Supporting Information

Actively Targeted *in vivo* Multiplex Detection of Intrinsic Cancer Biomarkers Using Biocompatible SERS Nanotags

Dinish U. S[§, #], Ghayathri Balasundaram[§, #], Young-Tae Chang[§, β] and Malini Olivo[§, γ, *]

[§]Singapore Bioimaging Consortium, Agency for Science Technology and Research (A*STAR), 11 Biopolis Way, Singapore 138667

[β]Department of Chemistry & MedChem Program of Life Sciences Institute, National University of Singapore, 117543 Singapore

[γ]School of Physics, National University of Ireland Galway, Galway, Ireland

* Corresponding Author: Malini_olivo@sbic.a-star.edu.sg

[#] These authors contributed equally and joint first authors

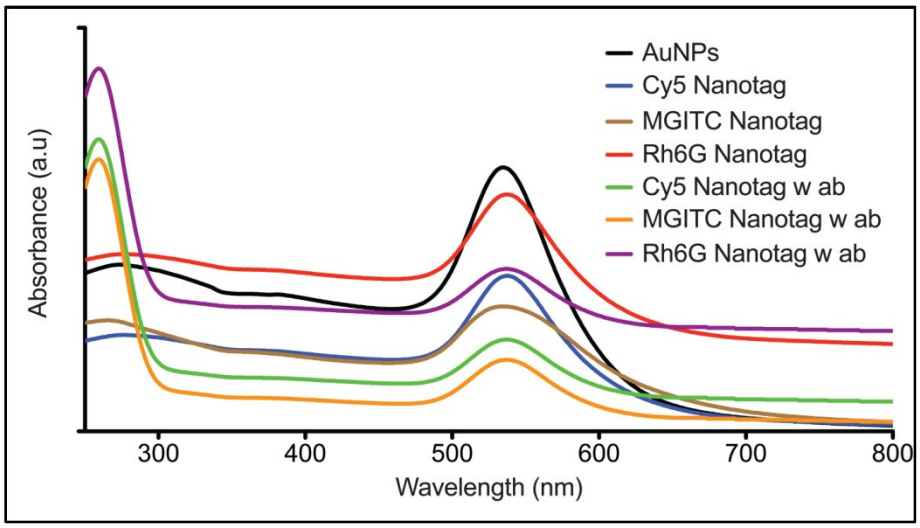

Fig. S1 Absorption spectrum of Pure AuNPs antibody conjugated and non conjugated three SERS nanotags constructed with RMs- Cy5, MGITC and Rh6G. 'w ab' refers to nanotags with antibody conjugation. Nanotags and pure AuNPs show the plasmon resonance peak at 536 nm. For the antibody conjugated nanotags, peak around 280 nm is contributed with the protein absorbance.

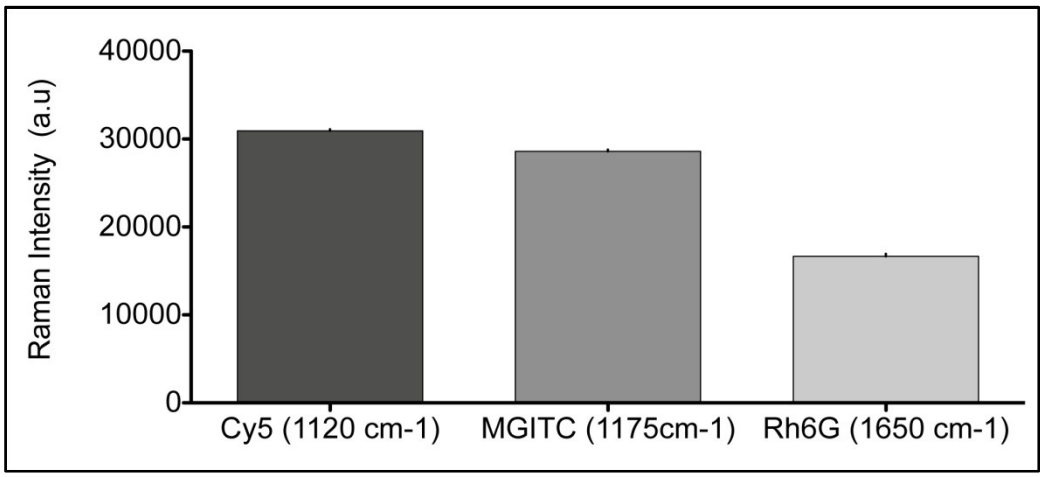

Fig. S2 Comparison of SERS intensities of the three nanoatgs-Cy5, MGITC and Rh6G at their characteristic multiplexing peak. Measurement was repeated three times and average intensity is plotted.

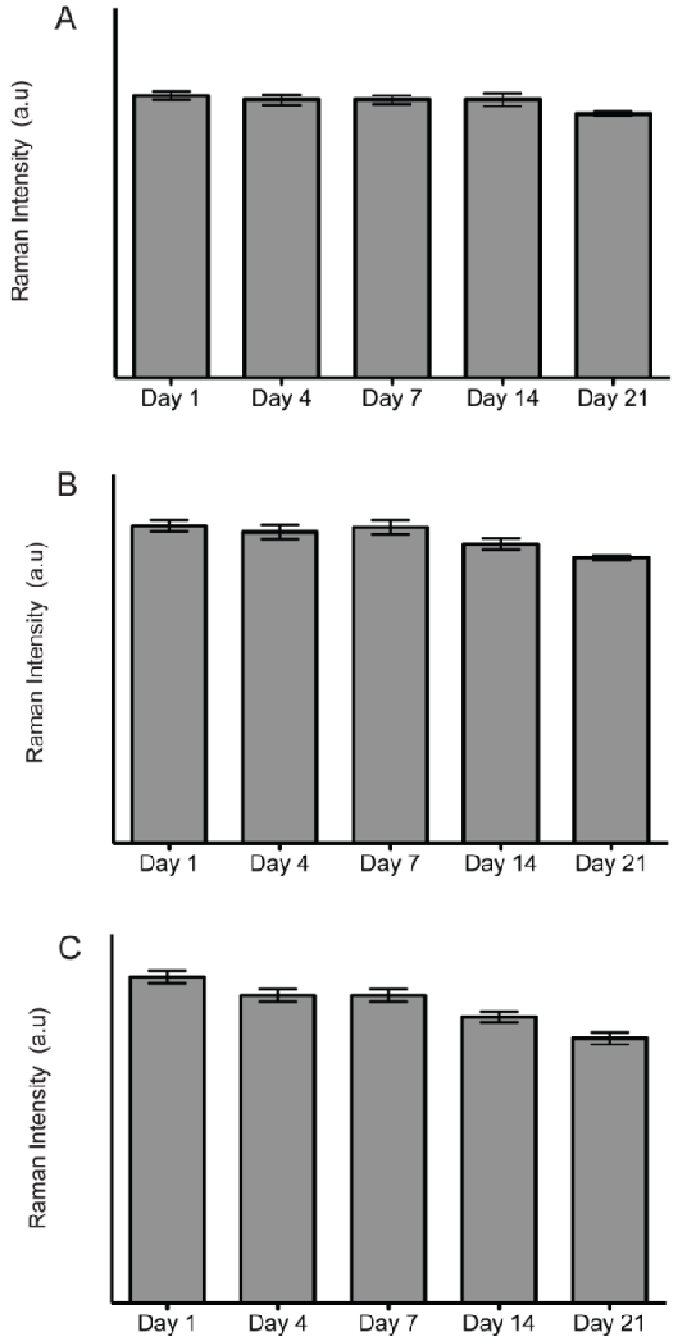

Fig. S3 Stability study of SERS intensity from bioconjugated nanotags for their characteristic multiplexing peaks. A: Cy5 (1120cm^{-1}), B: MGITC (1175 cm^{-1}) and C: Rh6G (1650 cm^{-1}).

SERS intensity study of SERS nanotags (monitored for its characteristic multiplexing peak) over a period of three weeks showed that all the nanotags exhibited excellent stability with minimal intensity reduction. Intensity from Cy5 nanotag is more or less same over a period of

three weeks. Since Cy5 can form covalent bonding with AuNPs, its intensity did not vary much during the course of the study. Intensity from MGITC was pretty stable up to 3 weeks with intensity reduction of only ~8% by 3rd week while in the case of Rh6G, a reduction in SERS intensity of only ~13% is observed at the end of 3 weeks.

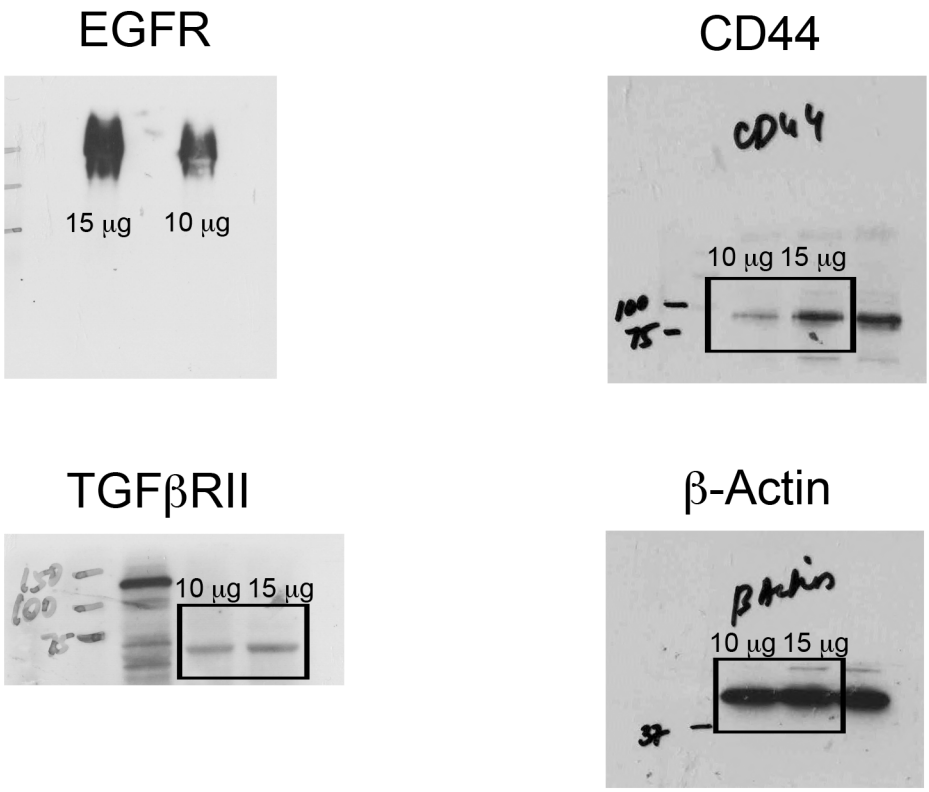

Fig. S4 Confirmation of the expression of intrinsic biomarkers-EGFR, CD44 and TGFβRII in total protein extracted from cultured MDA-MB-231 by western blot – Full blot image.

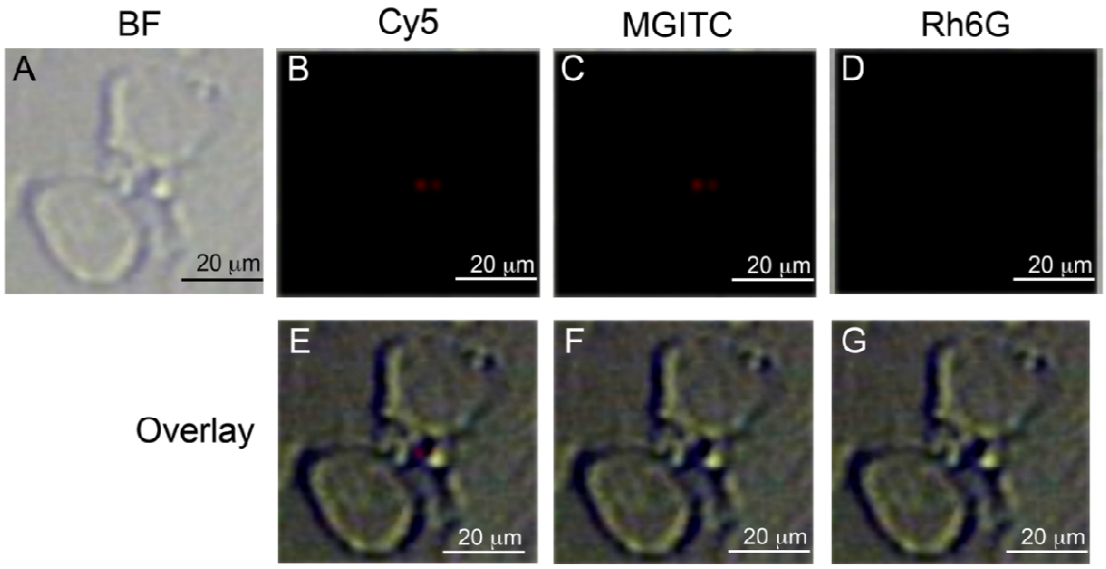

Fig. S5 SERS intensity mapping of nanotags on Jurkat cells (negative control that do not express the biomarkers, EGFR, CD44 and TGFβRII) using antibody conjugated three nanotags. A: bright field image of the cell. (B-D) Intensity map for Cy 5, MGITC and Rh6G nanotags respectively, while (E-G) shows the corresponding overlaid images (bright field and SERS map).

In this study, antibody conjugated nanotags do not bind to the Jurkat cell because it do not express the biomarkers- EGFR, CD44 and TGFβRII on the cell surface. Due to this, resultant Raman spectral measurement did not yield any peaks that led to the blank (dark) SERS image.

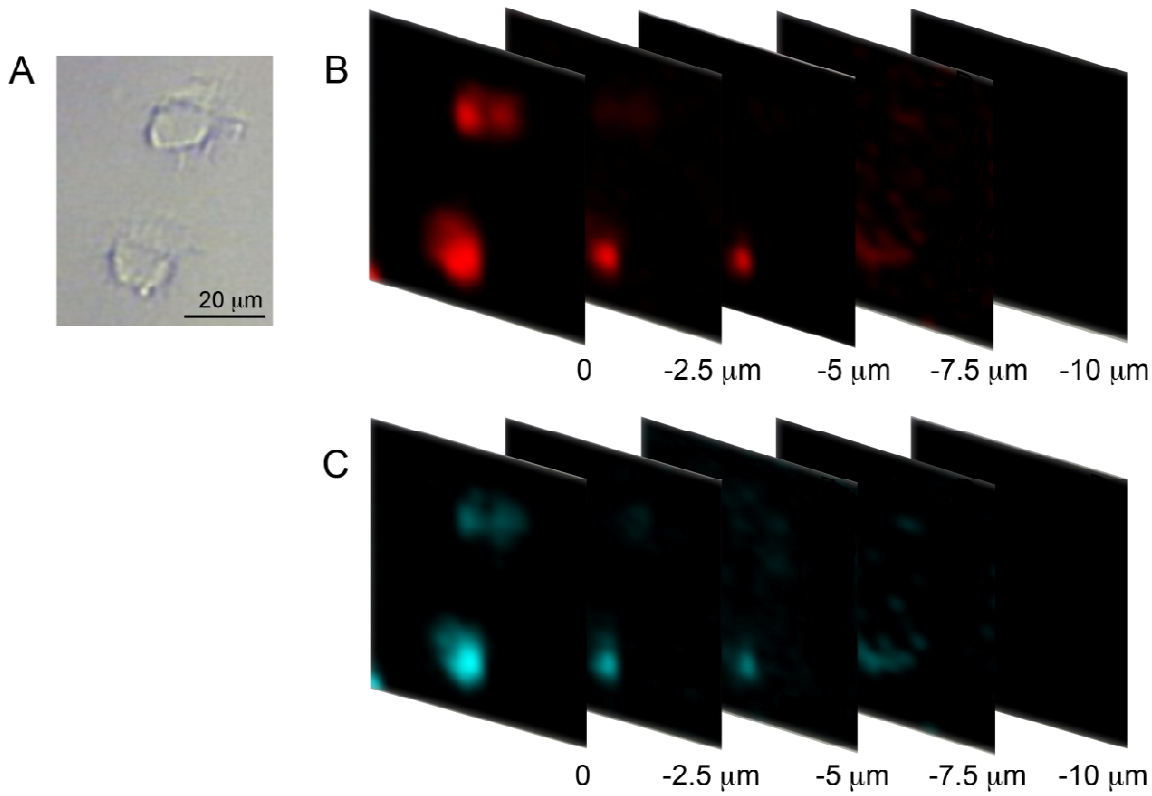

Fig. S6 SERS intensity map images of the z-series scan at different depth at an interval of 2.5 μm. A: bright field image of the cell, B: depth scan SERS image of the intensity from the MGITC nanotag (for CD44) and C: depth scan SERS image of the intensity from Rh6G nanotag (for EGFR).

Dark-field imaging with SERS nanotags

2 x10^4 MDA-MB-231 cells/well were seeded in a 8-well chamber slide (Millicell, Merck-Millipore, USA) and incubated overnight at 37 °C, 5% CO_2. 100 µL of 1 OD nanotag solution in 500 µL of medium was added to each well and incubated for 3 hours at 37 °C. Cells were washed with PBS to remove the tags and fixed with 4% paraformaldehyde (PFA) for 15 min. After rinsing with PBS to remove the PFA, the slide was mounted on a clear mount with Tris Buffer mounting medium (Electron Microscopy Sciences, Hatfield, PA). Nanotags were visualized via their resonant light scattering using an enhanced dark-field (EDF) illumination system (CytoViva, Auburn, AL) attached to a Nikon LV100 microscope. The system consists of a dark-field condenser (CytoViva) in place of the microscope's original condenser, attached via a fiber optic light guide to a 24-W metal halide light source (Solarc Lighting Technology). Images were acquired at 500 ms exposure time using a Nikon objective lens (100, NA 1.25, oil lens) and a Nikon DS-Fi1 camera with associated software (NIS-elements D).

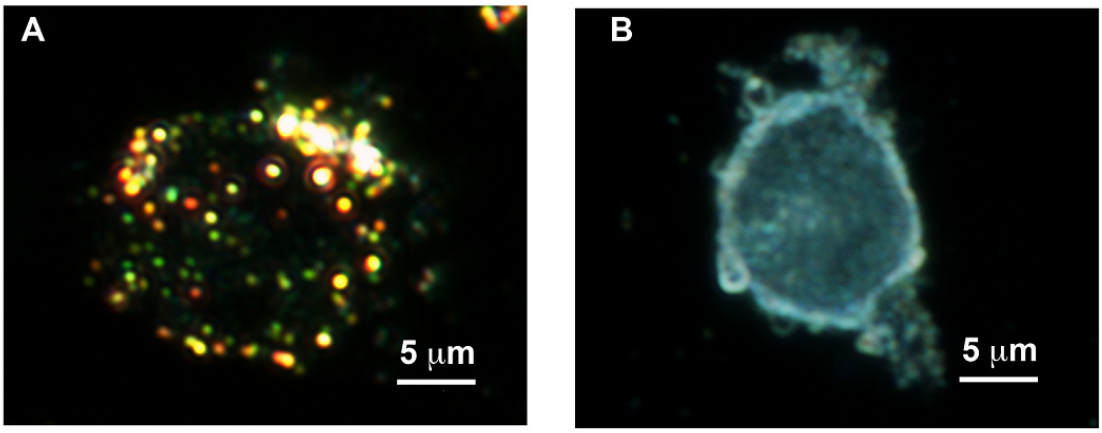

Fig. S7 *In vitro* dark field image of the MDA-MB-231 cells. A: cells treated with antibody conjugated three SERS nanotags. B: for the cells treated with SERS nanotags that are not conjugated with antibodies.

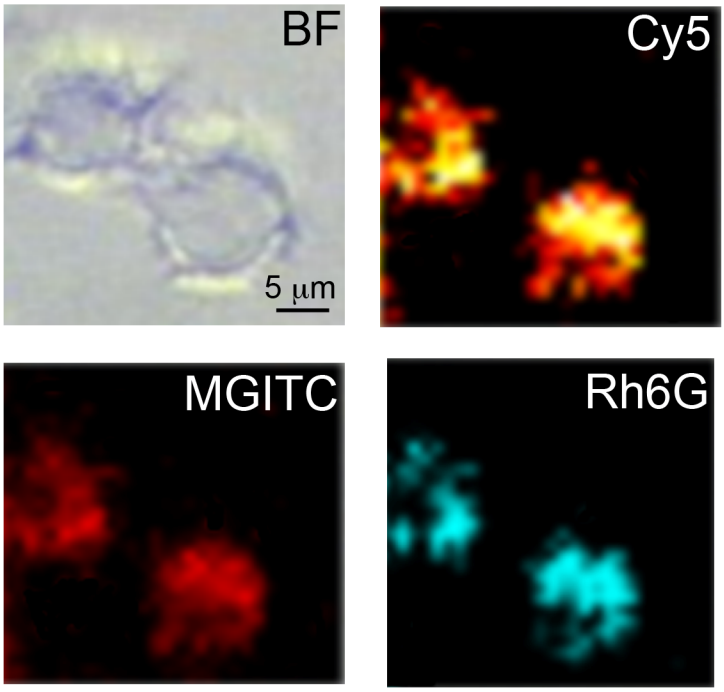

Fig S8. *In vitro* SERS mapping results of three SERS nanotags along with the corresponding bright field (BF) image of the cell. SERS mapping was carried out at 1120 cm^{-1} peak of Cy5, 1175 cm^{-1} of MGITC and 1650 cm^{-1} of Rh6G.