SUPPLEMENTARY INFORMATION

Ratiometric *in vivo* auditioning of targeted silver nanoparticles

Kadri Toome[a], Anne-Mari A. Willmore[a], Päärn Paiste[b], Allan Tobi[a], Kazuki N Sugahara [c,d], Kalle Kirsimäe[b], Erkki Ruoslahti[d,e], Gary B Braun*[d#], Tambet Teesalu[a,d,e]*

[a] Laboratory of Cancer Biology, Institute of Biomedicine and Translational Medicine, University of Tartu, Ravila 14B, Tartu, 50411, Estonia

[b] Department of Geology, University of Tartu, Ravila 14A, Tartu, 50411, Estonia

[c] Department of Surgery, Columbia University College of Physicians and Surgeons, New York, New York, USA.

[d] Cancer Research Center, Sanford Burnham Prebys Medical Discovery Institute, 10901 N. Torrey Pines Road, La Jolla, 92097 California, USA

[e] Center for Nanomedicine and Department of Cell, Molecular and Developmental Biology, University of California, Santa Barbara Santa Barbara, 93106 California, USA

[#] Present address: STEMCELL Technologies Inc., Vancouver, Canada.

AgNP mixes used for ratiometric homing studies.

IV injection mix 1:

1. Ag107 RPARPAR : 7.93E+11 particles, 53.16 mg
2. Ag109 Biotin: 6.66E+11 particles, 44.65 mg

IV injection mix 2:

1. Ag107 CAGALCY: 1.34E+12 particles, 89.84 mg
2. Ag109 Biotin: 1.13E+12 particles, 75.46 mg

SI Table 1. Experimental conditions of LA system.

Ablation Spot Diameter	40 µm
Scan rate	40 µm/s
Repetition frequency	20 Hz
Laser energy	0.62 J/cm^2
He flow rate	800 mL/min
Additional Ar flow rate	850 mL /min

SUPPLEMENTARY FIGURE LEGENDS

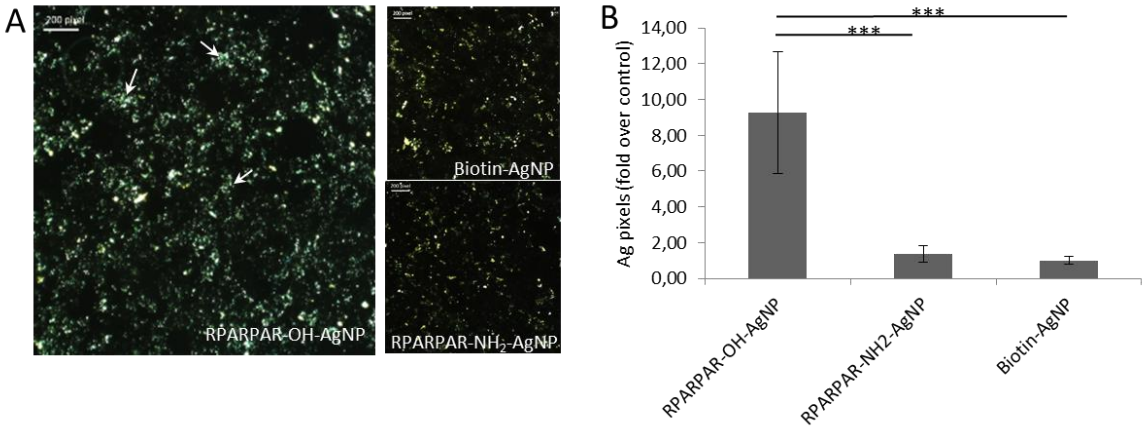

Figure S1 . Dark-field imaging of RPARPAR-AgNPs and control AgNPs in the lung tissue. Fluorescently labeled AgNPs in 200 µl of PBS were injected i.v. in balb/c mice. 5 h post i.v. administration, the animals were perfused with 15 ml PBS to remove free plasma AgNPs and the organs were snap-frozen. 10µm lung sections on Superfrost+ slides were subjected to silver enhancement procedure and dark-field imaging. (A) Microscopic imaging of the light refracted from silver grains in tissue sections by dark-field microscopy. Representative images from mice injected with RPARPAR-OH-AgNPs, or control nanoparticles loaded with RPARPAR-NH_2 or biotin, are shown. (B) Quantitation of the dark-field signal from 5 random fields per location. Data represent mean ± SD, *** $p < 0.001$.

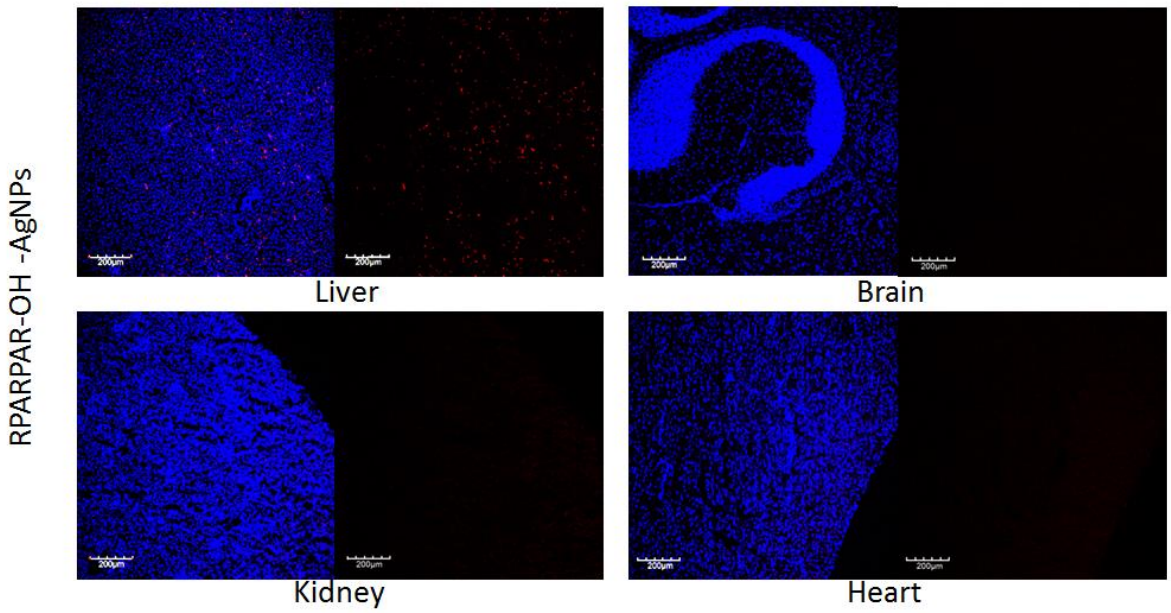

Figure S2. RPARPAR-OH-AgNP biodistribution in liver, brain, kidney, and heart.

Fluorescently labeled peptide-AgNPs in 200 µl of PBS were injected i.v. in balb/c mice. 5 h post i.v. administration, the animals were perfused with 15 ml PBS to remove free plasma AgNPs and the indicated organs were snap-frozen. 10µm tissue sections on Superfrost+ slides were subjected to confocal imaging. Representative matching images of CF555 alone (red) or CF555 + DAPI (blue) are shown. Scale bars = 200µm.

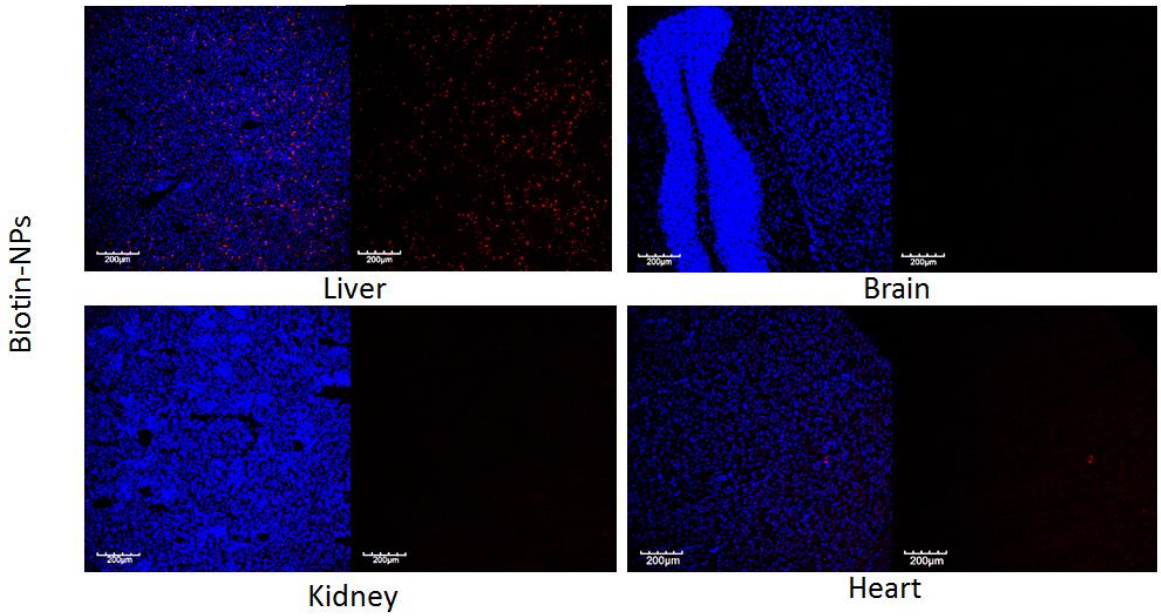

Figure S3. Biotin-AgNP biodistribution in in liver, brain, kidney, and heart.

Fluorescently labeled biotin-AgNPs in 200 µl of PBS were injected i.v. in balb/c mice. 5 h, the post i.v. administration the animals were perfused with 15 ml PBS to remove free plasma AgNPs and the indicated organs were snap-frozen. 10µm tissue sections on Superfrost+ slides were subjected to confocal imaging. Representative matching images of CF555 alone (red) or CF555 + DAPI (blue) are shown. Scale bars = 200µm.

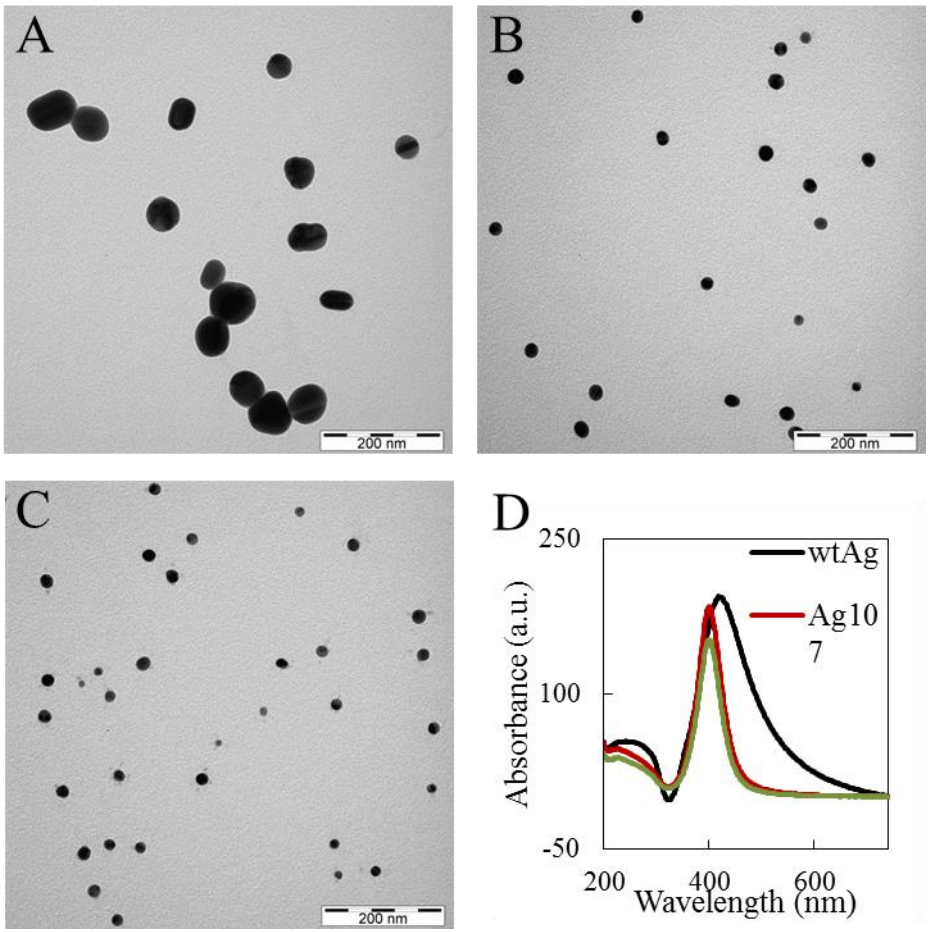

Figure S4 Characterization of AgNPs used in this study.

TEM images of A) wtAg; B) Ag107; and C) Ag109. Unfunctionalized AgNPs were diluted in DI water, dropped onto TEM grids, air-dried, and imaged at 80 kV. Scale bars: 200 nm; magnification 135,000. D) Spectral characterization of AgNPs in DI water by UV-Vis spectrometry. Peak absorbance is at 420 nm for wtAg, and at 400 nm for Ag107 and Ag109; the peak shift reflects a difference in size.

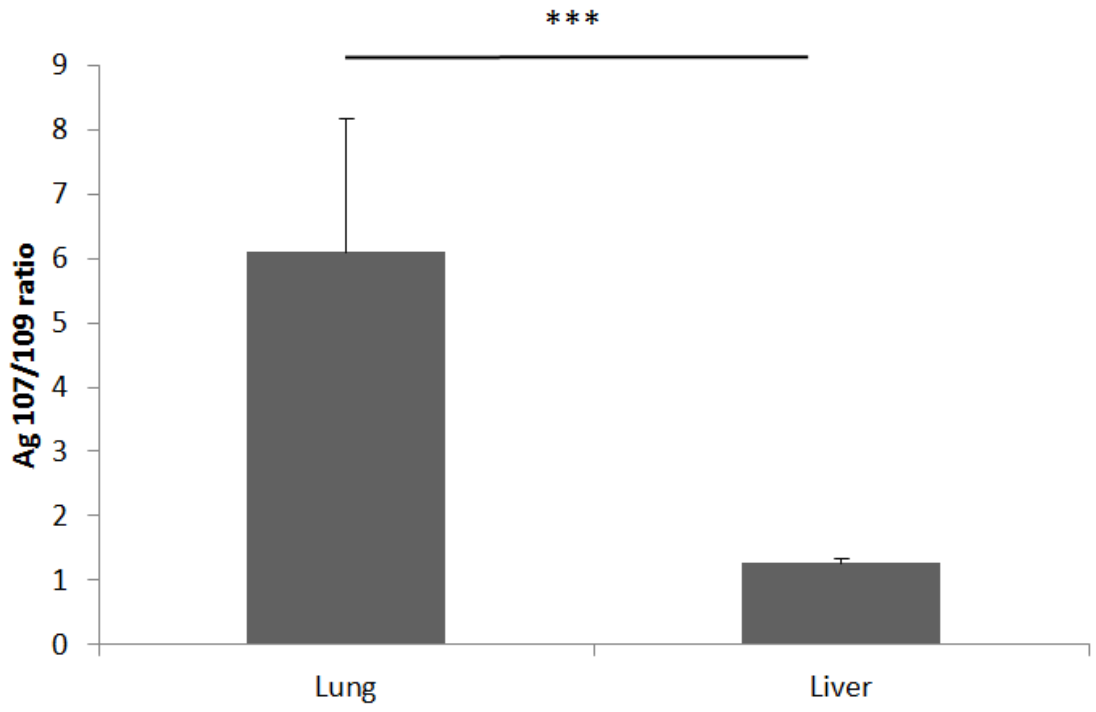

Figure S5. Averaged ratiometric LA-ICP-MS profiling on cryosections of lung and liver. Normal mice were injected i.v. with 200 µL of a mixture of RPARPAR-OH-AgNPs (prepared from 107Ag) and control biotin-AgNPs (prepared from 109Ag). At 5 h time point, the animals were perfused with 15 ml PBS to remove free plasma AgNPs and the indicated organs were snap-frozen, sectioned at 30 µm, and subjected to 40 µm line scans using a Cetac LSX-213 G2+ laser ablation system. Average $^{107}Ag/^{109}Ag$ ratio was estimated for lung and liver based on values of each data point for 16 ablation paths in lung and 13 laser ablation paths in liver. Data are expressed as a mean ± SD.

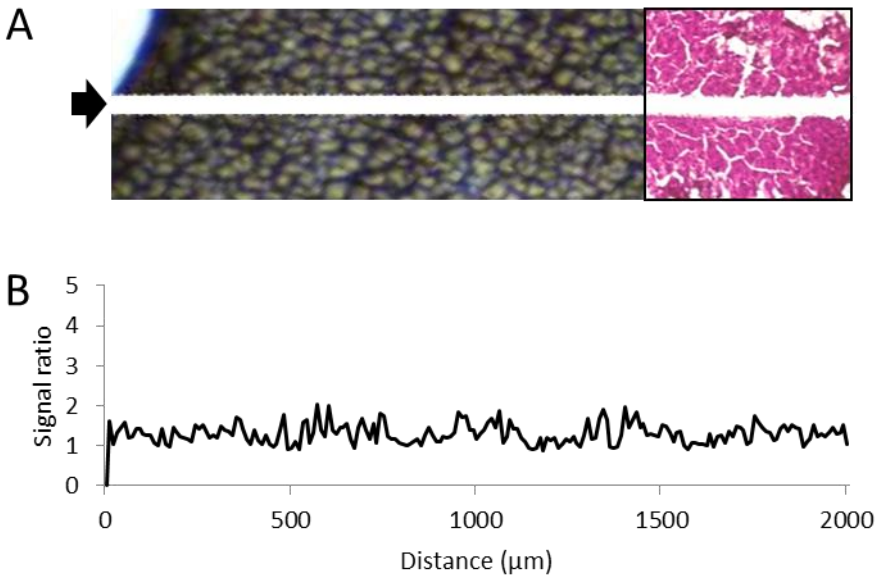

Figure S6. Ratiometric LA-ICP-MS profiling on section of liver.

Balb/c mice were injected i.v. with 200 µL of a mixture of RPARPAR-OH-AgNPs (prepared from 107Ag) and control biotin-AgNPs (prepared from 109Ag). At 5 h time point, tissues were snap-frozen, sectioned at 30 µm, and subjected to 40 µm line scans using a Cetac LSX-213 G2+ laser ablation system. (A) Phase contrast image of liver tissue used for LA-ICP-MS. The laser ablation path is indicated by arrow. Note the relative structural homogeneity of the liver sample. (B) $^{107}Ag/^{109}Ag$ and ^{13}C/Ag-total ratios along the laser ablation path (data are representative of 13 laser ablation paths). Scale bars: A, 200 µm; C, 100 µm.

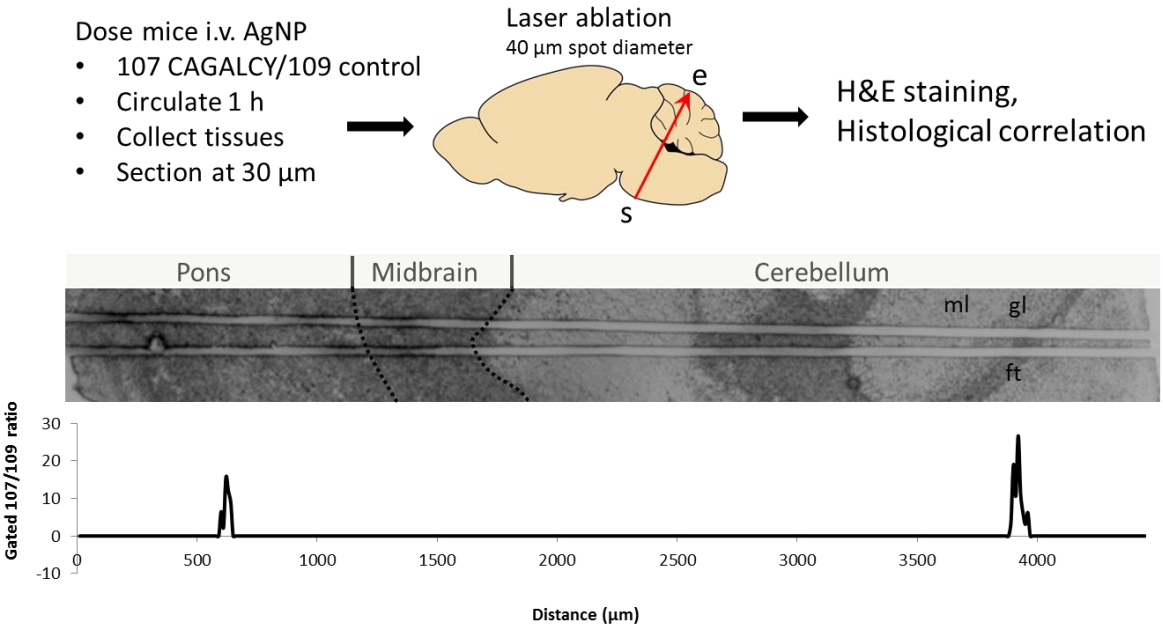

Figure S7. Ratiometric LA-ICP-MS profiling on section of brain.

Balb/c mice were injected i.v. with 200 µL of a mixture of CAGALCY-AgNPs (prepared from 107Ag) and control biotin-AgNPs (prepared from 109Ag). At 5 h time point, tissues were snap-frozen, sectioned at 30 µm, and subjected to 40 µm line scans using a Cetac LSX-213 G2+ laser ablation system.