Supporting Information for:

Specific targeting of ovarian tumor associated macrophages by large, anionic nanoparticles

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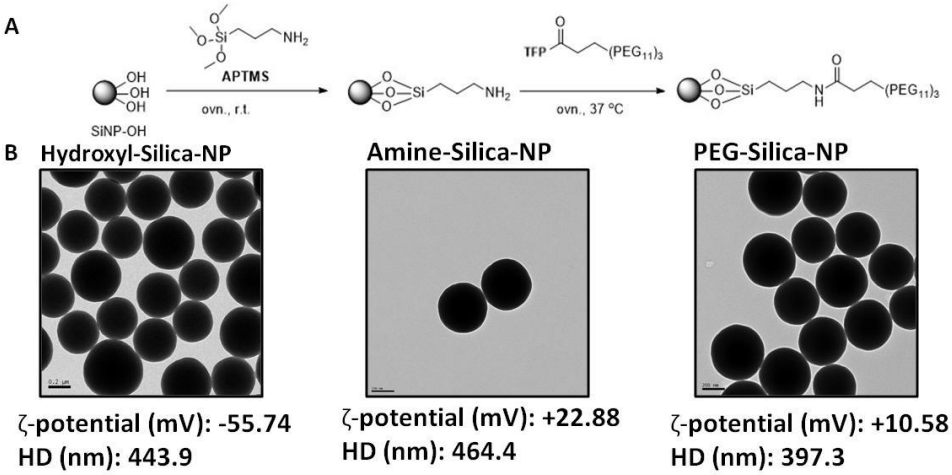


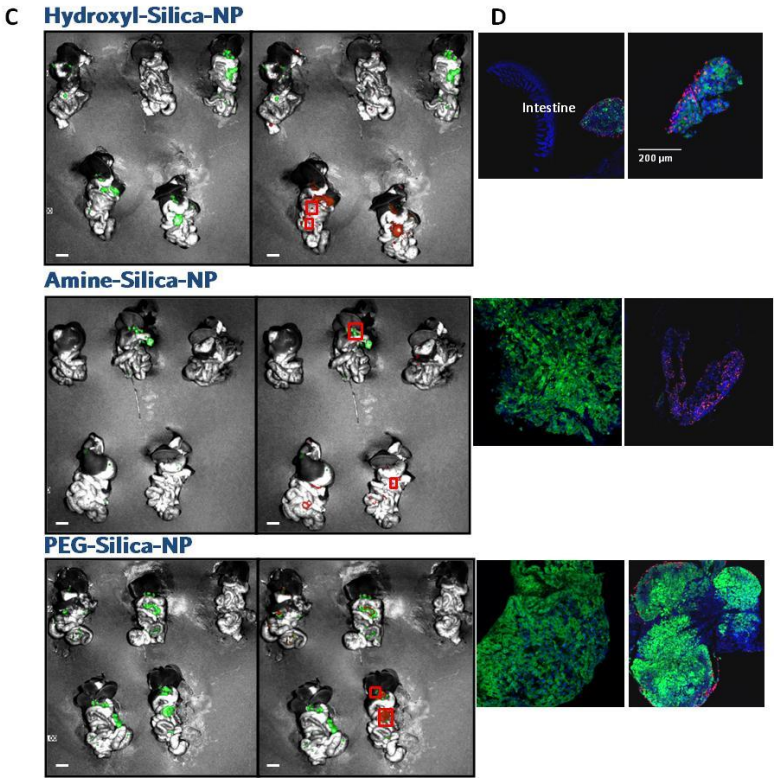
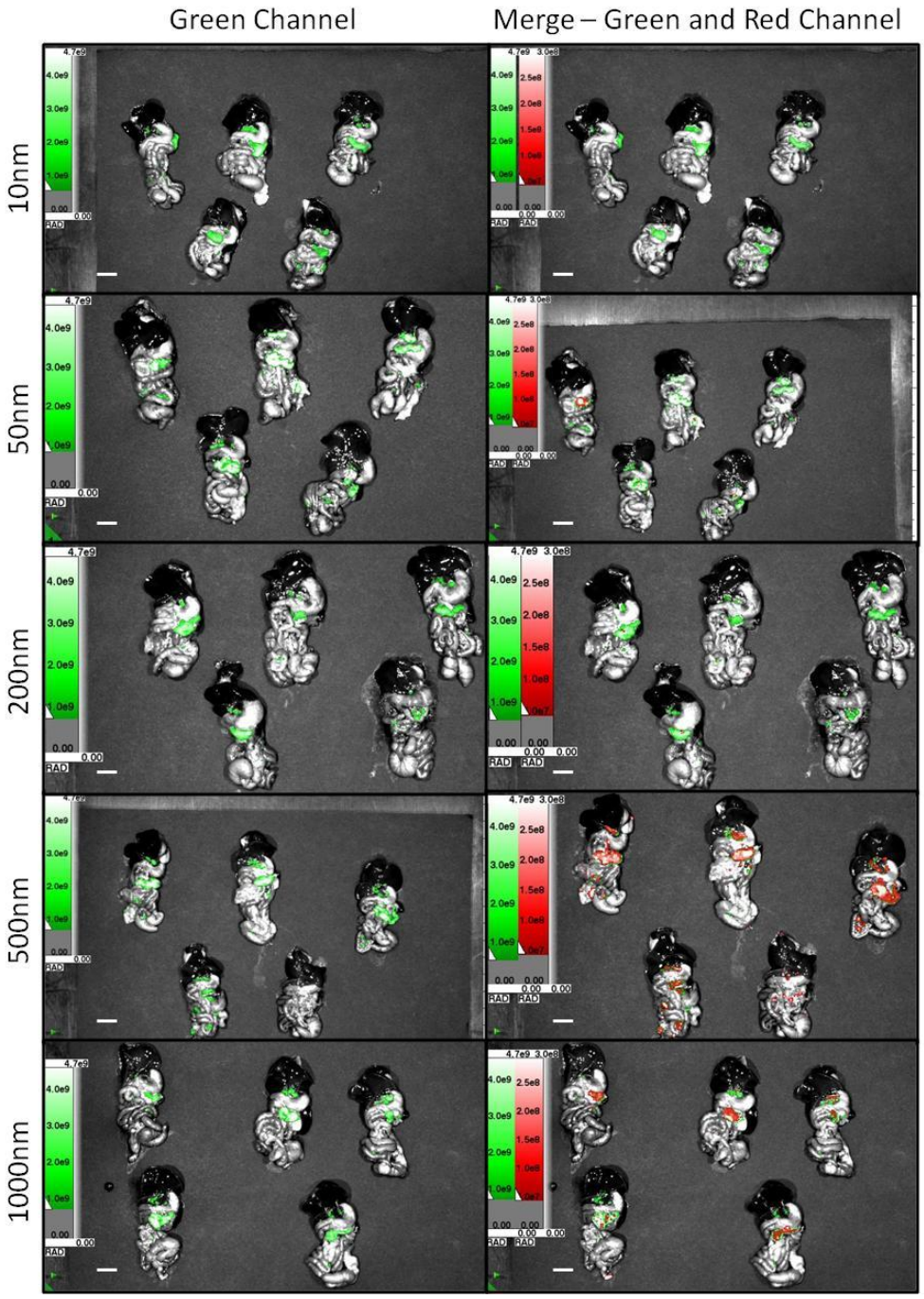
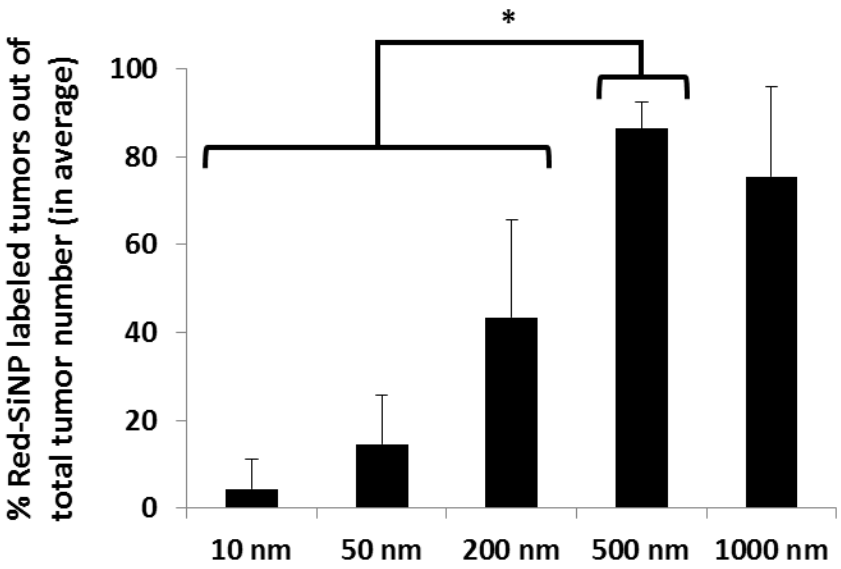


Figure S1. The effect of surface charge on the selective tumor targeting of SiNPs. (A) Schema of Hydroxyl-SiNPs, Amine-SiNPs and PEG-SiNPs preparation. **(B)** TEM images, zeta potential (mV) and hydrodynamic size (nm) of the different SiNPs. **(C)** Spectral Ami-X whole-body images of the IP cavity organ block. SiNPs red, tumors green. Scale bar = 1.0 cm. **(D)** Confocal images of the sectioned tumors (SiNPs red, eGFP tumors green/dense blue nuclei). Scale bar = 200 µm.

A


Green Channel
Merge – Green and Red Channel
10nm
50nm
200nm
500nm
1000nm

B



C

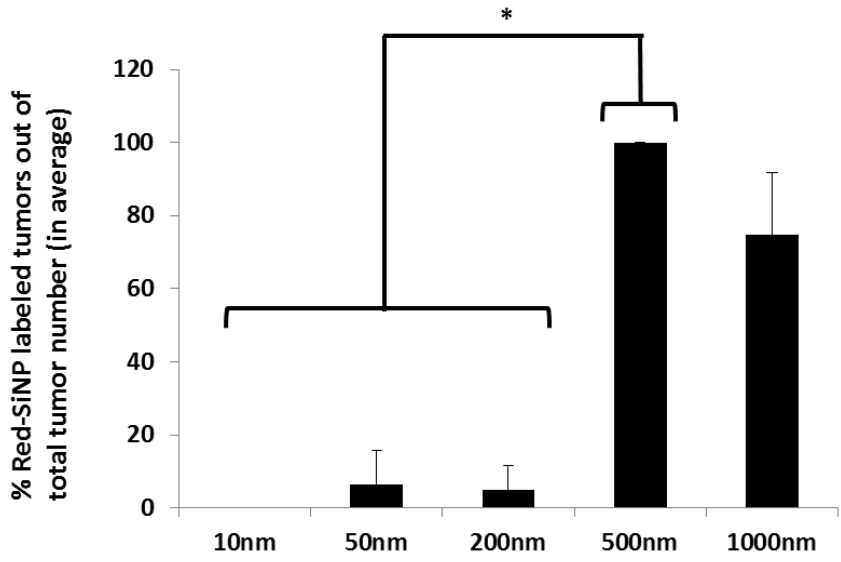


Figure S2. The effect of size on the selective tumor targeting of SiNPs. (A) Spectral Ami-X wide-field images of the IP cavity organ block 4 days after IP injection of 10, 50, 200, 500 and 1000 nm red-fluorescent-labeled SiNPs and no SiNP control (SiNPs red, eGFP tumors green). Scale bar = 1.0 cm. **(B)** Fluorescence co-localization analysis of the dissection macroscope images and **(C)** of the wide-field fluorescence images of the IP cavity organ block 4 days after IP injection of 10, 50, 200, 500 and 1000 nm red-fluorescent-labeled SiNPs and no SiNP control. For the comparison between the different groups ANOVA followed by a corrected bonferroni post hoc t-tests were performed. The ANOVA for the 500 nm group was significant and the corrected bonferroni post hoc t-tests indicated that the co-localization of the SiNPs and tumors in this group was significantly higher than groups 10, 50, 200 nm. (**(B)** P=0.003, **(C)** P<0.001), (using Image J software, NIH, USA).

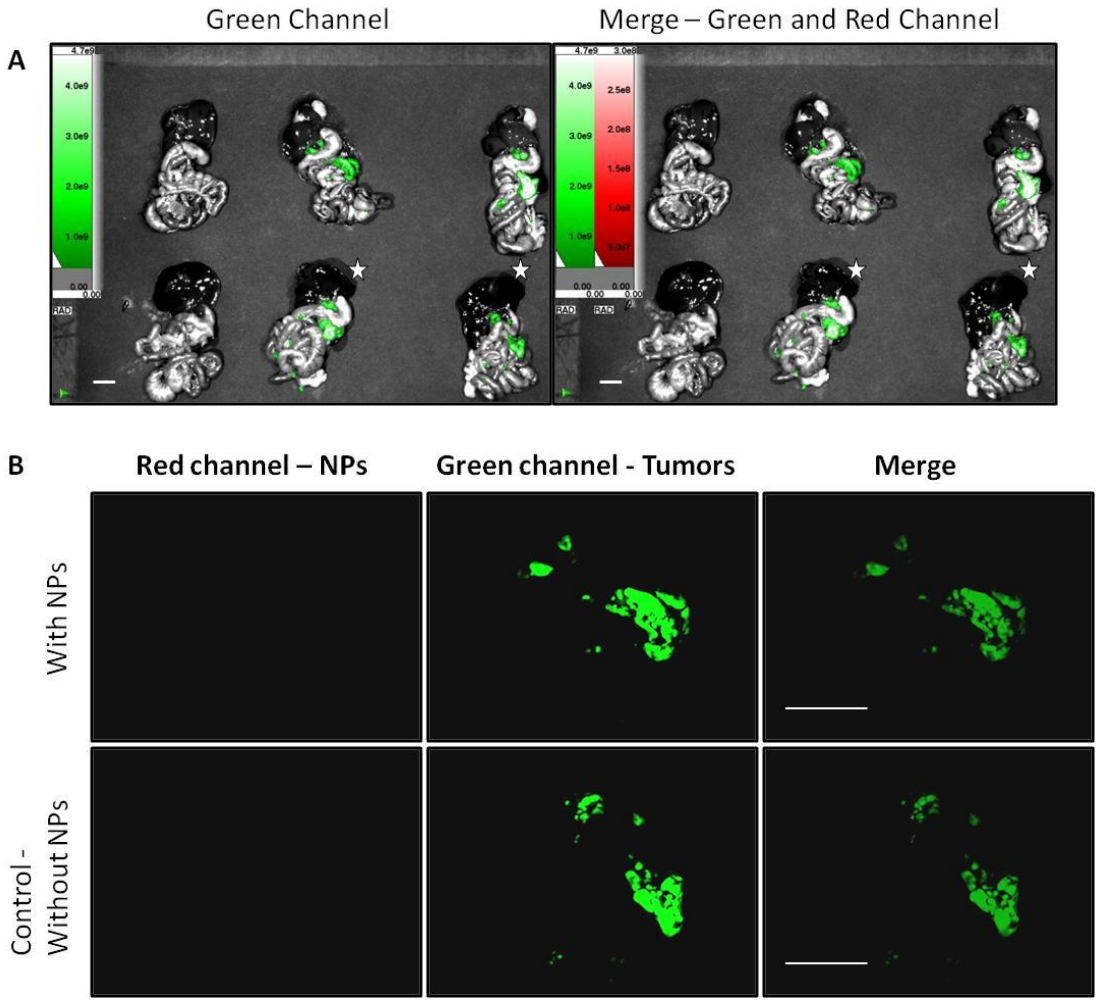
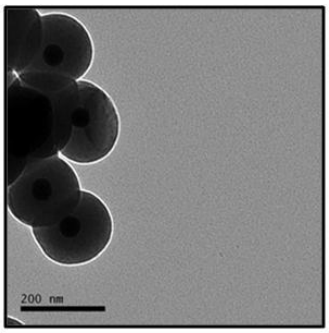
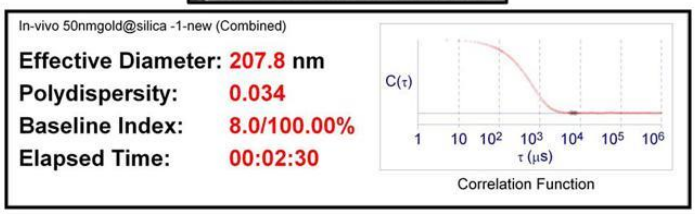


Figure S3. The effect of route of administration (IV administration) on the selective tumor targeting of SiNPs. (A) Spectral Ami-X wide-field images and **(B)** LeicaZ16 dissection macroscope images of the IP cavity organ block 4 days after IV injection (IV administration) of 500 nm red-fluorescent-labeled SiNPs (red). No SiNP controls (indicated by white stars). Scale bar = 1.0 cm.

A



B



C

Run	Mobility	Zeta Potential (mV)	Rel. Residual
1	-3.58	-45.85	0.0168
2	-3.43	-43.84	0.0282
3	-3.50	-44.81	0.0151
4	-3.56	-45.61	0.0174
5	-3.36	-42.98	0.0292
6	-3.58	-45.79	0.0218
7	-3.42	-43.74	0.0166
8	-3.26	-41.76	0.0246
9	-3.71	-47.43	0.0150
10	-3.80	-48.59	0.0271
Mean	-3.52	-45.04	0.0212
Std. Error	0.05	0.65	0.0018
Combined	-3.52	-45.01	0.0121

Figure S4. Characteristics of Au@SiNPs. (A) TEM images of 50 nm gold cores in 75 nm silica shell Scale bar =200 nm). **(B)** Hydrodynamic size (nm) of Au@SiNPs and **(C)** Zeta potential of Au@SiNPs (mV).

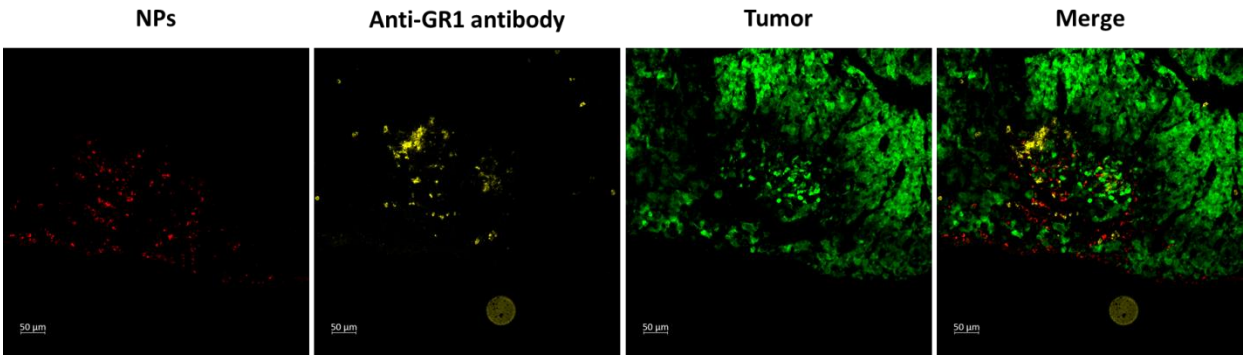


Figure S5. Myeloid-Derived Suppressor Cells (MDSCs) immunohistochemistry. Confocal image of sectioned tumor 4 days after IP injection of red fluorescent SiNPs. Red fluorescent labeled SiNPs - red, Anti-GR1 antibody – yellow, eGFP tumors - green. Scale bar = 50 µm.

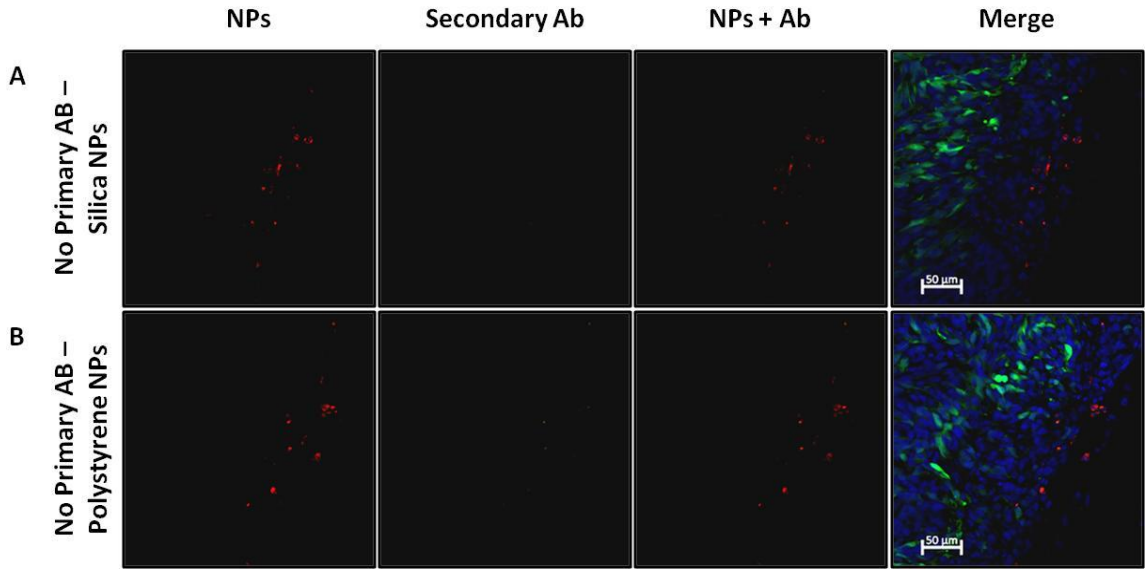


Figure S6. Confocal imaging of no primary antibody controls. (A) Red fluorescent labeled SiNPs, **(B)** Red fluorescent labeled polystyrene NPs. Secondary antibody Alexa Fluor 647 goat anti-rat (Invitrogen) yellow. eGFP tumors green, DAPI nuclei blue. Scale bar = 50 µm.

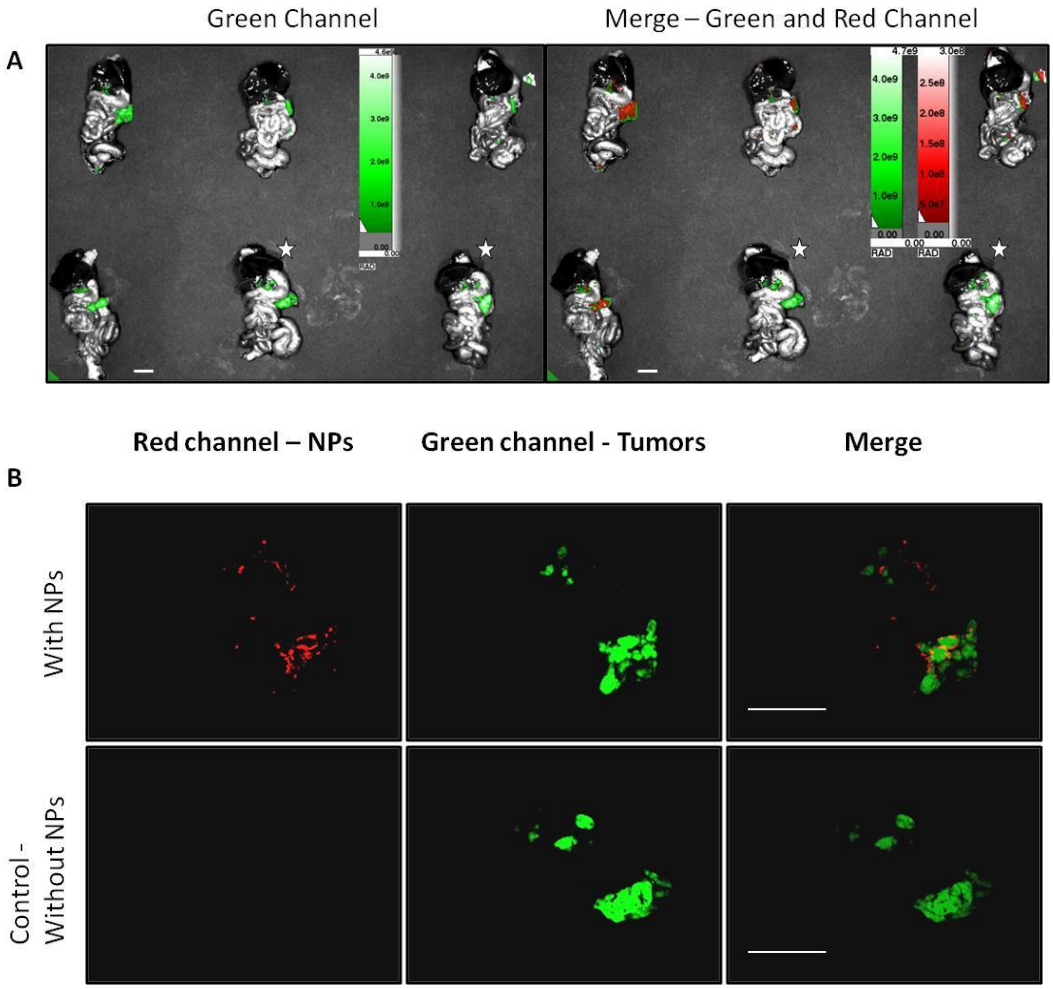
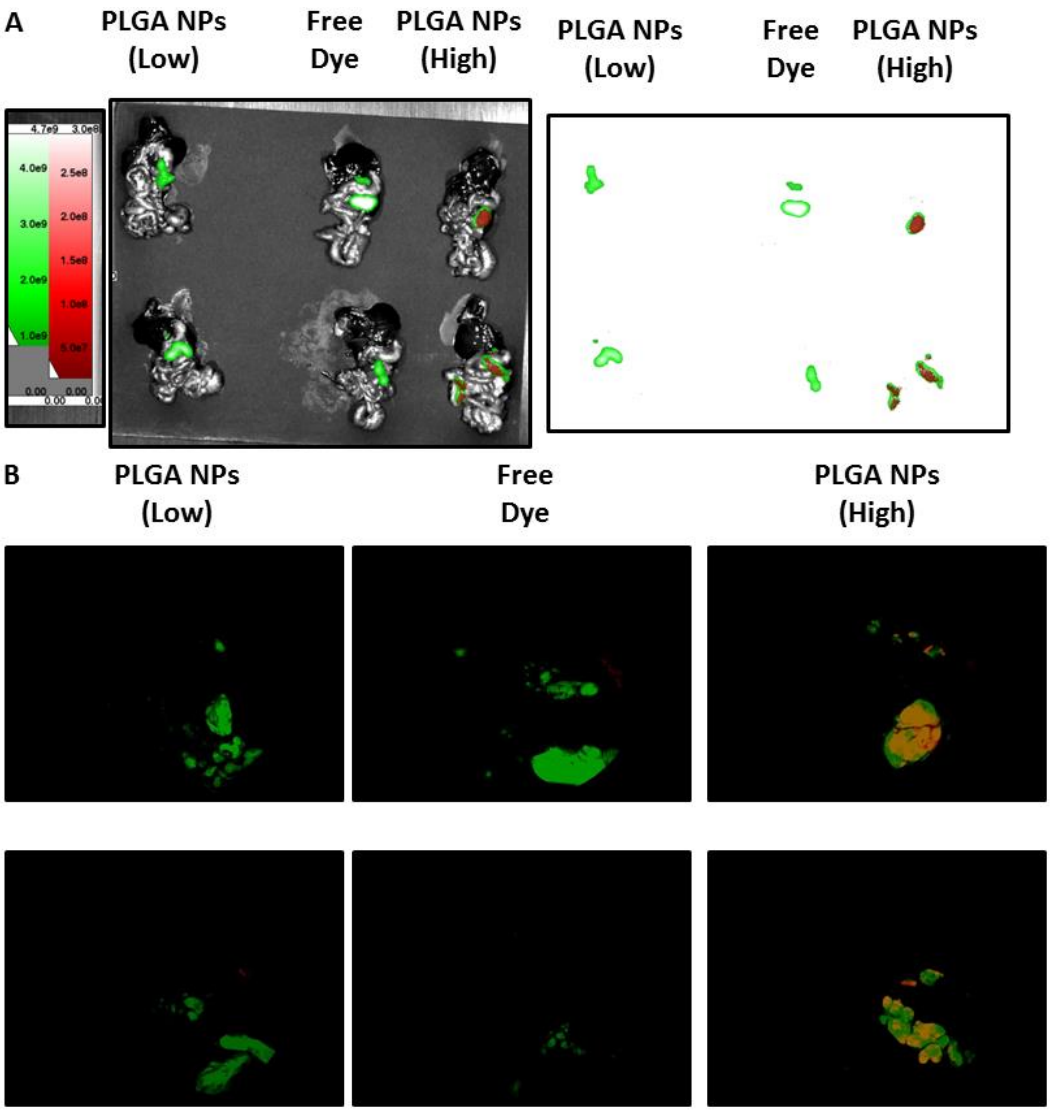


Figure S7. SiNPs target additional ovarian cancer cell line. Human SKOV-3.eGFP ovarian cancer cells were injected IP to generate abdominal metastases **(A)** Spectral Ami-X wide-field images and **(B)** Leica Z16 dissection macroscope images of the IP cavity organ blocks 4 days after the IP injection of 500 nm red fluorescent labeled SiNPs red, tumors green. No SiNP controls (indicated by white stars). Scale bar = 1.0 cm.


A
PLGA NPs (Low)
Free Dye
PLGA NPs (High)
PLGA NPs (Low)
Free Dye
PLGA NPs (High)
4.7e9
3.0e8
4.0e9
2.5e8
3.0e9
2.0e8
1.5e8
2.0e9
1.0e8
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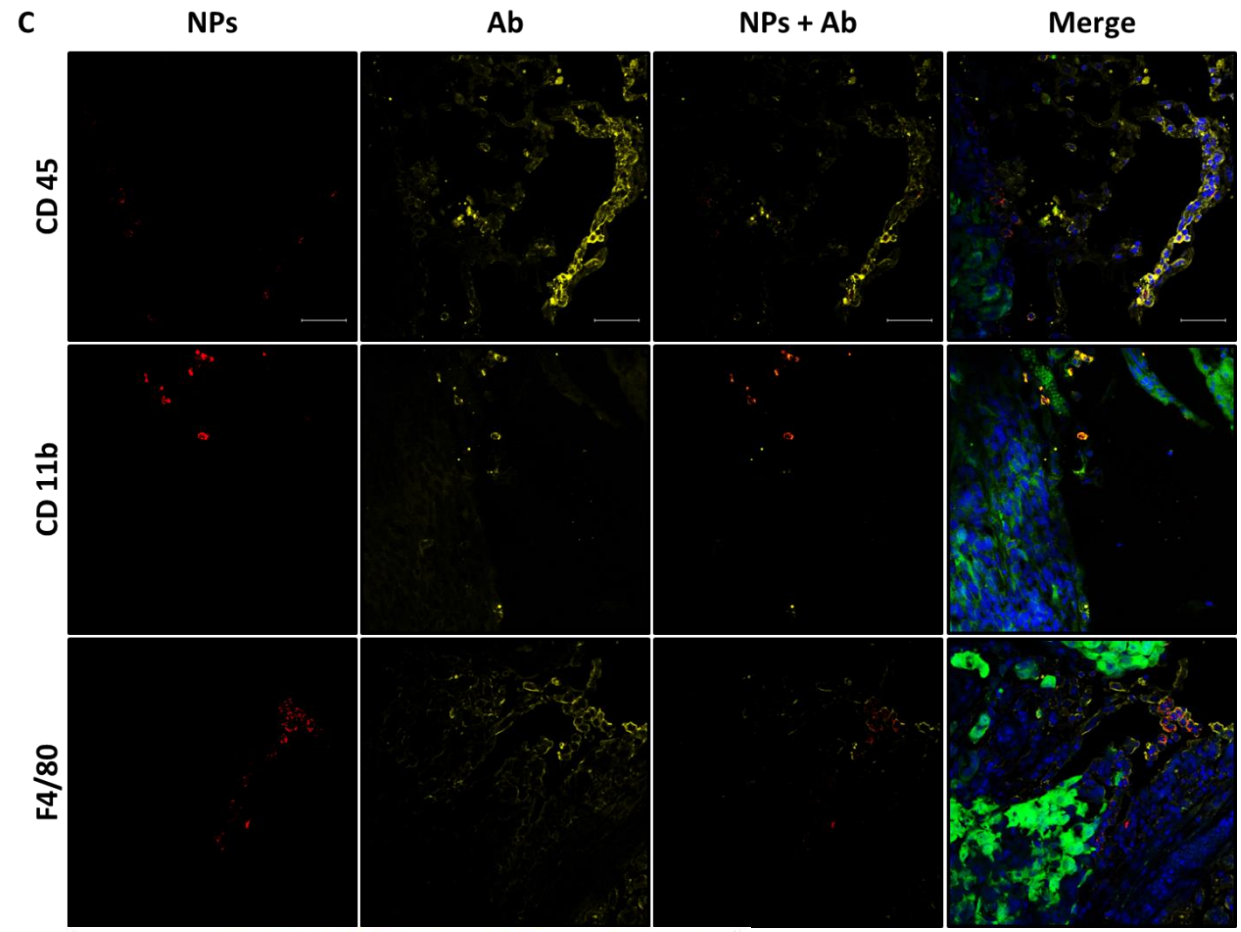
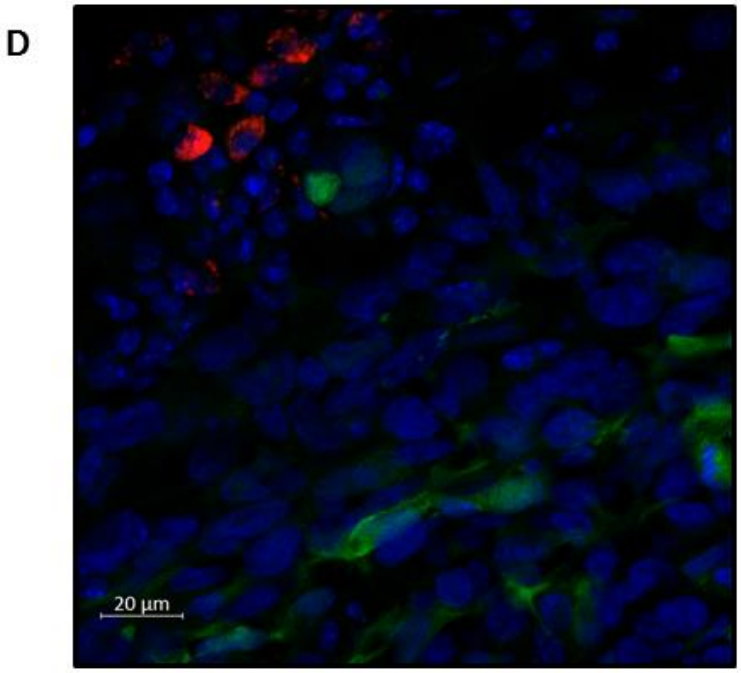
B
PLGA NPs (Low)
Free Dye
PLGA NPs (High)





X40

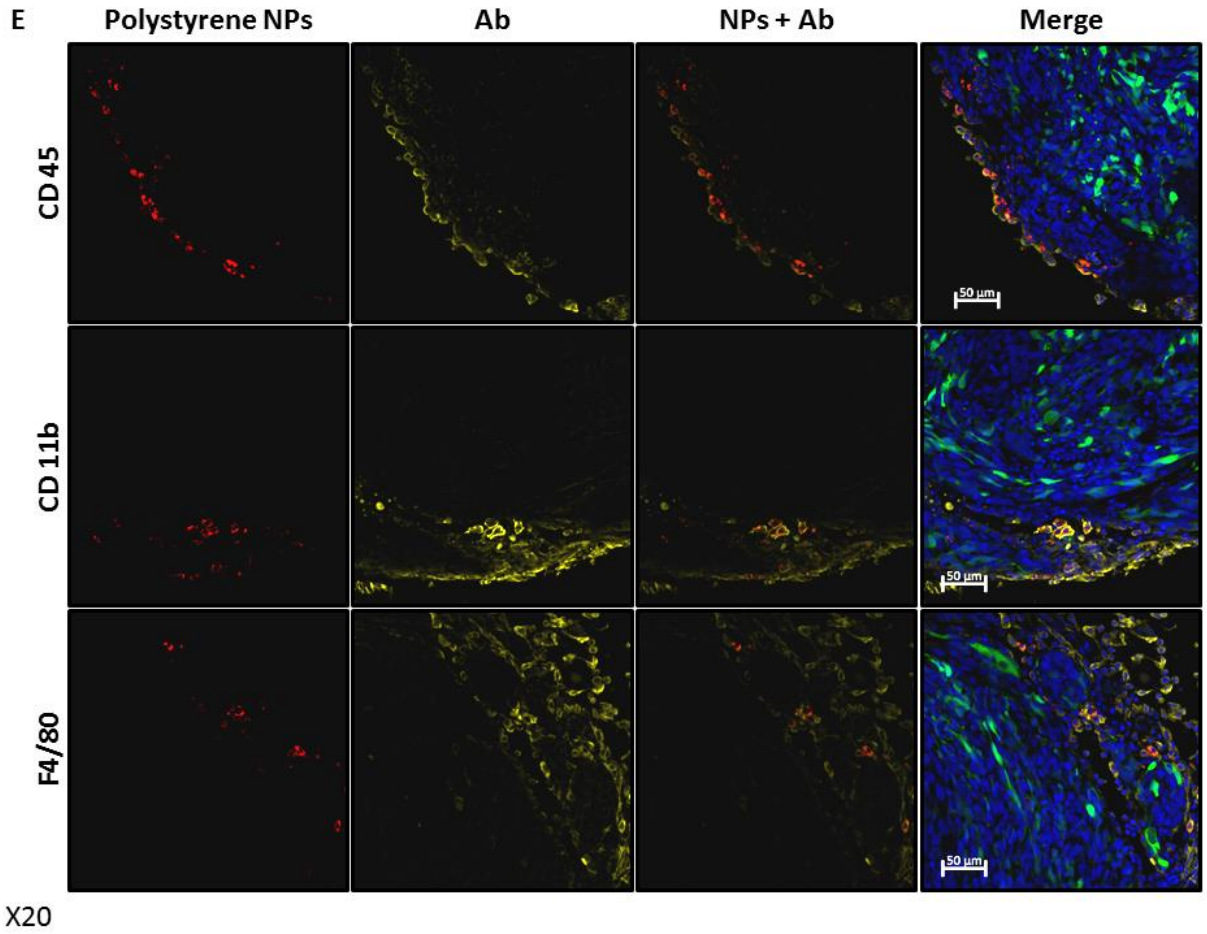
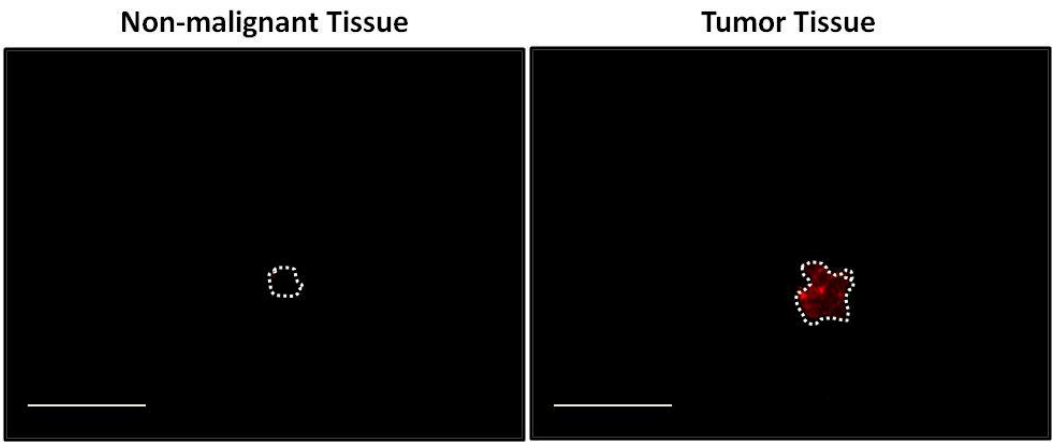
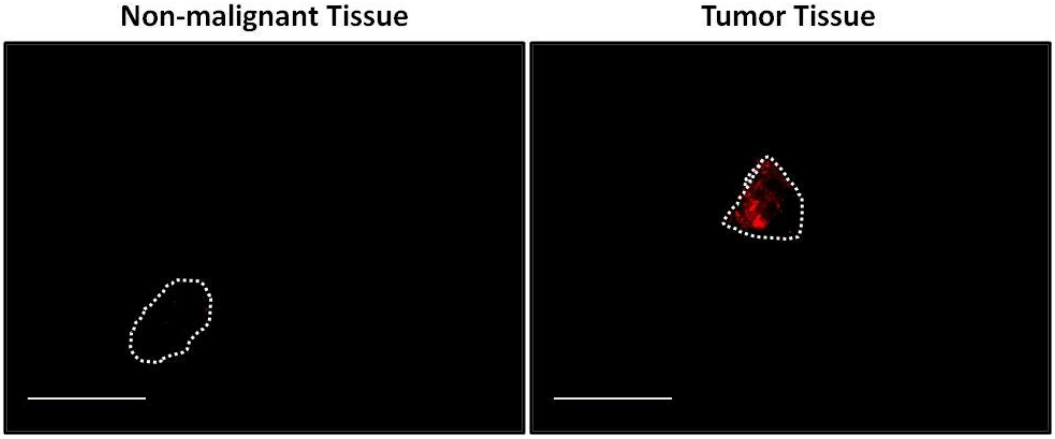


Figure S8. Other large anionic NPs also target ovarian TAMs following IP injection. (A) Ami-x and **(B)** dissection macroscope images of the IP cavity organ blocks 4 days after the IP injection of fluorescently labeled PLGA NPs (red), tumors green. **(C)** PLGA NPs red, tumors green, DAPI stained nuclei blue. Anti-CD45, Anti-CD11b and Anti-F4/80 antibody staining yellow to identify TAMs. Note merged images in far-right panels showing co-localization of polystyrene NPs and macrophages at tumor surface. **(D)** Confocal image of representative sectioned tumor 4 days after IP injection of red fluorescently polystyrene NPs. **(E)** Polystyrene NPs red, tumors green, DAPI stained nuclei blue. Anti-CD45, Anti-CD11b and Anti-F4/80 antibody staining yellow to identify TAMs. Note merged images in far right panels showing co-localization of polystyrene NPs and macrophages at tumor surface. Scale Bar = 50 µm.

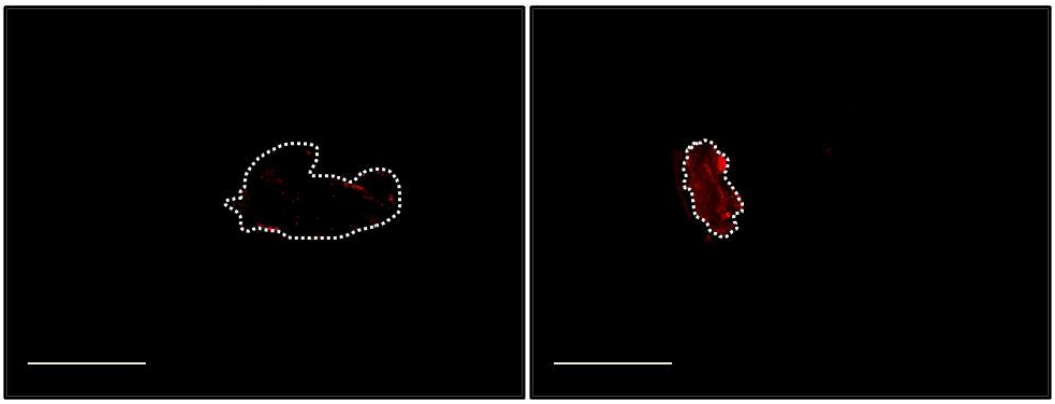
A


Non-malignant Tissue
Tumor Tissue

B


Non-malignant Tissue
Tumor Tissue

C


Non-malignant Tissue
Tumor Tissue

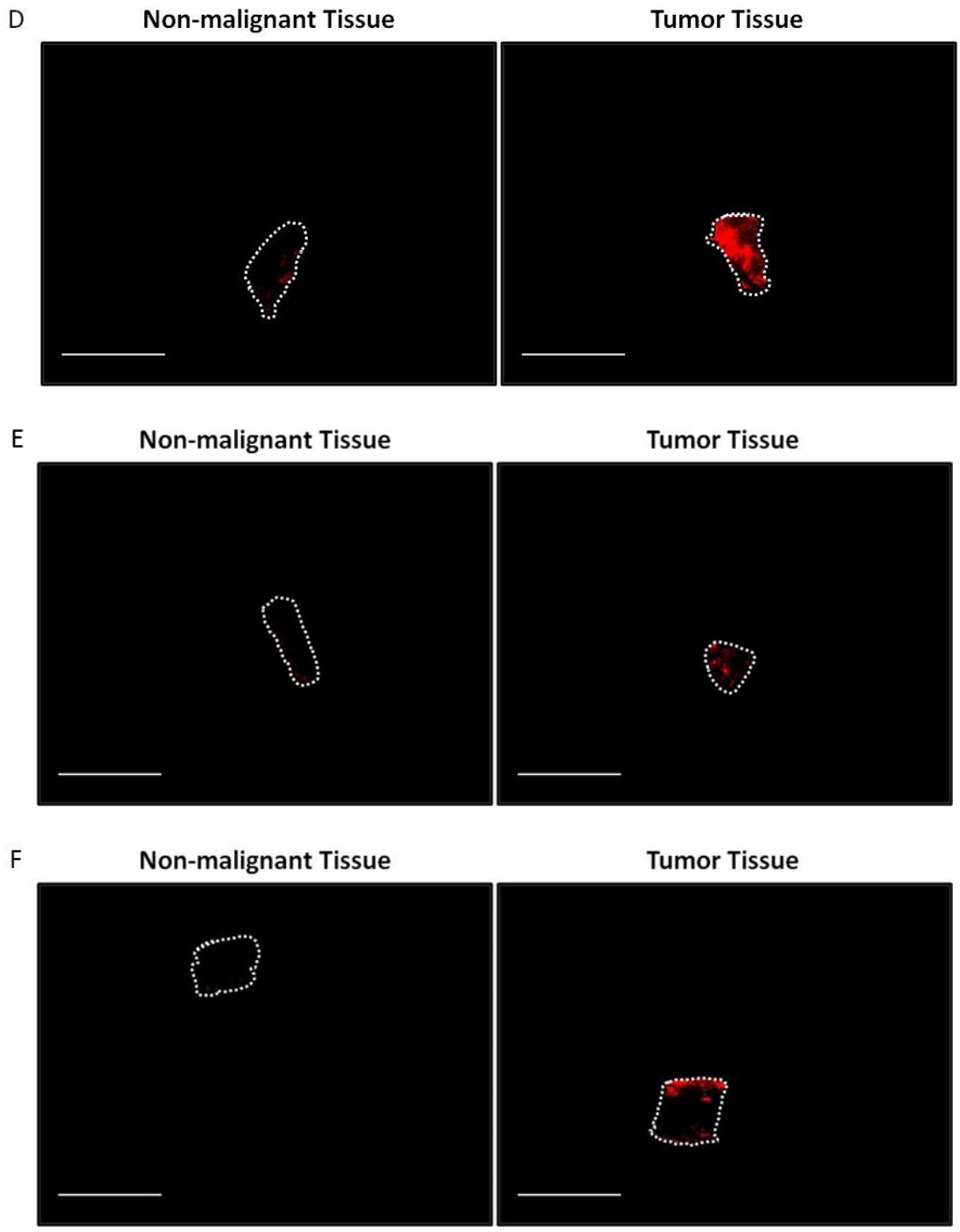


Figure S9. The red fluorescently-labeled nanoparticles can selectively detect human tumors. Fresh tumors (A-D) Omentum, (E) Rectus and non-malignant tissues were obtained from patients and incubated *ex-vivo* with red fluorescently-labeled silica nanoparticles and imaged after 4 days. Each row/letter is a different patient. (F) Fresh tumors (Omentum) and non-malignant tissues were obtained from a patient and incubated *ex-vivo* with red fluorescently-labeled polystyrene nanoparticles and imaged after 4 days. Tissues are marked in white dashed line, scale bar – 1cm (silica nanoparticles - red) (Leica Z16 dissection Macroscope).

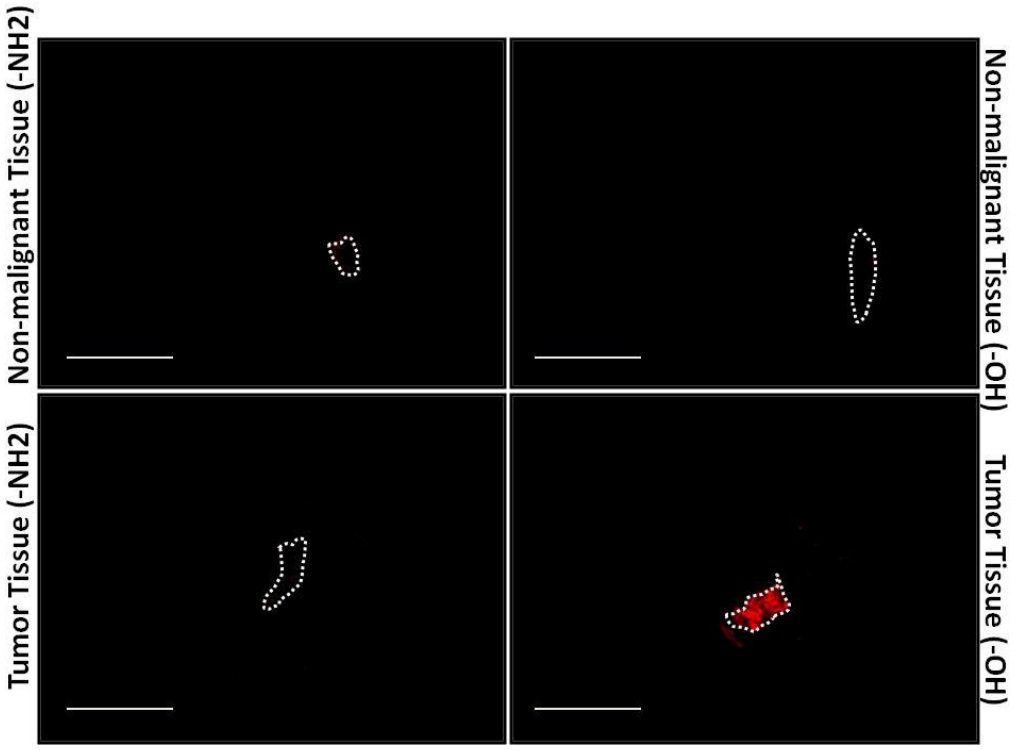


Figure S10. The effect of surface charge on the selective tumor targeting of silica nanoparticles in human samples. Fresh tumors and non-malignant tissues were obtained from patients and incubated *ex-vivo* with negatively (-OH) and positively (-NH2) surface charged red fluorescently-labeled silica nanoparticles and imaged after 4 days. Tissues are marked in white dashed line, scale bar – 1 cm (silica nanoparticles - red) (Leica Z16 dissection Macroscope).

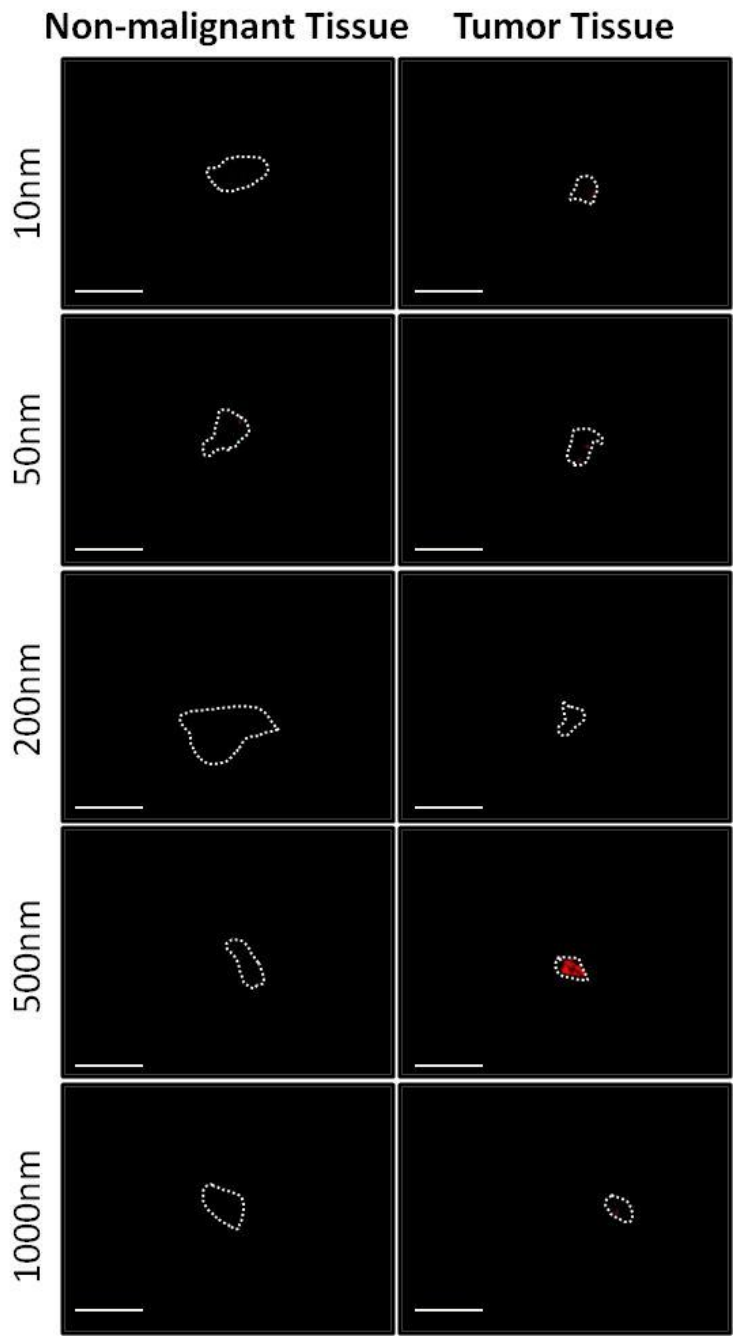
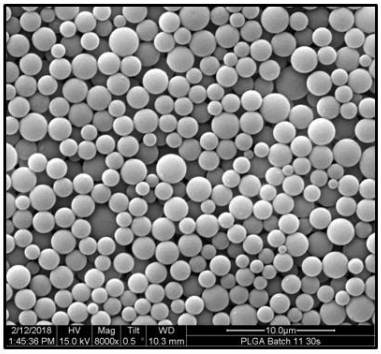


Figure S11. The effect of silica nanoparticles' size on the selective tumor targeting of silica nanoparticles in human samples. Fresh tumors and non-malignant tissues were obtained from patients and incubated *ex-vivo* with 10, 50, 200, 500 and 1000nm red fluorescently-labeled silica nanoparticles and imaged after 4 days. Tissues are marked in white dashed line, scale bar – 1 cm (silica nanoparticles - red) (Leica Z16 dissection Macroscope).

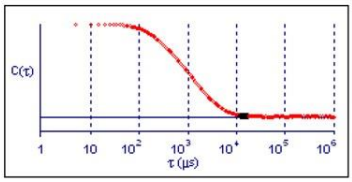
A



B

PLGA Batch 11 30 sec (Combined)

Effective Diameter:	**398.0 nm**
Polydispersity:	**0.355**
Baseline Index:	**4.3/100.00%**
Elapsed Time:	**00:07:30**



Correlation Function

C

Run	Mobility	Zeta Potential (mV)	Rel. Residual
1	-2.11	-26.99	0.0321
2	-1.79	-22.96	0.0214
3	-1.63	-20.92	0.0275
4	-1.57	-20.05	0.0347
5	-2.18	-27.91	0.0177
6	-1.59	-20.39	0.0235
7	-1.49	-19.11	0.0339
8	-1.51	-19.33	0.0420
9	-1.73	-22.18	0.0339
10	-1.64	-21.04	0.0409
Mean	-1.73	-22.09	0.0308
Std. Error	0.08	0.97	0.0026
Combined	-1.71	-21.89	0.0072

Figure S12. Characteristics of PLGA nanoparticles. (A) SEM images of PLGA nanoparticles Scale bar =10um). **(B)** Hydrodynamic size (nm) of PLGA nanoparticles and **(C)** Zeta potential of PLGA nanoparticles (mV).

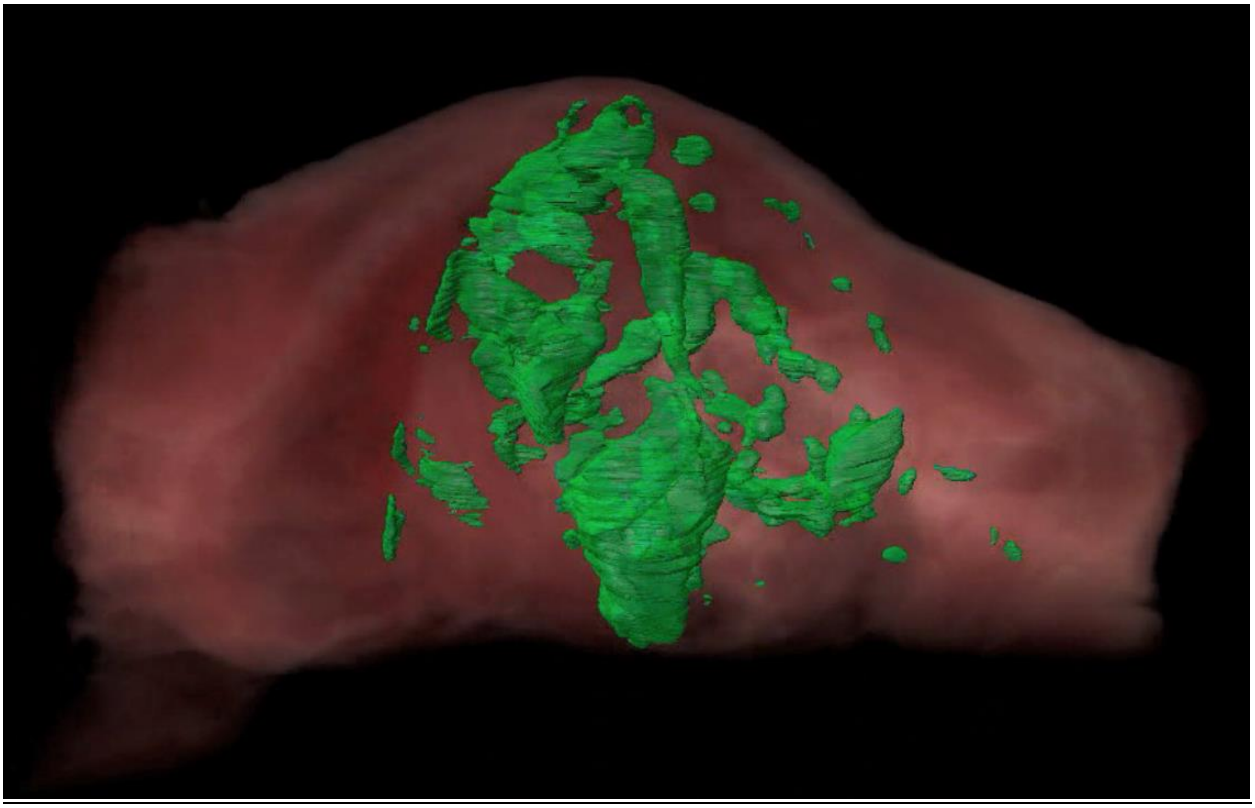


Figure S13. Imaging of tumor only mouse CryoVis reconstructed images of a whole mouse bearing EGFP-labelled ovarian tumors (green). The EGFP-expressing human ovarian cancer cells (OVCAR8) were injected IP. After 21 days, 1 mL PBS was injected IP and then 4 days later the mouse was euthanized and cryo-frozen for imaging.

Supplemental Information

Materials: ICP-MS grade nitric acid (70%), hydrochloric acid (37%), and hydrofluoric acid (50%) were purchased from BD Aristar. 50 mL and 15 mL metal-free plastic tubes were purchased from SCP Science. 100 μg mL^{-1} gold standard was purchased from Spex Certiprep.

Method

A stock solution of 2% HNO_3 1% HCl solution was made by adding 28 mL HNO_3 (70%) and 27 mL HCl (37%) to 945 mL milliQ H_2O and stored in a plastic bottle. A stock solution of concentrated acid (68% HNO_3 1% HCl) was made fresh by adding 0.27 mL HCl (37%) to every 10 mL HNO_3 (70%). To prepare the samples for ICP-MS analysis, the standard method was to add 500 µL of the acid blend, then 2 µL of HF (50%) directly into each tube containing sample. For the control, the 100% injected dose (1 mL) of Au@SiNPs solution was split into two 500 µL aliquots and similarly digested. For the tumors, liver, and intestines, additional acid was required to completely digest the organs; the additional acid was added at a fixed ratio of 500 µL acid blend plus 2µL HF (**Table S1**). The tubes containing samples in acid were incubated at 80 °C in an oil bath overnight to allow maximal digestion. After digestion, the samples were serially diluted 1000x with a 2% HNO_3 1% HCl solution by first diluting 10x in the same tube, then aliquoting 100 µL sample into a new 15 mL metal-free tube and diluting 100x to a final volume of 10 mL (**Table S1**). This dilution was chosen so that the final concentration of HF would be at the acceptable working limits for the ICP-MS instrument (0.0002%). For the intestines, undigestable material remained after overnight digestion which required a centrifugation step during serial dilution to remove. Briefly, 2 mL of sample was taken after the 10x dilution step and centrifuged at 21130 *g* for 5 minutes, then 100 µL of the supernatant was taken for the 100x dilution step. A standard curve ranging from 0.195 to 500 ppb (0.5 µg mL^{-1}) was made using a serial dilution of a 100 ppm (100 µg mL^{-1}) gold standard (Spex Certiprep) in a 2% HNO_3 1% HCl solution. Samples were analyzed on an Agilent 8800 ISIS (discrete sampling) in no gas mode to determine gold concentration. Rinse solution was 2% HNO_3 and carrier solution was 2% HNO_3 1% HCl. Each sample was measured by the instrument 5 times (technical replicates).

A blank solution and calibration standard was measured after approximately every 10 samples to ensure that there was no carry-over between samples, and to check for instrument consistency. The total amount of gold was calculated by multiplying the measured concentration (ppb) with the calculated total volume of sample after dilutions, then normalized to the 100% injected dose of Au@SiNPs. Measurements below the lower limit of the standard curve were considered to be zero.

			10x dilution		**100x dilution**		
Sample	**vol HNO_3 HCl (mL)**	**vol HF (uL)**	**vol added (mL)**	**final (mL)**	**vol taken (uL)**	**vol added (mL)**	**Final vol (mL)**
intestine	2	8	18	20	100	9.9	2000
liver	1	4	9	10	100	9.9	1000
tumor	1	4	9	10	100	9.9	1000
lung	0.5	2	4.5	5	100	9.9	500
kidney	0.5	2	4.5	5	100	9.9	500
spleen	0.5	2	4.5	5	100	9.9	500
stomach	0.5	2	4.5	5	100	9.9	500
injected NP dose (50%)	0.5	2	4.5	5	100	9.9	500 (1000 for 100% dose)

Table S1. Reagent calculations for the volumes of concentrated acid needed to digest each sample and the volume of 2% HNO3 1% HCl needed to dilute each sample. The calculated final volume after dilution was used to calculate the total amount (ng) of gold in each sample.