Supplementary Information For

Microparticle Uptake Reduces Efferocytic Capacity of Macrophages

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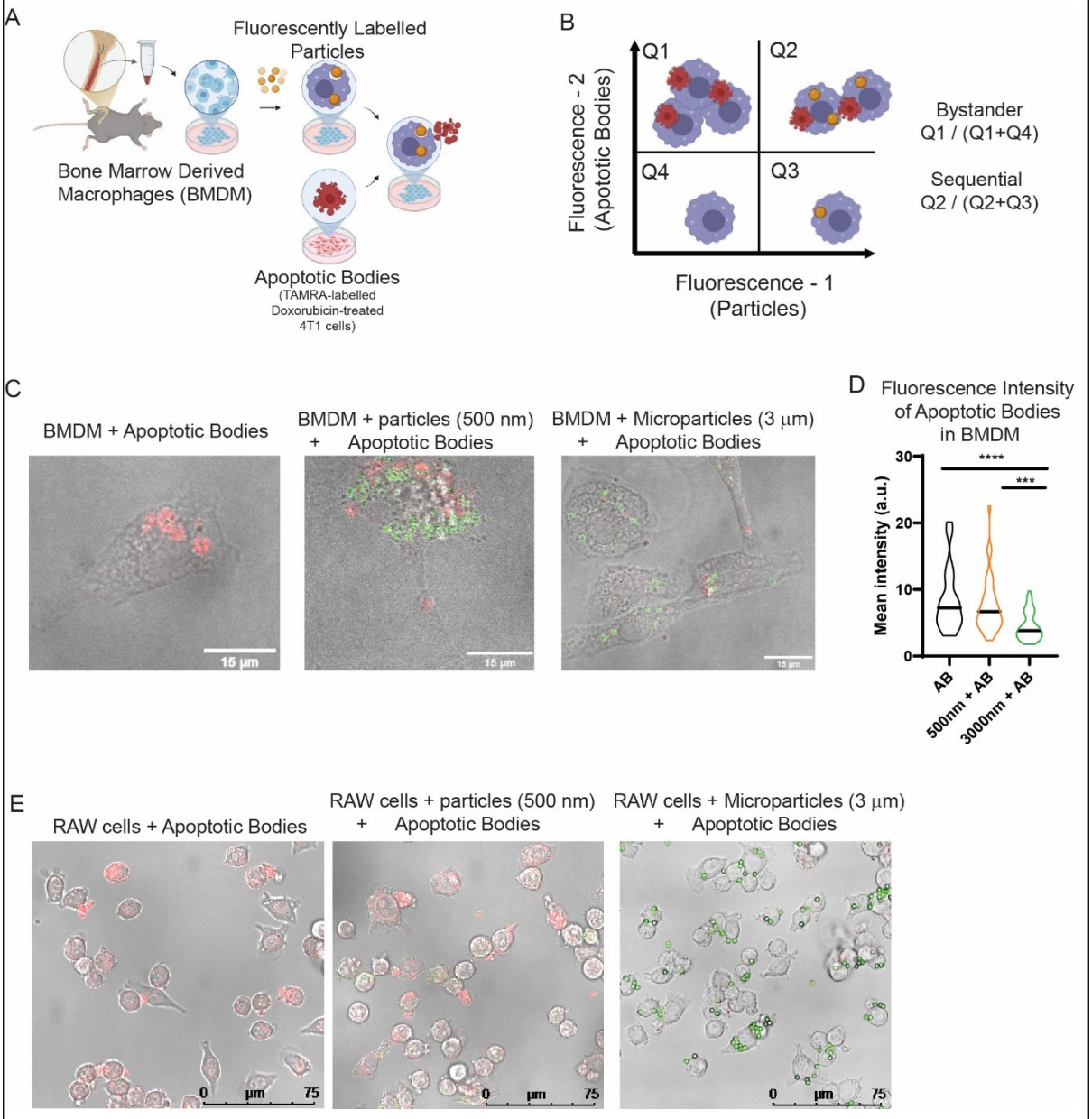


Supplementary Figure S1: Efferocytosis suppression in bone marrow derived macrophages (BMDMs) and RAW 264.7 cells.

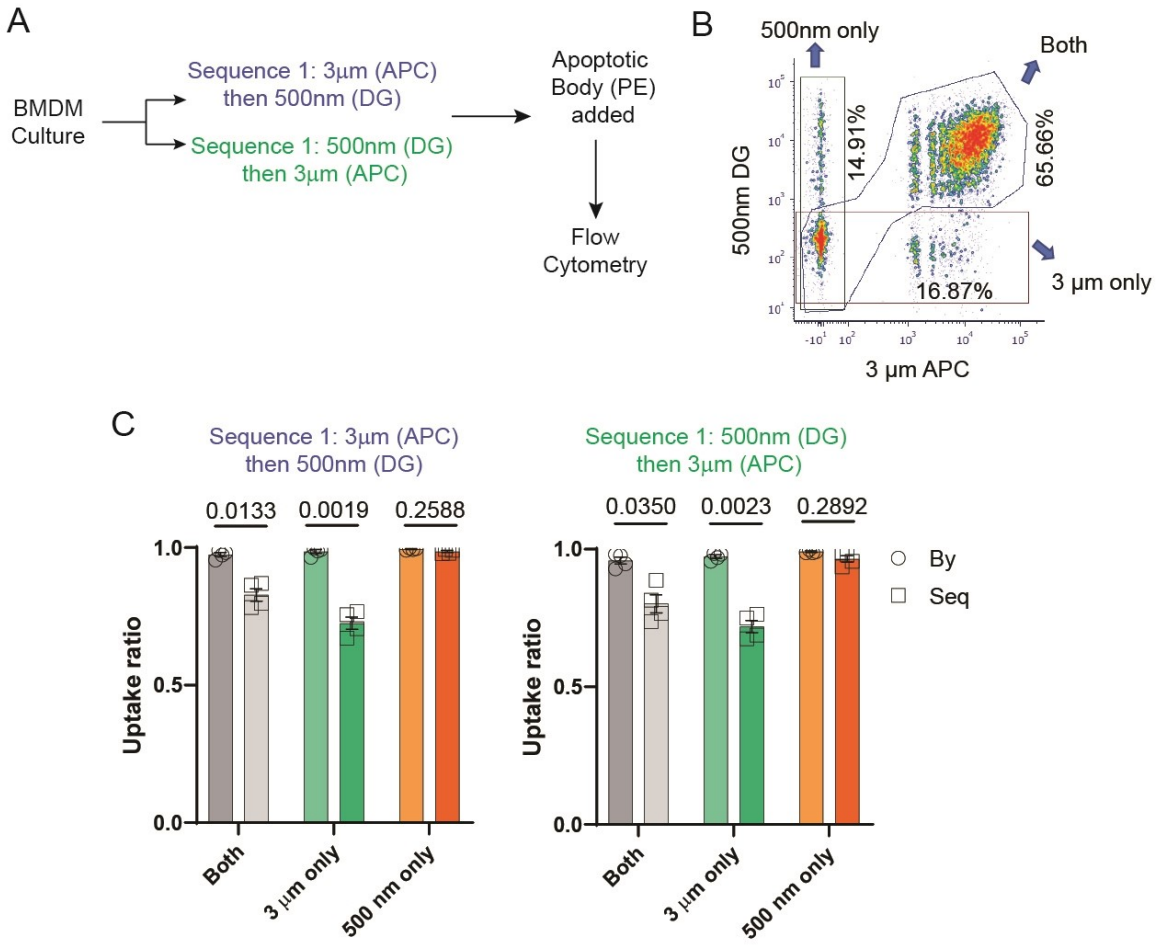
A – Schematic describing the experimental protocol followed to study how particle uptake affects the efferocytic capacity of BMDMs or RAW cells. The apoptotic bodies were obtained by labelling 4T1 cells with TAMRA followed by treatment with doxorubicin.

B – Cartoon showcasing the flow cytometry dot-plots that would be obtained following the sequential uptake experiment, and how bystander uptake and sequential uptake are calculated.

C – Representative microscopy images (merge of bright field (cells), particles in green and TAMRA-labelled AB in red) of BMDMs that have been incubated with 500 nm or 3 μm sized particles, followed by 30 min. AB exposure. BMDMs incubated with AB for 30 min. (without exposure to particles) served as controls. Scale bars as denoted in the figure.

D – Quantification of TAMRA-labelled AB fluorescence intensity; n indicates number of

cells that were imaged. Data sets are representative of N ≥ 3 (independent experiments). ***p < 0.001, ****p < 0.0001 (one-way ANOVA followed by post-hoc Tukey test.
E – Representative microscopy images (merge of bright field (cells), particles in green and TAMRA-labelled AB in red) of RAW cells similar to the data described in C.

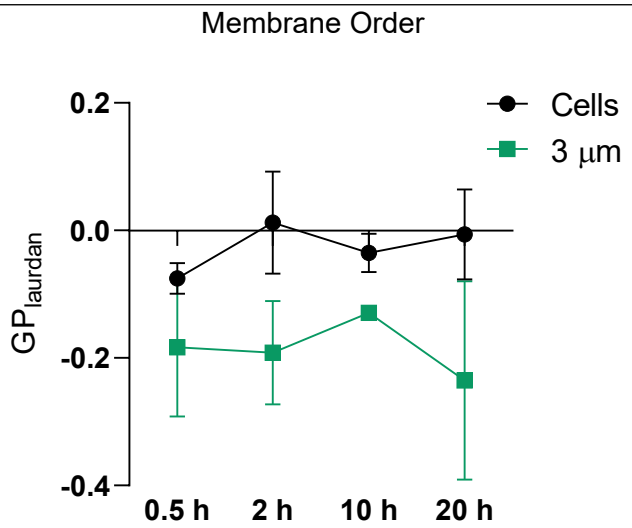


Supplementary Figure S2:

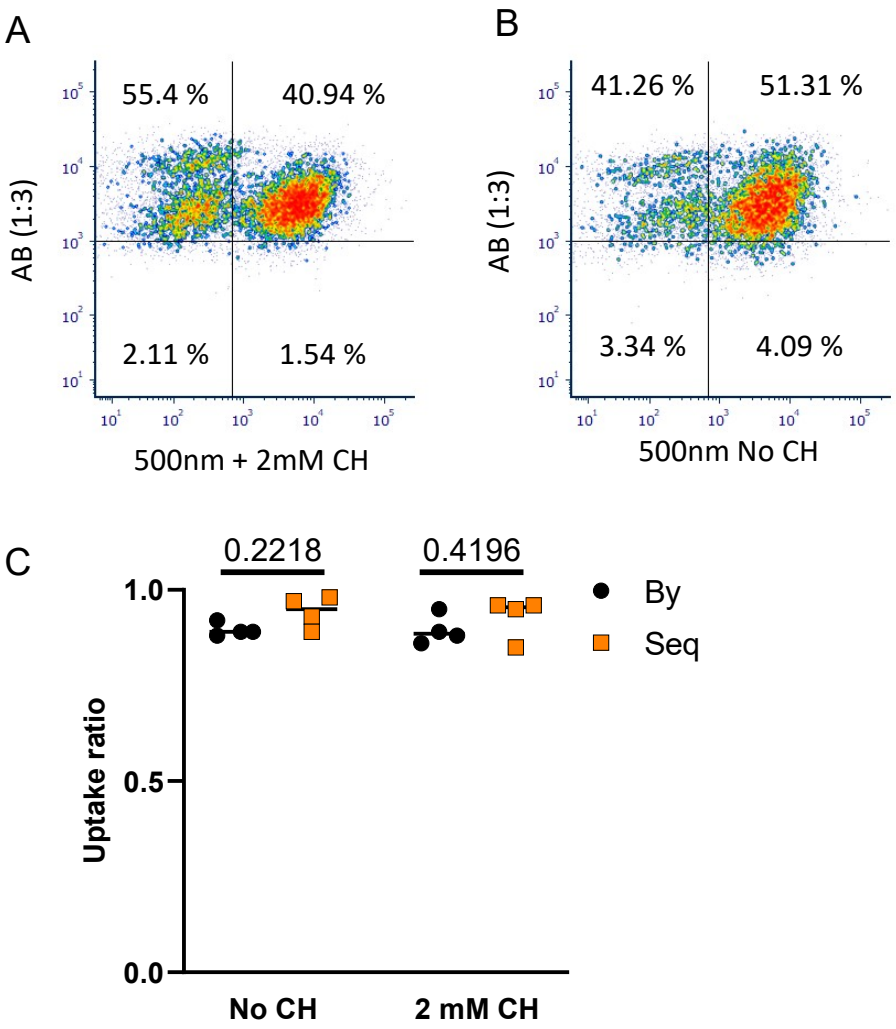
A − Schematic showing an experiment involving exposure of cells to particles of 2 sizes followed by apoptotic bodies (AB). The fluorophore used for each particle type and AB is indicated in parentheses. BMDM subpopulations pre-incubated with both particles for 1 hour each irrespective of the sequence followed by AB exposure (cell-to-AB ratio of 1:3 for 2 hours).

B − Representative flow cytometry plot showing gating strategy for particle positive and particle negative BMDMs. AB uptake was measured across particle-positive subpopulations including cells containing both particle sizes (500 nm and 3 µm), only 500 nm particles, or only 3 µm particles (sequential uptake) and compared to cells that did not take up particles (bystander uptake).

C − Quantification of the plots from B. p values are mentioned, which was calculated using a two-way ANOVA followed by Sidak test; N = 4 independent experiments.



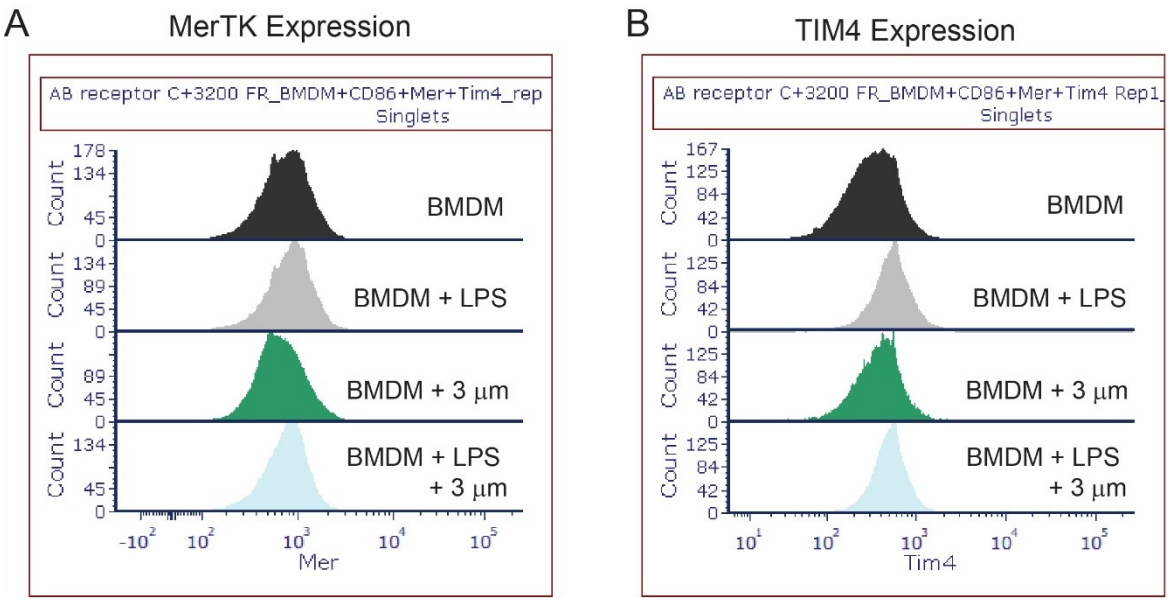
Supplementary Figure S3: Measurement of membrane order using the generalized polarization (GP) value for the membrane dye Laurdan in RAW 264.7 macrophages incubated with 3 μm sized particles compared to untreated cells over time. Data presented in figure 2A was for the 2 hours time point shown in this graph (h stands for hours).



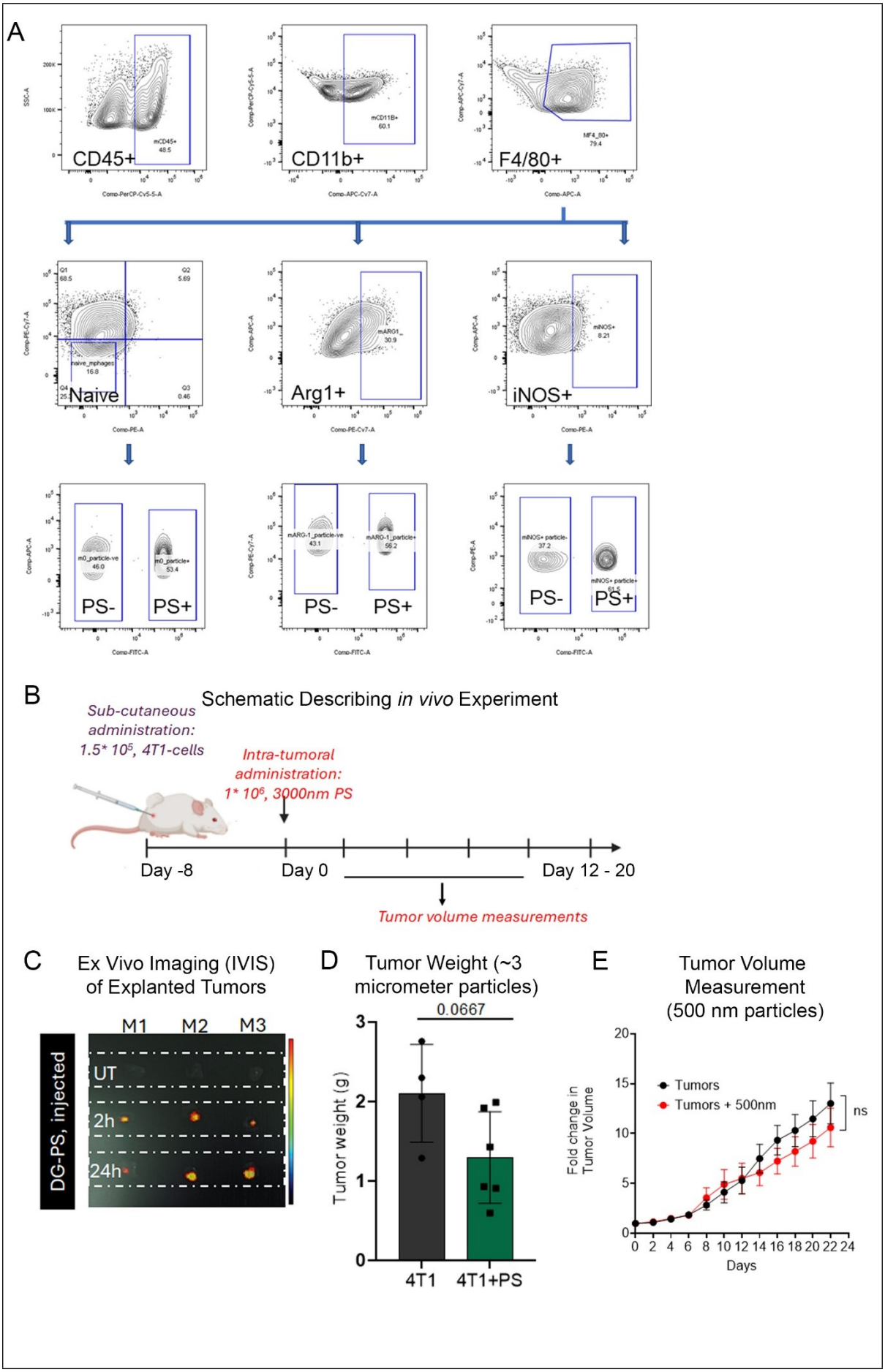
Supplementary Figure S4: Cholesterol supplementation does not alter efferocytosis in macrophages treated with sub-micron particles

A and B − Representative flow cytometry plots showing apoptotic body (AB) uptake in particle-treated macrophages with or without cholesterol supplementation.

C − Quantification of bystander (By) and sequential (Seq) uptake ratios among BMDMs that have been cultured with cholesterol (CH) or no CH (control) and subjected to 500nm PS treatment followed by the addition of apoptotic bodies. p values are mentioned, which were calculated using a two-way ANOVA followed by Sidak test; N = 4 independent experiments.



Supplementary Figure S5: Representative flow cytometry histograms supporting data presented in figure 4, showing the expression (fluorescence intensity in the x-axis) of MerTK (**A**) and Tim4 (**B**) in bone marrow derived macrophages (BMDMs) following various treatments.


A
CD45+
CD11b+
F4/80+
Naive
Arg1+
iNOS+
PS-
PS+
PS-
PS+
PS-
PS+
B
Schematic Describing in vivo Experiment
Sub-cutaneous administration: 1.5* 10^5, 4T1-cells
Intra-tumoral administration: 1* 10^6, 3000nm PS
Day -8
Day 0
Day 12 - 20
Tumor volume measurements
C
Ex Vivo Imaging (IVIS) of Explanted Tumors
M1
M2
M3
DG-PS, injected
UT
2h
24h
D
Tumor Weight (~3 micrometer particles)
0.0667
Tumor weight (g)
4T1
4T1+PS
E
Tumor Volume Measurement (500 nm particles)
Fold change in Tumor Volume
Tumors
Tumors + 500nm
ns
Days

Supplementary Figure S6: Ex vivo and in vivo studies in 4T1 tumors.
A – Flow cytometry plots showing macrophage subsets in ex vivo incubation of dissociated tumor cells with and without fluorescent PS particles.
B – Schematic of in vivo experimental design where BALB/c mice bearing >50 mm^3 (in volume) 4T1 tumors received single intratumoral dose of $1x10^6$, 3 μm sized fluorescent microparticles, while untreated tumor-bearing mice served as controls.
C – An independent experiment to determine if fluorescently-labelled ~3 μm sized PS microparticles injected in the tumor-bearing mice remain inside tumors. Imaging was performed using an in vivo imaging system (IVIS).
D - Measurement of the weight of excised tumors at the endpoint of the experiment from un-injected and ~3 μm sized PS microparticle injected tumors. p value was calculated by performing a Mann-Whitney test.
E – Fold change in tumor volume of untreated tumors versus 500 nm-sized particle-treated group. P=0.3009, two-way ANOVA followed by Sidak test