Supporting Information

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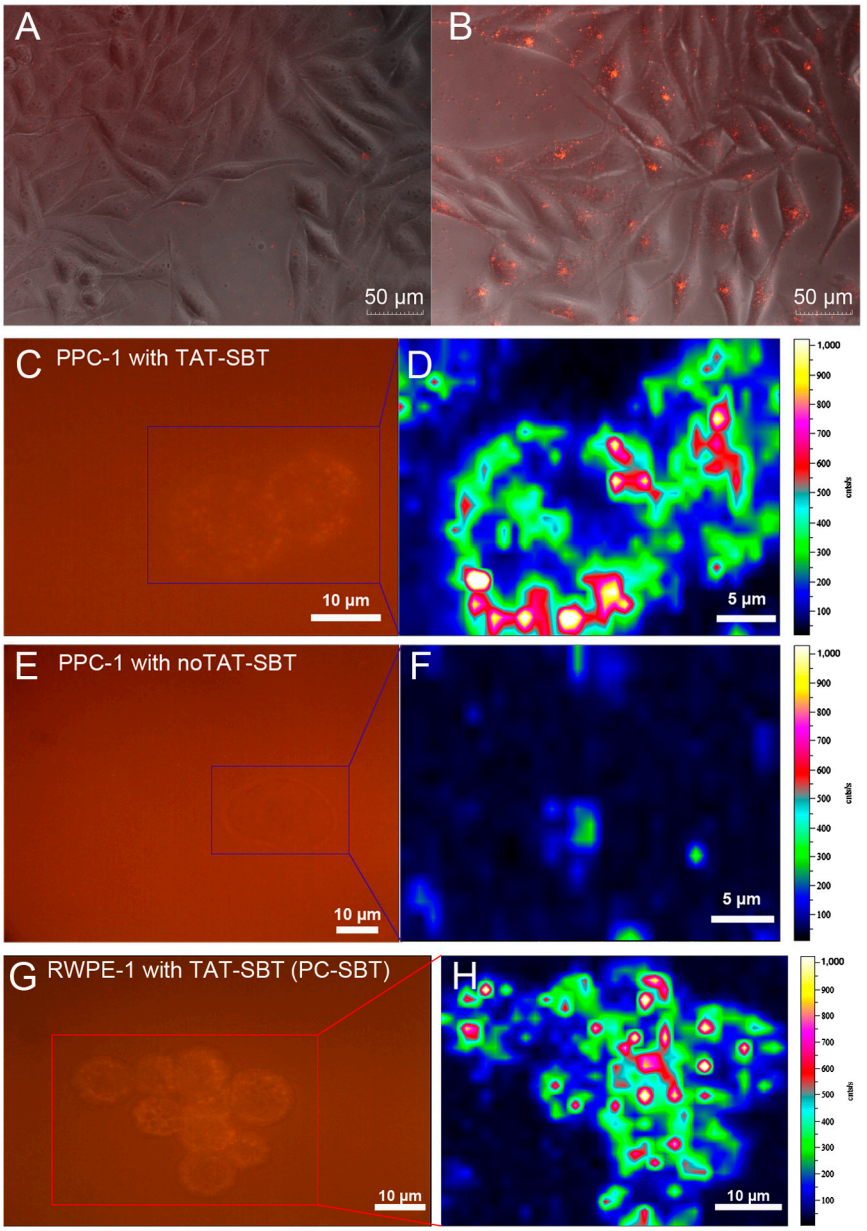

Fig. S1. Epifluorescence, bright field, and surface-enhanced Raman spectroscopy (SERS) maps of prostate cancer cells (PPC-1) and noncancerous prostate epithelial cells (RWPE-1) incubated with surface-enhanced resonance Raman scattering biotags (SBTs) with or without cys-TAT. (*A* and *B*) Epifluorescence images overlayed with bright field of PPC-1 cells incubated with rhodamine labeled non cys-TAT SBTs (*A*) and with cys-TAT SBTs (*B*) for 120 min in complete growth medium, at 37 °C, 5% CO_2. Bright field (*C* and *E*) images and corresponding SERS maps (*D* and *F*) of PPC-1 cells incubated for 30–60 min at room temperature in DMEM supplemented with 10% FBS with either TAT (*C* and *D*) or non-TAT (*E* and *F*) SBTs. The SERS maps represent the intensity of the band at 1,620 cm^{-1} of thionin across the mapped area. Positive control (PC) SERS biotags were also incubated with RWPE-1 cells in suspension at room temperature for 30–60 min in DMEM supplemented with 10% FBS. (*G*) Bright field image of the SERS mapped cells (the red rectangle represents the actual scanned area). (*H*) SERS map of the group of cells enclosed in the red rectangle in *G*. The map represents the intensity of the methylene blue band at 1,620 cm^{-1} across the scanned area.

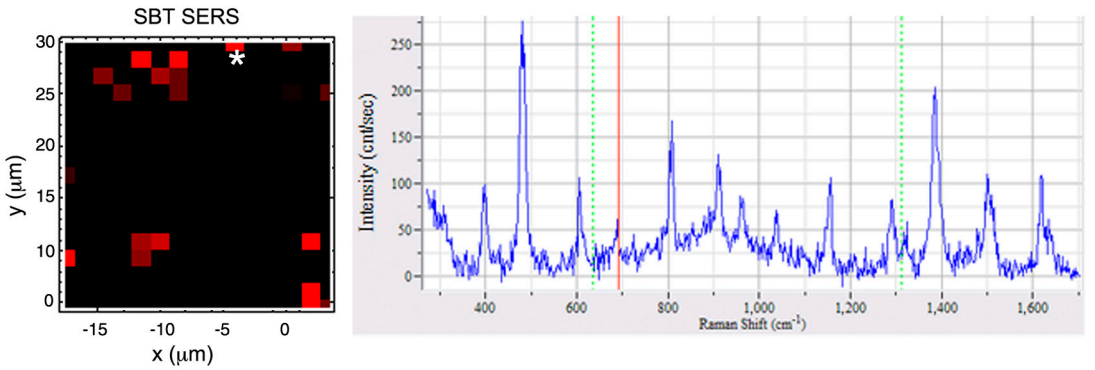

Fig. S2. SBTs were deposited on a glass slide and mapped using the same settings as for the cells. The signals from bright SBT were typically 100 counts or more at the most intense bands. The star indicates where in the map the spectrum comes from.

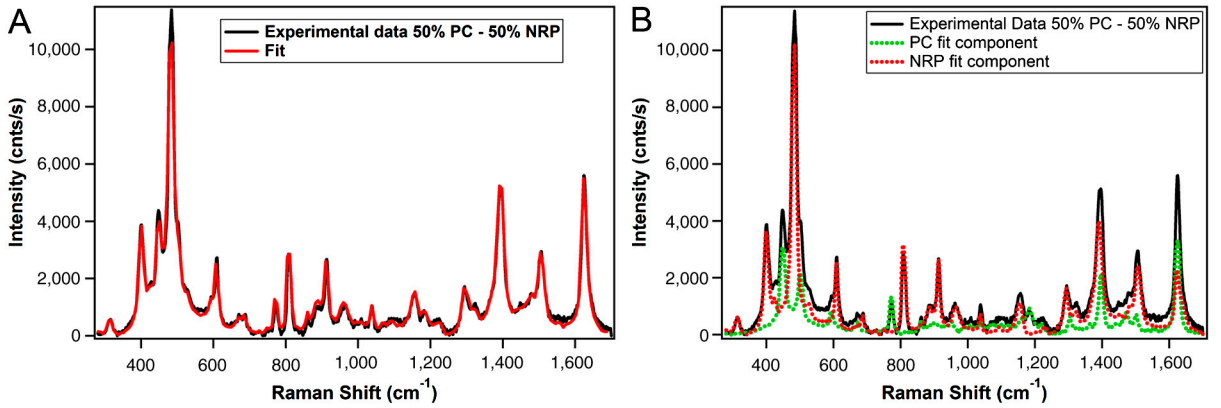

Fig. S3. Representative spectra and corresponding deconvolution for the calibration of the percentages of the two SBTs. Known volumes of the two SBTs were mixed in the following proportions: 90% neuropilin (NRP) (or PC)—10% PC (or NRP), 70% NRP (or PC)—30% PC (or NRP), and 50% NRP—PC. (*A*) Equal volumes of NRP and PC SERS biotags were mixed together, and the resulting composite spectrum is shown (black), together with the corresponding fit (red). (*B*) The same experimental spectrum with the deconvoluted single components calculated from the fit.

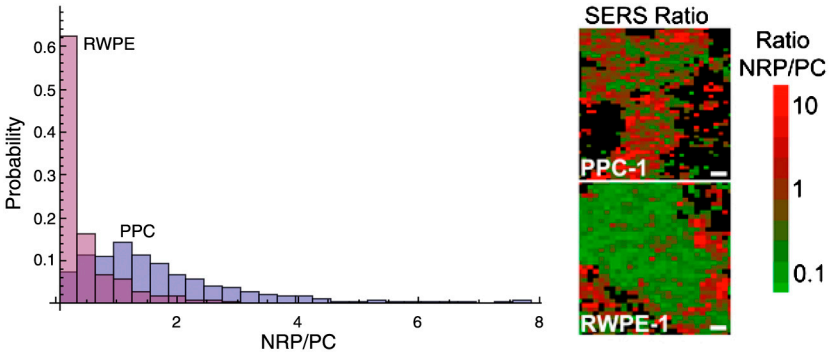

Fig. S4. The NRP/PC ratio from each point in the two maps (from Fig. 4 *A* and *D*), plotted as probability histograms. The tail of the RWPE is attributed to the nonspecifically associated particles around the cell periphery. The shape of these two distributions is consistent with the histogram of individual cell values in Fig. 5*A* that were obtained by averaging areas within maps.

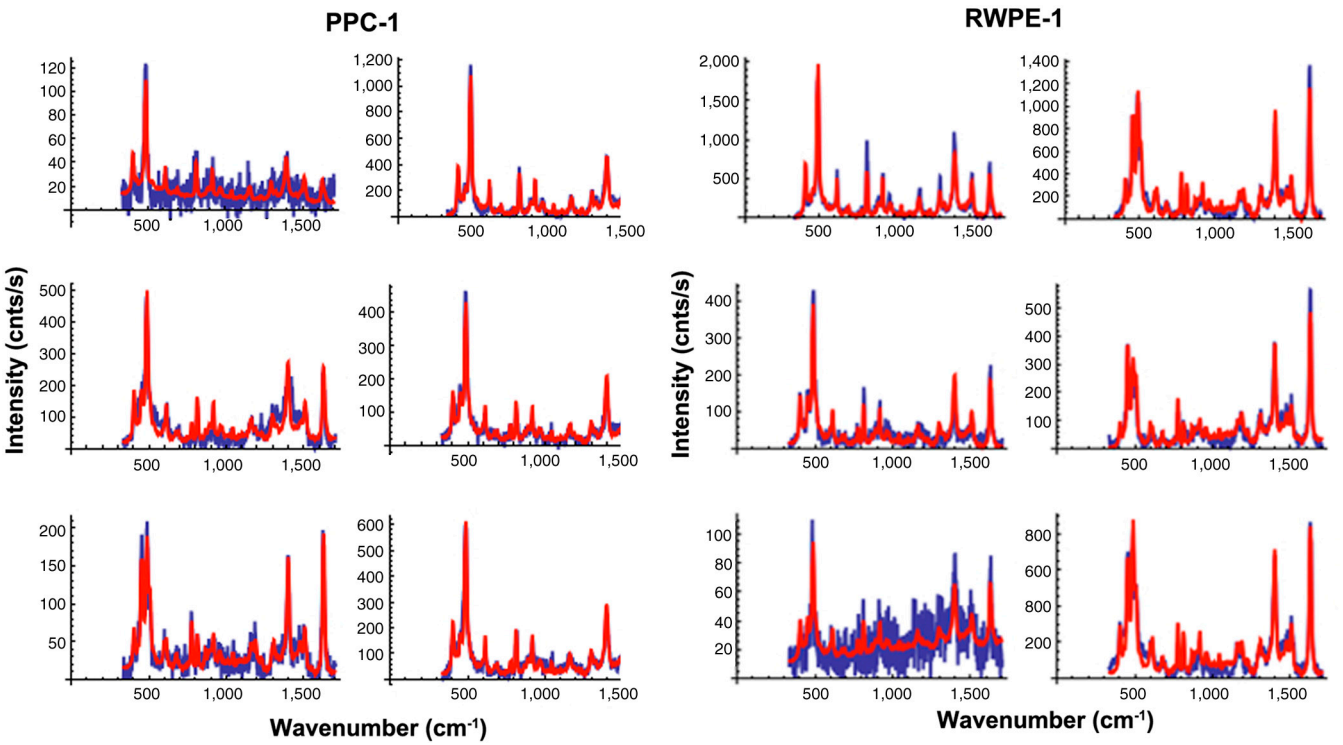

Fig. S5. Example fits from the Mathematica program for some points in the PPC-1 map Fig. 4A (*Left*), and for the RWPE-1 map Fig. 4D (*Right*), chosen across a range of intensity to demonstrate the quality of the fitting. For RWPE-1 the dominant signal is coming from the methylene blue PC-SBTs.

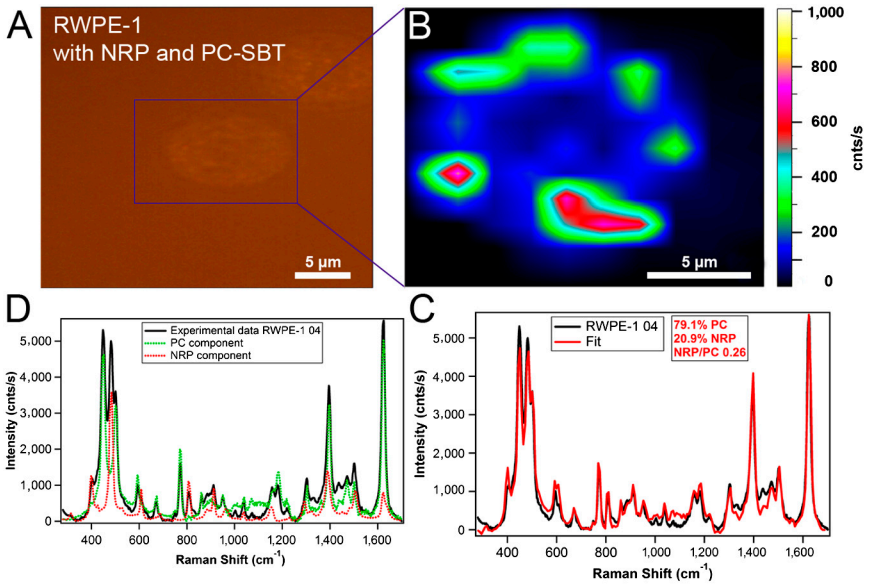

Fig. S6. Representative SERS map, whole cell spectrum, and deconvolution for RWPE-1 cells. (*A*) Bright field image of one RWPE-1 cell incubated with NRP and PC SERS biotags simultaneously for 60 min at room temperature in DMEM supplemented with 10% FBS. (*B*) SERS map of the area enclosed in the blue rectangle in *A* of the intensity of the 1,620 cm^{-1} band of thionin and methylene blue. (*C*) Full SERS spectrum averaged over the whole cell, after baseline subtraction and normalization (black trace) and corresponding fit (red trace). (*D*) Experimental SERS spectrum from the whole cell (black) and fit components corresponding to NRP (red) and PC (green) SERS biotags. The weight coefficients obtained from the fit algorithm were used together with the calibration curve of Fig. 2 to calculate the percentage of PC and NRP present on each cell and their ratio.

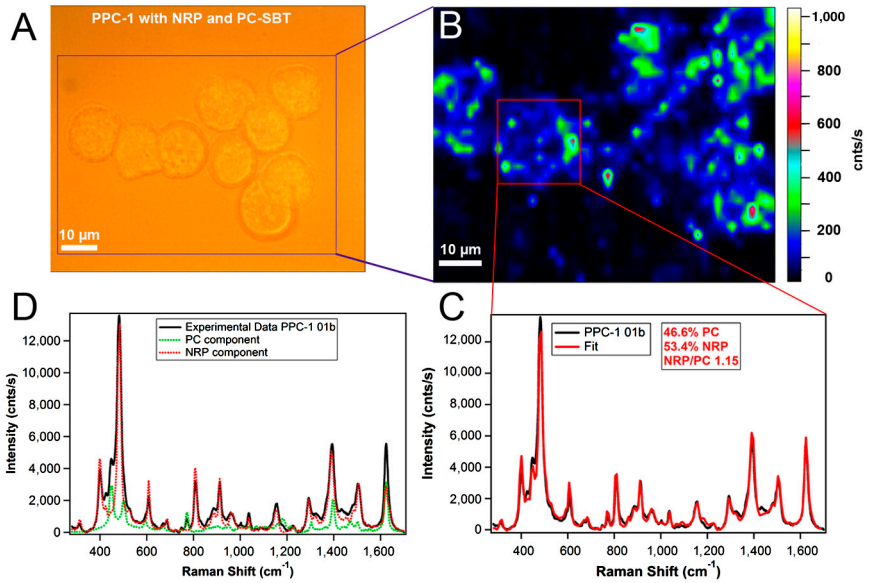

Fig. S7. Representative SERS map, whole cell spectrum, and deconvolution for PPC-1 cells. (*A*) Bright field image of a group of PPC-1 cells incubated with NRP and PC SERS biotags simultaneously for 60 min at room temperature in DMEM supplemented with 10% FBS. (*B*) SERS map of the area enclosed in the blue rectangle in *A* of the intensity of the 1,620 cm^{-1} band of thionin and methylene blue. (*C*) Full SERS spectrum averaged over the one whole cell, after baseline subtraction and normalization (black trace) and corresponding fit (red trace). (*D*) Experimental SERS spectrum from the whole cell (black) and fit components corresponding to NRP (red) and PC (green) SERS biotags. The weight coefficients obtained from the fit algorithm were used together with the calibration curve of Fig. 2 to calculate the percentage of PC and NRP present on each cell and their ratio.

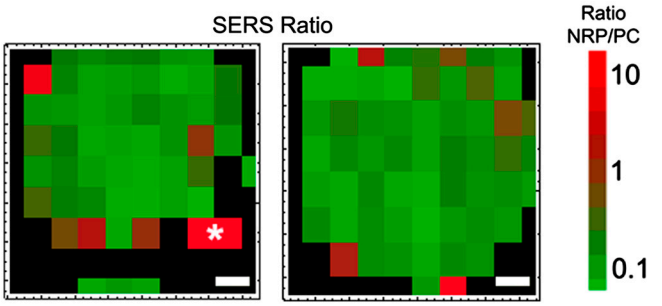

Fig. S8. Point-by-point SERS maps of the NRP/PC ratio on two RWPE-1 cells possible outliers. (*Left*) Map of the cell that caused the highest extreme value in which there are two spots very close to the cell boundary corresponding to high NRP signal, indicated by the asterisk (*). (*Right*) map of the cell that caused the lowest extreme value in which the positive control signal is very uniform and there is extremely low NRP signal, consistent with low nonspecific binding.