Supplementary Material

In Situ Quantification of Diverse Titanium Dioxide Nanoparticles Unveils Selective Endoplasmic Reticulum Stress-Dependent Toxicity.

Marina Simon[1,2]‡, Gladys Saez[1,2]‡, Giovanna Muggiolu[1,2], Magali Lavenas[3,4], Quentin Le Trequesser[1,2,3,4], Claire Michelet[1,2], Guillaume Devès[1,2], Philippe Barberet[1,2], Eric Chevet[5,6], Denis Dupuy[7,8], Marie-Hélène Delville[3,4] and Hervé Seznec[1,2*]

Exposure type	Ca content ($mg.g^{-1}$)	Ti content ($\mu g.cm^{-2}$)
Control	0.08 +/- 0.03	0.006 +/- 0.002
P25	0.09 +/- 0.02	1.67 +/- 1.03
INPs	0.08 +/- 0.03	1.64 +/- 0.74
NNs	0.09 +/- 0.02	0.57 +/- 0.22
TNs	0.091 +/- 0.05	0.50 +/- 0.5

Table S1: Single cell intracellular calcium and titanium concentrations (in $mg.g^{-1}$ and $\mu g.cm^{-2}$, respectively) measured by μ-PIXE in HUVEC cells exposed to 2 $\mu g.cm^{-2}$ of different TiO_2 NPs morphologies (P25, INPs, NNs and TNs). Results are expressed as median values +/-median absolute deviation.

	Exposure type	Ca content ($mg.g^{-1}$)	Ti content ($\mu g.cm^{-2}$)
0.06 mmol Calcium	*Control*	0.1 +/- 0.02	0.008 +/- 0.003
	P25	0.1 +/- 0.04	0.13 +/- 0.1
	INPs	0.14 +/- 0.07	0.2 +/- 0.17
	NNs	0.17 +/- 0.06	0.46 +/- 0.5
	TNs	0.14 +/- 0.05	0.045 +/- 0.05
1.2 mmol Calcium	*Control*	0.09 +/- 0.03	0.003 +/- 0.001
	P25	0.09 +/- 0.02	0.033 +/- 0.05
	INPs	0.08 +/- 0.02	0.035 +/- 0.05
	NNs	0.1 +/- 0.03	0.012 +/- 0.01
	TNs	0.1 +/- 0.04	0.003 +/- 0.002

Table S2: Single cell intracellular calcium and titanium concentrations (in $mg.g^{-1}$ and $\mu g.cm^{-2}$, respectively) measured by μ-PIXE in HEKn cells exposed to 2 $\mu g.cm^{-2}$ of different TiO_2 NPs morphologies (P25, INPs, NNs and TNs) in low- and high-calcium concentration culture media. Results are expressed as median values +/- median absolute deviation.

Table S3- RT*q*PCR Primers list.

Gene	GenBank	Forward primer	Reverse Primer
hBIP	NM_005347.4	5'-GCTTATGGCCTGGATAAGAGG-3'	5'-CCACAACTTCGAAGACACCAT-3'
hDDIT3*/*chop	NM_001195053.1	5'-ACCAAGGGAGAACCAGGAAACG-3'	5'-TCACCATTCGGTCAATCAGAGC-3'
hHERPUD1	NM_014685.3	5'-TCCTCCTCCTGACGTTGTAAA-3'	5'-TGCTCGCCATCTAGTACATCC-3'
hErdj4	NM_012328.2	5'-CTTCGTTGAGTGACAGTCCTGC-3'	5'-TGGTGGTTCCAGTAGACAAAGG-3'
hGAPDH	NM_001289746.1	5'-AAGGTGAAGGTCGGAGTCAA-3'	5'-CATGGGTGGAATCATATTGG-3'
h SOD1	NM_000454.4	5'-AAAGATGGTGTGGCCGATGT-3'	5'-CAAGCCAAACGACTTCCAGC-3'
***hSOD2* (*isof*. 1-2)**	NM_000636.2	5'-GGCCTACGTGAACAACCTGA-3'	5'-GTTCTCCACCACCGTTAGGG-3'
hCAT	NM_001752.3	5'-AGTGATCGGGGGATTCCAGA-3'	5'-AAGTCTCGCCGCATCTTCAA-3'
***hGPX1* (*isof*. 1)**	NM_000581.2	5'-TATCGAGAATGTGGCGTCCC-3'	5'-TCTTGGCGTTCTCCTGATGC-3'
***hGPX1* (*isof*. 2)**	NM_201397.1	5'-AACCAGTTTGGGCATCAGGT-3'	5'-CCAAGGGCGGAGAGGAATTT-3’
h18S	NR_046235.1	5'-GGCCGTTCTTAGTTGGTGGA-3'	5'-CCCGGACATCTAAGGGCATC-3'

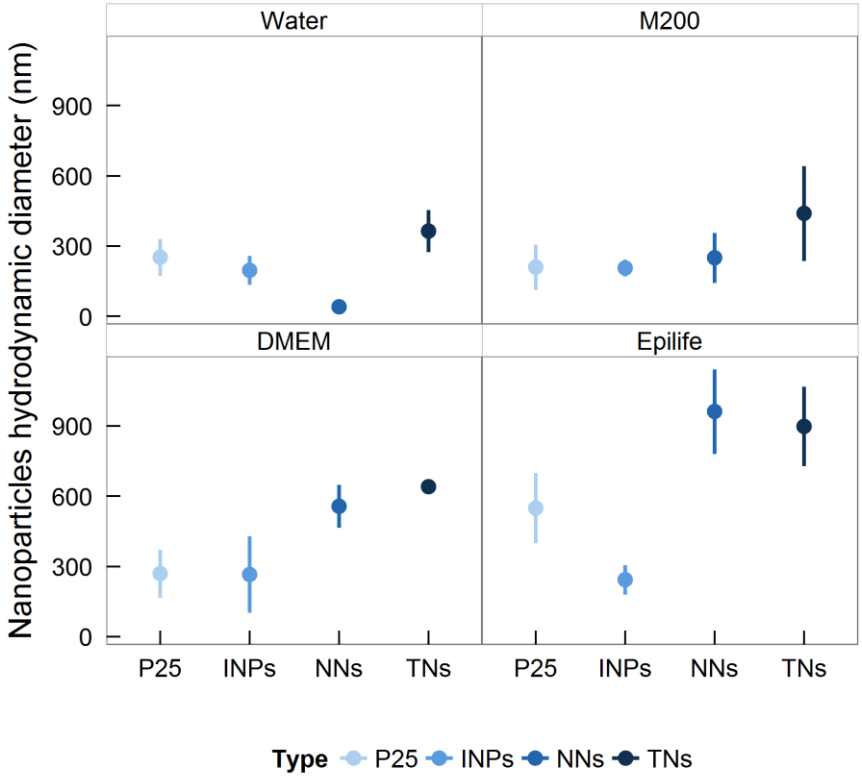

Figure S1. TiO_2 NPs behavior in biological media such as water, M200-PRF®, DMEM and Epilife®. The hydrodynamic diameter is expressed in nm and is shown to be medium-dependent. Water is used as synthesis medium and for stock solution composition. M200-PRF®, DMEM and Epilife® are sterile and defined culture media provided by the manufacturer and correspond to the culture media used for HUVEC, HeLa, and HEKn cell lines, respectively.

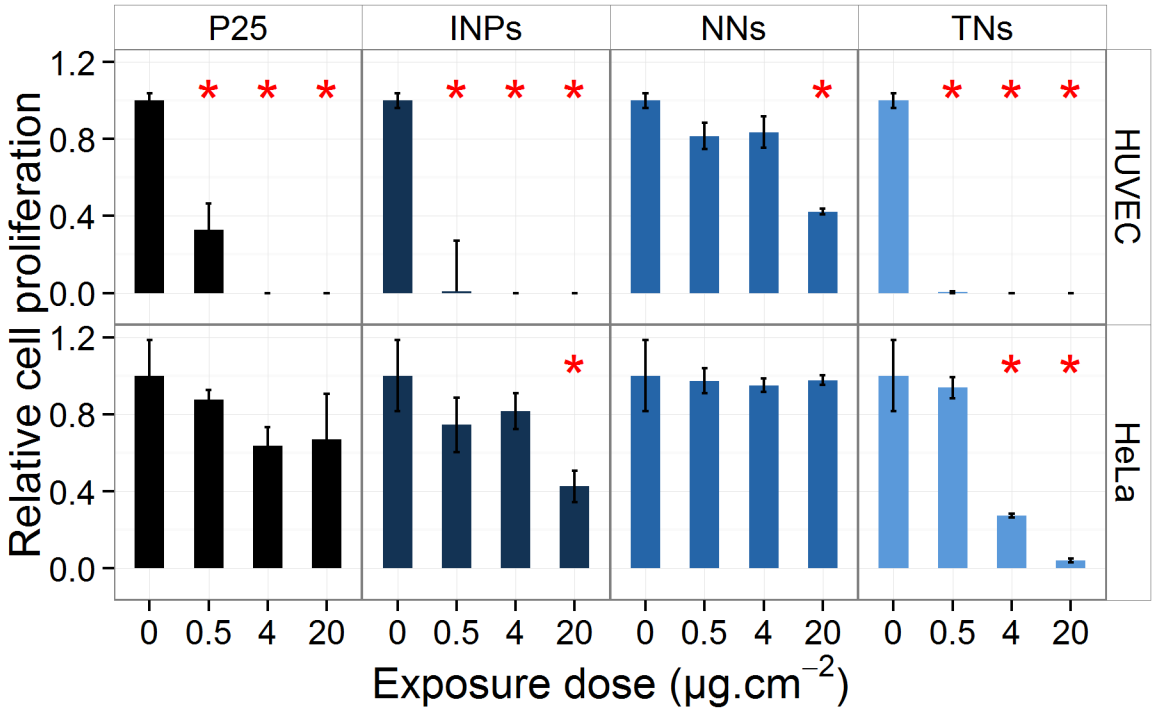

Figure S2. Dose-dependent effect TiO_2 NPs morphologies (P25, INPs, NNs, TNs) on human cell culture proliferation. Relative cell proliferation of human endothelial cells (HUVEC, top) and immortalized and cancerous cell line (HeLa, down). Measurements performed 8 days after exposure. Exposure doses: 0.5, 4 and 20 µg.cm^{-2}. Data expressed as mean+/- SD of at least three independent experiments normalized to untreated controls, * $P < 0.01$ compared to the control (*i.e.*, non-exposed cell populations).

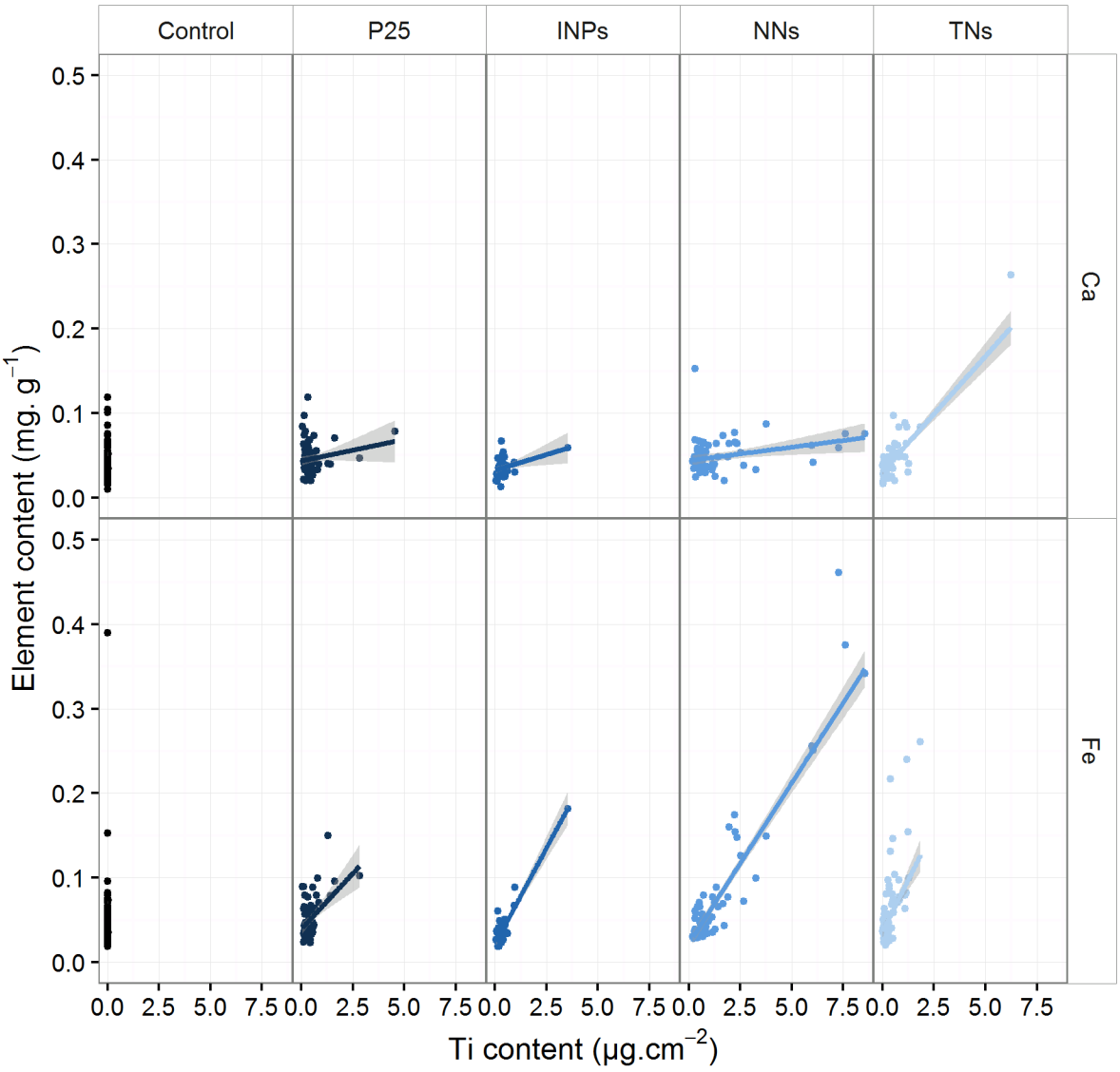

Figure S3. Relation between intracellular titanium content and intracellular chemical elements (iron and calcium). Single cell intracellular titanium (µg.cm^{-2}), calcium and iron contents (mg.g^{-1}) measured by µ-PIXE in human primary cell line (HEKn) exposed to 2 µg.cm^{-2} of different TiO_2 NPs morphologies (P25, INPs, NNs and TNs). The single cell quantitative analysis revealed a positive correlation between the intracellular titanium and intracellular calcium (top) and iron (down) contents. The heterogeneity of the titanium intracellular content per cell is also well depicted.

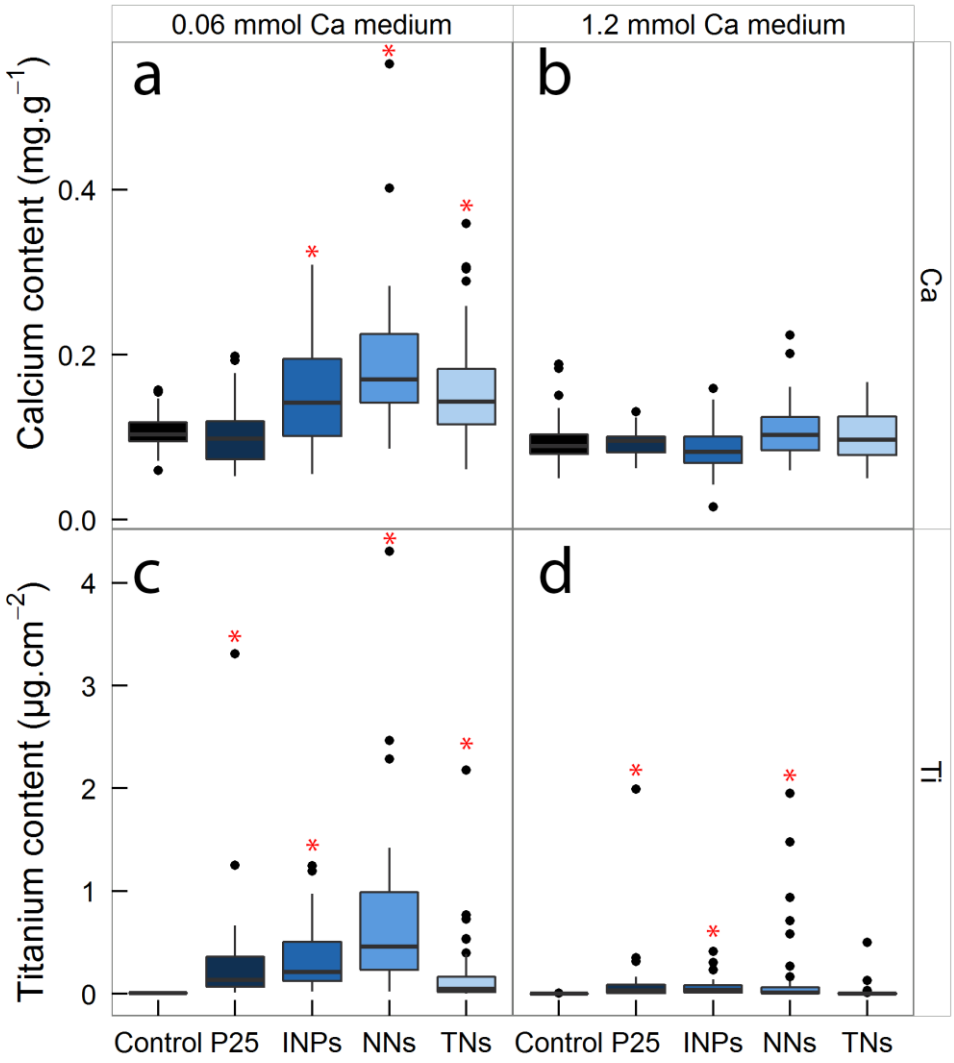

Figure S4. Effect of the extracellular calcium concentration on the intracellular TiO_2 NPs content in primary human keratinocytes (HEKn). Single cell quantitative analysis of the intracellular titanium and calcium concentrations (% dry mass per cell) were measured by µ-PIXE in cells exposed to 2 µg.cm^{-2} of different TiO_2 NPs morphologies (P25, INPs, NNs and TNs) in low- and high-calcium concentration culture medium (0.06 and 1.2 mM, respectively) a, c). Intracellular calcium and titanium contents in low-calcium condition. b, d) Intracellular calcium and titanium contents in high-calcium conditions. Asterisks over distributions indicate significant differences with untreated groups (Kruskal-Wallis test ($p<0.05$) and post hoc Nemenyi test ($p<0.05$))

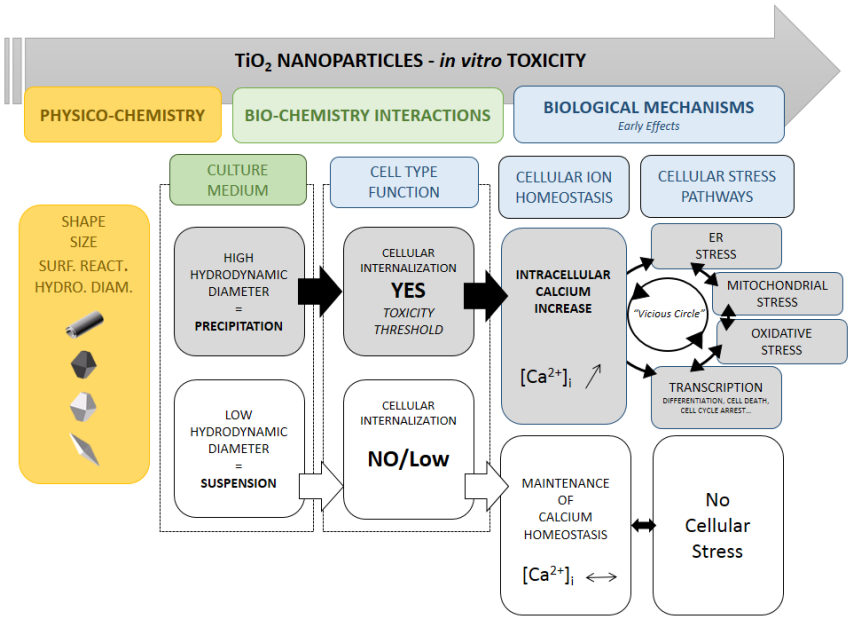

Figure S5. Schematic representation of the proposed methodology to evaluate TiO_2 NPs toxicity.