

Supporting Information

Diagnosis of Immunomarkers *in vivo* via Multiplexed Surface Enhanced Raman Spectroscopy with Gold Nanostars

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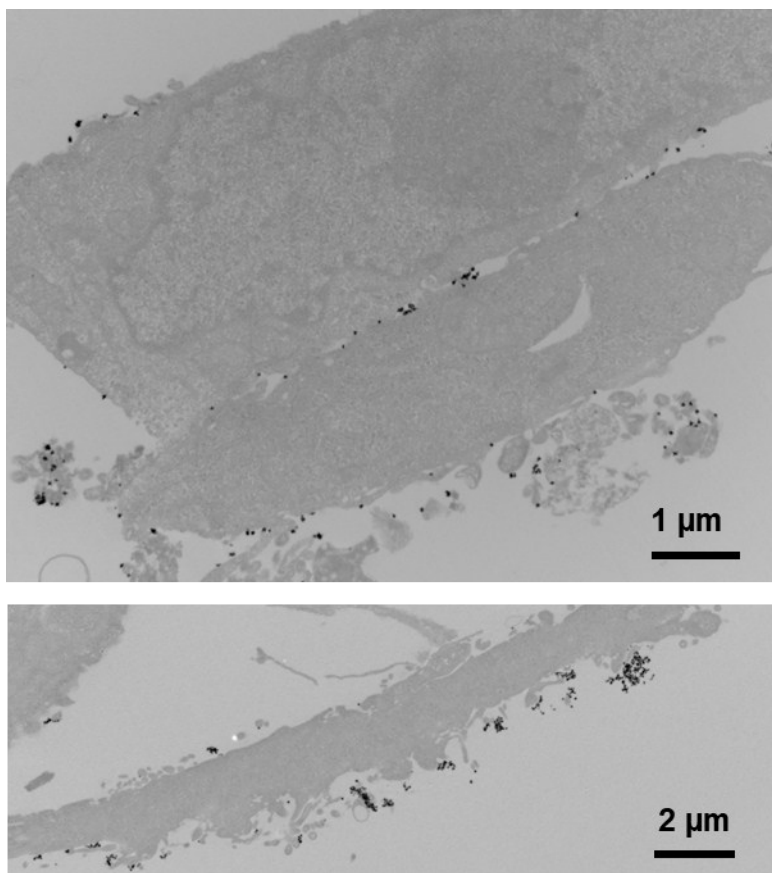


Figure S1. TEM images of MDA-MB-231 incubated with functionalized AuNS for 16 h. AuNS were bound to the surface of the cells via receptor-antibody interactions.

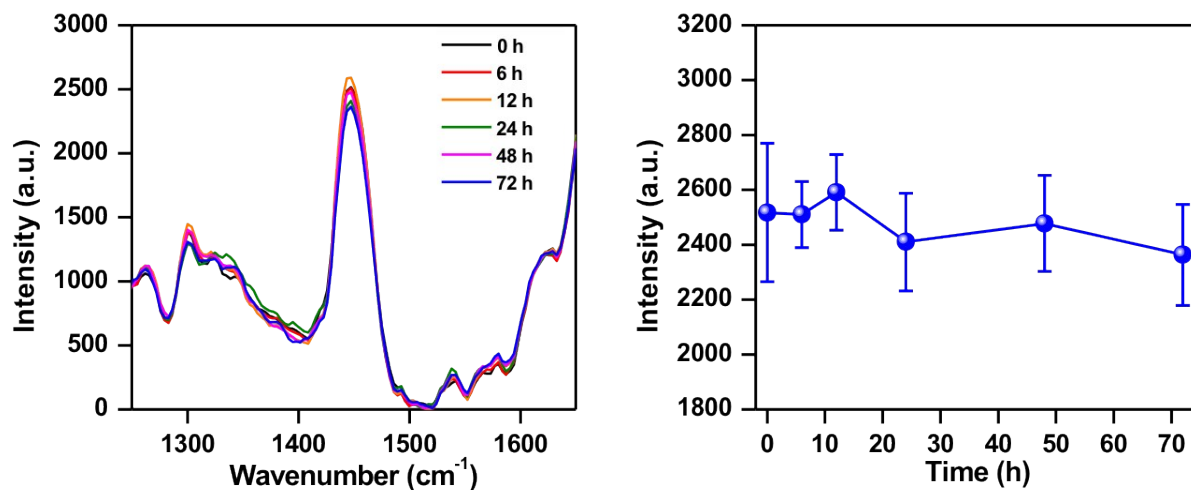


Figure S2. Raman spectra of tumor xenograft without functionalized AuNS injection showing the intensity of the lipid peak (1440 cm^{-1}) remains consistent over time.

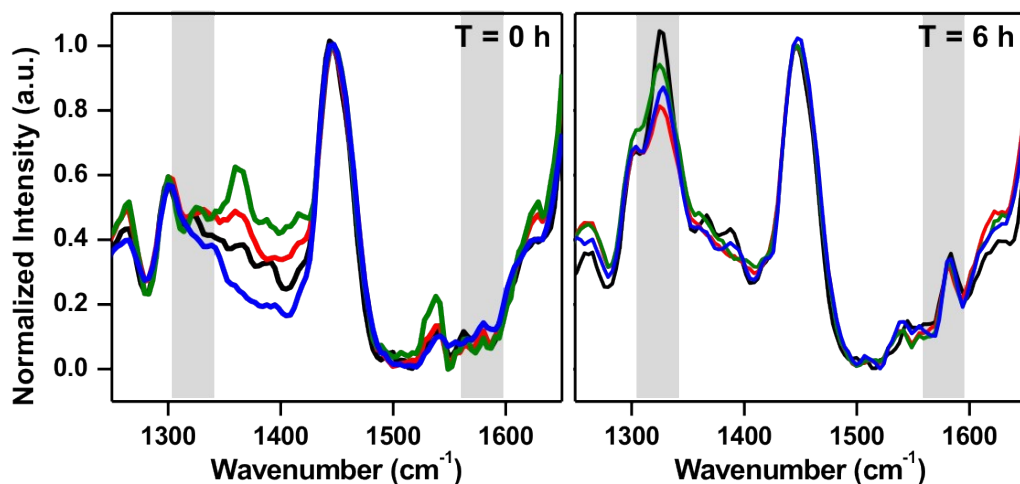


Figure S3. Mammary tumor xenografts were imaged before functionalized AuNS injection (0 h). Each color indicates averaged Raman spectrum of a single mouse ($n=4$). Both DTNB (at 1325 cm^{-1}) and pMBA's (at 1580 cm^{-1}) signature peaks, indicated by the grey box, were not observed at 0 h. Mice were then injected with 1.2 mg of a 2:1 mixture of anti-EGFR-pMBA-AuNS and antiPD-L1-DTNB-AuNS. The tumors were imaged at 6 h post particle injection.

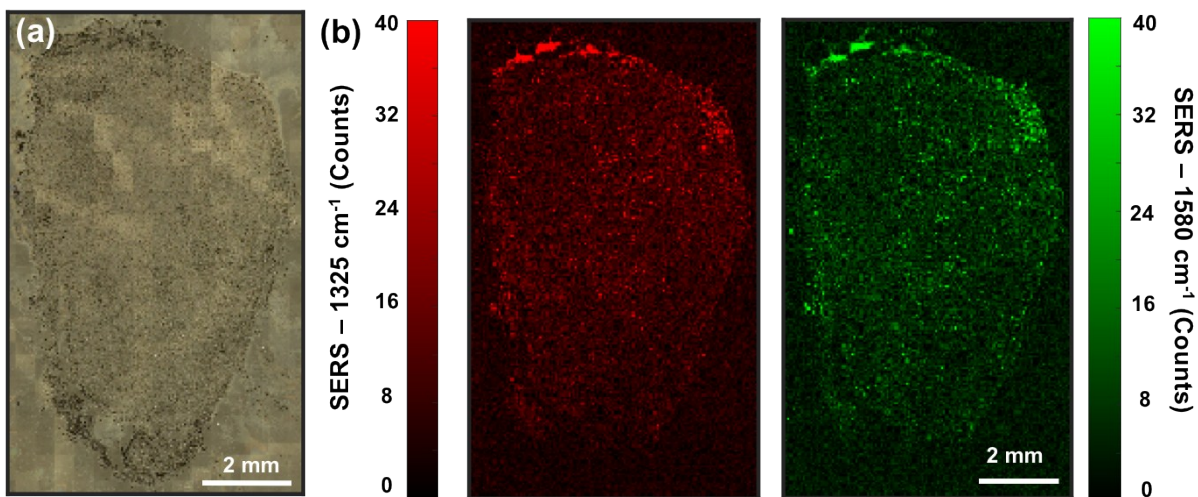


Figure S4. *Ex vivo* Raman mapping of MDA-MB-231 tumor xenograft. Tumor was collected 6 h post functionalized AuNS injection. Raman mapping was done at $50\text{ }\mu\text{m} \times 50\text{ }\mu\text{m}$ per pixel. (a) Brightfield imaging of the tissue. (b) The intensity map of anti-PDL1-DTNB-AuNS at 1325 cm^{-1} (left) is assigned with red while the intensity map of anti-EGFR-pMBA-AuNS at 1580 cm^{-1} (right) is assigned with green.

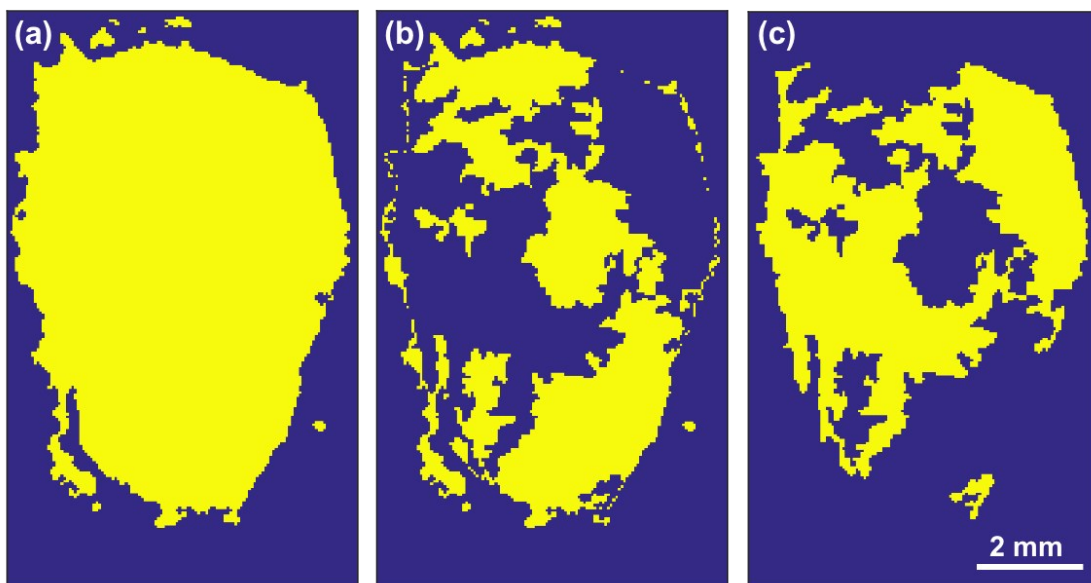


Figure S5. Tissue masks (a and b) and sub-masks (c) of the lipid peak at 1440 cm^{-1} were used to quantify *ex vivo* mapping.

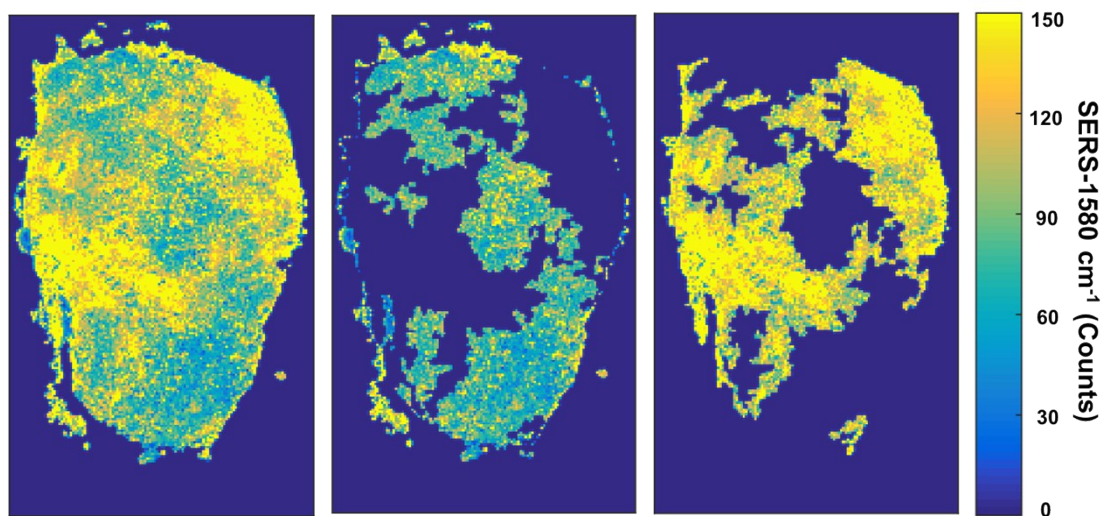


Figure S6. Quantitative analysis of *Ex vivo* Raman mapping at 1580 cm^{-1} (pMBA).

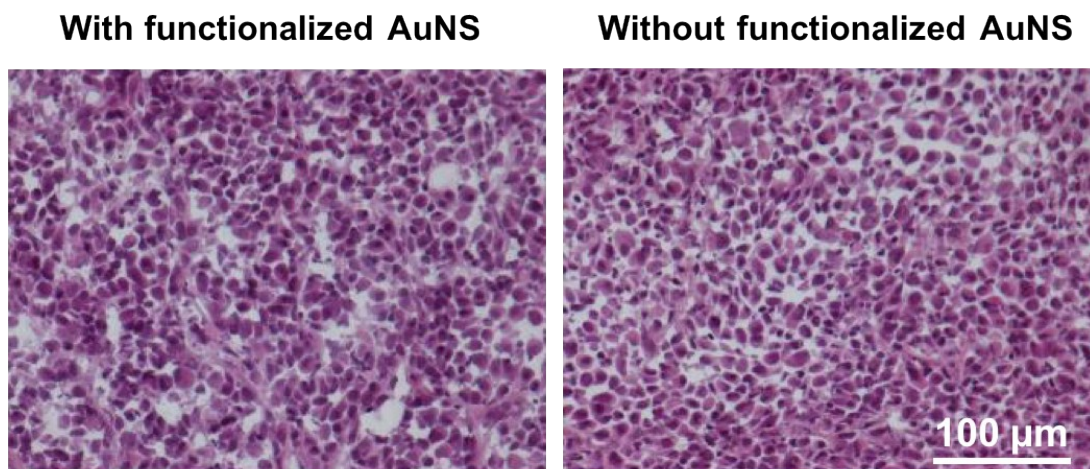


Figure S7. Haemotoxylin and Eosin (H&E) staining of MDA-MB-231 tumor xenograft with (left) and without (right) functionalized AuNS administration. The results show the morphology of tumor does not change with functionalized AuNS indicating the particles themselves are not detrimental to biological tissues.

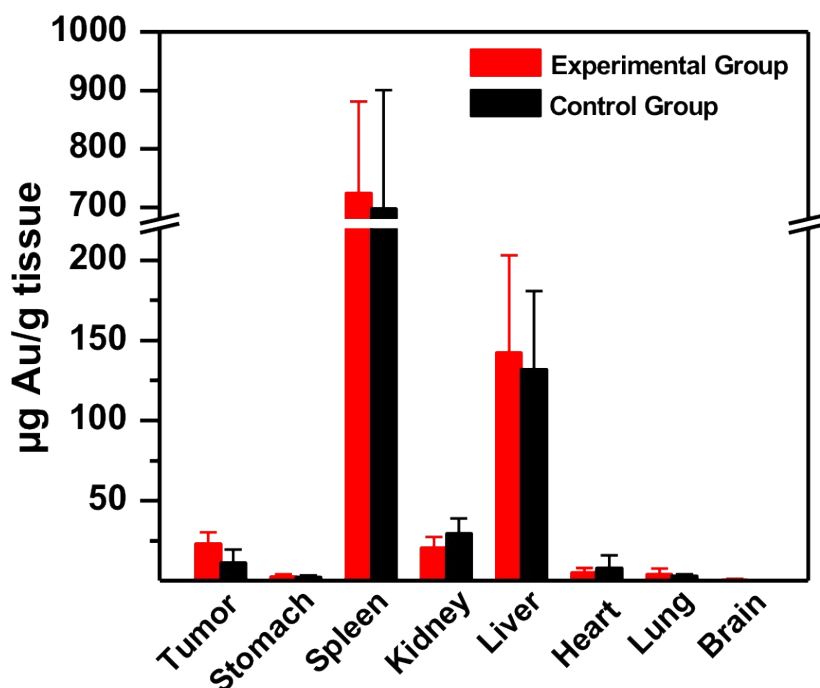


Figure S8. Biodistribution of AuNS functionalized with Raman tags, antibodies, and PEG at the end of the study, 72 h time point in experimental group mice (n = 4). The control group (n=3) was pre-blocked with IP delivery of anti-EGFR + anti-PD-L1 antibodies prior to functionalized AuNS injection.