## Supporting Information

Imaging of Liver Tumors Using
Surface-Enhanced Raman Scattering Nanoparticles

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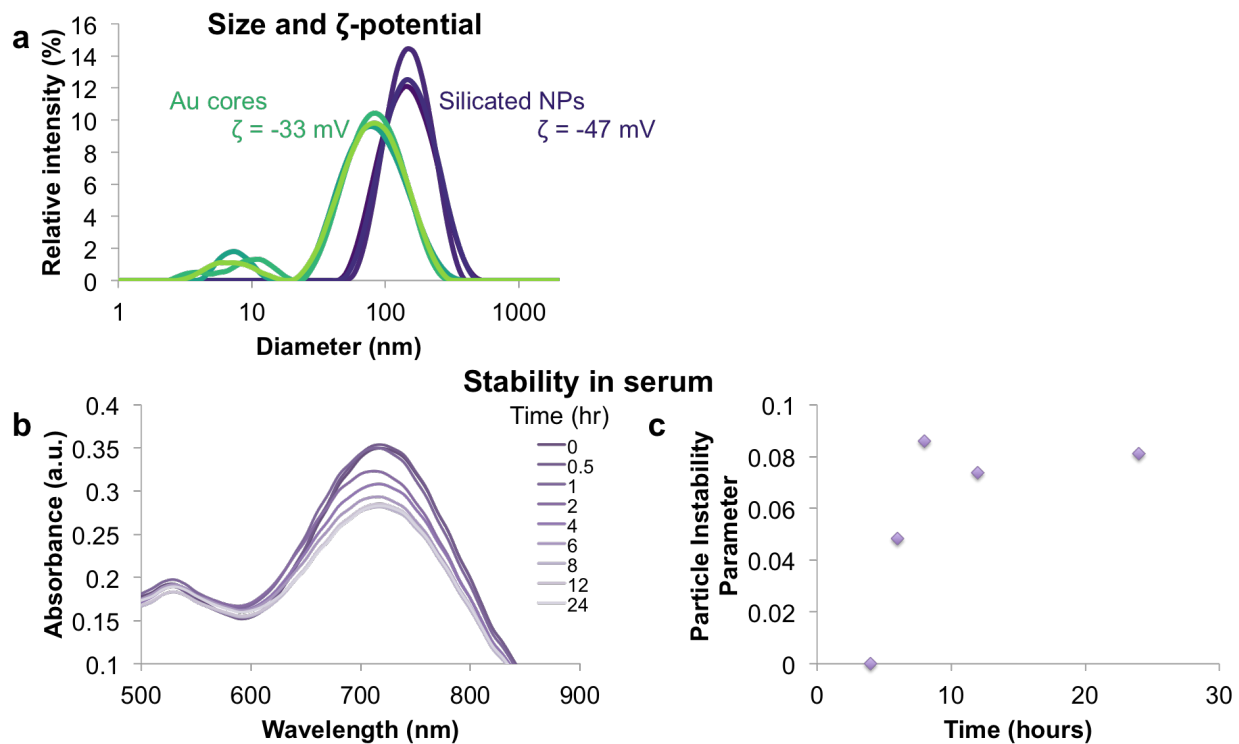
Figure S1. Nanoparticle characterization. (a) Size distribution and zeta-potential as measured by DLS. (b) Stability in serum was established by monitoring absorbance over 24 hours. (c) Absorbance initially decreased, but stabilized after 4 hours as determined by the particle instability parameter.[26]
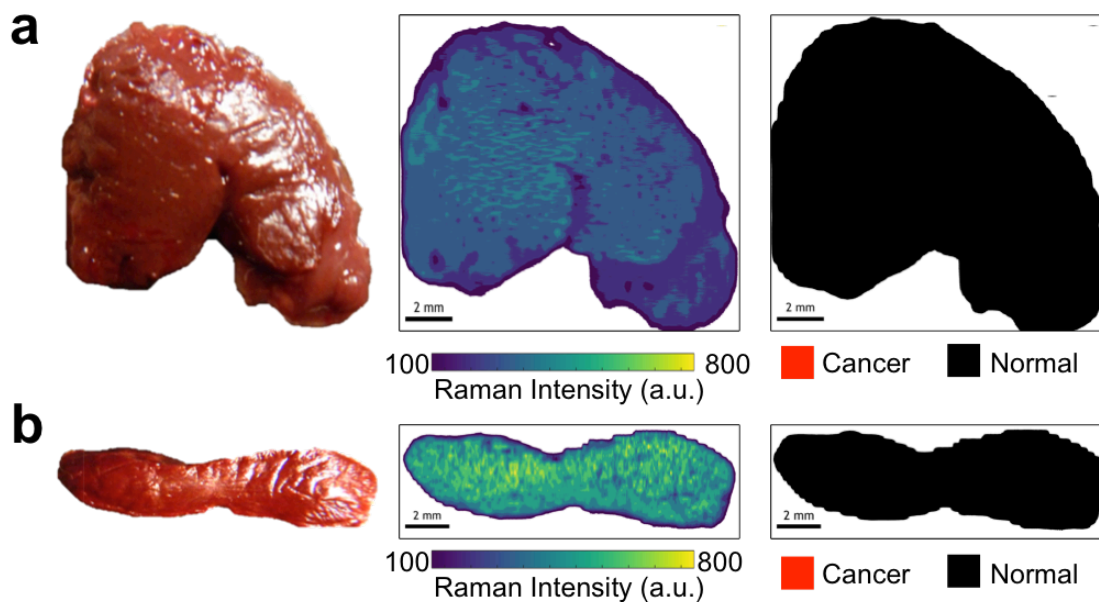
Figure S2. SERS nanoparticle accumulation in liver and spleen of healthy mice.
(a) Photograph of excised liver (left), map of the intensity of the 1215 cm^{-1} Raman band (middle), and Raman map derived from DCLS analysis shows the presence of the SERS NP spectrum throughout the liver (right). In this healthy control no signal for cancer is detected. (b) Excised spleen of the same animal, presented as in (a).
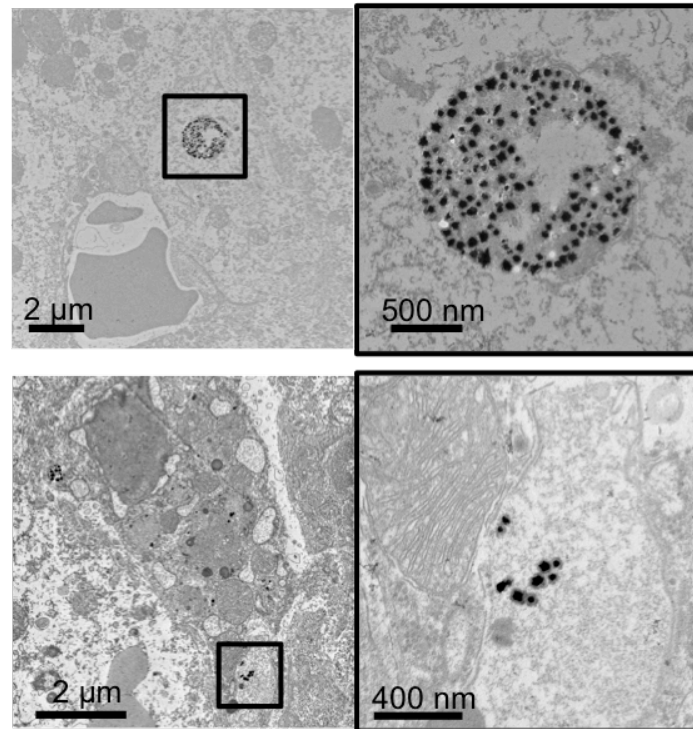
Figure S3. TEM of SERS nanoparticles in liver. TEM micrographs of tissue slices from normal liver from a Myc-driven HCC mouse model. Nanoparticles were usually detected in circular clusters (top), or small groups (bottom) throughout the normal liver tissue.

Procedure for creation of Raman maps to discriminate cancerous from healthy liver tissue

Each spectral image carries a wealth of information. First, it signifies whether the Raman signature specific to the SERS NPs is present. Second, any innate Raman spectra emanating from tissues will also be collected, although these are typically of too low an intensity to be meaningful when using rapid scanning parameters. Third, the Raman spectrometer also detects fluorescence present in the interrogated wavelengths, including tissue autofluorescence. An image showing the mean intensity of the raw spectrum at each pixel, which includes both the SERS signal as well as fluorescence, is shown in Supporting **Figure S4b**. We can apply an algorithm based on DCLS to identify the points that show the Raman signature of our SERS NPs, which indicate healthy liver tissue, and—as per our convention—denote them in black. By readjusting the image contrast the different tissue morphologies, based on autofluorescence, can now be visualized (Supporting **Figure S4c**). This allows us to track the extent of the healthy liver parenchyma, and outline the surrounding tissues, including the tumors. Once this information is obtained, the tumors are easily identified and denoted in red to create the final image shown in Supporting **Figure S4d**.
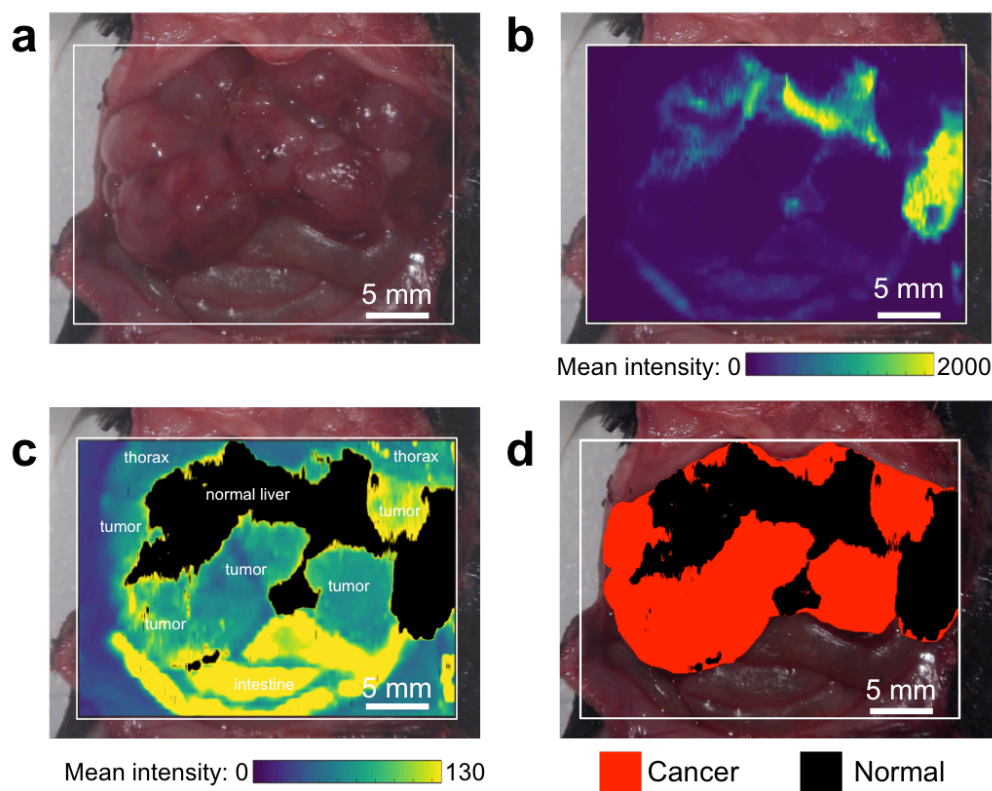
Figure S4. In vivo Raman scan of a Myc-driven HCC mouse model. (a) The area of interest, featuring multiple tumors, is imaged. (b) A spectrum is collected at each interrogated point. The acquired spectra include both the Raman signal, as well as any fluorescence. (c) By using DCLS to identify the Raman signal of our SERS NPs, and readjusting the contrast of the image to visualize authofluorescence, we can delineate and help identify tissues (including tumors). (d) The resulting qualitative map of the liver with normal liver tissue shown in black, and tumors in red.
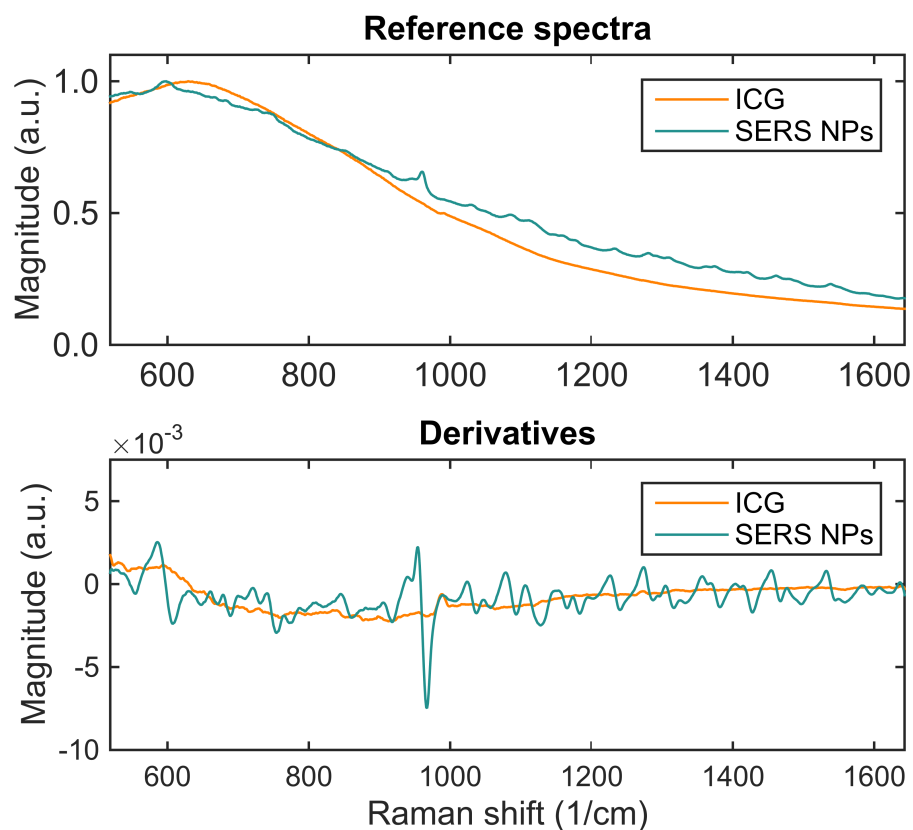
Figure S5. Reference spectra used for DCLS model. Top: the spectra for ICG and IR780-SERS NPs normalized to unit maximum intensity. They both exhibit a large fluorescence peak, but only the SERS NPs have small sharp Raman peaks. Bottom: the derivatives of the spectra shown on top. The Raman peaks are amplified and thus the spectra become distinguishable.
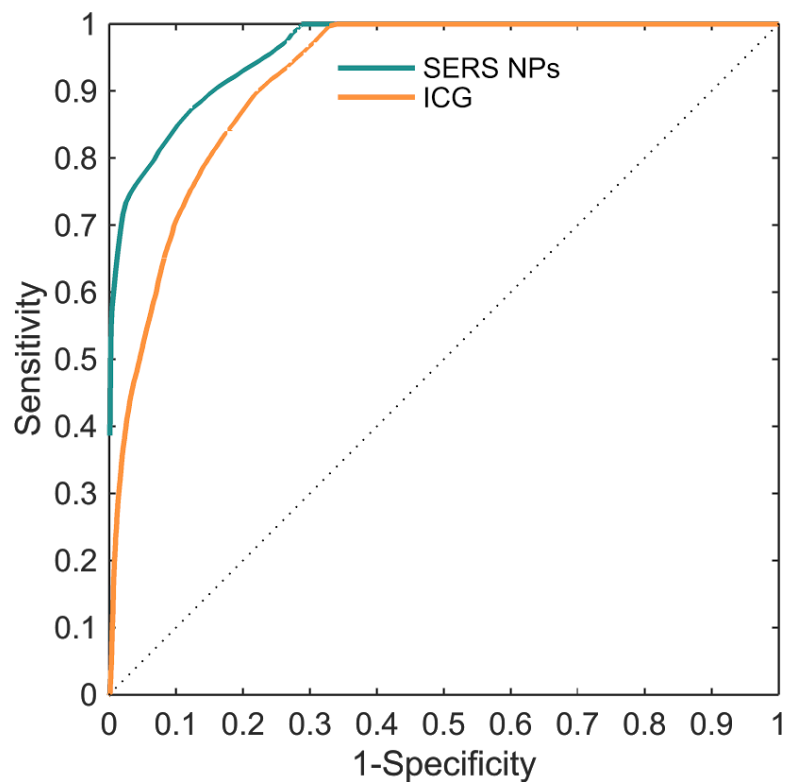
Figure S6. Receiver operating characteristic curves for SERS NPs and ICG. The ROCs were generated under the strict assumption that true positives are points identified as tumor by both imaging agents.