Electronic Supplementary Material (ESI) for RSC Advances.

Electronic Supplementary Information

TiO_2 nanoparticles generate superoxide and alter gene expression in human lung cells

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Table S1. Sequencing reads summary of RNA-Seq libraries.

Sample	Total sequencing reads	Uniquely mapped reads	Uniquely mapped reads (% of total reads)	Unmapped reads	Unmapped reads (% of total reads)	Total mapped reads	Total mapped reads (% of total reads)
Generation 1							
Ctrl(G1)_1	59,751,708	48,181,700	80.64	386,879	0.65	59,364,829	99.35
Ctrl(G1)_2	64,587,351	54,215,971	83.94	422,184	0.65	64,165,167	99.35
Ctrl(G1)_3	49,945,215	41,444,145	82.98	304,587	0.61	49,640,628	99.39
G1_1	50,098,890	42,289,847	84.41	385,139	0.77	49,713,751	99.23
G1_2	49,555,439	41,726,349	84.20	394,522	0.80	49,160,917	99.20
G1_3	48,412,083	39,612,225	81.82	395,774	0.82	48,016,309	99.18
Generation 10							
Ctrl(G10)_1	41,819,285	33,728,982	80.65	805,253	1.93	41,014,032	98.07
Ctrl(G10)_2	46,062,713	37,682,665	81.81	633,069	1.37	45,429,644	98.63
Ctrl(G10)_3	46,412,991	37,832,576	81.51	637,844	1.37	45,775,147	98.63
G10_1	40,197,773	32,578,151	81.04	618,449	1.54	39,579,324	98.46
G10_2	41,105,591	33,348,713	81.13	633,362	1.54	40,472,229	98.46
G10_3	40,947,519	33,971,224	82.96	589,528	1.44	40,357,991	98.56
Re-challenge							
Ctrl (ReC)_1	46,380,195	36,327,224	78.32	715,819	1.54	45,664,376	98.46
Ctrl (ReC)_2	58,541,006	47,129,751	80.51	818,210	1.40	57,722,796	98.60
Ctrl (ReC)_3	48,280,217	36,644,376	75.90	647,652	1.34	47,632,565	98.66
ReC_1	55,447,838	39,019,388	70.37	608,370	1.10	54,839,468	98.90
ReC_2	45,279,571	32,152,960	71.01	617,851	1.36	44,661,720	98.64
ReC_3	43,690,593	30,451,739	69.70	717,512	1.64	42,973,081	98.36

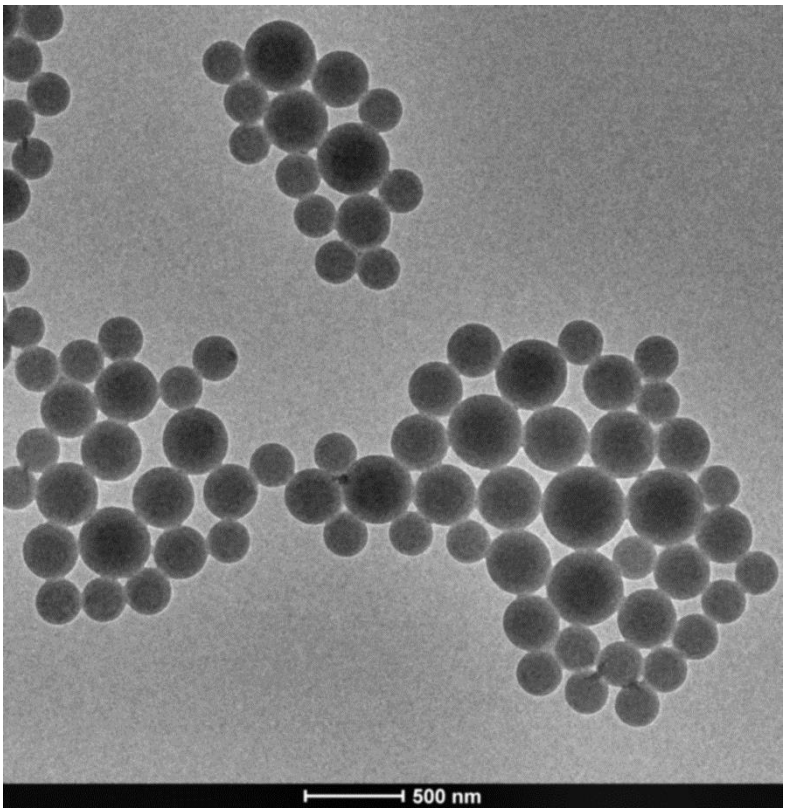
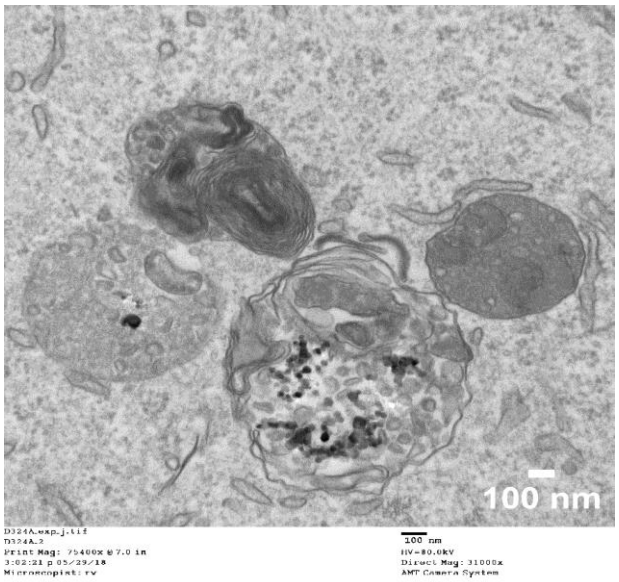


Figure S1. Representative TEM image of the polystyrene NPs (#F8806, Life Technologies, Carlsbad, CA) used in experiments.

A



B

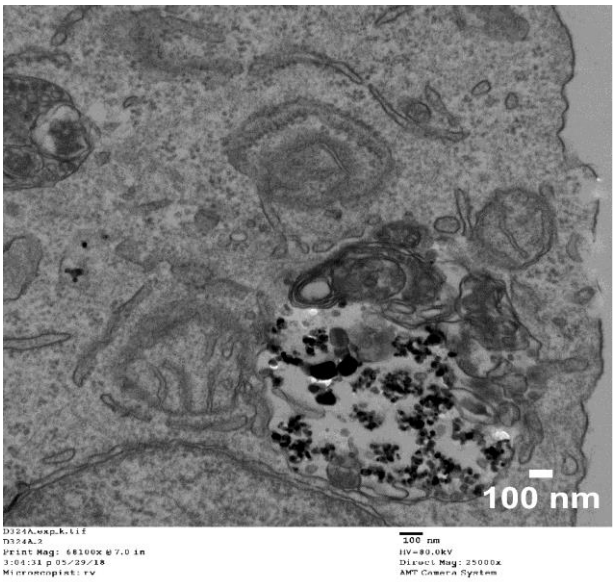


Figure S2. TEM images of A549 cells incubated with TiO_2 NPs (800 μg/mL, 24 hr, 37°C) shows association with myelin-like figures. A and B are representative images.

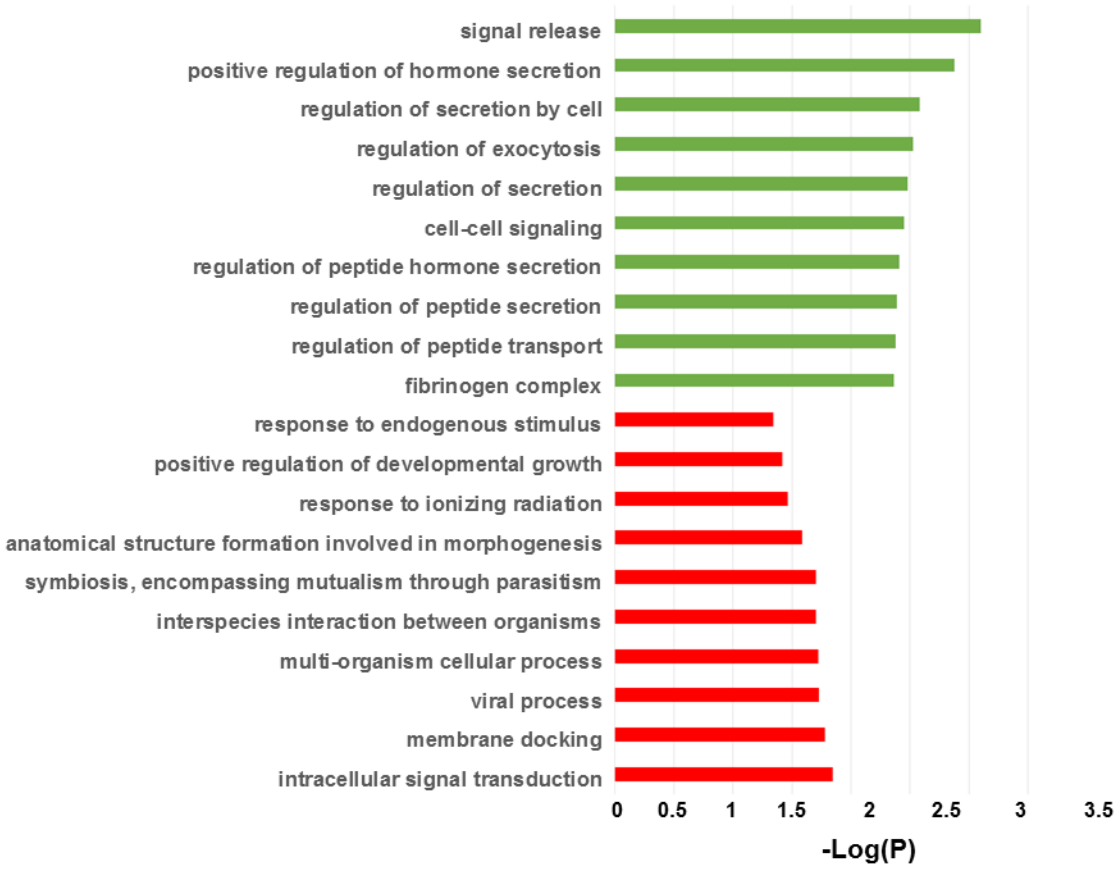


Figure S3. Gene ontology analysis of differentially expressed genes common to Generation 10 (G10) and re-challenged (ReC) cells. Top enriched GO terms of UpUp genes (red) and DownDown genes (green) are shown.

Table S2. Differentially expressed genes (DEGs) and corresponding fold change (FC) related to oxidative stress at Generation 1 (G1), Generation 10 (G10), and following a re-challenge (ReC). These genes had FC<2, the cutoff used for results presented in the main text, but were of particular interest based on previous results. DEGs with p<0.01 are shaded.

Oxidative stress-related DEG	G1 FC	G10 FC	ReC FC
PRDX1	-1.65	-1.11	-1.17
PRDX2	-1.55	1.04	-1.17
PRDX3	-2.05	-1	-1.07
PRDX4	-1.04	1.05	-1.14
PRDX5	-1.13	1.03	1
PRDX6	-1.37	-1.04	-1.08
SOD1	-1.82	-1.01	-1.31
SOD2	-1.01	1.01	1.25
SOD3	NA	NA	1.01

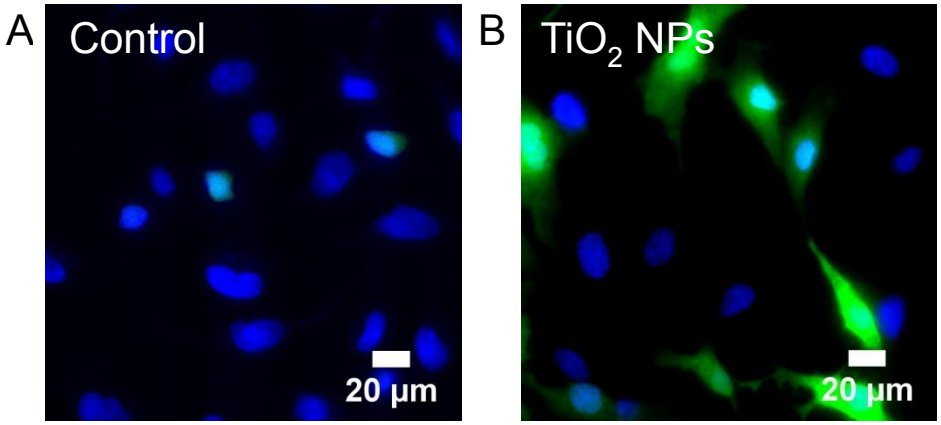


Figure S4. H_2DCF, a non-specific probe of intracellular ROS, was used to image ROS in Generation 1 HeLa cells. H_2DCFDA (5 µM) was incubated with cells for 30 min prior to imaging and then rinsed with PBS. A. Untreated control cells. B. TiO_2 NP-treated cells (773 µg/mL, 24 hr, 37°C). This concentration of TiO_2 NPs was shown previously to be non-cytotoxic with HeLa cells.[1] DAPI (blue) was used to label nuclei (50 µM, 30 min).

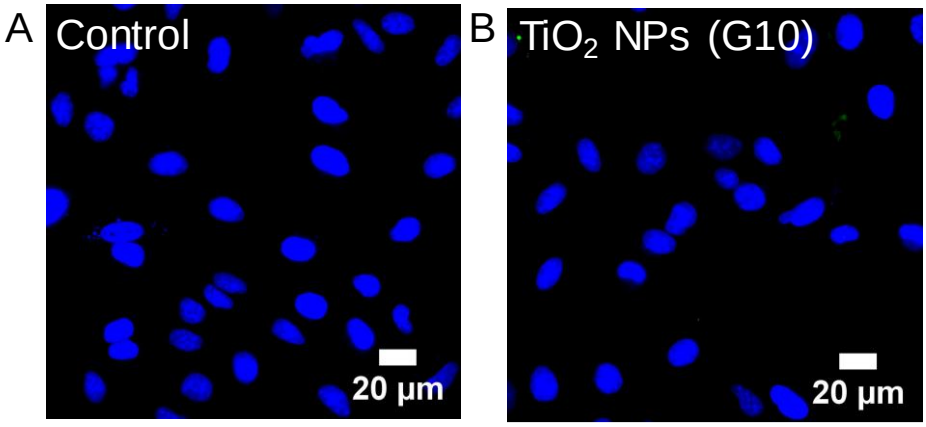


Figure S5. H_2DCF was used to image ROS in Generation 10 A549 cells. H_2DCFDA (5 μM) was incubated with cells for 30 min prior to imaging and then rinsed with PBS. A. Untreated control cells at Generation 10. B. TiO_2 NP-treated (994 μg/mL, 24 hr, 37°C) cells at Generation 10. DAPI (blue) was used to label nuclei (50 μM, 30 min).

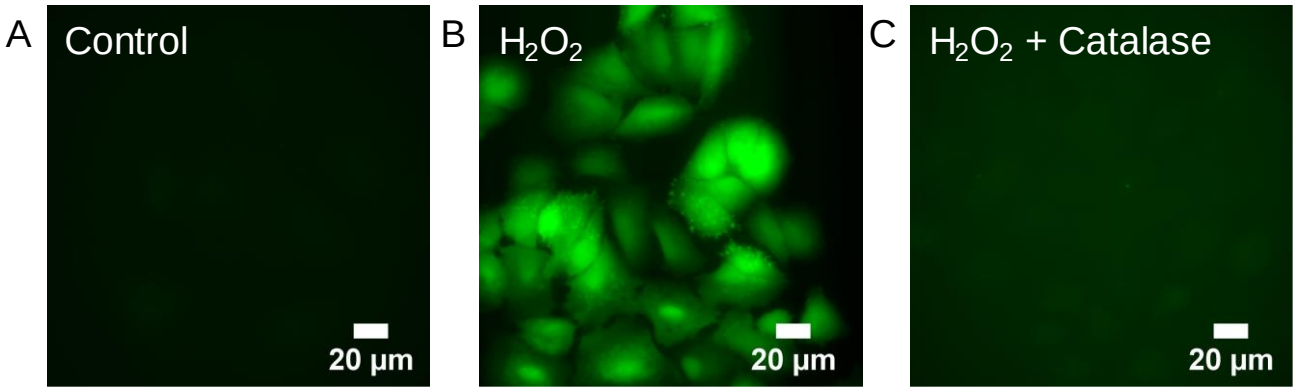


Figure S6. H_2DCFDA assay to confirm catalase activity. H_2DCFDA (5 µM) was incubated with cells for 30 min prior to imaging and then rinsed with PBS. A. Untreated control cells. B. H_2O_2 treated cells (50 µM, 30 min, 37°C). C. Co-incubation of H_2O_2 (50 µM, 30 min, 37°C) with catalase (50 U/mL).

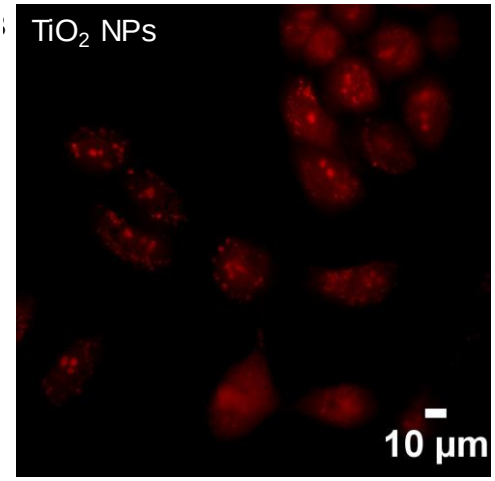


Figure S7. DHE assay with cold-bound TiO_2 NPs (994 μg/mL, 10 min, 4°C), which allows binding, but not internalization of NPs. DHE (10 μM) was incubated with cells for 30 min prior to imaging and then rinsed with clear MEM. Figure 9A shows a DHE assay for an untreated control and Figure 9B shows a 24 hr, 37°C TiO_2 NP incubation.

References

[1] S. Runa, D. Khanal, M. L. Kemp, C. K. Payne, *J. Phys. Chem. C* **2016,** *120*, 20736.