

Supplementary information

Bioaccumulation of polystyrene nanoplastics and their effect on the toxicity of Au ion in zebrafish embryo

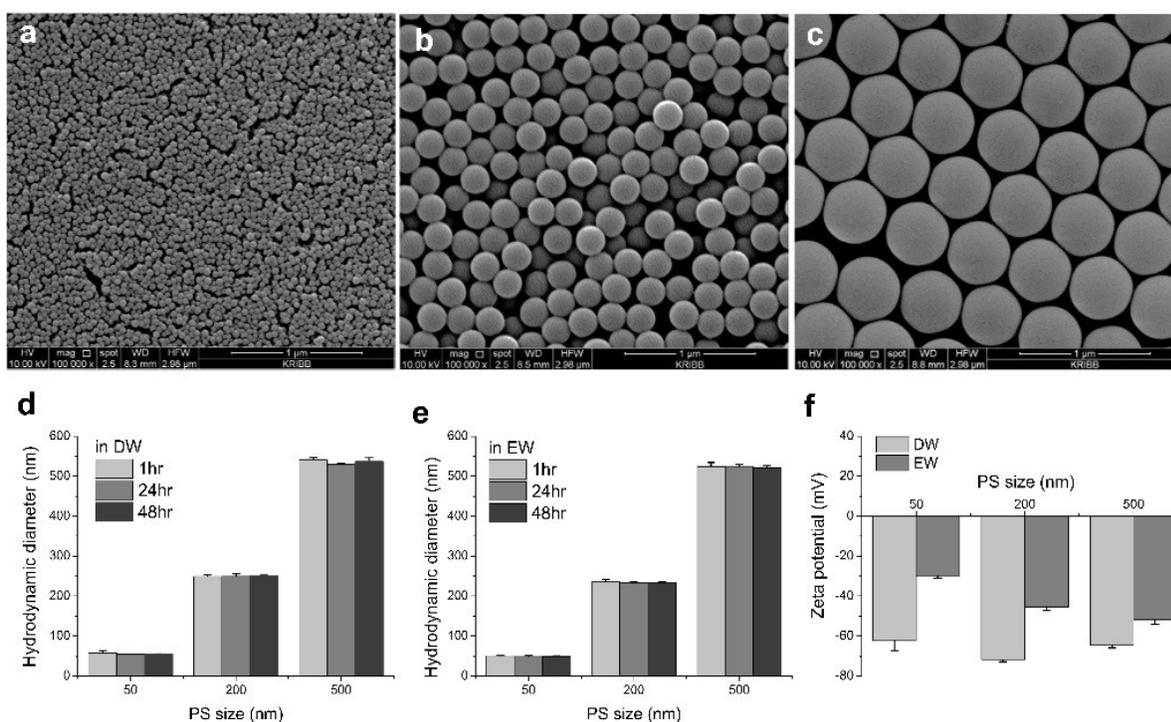


Fig. S1. Characterization of three different sized PS nanoplastics. SEM images of a) PS_{50nm}, b) PS_{200nm}, and c) PS_{500nm}, respectively. All images were obtained at 10kV and 10K magnification after gold sputtering. The scale bars indicate 1 micrometer. Hydrodynamic diameter of PS_{50nm}, PS_{200nm}, and PS_{500nm} in d) DW and e) EW, respectively at time point of 1, 24 and 48 hrs incubation. f) Zeta-potential values of three PS nanoplastics in DW and EW. Each PS nanoplastics were measured at the concentration of 0.1 mg/ml.

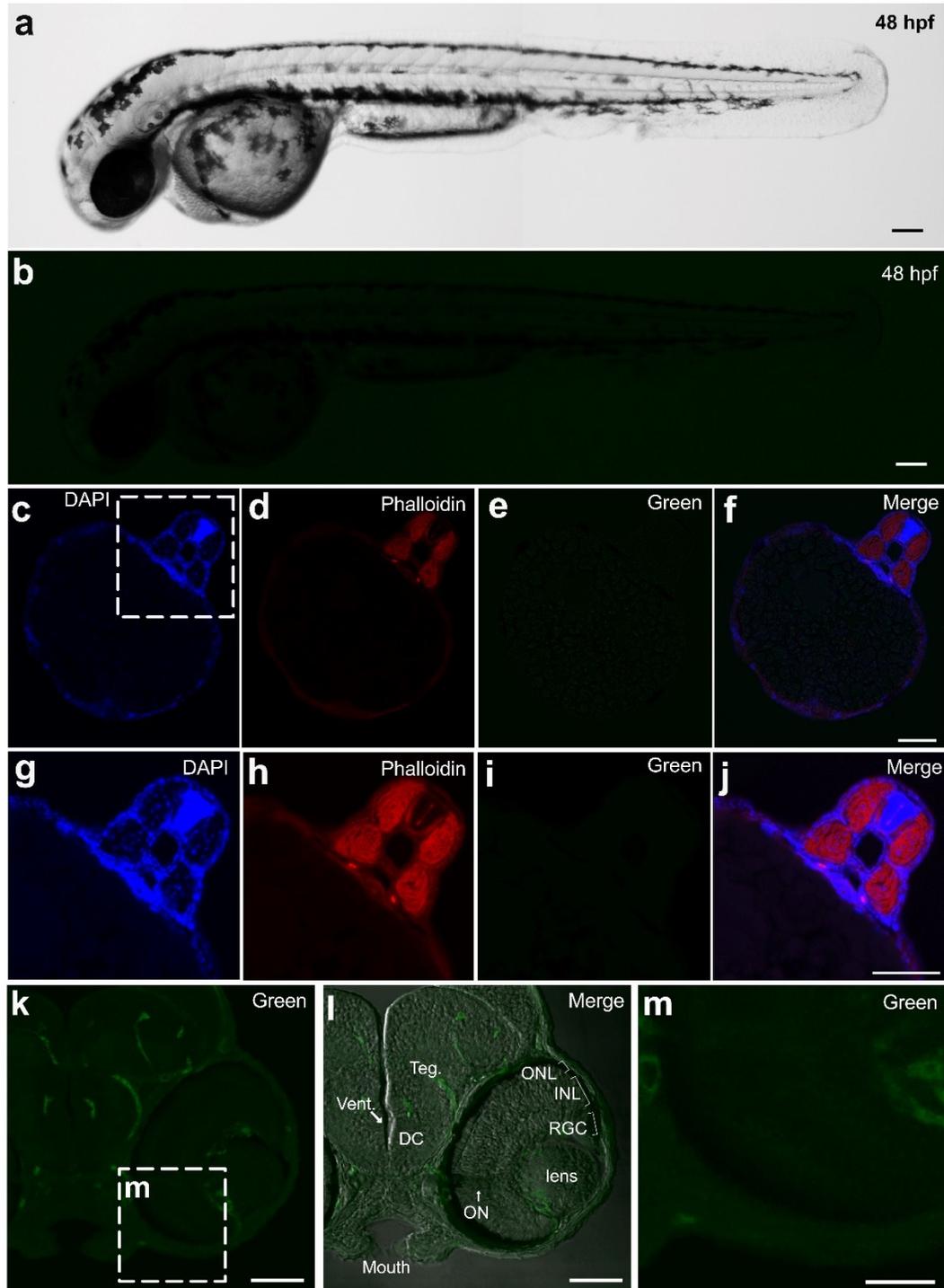


Fig. S2. Optical and fluorescent images of control ZFEs. (a) DIC image and (b) green fluorescent image of control ZFEs at 48 hpf after dechorionation. (c-f) confocal images of cross-sectioned control ZFEs with (c) blue filter for DAPI, (d) red filter for Phalloidin (e) green filter and (f) merged image. (g-j) the extended images of the white box of (c). (k-i) confocal images of cross-sectioned control ZFEs in retina region. (m) the extended image of the white box in (k). Scale bars: 100 μm (a-f); 200 μm (g-j); 50 μm (k,l) and 20 μm (m).

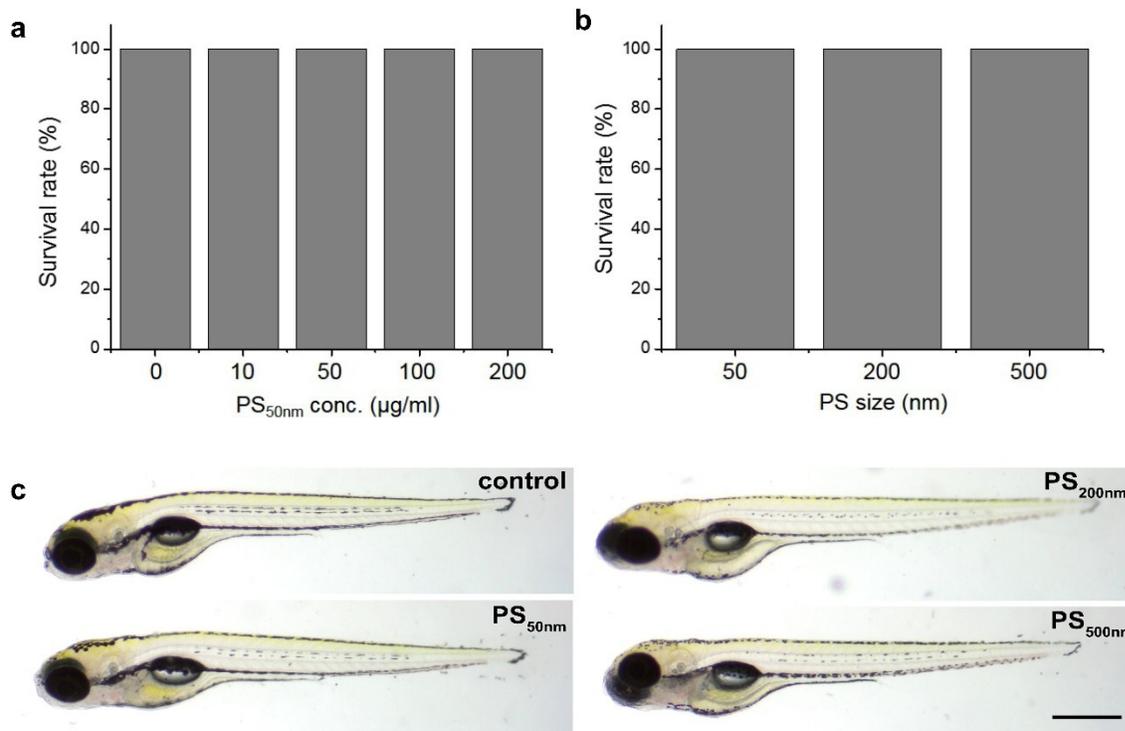


Fig. S3. (a) Survival rate of ZFEs exposed to various concentration of PS_{50nm} (0, 10, 50, 100, 200 µg/ml). (b) Survival rate of ZFEs exposed to various sized PS nanoplastics (50, 200, and 500 nm). All experiments were performed triplicate (n=10). (c) Optical images of 5 dpf ZFEs exposed to none as control, PS_{50nm}, PS_{200nm}, and PS_{500nm} at fixed concentration (0.1 mg/ml).

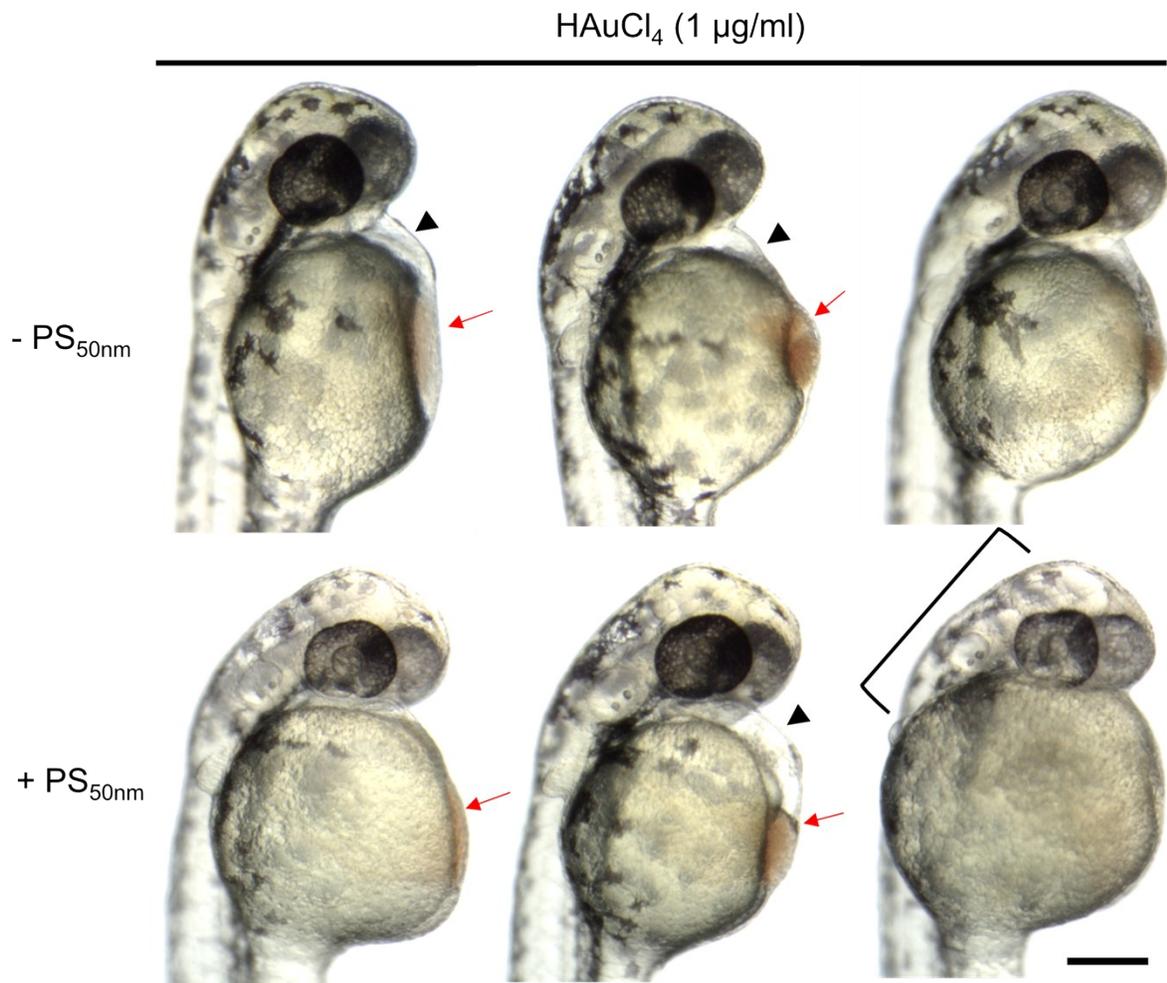


Fig. S4. Optical images of dechorionated ZFEs after 24 hrs exposure to HAuCl₄ (1 µg/ml) without or with PS_{50nm} nanoplastics (0.1 mg/ml) at 48 hpf. These ZFEs were exposed to each sample at 24 hpf. Scale bar: 500 µm.

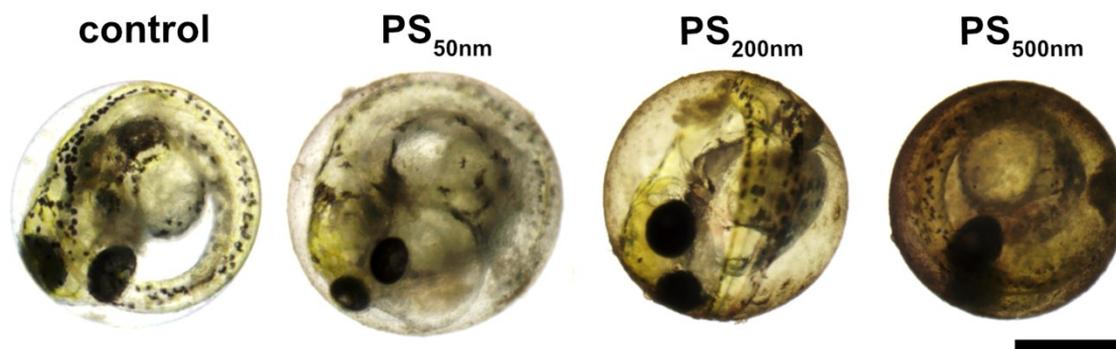


Fig. S5. Optical images of ZFEs after exposure to H₂AuCl₄ (1 μ g/ml) with three different sized PS nanoplastics (0.1 mg/ml) at 120 hpf. These ZFEs were exposed to each sample at 24 hpf. Scale bar: 500 μ m.

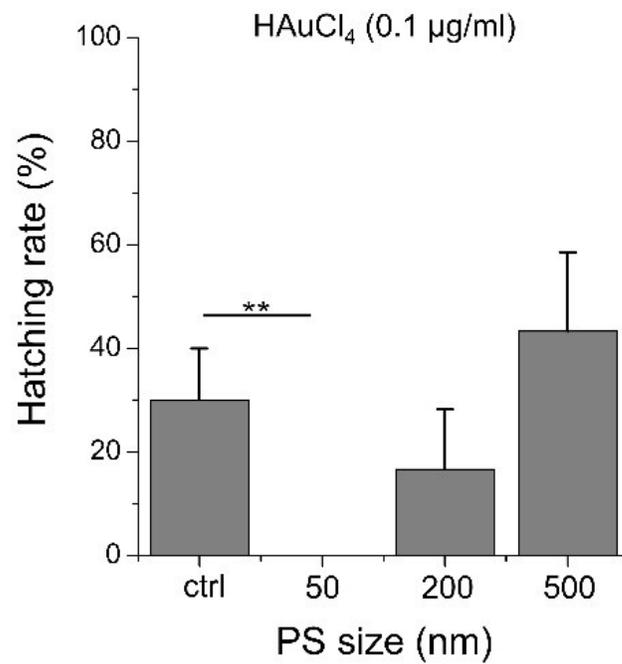


Fig. S6. Hatching rate of ZFEs at 120 hpf after exposure to HAuCl_4 ($0.1 \mu\text{g/ml}$) with three different sized PS nanoparticles (0.1 mg/ml). The ZFEs were exposed to each sample at 24 hpf. The asterisks (**) indicate a significant difference between the treatment group and control ($p < 0.01$)

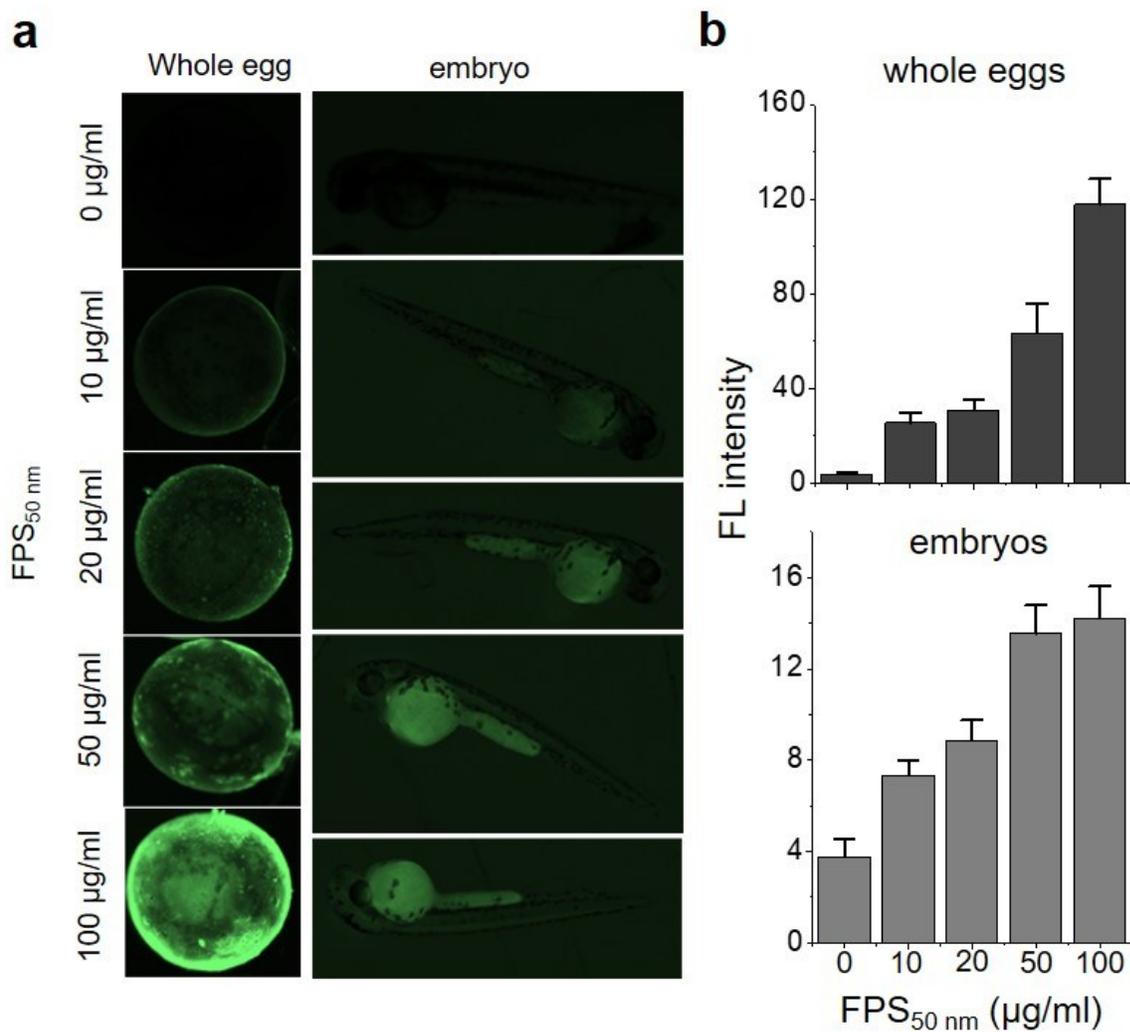


Fig. S7. (a) Fluorescent images of ZFEs after exposure to HAuCl_4 ($1\mu\text{g/ml}$) and various concentration of FPS_{50 nm} at 0, 10, 20, 50, 100 $\mu\text{g/ml}$. These ZFEs were exposed to each sample at 24 hpf for 24 hrs and the images were taken before and after dechoriation. (b) Quantitative comparison of accumulated FPS_{50 nm} nanoplastics in whole eggs and embryos by analyzing using ImageJ software (n=8).

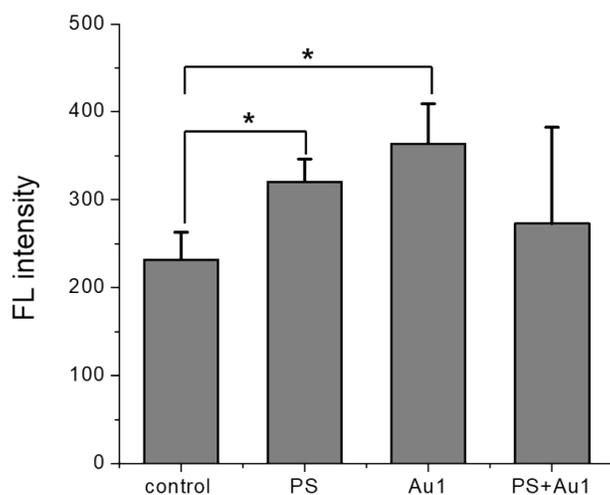


Fig. S8. Mitochondrial ROS generation of ZFEs either in absence or presence of PS_{50nm} (100µg/ml) nanoplastics and HAuCl₄ (1µg/ml) using MitoSOX™ Red (Molecular Probes, OR, USA) which is mitochondrial superoxide indicator. Ten ZFEs were treated PS_{50nm} or HAuCl₄ for 24 hrs at 24 hpf. After washing with fresh E3 egg water at 48 hpf, the ZFEs were stained by 5µM MitoSOX™ Red for 30 min. Then, the fluorescence was measured with excitation/emission set at 510nm/580nm after washing three times.