Thermal annealing of carbon nanotubes reveals a strong toxicological impact of the structural defects

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SUPPLEMENTARY DATA

THERMAL ANNEALING OF CARBON NANOTUBES REVEALS A STRONG TOXICOLOGICAL IMPACT OF THE STRUCTURAL DEFECTS

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1. Thermal desorption

Thermal desorption allows a detection of the elements thermally desorbed from a sample (**Figure S1**). The volatilized elements are analyzed by a mass spectrophotometer. The obtained graphs show the intensity variation depending on the temperature for a set molecular mass.

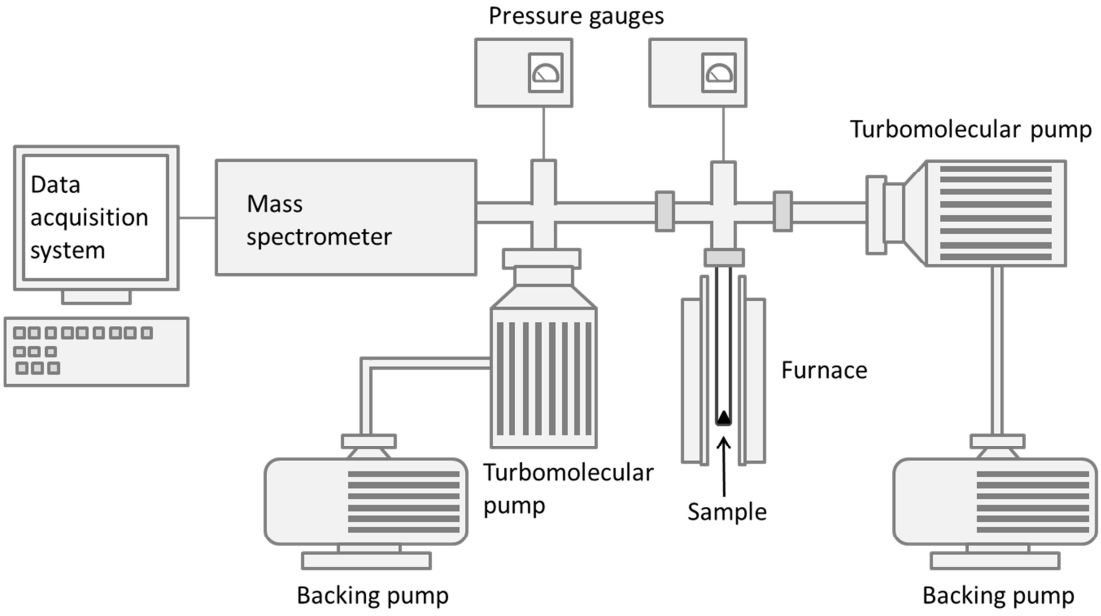

Figure S1: Thermal desorption device

2. 6. Corrections of the ROS assay

An experiment was carried out to evaluate the biases induced by the MWCNT and due to fluorescence absorption. A standard curve of the fluorescent probe DCF (2',7'-dichlorodihydrofluorescein) with concentrations of 0, 10, 100, 1000 nM was realized in complemented culture medium without any cells. The same experiment was conducted with CNT or CNTa at concentrations of 15, 30, 60, and 120 $\mu g.mL^{-1}$. The difference between the fluorescence without and with MWCNT was calculated for each MWCNT sample. **Figure S2** shows an example of the obtained correction curves, and

Table S1 sums up the corrective equations for each MWCNT sample. These corrections were applied to the data from the ROS (reactive oxygen species) assay to obtain corrected values.

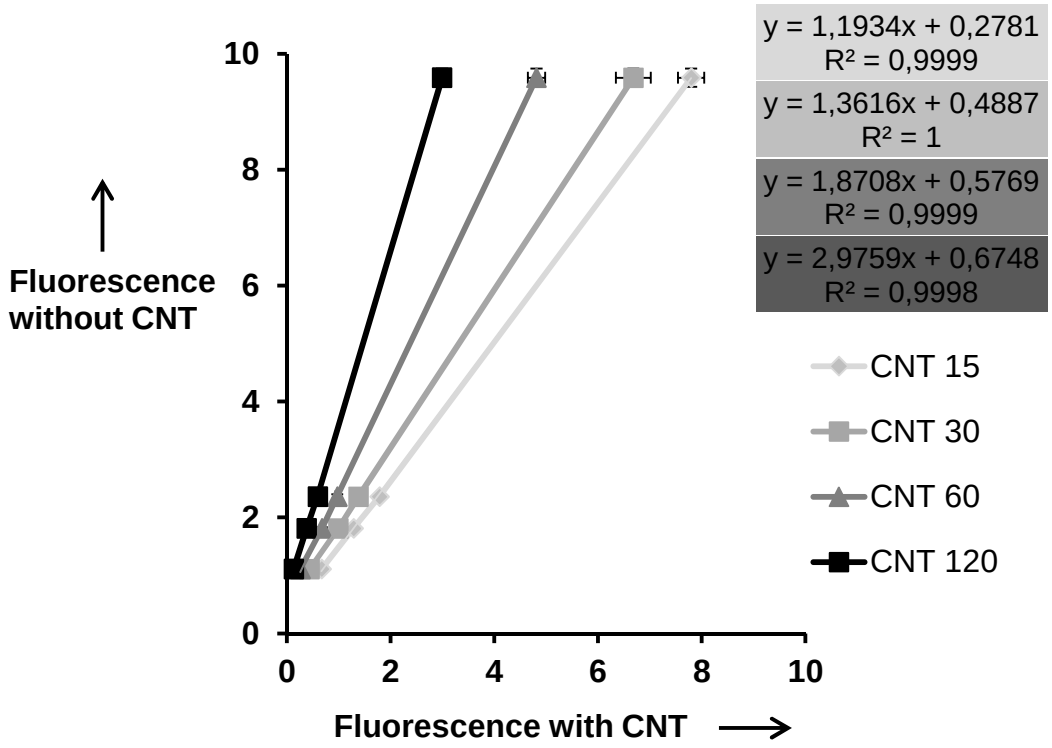

Figure S2: Example of the correction curves of DCF with and without CNT

Table S1 : Corrective equations for each sample of CNT and CNTa used for the ROS assay (repeated n=3)

Powder	**Correction at 15 µg.mL^{-1}**	**Correction at 30 µg.mL^{-1}**	**Correction at 60 µg.mL^{-1}**	**Correction at 120 µg.mL^{-1}**
CNT n°1	y = 1.19x + 0.28	y = 1.36x + 0.49	y = 1.87x + 0.58	y = 2.98x + 0.67
CNT n°2	y = 1.26x + 0.25	y = 1.49x + 0.41	y = 1.96x + 0.54	y = 3.14x + 0.65
CNT n°3	y = 1.29x + 0.44	y = 1.50x + 0.64	y = 2.17x + 0.74	y = 3.47x + 0.86
CNTa n°1	y = 1.24x + 0.24	y = 1.38x + 0.47	y = 1.78x + 0.62	y = 2.60x + 0.78
CNTa n°2	y = 1.17x + 0.27	y = 1.29x + 0.445	y = 1.63x + 0.61	y = 2.30x + 0.74
CNTa n°3	y = 1.31x + 0.32	y = 1.36x + 0.61	y = 1.68x + 0.79	y = 2.39x + 0.97

3. Diameter distribution

The diameters from the 300 measurements conducted on the FEG-SEM images are presented as a distribution in number in **Figure S3**. No major difference could be detected between both diameter distributions.

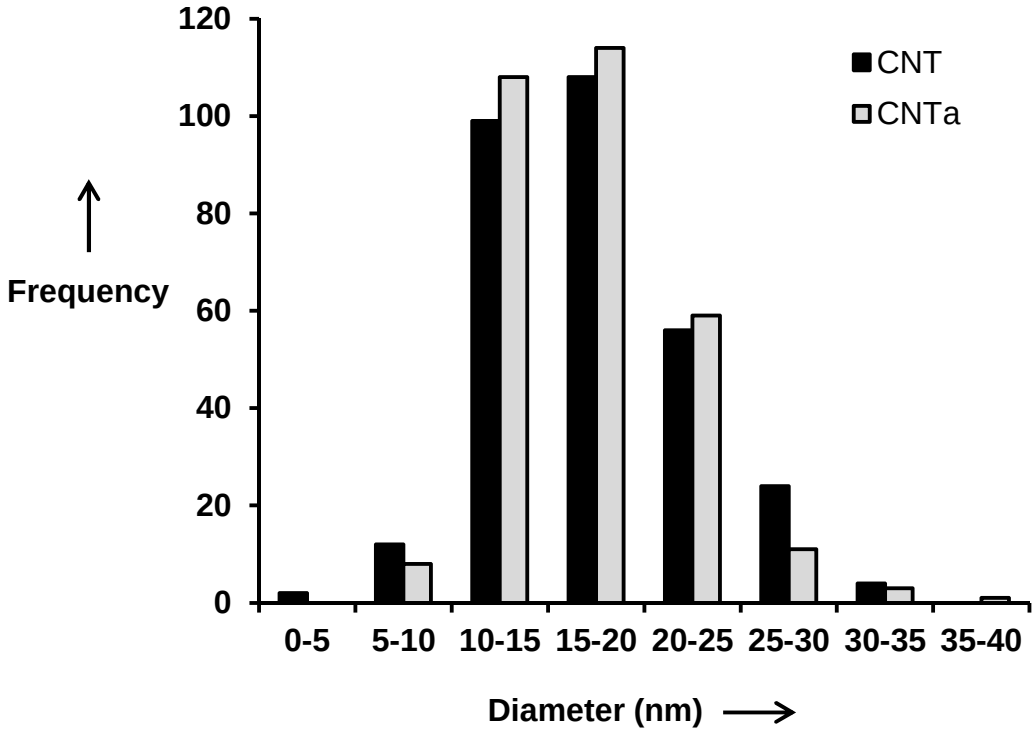

Figure S3: Distribution of the MWCNT diameters, CNT: as-produced, CNTa: annealed at 2125°C 1h under Ar

4. X-ray diffraction

An X-ray diffractometer (D5000 Siemens) was used to assess the crystallinity of the MWCNT powders. A Cu Kα X-ray source was used. **Figure S4** and **Figure S5** show the XRD spectra of the pristine CNT and the annealed CNTa. A high background noise was detected for both samples, meaning that the crystallinity was poor even after annealing.

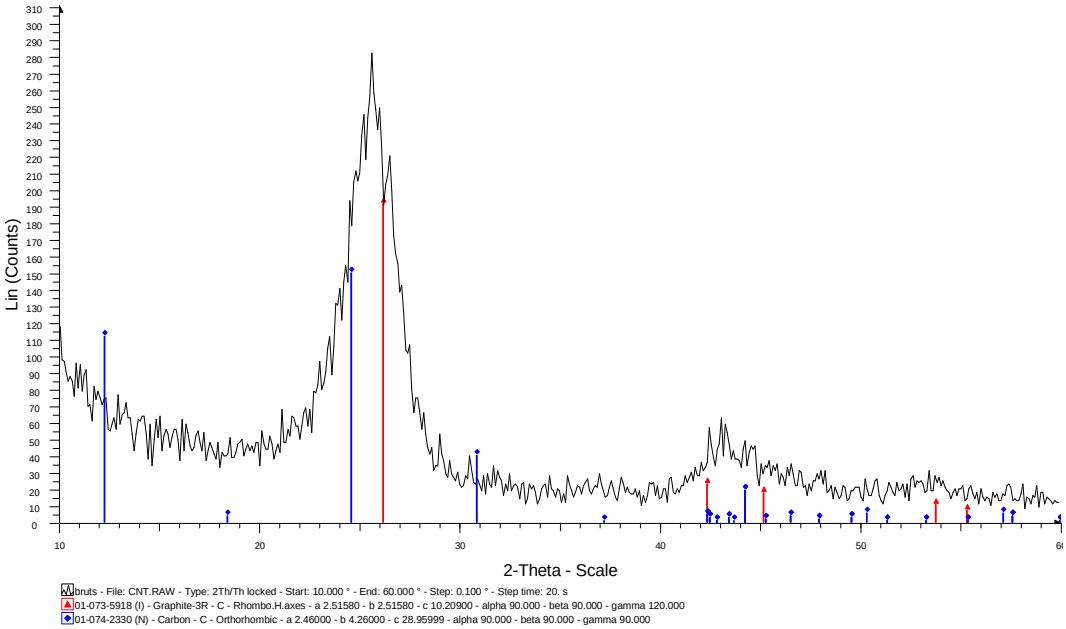

Figure S4: XRD spectrum of the as-produced MWCNT (CNT)

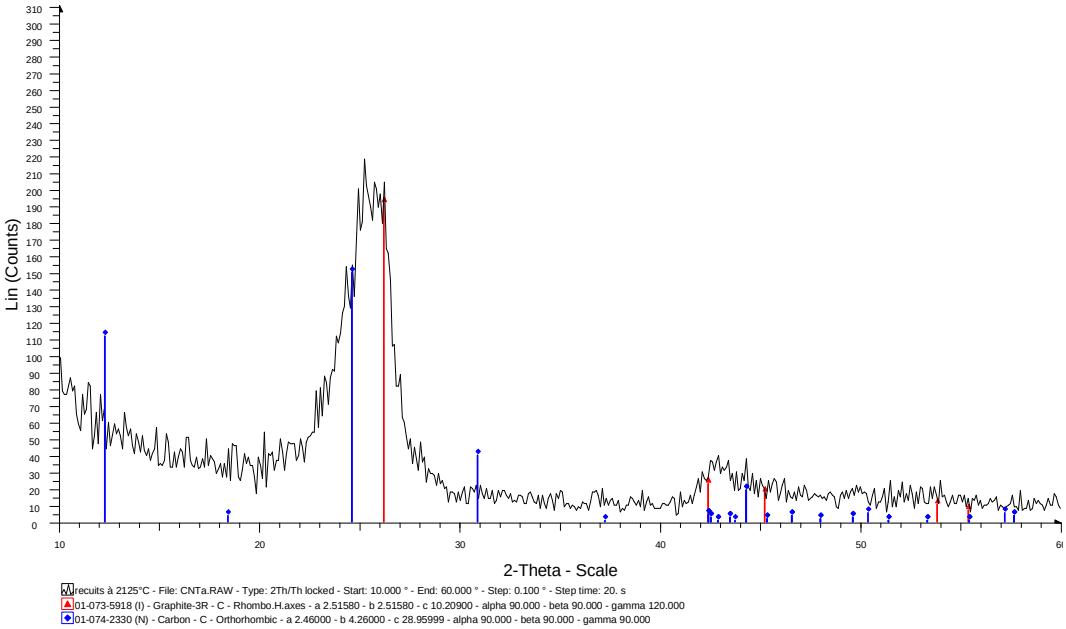

Figure S5: XRD spectrum of the annealed MWCNT (CNTa)

5. HR-TEM, presence of catalytic impurities

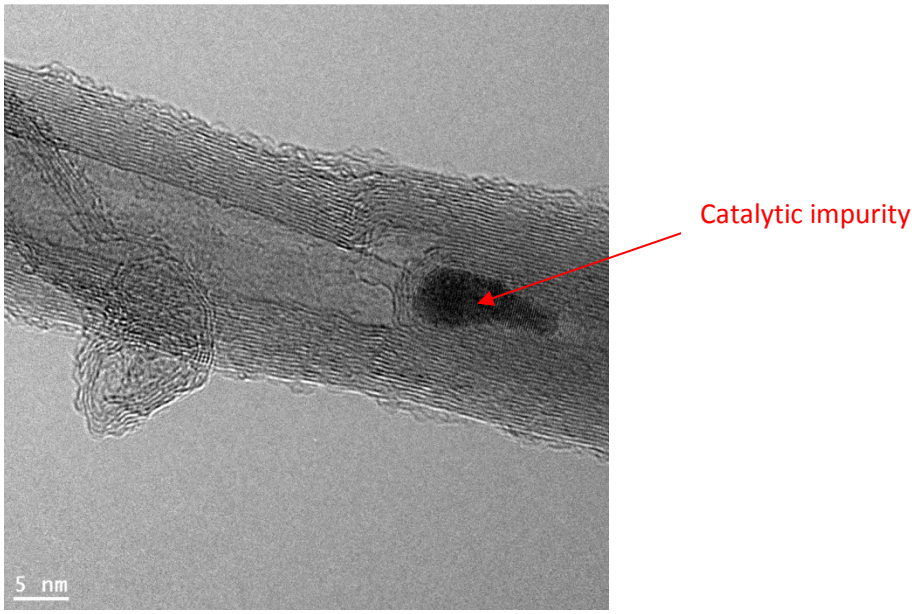

Figure S6: HR-TEM images of multi-walled carbon nanotubes: as-produced (CNT) with a catalytic impurity.

6. Chemical modifications by thermal annealing

The chemical properties of the CNT and CNTa were assessed by X-ray photoelectron spectroscopy (XPS) (**Figure S7**).

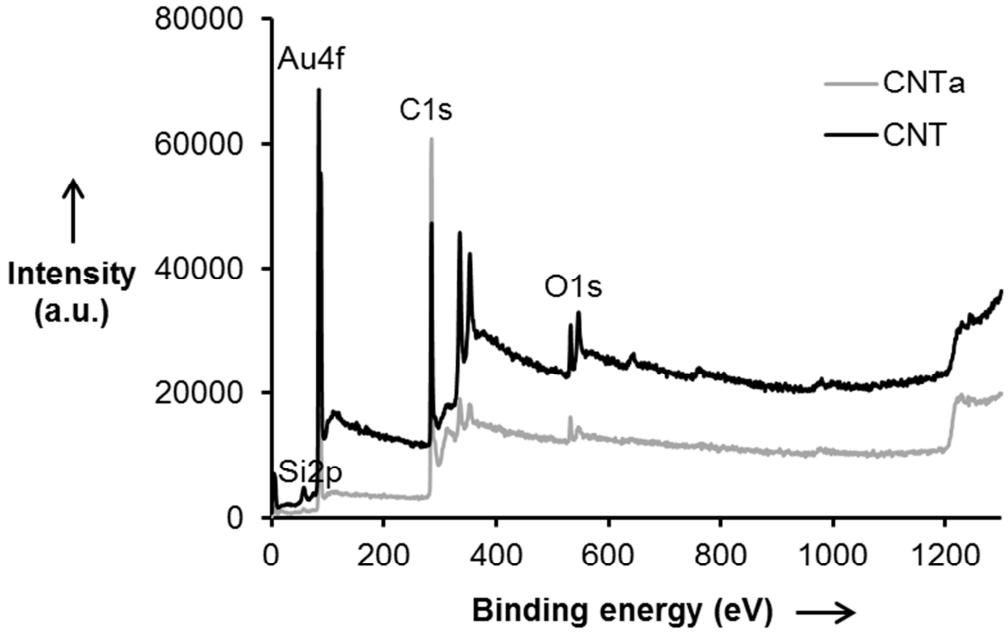

Figure S7: XPS spectra of the pristine CNT and annealed CNTa.

Furthermore, it was possible to identify a decrease in oxygenated groups by thermal desorption. In the main text, the **Figure 3** shows a different behavior regarding the desorption of CO. In **Figure S8** and **Figure S9** are presented the thermal desorption of H_2O and CO_2. These graphs show strong releases of CO for both materials, CO_2 and H_2O for CNTa at high temperatures (over 700°C). They are believed to come from the degradation of the nanotubes. The vacuum may be not high enough and no Argon is added (unlike for the annealing treatment) to prevent it from happening.

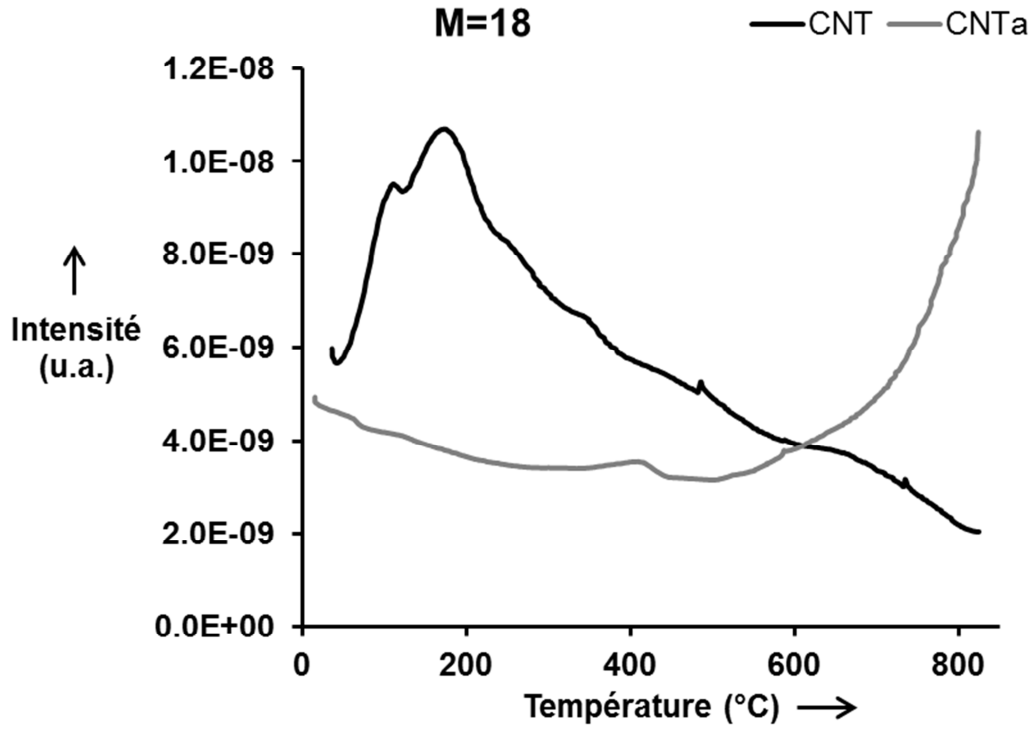

Figure S8: Thermal desorption of carbon nanotubes, following the M=18 related to H_2O

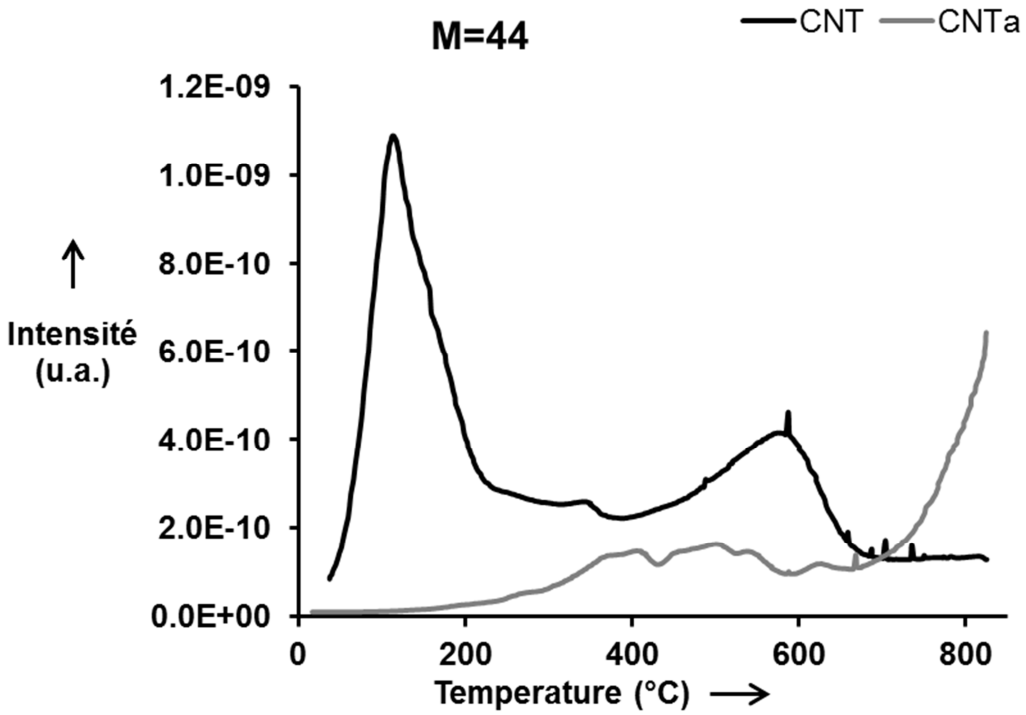

Figure S9: Thermal desorption of carbon nanotubes, following the M=44 related to CO_2

7. Microscopic observations Microscopic observations have been conducted after a 24 h exposition of the murine macrophages to 15 $\mu g.mL^{-1}$ of CNT or CNTa. Cells were seeded directly with the MWCNT on a 8-well chambered coverglass (Lab-Tek®) at a concentration of 25,000 cells/well. After 24 h, the cells were then rinsed twice with PBS, dried and kept at -20°C before a May-Grünwald Giemsa staining (MGG) (**Figure S10**).

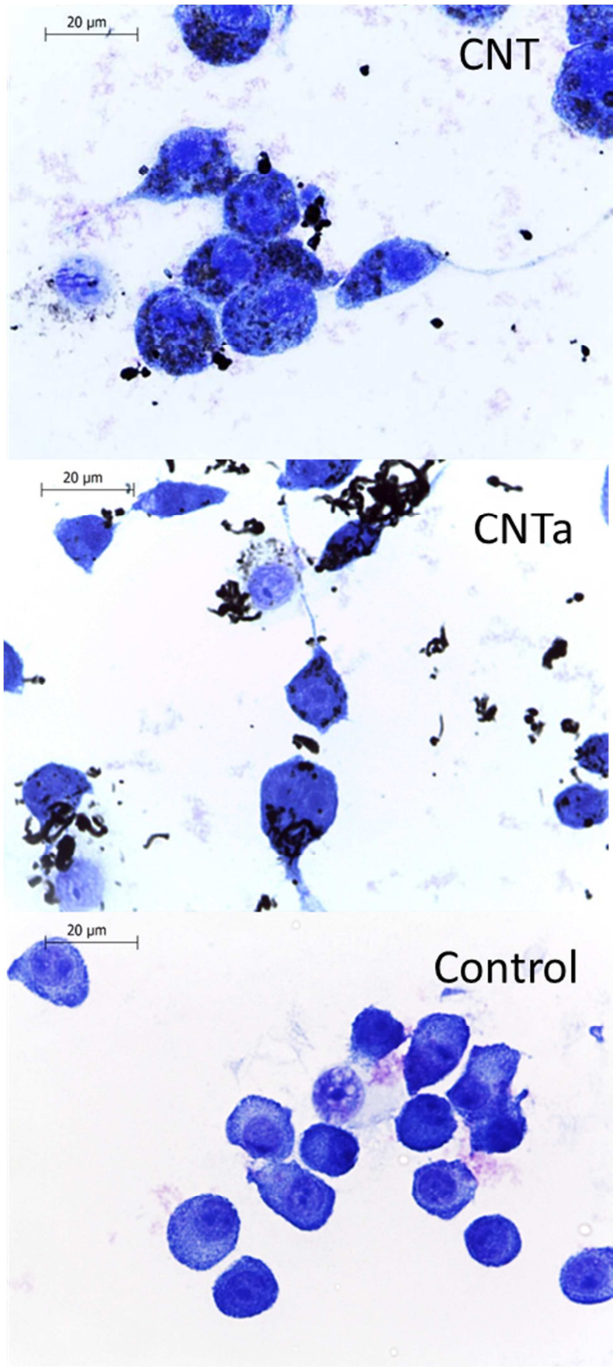

Figure S10: Microscopic observations of RAW 264.7 macrophages exposed for 24h to 15 µg.mL^{-1} of CNT or CNTa or culture medium only, May-Grünwald Giemsa staining.