  2 cm.

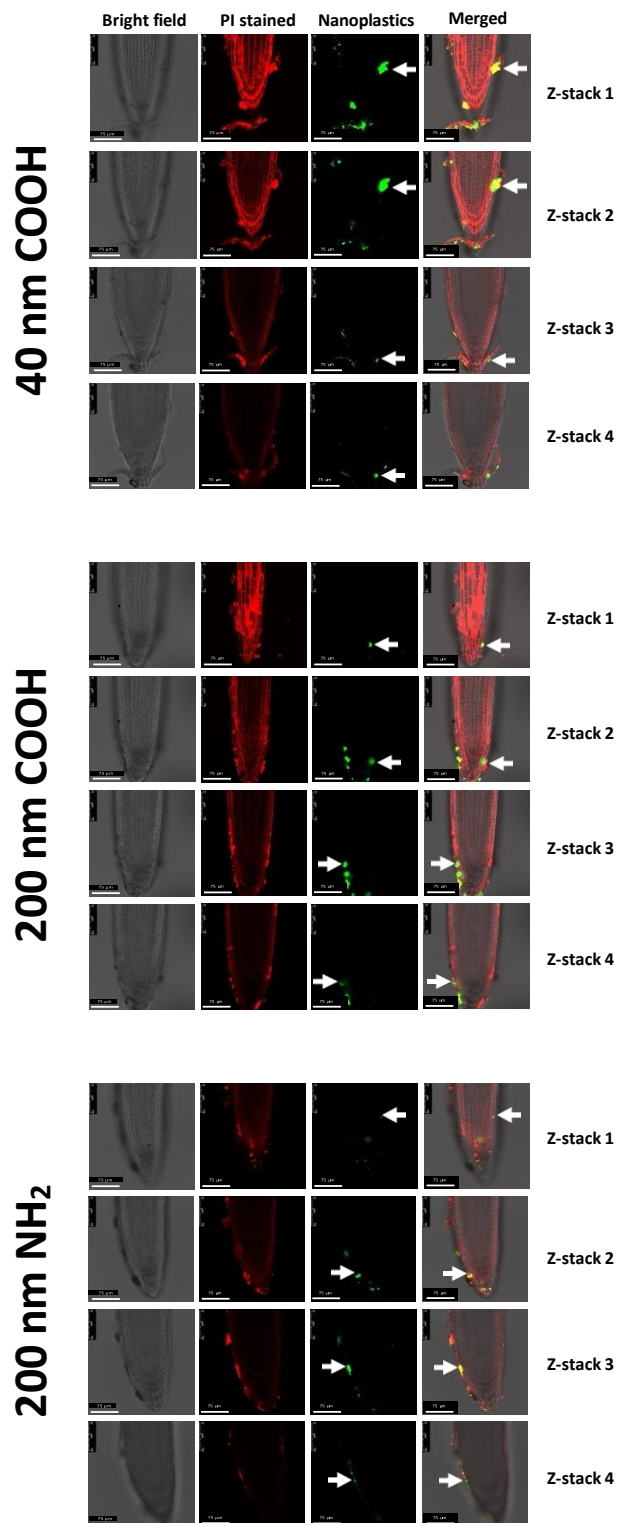


**Figure S4.** *Arabidopsis thaliana* grown in hydroponics for 20 days used for plant biomass and ROS measurements. Left panel shows the Magenta boxes with 20 days grown plants, and right panel shows the representative plants from each replication (scale bar = 2 cm).

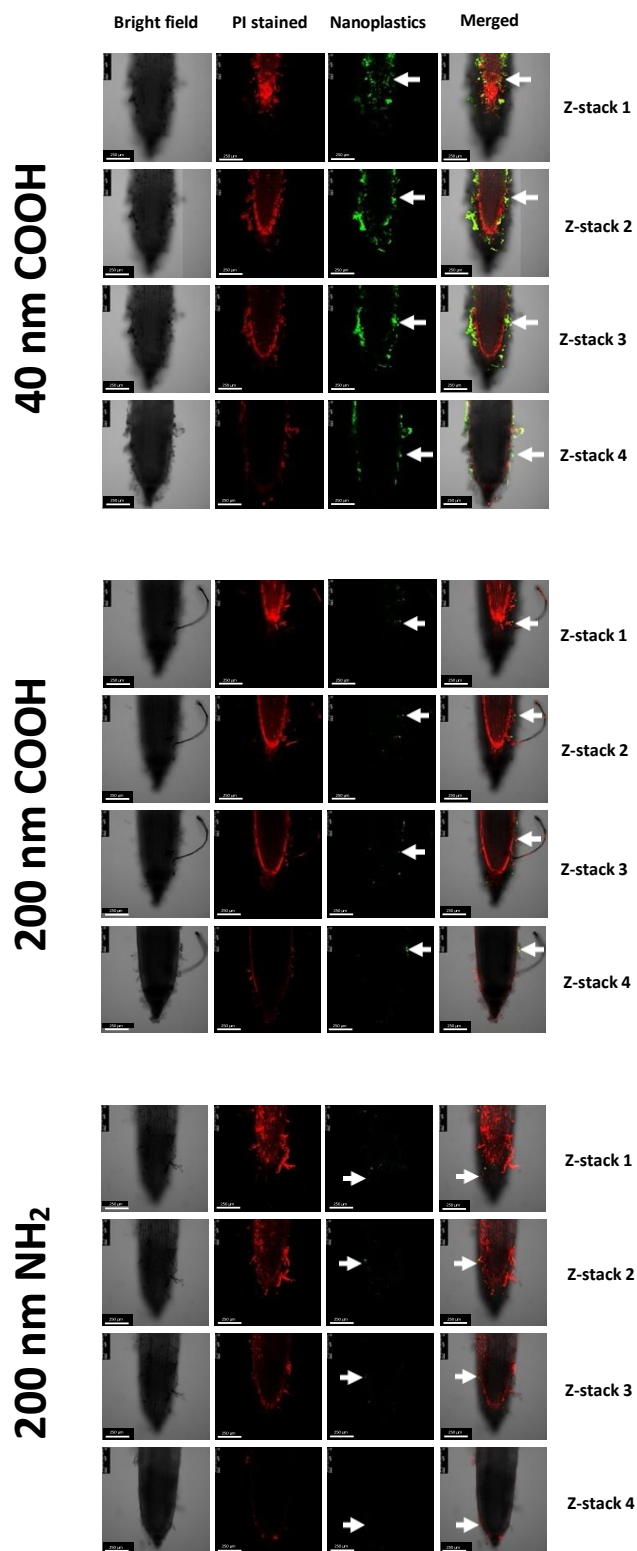




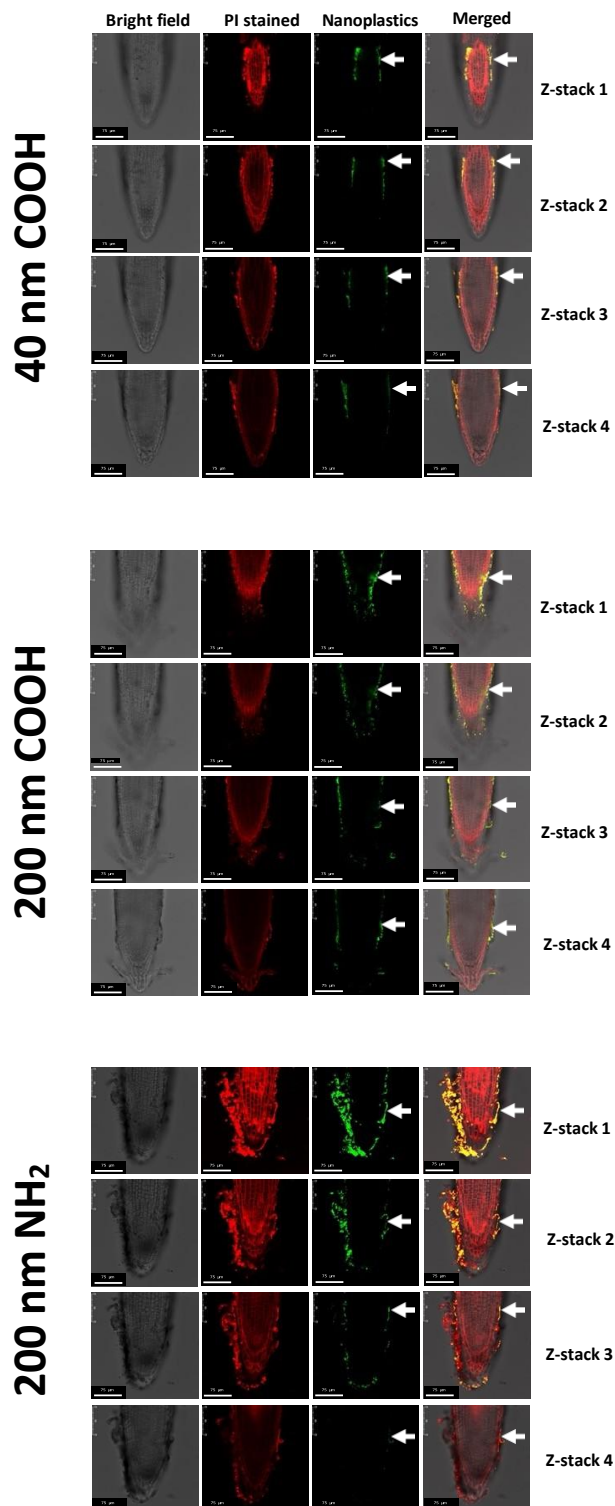
**Figure S5. Wheat in agar medium:** Confocal Z-stack images of wheat roots from agar media for 40 nm carboxylate-modified polystyrene nanospheres, 200 nm carboxylate-modified polystyrene nanospheres, and 200 nm amino-modified polystyrene nanospheres. Arrows indicate nanospheres attached to the root cap cells. PI: propidium iodide.



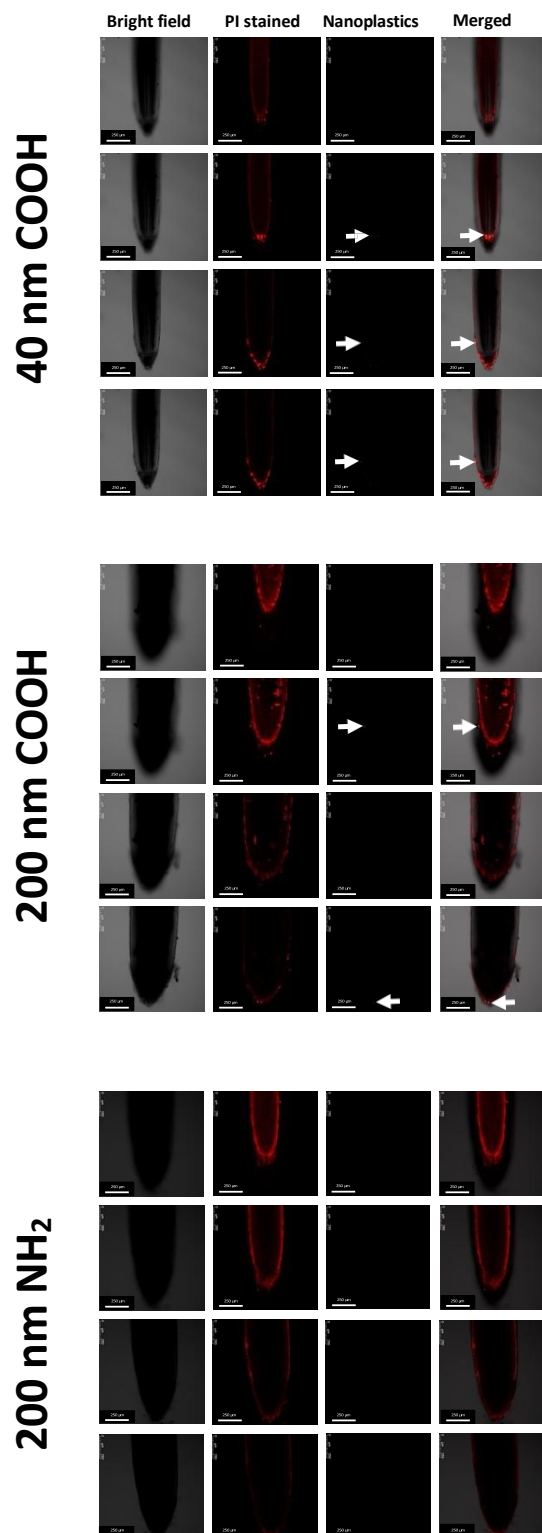
**Figure S6. Arabidopsis thaliana in agar medium:** Confocal z-stack images of *A. thaliana* roots for 40 nm carboxylate-modified polystyrene nanospheres, 200 nm carboxylate-modified polystyrene nanospheres, and 200 nm amino-modified polystyrene nanospheres. Arrows indicate nanospheres attached to the root cap cells. PI: propidium iodide.



**Figure S7. Wheat in hydroponics medium:** Confocal Z-stack images of wheat roots for 40 nm carboxylate-modified polystyrene nanospheres, 200 nm carboxylate-modified polystyrene nanospheres, and 200 nm amino-modified polystyrene nanospheres. Arrows indicate nanospheres attached to the root cap cells. PI: propidium iodide.



**Figure S8. Arabidopsis thaliana in hydroponics medium:** Confocal z-stack images of *A. thaliana* roots for 40 nm carboxylate-modified polystyrene nanospheres, 200 nm carboxylate-modified polystyrene nanospheres, and 200 nm amino-modified polystyrene nanospheres. Arrows indicate nanospheres attached to the root cap cells. PI: propidium iodide.



**Figure S9. Wheat in soil medium:** Confocal Z-stack images of wheat roots for 40 nm carboxylate-modified polystyrene nanospheres, 200 nm carboxylate-modified polystyrene nanospheres, and 200 nm amino-modified polystyrene nanospheres. Arrows indicate nanospheres attached to the root cap cells. PI: propidium iodide.

