Electronic Supplementary Material (ESI) for Nanoscale.

Functionalized Iron Oxide Nanoparticles for Controlling the Movement of Immune Cells

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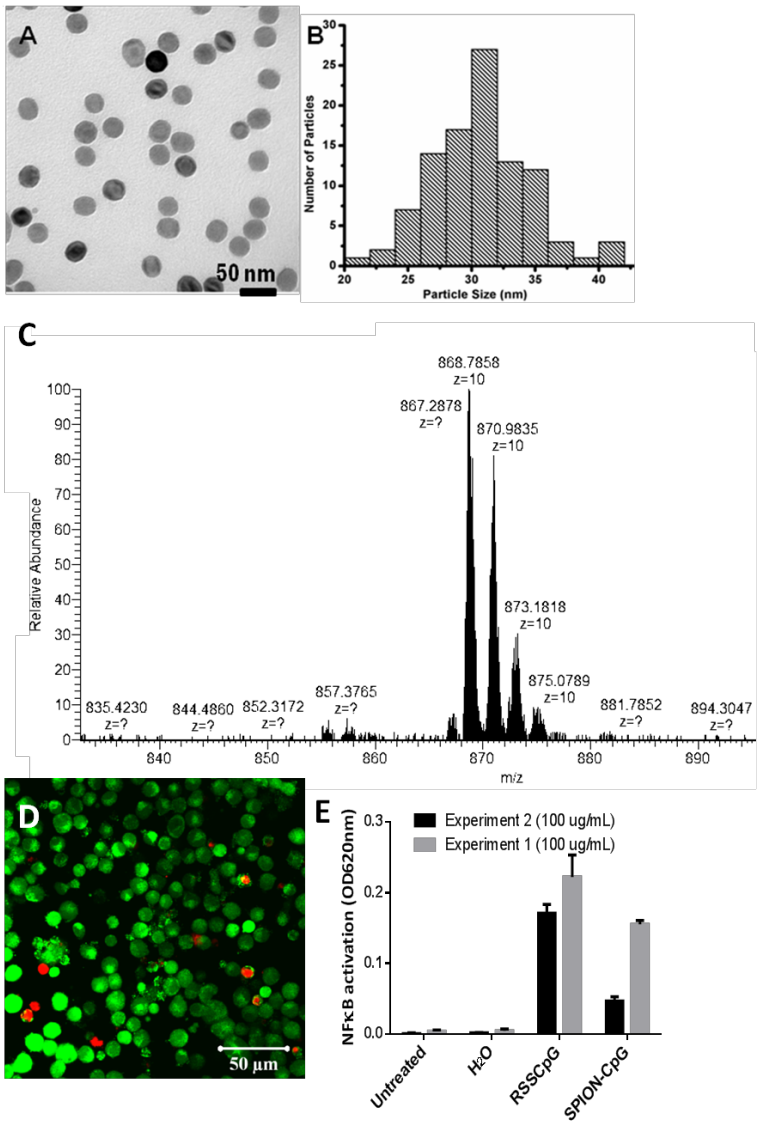

Figure S1. (A) Transmission electron microscopy image of the SPION. (B) Histogram size distributions obtained by counting approximately 100 particles. (C) Negative ion high-resolution MS of the product from RSSCpG reduction. The exact mass of reduced HS-CpG is 8559.8 (MW = 8566.0) while unreduced RSSCpG starting material has an exact mass of 8691.9 (MW=8698.3). (D) LIVE/DEAD staining of untreated control cells. (E) Raw data from two independent NFκB activity assays. Normalized, averaged data from these experiments is shown in Figure 1.

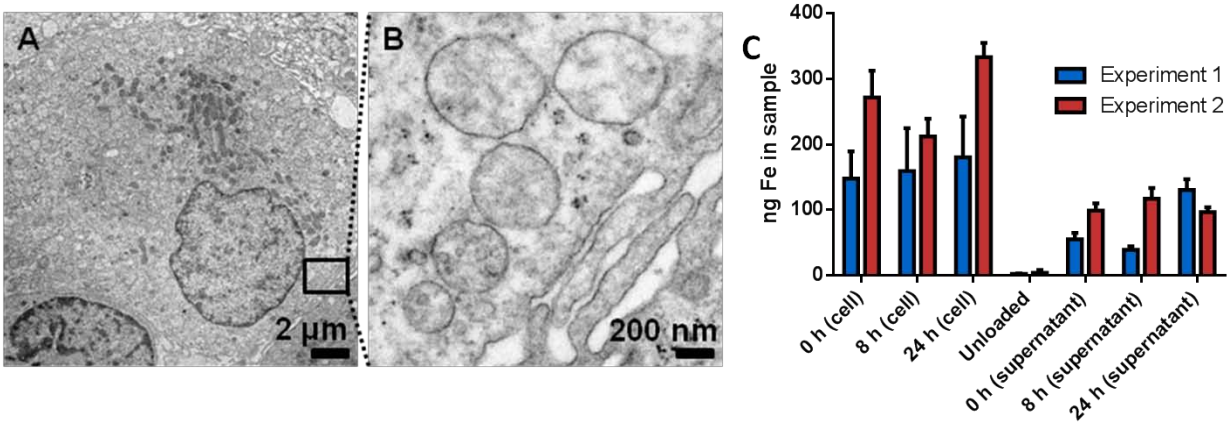

Figure S2. (A-B) Transmission electron microscopy images of an untreated control cell at different magnifications. (C) Data from the two independent exocytosis experiments displayed side by side (treatments performed in triplicate). Error bars represent standard deviations of iron content in replicate wells.

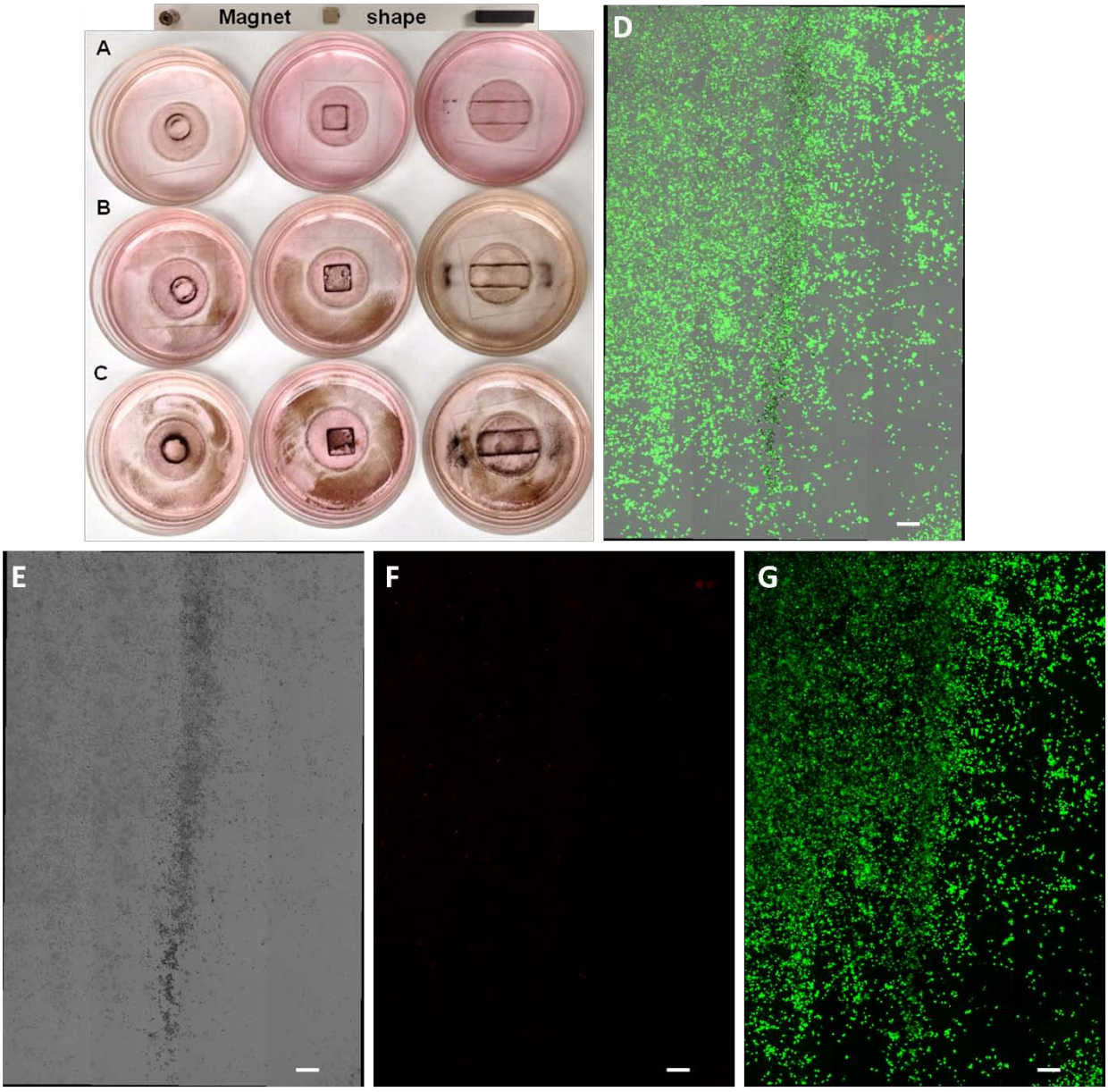

Figure S3: (A-C) Magnetically induced movement of the cells loaded with increasing concentrations of SPION-CpG. Color images of loaded cells exposed to differently-shaped magnets for 20 h. Increasing concentrations of SPION-CpG were used (0.1, 0.3 and 0.5 mg/mL for A, B and C respectively). Data from Fig S3A is shown in Fig 3A. (D-G) Uncropped, tiled images from the LIVE/DEAD assay shown in Fig 3 G-H. (D) is the merged image while (E-G) are individual channels. Scale bar = 200 μm.

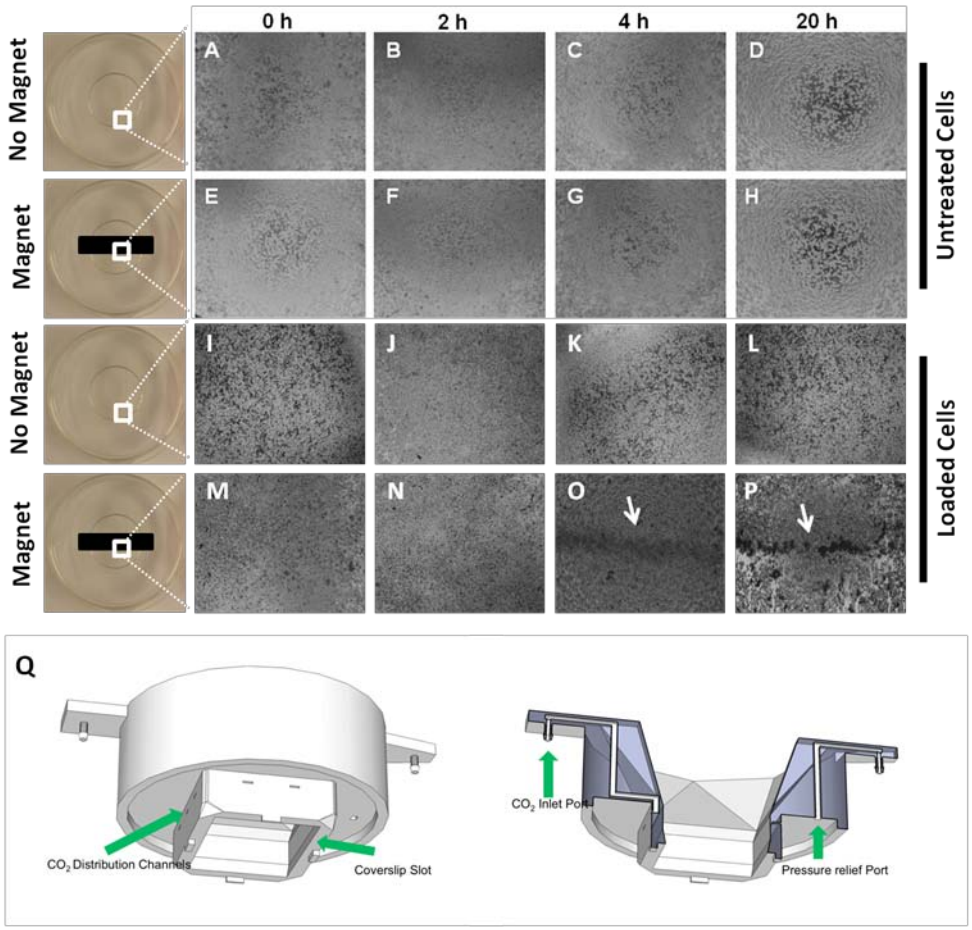

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Figure S4: (A-P) Time-lapsed imaging of magnetically induced movement of the cells. Bright field microscopy images at different time points of the cells loaded with 0.1 mg/mL SPION-CpG conjugates (I-L) without or (M-P) with magnet exposure. As cell-only controls, untreated N9 cells were also imaged (A-D) without or (E-H) with magnet exposure. (Q) Custom cell box imaging apparatus. The cell box aids in incubating, imaging, and magnetically manipulating adherent cells. The cell box attaches onto a standard petri dish filled with cell media. A coverslip is attached to the coverslip slot and used as a substrate for cell growth. The hollow center provides a light path for brightfield illumination. An atmosphere distribution channel is routed through the internal structure of the cell box. A pressure relief port prevents accumulation of atmosphere inside the petri dish.

Table S1. Viability analysis of microglia N9 cells alone or treated with SPION-CpG [0.5 mg/mL] obtained from confocal microscopy images using Image J (Version 1.4.3) software.

	Microglia N9 cells		
Sample	**Dead Cells (Red)**	**Live Cells (Green)**	**Total cells**
Cells Alone	14	171	185
Cells+SPION-CpG	26	158	184