Electronic Supplementary Material (ESI) for Nanoscale.

Nanodelivery Vehicles Induce Remote Biochemical Changes *in vivo*

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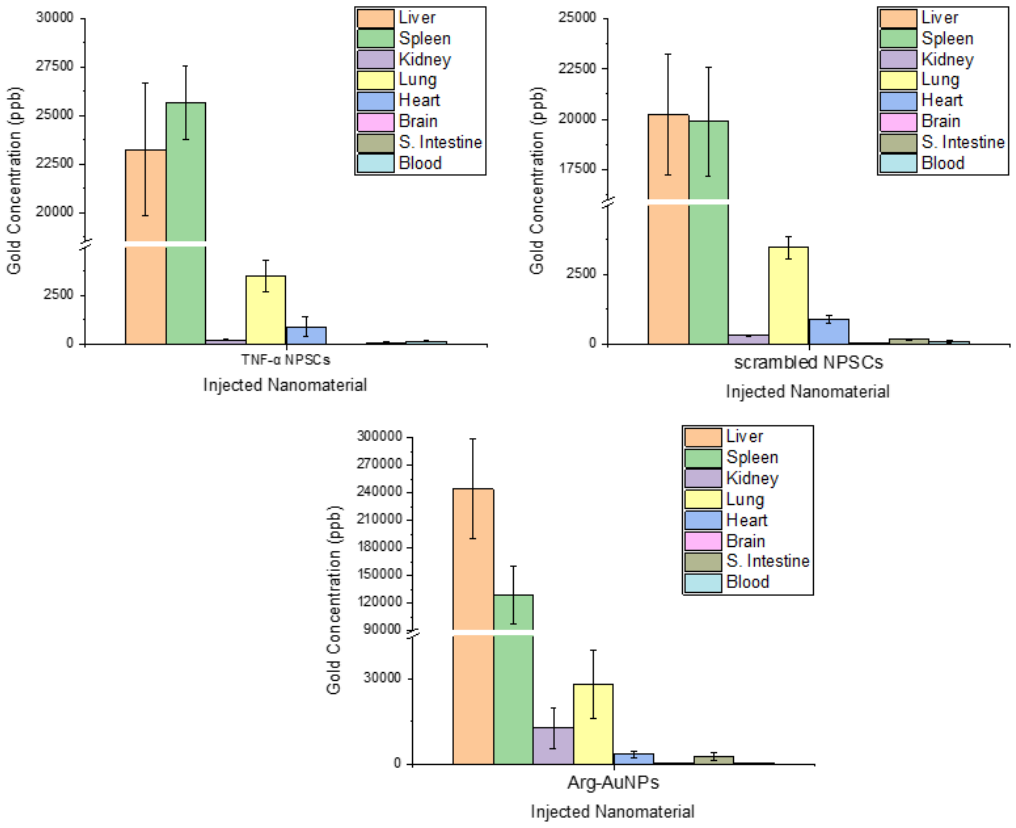

Figure S-1. ICP-MS analysis of gold in digested tissues from mice injected with anti-TNF-α NPSCs, scrambled NPSCs, and Arg-AuNPs. The ICP-MS data for the anti-TNF-α NPSCs and scrambled NPSCs was also reported previously.[1] All indicated concentrations are average values from three mice. Error is reported as standard deviation. Note that the injected concentration of gold for the Arg-AuNPs was 10-fold higher than the gold injected in the NPSC-injected mice.

Table S-1. MALDI-MSI detected lipid changes in splenic tissues of mice injected with anti-TNF-α NPSCs, scrambled siRNA NPSCs, or arginine AuNPs as compared to control mouse splenic tissues. Lipid responses in the TNF-α NPSC column are highlighted as follows: green for "predicted" responses, red for "unexpected" responses, and white for no observed response, based on TNF-α knockdown. Lipids are highlighted in scrambled NPSCs or arginine AuNP column if they share a response with the TNF-α column. (Abbreviations: LPC – lysophosphatidylcholine; PC – phosphatidylcholine; Cer – ceramide; SM – sphingomyelin; PE – phosphatidylethanolamine; CAR – carnitine; "p-" – plasmalogen)

		ROC AUC Values from Comparisons to Control Tissues		
Lipid I.D.	**Detected m/z**	**TNF-α NPSCs[a]**	**Scrambled NPSCs[a]**	**Arginine AuNPs**
CAR (16:0)	400.6	0.293	0.508	0.689
CAR (18:1)	426.4	0.318	0.420	0.533
LPC (16:0)	496.3 519.3	0.715	0.513	0.620
LPC (18:0)	524.4	0.683	0.515	0.497
LPC (18:2)	520.3	0.501	0.553	0.502
PC (30:0)	706.5	0.55	0.515	0.569
PC (p-32:0)	756.5	0.706	0.652	0.463
PC (34:0)	762.6	0.785	0.773	0.603
PC (p-34:0)	746.6	0.692	0.686	0.602
PC (34:1)	760.6 798.5	0.531	0.501	0.466
PC (p-34:1)	744.6	0.616	0.683	0.662
PC (34:2)	780.5	0.446	0.478	0.426
PC (p-36:5)	786.5 802.5	0.727	0.543	0.608
PC (p-36:4)	788.6 804.5	0.684	0.689	0.544

PC (p-36:2)	770.6 792.6 808.6	0.739	0.510	0.627
PC (36:0)[d]	790.6 812.6 828.6	0.717	0.715	0.509
PC (p-38:6)[d]	790.6	0.717	0.719	0.549
PC (p-38:5)	792.6 830.5	0.77	0.490	0.581
PC (p-38:4)	794.6 816.6 832.6	0.717	0.633	0.546
PC (p-40:5)	820.6 858.6	0.81	0.610	0.527
2H OH Cer (d18:1/20:0)	650.5	0.301	0.437	0.596
SM (d18:1/17:0)	739.6 755.6	0.737	0.557	0.584
SM (d18:1/20:0)	759.6	0.767	0.627	0.403
SM (d18:1/21:1)	771.6 809.7	0.722	0.681	0.591
SM (d18:1/23:2)	820.6	0.283	0.627	0.531
SM (d18:1/24:0)	853.8	0.74	0.342	0.469
PE (26:4)	608.4	0.745	0.669	0.592
PE (p-34:3)	698.5	0.664	0.531	0.697
PE (p-34:2)	700.5 722.5	0.476	0.583	0.441
PE (p-34:1)	732.5	0.661	0.523	0.398
PE (p-36:4)	724.5	0.661	0.469	0.508

PE (p-36:3)	726.5	0.693	0.626	0.566
PE (38:2)	794.6	0.653	0.302	0.514
LPC (p-18:0)	508.2	0.306	0.342	0.459
LPC (20:4)	544.3	0.345	0.488	0.350
PC (32:0)	734.6 772.7	0.724	0.547	0.656
PC (34:3)	756.7	0.718	0.682	0.563
2H Cer (d18:1/25:1)	681.7	0.331	0.527	0.547
2H Cer (d18:1/20:1)	632.7	0.343	0.565	0.498
PE (p-34:1)	583.5	0.324	0.468	0.501
PE (p-36:4)	583.5	0.324	0.468	0.396
PE (38:1)	671.5	0.546	0.583	0.527
PE (p-40:5)	671.5	0.546	0.583	0.496
Glucosylceramide	806.5	0.756	0.774	0.691
SM (d18:1/24:3)	809.6 847.7	0.706	0.788	0.670

[a] ROC AUC values for the anti-TNF-α NPSCs and scrambled siRNA NPSCs are similar to those reported previously.[1]

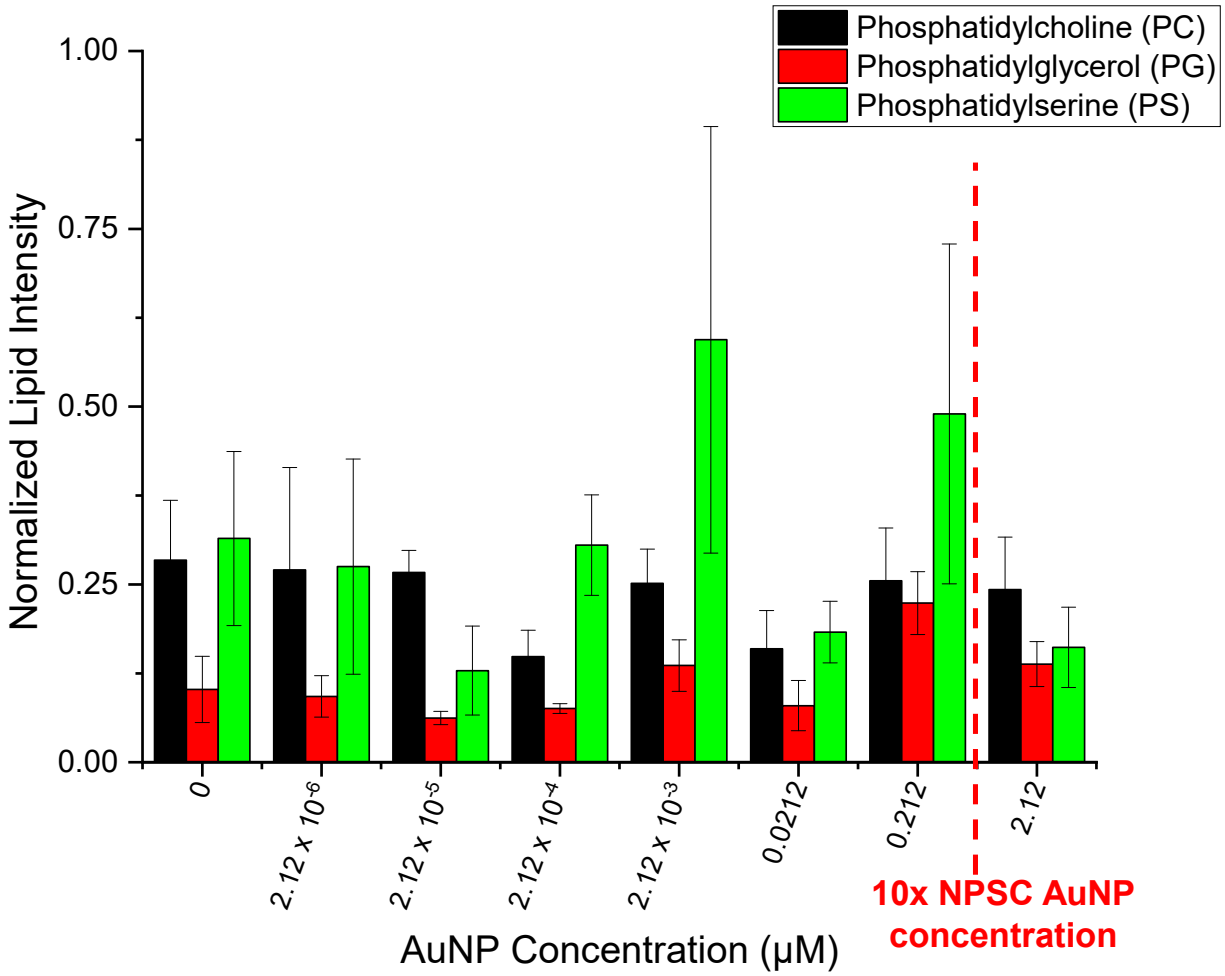

Figure S-2. MALDI-MS signals of lipids in the presence of the AuNPs used in this study. Representative lipids of different polarities (positive – PC; neutral – PG; negative – PS) were measured in combination with DHB as a matrix and increasing concentrations of AuNPs. Lipid ion intensities were normalized to the ion intensity of the $[M+H]^+$ peak of the DHB matrix in each spectrum. Each lipid ion intensity was calculated from the average of ten measurements acquired from three sample replicates. Error bars represent the standard deviation of 30 measurements. The AuNP concentrations that were used covered the range of AuNP concentrations that were detected in different tissues. The concentrations were calculated by dividing the Au concentrations (as measured by ICP-MS) by the average number of Au atoms in each NP (~200).[2] Even at AuNP concentrations 10-fold higher than those detected in NPSC tissues (represented by the red line at ~600 nM, see Figure S1) the AuNPs did not significantly affect lipid ionization, suggesting that all lipid level increases are unrelated to the presence of AuNPs. Significance was determined by pairwise t-tests of each of the signals, and every pair of signals for a given lipid was found to be different at a 99% confidence interval.

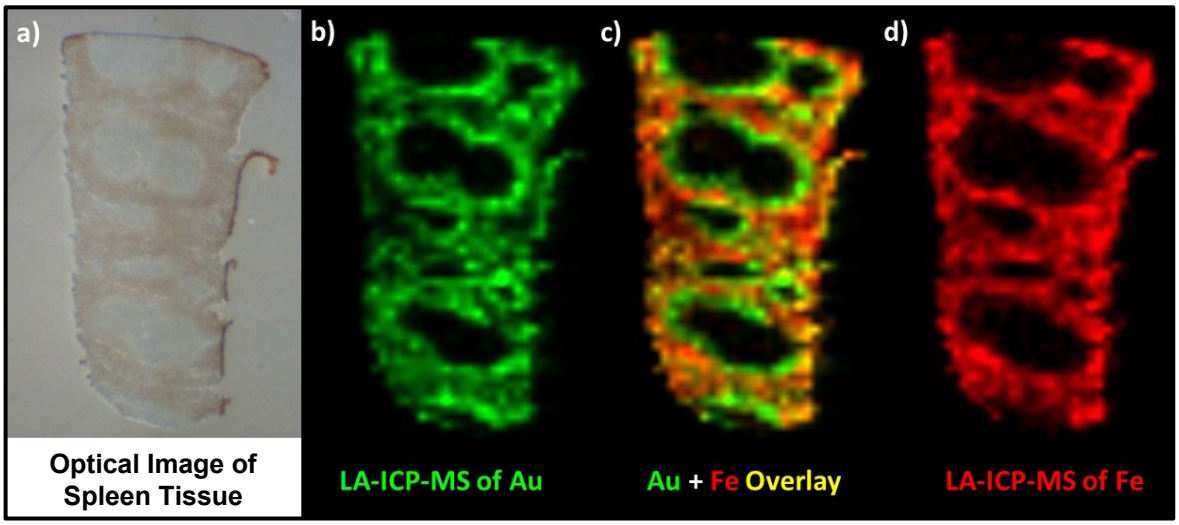

Figure S-3. Representative optical and LA-ICP-MS images of scrambled NPSC-injected mouse spleen. (a) Optical camera images. (b) Reconstructed LA-ICP-MS image of gold, single color scale at 50 μm resolution. (c) Gold (green) and iron (red) LA-ICP-MS overlay images. Yellow pixels indicate analyte overlap. (d) Reconstructed LA-ICP-MS image of iron, single color scale at 50 μm.

Table S-2. MALDI-MSI detected lipid changes in liver tissues of mice injected with anti-TNF-α NPSCs, scrambled siRNA NPSCs, or arginine AuNPs as compared to control mouse liver tissues. Lipid responses in the TNF-α NPSC column are highlighted as follows: green for "predicted" responses, red for "unexpected" responses, and white for no observed response, based on TNF-α knockdown. Lipids are highlighted in scrambled NPSCs or arginine AuNP column if they share a response with the TNF-α column. (Abbreviations: LPC – lysophosphatidylcholine; PC – phosphatidylcholine; Cer – ceramide; SM – sphingomyelin; PE – phosphatidylethanolamine; CAR – carnitine; "p-" – plasmalogen)

		ROC AUC Values from Comparisons to Control Tissues		
Lipid I.D.	**Detected m/z**	**TNF-α NPSCs**	**Scrambled NPSCs**	**Arginine AuNPs**
CAR (16:0)	400.6	0.293	0.378	0.420
LPC (16:0)	496.3 519.3	0.715	0.593	0.584
LPC (18:2)	520.3	0.501	0.433	0.474
PC (30:0)	706.5	0.550	0.366	0.370
PC (34:0)	762.6	0.779	0.723	0.644
PC (34:1)	760.6 798.5	0.531	N/A	N/A
PC (p-34:1)	744.6	0.616	0.561	N/A
PC (34:3)	756.6	0.446	0.438	0.432
PC (p-36:5)	786.5 802.5	0.727	0.447	0.425
PC (p-36:4)	788.6 804.5	0.684	0.583	0.606
PC (p-36:2)	770.6 792.6 808.6	0.739	0.643	0.519
PC (36:0)	790.6	0.717	0.566	0.502

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PC (p-38:6)[d]	790.6	0.717	0.566	0.502
PC (p-38:4)	794.6 816.6 832.6	0.717	0.456	0.377
PC (p-40:5)	820.6 858.6	0.81	0.390	0.378
2H OH Cer (d18:1/20:0)	650.5	0.301	0.453	0.431
SM (d18:1/21:1)	771.6 809.7	0.722	0.519	0.522
LPC (18:0)	524.4	0.334	0.465	0.445
LPC (p-18:0)	508.2	0.306	0.416	0.527
PC (32:0)	734.6 772.7	0.724	0.740	0.554
PC (p-32:0)	756.7	0.506	0.544	0.555
PC (p-34:0)	746.6	0.598	0.381	0.359
PC (34:2)	780.5	0.718	0.639	0.617
PC (p-38:5)	792.6 830.5	0.633	0.437	0.395
2H Cer (d18:1/20:1)	632.7	0.343	0.487	0.492
PE (p-34:1)	583.5	0.560	0.450	0.478
PE (38:1)	671.5	0.301	0.326	0.344
PE (p-40:5)	671.5	0.546	0.326	0.344
Glucosylceramide	806.5	0.728	0.762	0.796
SM (d18:1/17:0)	739.6 755.6	0.506	0.611	0.512

SM (d18:1/24:3)	809.6 847.7	0.706	0.795	0.784

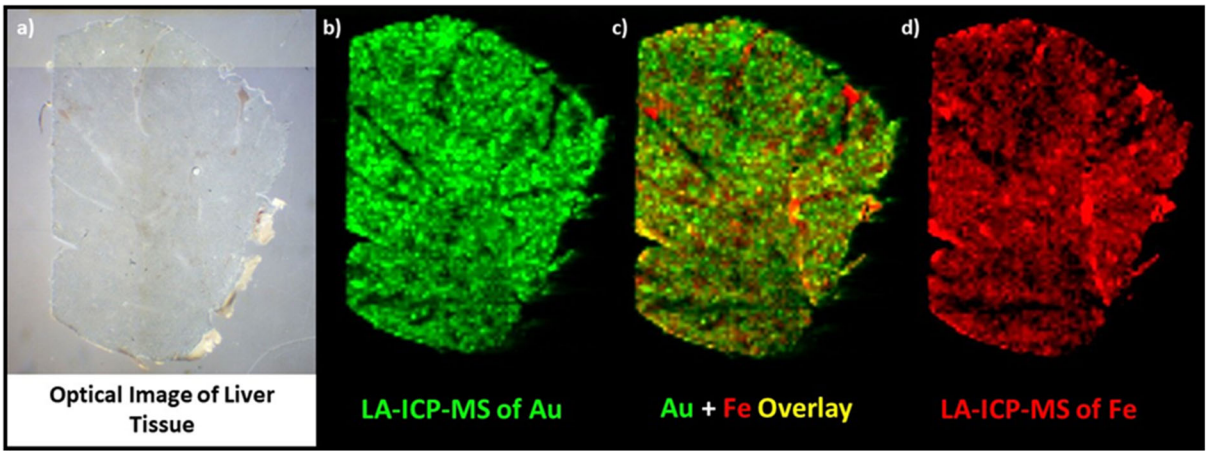

Figure S-4. Representative optical and LA-ICP-MS images of scrambled-NPSC-treated mouse liver. (a) Optical camera images from Teledyne CETAC LSX-213 G2 laser ablation system. (b) Reconstructed 50 µm LA-ICP-MS image of gold heat map. (c) Reconstructed 50 µm LA-ICP-MS image of gold, single color scale. (d) Gold (green) and iron (red) LA-ICP-MS overlay images. (e) Reconstructed 50 µm LA-ICP-MS image of iron, single color scale.

Table S-3. Physical characteristics of anti-TNF-α NPSCs, scrambled NPSCs, and Arg-AuNPs measured by dynamic light scattering (DLS).

Particle Type	Hydrodynamic Diameter (nm)	Polydispersity Index	Zeta Potential (mV)
Anti-TNF-α NPSCs	179 ± 3	0.24 ± 0.01	-3.3 ± 0.1
Scrambled NPSCs	181 ± 1	0.30 ± 0.02	-1.1 ± 0.1
Arg-AuNPs	11 ± 1	0.26 ± 0.01	+15.1 ± 0.2

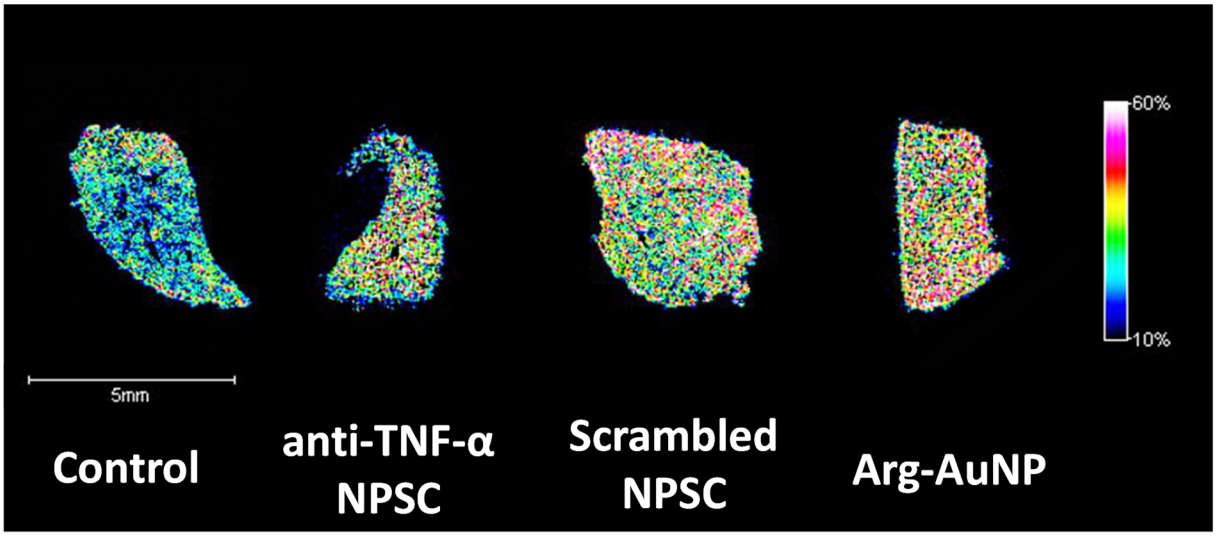

Figure S-5. Representative MALDI-MS images of glucosylceramide response in anti-TNF-α NPSC, scrambled NPSC, and Arg-AuNP-injected mouse liver tissues compared to control liver tissues. When compared to the control tissue, the anti-TNF-α-NPSC tissue exhibits an ROC AUC of 0.728, the scrambled NPSC tissue an ROC AUC of 0.762, and the Arg-AuNP tissue an ROC AUC of 0.798.

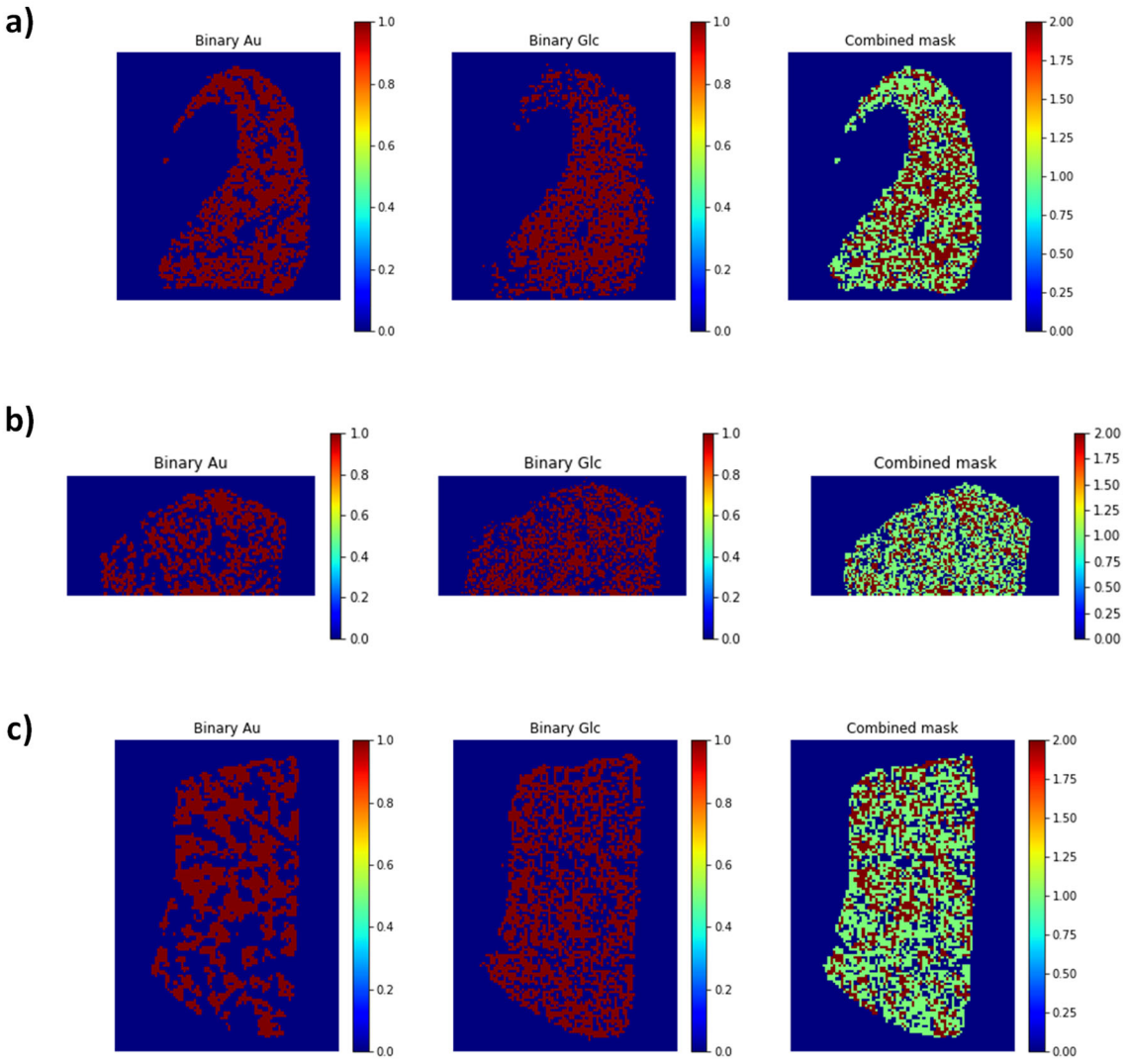

Figure S-6. LA-ICP-MS gold (Au) (left column) and MALDI-MS glucosylceramide (Glc) (middle column) overlaid in a combined binary mask (right column) in liver tissues of mice treated with: (a) TNF-α NPSCs, (b) scrambled NPSCs, and (c) arginine AuNPs. In the Au and Glc figures, a value of 0 (blue) indicates no signal and a value of 1 (red) indicates true analyte signal above the noise. In the combined mask, a value of 0 (blue) indicates no signal from either analyte, a value of 1 (red) indicates signal from either Au or Glc, and a value of 2 (green) indicates signal from both Au and Glc.

1. K.N. Sikora, J. M. Hardie, L. J. Castellanos-García, Y. Liu, B. M. Reinhardt, M. E. Farkas, V. M. Rotello and R. W. Vachet, Dual Mass Spectrometric Tissue Imaging of Nanocarrier Distributions and Their Biochemical Effects. *Anal. Chem.* 2020, **92**, 2011–2018.
2. S. G. Elci, B. Yan, S. T. Kim, K. Saha, Y. Jiang, G. A. Klemmer, D. F. Moyano, G. Y. Tonga, V. M. Rotello and R. W. Vachet, Quantitative Imaging of 2 nm Monolayer-Protected Gold Nanoparticle Distributions in Tissues Using Laser Ablation Inductively-Coupled Plasma Mass Spectrometry (LA-ICP-MS). *Analyst* 2016, **141**, 2418–2425.