

## Supplementary Information

### **Exploring the efficacy and cellular uptake of sorafenib in colon cancer cells by Raman micro-spectroscopy**

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### **Real-time cell analysis (RTCA)**

The RTCA assay was acquired using the xCELLigence DP instrument (Bioscience, Germany). Cells suspension was diluted to a concentration of 10000 cells/100  $\mu$ l in case of HT29 and SW480 cells and 20000 cells/100 $\mu$ l in case of SW48 cells in each well of 16-well E-plates and was incubated for 24 hours at 37°C. Afterwards, 50  $\mu$ l of the medium was then removed from each well, and 50  $\mu$ l of different sorafenib concentrations (15, 25, and 50  $\mu$ M) were added to the wells. The cellular proliferation was detected by xCELLigence system software. The quantification of cellular proliferation was calculated by the cell index value based on the detected cell-electrode impedance in each well. The cell index values were normalized using cell index value at the time point of adding sorafenib to cells and measured every two minutes for around 75 hours.

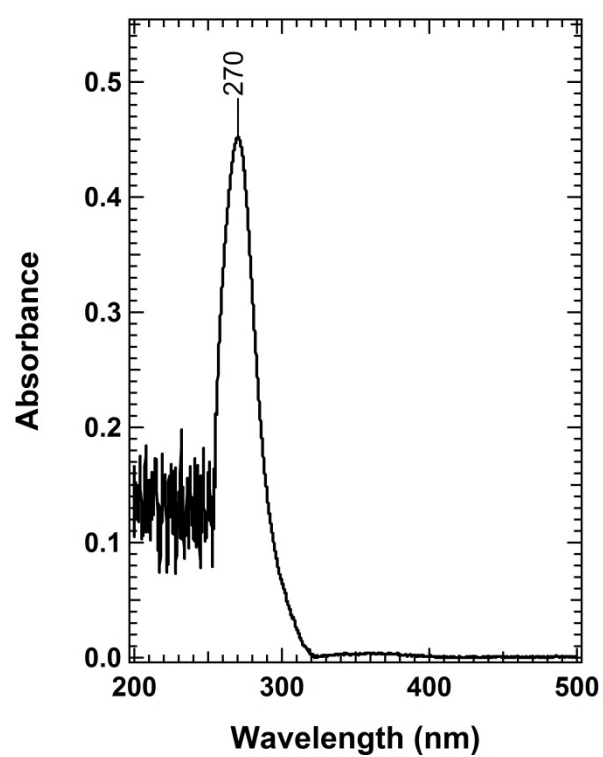
### **Western blot analysis**

A buffer containing phosphatase inhibitor mixture II (Sigma-Aldrich, München, Germany) and protease inhibitor cocktail (Roche, Mannheim, Germany) was used to harvest cells. Cells were resolved by SDS-PAGE using 10% polyacrylamide gels and transferred to Immobilon-P (Millipore, Hessen, Germany) membranes. The membranes were then incubated with antibodies to phosphorylated ERK1 and ERK2 (p-ERK; Cell Signaling Technology, Danvers, MA, USA) and total ERK1 and ERK2 (Cell Signaling Technology, Danvers, MA, USA). Antibodies were detected with an anti-mouse horseradish peroxidase-conjugated secondary antibody enhanced by chemiluminescence (Pierce, Life Technologies GmbH, Darmstadt, Germany). Images were captured with the Versa Doc 5000 imaging system (Bio-Rad, München, Germany).  $\beta$ -actin (Cell Signaling Technology, Danvers, MA, USA) was used as a loading control.

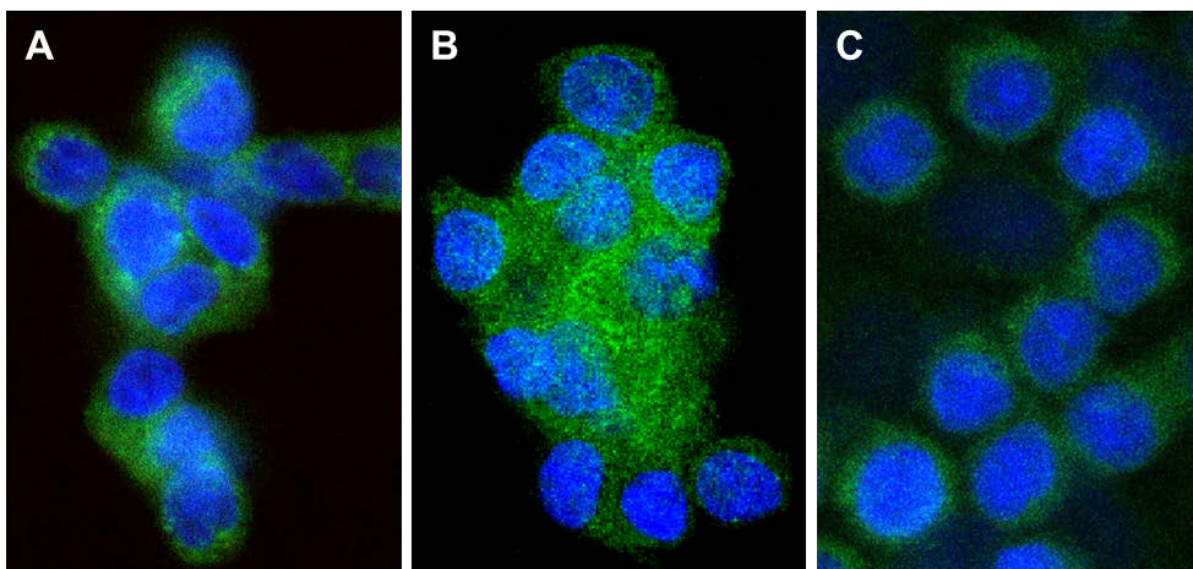
**Immunofluorescence staining and fluorescence microscopy.**

Cells fixed with formalin were permeabilized with 0.2% Triton X-100 for 5 min at room temperature, washed with PBS, and blocked with 1% bovine serum albumin (BSA) for 30 min. Cells were incubated with the primary mouse monoclonal anti-B-RAF (Santa Cruz Biotechnology, California, USA, sc-5284)) overnight at 4°C, followed by washing with PBS buffer and incubation for 1 h at room temperature with fluorescein- (FITC; Jackson ImmunoResearch, West Grove, USA) conjugated secondary antibodies. Excess antibodies were removed by washing with PBS buffer. Then, cells were further incubated with 1,5-bis{[2-(dimethylamino)ethyl]amino}-4,8 dihydroxyanthracene-9,10-dione (DRAQ-5; Cell Signaling Technology, Danvers, USA) for 15 min. Afterwards, cells were washed with PBS buffer.

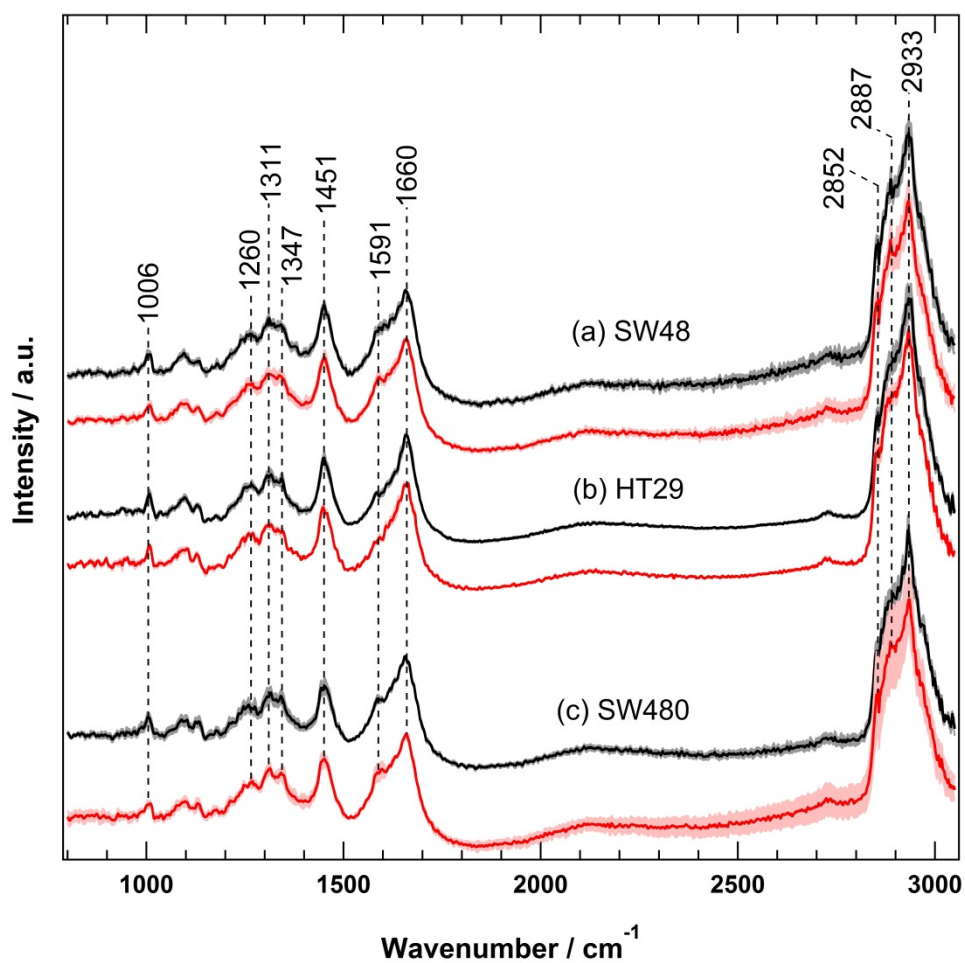
Fluorescence measurements were acquired with a confocal microscope (Leica TCS SP5 II) using a Leica HCX PL APO (25×/0.95 NA) water-immersion objective. The fluorescence of nucleus and B-RAF was imaged by exciting with the 633 and 488 nm lasers, respectively.



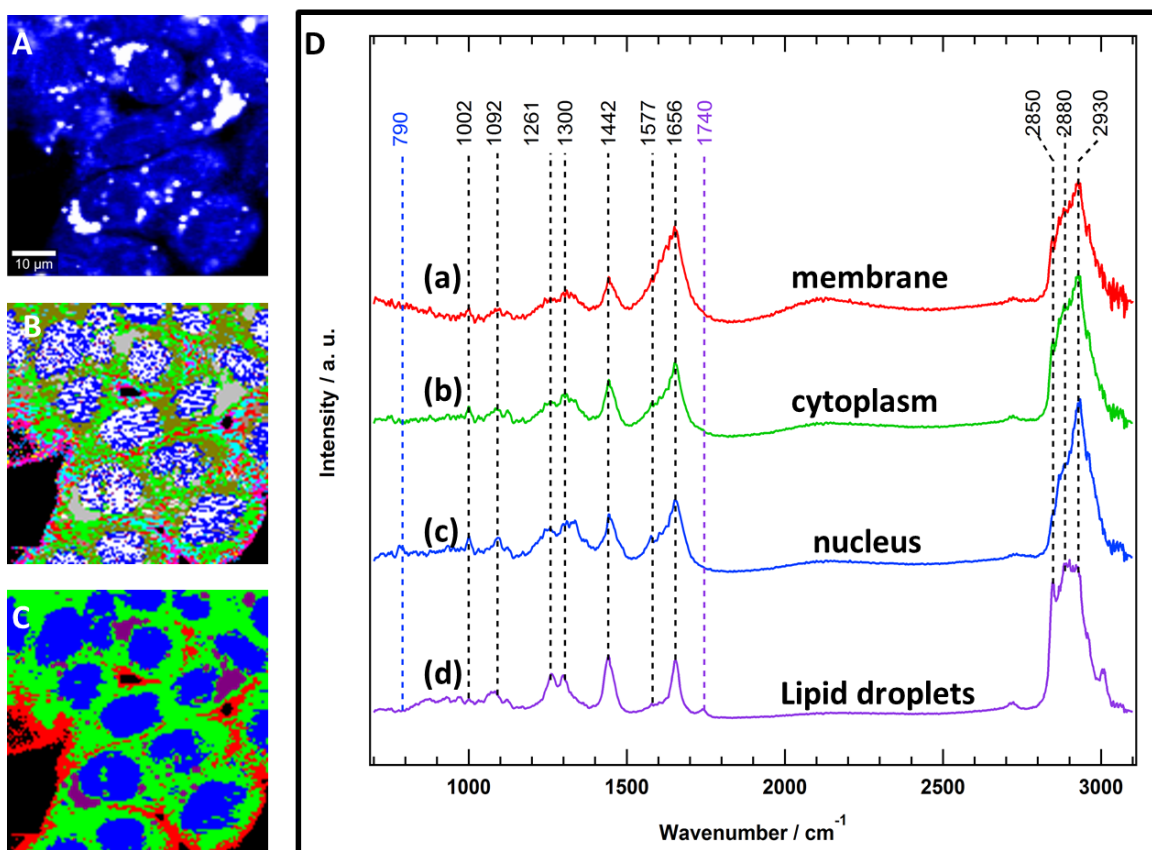
**Figure S1.** The UV-visible absorption spectrum of 10  $\mu$ M sorafenib dissolved in DMSO. Sorafenib has an absorption band at 270 nm.



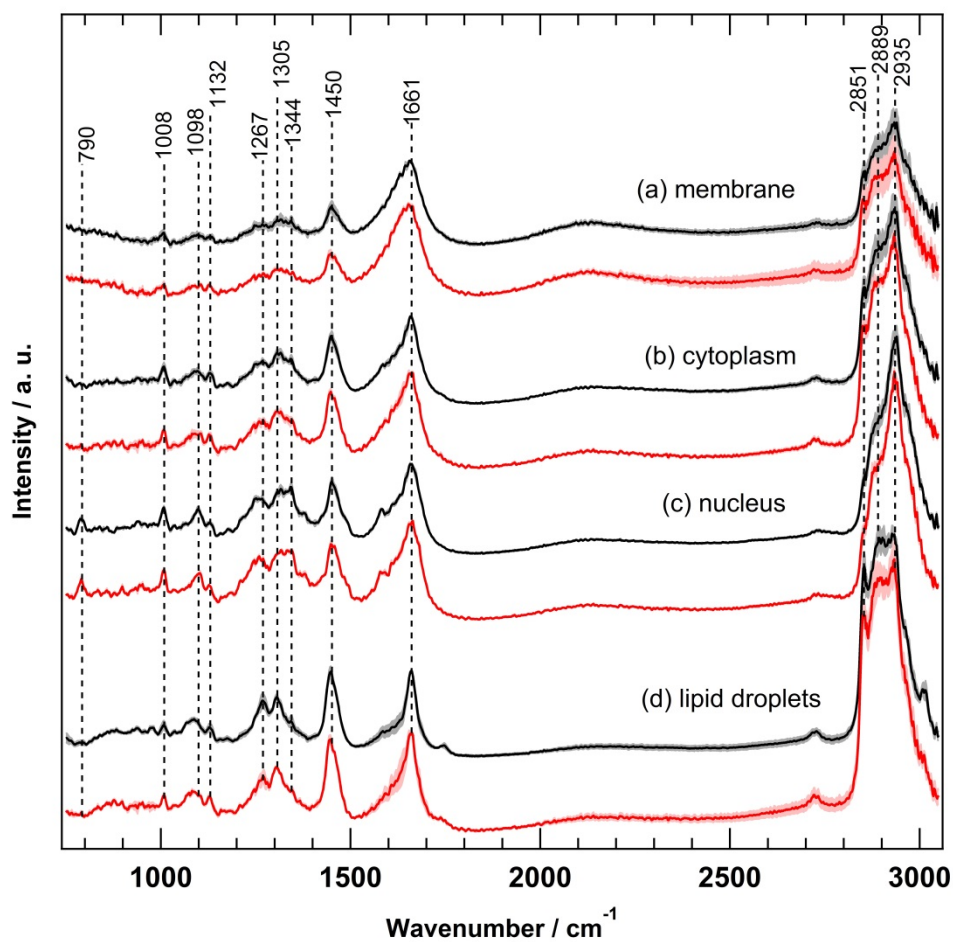
**Figure S2.** (a) Immunofluorescence staining of SW48 (A), HT29 (B), and SW480 (C) cells. The fluorescence image displays the nucleus in blue and B-RAF in green.



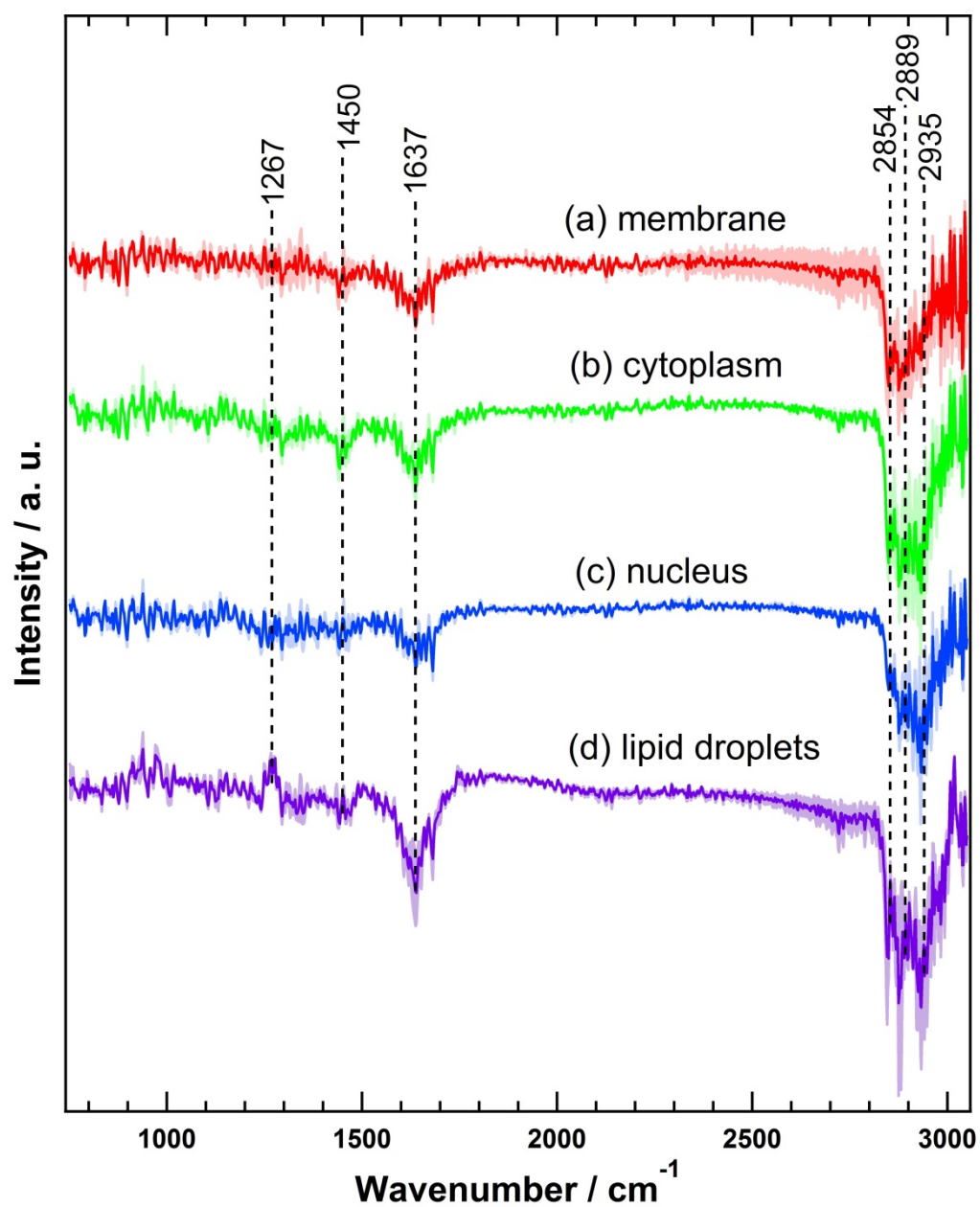
**Figure S3.** The average Raman spectra of SW48 (a), HT29 (b), and SW480 (c) cells in the 800–3050  $\text{cm}^{-1}$  region. The spectra of control and sorafenib-treated cells are shown in black and red, respectively. Shading represents the standard deviation.



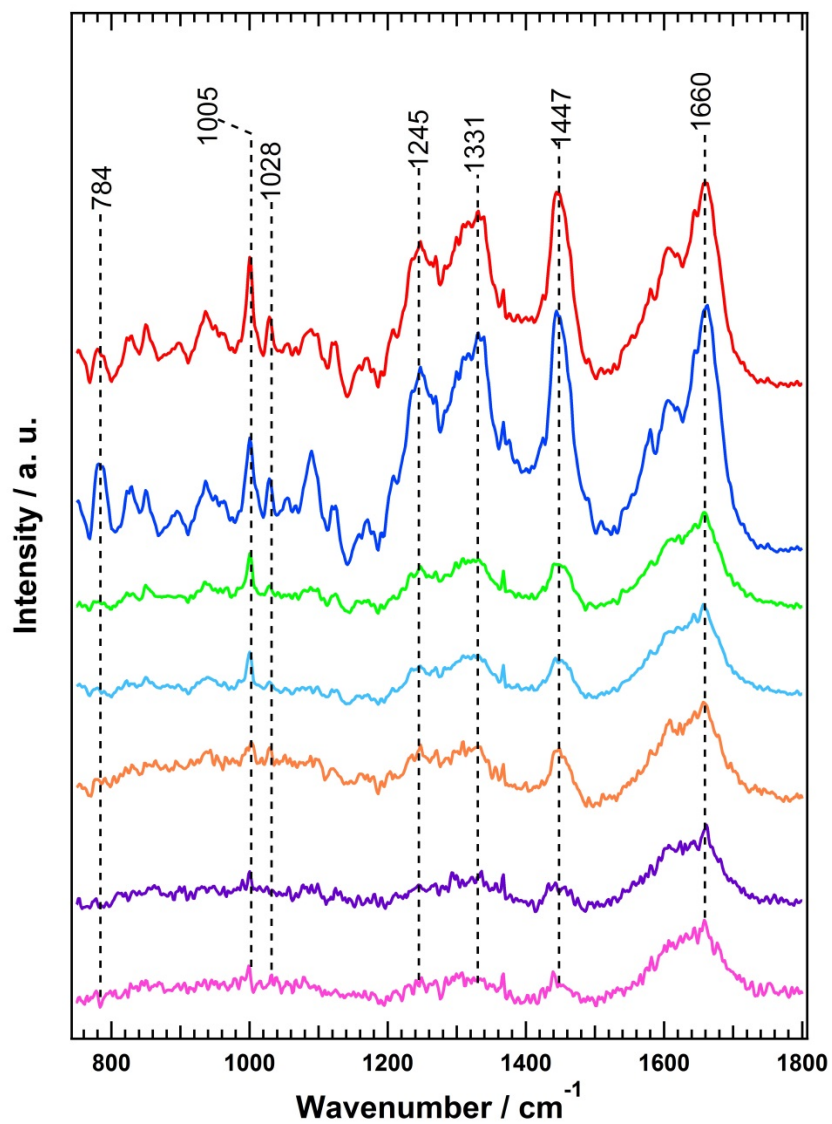
**Figure S4.** (A) Integrated Raman intensity image in the 2800-3050  $\text{cm}^{-1}$  region of HT29 cells. (B) HCA image using 15 clusters. (C) HCA image of only four components, the plasma membrane (red), cytoplasm (green), nucleus (blue), and lipid droplets (magenta) after merging the clusters of similar spectra. (D) The average cluster Raman spectra of the plasma membrane (a), cytoplasm (b), nucleus (c), and lipid droplets (d) of SW48 cells in the 700–3050  $\text{cm}^{-1}$  region.



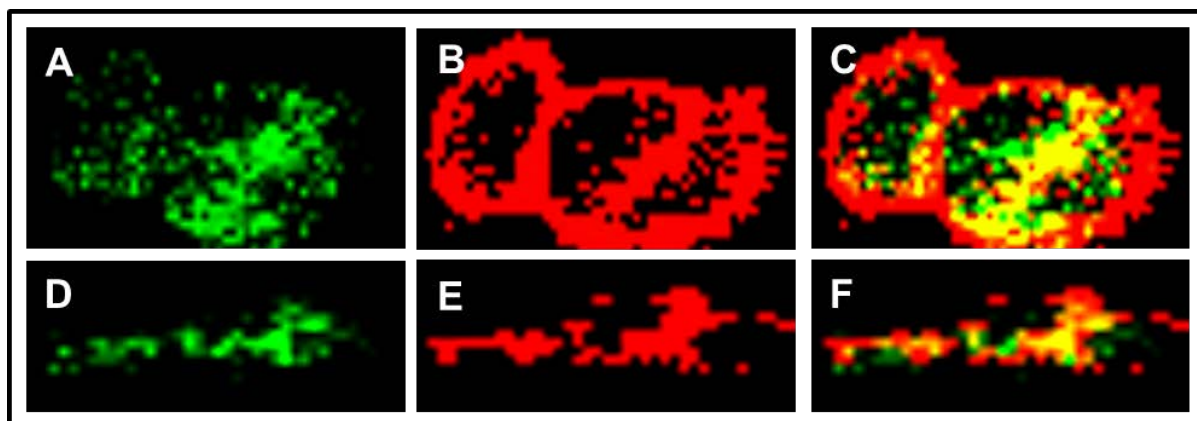
**Figure S5.** The average cluster Raman spectra of the plasma membrane (a), cytoplasm (b), nucleus (c), and lipid droplets (d) of HT29 cells in the 750–3050  $\text{cm}^{-1}$  region. The spectra of control and sorafenib-treated cells are shown in black and red, respectively. Shading represents the standard deviation.



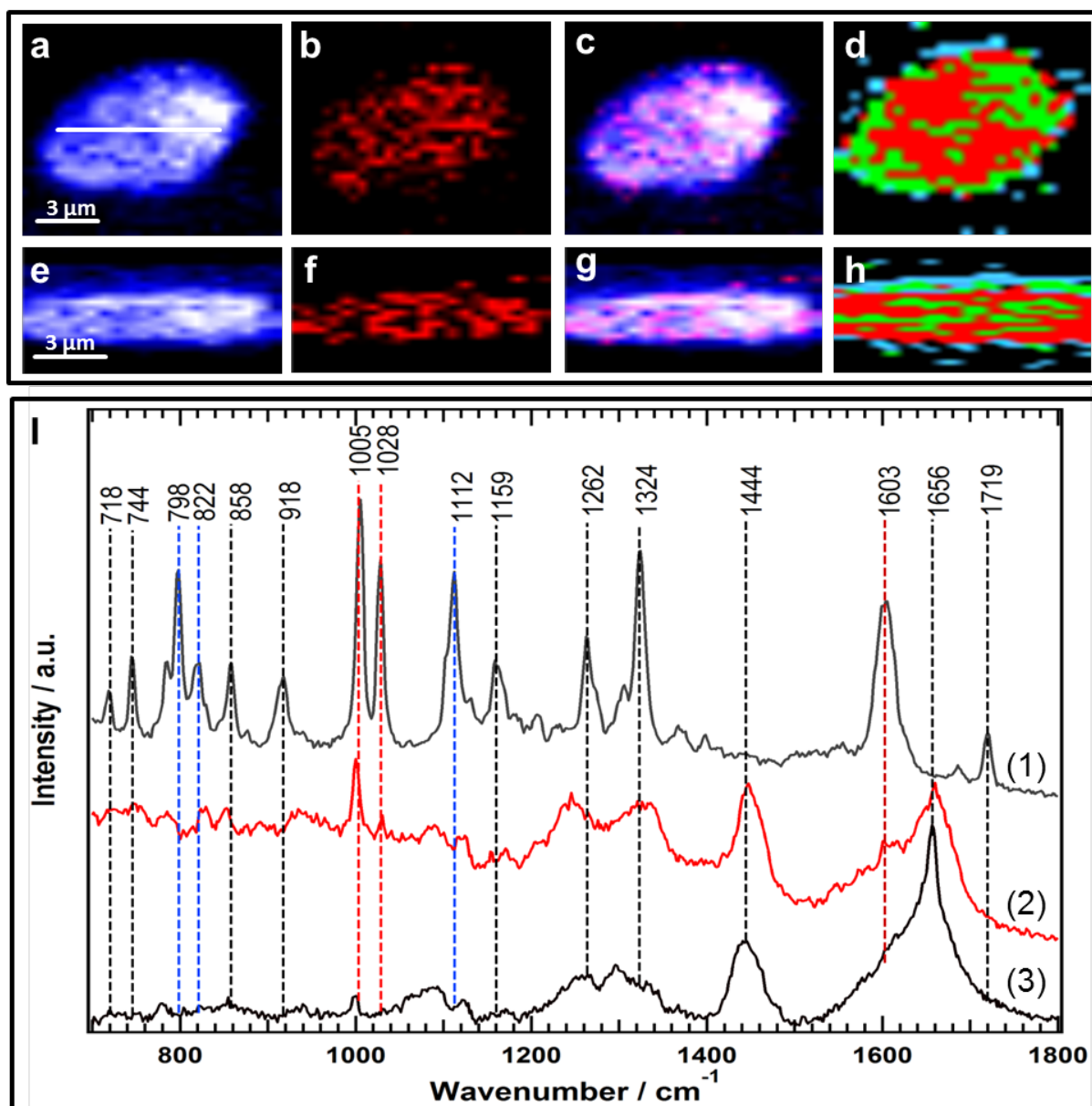
**Figure S6.** Effect of sorafenib on HT29 subcellular components. Raman difference spectra of control cells versus cells exposed to sorafenib for the plasma membrane region (a), cytoplasm (b), nucleus (c), and lipid droplets (d). Shading represents the standard deviation.



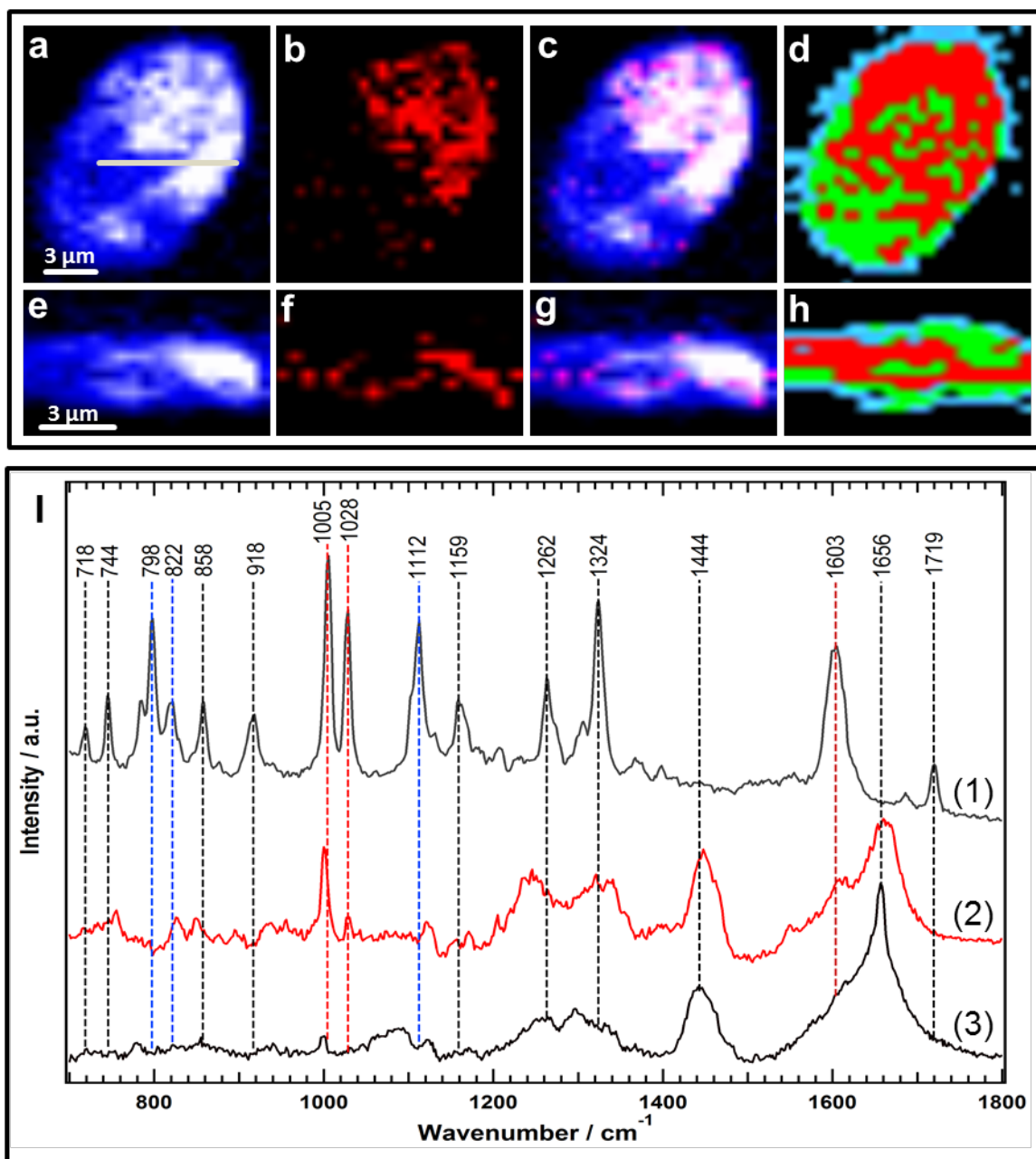
**Figure S7.** The average Raman spectra of HCA shown in Figure 6E. The colour of the spectra is the same of their corresponding clusters in the HCA image.



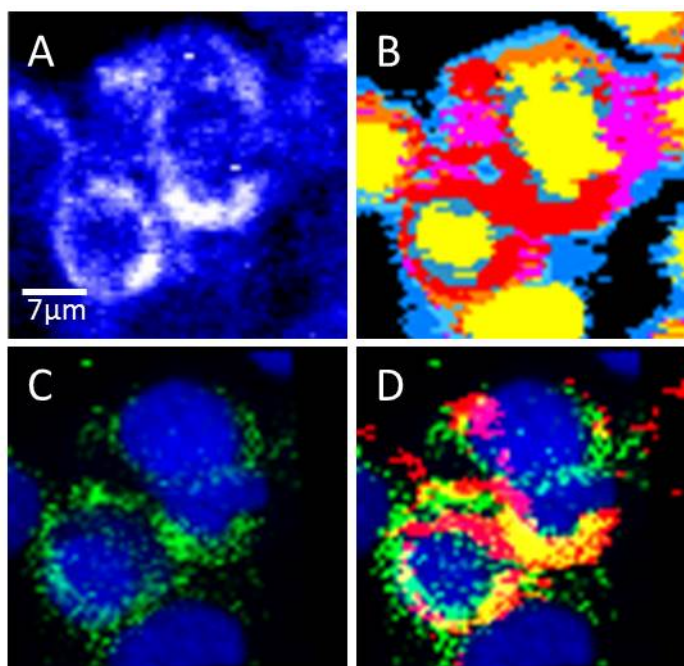
**Figure S8.** Raman imaging of HT-29 cells treated with sorafenib and this result is shown in Figure 6. (A) Raman image reconstructed from the band intensity of sorafenib at 1015-1040  $\text{cm}^{-1}$ . (B) Sorafenib-containing cluster obtained by HCA of the Raman data shown in Panel (A). (C) Overlay of Panels (A) and (B) and the overlaid regions are displayed in yellow. (D) Cross-section Raman images along the x-z axis of the same cells shown in Panel (A). (E) Sorafenib-containing cluster obtained by HCA of the Raman data shown in Panel (D). (F) Overlay of Panels (D) and (E) and the overlaid regions are displayed in yellow.



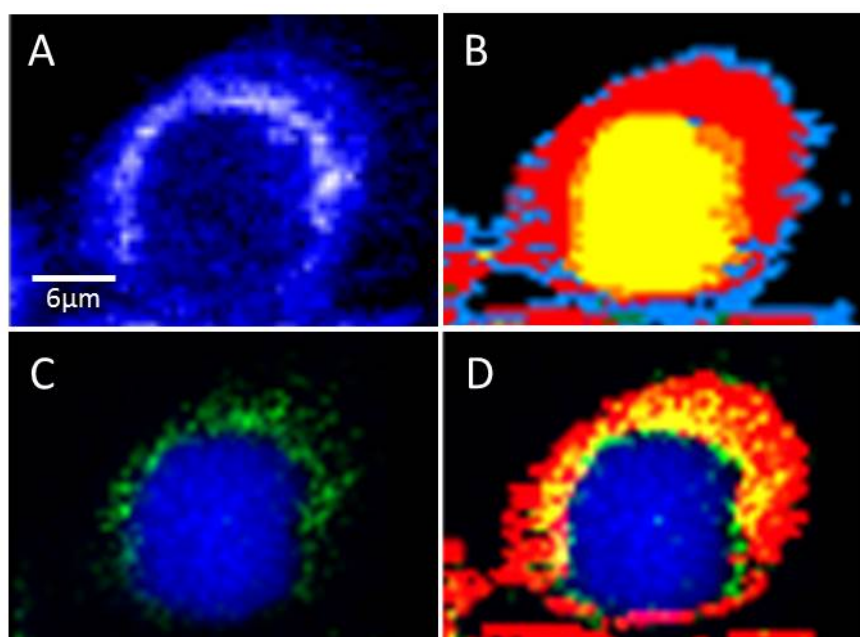
**Figure S9.** Raman imaging of HT-29 cells treated with 50  $\mu\text{M}$  sorafenib for 4 h. Raman images reconstructed at 1420-1470  $\text{cm}^{-1}$  (a) and at 1028  $\text{cm}^{-1}$  (b) intensities. (c) Overlay of panels (a) and (b). (e-g) Cross-section Raman images along the x-z axis of the same cell. Scanning positions are indicated by the white line in Panel (a). (d,h) HCA results based on the Raman data shown in Panels (a,e). (i) Raman spectra of free sorafenib (1), sorafenib-containing cluster within the HT-29 cell (2), and untreated cells (control, 3) are displayed.



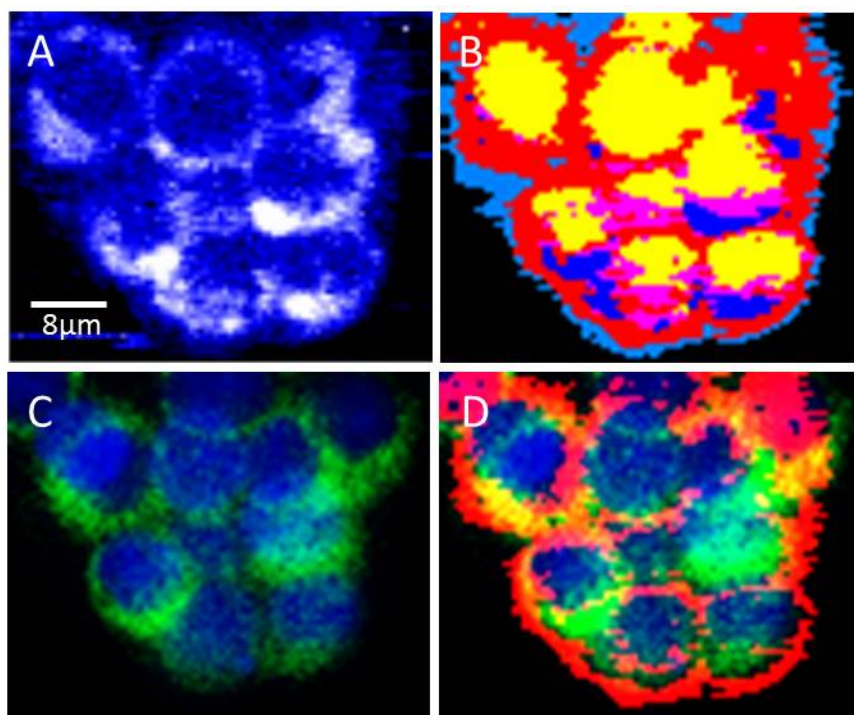
**Figure S10.** Raman imaging of HT-29 cells treated with 50  $\mu\text{M}$  sorafenib for 4 h. Raman images reconstructed at 1420-1470  $\text{cm}^{-1}$  (a) and at 1028  $\text{cm}^{-1}$  (b) intensities. (c) Overlay of panels (a) and (b). (d-f) Cross-section Raman images along the x-z axis of the same cell. Scanning positions are indicated by the white line in Panel a. (I) Raman spectra of free sorafenib (1), sorafenib-containing cluster within the HT-29 cell (2), and untreated cells (control) (3) are displayed.



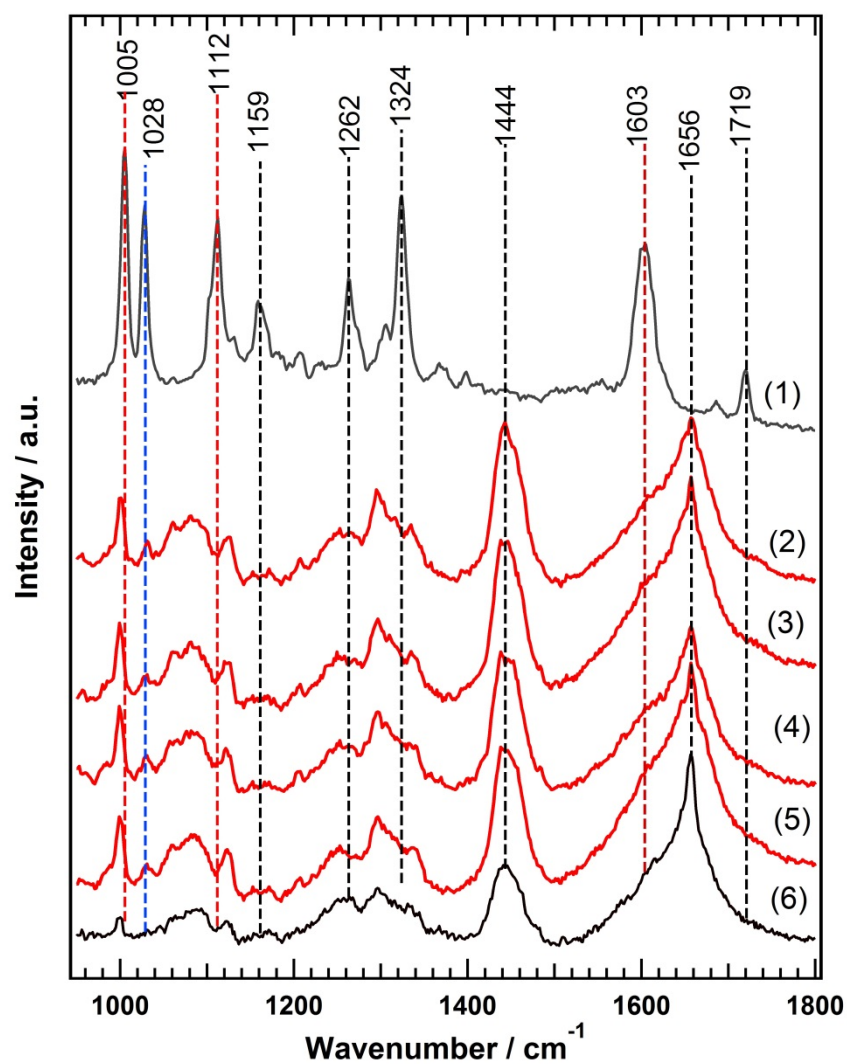
**Figure S11.** (A) Integrated Raman image of HT29 cell treated with sorafenib. (B) HCA of the Raman data shown in (A) containing sorafenib (red). (C) Fluorescence image of the same cell that shows the B-RAF (green) and the nucleus (blue). (D) Overlay of the sorafenib-containing cluster (red) from (B) and B-RAF (green) from (C) and the overlaid regions are shown in yellow.



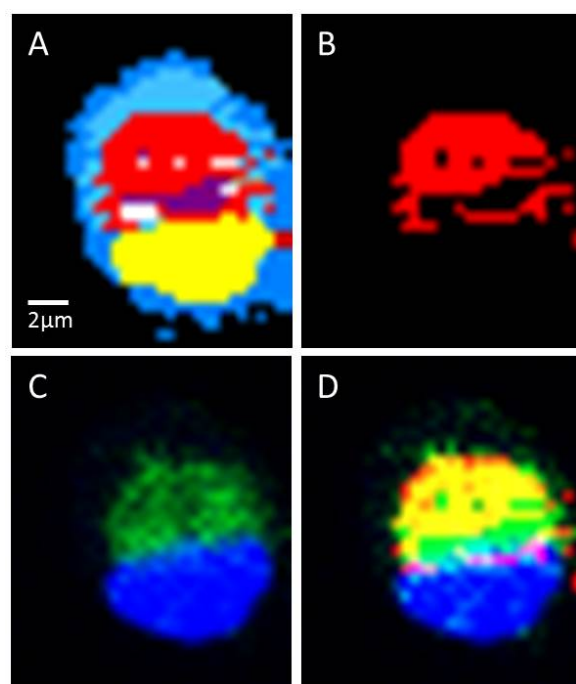
**Figure S12.** (A) Integrated Raman image of HT29 cell treated with sorafenib. (B) HCA of the Raman data shown in (A) containing sorafenib (red). (C) Fluorescence image of the same cell that shows the B-RAF (green) and the nucleus (blue). (D) Overlay of the sorafenib-containing cluster (red) from (B) and B-RAF (green) from (C) and the overlaid regions are shown in yellow.



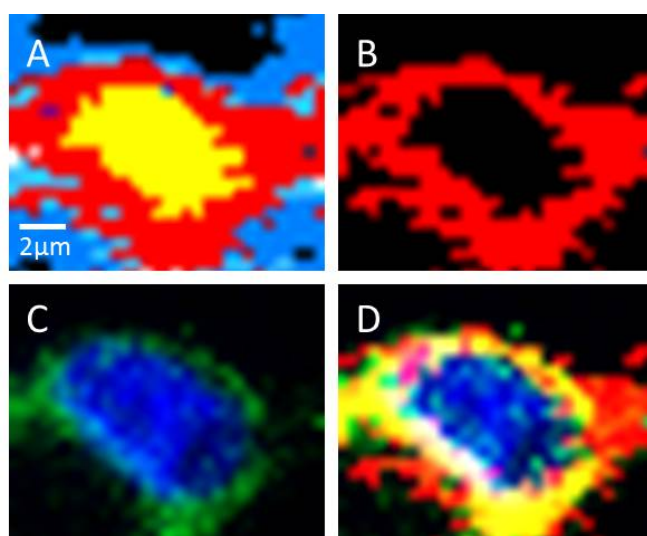
**Figure S13.** (A) Integrated Raman image of HT29 cell treated with sorafenib. (B) HCA of the Raman data shown in (A) containing sorafenib (red). (C) Fluorescence image of the same cell that shows the B-RAF (green) and the nucleus (blue). (D) Overlay of the sorafenib-containing cluster (red) from (B) and B-RAF (green) from (C) and the overlaid regions are shown in yellow.



**Figure S14.** The average Raman spectra of sorafenib containing clusters (2-4) shown in Figures 7 and S11-13 are compared with the spectra of pure sorafenib (1) and HT29 cells (6, control).



**Figure S15.** (A) HCA of the Raman data of SW48 cell. (B) HCA of only sorafenib-containing sorafenib cluster. (C) Fluorescence image of the same SW48 cell that shows the B-RAF (green) and the nucleus (blue). (D) Overlay of the sorafenib-containing cluster (red) from (B) and B-RAF (green) from (C) and the overlaid regions are shown in yellow.



**Figure S16.** (A) HCA of the Raman data of SW48 cell. (B) HCA of only sorafenib-containing sorafenib cluster. (C) Fluorescence image of the same SW48 cell that shows the B-RAF (green) and the nucleus (blue). (D) Overlay of the sorafenib-containing cluster (red) from (B) and B-RAF (green) from (C) and the overlaid regions are shown in yellow.