Electronic Supplementary Information

Unveil Early-Stage Nanocytotoxicity by a Label-Free Single Cell pH Nanoprobe

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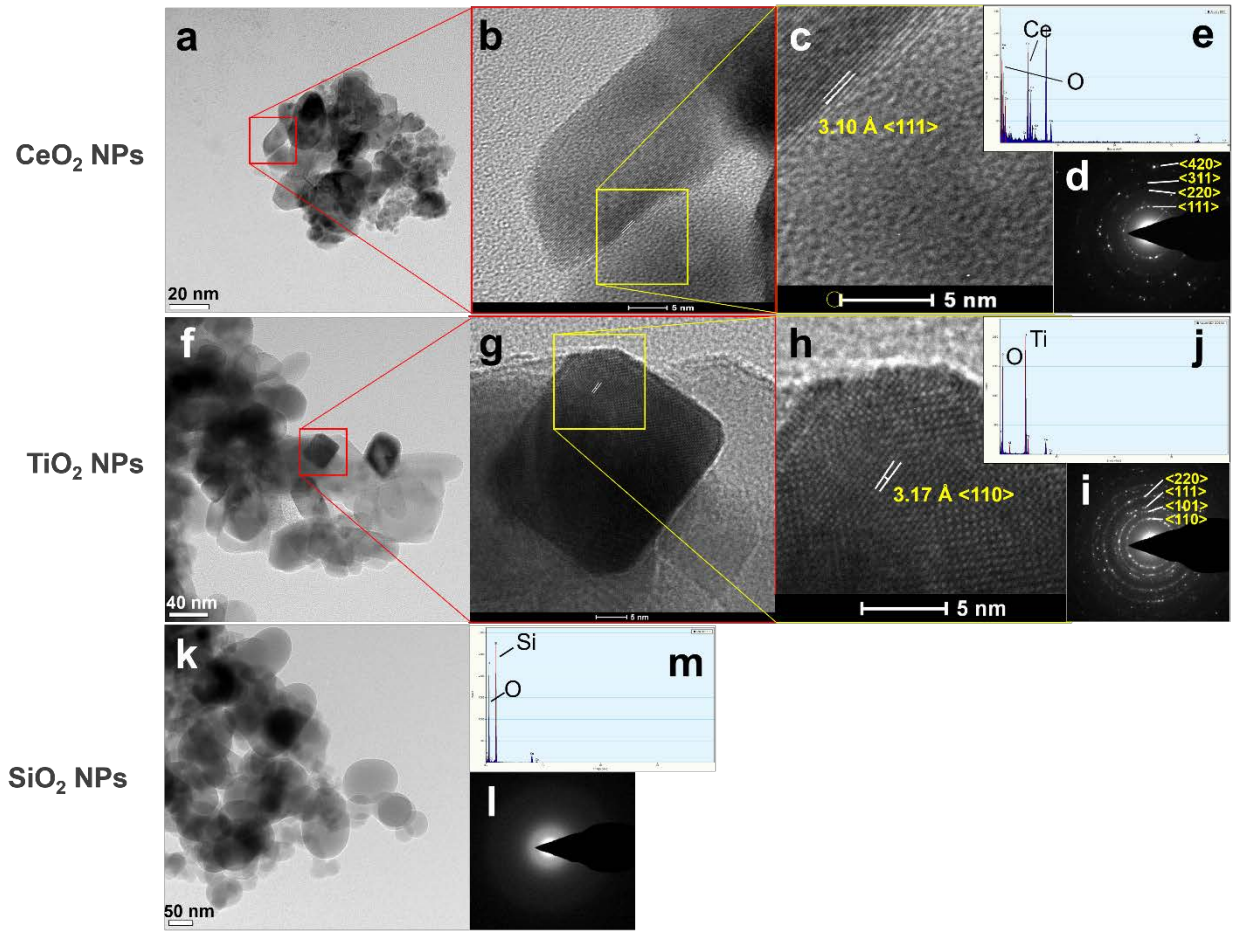


Figure S1 TEM images and HRTEM analysis of three selected NPs for this study, CeO_2, TiO_2 and SiO_2, with averaged sizes of 20 nm, 40 nm and 46 nm, respectively, based on multiple (n = 50) measurements and distribution patterns. (a, f and k) are TEM images of the three NPs. HRTEM images of CeO_2 and TiO_2 NPs (b, c, g and h) showing lattice fringes corresponding to (111) and (110) planes. Bottom insets (d and i) show typical SAED patterns with spots corresponding to (111) and (110) planes. HRTEM image (l) showing the amorphous status of the SiO_2 NPs. EDX analysis of three NPs (e, j, m) showing their chemical composition and good purities.

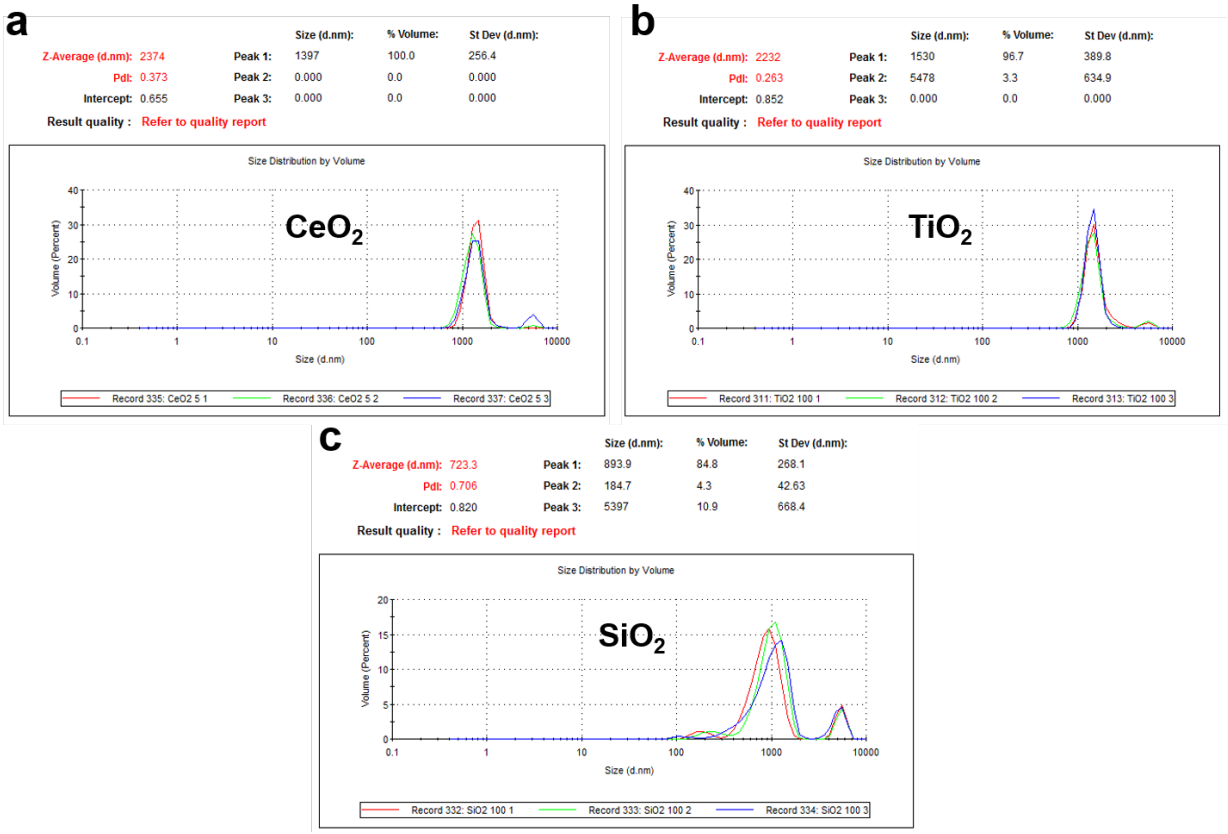


Figure S2 DLS-based hydrodynamic size determination using Zeta-Sizer on (a) CeO_2, (b) TiO_2 and (c) SiO_2 NPs at 100 μg/mL original dosage in pH 7.4 serum-free culture medium F-12K. Large agglomerates with sizes that peaked at 1397 nm in CeO_2 NPs, and at 1530 nm and 5478 nm in TiO_2 NPs were observed, while relatively smaller agglomerates with sizes that peaked at 893.9 nm and 184.7 nm were observed in SiO_2 NPs.

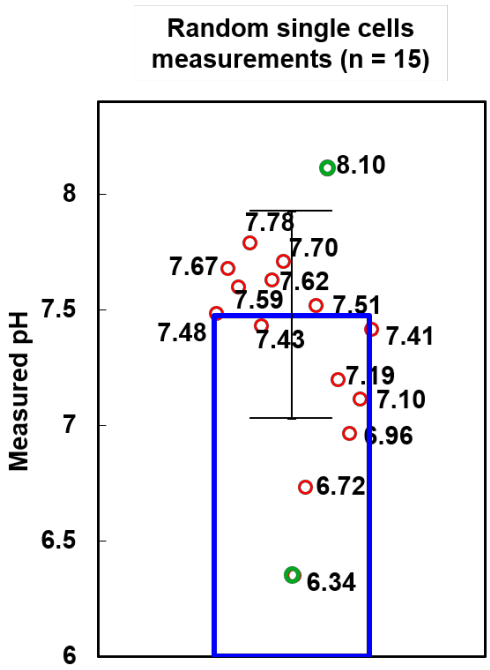
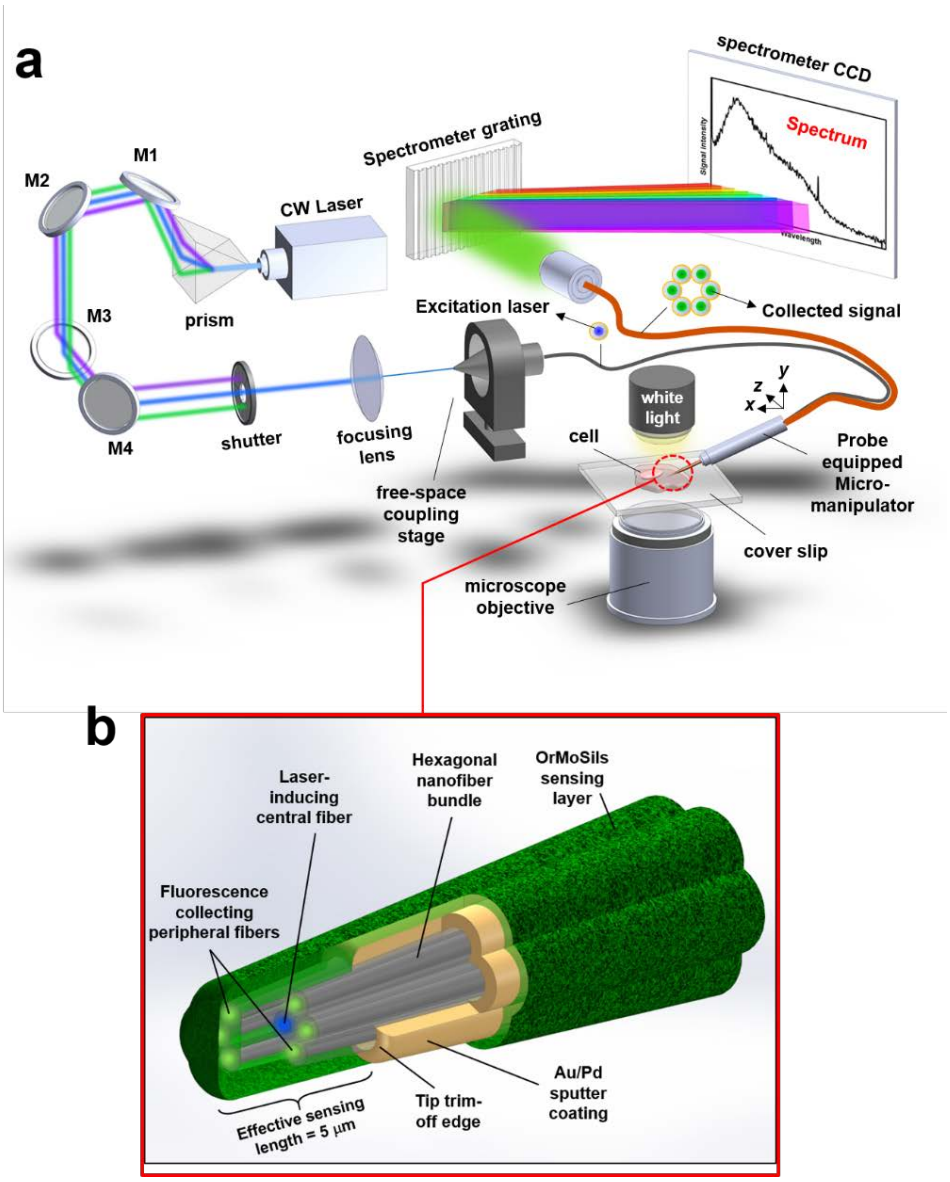


Figure S3 Intracellular pH measurement using our developed pH micro-probe on 15 randomly selected single cells (red and green dots), that cultured under same 37 °C, 5% CO_2 condition with 95% humidity. Blue bar in the graph represents the calculated median pH value (pH 7.47) that acquired out of 13 cells, and error bar stand for the Standard Deviation of all data points which equals to 0.44 pH unit. Two points, pH 8.10 a pH 6.34 (green dots), that far from a normal cell pH level, was considered as outliers and have been taken out from the calculation.



Scheme S1 Schematics of the sensing system setup and structure of the probe sensing head. (a) Schematic illustration of the single cell pH probing system assisted with an inverted epifluorescence microscope. An Argon-ion laser was used as excitation laser source. After purification down to one wavelength (488 nm) laser beam was free-space coupled into the central fiber for tip-embedded HPTS dye excitation. Surrounding six fibers collect fluorescent emission back to a spectrometer (USB2000, Ocean Optics). Accurate single cell probe insertion was achieved by a home-built oil-pressured single cell capturing device and a 3-D micro-probe manipulation platform. (b) The probe consisted of a bundle of seven highly tapered, hexagonally arranged multimode optical fibers. Fabricated by a home-built coaxial-twisting and gravitational-

stretching system. A gold (Au)/palladium (Pd) shielding layer was deposited onto the peripheral six fibers, however only trimmed off at the tip region (≤ 5 μm in length), to expose the inner fibers and finally coated by the HPTS dye embedded OrMoSil.

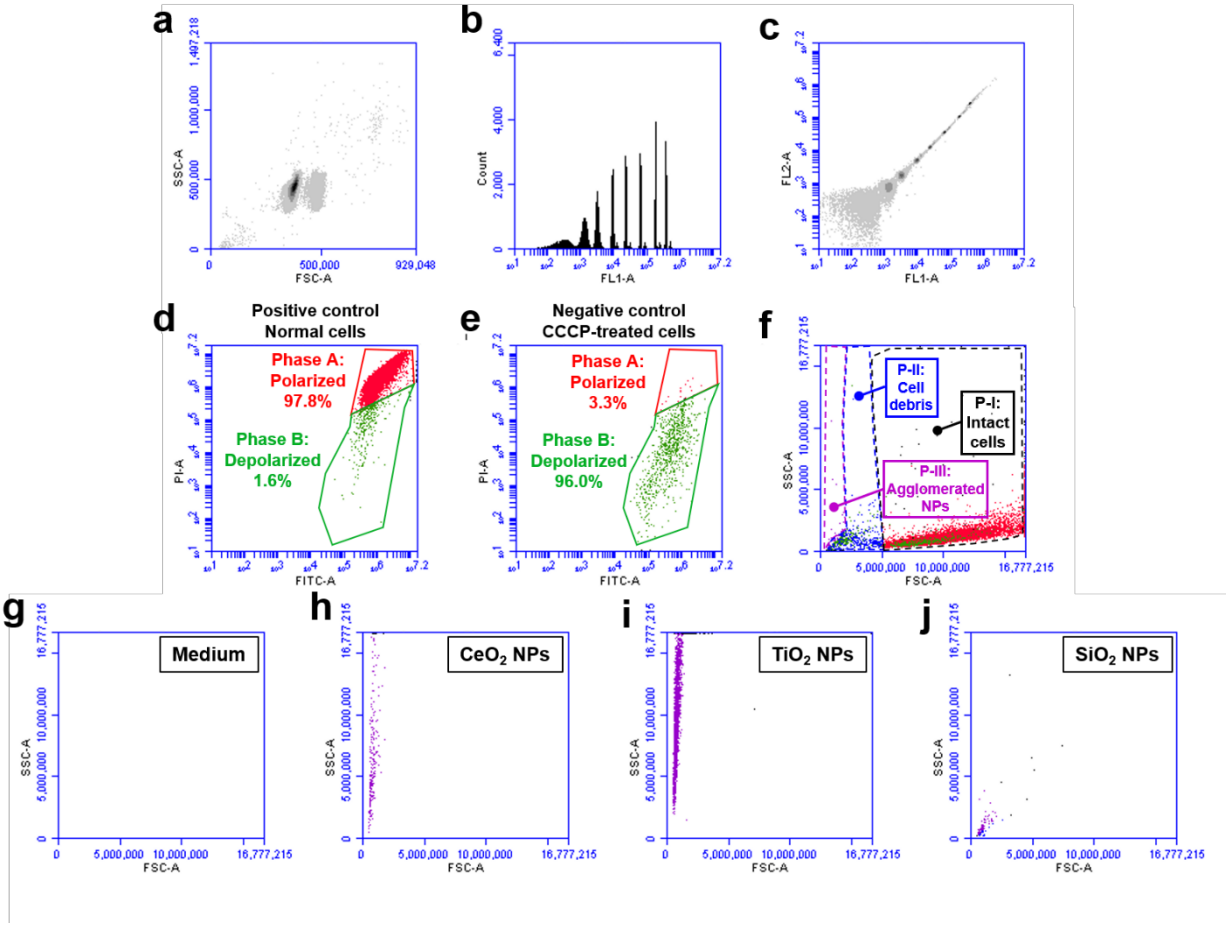


Figure S4 FACs-based flow cytometry experiments running on eight rainbow beads as standard control, where (a) shows the particle size distribution at mainly two close spots located in the middle of SSC-FSC plot, with equal surface roughness. Particle counts at each fluorescence channel (b) and fluorescence intensity linear distribution (c) were also successfully detected and confirmed. Normally cultured A549 cell staining with JC-1 dye were subjected to (d) direct flow cytometry measurement, or (e) 50 μM CCCP treatment for 30 minutes and then flow cytometry measurement. Resulted data spots were categorized as Phase A and Phase B that represents for polarized (red) and depolarized (green) mitochondrial inner membrane potential. Besides, intact cells (P-I, black), cell debris (P-II, blue) and agglomerated NPs (P-III, purple) were also characterized and designated within different phases in the SSC-FSC plot (f). The serum-free F-

12K cell culture medium (g) and the three types of NPs: CeO_2 (h), TiO_2 (i), and SiO_2 (j), were also examined and showed in the SSC-FSC plots.

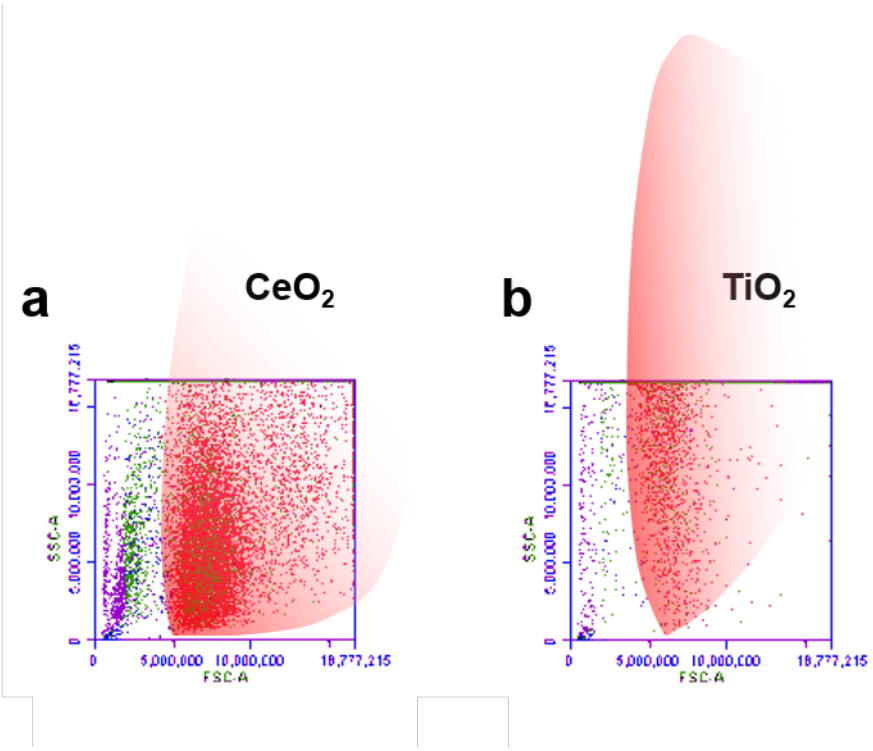


Figure S5 Extrapolated covering area and distribution density of the untracked cell parts outside the detection range of SSC-FSC plot, for the CeO_2 (a) and TiO_2 (b) NP treated cell group, as schematically shown in the red “plume” areas.

Table S1 SP-ICP-MS analysis on digested A549 cells that have uptaken CeO_2 NPs for 24 hours.

Original NPs conc. (ppm)	Detected NPs concentration (ppb)	NO. of Particles (× 10^{13})	NO. of cells (× 10^{6})	Particles per cell (× 10^{8})	Uptaking %
5 ppm	3.0 ± 0.4	6.4 ± 0.9	1.6	0.40	0.3
20 ppm	17.4 ± 2.6	37.4 ± 5.5	0.98	3.82	0.43
100 ppm	102.9 ± 1.9	221 ± 4.0	0.85	26.0	0.51

Table S2 Detailed SP-ICP-MS running parameters for examining cells that have exposed to CeO_2 NPs for 24 hours.

NexION™ 300 ICP-MS conditions

ICP-MS Operating Condition	
Nebulizer Gas Flow (L/min)	1.02*
Auxiliary Gas Flow (L/min)	1.2
Plasma Gas Flow (L/min)	18
ICP RF Power (W)	1600
Analog Stage Voltage (V)	-1675
Pulse Stage Voltage (V)	1250
Cell Entrance Voltage (V)	-6
Cell Exit Voltage (V)	-6
Cell Rod Offset	-15
Sampler Cones	Platinum
Skimmer Cones	Platinum
Sample Introduction System	Cyclonic Spray Chamber with Meinhard Nebulizer
Analyte	Ce-140
Dwell Time (ms)	100

*Optimized daily

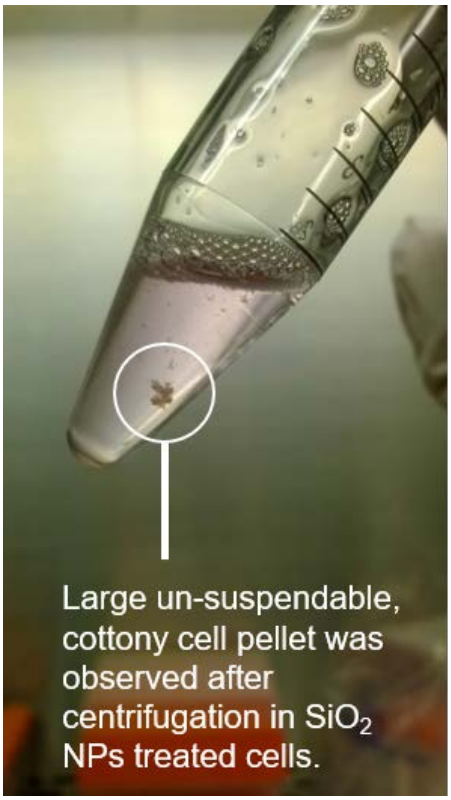


Figure S6 A piece of un-suspendable cell pellet found after resuspension of the centrifugated cells that has been exposed to SiO_2 NPs for 12 hours.

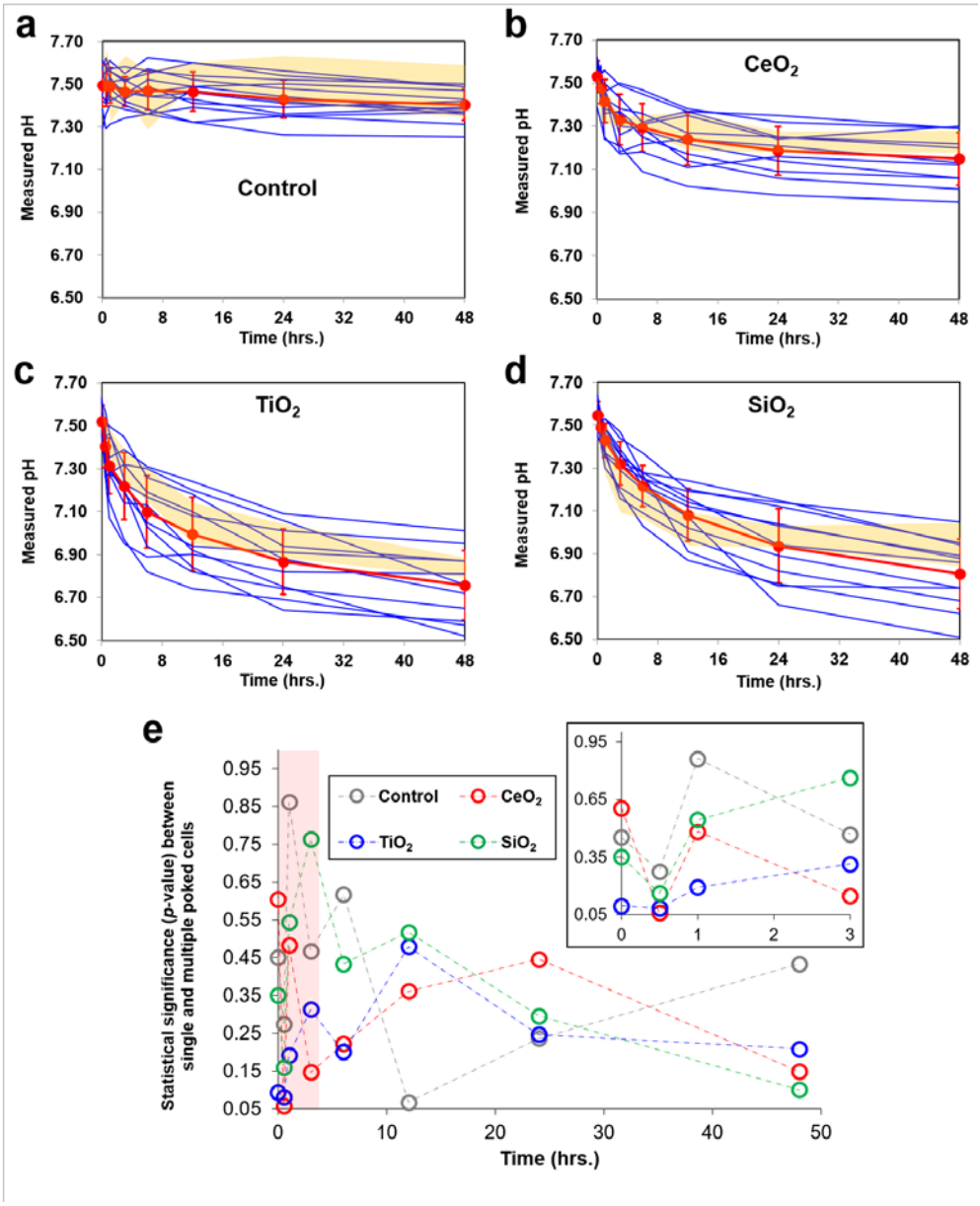


Figure S7 (a) Combination of all 48-hour data in single cell pH probing. Each red line in (a - d) represents averaged values out of at least 11 single cell measurements. Error bars indicate standard deviation. Yellow polygon in each plot stands for at least 3 separately probed single cells. (e) Statistical analysis between repeatedly poked and single poked cells. Data points representing *p*-values are calculated based on one-way ANOVA between multiple poked and single poked cell data, where all data points are located above the x-axis which equals to a threshold *p*-value of 0.05.