Supplementary Information

Long-Term Exposure to Nanoparticles Alters Senescence Markers and Immune Responses in Macrophages

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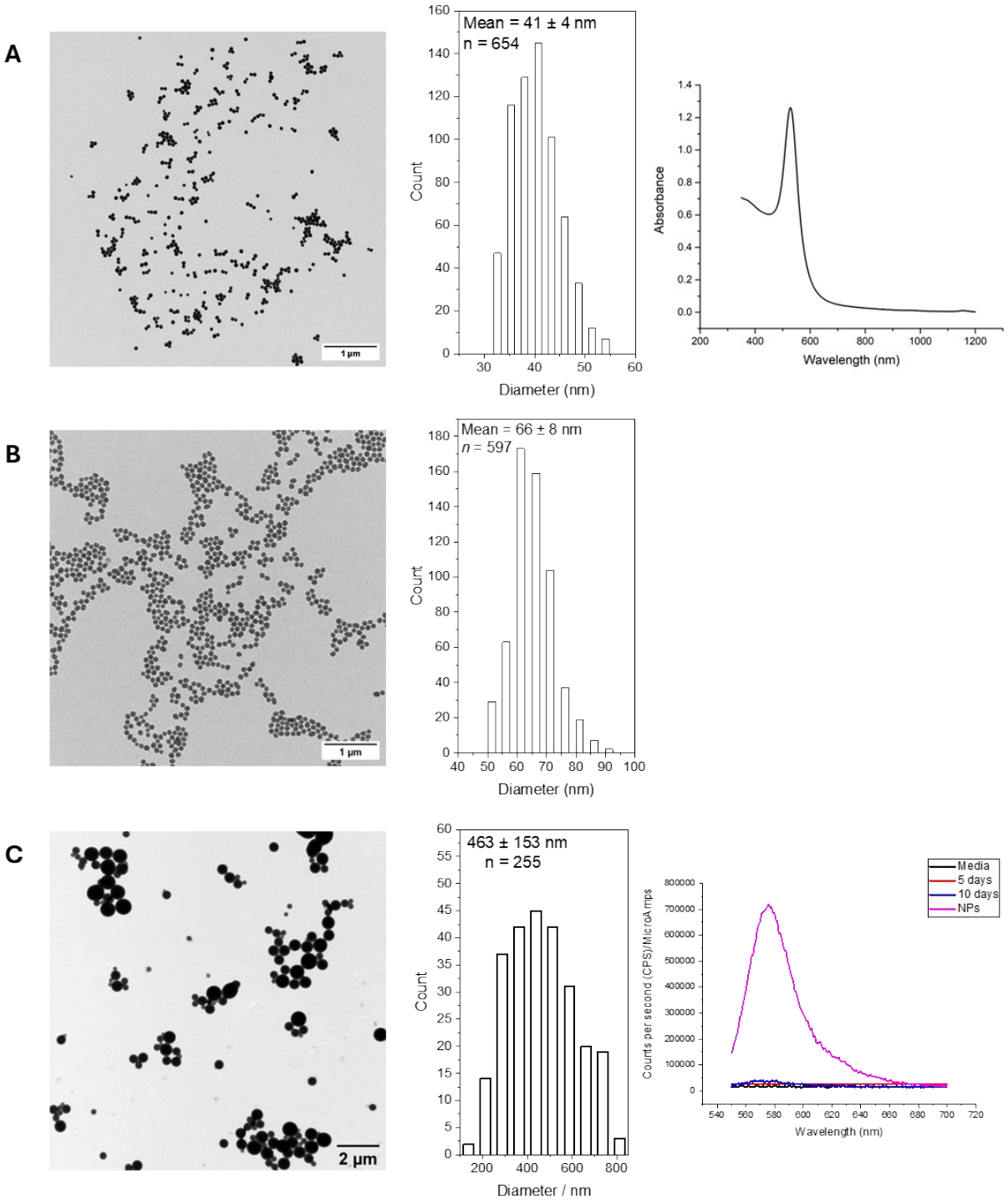

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16 *Figure S 1* Characterization of NPs. (A) Representative TEM micrograph showing morphology and size distribution
17 histogram of AuNPs indicating a mean diameter of 41 nm ± 4 nm, and UV-Vis absorption spectrum highlighting the
18 plasmon resonance peak at 528 nm. (B) TEM micrograph displaying morphology and size distribution histogram of
19 SiO_2 NPs with an average diameter of 66 nm ± 8 nm. (C) TEM micrograph illustrating PET NPs and size distribution
20 histogram with a mean size of 463 nm ± 153 nm. Fluorimeter measurement showing emission profiles of media
21 alone, PET NPs after 5 and 10 days of incubation in media followed by filtration, and PET NPs at 20 µg/mL. The

graph highlights fluorescence intensity (counts per second/microamps) as a function of wavelength (nm), with PET NPs showing a pronounced peak compared to the control groups.

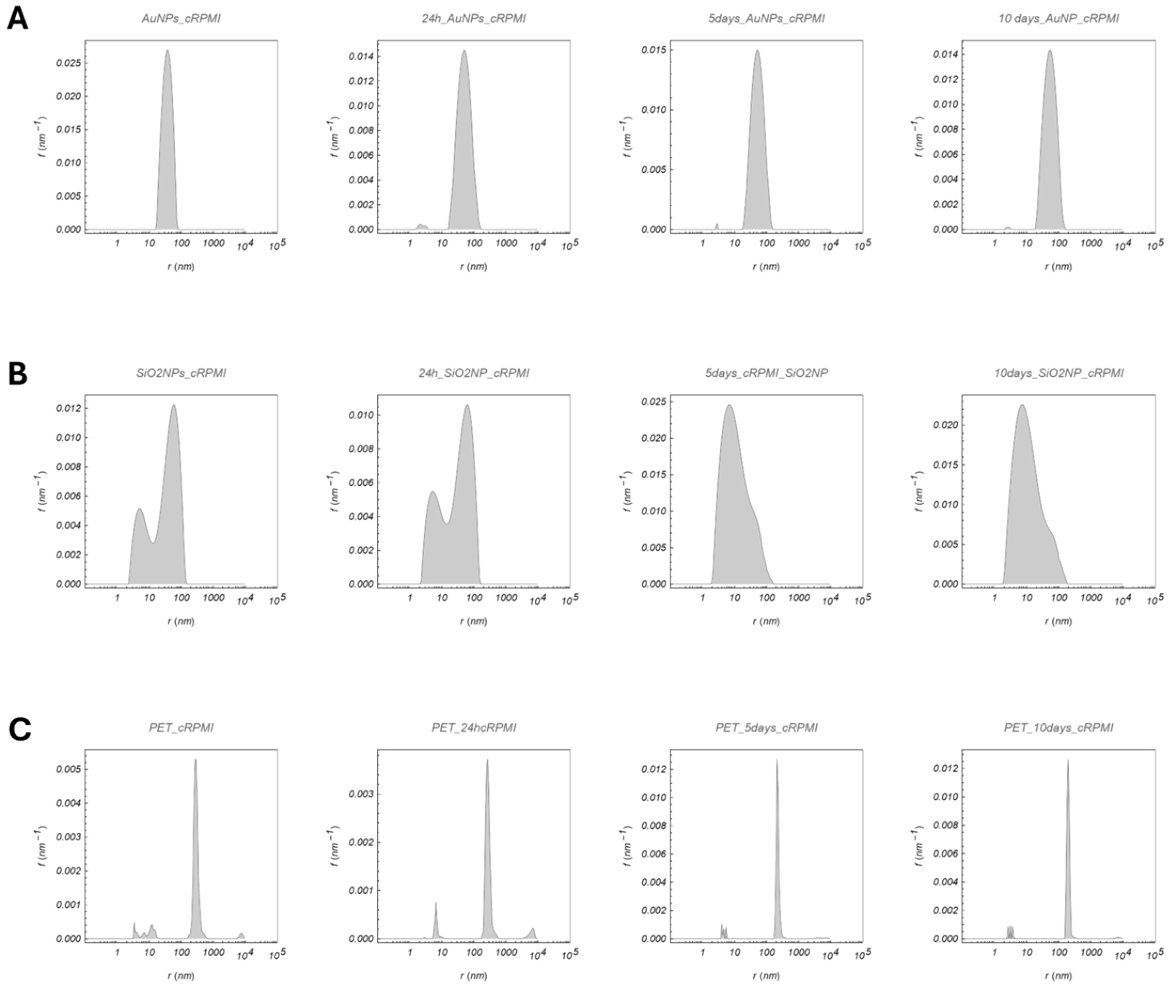


Figure S 2 Size distribution of NPs in complete Roswell Park Memorial Institute medium (cRPMI) as determined by dynamic light scattering (DLS). The distributions are shown for AuNPs (row A), SiO_2 NPs (row B), and PET NPs (row C), organized by columns for measurements taken immediately after dispersion and after 24 hours, 5 days, and 10 days.

Table S 1 Physicochemical characterization of NPs.

NPs type	NPs core size (TEM)	Zeta potential	Hydrodynamic diameter (DLS) nm

	nm	(DLS) mV			
		H_2O	H_2O	cRPMI (t=0h)	cRPMI (t=10d)
AuNPs	41± 4	-29 ± 2	57 ± 6	74 ± 9	115 ± 7 nm
SiO_2 NPs	66 ± 8	−52 ± 2	83 ± 3	108 ± 5	35 ± 4 nm
PET NPs	463 ± 153	-35 ± 3	555 ± 14	580 ± 44	468 ± 57 nm

Table S 2 Determination of Size distribution and concentration of PET NPs by NTA analysis.

	NTA analysis*						
	Particles ($x10^7$) / mL	Particles / Frame	Mean	Mode	D10	D50	D90
PET	608 ± 51	13 ± 1	432 ± 13	341 ± 49	214 ± 14	448 ±	626 ± 26

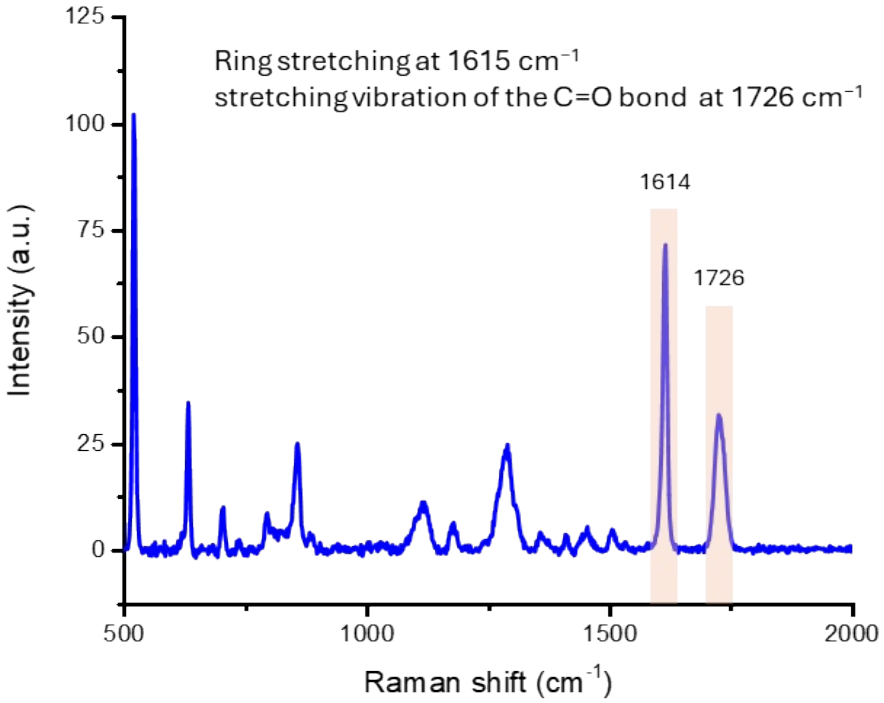
	(0.44 mg/mL**)					12	

***** No particles were detected in the Milli-Q water when analyzed using the same parameters utilized for measuring the PET NPs.

** The concentration of PET NPs expressed in mg/mL was calculated using the following equation:

$$[PET]_{mg/mL} = \frac{\rho_{PET} \cdot 4/3\pi r^3 \cdot Number\ Particles/mL \cdot 1 \cdot 10^{-18}}{Volume\ in\ mL}$$

The ρ_{PET} = 1.39 g cm^{-3}; $608 \cdot 10^7$ particles/mL and diameter = 463 nm.



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56 *Figure S 3* Raman spectrum of PET NPs displaying the chemical composition of the particles. The highlighted
57 peaks correspond to characteristic stretching modes of polyethylene terephthalate.

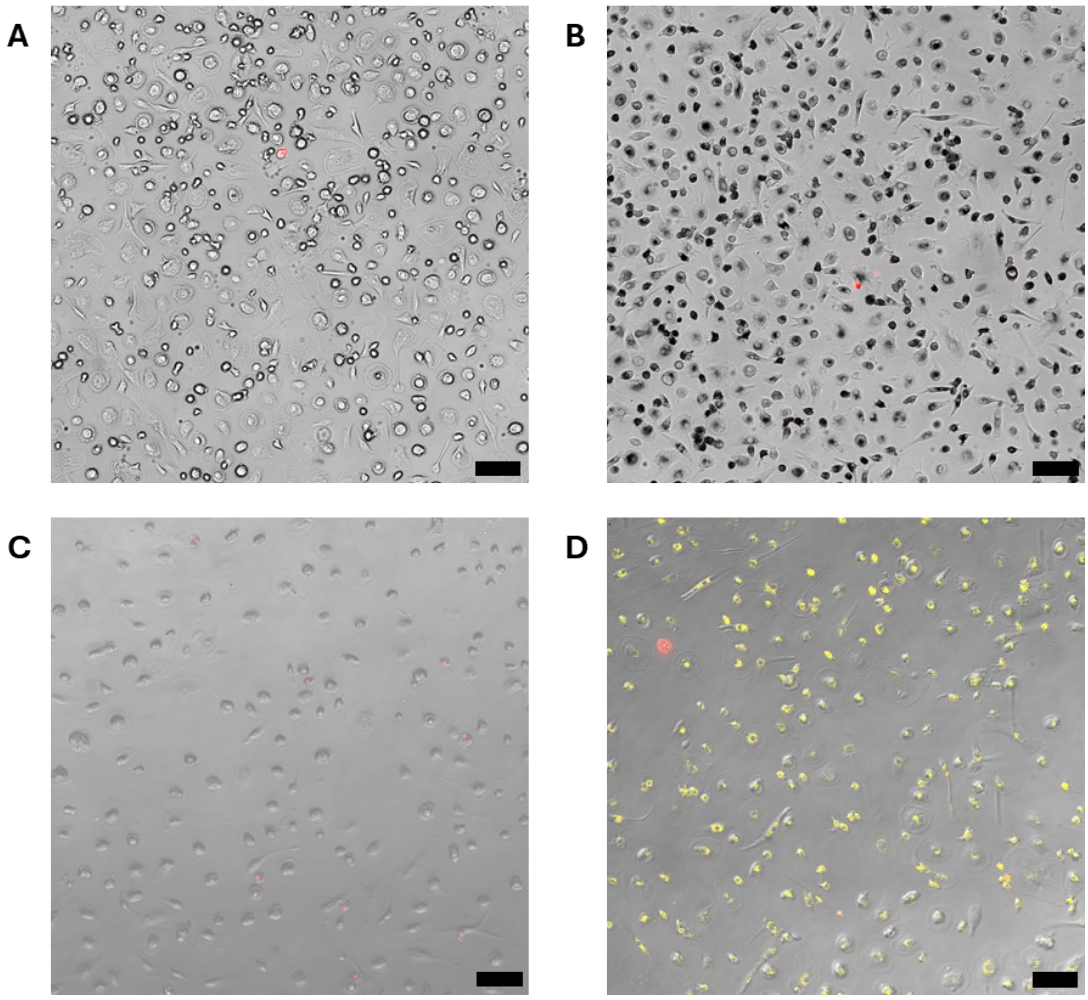

Figure S 4 Live/dead staining of macrophages after 10 days of continuous exposure to nanoparticles at a concentration of 20 µg/mL, without media change. (A) shows untreated cells, while (B), (C), and (D) represent macrophages exposed to AuNPs, SiO_2 NPs, and PET NPs, respectively. Dead cells are stained in red. Scale bar: 20 µm.

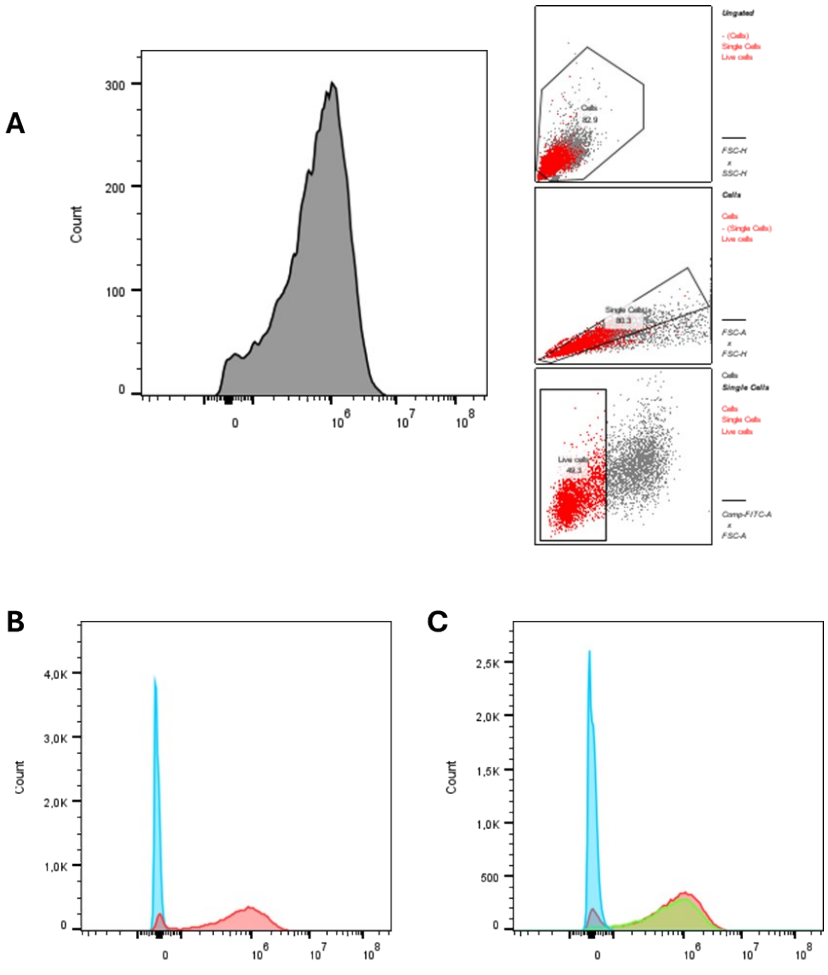

Figure S 5 Representative flow cytometry gating strategy (A) was applied to evaluate nanoparticle-derived median fluorescence intensity in macrophages. Histograms show the fluorescence intensity of samples after 5 days (B), with control samples in blue and exposed samples in red. For 10-day samples, histograms display controls (blue), acutely exposed cells (red), and continuously exposed cells (green), illustrating differences in fluorescence distribution based on exposure conditions.

Table S 3 Information about the primers used for Real-time qRT-PCR. FW: Forward. RV: Reverse.

Primers	**Gene Symbol**	**Gene name**	***Sequence (5'->3')***	***Product length***	***Efficiency (%)***
p16INK4A	CDKN2A	cyclin-dependent kinase inhibitor 2A	FW: CCAGAGGCAGTAACCATGCC RV: TTTCCCGAGGTTTCTCAGAGC	96	105
IL-6	IL6	Interleukin 6	PrimePCR™ SYBR® Green Assay: IL6, Human (Bio Rad; RedSeq: NC000007)		98
IL-8	CXCL8	C-X-C motif chemokine ligand 8	FW: GAGAAGTTTTTGAAGAGGGCTGA RV: GCTTGAAGTTTCACTGGCATCT	91	102
Housekeeping	GAPDH	Glyceraldehyde-3-phosphate dehydrogenase	FW: GTCGGAGTCAACGGATTTGG RV: GCCATGGGTGGAATCATATTGG	147	101
	YWHAZ	Tyrosine 3-monooxygenase /tryptophan 5-monooxygenase activation protein zeta	FW: GCTGGTGATGACAAGAAAGGGAT RV: GTTAAGGGCCAGACCCAGTC	120	97