

1                             **Supplementary Information for**  
2                             **Poly (acrylic acid)-coated titanium dioxide nanoparticle and ultraviolet light co-exposure has**  
3                             **minimal effect on developing zebrafish (*Danio rerio*)**

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18 Fig. S1 Schematic of experimental design and endpoints examined. Zebrafish embryos (24 hpf)  
19 were exposed to uncoated TiO<sub>2</sub> NPs, polymer-coated TiO<sub>2</sub> NPs, or polymer NPs from 24 to 168  
20 hpf. NP suspensions were renewed at 74 and 122 hpf. Larvae (168 hpf) were washed thrice with  
21 DTW, snap frozen in liquid nitrogen, and stored at -80 °C immediately after the experimental  
22 period. Measured endpoints included survival (S), hatching success (H), malformation (M),  
23 TBARS, Cat activity, TG levels, Sod activity, as well as alteration of gene expression (GE). A gray  
24 rectangle indicates an 8 h UV light exposure period.

25

26 Fig. S2 Representative micrographs of 144 hpf larvae showing (A) normal development and  
27 incidences of malformation including (B) bs, (C) pe, and yse. Scale bars are 1 mm.

28

29 Fig. S3 Representative TEM images of (A) uncoated TiO<sub>2</sub> NPs (scale bar = 100 nm) and (B)  
30 polymer-coated TiO<sub>2</sub> NPs (scale bar = 20 nm). White arrow denotes an individual particle.

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32 Fig. S4 Percent survival of zebrafish exposed to NPs in the absence (-) or presence (+) of UV  
33 illumination. Embryos were exposed to 0 (DTW), 0.1, 1 or 10 mg/L (A, B) uncoated TiO<sub>2</sub> NPs, (C,  
34 D) polymer-coated TiO<sub>2</sub> NPs, or (E, F) polymer NPs ( $n = 7$ ) for 6 d from 24 hpf. Half of the NP  
35 exposed zebrafish were illuminated for 8 h / d for 5 subsequent days, as indicated by the gray  
36 bars. The other half was kept under ambient fluorescent lighting. Each  $n$  represents 4-well plate  
37 replicate consisting of 80 embryos (20 embryos/well). Values are mean  $\pm$  SEM (two-way  
38 ANOVA, Dunnett's, unpaired t-test,  $p > 0.05$ ).

39

40 Fig. S5 Malformations caused by NP and UV light co-exposure. Percent incidence of  
41 malformation observed in 144 hpf larvae exposed to 0 (DTW), 0.1, 1 or 10 mg/L (A, B) uncoated  
42 TiO<sub>2</sub> NPs, (C, D) polymer-coated TiO<sub>2</sub> NPs, or (E, F) polymer NPs (n = 4) in the absence (–) and  
43 presence (+) of UV illumination. Each n represents 4-well plate replicate consisting of 80  
44 embryos (20 embryos/well). Malformations were categorized as normal, bs, pe, or yse. Values  
45 are mean ± SEM (two-way ANOVA, Dunnett's, unpaired t-test, p > 0.05).

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47 Fig. S6 Uncoated TiO<sub>2</sub> NPs absorb at wavelengths used for TG (412 nm), Sod (450 nm), TBARS  
48 (531 nm), Cat (560 nm), and protein (562 nm) assay measurements. Representative absorption  
49 spectral scans (200-800 nm) of 100 mg/L uncoated TiO<sub>2</sub> NP, polymer-coated TiO<sub>2</sub> NP and  
50 polymer NPs. Spectral scans of NPs diluted with phosphate, 1x reaction, or sucrose buffer were  
51 similar. Uncoated and polymer-coated TiO<sub>2</sub> NPs had maximum absorption peaks at 300 and  
52 292 nm, respectively, as indicated by the black arrows.

53

54 Fig. S7 NP interference with biochemical assay components, without (–) and with (+) analyte.  
55 Difference in reported (A) TBARS, (C) Cat, (E) TG, (G) Sod, and (I) protein without analyte  
56 addition in the presence of 0.1, 1 or 10 mg/L uncoated TiO<sub>2</sub> NPs, polymer-coated TiO<sub>2</sub> NPs, or  
57 polymer NPs (n = 3). Analytes tested included 3.125 uM TEP, 2 U/mL Cat, 20 μmol/L GSSG,  
58 100 U/mL Sod from horseradish, 1000 μg/mL bovine serum albumin (BSA), and 168 hpf  
59 zebrafish larvae (n = 11-20). Difference in reported (B) TBARS, (D) Cat, (F) TG, (H) Sod, and (J)  
60 protein with analyte addition in the presence of 10 mg/L uncoated TiO<sub>2</sub> NPs (n = 3). Each n  
61 represents either a 96-well microplate replicate or pooled 168 hpf larvae per treatment. An

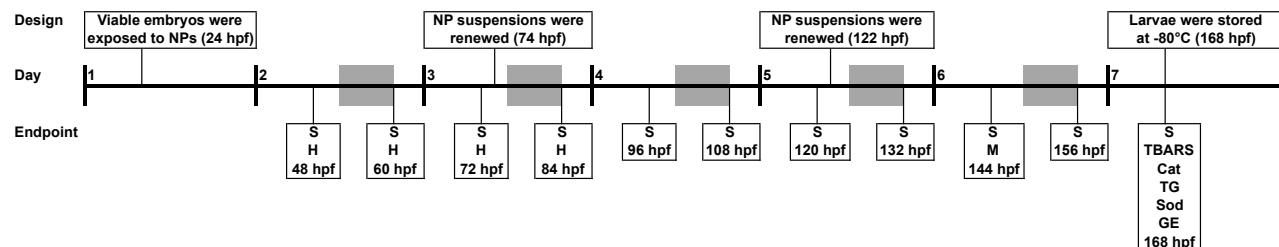
62 asterisk (\*) indicates significant difference compared to DTW control (one-way ANOVA,  
63 Dunnett's,  $p < 0.05$ ) or between treatment groups (unpaired t-test,  $p < 0.05$ ). Values are mean ±  
64 SEM.

65

66 Fig. S8 Changes in endogenous control gene expression patterns. Relative fold change of *ef1α* in  
67 zebrafish larvae exposed to 0 (DTW), 0.1, 1 or 10 mg/L uncoated TiO<sub>2</sub> NPs, polymer-coated TiO<sub>2</sub>  
68 NPs, or polymer NPs ( $n = 3$ ) in the absence (–) or presence (+) of illumination for 6 d from 24  
69 hpf. Data are relative to unexposed DTW control larvae (dotted line;  $n = 3$ ). Each  $n$  represents  
70 five randomly pooled 168 hpf larvae per treatment. Values are mean ± SEM (two-way ANOVA,  
71 Tukey's,  $p > 0.05$ ).

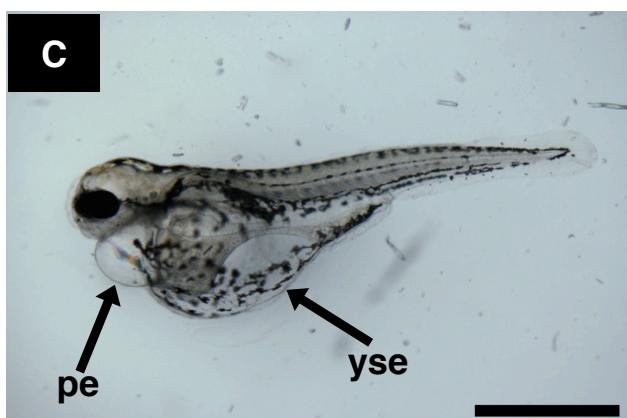
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73 Fig. S1



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75 Fig. S2



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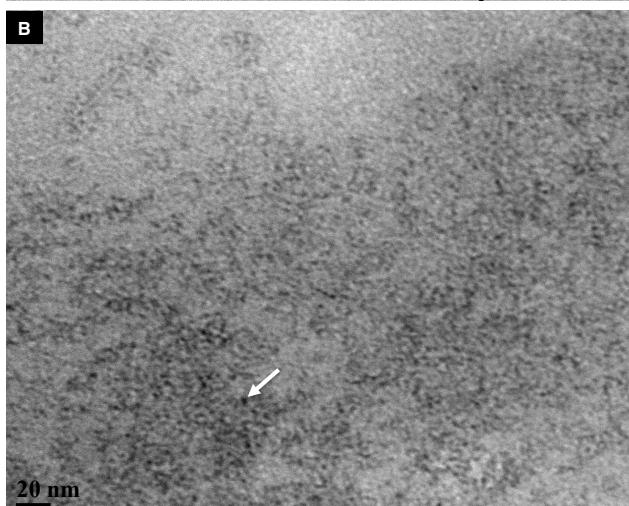
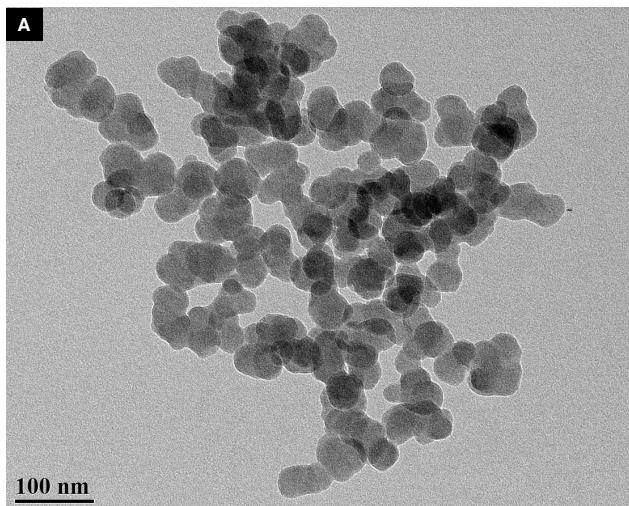
77 Table S1. Gene-specific primers for zebrafish.

Gene Name (Abbreviation)	Reference Sequence	F / R	Primer Sequence (5' – 3')	Length	E
<i>Elongation factor 1 alpha (ef1α)</i>	NM_131263.1	F	TTCTCAGGCTGACTGTGCTG	83	2.01
		R	GGGTCTGTCCGTTCTGGAG		
<i>Catalase (cat)</i>	NM_130912.1	F	AACAACCCTCCAGACAGACC	115	1.92
		R	TCCGTCGACTTTCTCTGTCG		
<i>Glutathione peroxidase 1a (gpx1a)</i>	NM_001007281.2	F	TTTACGACCTGTCCCGAAA	108	2.02
		R	CTGTTGTGCCTCAAAGCGAC		
<i>Superoxide dismutase 2 (sod2)</i>	NM_199976.1	F	GAGCCTCACATCTGTGCTGA	111	2.04
		R	CTTGGCCAGAGCCTCTTGAT		

78 NCBI reference sequence, forward (F) and reverse (R) primer sequences (5' – 3') designed for  
 79 this study, amplicon length in base pairs, and amplification efficiency (E) of endogenous control  
 80 gene (*ef1α*) and genes of interest (*cat*, *gpx1a*, and *sod2*) measured using quantitative  
 81 polymerase chain reaction.

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83 Fig. S3



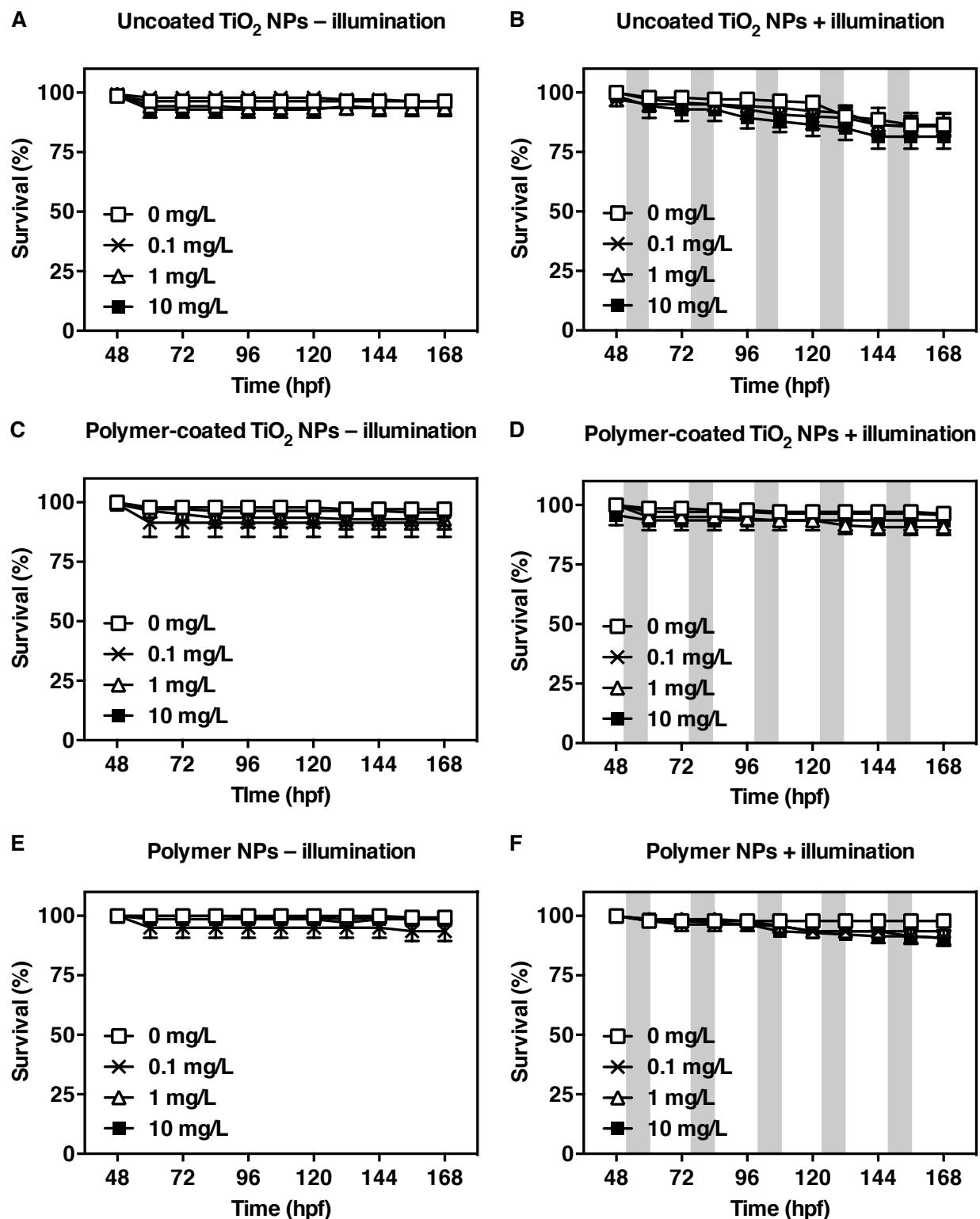
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85 Table S2. Physicochemical characteristics of NP suspensions over time. Time-dependent  
 86 changes in hydrodynamic diameter (nm), polydispersity index, and  $\zeta$  potential (mV) of uncoated  
 87 TiO<sub>2</sub> NPs (pH 7.97), polymer-coated TiO<sub>2</sub> NPs (pH 7.95), and polymer NPs (pH 7.70) diluted to  
 88 10 mg/L with DTW (pH 8.00) at 0, 24, and 48 h. Data are presented as mean  $\pm$  SEM of three  
 89 independent replicates.

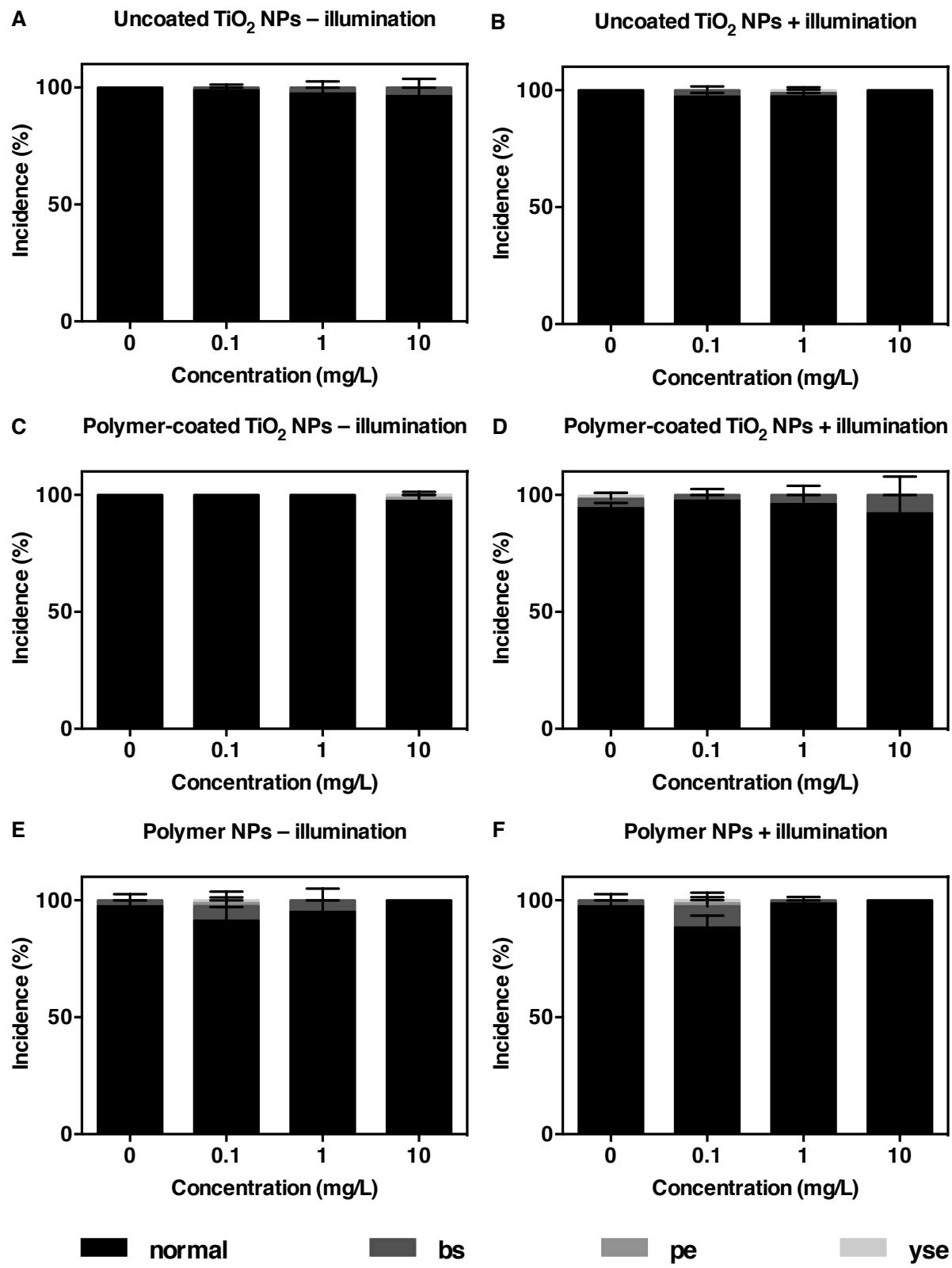
NP Type	Time (h)	Hydrodynamic Diameter (nm)	Polydispersity Index	$\zeta$ Potential (mV)
Uncoated TiO <sub>2</sub> NPs	0	2460.00 $\pm$ 25.51	0.71 $\pm$ 0.04	-14.57 $\pm$ 0.43
	24	5974.33 $\pm$ 610.72	1.00 $\pm$ 0.00	-13.77 $\pm$ 0.20
	48	5300.67 $\pm$ 1047.48	1.00 $\pm$ 0.00	-13.80 $\pm$ 0.21
Polymer-coated TiO <sub>2</sub> NPs	0	1165.00 $\pm$ 15.04	0.43 $\pm$ 0.01	-14.50 $\pm$ 0.21
	24	826.90 $\pm$ 28.37	0.67 $\pm$ 0.07	-13.80 $\pm$ 0.50
	48	814.27 $\pm$ 29.76	0.74 $\pm$ 0.06	-14.43 $\pm$ 0.22
Polymer NPs	0	84.82 $\pm$ 0.97	0.15 $\pm$ 0.04	-12.73 $\pm$ 0.23
	24	103.80 $\pm$ 1.06	0.04 $\pm$ 0.01	-13.13 $\pm$ 0.67
	48	111.00 $\pm$ 1.40	0.05 $\pm$ 0.01	-13.63 $\pm$ 0.19

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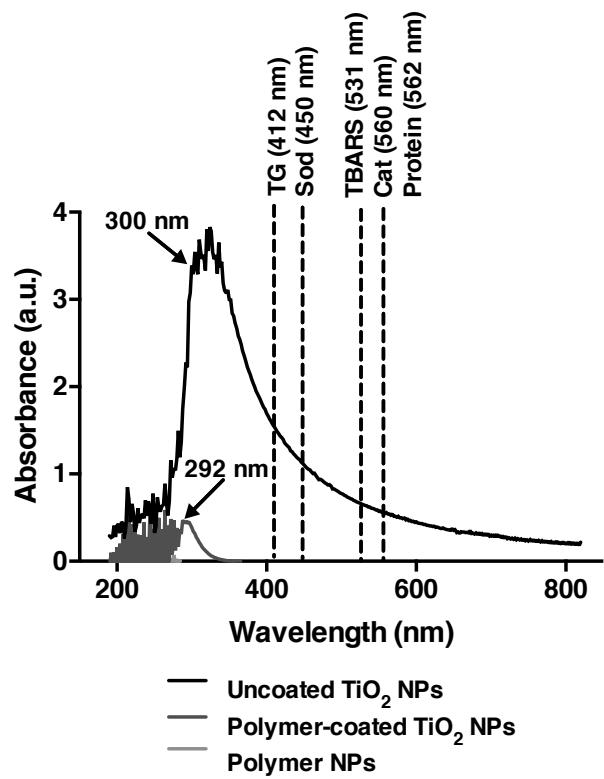
91 Fig. S4



93 Fig. S5

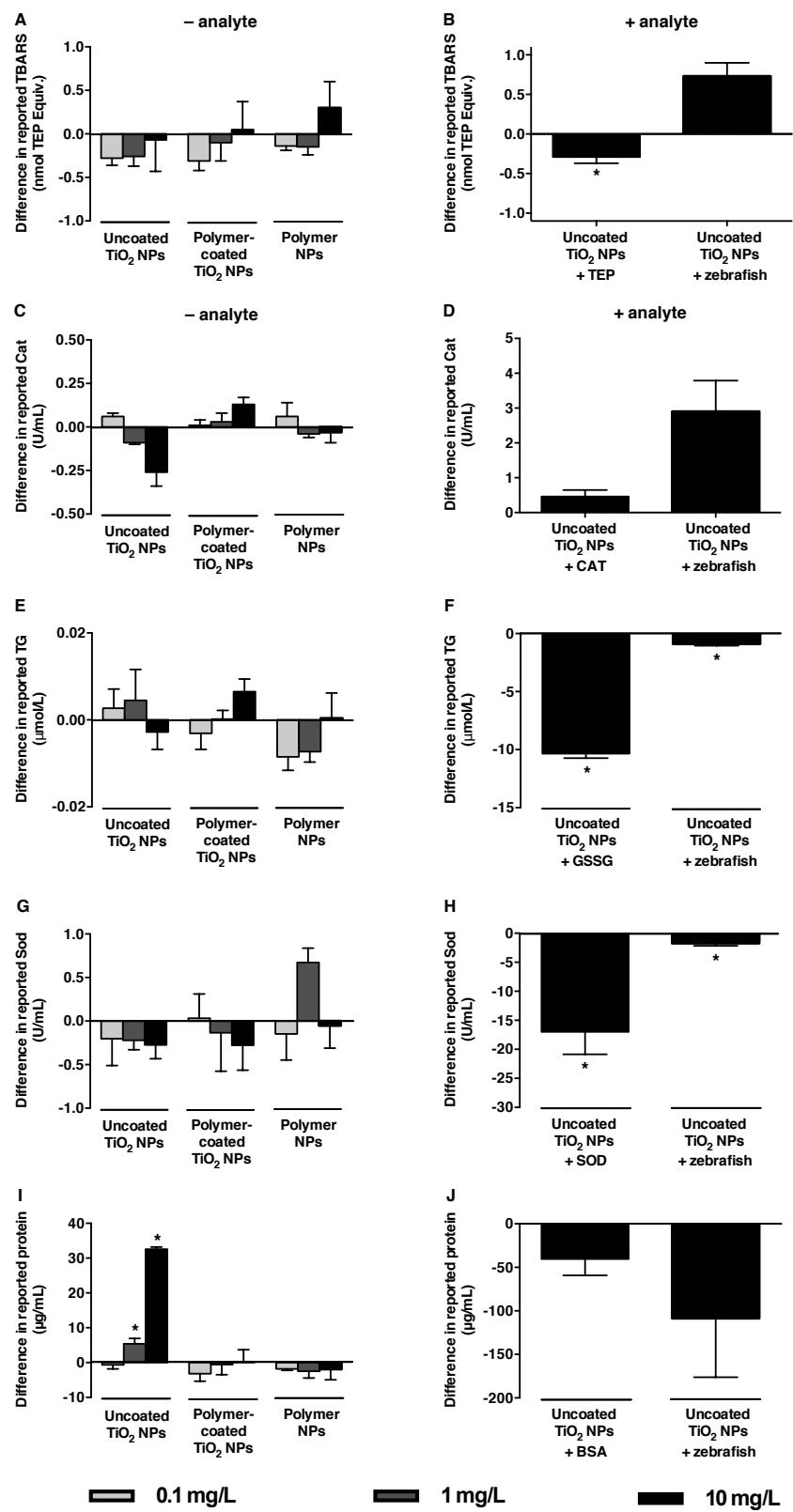


95 Fig. S6

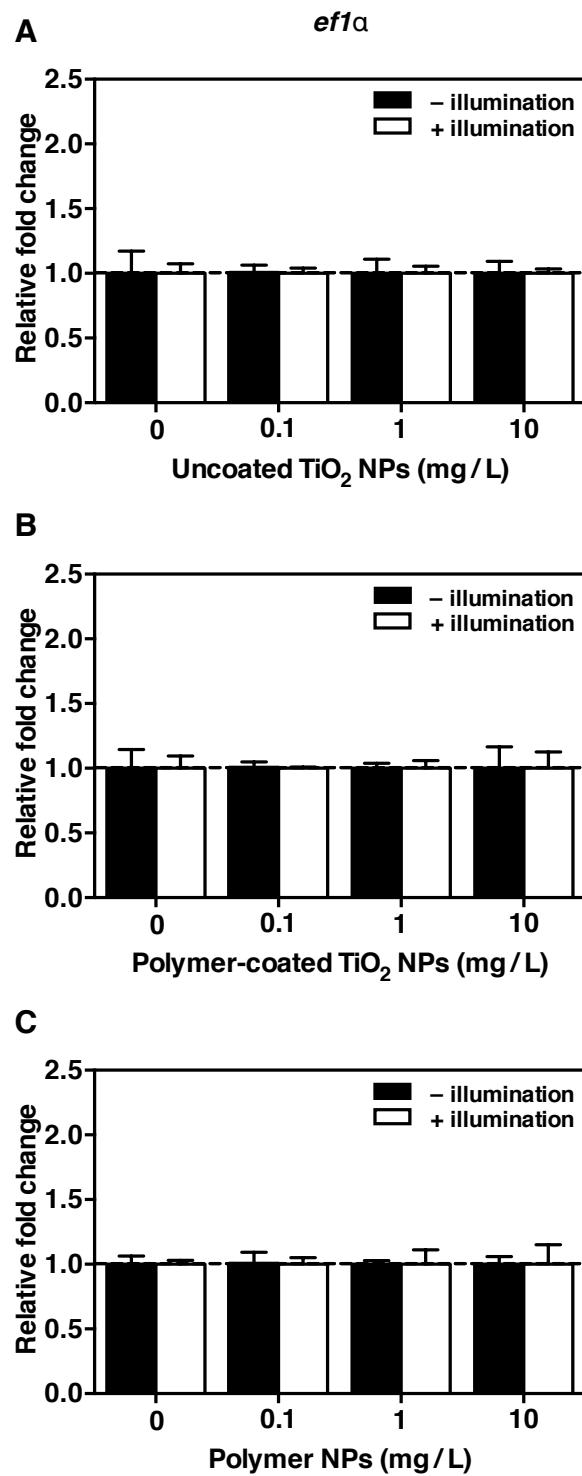


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97 Fig. S7



99 Fig. S8



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101 Table S3. Mean, SD, and CV of Ct values among treatment groups and over the entire data set.

NP Type	+/- Illumination	Mean	SD	CV (%)
Uncoated TiO <sub>2</sub> NPs	+	17.14	0.04	0.22
	-	17.24	0.05	0.29
Polymer-coated TiO <sub>2</sub> NPs	+	17.67	0.06	0.31
	-	17.92	0.07	0.42
Polymer NPs	+	17.16	0.07	0.43
	-	17.28	0.06	0.33
All Data	+/-	17.40	0.36	2.04

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