Methods

EROD activity in Gill

Gill arches were dissected from the gill and placed in 1 mL of tissue buffer containing ice-cold 1x Hanks' Balanced Salt Solution with calcium, magnesium and glucose (HBSS; ThermoFisher, 14025092) and 1.43 g L^{-1} HEPES (Fisher BioReagents, BP310-1) at pH 7.7 in wells of a 12-well plate. Duplicate groups of ten 2 mm-long filament tips were cut from gill arches and transferred into 1 mL fresh tissue buffer in wells of a 24-well plate. The tissue buffer was replaced with 1 mL of reaction buffer consisting of tissue buffer, 0.603 mg L^{-1} 7-ethoxyresorufin (7-ER) and 3.363 mg L^{-1} dicumarol. Gill filament tips were incubated at room temperature on a shaking plate in the dark for 30 min. After incubation, a triplicate of 0.2 mL aliquots from each well was transferred to a black-walled 96-well plate (Greiner Cellstar, Frickenhausen, Germany) and triplicate 0.2 mL aliquots of resorufin standards (0-1000 pM) were transferred to the same plate to generate a standard curve. The fluorescence was measured by a microplate reader (1420 Multilabel Counter, VICTOR 3V, PerkinElmer, MA, USA) at an excitation wavelength of 353 nm and an emission wavelength of 595 nm. EROD activity was calculated and expressed as relative fold change to the control group.

EROD activity in liver

Approximately 0.15 g liver was dissected and placed in 2 mL microfuge tubes with 0.5 mL ice-cold KCl-HEPES homogenization buffer (11.18 g L-1 KCl, 4.77 g L-1 HEPES, pH 7.4). Liver tissue was homogenized on ice using a hand-held tissue pestle homogenizer on ice for 15 s and then centrifuged at 10,000g at 4 °C for 20 min. Fifty µL ice-cold supernatant was transferred into a 96-well and then 200 µL ice-cold EROD buffer consisting of 11.12 g L^{-1} Tris-HCl, 0.292 g

L^{-1} EDTA and 0.603 mg L^{-1} 7-ER at pH 7.4 and resorufin standards (0-1000 pM) were added into each well. Ten µL of 3.72 g L^{-1} NADPH solution was added into each well to start the reaction. The reaction was incubated at room temperature on a shaking plate in the dark for 20 min. After the incubation, fluorescence was measured at 535/595 (excitation/emission) by the microplate reader. Protein concentration in each liver sample was determined by the Pierce BCA protein assay kit (Thermo Scientific, 23227). EROD activity was normalized based on the protein concentration and expressed as relative fold change to control.

Confocal slides preparation

Tissues were fixed in 4% paraformaldehyde (pH 7.4, diluted from 32% paraformaldehyde, Electron Microscopy Sciences, 15714-S) at 4 °C overnight. The paraformaldehyde was removed and cold 70% ethanol was added to cover the tissues at 4 °C. After 30 min, ethanol was replaced by fresh, cold 70% ethanol. The samples were serially dehydrated in a graded ethanol series followed by being embed into paraffin wax. Tissue blocks were sectioned at 7 µm by microtome (Leica Biosystems) and placed on Superfrost Plus microscope slides (Fisher Scientific, 12-550-15). Slides were deparaffinized by toluene for 10 mins thrice and immersed in 100%, 95% and 70% ethanol for 10 min once and then washed with phosphate-buffered saline (PBS) for 10 min thrice. Tissues were incubated in 1:1000 diluted (in PBS) Hoechst 33342 nuclear stain (ThermoFisher Scientific, H3570, Excitation/Emission maxima: 352 nm/461 nm) for 10 mins and then slides were washed with PBS for 10 mins trice. Tissues were incubated in 1:1000 diluted (in PBS) CellMask™ Orange Plasma membrane Stain (ThermoFisher Scientific, C10045, Excitation/Emission maxima: 554 nm/567 nm) for 10 mins and then slides were washed with PBS for 10 mins trice. Coverslips were mounted with Fluoro-Gel mounting medium (Fisher Scientific, 50-247-04) and slides were stored at 4 °C.

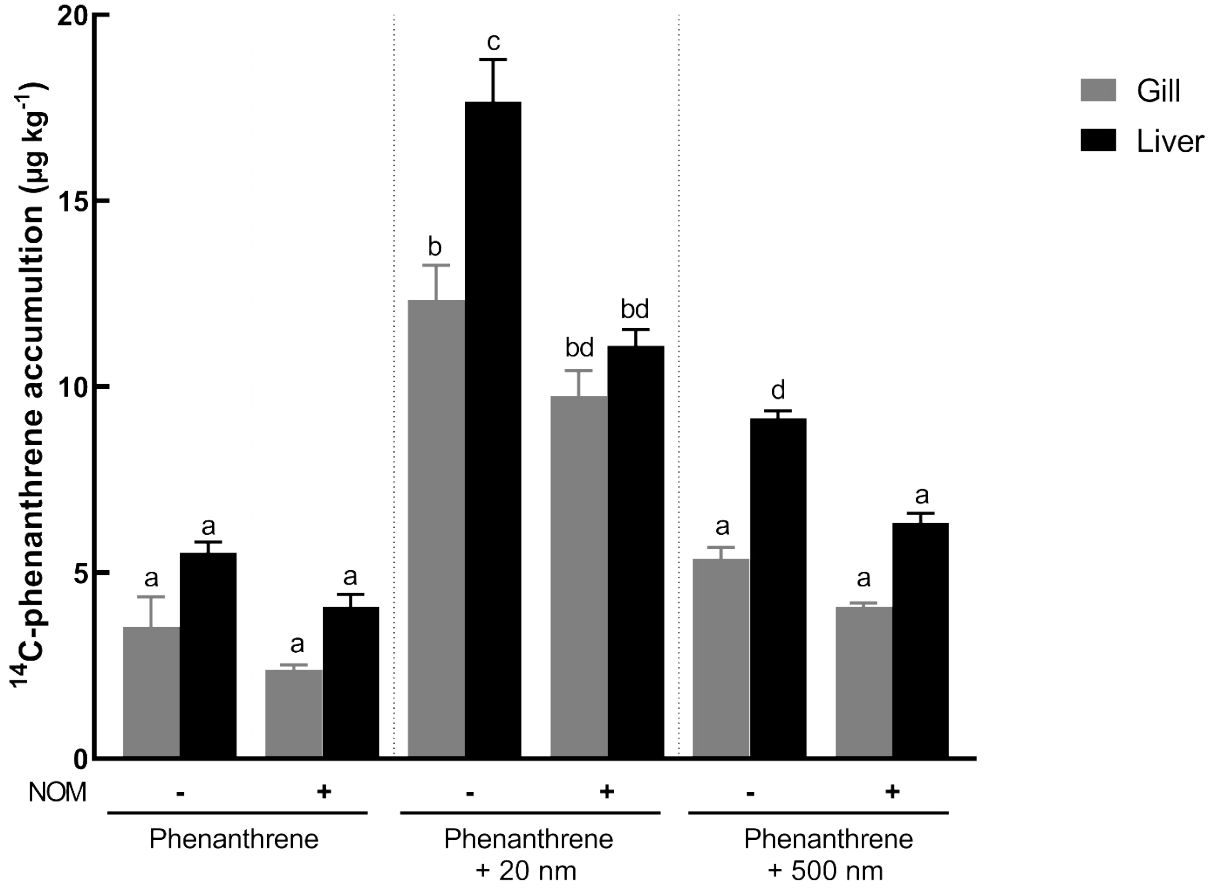
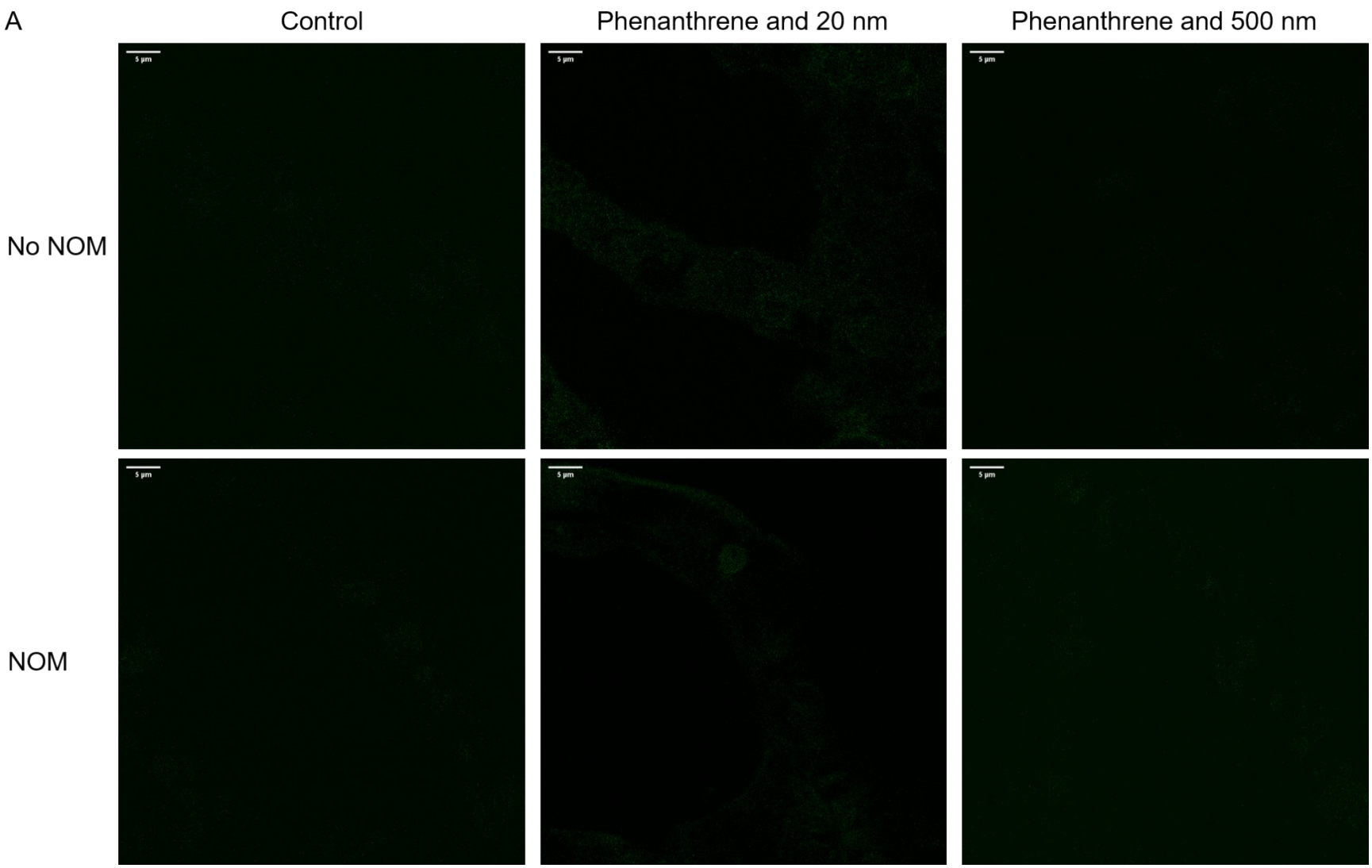
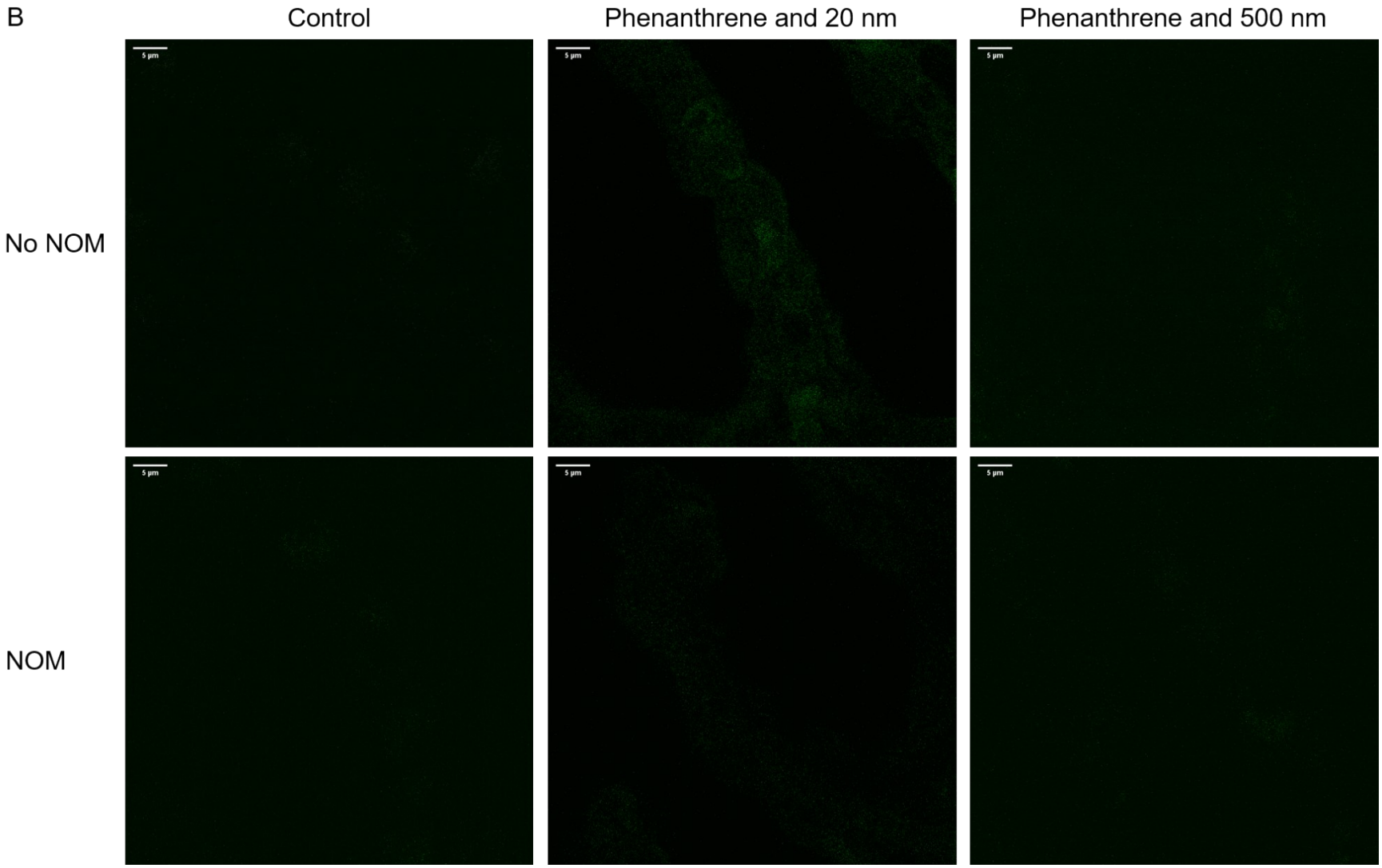


Figure S1. Residual ^{14}C-labeled phenanthrene detected in rainbow trout fingerlings gills and livers following 24 h recovery in clean RO water. Means sharing the same letter are not significantly different from each other ($p > 0.05$). Values are mean ± SEM. n = 4.


A
Control
Phenanthrene and 20 nm
Phenanthrene and 500 nm
No NOM
NOM
5 µm


B
Control
Phenanthrene and 20 nm
Phenanthrene and 500 nm
No NOM
NOM
5 µm

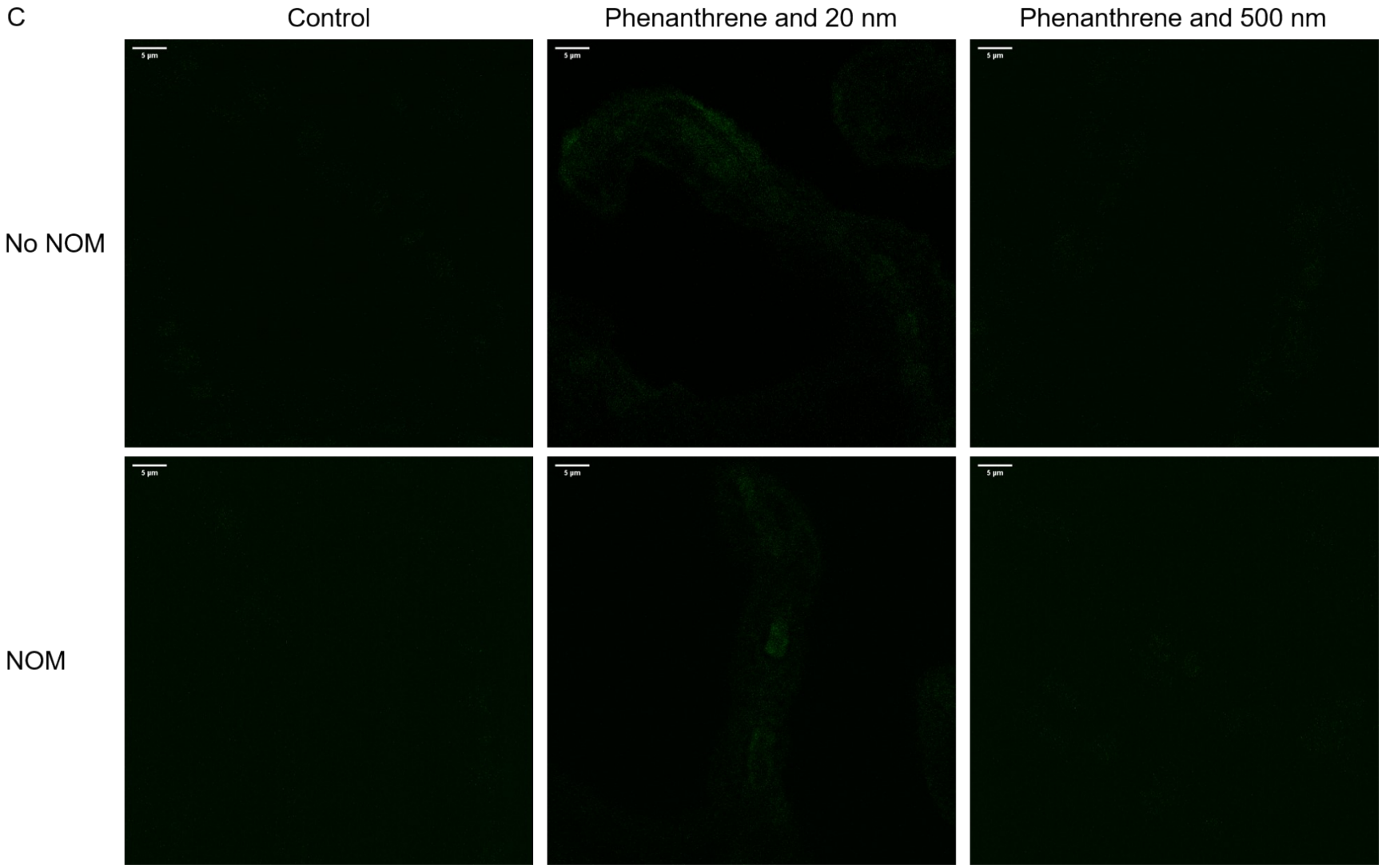
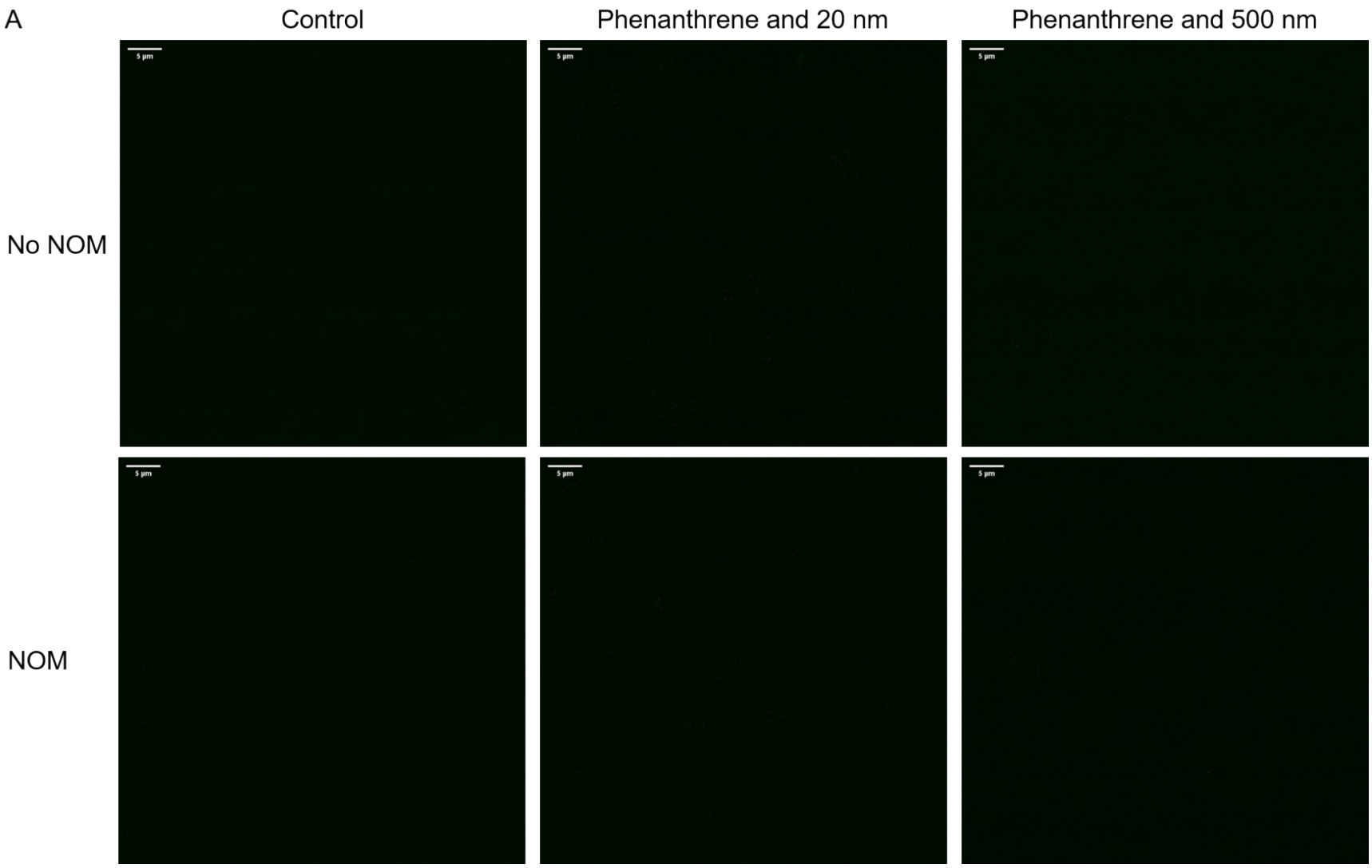
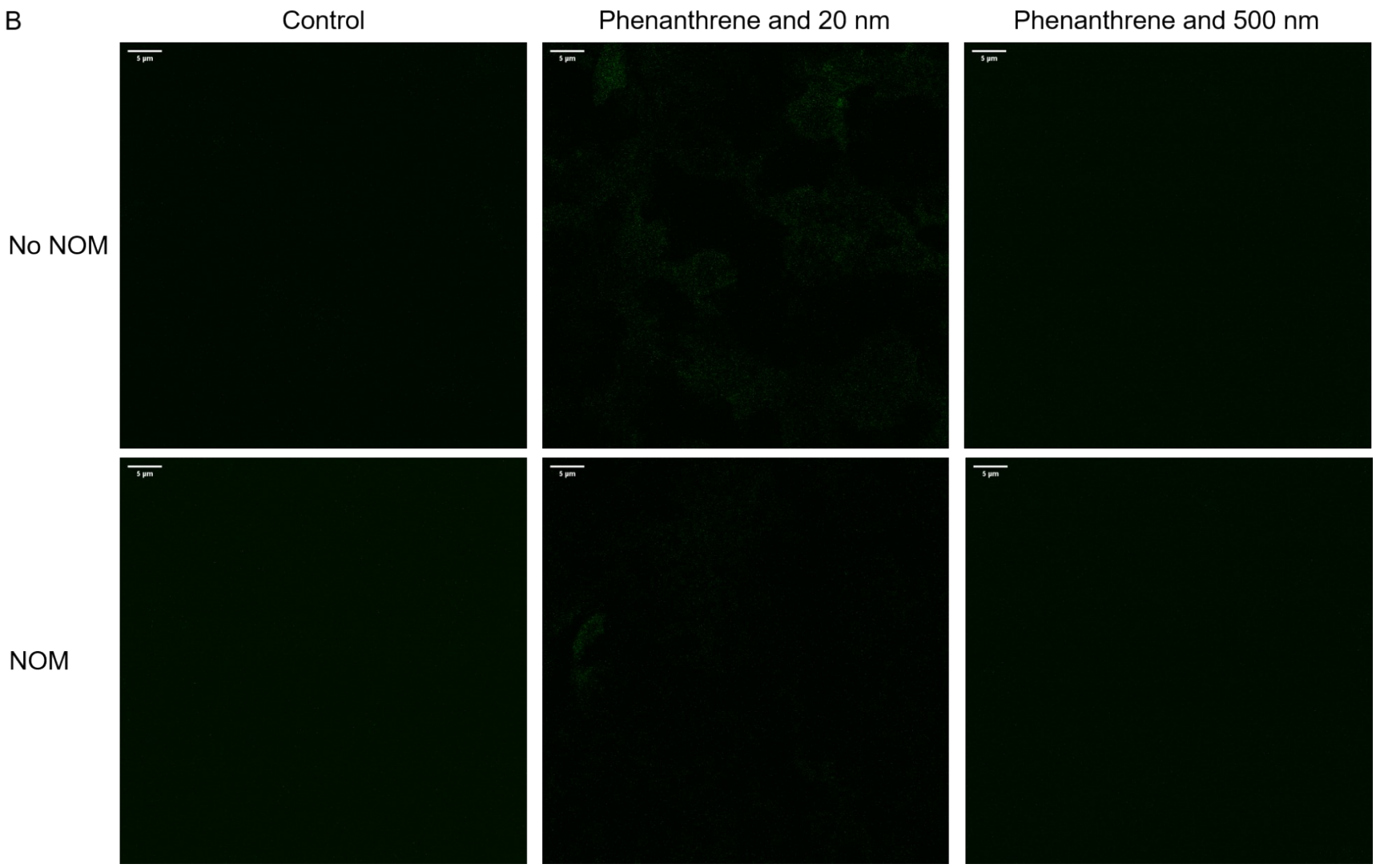

C
Control
Phenanthrene and 20 nm
Phenanthrene and 500 nm
No NOM
NOM
5 µm

Figure S2. Unprocessed confocal images (PS-NPs green fluorescence) of gills of rainbow trout fingerlings exposed to control, NOM, phenanthrene in the presence of 20 nm PS-NPs with or without NOM and phenanthrene in the presence of 500 nm PS-NPs with or without NOM at 4h (A), 24h (B) and 24 h after recovery (C). Scale bars are 5 μm.


A
Control
Phenanthrene and 20 nm
Phenanthrene and 500 nm
No NOM
NOM
5 µm


B
Control
Phenanthrene and 20 nm
Phenanthrene and 500 nm
No NOM
NOM
5 µm

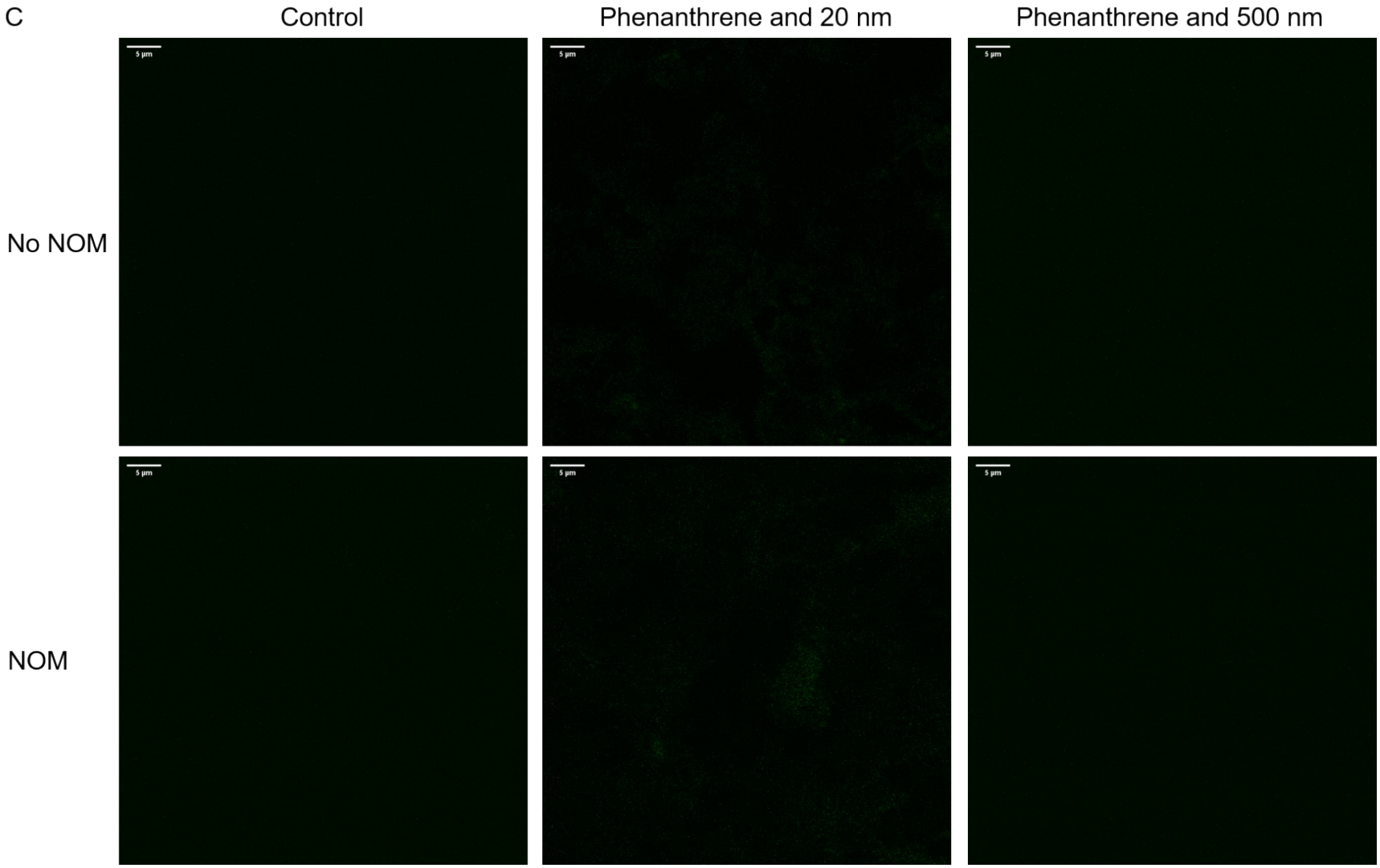

C
Control
Phenanthrene and 20 nm
Phenanthrene and 500 nm
No NOM
NOM
5 µm

Figure S3. Unprocessed confocal images (PS-NPs green fluorescence) of livers of rainbow trout fingerlings exposed to control, NOM, phenanthrene in the presence of 20 nm PS-NPs with or without NOM and phenanthrene in the presence of 500 nm PS-NPs with or without NOM at 4h (A), 24h (B) and 24 h after recovery (C). Scale bars are 5 μm.

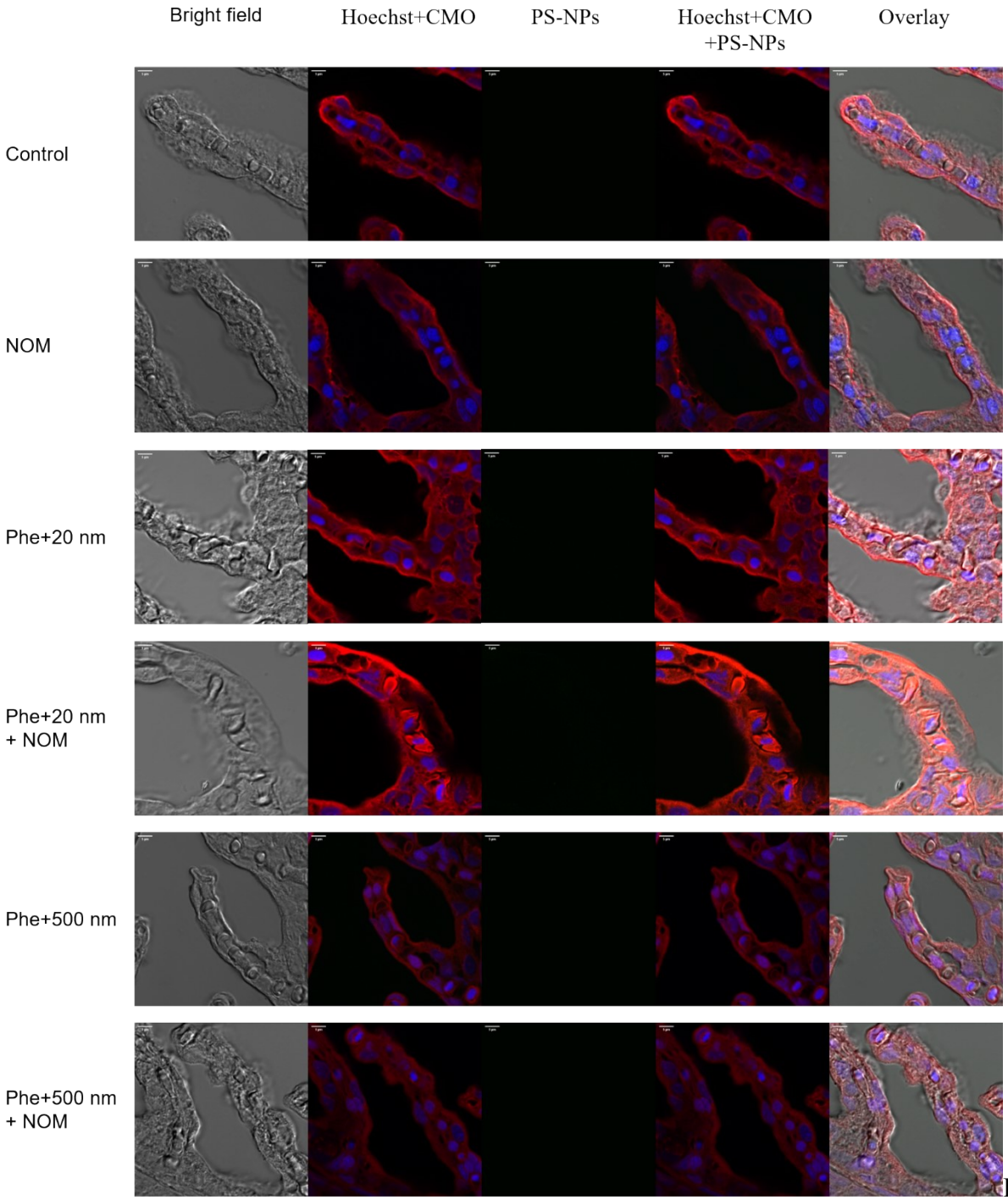


Figure S3. Confocal images (bright field, Hoechst+CMO, PS-NP fluorescence, Hoechst+CMO+PS-NPs fluorescence and overplay) of gills of rainbow trout fingerlings exposed to control, NOM, phenanthrene in the presence of 20 nm PS-NPs with or without NOM and phenanthrene in the presence of 500 nm PS-NPs with or without NOM at 4h. Scale bars are 5 µm.

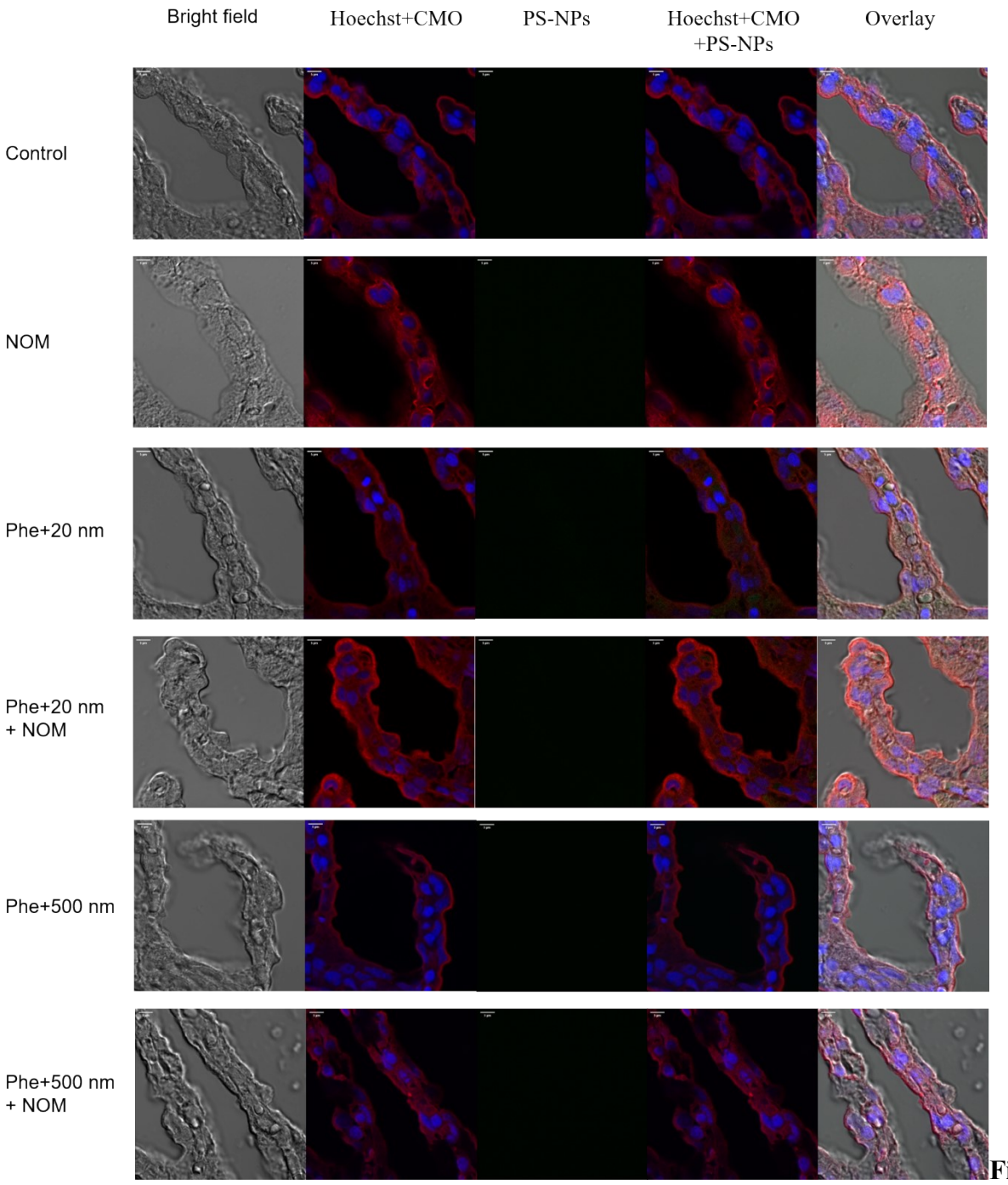


Figure S4. Confocal images (bright field, Hoechst+CMO, PS-NP fluorescence, Hoechst+CMO+PS-NPs fluorescence and overplay) of gills of rainbow trout fingerlings exposed to control, NOM, phenanthrene in the presence of 20 nm PS-NPs with or without NOM and phenanthrene in the presence of 500 nm PS-NPs with or without NOM at 24h. Scale bars are 5 µm.

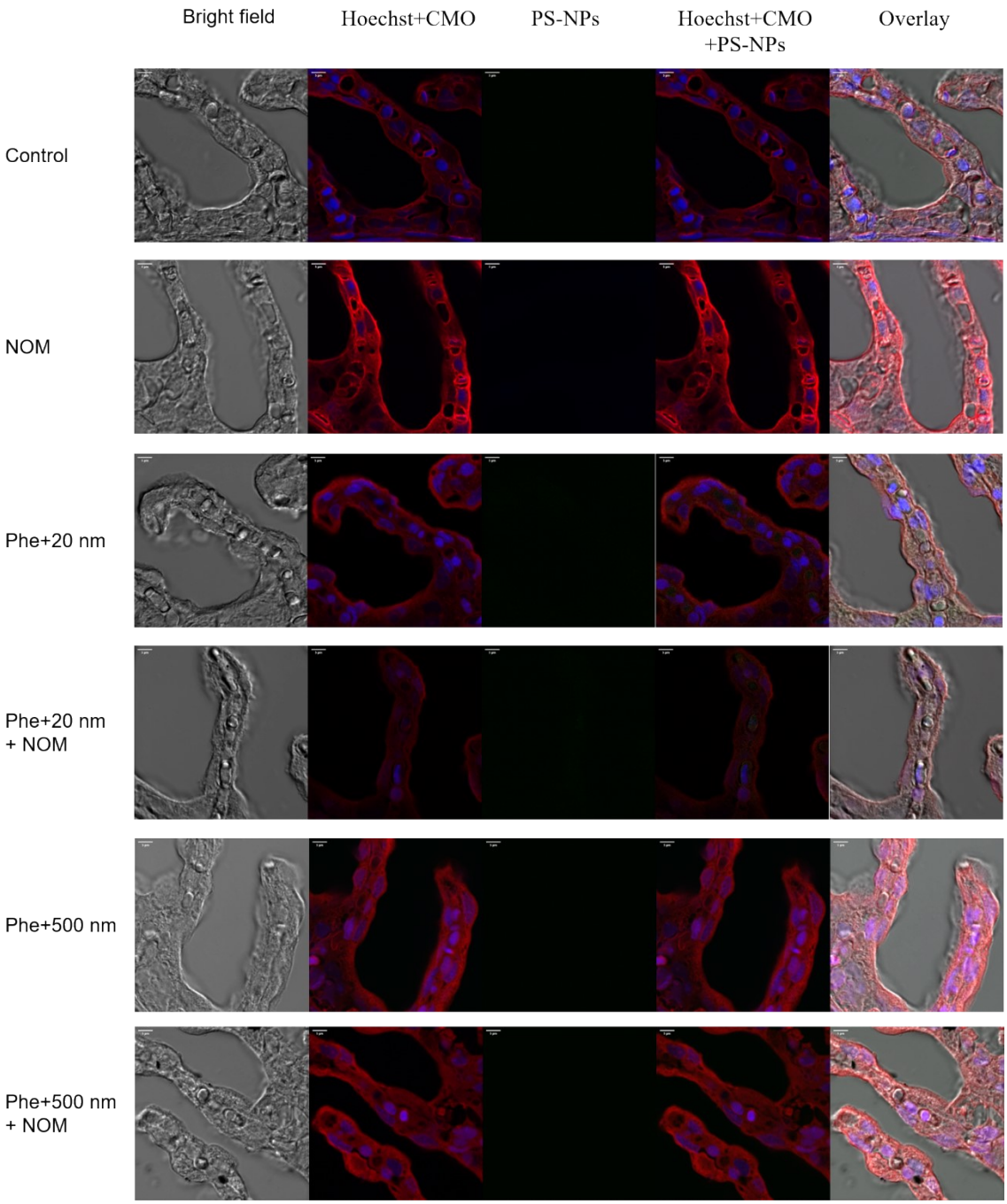


Figure S5. Confocal images (bright field, Hoechst+CMO, PS-NP fluorescence, Hoechst+CMO+PS-NPs fluorescence and overplay) of gills of rainbow trout fingerlings exposed to control, NOM, phenanthrene in the presence of 20 nm PS-NPs with or without NOM and phenanthrene in the presence of 500 nm PS-NPs with or without NOM at 24 h after recovery. Scale bars are 5 μm.

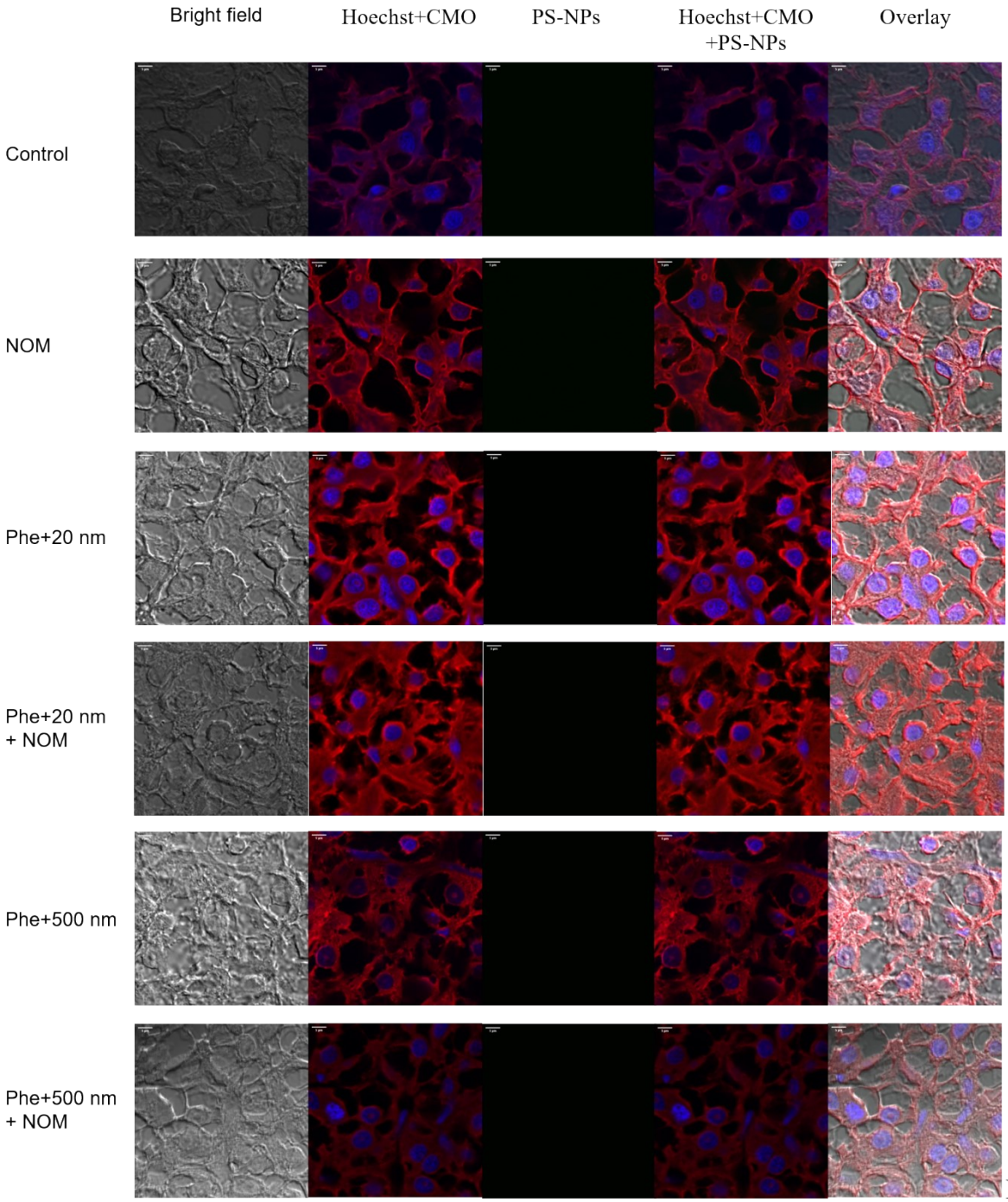


Figure S6. Confocal images (bright field, Hoechst+CMO, PS-NP fluorescence, Hoechst+CMO+PS-NPs fluorescence and overplay) of livers of rainbow trout fingerlings exposed to control, NOM, phenanthrene in the presence of 20 nm PS-NPs with or without NOM and phenanthrene in the presence of 500 nm PS-NPs with or without NOM at 4h. Scale bars are 5 μm.

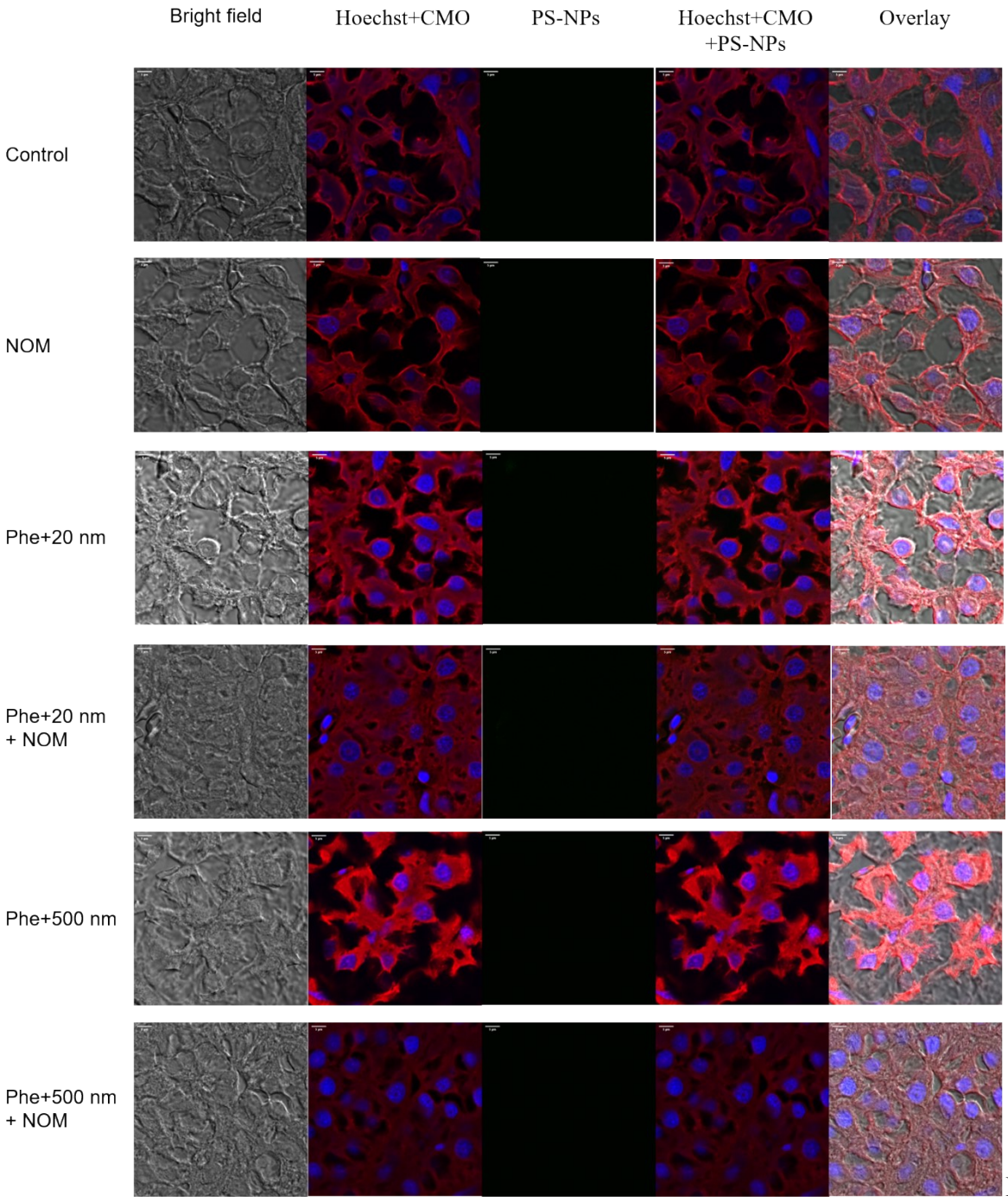


Figure S7. Confocal images (bright field, Hoechst+CMO, PS-NP fluorescence, Hoechst+CMO+PS-NPs fluorescence and overplay) of livers of rainbow trout fingerlings exposed to control, NOM, phenanthrene in the presence of 20 nm PS-NPs with or without NOM and phenanthrene in the presence of 500 nm PS-NPs with or without NOM at 24h. Scale bars are 5 µm.

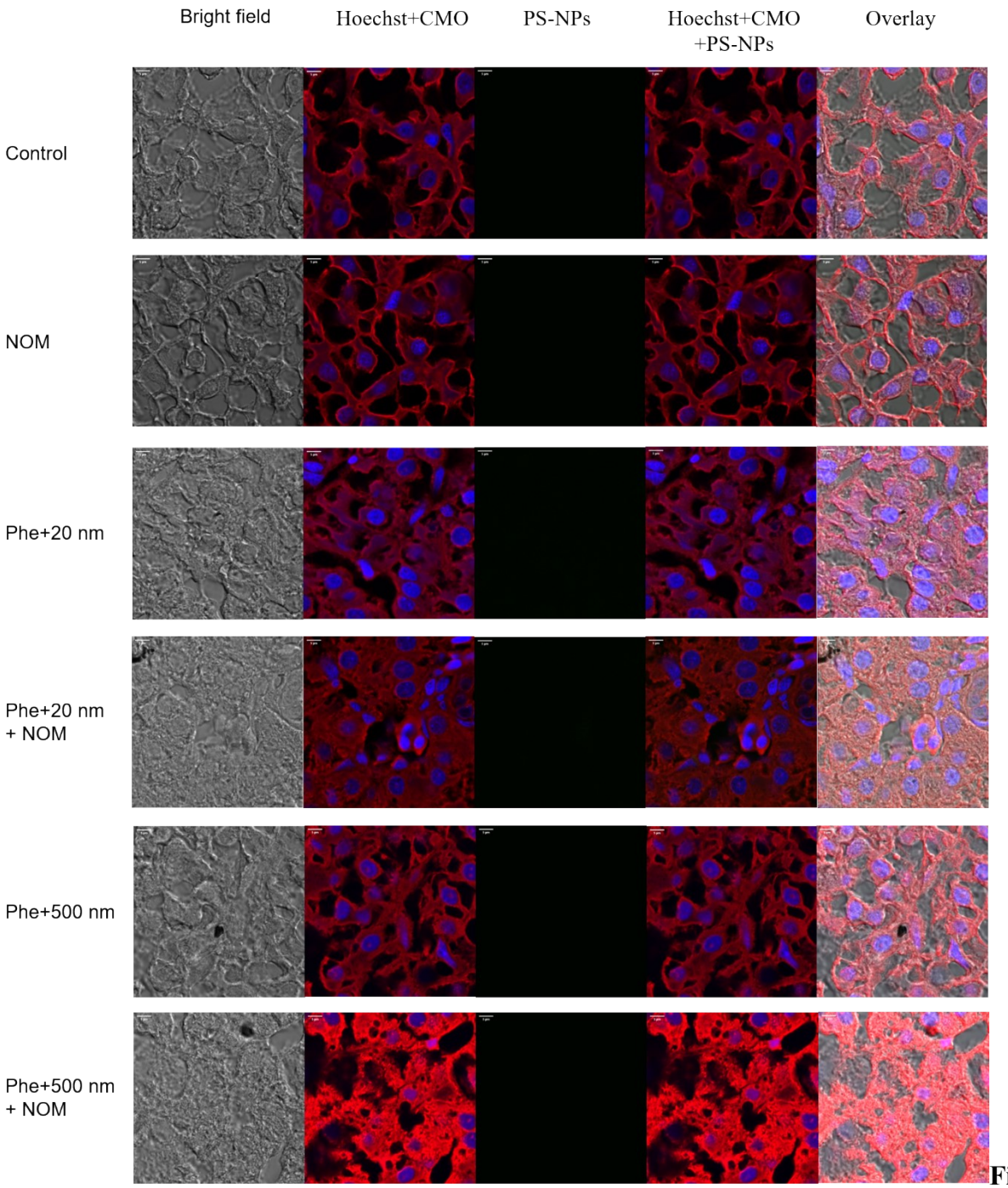


Figure S8. Confocal images (bright field, Hoechst+CMO, PS-NP fluorescence, Hoechst+CMO+PS-NPs fluorescence and overplay) of livers of rainbow trout fingerlings exposed to control, NOM, phenanthrene in the presence of 20 nm PS-NPs with or without NOM and phenanthrene in the presence of 500 nm PS-NPs with or without NOM at 24 h after recovery (C). Scale bars are 5 µm.

Table S1. The hydrodynamic diameters (HD), polydispersity index, and zeta-potential of dialyzed PS-NPs at 0, 4 and 24 h. Lowercase letters were used to compare 20 nm with or without NOM, while uppercase letters were used to compare 500 nm with or without NOM. Means sharing the same letter are not significantly different from each other ($p > 0.05$). Values are mean ± SD. n=3.

PS-NPs	HD (nm)			PDI			Zeta-potential (mV)		
	0 h	4 h	24h	0 h	4 h	24h	0 h	4 h	24h
20 nm	37.3 ± 1.0^{a}	37.6 ± 0.7^{a}	41.0 ± 1.5^{a}	0.148 ± 0.004^{a}	0.150 ± 0.002^{a}	0.159 ± 0.008^{a}	-12.1 ± 0.7^{a}	-12.3 ± 0.3^{ab}	-12.7 ± 0.3^{bc}
20 nm + NOM	35.6 ± 2.5^{a}	36.7 ± 0.4^{a}	40.4 ± 2.1^{a}	0.144 ± 0.005^{a}	0.145 ± 0.004^{a}	0.148 ± 0.004^{a}	-12.8 ± 0.4^{bc}	-12.6 ± 0.3^{bc}	-13.0 ± 0.3^{c}
500 nm	556 ± 8^{A}	560 ± 6^{A}	562 ± 5^{A}	0.158 ± 0.010^{A}	0.162 ± 0.003^{A}	0.168 ± 0.008^{A}	-12.1 ± 0.3^{A}	-12.2 ± 0.3^{AB}	-12.6 ± 0.4^{AB}
500nm + NOM	524 ± 14^{B}	526 ± 5^{B}	530 ± 8^{B}	0.149 ± 0.007^{A}	0.152 ± 0.002^{A}	0.155 ± 0.007^{B}	-11.9 ± 0.3^{A}	-12.4 ± 0.4^{AB}	-13.2 ± 0.3^{B}