Electronic Supplementary Material (ESI) for Biomaterials Science.

Supplementary Information

"Disparate effects of PEG or albumin based surface modification on uptake of nano- and micro-particles"

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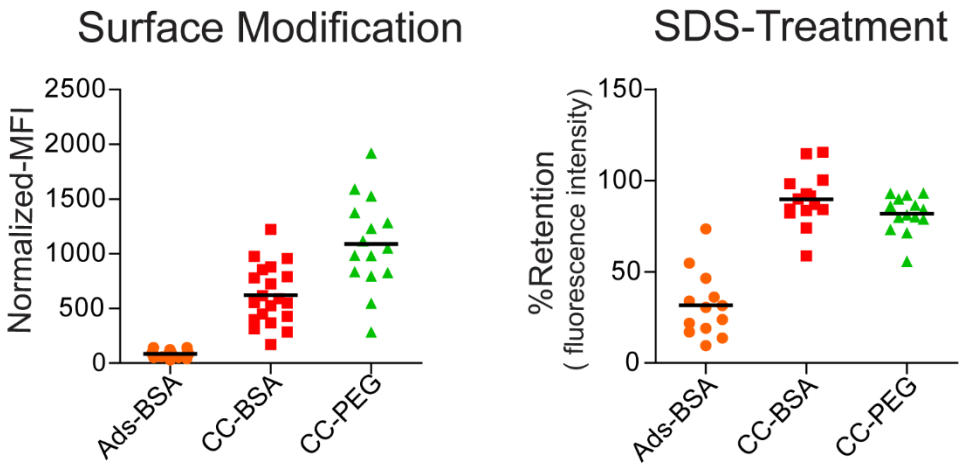
Supplementary Figure 1

A



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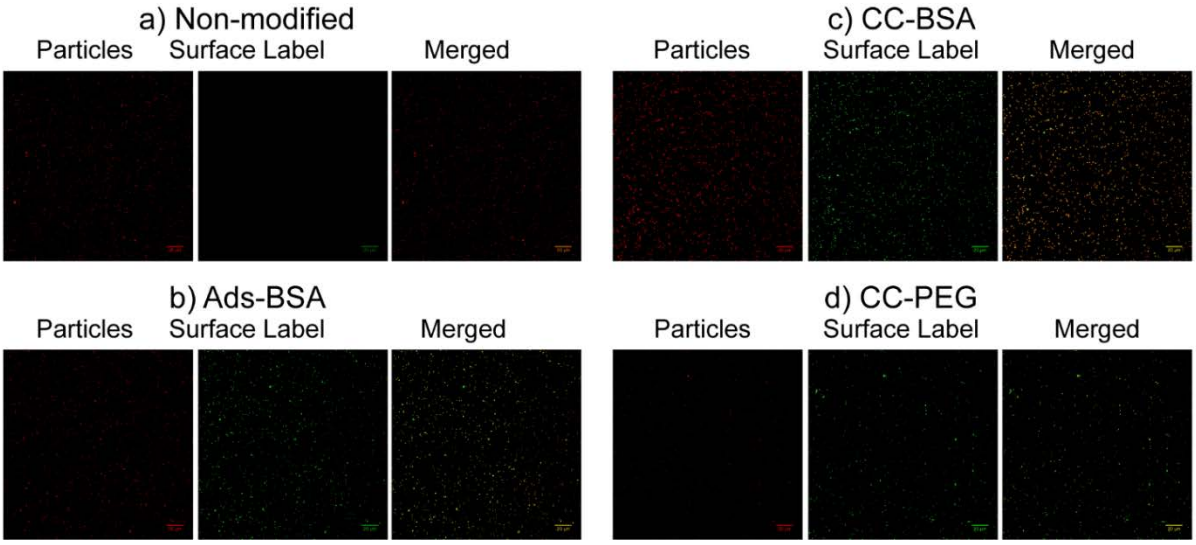
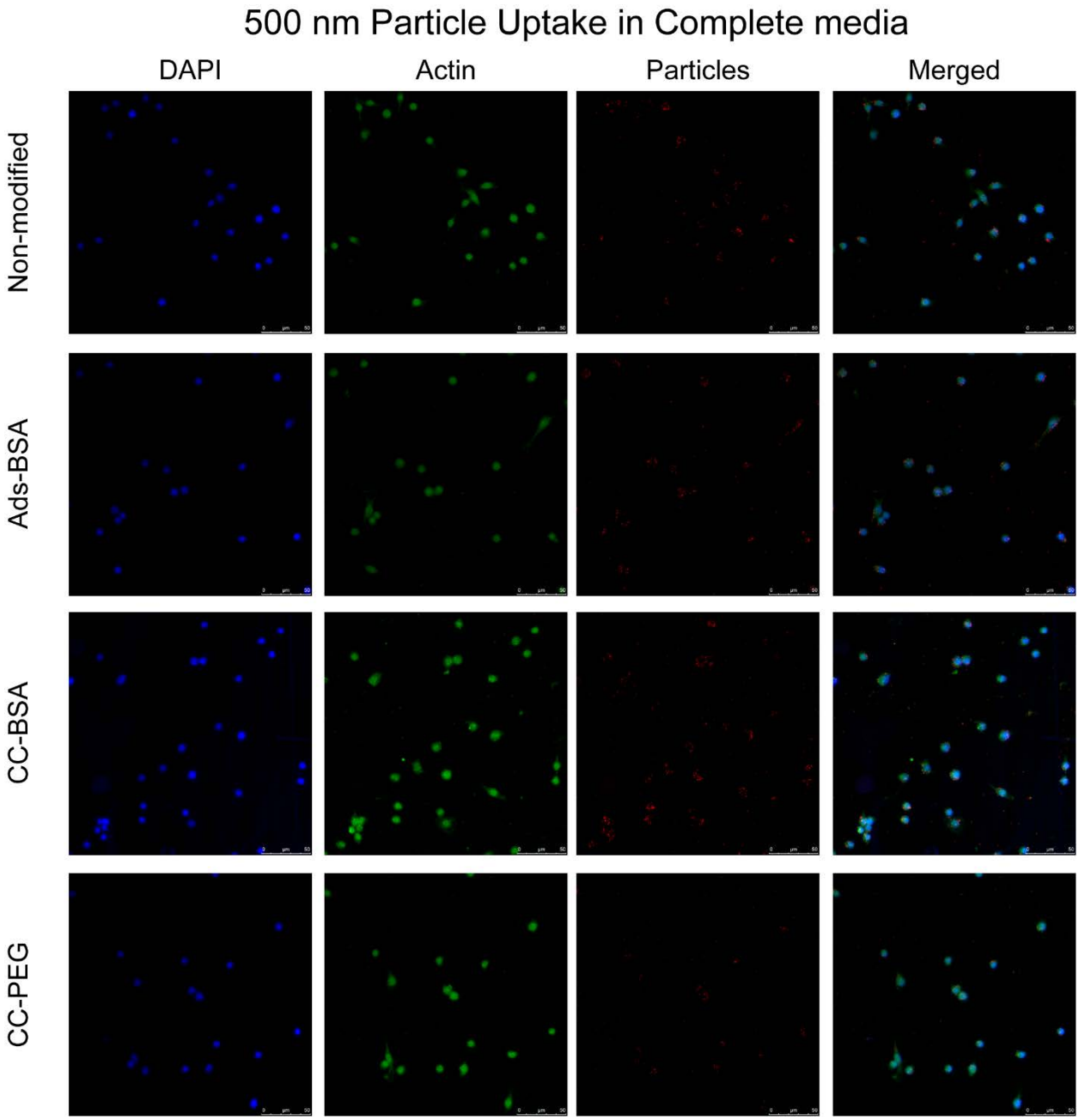
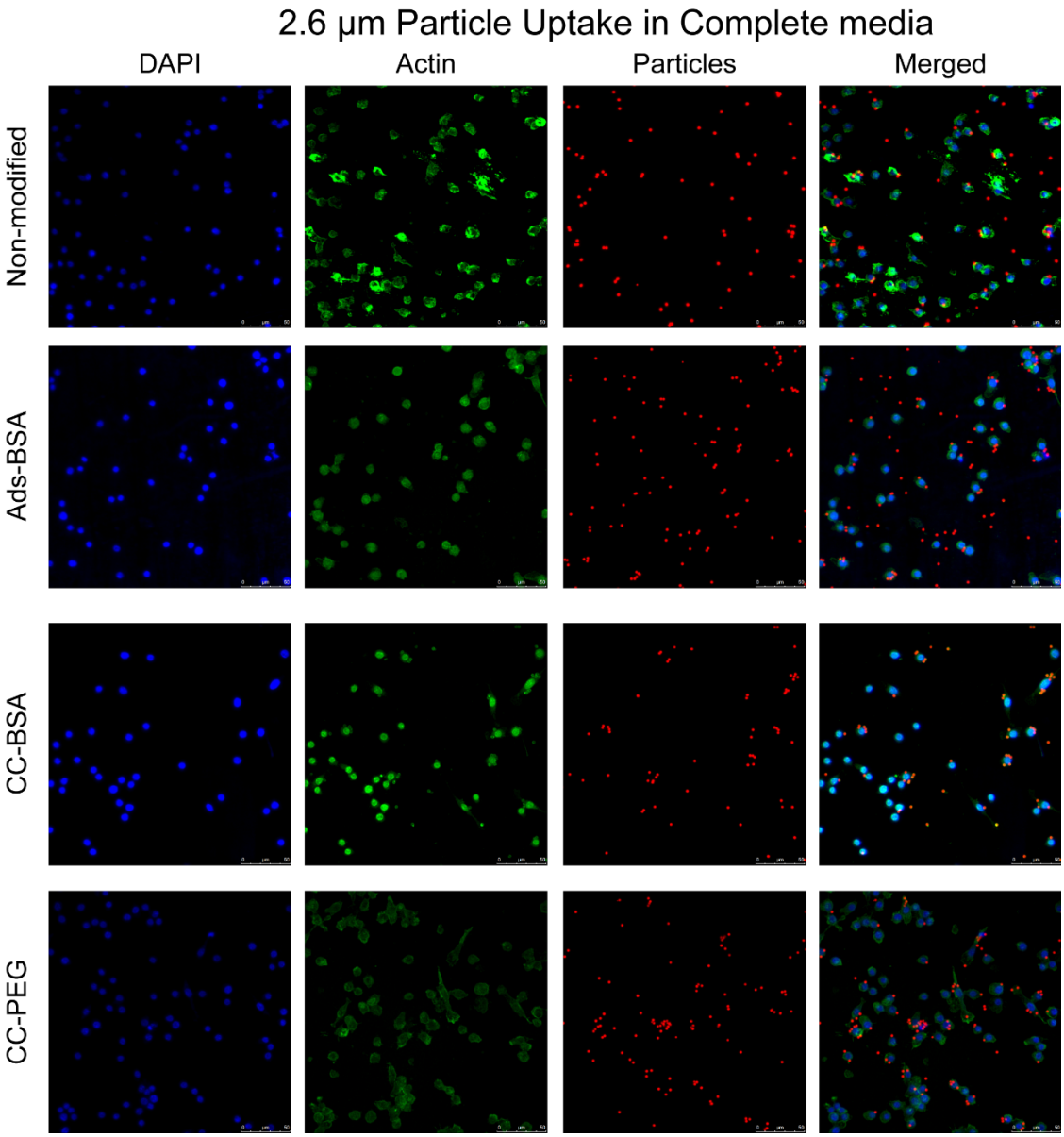


Supplementary Figure 1: Surface modification of micro-particles. A – (*Left)* Surface labeling of 2.6 µm polystyrene particles with albumin-FITC/PEG-FITC. Normalized-MFI represents the ratio of median fluorescence intensity (MFI) of labeled particles to that of non-modified particles. (*Right*) Determination of particle MFI following treatment with 1% SDS (in PBS) for one hour, and represented as a percentage retention of fluorescence intensity when compared to original MFI. Data are based on n>8 independent experiments. **B** – Images of 500 nm particle post surface modification (fluorescent imaging). Presence of BSA/PEG on the surface can been seen as green fluorescence (surface label) associated with red fluorescence (Particles). 'Ads' represents Physical Adsorption and 'CC' represents Covalent Conjugation.

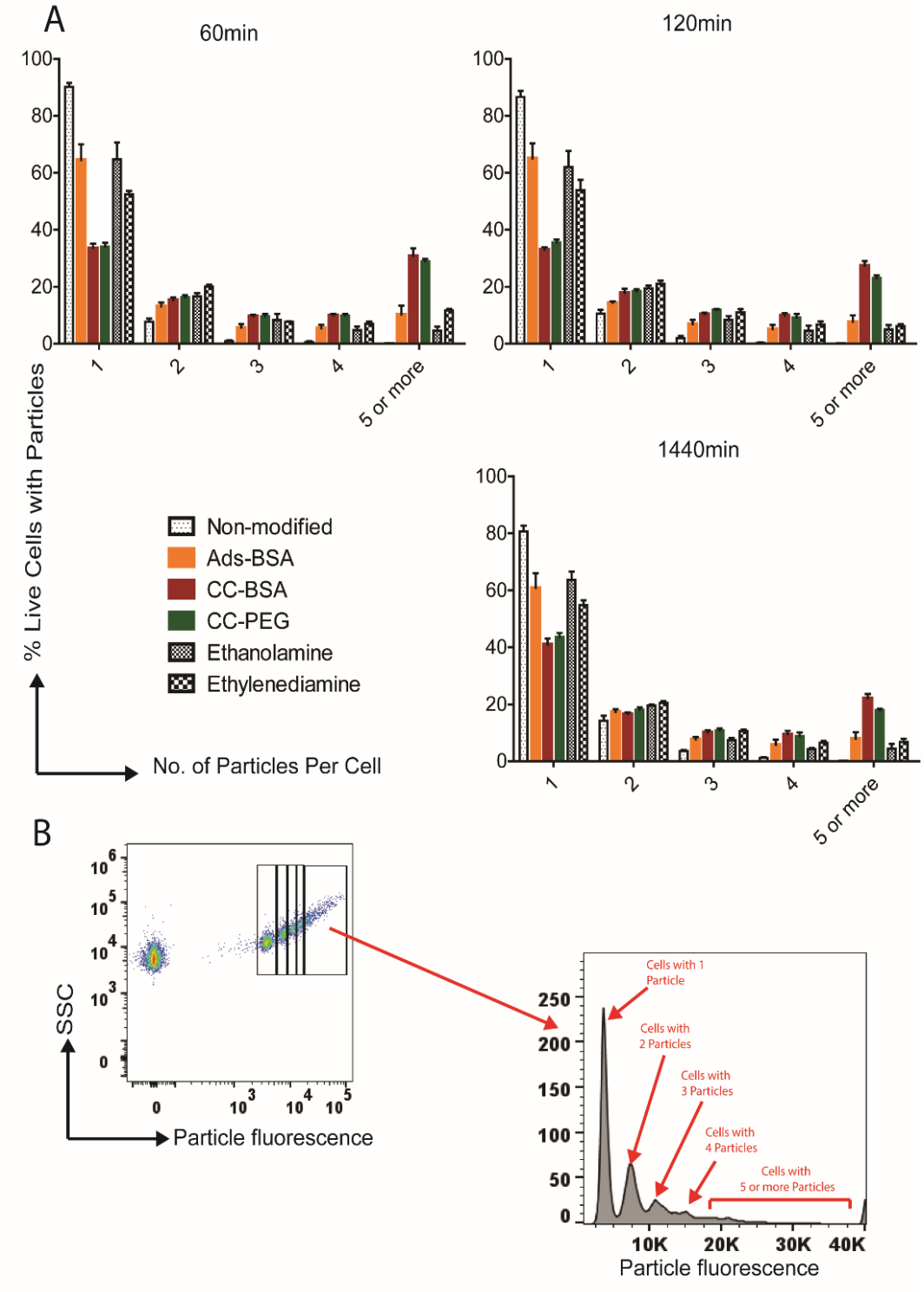
Supplementary Figure 2





Supplementary Figure 2: Immunofluorescence images of RAW 264.4 cells 2-hour post particle addition. Cells were fixed, permeabilized and stained with phalloidin (for staining actin) and DAPI. Stacks at different z-positions (distance between stacks is 1 µm) were captured by Leica DMI6000 B at a magnification of 63X, and maximum intensity projection is shown. Images demonstrate that at 2 hours majority of the particles are inside the cells

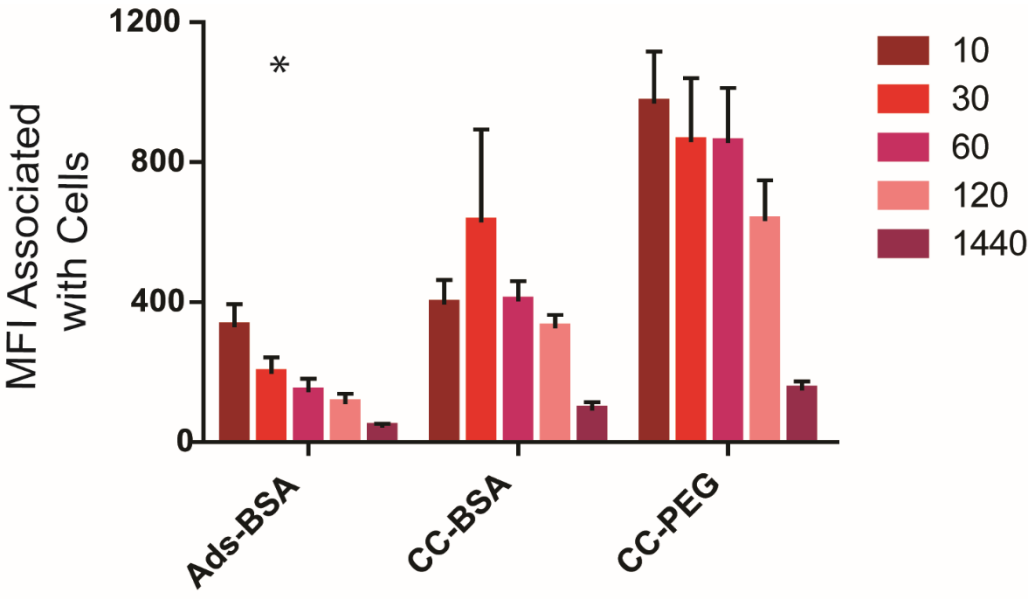
Supplementary Figure 3



Supplementary Figure 3: Number of particles taken up by phagocytic cells. A – Quantification of the percentage of cells with different numbers (1, 2, 3, 4, 5 or more) of particles

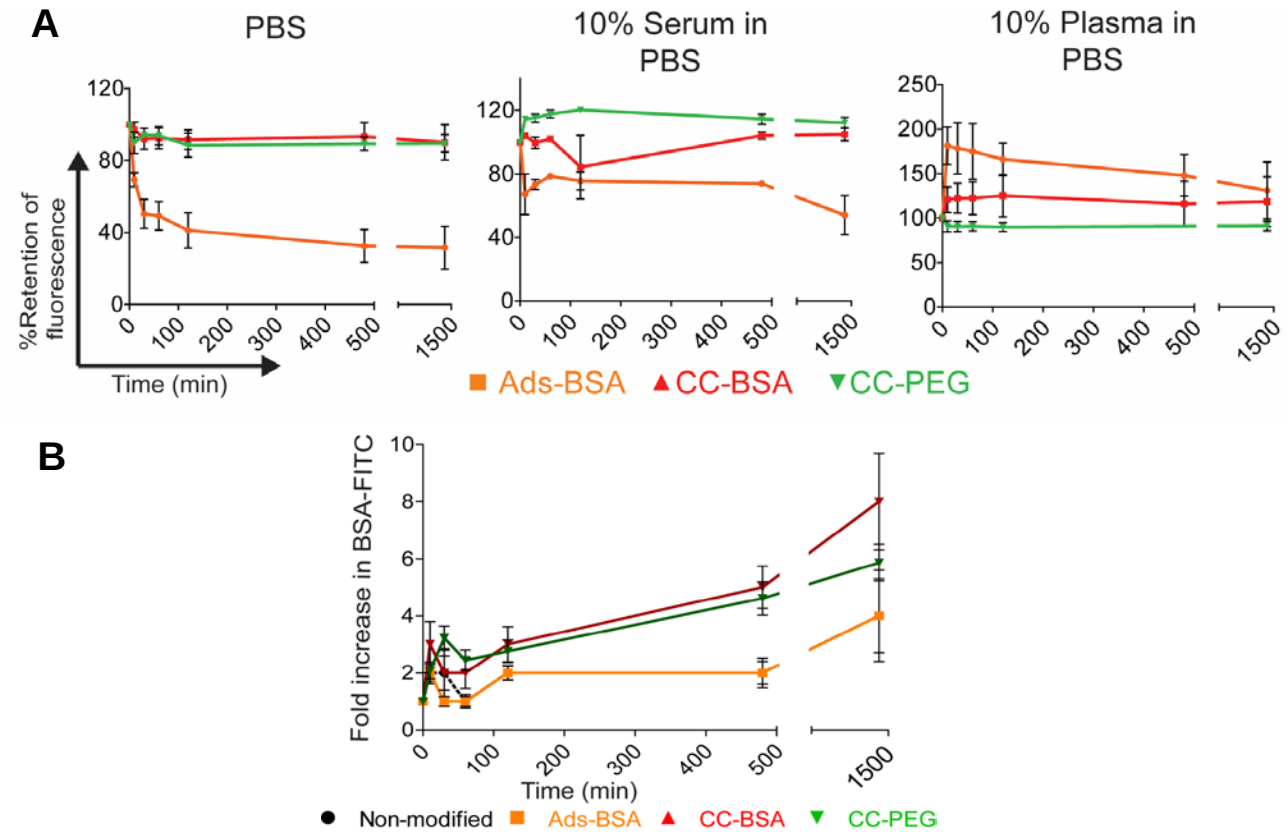
among cells that have taken up 2.6 µm particles. **B** – Flow plots showing how the determination of cells with one or more particles was performed for 2.6 µm particles

Supplementary Figure 4



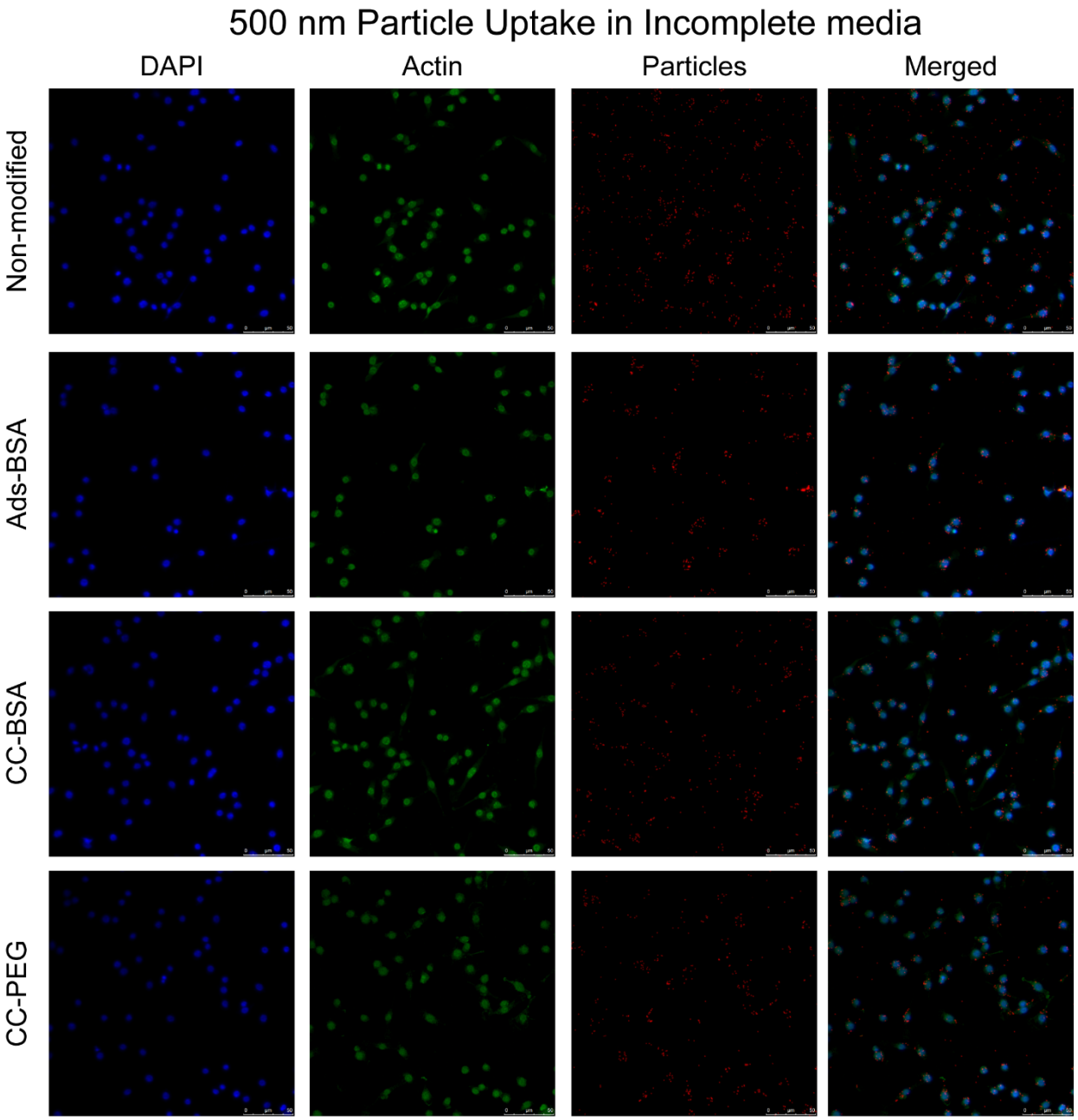
Supplementary Figure 4: PEG/Albumin (FITC) MFI in cells that have taken up particles. MFI of FITC (corresponding to the PEG or albumin present on 2.6 µm PS particle surface) was measured in cells that have taken up particles. The number of cells with particles continues to increase (figure 2 of paper), but the total FITC MFI in these cells decreases steadily. While this could be due to quenching of FITC following particle uptake, it could also imply processing or degradation of PEG/albumin or FITC itself. The MFI at 1440 min in all three surface modifications is significantly lower than the MFI at 10 min.

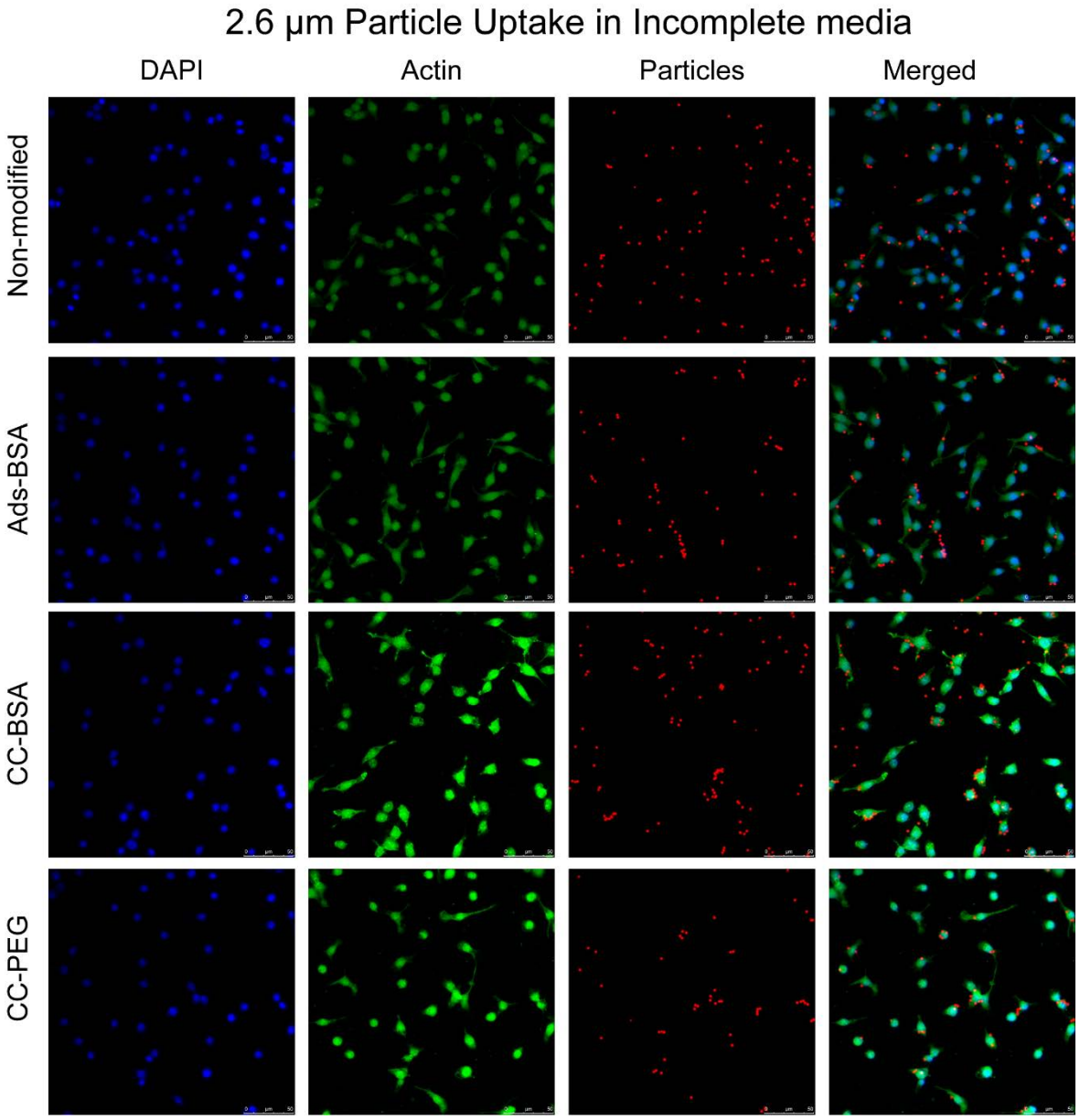
Supplementary Figure 5



Supplementary figure 5: Stability of surface label in biological fluid mimics. A – Incubation of surface modified particles 3 µm PS particles in PBS, 10% Serum (in PBS) and 10% Plasma (in PBS). The MFI of labeled particles is shown as 100 percent at 0 min, and the percentage of MFI retained over time (10, 30, 60, 120 and 1440 min) is plotted. Data are based on n≥3 independent experiments and are plotted as Mean ± SEM. **B** – Surface modified and non-modified particles were incubated in PBS containing 100 µg/ml of albumin-FITC. Following incubation, FITC MFI on particles was measured at different times (10, 30, 60, 120 and 1440 min), and is reported as fold increase over the FITC MFI of particles prior to incubation. Data are for n ≥ 3 and are plotted as Mean ± SEM.

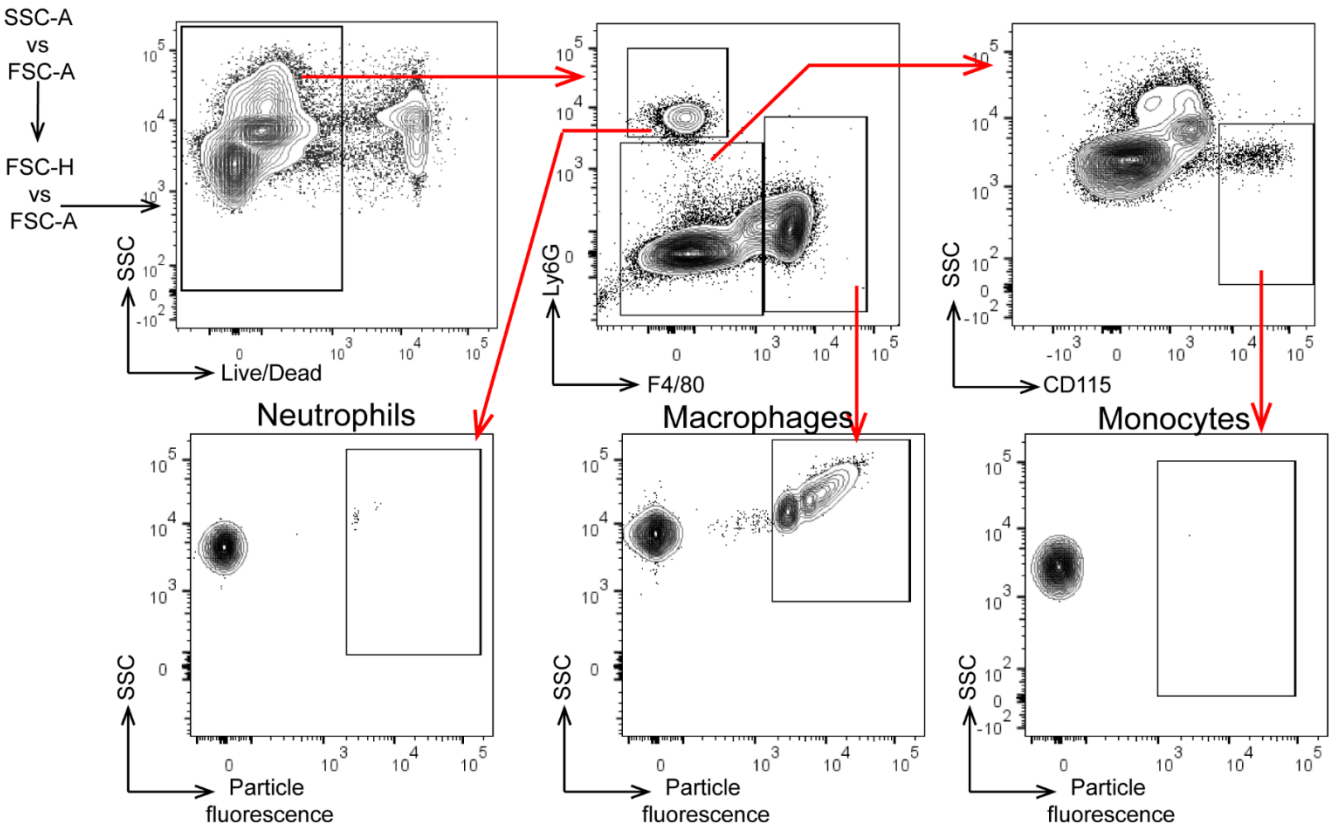
Supplementary Figure 6





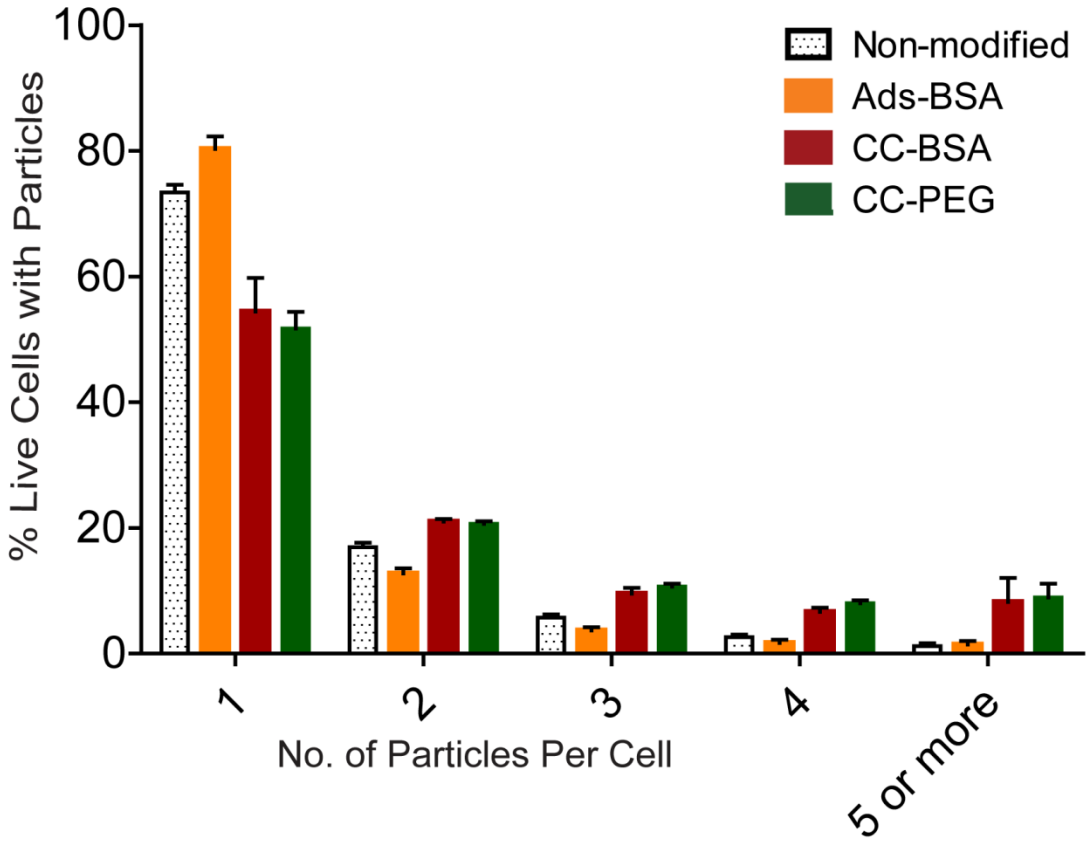
Supplementary Figure 6: Immunofluorescence images of cells cultured in absence of serum and 2-hour post particle addition. Incomplete media (absence of serum) cultures of cells with particles showing that the particles are taken up inside cells even in the absence of serum. Phalloidin (for staining actin) and DAPI (nucleus). Stacks at different z-positions (distance between stacks is 1 µm) were captured by Leica DMI6000 B at a magnification of 63X, and maximum intensity projection is shown.

Supplementary Figure 7



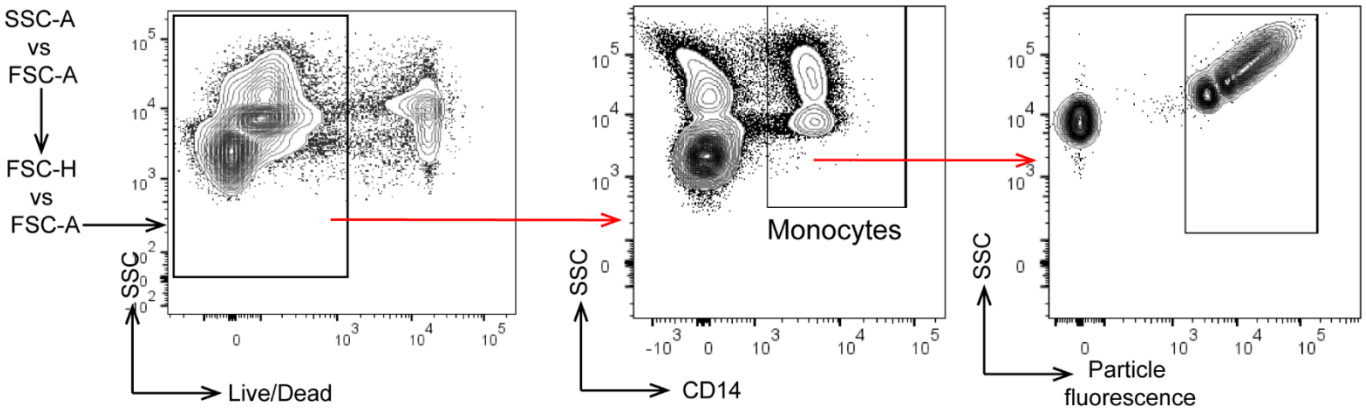
Supplementary Figure 7: Flow cytometry schematics for in vivo uptake experiments. Representative flow cytometry contour plots describing the gating scheme used for *in-vivo* uptake experiments. F4/80 is a marker for macrophages, Ly6G is a marker for neutrophils, and CD115 expressing cells that do not express F4/80 and Ly6G are monocytes.

Supplementary Figure 8



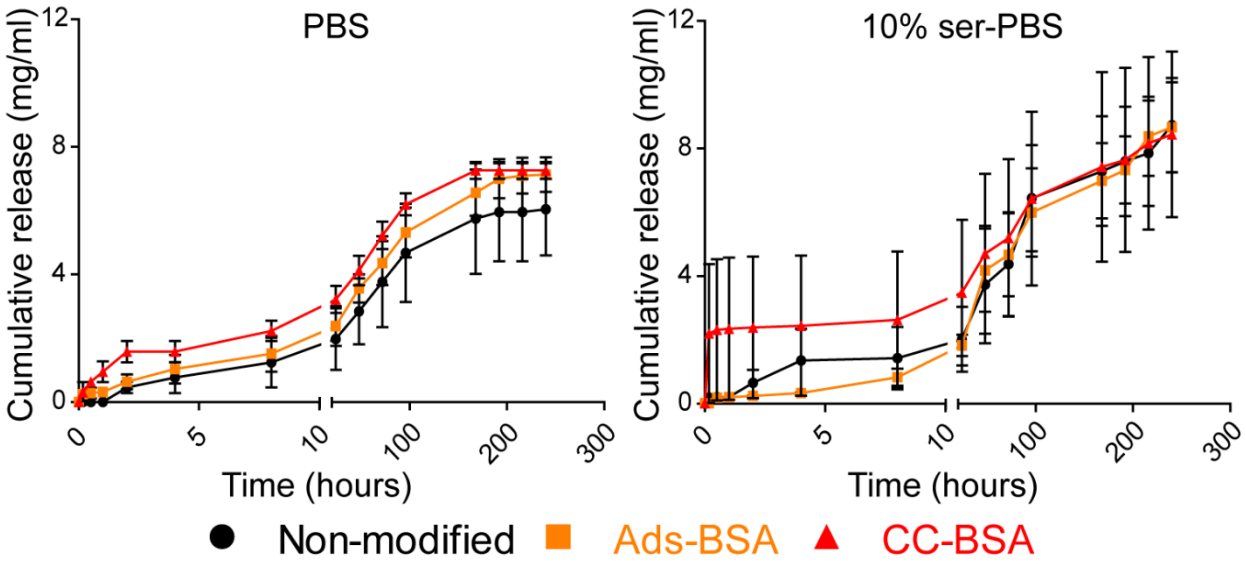
Supplementary Figure 8: Number of particles associated with cells. Similar to *in vitro* experiments, the percentage of cells with differring numbers (1, 2, 3, 4, 5 or more) of particles was measured following *in vivo* uptake.

Supplementary Figure 9



Supplementary Figure 9: Flow cytometry schematics. Representative flow cytometry contour plots showing the gating scheme used for *ex-vivo* uptake experiments. CD14 is a marker for monocytes in human PBMC.

Supplementary Figure 10



Supplementary Figure 10: Release kinetics of rhodamine encapsulated PLGA particles Release kinetics of rhodamine from rhodamine-loaded and surface modified (or non-modified) PLGA particles. Data set is based on n=3 and are plotted as Mean ± SEM. No significant difference was observed in the release kinetics between non-modified and surface modified particles.

Supplementary Table 1: Percentage uptake data (from in vitro RAW cell experiments) represented as mean ± SD for particles of different size and surface modification.

60 min				
Particle Size				
	Non-Modified	***Ads-BSA***	***CC-BSA***	***CC-PEG***
30 nm	14.56 ± 10.99	12.43 ± 10.04	1.58 ± 1.25	0.83 ± 0.68
100 nm	69.67 ± 8.99	98.17 ± 0.86	5.73 ± 2.42	2.44 ± 0.57
500 nm	56.36 ± 4.05	59.97 ± 2.84	45.96 ± 9.25	32.33 ± 7.22
2.6 µm	11.44 ± 8.81	8.15 ± 3.87	10.05 ± 4.84	14.33 ± 7.16
120 min				
	Non-Modified	***Ads-BSA***	***CC-BSA***	***CC-PEG***
30 nm	33.11 ± 22.81	26.71 ± 18.19	4.03 ± 2.49	1.26 ± 0.89
100 nm	84.47 ± 6.85	98.97 ± 1.04	10.17 ± 4.20	6.61 ± 1.97
500 nm	66.13 ± 6.36	79.56 ± 2.70	64.46 ± 8.60	43.53 ± 5.49
2.6 µm	19.43 ± 12.75	15.11 ± 6.58	14.89 ± 5.44	19.44 ± 7.70
1440 min				
	Non-Modified	***Ads-BSA***	***CC-BSA***	***CC-PEG***
30 nm	87.90 ± 16.29	84.17 ± 15.91	15.83 ± 0.68	3.07 ± 1.84
100 nm	98.9 ± 0.62	99.50 ± 0.40	36.62 ± 35.21	21.50 ± 15.71
500 nm	91.96 ± 11.24	97.56 ± 2.41	96.43 ± 4.20	76.26 ± 7.25
2.6 µm	22.73 ± 7.99	27.59 ± 8.25	20.07 ± 5.45	23.67 ± 10.39

Supplementary Table 2: Summary of statistical analysis performed for data shown in figure 2. One-way ANOVA comparing uptake of particles with different modifications (or no modification) for a specific time point (and specific particle size) followed by Bonferroni post-hoc testing. * indicates $p < 0.05$, ** indicates $p < 0.01$, *** indicates $p < 0.001$. Majority of the differences (especially lowering of uptake following PEG or albumin) was observed in the 30 nm and 100 nm particles.

30 nm	60 min	120 min	1440 min
Non-modified vs Ads-BSA	ns	ns	ns
Non-modified vs CC-BSA	*	ns	***
Non-modified vs CC-PEG	*	**	***
Ads-BSA vs CC-BSA	*	ns	***
Ads-BSA vs CC-PEG	*	**	***
CC-BSA vs CC-PEG	ns	ns	ns

100 nm	60 min	120 min	1440 min
Non-modified vs Ads-BSA	***	**	ns
Non-modified vs CC-BSA	***	***	***
Non-modified vs CC-PEG	***	***	***
Ads-BSA vs CC-BSA	***	***	***
Ads-BSA vs CC-PEG	***	***	***
CC-BSA vs CC-PEG	ns	ns	ns

500 nm	60 min	120 min	1440 min
Non-modified vs Ads-BSA	ns	ns	ns
Non-modified vs CC-BSA	ns	ns	ns
Non-modified vs CC-PEG	**	**	ns
Ads-BSA vs CC-BSA	ns	*	ns
Ads-BSA vs CC-PEG	**	***	ns
CC-BSA vs CC-PEG	ns	*	ns

2.6 µm	60 min	120 min	1440 min
Non-modified vs Ads-BSA	ns	ns	ns
Non-modified vs CC-BSA	ns	ns	ns
Non-modified vs CC-PEG	ns	ns	ns
Ads-BSA vs CC-BSA	ns	**	**
Ads-BSA vs CC-PEG	**	ns	ns
CC-BSA vs CC-PEG	*	ns	ns

Supplementary Table 2: Following intraperitoneal injection of 2.6 µm PS particles (*in vivo* in BALB/c mice), immune cells in the peritoneal fluid were analyzed. Data reported here are percentages of specific sub-populations of immune cells (among all live cells), and the percentage of a specific sub-population that is associated with the PS particles (among all live cells). Data represented as Mean ± SD, based on n ≥ 4 mice per group.

	$F4/80^+Ly6G^-$ $CD115^-$ (Macrophages)		**$Ly6G^+$ $CD115^-$ $F4/80^-$ (Neutrophils)**		**$CD115^+$ $Ly6G^-$ $F4/80^-$ (Monocytes)**	
Particle Type	*%* **(of all live cells)**	*% with Particles* **(of all live cells)**	*%* **(of all live cells)**	*% with Particles* **(of all live cells)**	*%* **(of all live cells)**	*% with Particles* **(of all live cells)**
Non-modified	11.7±3.2	3.1±1.2	0.17±0.06	0.01±0.005	0.3±0.2	Negligible
Ads-BSA	6.2±3.3	2.2±1.4	0.4±0.2	0.02±0.02	1±1	Negligible
CC-BSA	13.8±5.7	4.36±0.09	1.1±0.8	0.07±0.04	0.5±0.4	Negligible
CC-PEG	13.6±4.8	4.5±1.3	1±1	0.05±0.04	0.5±0.3	Negligible