

## Supplementary

# Comparing the Effects of Intracellular and Extracellular Magnetic Hyperthermia on the Viability of BxPC-3 Cells

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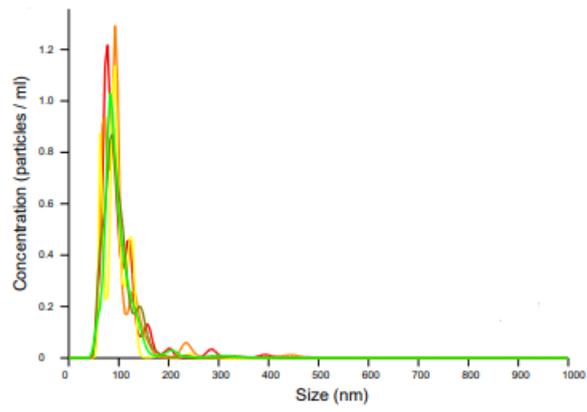
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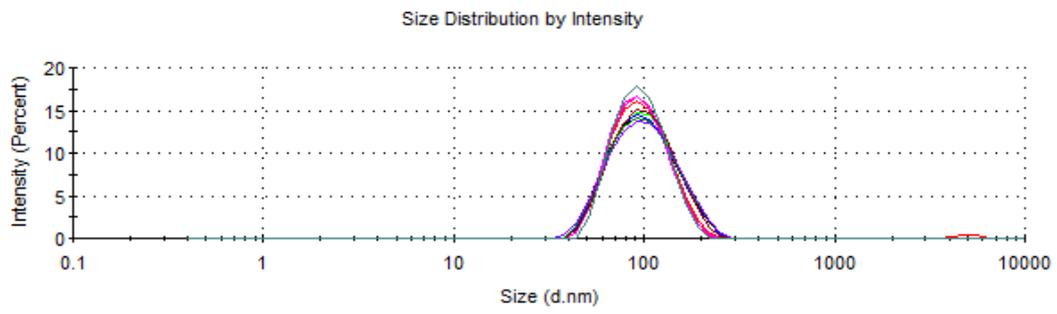
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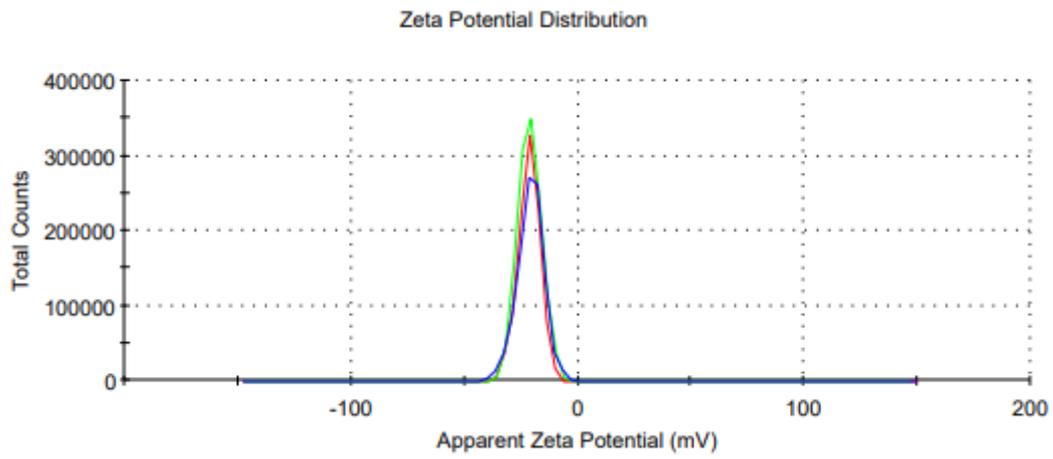
A.



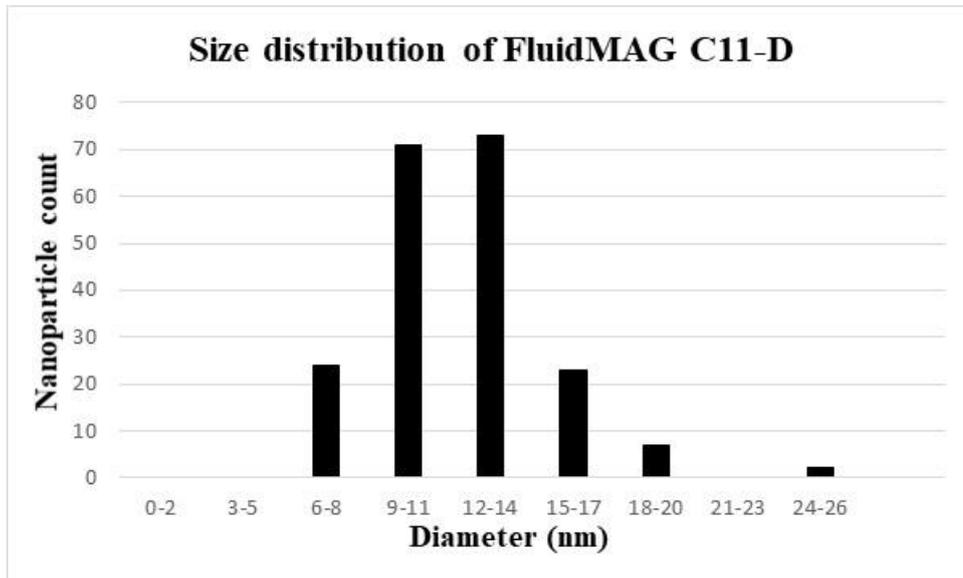
B.



C.

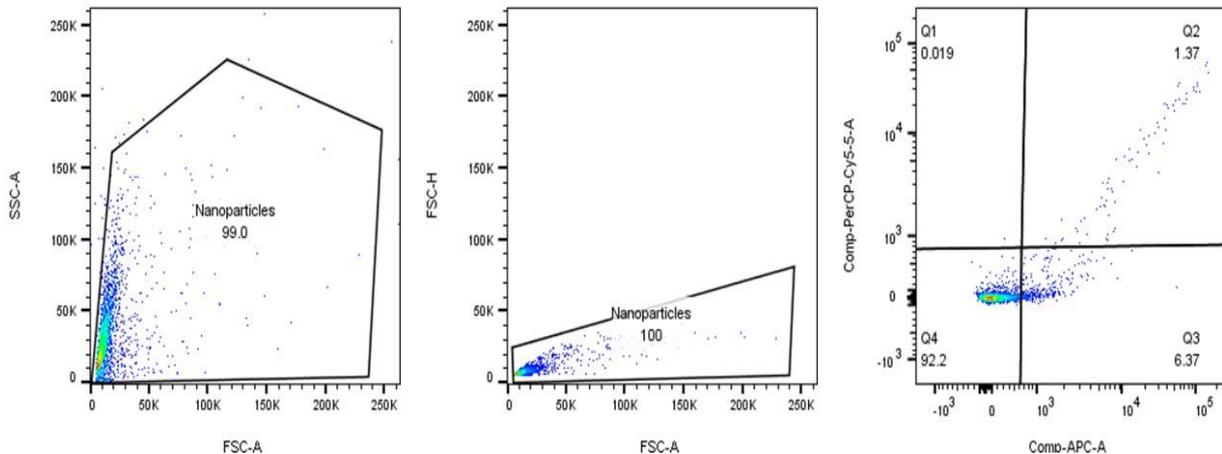


D.



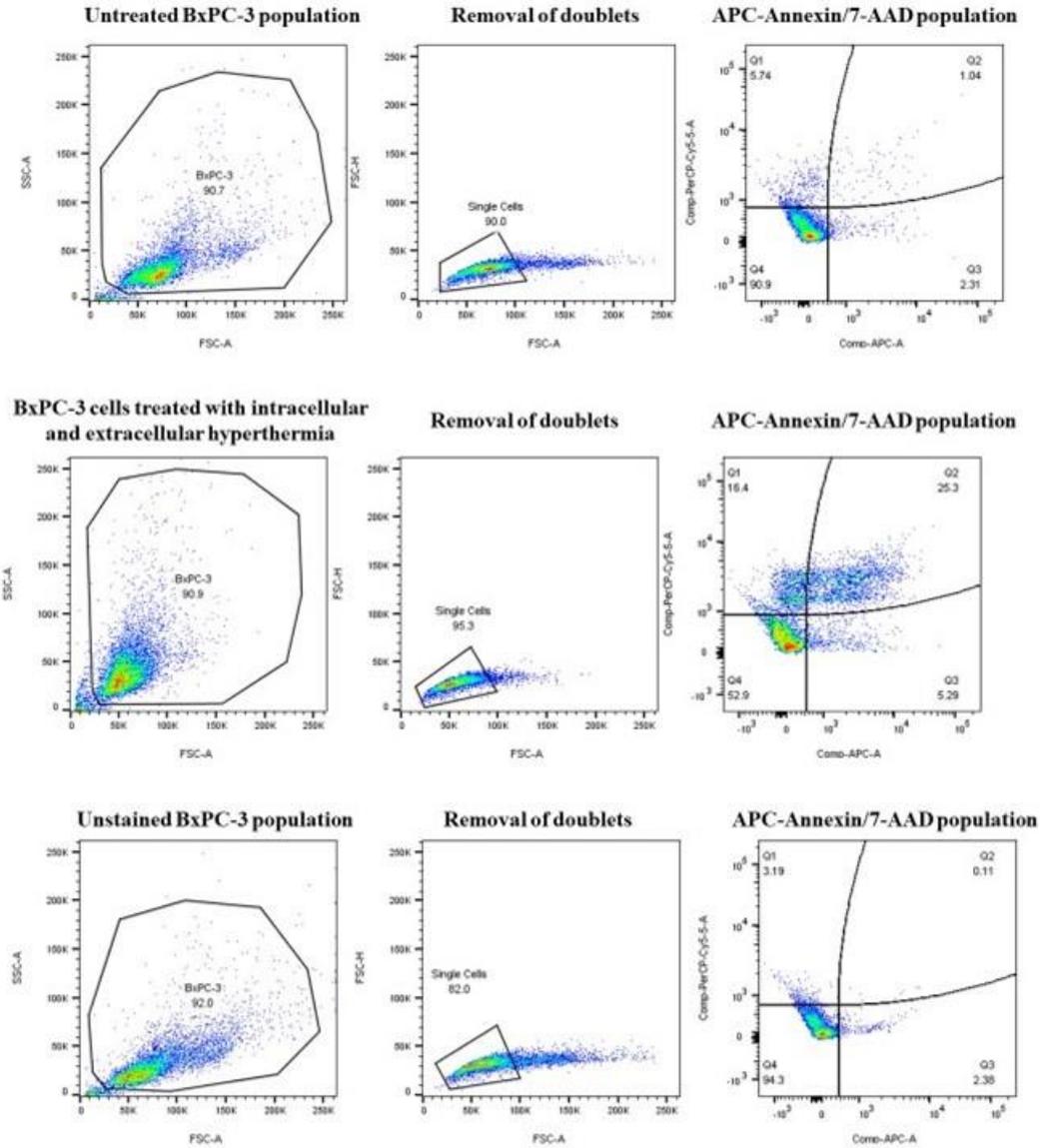
**Figure S1:** Nanoparticle tracking analysis, dynamic light scattering and transmission electron microscopy graphs for fluidMAG/C11-D.

A. Nanoparticle tracking analysis size versus concentration graph. Here, the nanoparticles were diluted to 10  $\mu\text{g}/\text{mL}$  in particle-free water (Sigma Aldrich, Ireland) and analysed through 5, 60 s recordings. B. Dynamic light scattering graphs depicting size distribution and apparent zeta potential at pH 7. Zeta potential data was provided by the suppliers, Chemicell (C). For size measurements with DLS, the nanoparticles were diluted to 50  $\mu\text{g}/\text{mL}$  in particle-free water. The nanoparticles are then analysed with 10 measurements of 12 runs. D. Size distribution graph of 200 individual nanoparticles imaged via transmission electron microscopy and analysed through ImageJ software.



**Figure S2:** Nanoparticle shows little interference with APC or PerCP-Cy5.5 channels.

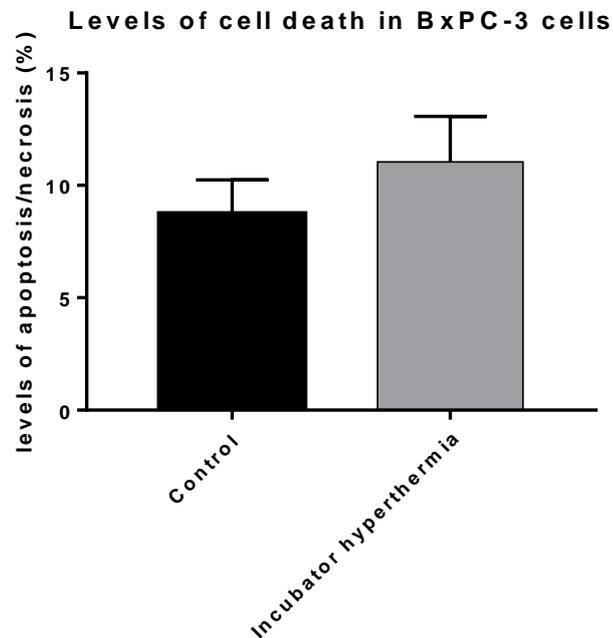
After gating for 99% of nanoparticles, less than 10% of the population were shown to interfere with APC and PerCP-Cy5.5 channels. It is also important to note that care was taken to avoid any nanoparticles when gating BxPC-3 cells in these experiments.



**Figure S3:** Gating strategy for BxPC-3 cells.

BxPC-3 treated with nanoparticles were gated to ensure nanoparticle avoidance. Doublets are removed through forward scatter height versus forward scatter area. BxPC-3 cells APC-/PerCP-Cy5-5-

were considered vital, APC<sup>+</sup>/PerCP-Cy5-5<sup>-</sup> were considered early apoptotic, APC<sup>+</sup>/PerCP-Cy5-5<sup>+</sup> were considered late apoptotic and APC<sup>-</sup>/PerCP-Cy5-5<sup>+</sup> were considered necrotic.



**Figure S4:** Annexin V/7-AAD staining of cells treated with incubator hyperthermia (42.5 °C for 30 min) versus untreated.

The levels of apoptosis and necrosis in incubator heated cells were not significantly different to untreated cells. N = 5 (triplicate). Analysed with unpaired t-test.