ADVANCED HEALTHCARE MATERIALS

Supporting Information

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Noninvasive Treatment of Alzheimer's Disease with Scintillating Nanotubes

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Table of Contents

Experimental Methods

Diffraction experiment (XRD). Powder XRD patterns were acquired in Bragg−Brentano geometry with Cu Kα radiation (analytical X'Pert Pro powder diffractometer).

ATR-FTIR (attenuated total reflection Fourier transform infrared spectroscopy). ATR-FTIR spectra of dried samples were obtained on a Thermo Scientific™ Nicolet™ iS20 FTIR spectrometer.

Optical studies. Absorption spectra were recorded using a Cary Lambda 900 spectrophotometer at normal incidence with Suprasil quartz cuvettes with a 0.1 cm optical path length. Steady-state PL and PLE spectra were recorded using a xenon lamp as an excitation source together with a double monochromator (Jobin-Yvon Gemini 180 with a 1200 grooves/mm grating) and recorded with a nitrogen-cooled CCD detector coupled to a monochromator (Jobin-Yvon Micro HR). Under CW laser excitation, signals were recorded using a nitrogen-cooled CCD coupled with a double monochromator, Triax-190 (Horiba Jobin-Yvon) with a spectral resolution of 0.5 nm. Spectra were corrected for the setup optical response. Time resolved PL spectra were recorded using a pulsed LED at 250 nm (3.65 eV, EP-LED 340 Edinburgh Instruments, pulse width of 700 ps) or a pulsed laser at 405 nm (3.06 eV, EPL-405 Edinburgh Instruments, pulse width of 150 ps). Data were obtained with an Edinburgh Instruments FLS-980 spectrophotometer with 5 nm bandwidth and 0.1 ns time resolution.

Circular dichroism (CD) spectroscopy. Experiments were performed using a Chirascan spectrometer (Applied Photophysics, UK). Samples were prepared in PB similar to the ThT aggregation assay, irradiated with 2 Gy X-rays and incubated for 48 h. Measurements were performed at room temperature in a 2 mm optical path length cell, and spectra were recorded from 260 to 190 nm with step size and bandwidth of 1 nm. Spectra are the average of three measurements after background subtraction.

Cell viability MTT assay. Human neuroblast SH-SY5Y cell lines were cultured in low-glucose DMEM supplemented with horse serum (10%), fetal bovine serum (FBS; 5%), L-glutamine, penicillin, and streptomycin at 37 °C in an incubator containing 5% of CO_2. To evaluate cytotoxic effects of different NT samples, cells were seeded 10,000 per well (100 µL) in 96-well plates for 24 h. Cell medium was then replaced with fresh DMEM containing different NT samples (1, 10 and 50 µM). Cells treated with PBS were used as control (100% cell viability). After 48 h of incubation, cell viability was determined using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.[1] Each experiment was performed in triplicate and repeated twice.

C. elegans lifespan toxicity assay. Age-synchronized WT CL802 animals were cultured on 60 mm NGM plates at 16 °C. For each treatment group, a total of 100 synchronized L4 worms were seeded (25 worms/plate) and fed with heat inactivated OP50 solution containing the vehicle (PBS), or 2 and 20 µM of the NTs. Newly hatched worms were removed from the plates and growing worms scored in each day. The experiment was terminated when all the worms were scored as dead or censored. Survival plot was obtained from Kaplan–Meier survival analysis method and lifespan data and statistics were analyzed by a one-way ANOVA followed by Tukey's multiple comparison test.

Supplementary Data

Table S1. Mass spectrometry analysis of Aβ40 after incubation with Ce6/PEG-NTs and irradiation with 2 Gy X-rays and the putative oxidation site.

Aβ40 amino acid sequence:
DAEFR**H**DSG**Y**EV**HH**QKLVFFAEDVGSNKGAIIGL**M**VGGVV

Species	Calculated m/z	Found m/z
$[Aβ40 + 4H]^{4+}$	1083.30	1083.29
$[Aβ40 + 4H + 1 \text{ His Ox }]^{4+}$	1086.79	1086.79
$[Aβ40 + 4H + 2 \text{ His} + 1 \text{ Met Ox }]^{4+}$	1094.28	1094.29
$[Aβ40 + 4H + 2 \text{ His} + 1 \text{ Met} + 1 \text{ Tyr Ox }]^{4+}$	1098.28	1098.29
$[Aβ40 + 4H + 3 \text{ His} + 1 \text{ Met} + 1 \text{ Tyr Ox}]^{4+}$ $[Aβ40 + 4H + 2 \text{ His} + 1 \text{ Met} + 1 \text{ Na}^{+}]^{4+}$	1101.78 1100.03	1102.29
$[Aβ40 + 4H +2 \text{ His} + 1 \text{ Met} + 1 \text{ Tyr Ox} + 1$ His peroxide$]^{4+}$ $[Aβ40 + 4H +2 \text{ His} + 1 \text{ Met} + 2 \text{ Na}^{+}]^{4+}$	1106.78 1106.28	1106.29
$[Aβ40 + 4H + 1 \text{ His} + 1 \text{ Met} + 1 \text{ Tyr Ox} + 2$ His peroxide$]^{4+}$ $[Aβ40 + 4H +2 \text{ His} + 1 \text{ Met} + 3 \text{ Na}^{+}]^{4+}$	1111.27 1111.78	1111.29

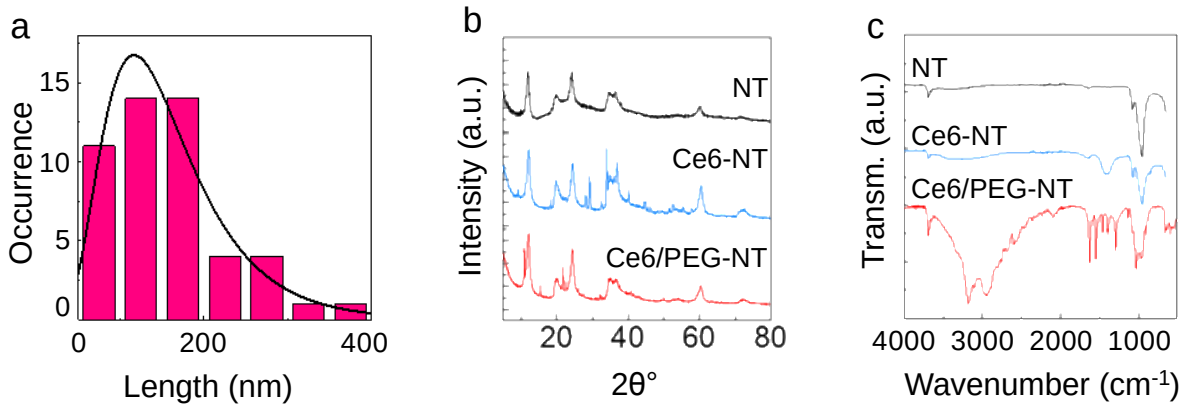

Figure S1. Chemophysical characterization of hydrated magnesium silicate nanotubes (NTs). A) NT length distribution obtained by analysis of TEM images. b) Powder X-ray diffraction (PXRD) patterns of bare NTs, Ce6-NTs and Ce6/PEG-NTs. The PXRD pattern displays the characteristic reflections of high crystalline hydrated magnesium silicate as a unique crystalline phase.[2] c) ATR-FTIR spectra of the NTs. All spectra show the presence of the main chrysotile vibrational peaks: Mg-OH stretching between 3600–3700 cm^{-1}, Si-O-Mg, Si-O-Si and Si-O stretching in the region around 1000 cm^{-1}. The characteristic peaks related to the organic compounds in the region of 1000–2000 cm^{-1} confirm the Ce6 functionalization, while the vibrational peaks of PEG chains (CH_2-CH_2O) appear around 2800–3000 cm^{-1}.

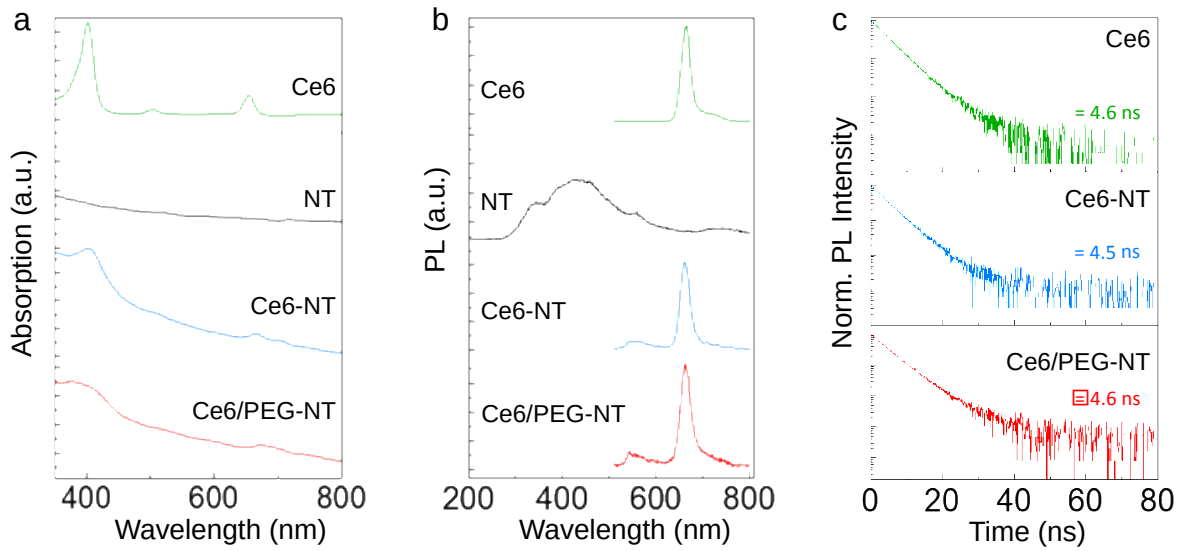

Figure S2. Optical and luminescence properties of multicomponent NTs. a) Absorption spectra of Ce6, bare NTs, Ce6-NTs and Ce6/PEG-NTs dispersion (1.7 mg/ml) in aqueous phosphate buffer saline (PBS, pH 7.4). b) Photoluminescence (PL) and c) time dependent PL intensity decay of Ce6 solution, bare NTs and the functionalized NTs dispersions in PBS, explored under optical excitation at 250 nm to excite both the NT and the Ce6. Time-resolved PL spectra are recorded at the maximum of the PL spectrum under pulsed excitation at 405 nm. Ce6-NTs and Ce6/PEG-NTs show photoluminescence and time resolved spectra similar to those of Ce6, suggesting the absence of undesired processes on the NTs surface that could compromise the performance of the dye.

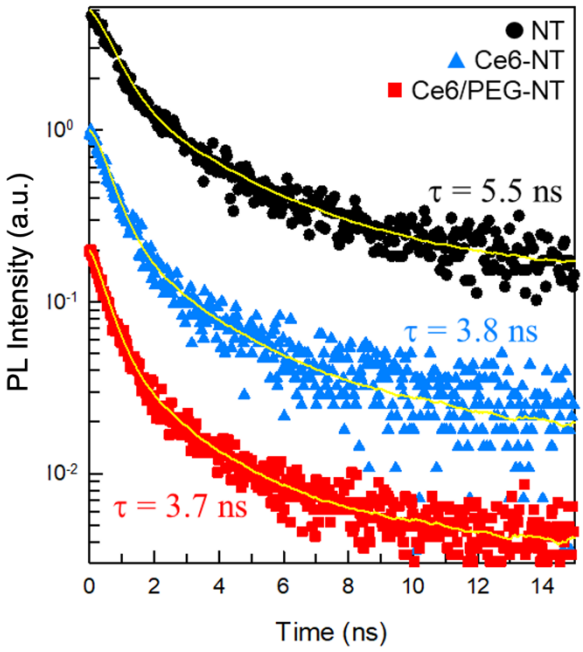

Figure S3. NT-to-Ce6 non radiative energy transfer. Photoluminescence (PL) intensity decay at 430 nm for tested NTs in water dispersion under pulsed excitation at 250 nm. Solid lines are the fit of data with a multi-exponential decay functions with characteristic average decay time τ of 5.5, 3.8 and 3.7 ns for bare NTs, Ce6-NTs and Ce6/PEG-NTs, respectively. The decay time is estimated as the weighted average of the decay components given by the fit. The non-radiative energy transfer yield ϕ_{ET} for sample *i* is calculated as $\phi_{ET} = 1- \tau_i/\tau_{NT}$. The calculation yields a ϕ_{ET} of about 0.32 for both Ce6 functionalized NT samples.

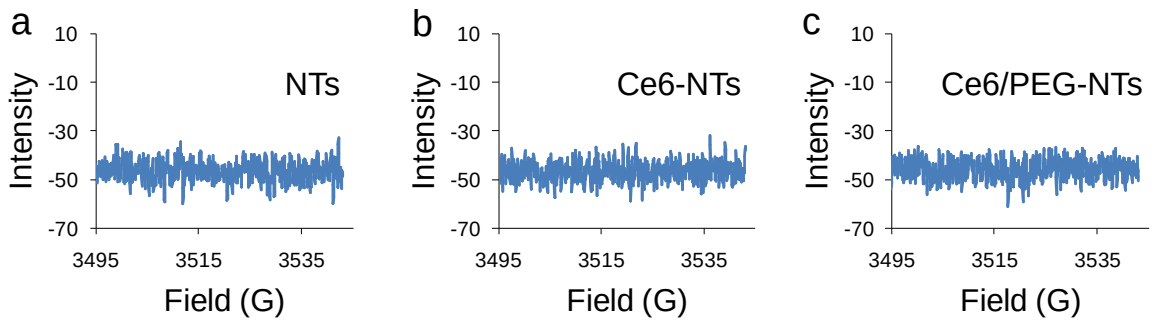

Figure S4. Generation of singlet oxygen (SO) by different NTs without X-ray irradiation. Control EPR spectra of NTs, Ce6-NTs and Ce6/PEG-NTs (5 μM each) and TEMP (40 μM) suspension in PBS/D_2O (1:9) under dark conditions. SO is not detected without irradiation.

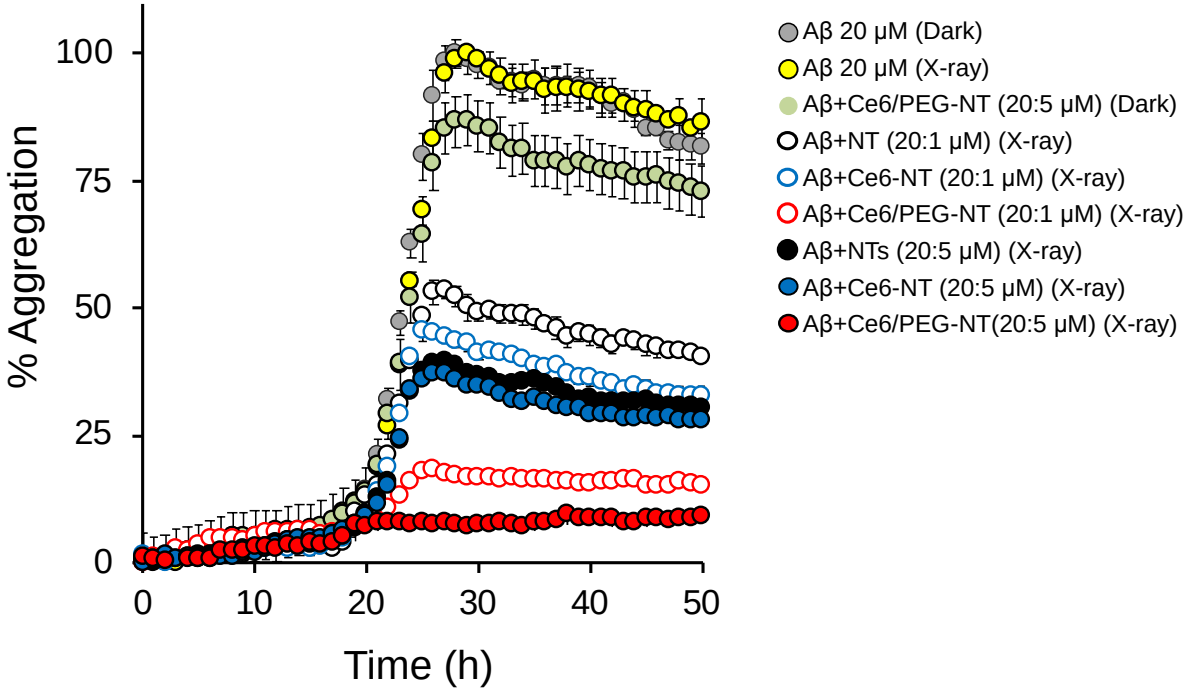

Figure S5. Effect of NTs on aggregation of Aβ40 using the Thioflavin T (ThT) aggregation assay. Monomeric Aβ40 (20 μM) was incubated in the absence or presence of NTs, Ce6-NTs and Ce6/PEG-NTs (1 and 5 μM) in PBS (50 mM, pH 7.2) with or without exposure to X-ray (2 Gy) and 100% aggregation was determined based on Aβ ThT fluorescence. Data are presented as mean ± SD of experiments carried out in triplicate and repeated twice.

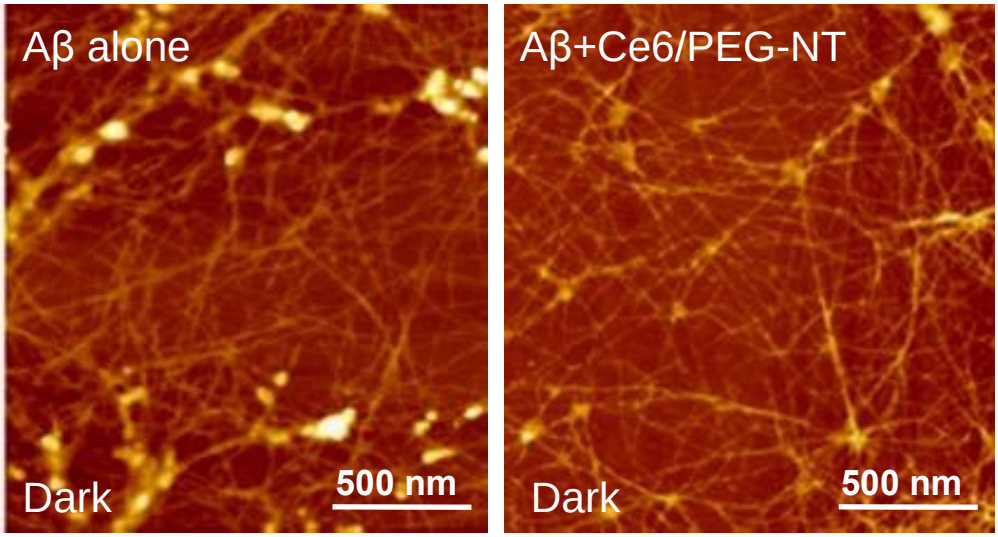

Figure S6. Atomic force microscope (AFM) images of Aβ alone (20 μM, left) and after treatment with Ce6/PEG-NTs (1 μM, right) under dark conditions.

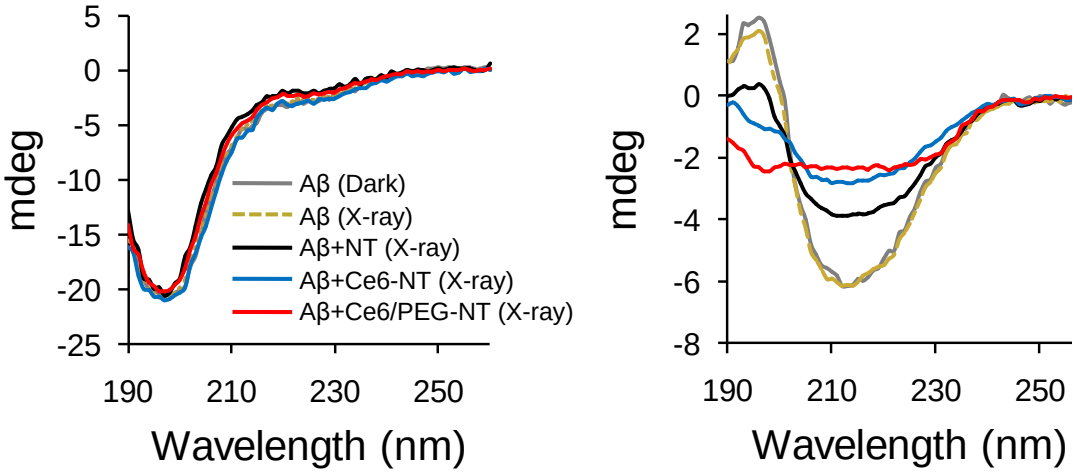

Figure S7. Effect of X-ray excited NTs on the secondary structure of Aβ40. Time dependent far-UV CD spectra of freshly prepared monomeric Aβ40 (10 μM) incubated in the absence or presence of 1 μM or equivalent amount of NTs, kept in dark or irradiated with 2 Gy X-rays in phosphate buffer (50 mM, pH 7.4), and analyzed after a) 0 and b) 48 h. Data are representative of three independent experiments after background subtraction.

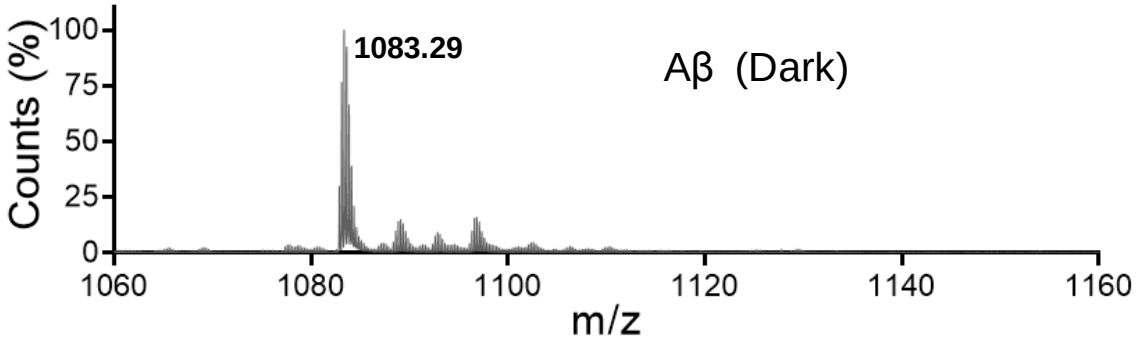

Figure S8. Representative ESI-MS spectrum of non-irradiated Aβ40 amyloid (40 μM).

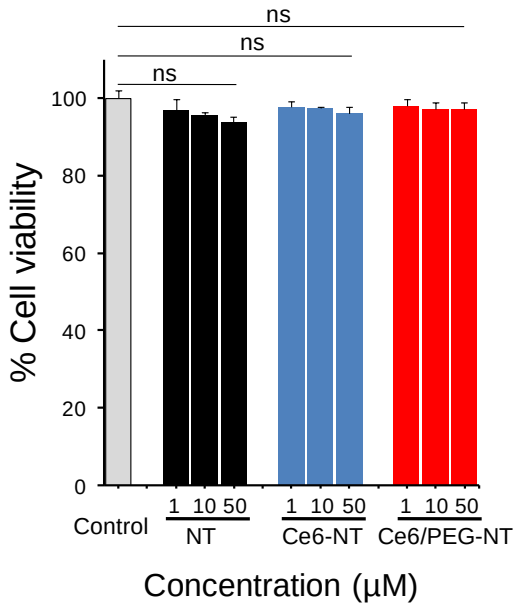

Figure S9. Toxicity effect of NTs on human neuroblastoma SH-SY5Y cells. *In vitro* cell viability study of human neuroblastoma SH-SY5Y cells after 48 h incubation with increasing concentration of NTs (1, 10 and 50 µM) and irradiation with 2 Gy X-rays, using the MTT assay. Results are expressed as percentage of the control (untreated) cells and are reported as the mean ± SD from two experiments (n = 3 each). Statistical analysis was performed using Student's t-test, $p \geq 0.05$ was considered as non-significant (ns).

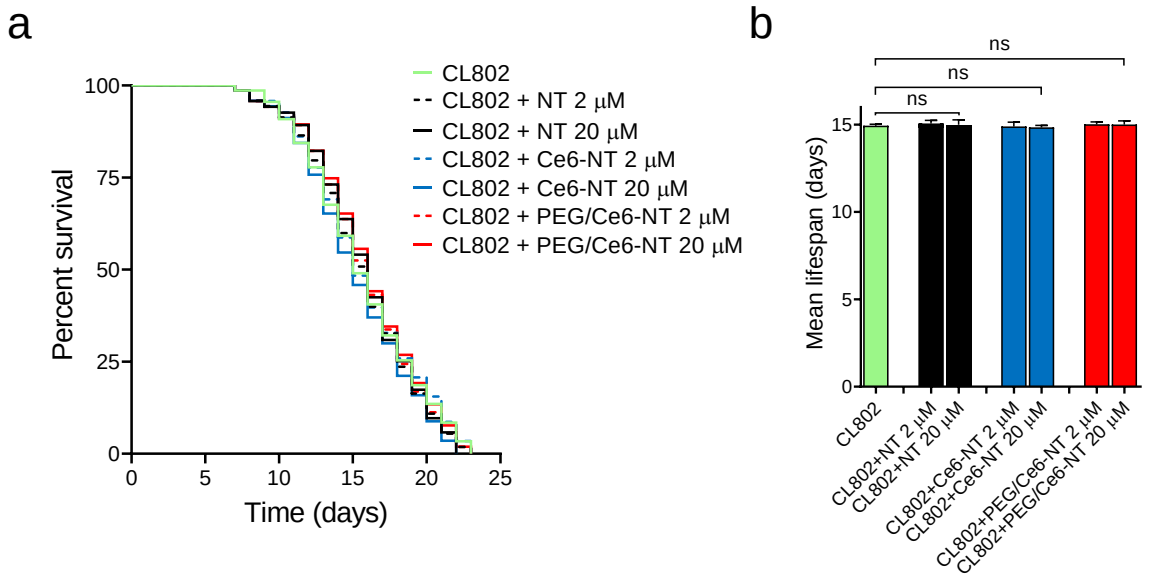

Figure S10. *In vivo* toxicity of NTs on WT *C. elegans*. a) Kaplan-Maier survival plots of WT *C. elegans* CL802 fed without or with 2 and 20 µM of the NTs. b) Median lifespan of WT CL802 *C. elegans* model treated with the NTs. Data presented are the mean ± standard deviation (SD) from three experiments and were analyzed by one-way ANOVA followed by Tukey's multiple comparison test. $p \geq 0.05$ was considered as not-significant (ns).

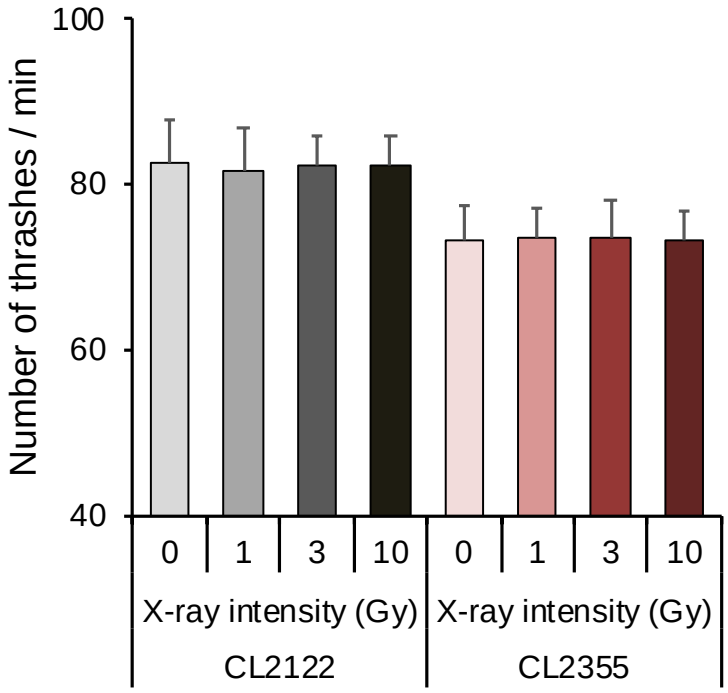

Figure S11. Effect of increasing X-ray intensity on mobility (thrashes per min) of transgenic CL2355 and control WT CL2122 worms. Results are mean ±SD from three independent experiments carried out with 20 worms per group. Statistical significance was analyzed by one-way ANOVA followed by Tukey's multiple comparison test (ns = not significant).

Supplementary References

[1] T. Mosmann, *J. Immunol. Methods* **1983**, 65, 55.
[2] G. Falini, E. Foresti, M. Gazzano, A. F. Gualtieri, M. Leoni, I. G. Lesci, N. Roveri, *Chem. Eur. J.* **2004**, 10, 3043.