Supporting information

Zebrafish High Throughput Screening to Study the Impact of Dissolving Metal Oxide Nanoparticles on the Hatching Enzyme, ZHE1

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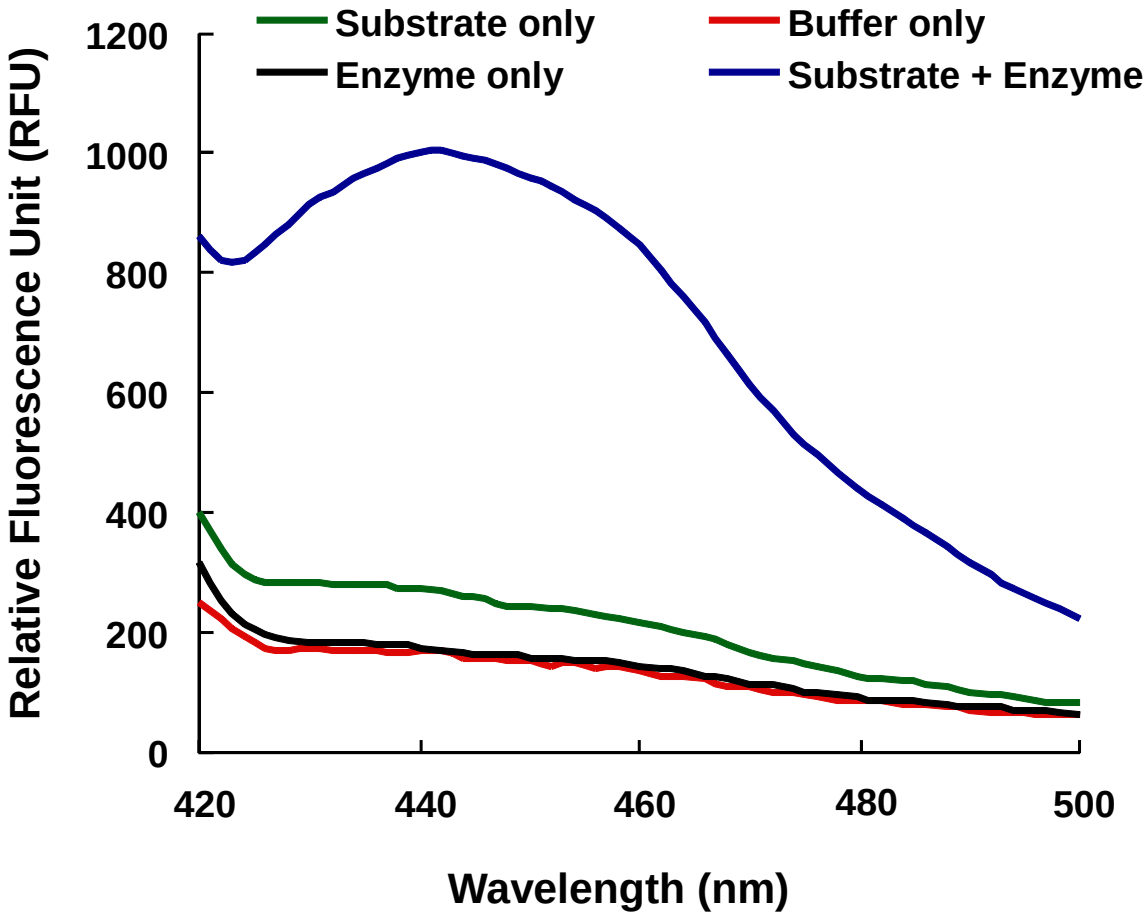

Figure S1A. Fluorescence spectra of samples containing a mixture of rec. ZHE1 and MCA (blue), enzyme alone (black), MCA substrate alone (green), or buffer alone (red). The increased fluorescence intensity at 460 nm is the result of substrate cleavage, leading to the release of a fluorescent fragment.

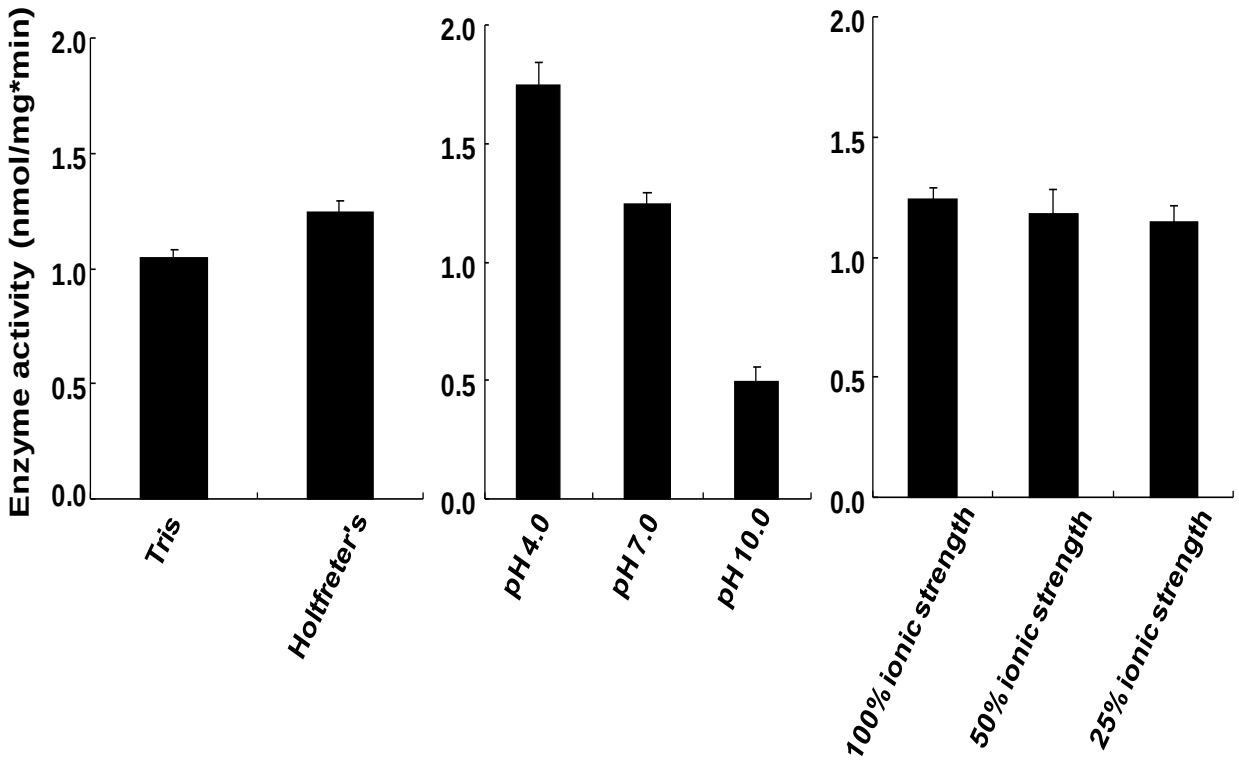

Figure S1B. Quantification of enzyme activity in different media. Left panel: rec. ZHE1 activity was similar in Tris-HCl buffer and Holtfreter's medium; Middle: Increase in rec. ZHE1 activity upon acidification of Holtfreter's medium; Right panel: rec. ZHE1 activity was not affected by the ionic strength of Holtfreter's medium.

Figure S2. Picture of the robotic pick-and-plate system. The integrated robotic system is comprised of a 6-axis robotic arm (Denso Robotics), a vision-recognition camera (Cognex Corporation) mounted on the robotic arm, a serological pipette and a liquid handling syringe pump (Hamilton Company USA). This integrated system speeds up the embryo pick-and-plate process to the extent that we can prepare 20~25 96-well plates in 2 hours. This allows us to increase the number of materials that can be investigated in a single experiment, as demonstrated in Figure 4B that shows the concurrent analysis of the hatching rate of zebrafish embryos in response to 24 metal oxides nanoparticles.

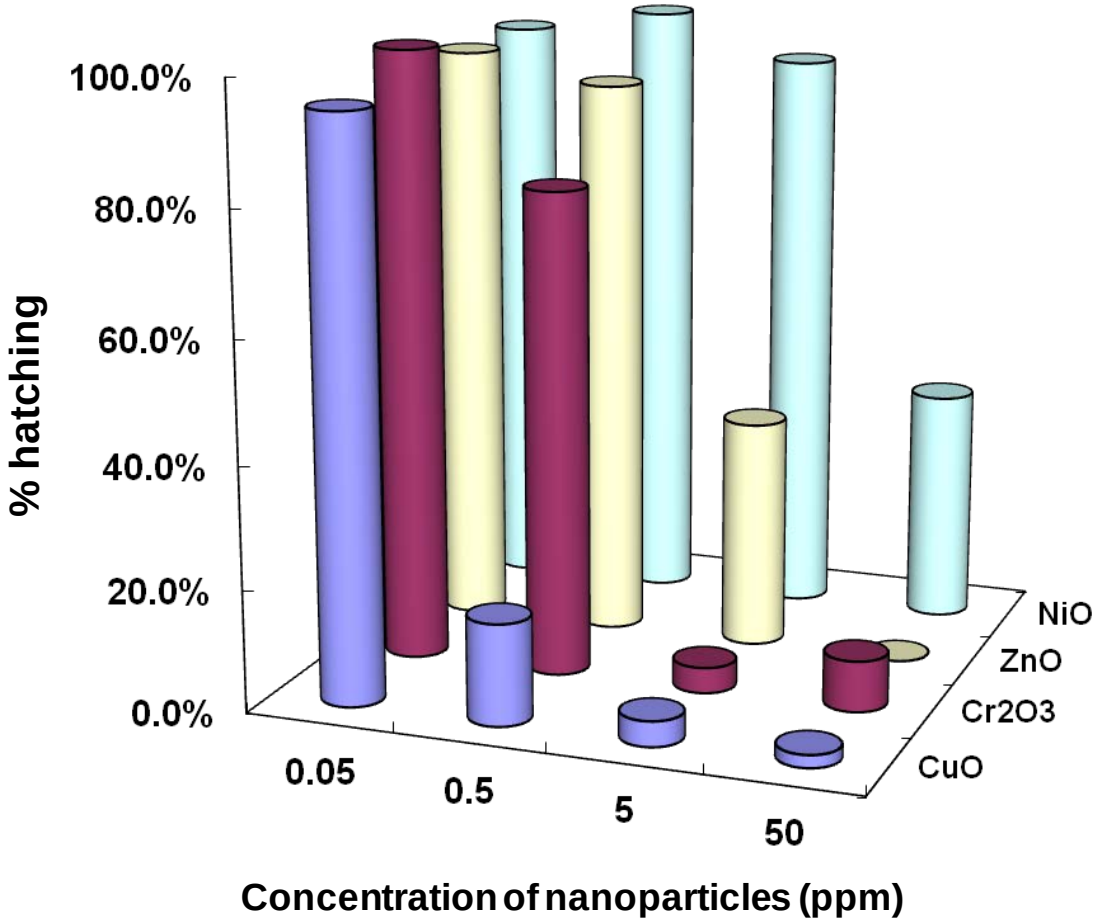

Figure S3. Dose-dependent hatching interference in embryos exposed to CuO, Cr_2O_3, ZnO and NiO nanoparticles. Zebrafish embryos (4 hpf) were exposed to CuO, Cr_2O_3, ZnO and NiO nanoparticles at concentrations of 0.05, 0.5, 5 and 50 ppm. Significant hatching interference of zebrafish embryos was observed at concentrations of: CuO >0.5 ppm, ZnO and Cr_2O_3 > 5 ppm and NiO nanoparticles > 50 ppm.

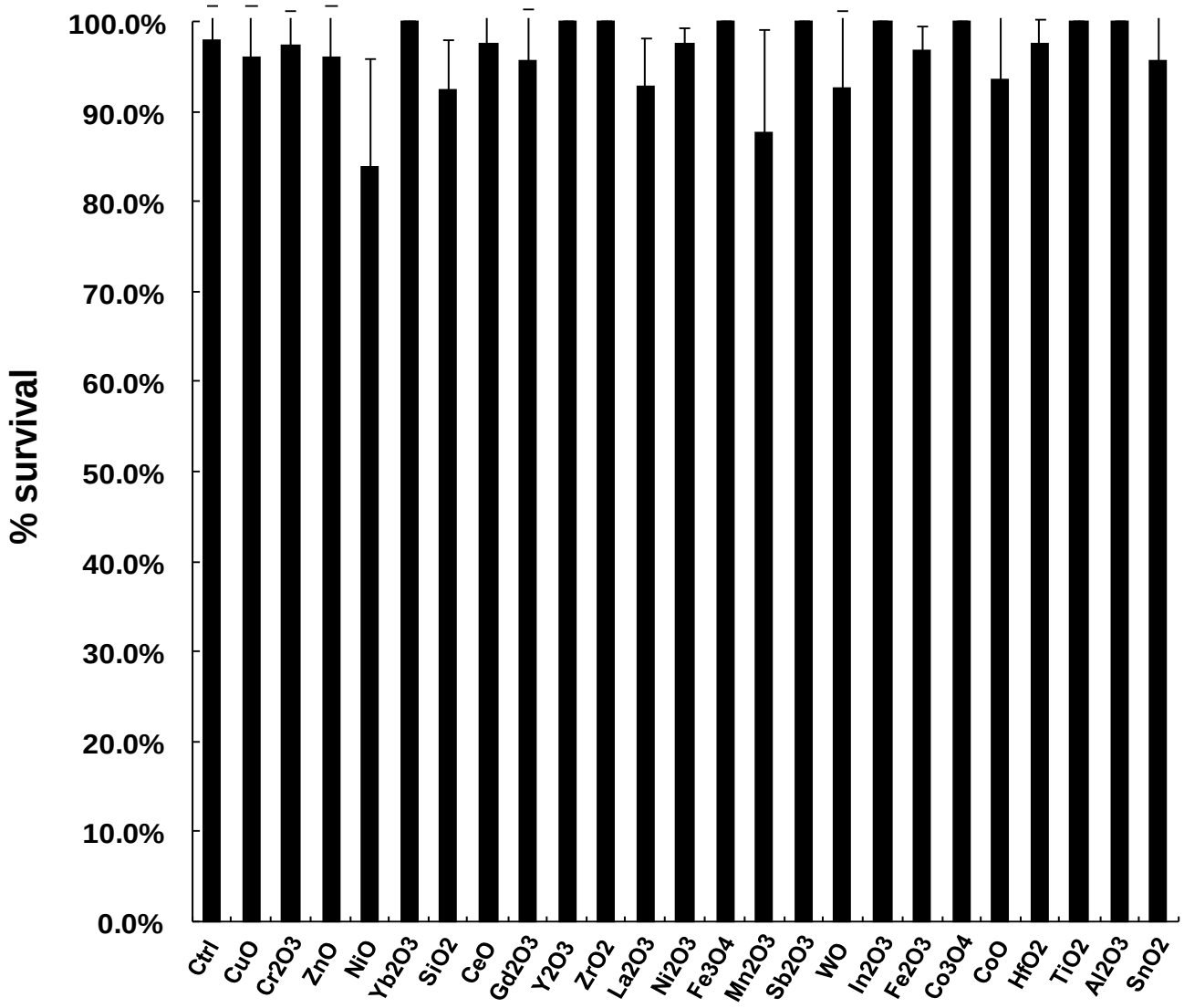

Figure S4. The survival rate of zebrafish embryos exposed to the 24 metal oxide nanoparticles, each at 50 ppm. The nanoparticle exposure did not affect the survival rate of zebrafish embryos. Survival rates were assessed by using high content imaging at 72 hpf.

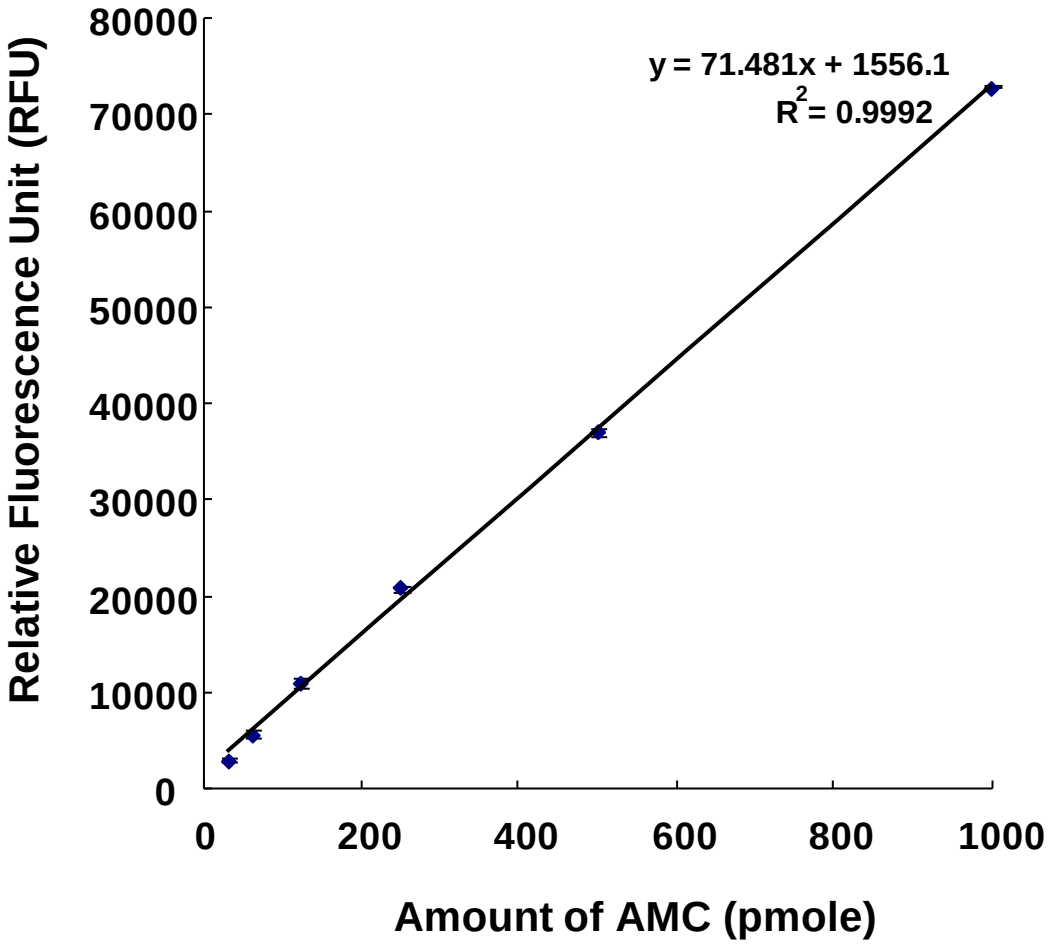

Figure S5. Calibration curve for the calculation of rec. ZHE1 activity. The rec. ZHE1 activity was experimentally measured based on the rate of increasing fluorescence intensities per minute (RFU/mg*min) by a microplate spectrophotometer. In order to convert RFU to the molar quantities of substrate being digested by rec. ZHE1, a calibration curve was constructed by measuring the RFU of known quantities (31.25, 62.5, 125, 250, 500, 1000 pico-mole) of AMC molecules (the fluorescent compound after enzyme digestion) and fit by linear regression. The slope of the resulted linear curve that was calculated as 71.481 corresponds to RFU per unit amount (pico-mole) of consumed substrate. The rec. ZHE1 activity was then converted from RFU/mg*min to nmole/mg*min.