Supplemental Materials

Uptake, tissue distribution, and toxicity of polystyrene nanoparticles in developing zebrafish (*Danio rerio*)

Jordan A. Pitt[1,2,*], Jordan S. Kozal[1], Nishad Jayasundara[1,3], Andrey Massarsky[1], Rafael Trevisan[1], Nick Geitner[4], Mark Wiesner[4], Edward D. Levin[5] & Richard T. Di Giulio[1]

[1]Nicholas School of the Environment, Duke University, Durham, NC 27708, USA.

[2]College of Environmental Science and Forestry, State University of New York, Syracuse, NY 13210, USA.

[3]School of Marine Sciences, University of Maine, Orono, Me 04469, USA

[4]Department of Civil and Environmental Engineering and the Center for the Environmental Implications of NanoTechnology, Durham, NC 27708, USA

[5]Department of Psychiatry and Behavioral Sciences, Duke University Medical Center, Durham, NC 27710, USA

***Corresponding author**: jopitt@syr.edu

1. Methylene blue does not increase the toxicity of PS NPs

To evaluate the potential of PS NPs to act as carriers of methylene blue, thereby mediating the toxic effects that were observed with PS NPs exposure, we have compared the toxicity of 10 ppm PS NPs in media supplemented (0.00003%) and non-supplemented with methylene blue. The treatment groups included control, vehicle control (control veh; contains an equivalent concentration of SDS and sodium azide that is present in 10 ppm PS NPs group – 0.0001% and 0.00009%, respectively), and 10 ppm PS NPs. Exposure was carried out as described at section 2.4. The following endpoints were evaluated at 72 hpf: viability, hatching success, heart rate, and pericardial area. The results demonstrate that (1) methylene blue does not affect the toxicity of PS NPs, and (2) control and vehicle control group are not significantly different (Fig. S1). The results also confirm that exposure to 10 ppm PS NPs reduces the heart rate (Fig S1).

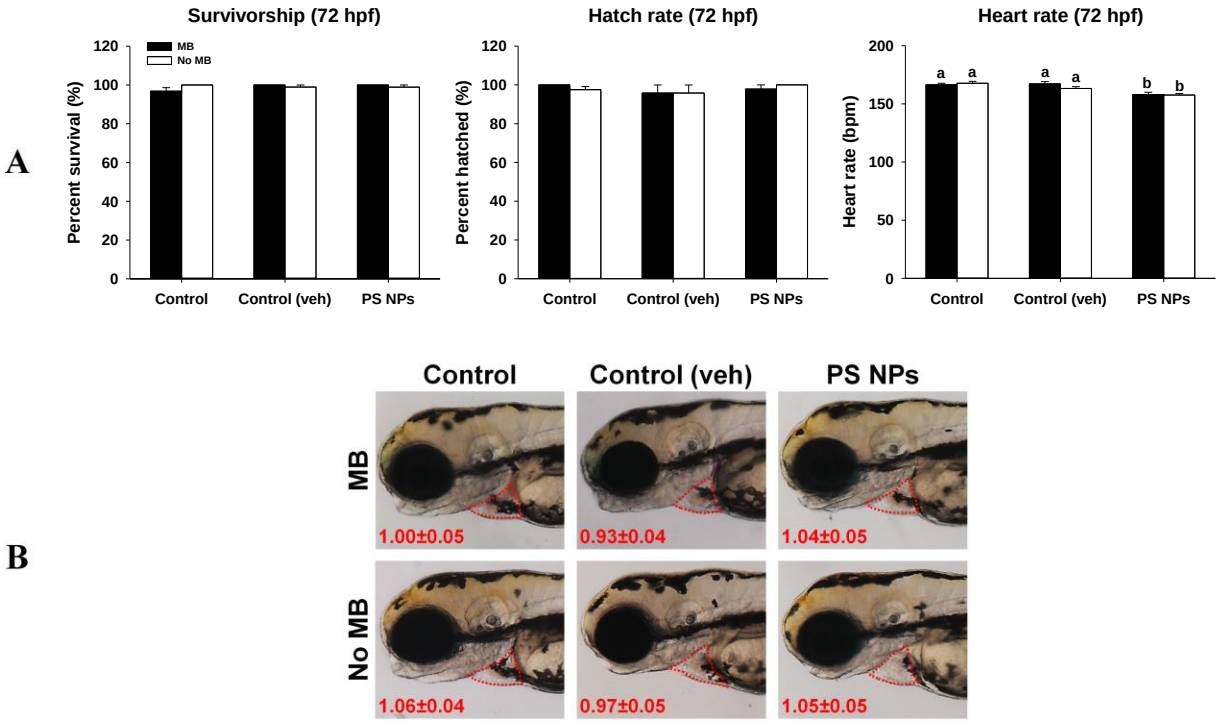

Figure S1. Toxicity of PS NPs in presence or absence of 0.00003% methylene blue (MB) in embryo medium. A. Survivorship, hatch rate, and heart rate were evaluated at 72 hpf in control, vehicle control (control veh; contains an equivalent concentration of SDS and sodium azide that is present in 10 ppm PS NPs group), and 10 ppm PS NPs-exposed larvae. Groups not sharing letters represent statistical differences assessed using a two-way ANOVA with a Fisher's LSD post hoc method ($p < 0.05$ was considered significant). B. Pericardial area (red dotted line) was assessed at 72 hpf and is indicated as fold change to control (with MB) in left bottom corner.

2. SDS and sodium azide do not increase toxicity and/or uptake of PS NPs

To evaluate the potential of SDS and sodium azide to affect the toxicity and uptake of PS NPs, we have compared the toxicity and uptake of 1 ppm PS NPs in presence of various concentrations of SDS and sodium azide. The treatment groups included control, vehicle control 1 (control veh 1; contains an equivalent concentration of SDS and sodium azide that is present in 1 ppm PS NPs group – 0.00001% and 0.000009%, respectively), vehicle control 2 (control veh 2; contains an equivalent concentration of SDS and sodium azide that is present in 10 ppm PS NPs group – 0.0001% and 0.00009%, respectively), 1 ppm PS NPs 1 (PS NPs 1; no additional SDS and sodium azide), and 1 ppm PS NPs 2 (PS NPs 2; contains an equivalent concentration of SDS and sodium azide that is present in 10 ppm PS NPs group). Exposure was carried out as described at section 2.4. The following endpoints were evaluated at 72 hpf: viability, hatching success, heart rate, pericardial area, and uptake. The results demonstrate that higher concentrations SDS and sodium azide do not increase the toxicity and uptake of PS NPs (Fig S2).

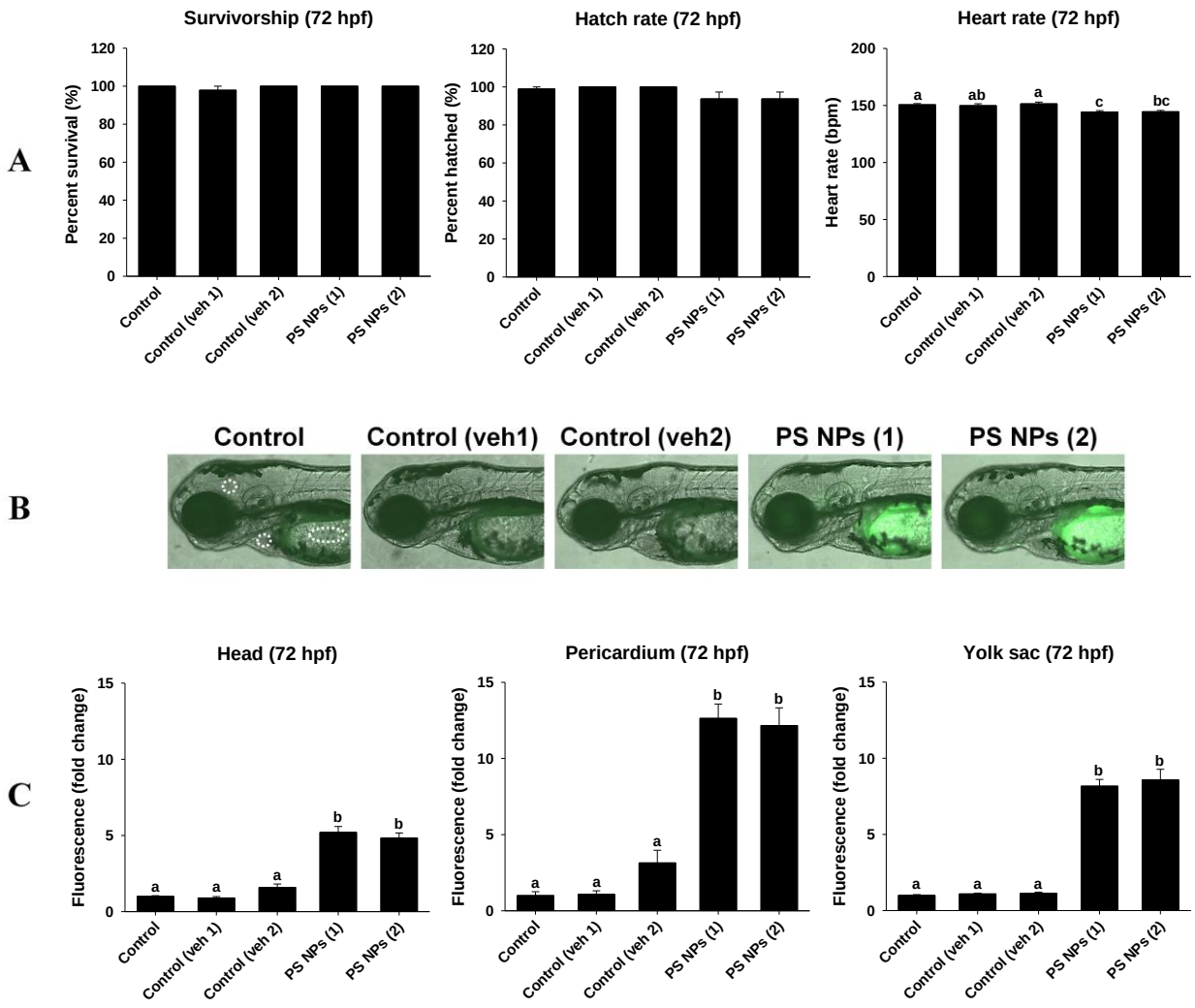

Figure S2. Toxicity and uptake of PS NPs in presence various concentrations of SDS and sodium azide. A. Survivorship, hatch rate, and heart rate were evaluated at 72 hpf in control, vehicle control 1 (control veh 1; contains an equivalent concentration of SDS and sodium azide that is present in 1 ppm PS NPs group), vehicle control 2 (control veh 2; contains an equivalent concentration of SDS and sodium azide that is present in 10 ppm PS NPs group), 1 ppm PS NPs 1 (PS NPs 1; no additional SDS and sodium azide), and 1 ppm PS NPs 2 (PS NPs 2; contains an equivalent concentration of SDS and sodium azide that is present in 10 ppm PS NPs group). B, C. Uptake of PS NPs was assessed at 72 hpf by quantifying fluorescence (fold change) in the head, pericardium, and yolk sac regions. Groups not sharing letters represent statistical differences assessed using a two-way ANOVA with a Fisher's LSD post hoc method ($p < 0.05$ was considered significant).

3. Fluorescence analysis of PS NPs uptake by zebrafish embryos and larvae.

To evaluate the uptake of PS NPs, analysis of fluorescence microscopy was carried out at different stages of the zebrafish development: 24 hpf (Fig. S3), 48 hpf (Fig. S4), and 144 hpf (Fig. S5). The treatment groups included control, 0.1 ppm PS NPs, 1ppm PS NPs, and 10 ppm PS NPs. Exposure was carried out as described at sections 2.4 and 2.6. Fluorescence was quantified at the indicated anatomical structures using ImageJ software.

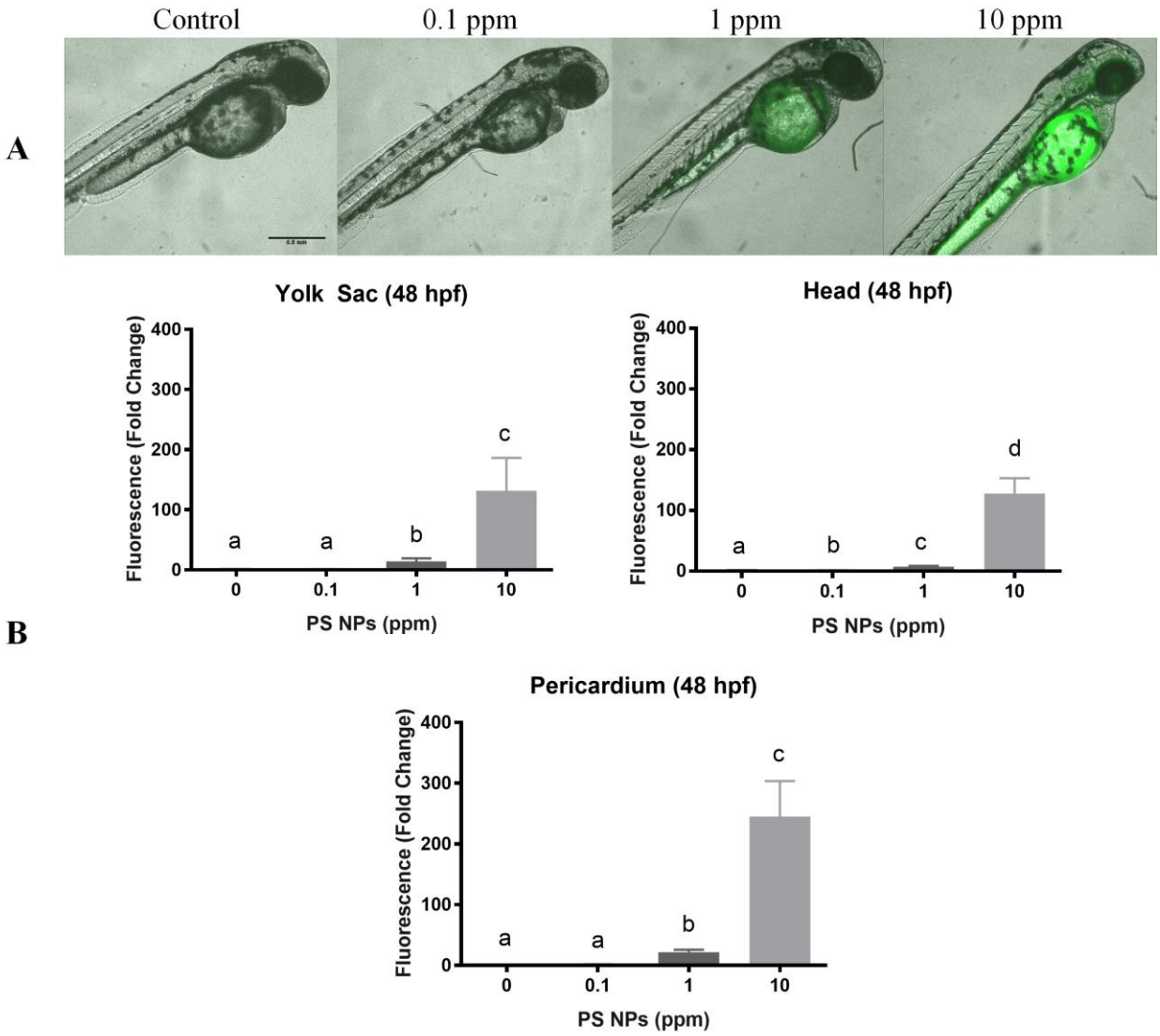

Figure S3. PS NPs fluorescence and distribution in 48 hpf zebrafish embryos
PS NPs fluorescence in zebrafish embryos at 48 hpf. (A) Fluorescence in the zebrafish larvae. (B) Fluorescence in the head, pericardial area, and yolk sac of zebrafish larvae expressed as fold change (means ± SEM). Significance was accepted if $p<0.05$. Different letters denote statistical differences across treatments.

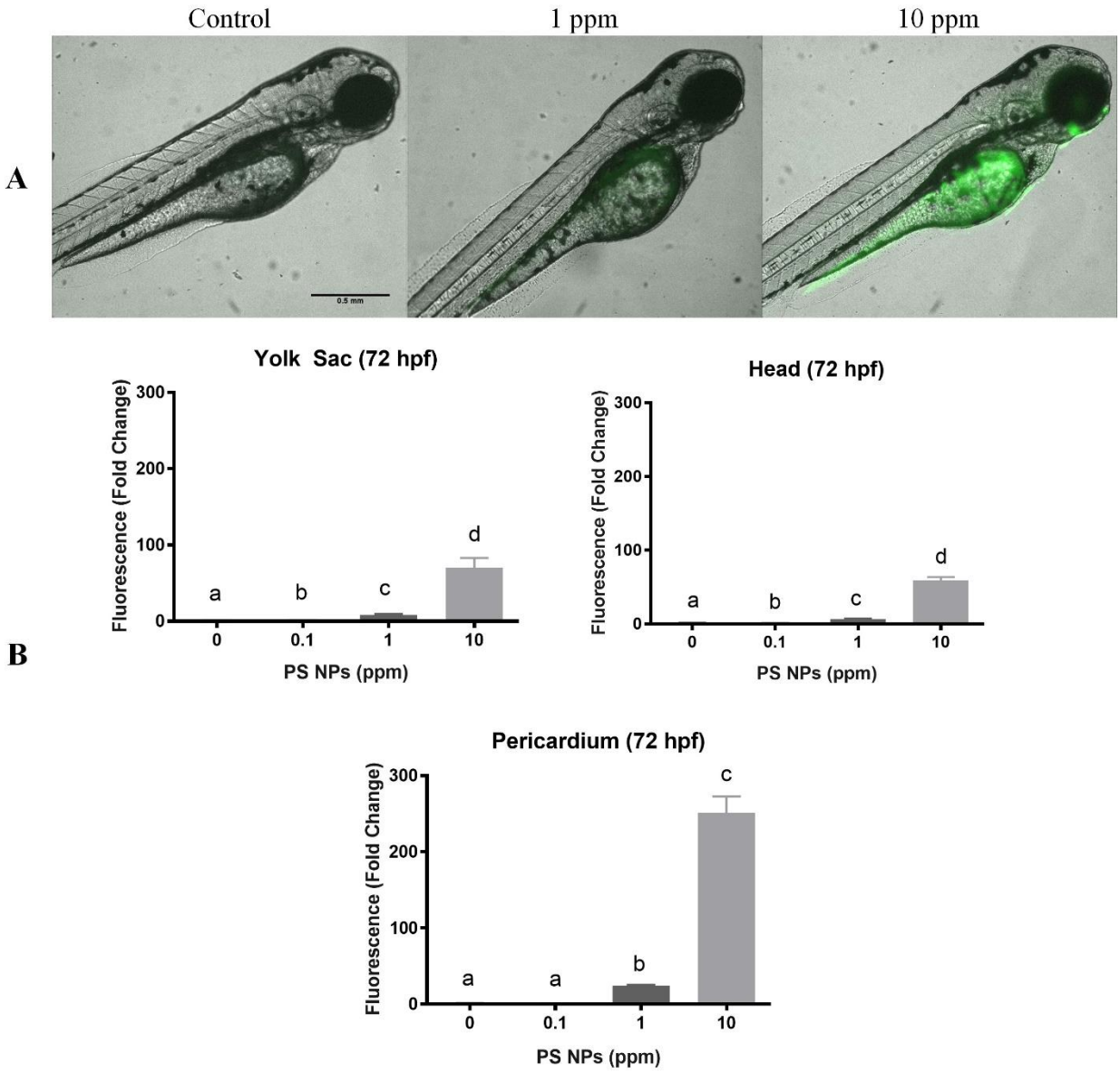

Figure S4. PS NPs fluorescence and distribution in 72 hpf zebrafish embryos
PS NPs fluorescence in zebrafish embryos at 72 hpf. (A) Fluorescence in the zebrafish larvae. (B) Fluorescence in the head, pericardial area, and yolk sac of zebrafish larvae expressed as fold change (means ± SEM). Significance was accepted if $p<0.05$. Different letters denote statistical differences across treatments.

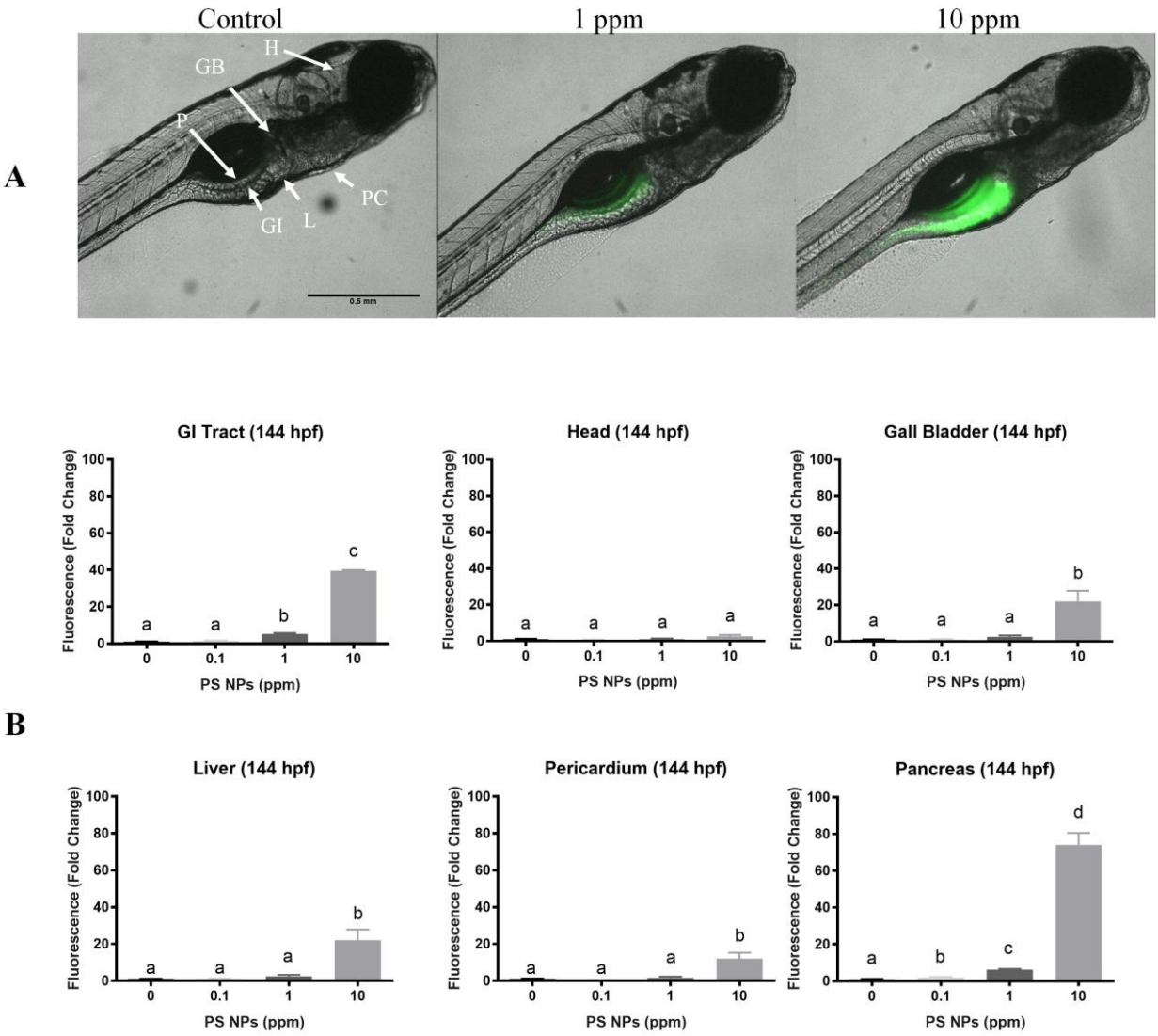

Figure S5. PS NPs fluorescence and distribution in 144 hpf zebrafish larvae

PS NPs fluorescence in zebrafish larvae at 144 hpf after 24 h of depuration (A) Fluorescence in the zebrafish larvae. The letters in the control image correspond to the various organs analyzed: (H) head, (GB) gall bladder, (P) pancreas, (PC) pericardium, (L) liver, (GI) gastrointestinal tract. (B) Fluorescence in the gastrointestinal tract, head, gall bladder, liver, pericardial area, and pancreas of zebrafish larvae presented as fold change (means ± SEM). Significance was accepted if $p<0.05$. Different letters denote statistical differences across treatments.

4. Effects of PS NPs on zebrafish larvae bioenergetics.

To evaluate the potential effects of PS NPs on zebrafish bioenergetics, oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) were assessed using the XFe24 Extracellular Flux Analyzer in 144 hpf larvae. The treatment groups included control, 0.1 ppm PS NPs, 1 ppm PS NPs, and 10 ppm PS NPs. Exposure was carried out as described at sections 2.4 and 2.8.

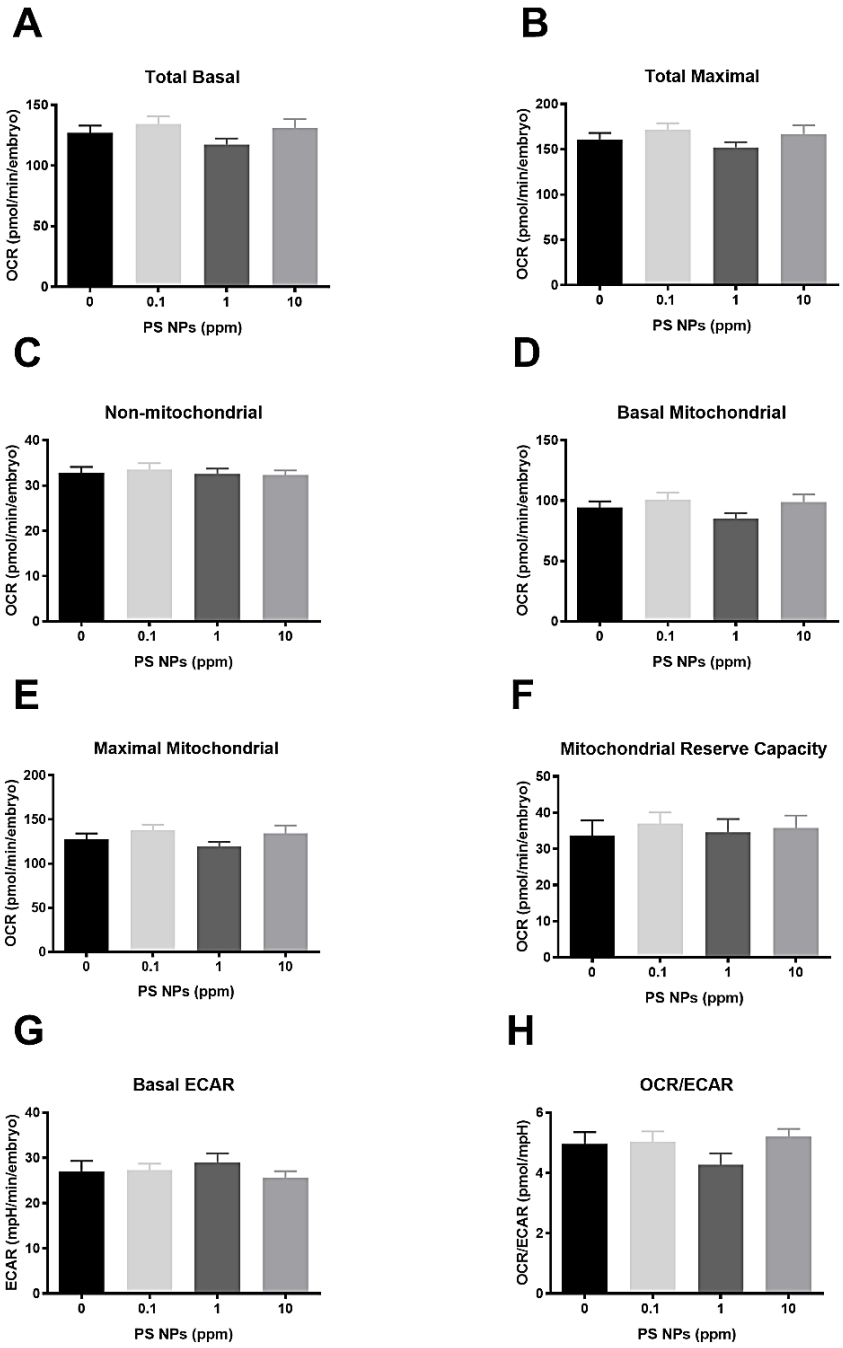

Figure S6. Mitochondrial function and metabolic partitioning in zebrafish embryos (24 hpf) Respiration (oxygen consumption rate; OCR) due to various bioenergetic parameters measured *in vivo* in 24 hpf zebrafish embryos exposed to PS NPs (n=10). (A) Total basal respiration. (B) Total maximal respiration (in the presence of FCCP). (C) Non-mitochondrial respiration (in the presence of sodium azide). (D) Basal mitochondrial respiration (total basal – non-mitochondrial). (E) Maximal mitochondrial respiration (total maximal – non-mitochondrial). (F) Mitochondrial reserve capacity (total maximal – total basal). (G) Basal extracellular acidification rate (ECAR). (H) Ratio of basal OCR to ECAR. Data are presented as means ± SEM. Significance was accepted if $p<0.05$.