**Supplemental Figure 1**

**A. Conventional determination of number of associated cells per FACS**

$$n[P/cell] = \frac{MF_{cell} - MF_{neg\_cell}}{MF_{Particle}} \qquad \textbf{S 1.1}$$

**B. Proposed determination of number of associated cells per FACS**

$$n[P/cell] = \frac{MF_{cell} - MF_{neg\_cell}}{\left[\left(1 - f_i\right) + f_i \times \frac{1}{FR_{o/i}}\right] MF_{Particle}} \qquad \textbf{S 1.2}$$

See Methods section for calculation of $f_i$ and $FR_{o/i}$.

**C. Hypothetical Example:**

Assume: The mean fluorescence intensity (MFI) of particles $MF_{Particle}$ (measured outside the cell) is 1000. Assume further that there are on average **100 particles** per cell associated, 90 inside the cell and 10 outside the cell -> $f_i$ = 0.9. The $FR_{o/i}$ for this fluorophore is 1.9. The MFI of the negative control (cells without particles) is 200.

The expected average MFI per cell (incubated with particles) would then be 57568:

| | |
|---|---|
| 10 particles with MFI 1000: | 10000 |
| 90 particles with MFI 526 : | 47368 |
| Autofluorescence of cell: | 200 |
| | Σ = 57568 |

Now apply formula S 1.1 *versus* formula S 1.2 to calculate average number of particles per cell:

a) Formula S1.1:

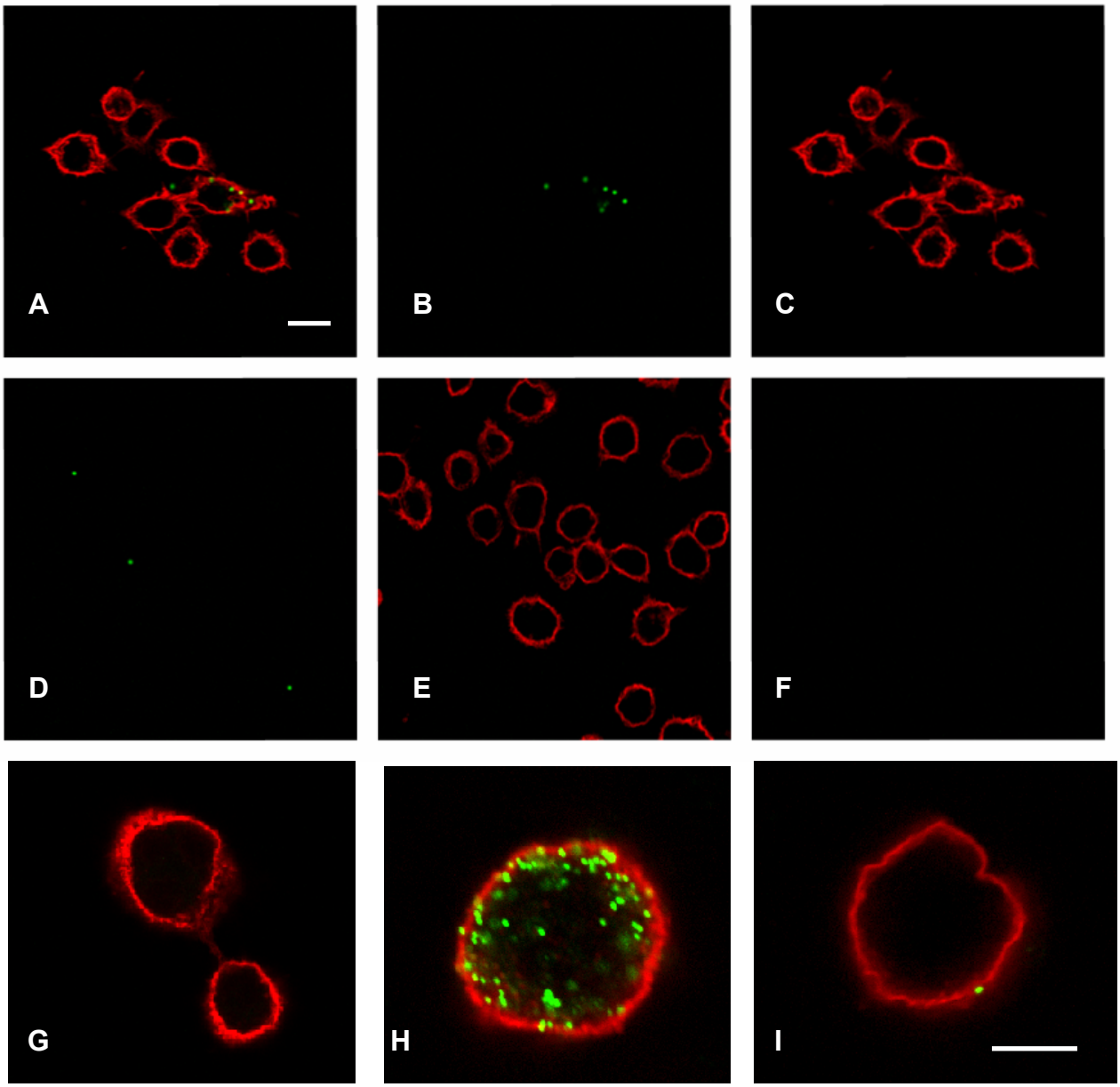
$$n[P/cell] = \frac{57568 - 200}{1000} = 57.6$$

b) Formula S1.2:

$$n[P/cell] = \frac{57568 - 200}{\left[\left(1 - 0.9\right) + 0.9 \times \frac{1}{1.9}\right] \times 1000} = 100$$

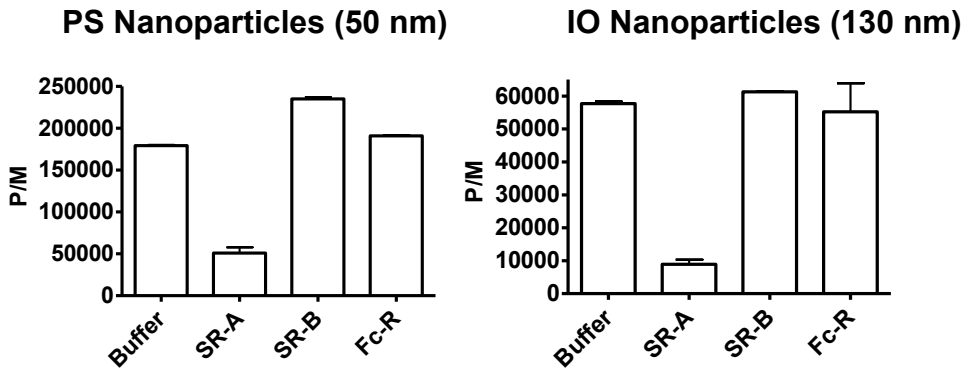
Conclusion: Using the (conventional) equation S1.1 gives a false result that differs significantly from the true value.

**Supplemental Figure 2**



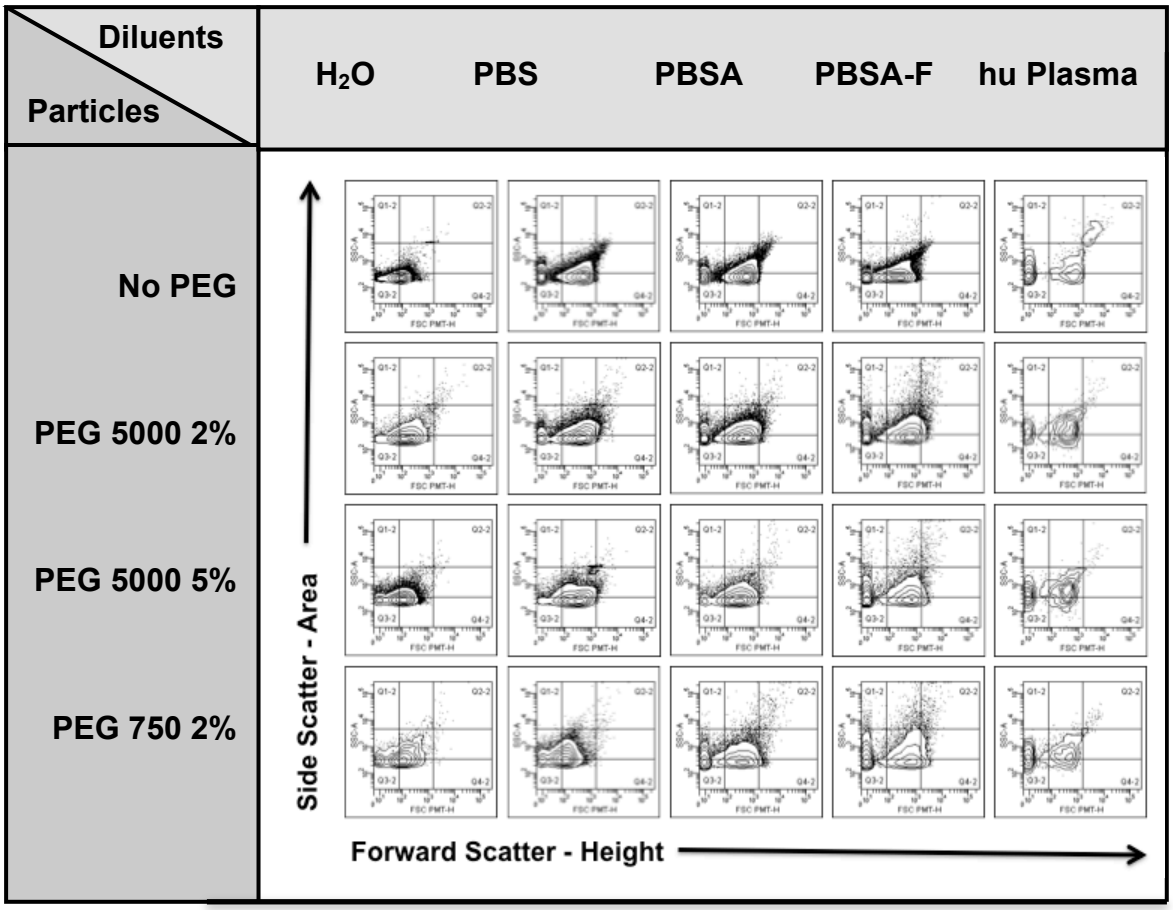

***Supplemental Figure 2: Confocal microscopy of murine macrophages after internalization of polystyrene particles - negative controls.*** *A-C: J774 murine macrophage cells incubated with polystyrene particles (1 micron diameter, dyed with green fluorescent Firefli™ green), cell membranes stained with red fluorescent wheat germ agglutinin-Alexa 594; A: FITC and Cy3.5 channel overlay; B: FITC channel; C: Cy3.5 channel. D: as A, but no WGA-Alexa 594 stain; E: as A, but no particles added; F: as A, but without addition of particles and without membrane stain. Microscope and camera settings were identical for A-F. G-I: Internalization of 0.4 µm PS particles dyed with Firefli™ green after incubation at different temperatures. G: no particle-control ; H: incubation at 37°C ; I: incubation at 4°C. Size bars A-G: 10 µm; H, I: 5 µm.*

**Supplemental Figure 3:**



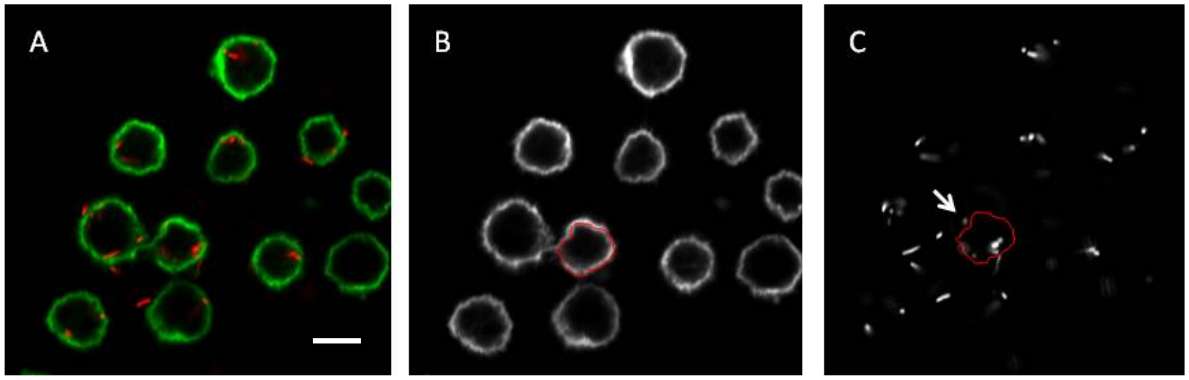

**Supplemental Figure 3: Flow cytometry analysis of adsorbed/internalized particles in the presence and absence of inhibitors for specific uptake receptors.** *J774 macrophages were incubated with polystyrene (PS) nanoparticles (50 nm diameter, internally-dyed with Firefli™-Red) or dextran-coated iron oxide (IO) nanoparticles (130 nm diameter, surface labeled with fluorescein isothiocyanate); the particles were diluted in phosphate buffered saline containing 2% w/v bovine serum albumin and 30% v/v fetal calf serum to prevent aggregation and unspecific binding. Inhibitors for scavenger receptor A (SR-A), scavenger receptor B (SR-B) and Fcγ-Receptor (Fc-R) were added during the incubation time. SR-A inhibitor: polyinosinic acid at 200μg/ml final concentration; SR-B inhibitor: phosphatidylserine vesicles at 200 μg/ml final concentration; Fc-R inhibitor: anti Fcγ-R antibody (Pharmingen 553140) at 30 μg/ml final concentration. Results are shown as average and standard deviation. P/M: average number of particles adsorbed/internalized per macrophage (uncorrected for intracellular fluorescence change). Inhibition of binding/uptake by SR-A inhibitor was significant for PS nanoparticles (p=0.02) and for IO nanoparticles (p=0.01) as determined by two-tailed t-test with Welch's correction. This shows that SR-A plays a role in PS and IO nanoparticle uptake by macrophages, and that it is still accessible after pre-dilution in blocking buffer.*

**Supplemental Figure 4:**



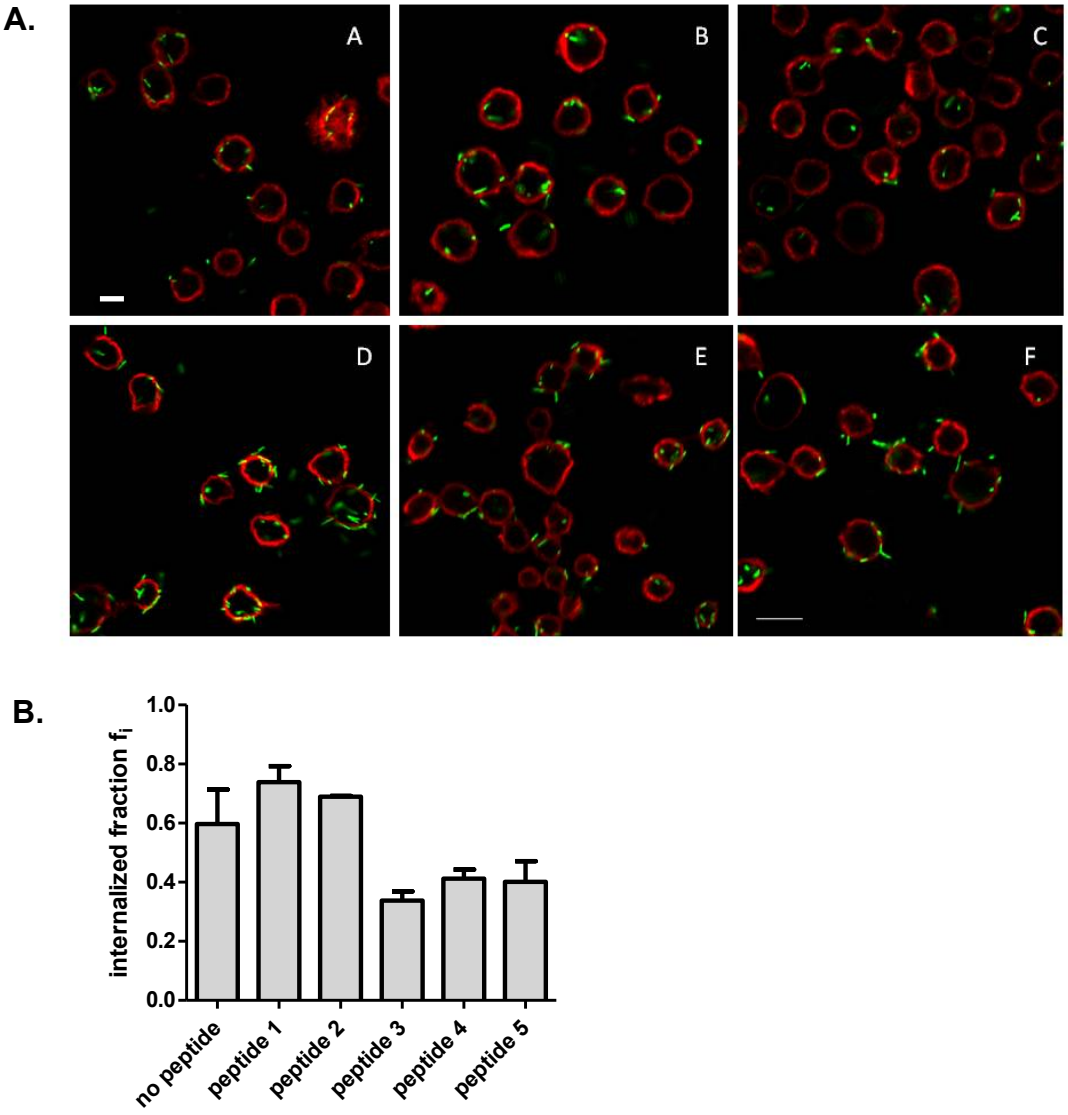

**Supplemental Figure 4: Vesosomes do not aggregate in the presence of bovine serum albumin (BSA).** *Flow cytometry of vesosomes in different physiological diluents. In contrast to polystyrene particles (Figure 7), vesosomes did not aggregate in the presence of BSA. Shown here are contour plots of forward scatter height (x-axis of all plots) versus side scatter area (y-axis of all plots). Grids are provided to guide the eye. There is no shift to the right and up in buffer containing 2% (w/v) BSA. Abbreviations: PBS: phosphate buffered saline; PBSA: PBS with 2% (w/v) BSA; PBSA-F: PBSA with 2% (w/v) BSA and 30% (v/v) fetal calf serum; hu: human; PEG: polyethylene glycol.*

**Supplemental Figure 5:**



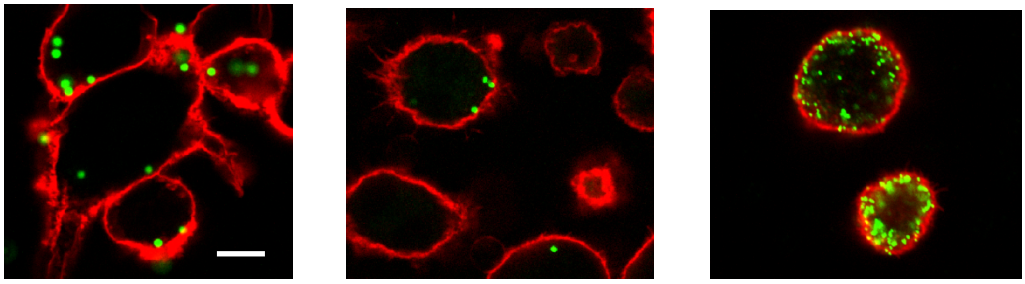

***Supplemental Figure 5:*** *Unmasking of fluorescence signals in single fluorescence channel. Macrophages (Membrane stain green in A, white in B) after phagocytosis of bacteria (red in A, white in C). Red line in B, C circles the interior of one cell (region of interest). Arrow in C points at bacterium that cannot be seen in the overlay image, but is clearly visible in the single, monochromatic channel. Size bar 10 μm.*

**Supplemental Figure 6:**

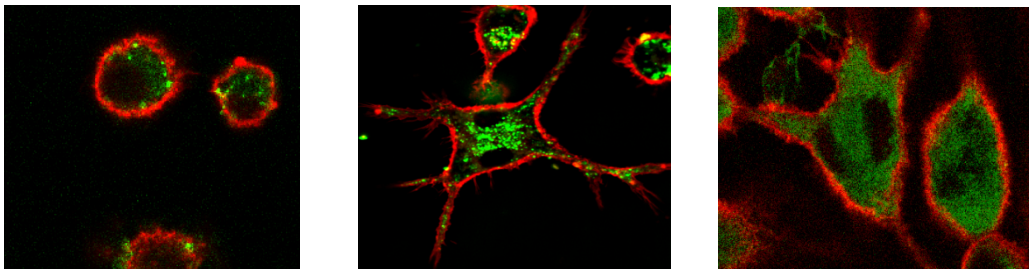



***Supplemental Figure 6: Phagocytosis of peptide displaying bacteria by macrophages.*** *Peptides binding to the surface of macrophages were selected from a combinatorial bacterial surface display library (described in Dane, K.Y., Gottstein, C., Daugherty, P.S. Mol. Cancer Ther.* ***8****, 1312-18 2008). Internalized fraction $f_i$ of green fluorescent E.coli bacteria displaying these peptides was measured by quantitative confocal microscopy. A: Confocal images of macrophages (red) after phagocytosis of bacteria (green). Panels A-F: Macrophages incubated with bacteria expressing no specific peptide (A), peptide 1 (B), peptide 2 (C) peptide 3 (D), peptide 4 (E) or peptide 5 (F). Size bar 20 $\mu$m. B: Corresponding $f_i$ values. Bars represent average and SEM of 2-3 images with approximately 20 macrophage cells analyzed per image. Size bar 10 $\mu$m.*

**Supplemental Figure 7:**



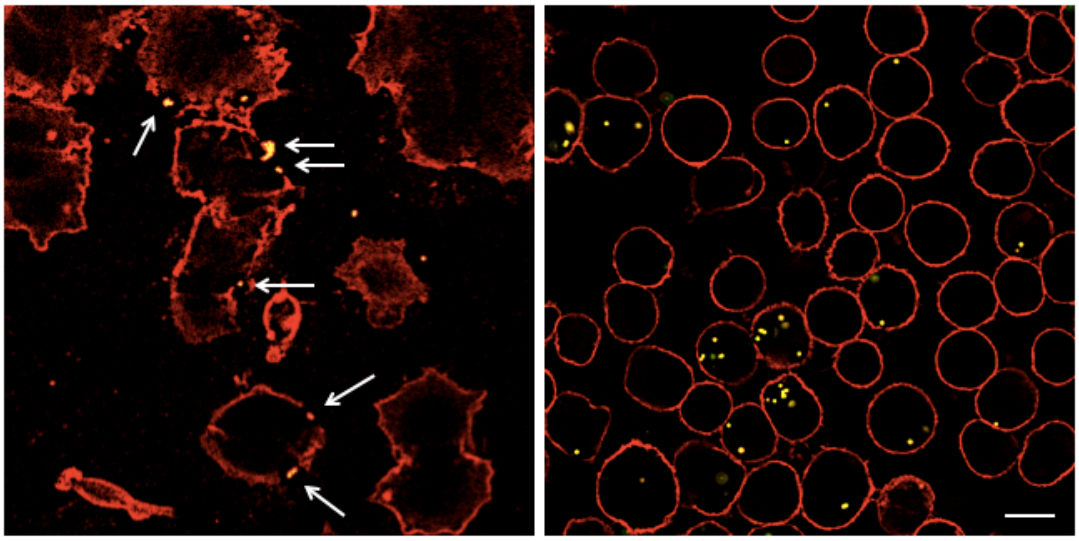
| Polystyrene 1 micron | Vesosomes 1 micron | Polystyrene 400 nm |
|---|---|---|



| Vesosomes 400 nm | Iron oxide 130 nm | Polystyrene 50 nm |
|---|---|---|

**Supplemental Figure 7: Confocal images of cells incubated with fluorescent particles of different sizes and materials.** *Polystyrene particles were internally-labeled with Firefli™ green, vesosomes were surface labeled with dialkylcarbocyanine (DiO), and iron oxide particles with fluorescein isothiocyanate (FITC). Number of internalized particles was determined as described in Methods. The method is generic, as long as particles can efficiently be labeled to overcome cellular autofluorescence at a given particle:cell ratio. Size bar 10 μm.*

**Supplemental Figure 8:**



**Supplemental Figure 8: Confocal images of cells incubated with fluorescent particles binding to breast cancer cells.** *A. MCF-7 breast cancer cells and B. J774 macrophages were incubated at 37°C with yellow-green polystyrene particles (size: 1 micrometer diameter), which had been functionalized with a peptide binding to breast cancer cells. For cancer cells (A) only surface binding is detected (arrows), while macrophages (B) internalized the particles. Size bar 10 μm.*