Supporting Information

Physicochemical and Toxicological Properties of Commercial Carbon Blacks Modified by Reaction with Ozone

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Table S1. Raw Data from EPR Spectroscopy of F101 and P90.

Spectra	*Mass (mg)*	*g-values*[a]	*Peak Area*	*Receiver Gain*	*Radical Conc. (spins/mg)*[b]
F101-a *(untreated)*	2.5	2.0022	5.32×10^{10}	1.00×10^{2}	4.06×10^{8}
F101-b *(untreated)*	3.8	2.0022	5.07×10^{10}	1.00×10^{2}	2.55×10^{8}
F101-c *(untreated)*	4.2	2.0022	4.09×10^{10}	1.00×10^{2}	1.86×10^{8}
F101-d *(ozonated)*	2.5	2.0024	8.63×10^{10}	1.00×10^{2}	6.59×10^{8}
F101-e *(ozonated)*	4.3	2.0024	1.11×10^{11}	1.00×10^{2}	4.93×10^{8}
F101-f *(ozonated)*	2.8	2.0024	8.67×10^{10}	1.00×10^{2}	5.91×10^{8}
F101-g *(ozonated aqueous)*	2.5	2.0024	1.47×10^{10}	1.00×10^{2}	1.12×10^{8}
F101-h *(ozonated aqueous)*	2.2	2.0024	1.34×10^{10}	1.00×10^{2}	1.16×10^{8}
F101-i *(ozonated aqueous)*	4.7	2.0024	2.10×10^{10}	1.00×10^{2}	8.53×10^{7}
P90-a *(untreated)*	2.5	-	1.34×10^{8}	1.00×10^{5}	1.03×10^{3} [c]
P90-b *(untreated)*	2.8	-	3.53×10^{8}	1.00×10^{5}	2.41×10^{3} [c]
P90-c *(untreated)*	3.6	-	5.90×10^{8}	1.00×10^{5}	3.13×10^{3} [c]
P90-d *(ozonated)*	2.9	2.0028	8.86×10^{9}	1.00×10^{5}	5.83×10^{4}
P90-e *(ozonated)*	2.9	2.0027	6.40×10^{9}	1.00×10^{5}	4.21×10^{4}
P90-f *(ozonated)*	3.9	2.0028	6.54×10^{9}	1.00×10^{5}	3.20×10^{4}
P90-g *(ozonated aqueous)*	5.1	2.0027	4.61×10^{10}	1.00×10^{4}	1.72×10^{6}
P90-h *(ozonated aqueous)*	5.2	2.0026	4.24×10^{10}	1.00×10^{4}	1.56×10^{6}
P90-i *(ozonated aqueous)*	2.0	2.0027	1.75×10^{10}	1.00×10^{4}	1.67×10^{6}

[a] *Values obtained using automatic fitting program* (1)
[b] *Concentrations determined using DPPH standard*
[c] *Negligible*

Table S2. DEPMPO Adduct Quantitation

Carbon System	Adduct Concentration *(μM / mg carbon)*
F101 – Untreated	0.62
F101 – Ozonated	10.9
P90 – Untreated	0.22
P90 – Ozonated	6.1
P90 – Untreated + EtOH	0.88
P90 – Ozonated + EtOH	12.5

Procedure for EPR Adduct Quantitation.

Prior to experiments, a known concentration of 3-CP was run through EPR for 10 scans. For each spin trapping experiment, a known mass of carbon was weighed (in mg) and added to a solution of 100 mM DEPMPO. Each set of spectra were recorded for 10 scans, and then integrated. A 3-CP EPR spectra is generated by the one unpaired electron per molecule split by a single nitrogen to give a triplet. A single peak height intensity was measured and divided to give the peak height per 1 μM 3-CP. Since the single electron of a DEPMPO spin adduct is divided over 12 peaks, (for DEPMPO-R, they are roughly equal and vary by the *cis* vs. *trans* conformers, and for DEPMPO-OH, the peaks are arranged in a 1:2:2:1:1:2:2:1 ratio to give a total of 12) and 3-CP is divided over 3, the ratio between the two is 1:4. So the intensity of a single peak of a DEPMPO adduct spectra is multiplied by 4 to allow it to be divided by the intensity of the 3-CP peak given per μM. This is equivalent to multiplying the 3-CP peak intensity by 3 to give the total intensity, then dividing by 12 to get the intensity per peak of DEPMPO adduct per μM. This gives the concentration of DEPMPO spin adducts present in solution in terms of μM. This number is then divided by the known concentration of carbon added to give the final concentration of adducts in μM / mg carbon.

Procedure for testing carbon interference with LDH enzyme activity

MH-S cells were cultured with RPMI + 10% FBS and 0.1% Pen Strep and seeded into 2 175 cm2 flasks and grown to confluency (4 days). Media was removed from the flask and the cells were lysed using 25 mL of RPMI + 10% FBS and 0.1% Penstrep containing 1% Triton 100. After 1 hour, cell supernatants containing LDH were collected and 1 mL was plated in each well of a 24 well plate. Carbon nanoparticles were suspended in DPBS and added in triplicate to designated wells at 2.5, 5 and 10 ug/cm2. Flask supernatants alone served as the negative control. LDH containing supernatants containing carbon nanoparticles were then incubated in a humidified environment at 37.0 C and 5% CO2 for 24 hours to determine if Carbon interferes with LDH detection. Triplicate samples from each well (100 uL) were collected and assayed for LDH activity using the LDH Cytotoxicity Detection Kit (Clontech) according to manufacturer's instructions. Plates were read on a Biotek plate reader and an absorbance wavelength of 490 nm, with a reference wavelength of 600 nm. The graphs in Figure S10 are absorbance values.

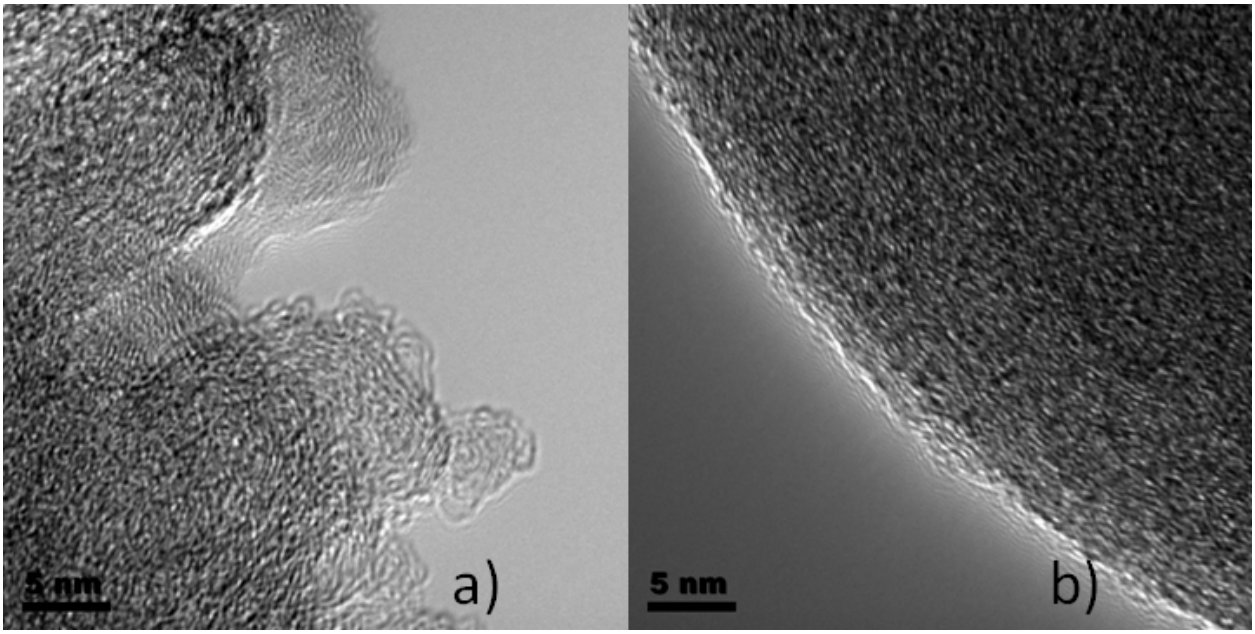


Figure S1: TEM images of (a) P90 and (b) F101 after treatment with ozone

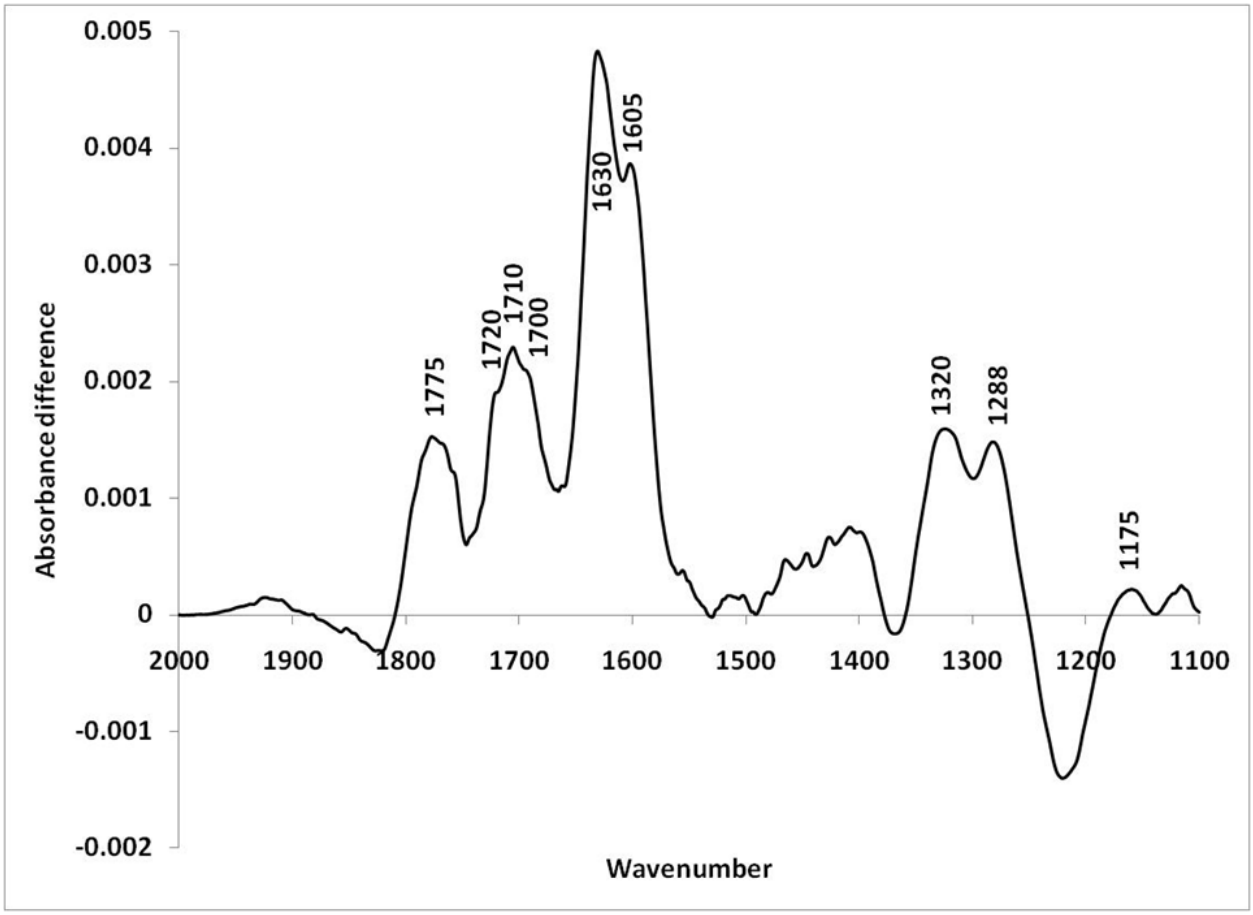


Figure S2: Difference spectrum of F101 exposed to ozone for 240 minutes

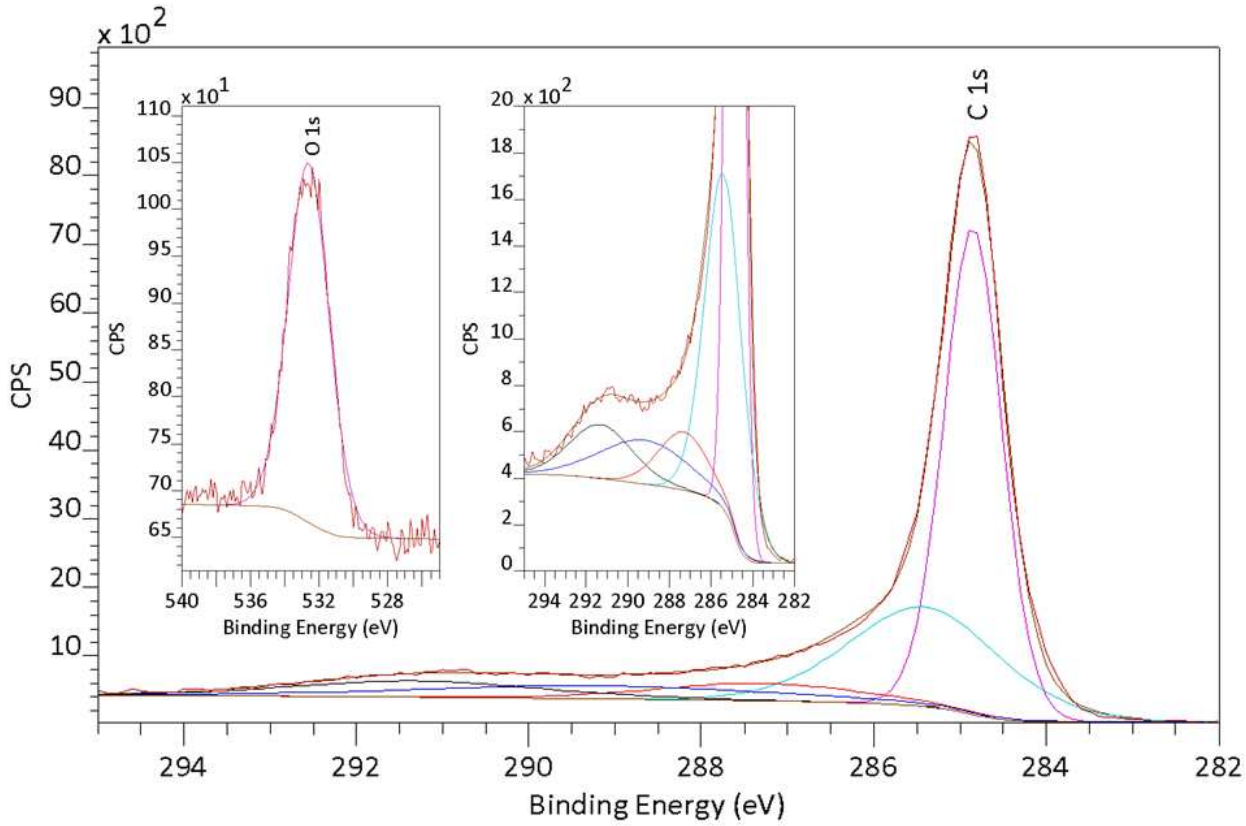


Figure S3: Representative XPS spectra of carbon blacks, shown as P90 after 240 minutes of ozone exposure. Inset: the O 1s region (left), and the C1s region rescaled to clarify peaks showing oxygen functionality (right).

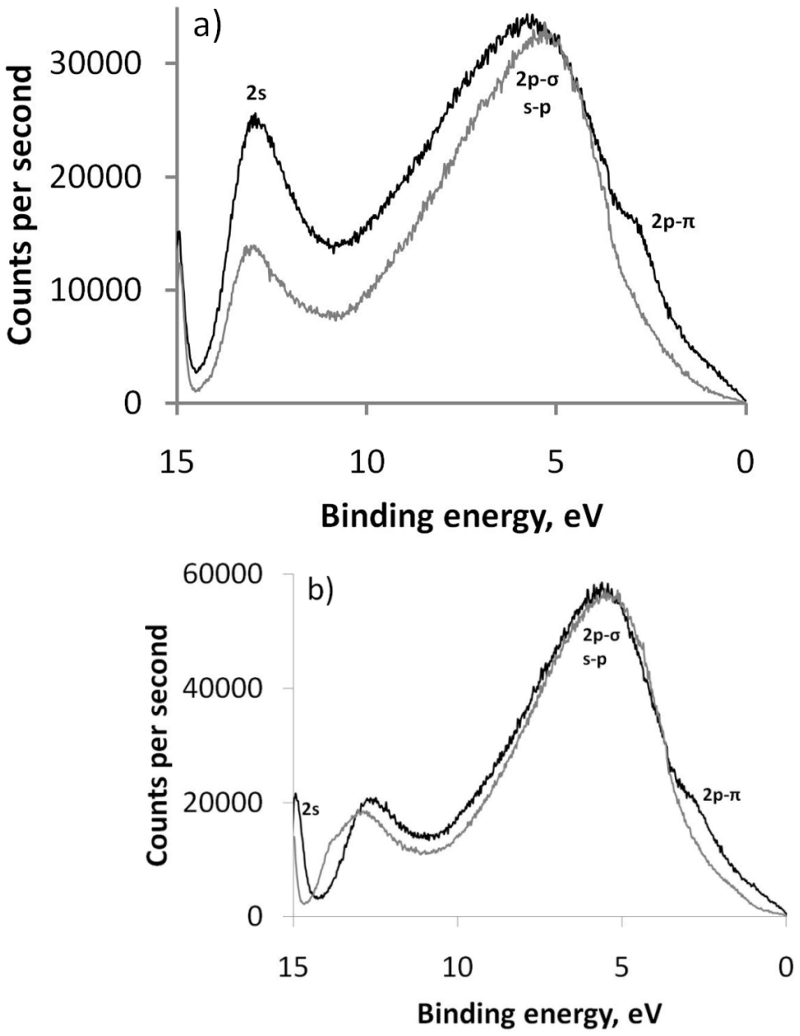


Figure S4: Ultraviolet photoelectron spectra of P90 (a) and F101 (b), before ozonolysis (black) and after ozonolysis (gray).

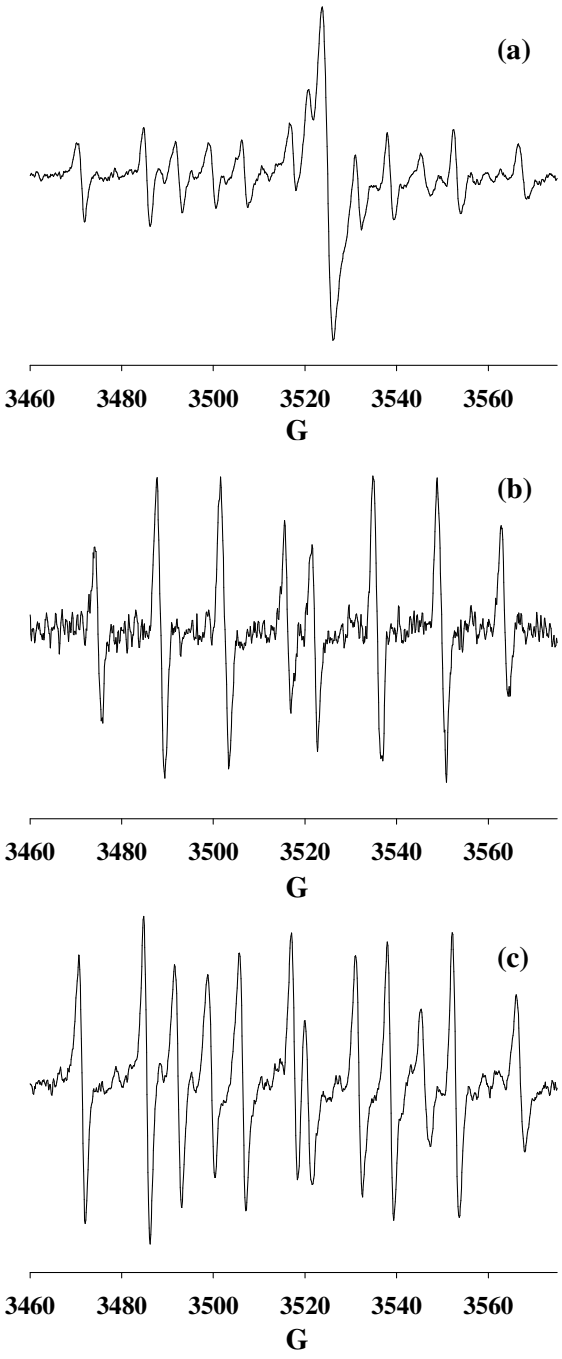


Figure S5. X-band spectra of DEPMPO in the presence of (a) untreated aqueous F101, (b) untreated aqueous P90, and (c) untreated aqueous P90 in ethanol.

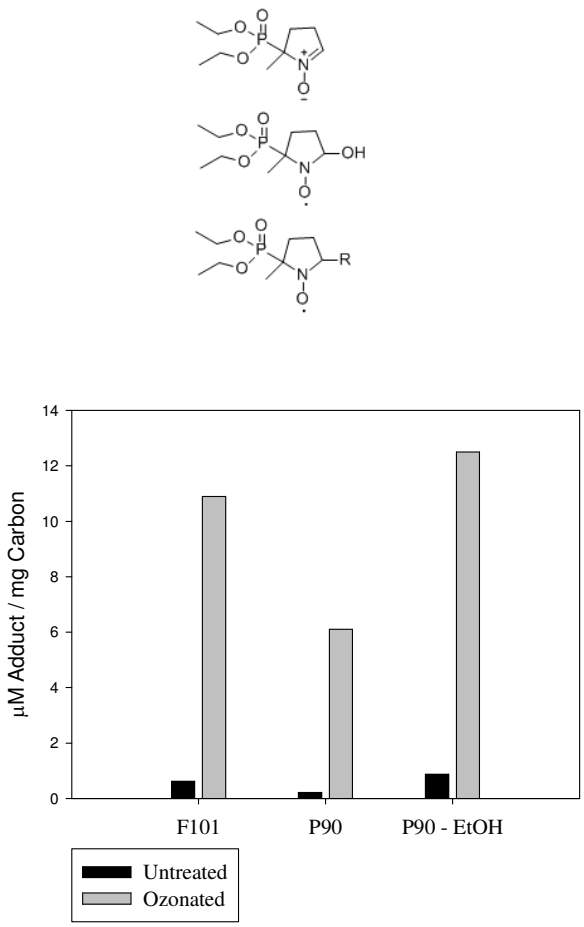
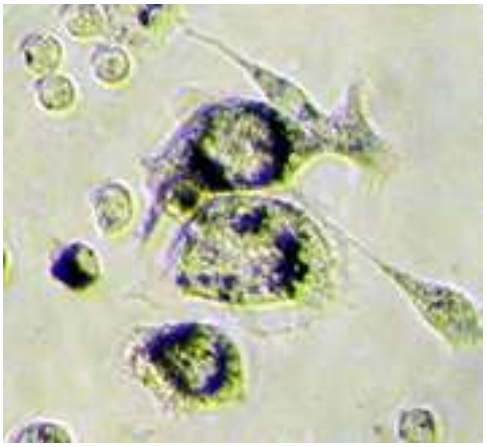


Figure S6. Quantitation of DEPMPO adduct per mg carbon generated in the presence of untreated or ozonated F101 and P90 in solution.

A)



B)

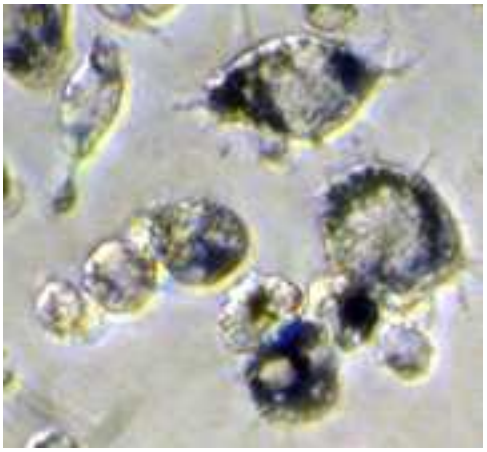


Figure S7. Phase contrast micrographs of MH-S murine macrophages incubated for 24 hours with 10 µg/cm^2 Printex (A) or Flammruss (B). Ozonated particles were internalized by macrophages to an equivalent extent (data not shown). Original magnification: 250x

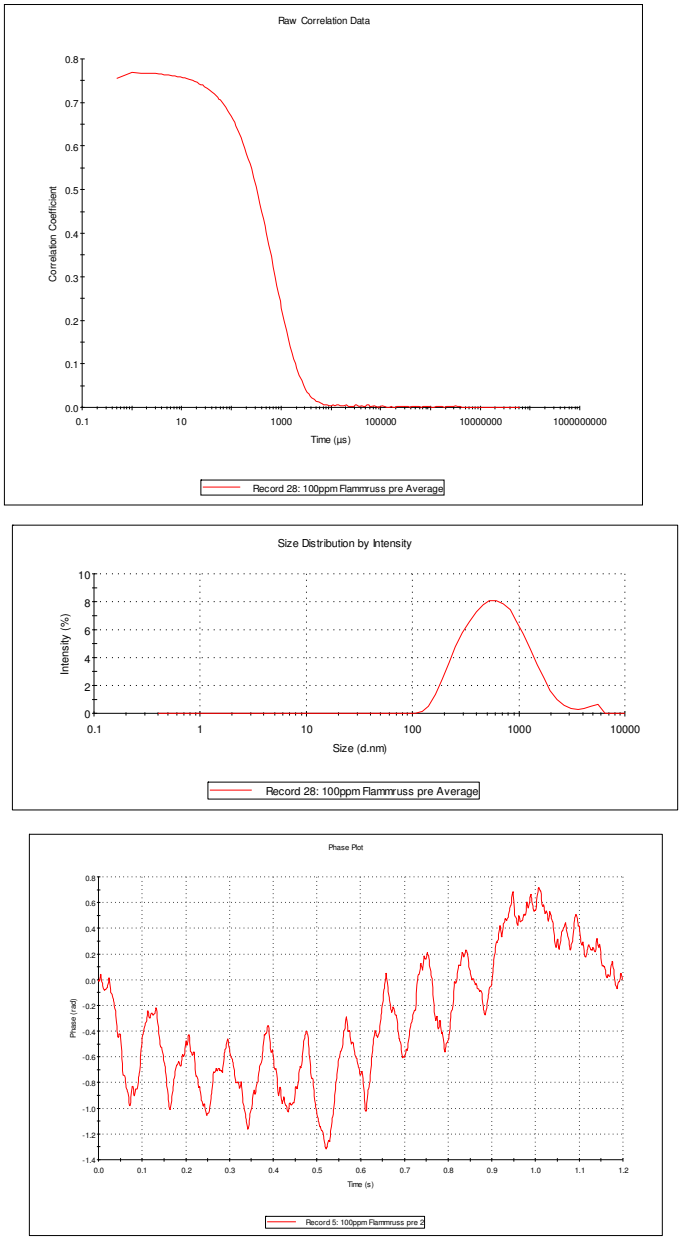


Figure S8. Characteristic correlation function for non-ozonated Flamruss 101 in media and the corresponding intensity distribution. Bottom plot depicts the phase plot from which the zeta potential was derived.

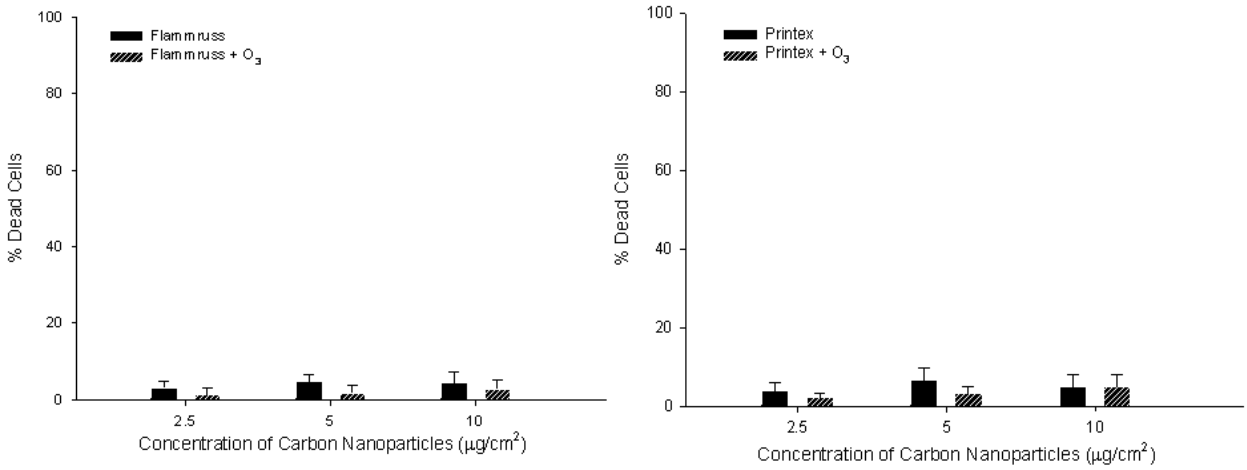


Figure S9. LDH assays for macrophages exposed to P90 (left) and F101 (right) with and without prior particle ozonolysis.

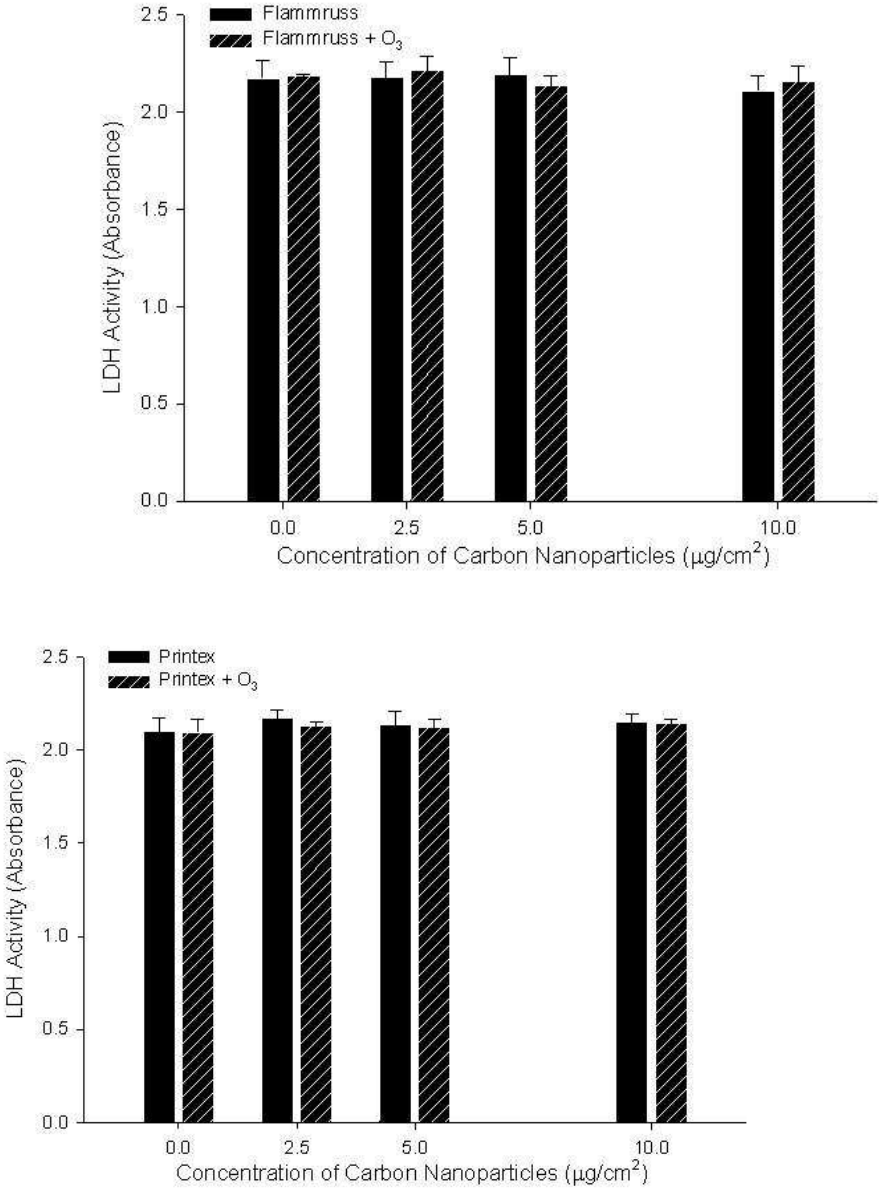


Figure S10. Data showing the lack of CB interference in the LDH assay.