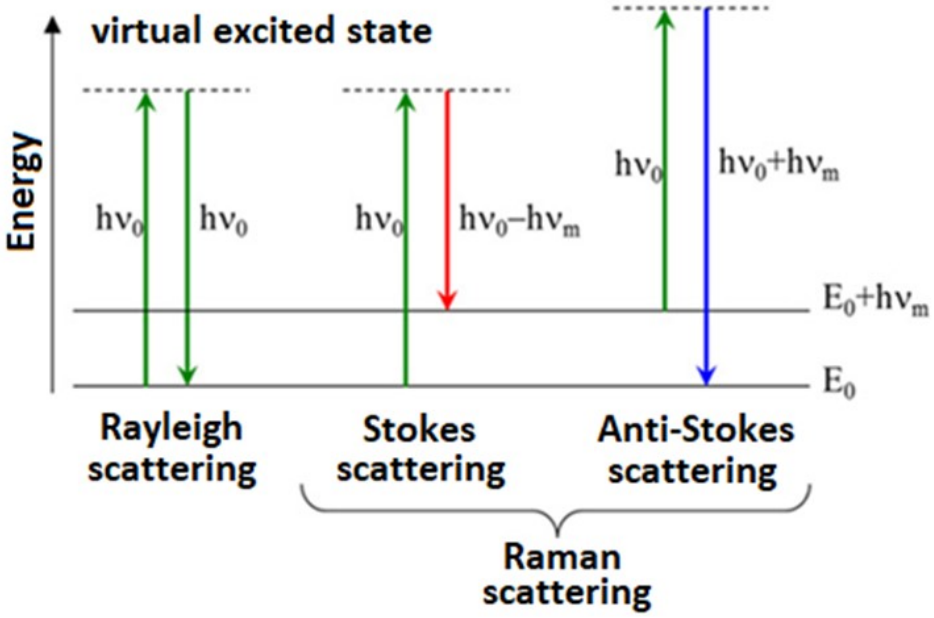
# SUPPLEMENTARY INFORMATION

## Raman Spectroscopy and Imaging

Raman spectroscopy is an analytical technique where inelastic scattered light is used to obtain the information about the vibrational energy of analysed molecules. In the vast majority of scattering events, the energy of the molecule is unchanged after its interaction with the photon; and the energy, and therefore the wavelength, of the scattered photon is equal to that of the incident photon. This is called elastic (energy of scattering particle is preserved) or Rayleigh scattering and is the dominant process during interaction of photon with the molecule.

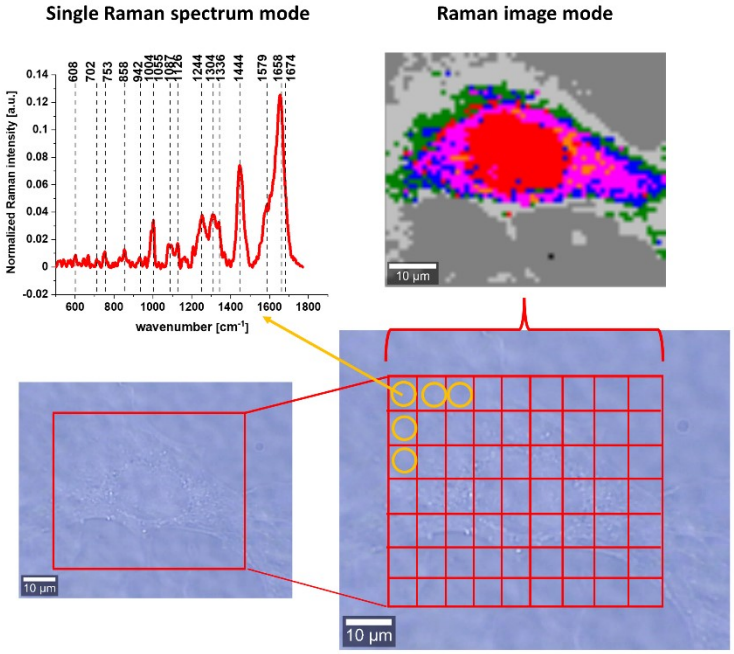
In a much rarer event (approximately 1 in 10 million photons) Raman scattering occurs, which is an inelastic scattering process with a transfer of energy between the molecule and scattered photon. If the molecule gains energy from the photon during the scattering (excited to a higher vibrational level) then the scattered photon loses energy and its wavelength increases which is called Stokes Raman scattering. Inversely, if the molecule loses energy by relaxing to a lower vibrational level the scattered photon gains the corresponding energy and its wavelength decreases; which is called Anti-Stokes Raman scattering. Quantum-mechanically, Stokes and Anti-Stokes are equally probable processes. However, with an ensemble of molecules, the majority of molecules will be in the ground vibrational level (Boltzmann distribution) and Stokes scattering is statistically more probable process. In consequence, the Stokes Raman scattering is always more intense than the Anti-Stokes component and for this reason, it is nearly always the Stokes Raman scattering that is measured wherewithal Raman spectroscopy.




**Scheme S1:** *Schematic illustration of Rayleigh, Stokes and Anti-Stokes Raman Scattering.*

Raman imaging is a technique based on Raman scattering allowing not only a single spectrum acquisition characteristic for a single point of the sample but also the analysis of

vibrational spectra of any sample area. The imaging mode allows the analysis of distribution of different chemical molecules inside the sample. Using algorithms such as Cluster Analysis (see section Materials and Methods) based on 2D data obtained during experiments Raman imaging mode makes possible to create Raman maps to visualize cell's substructures: nucleus, mitochondria, lipid structures, cytoplasm, cell membrane and learn about their biocomposition.




**Scheme S2:** Schematic comparison of Raman single spectra and Raman imaging modes of data acquisition.

## ROC - Receiver Operating Characteristic curves

Thanks to the PLSDA analysis we estimated the potential of Raman spectroscopy to differentiate all types of analyzed human colon cells (normal, cancer and cancer upon mevastatin supplementation). We calculated ROC curves (Receiver Operating Characteristic curves) presented on Figure S1. The area under curve (AUC) supports the discriminatory quality of the Raman analysis. For the random test an AUC =0.5, and represents the area under the diagonal from the origin of the plot (dashed line). The ROC curve in Figures S2A and S2C is shifted towards the upper left and top corner indicating the discriminatory performance of the Raman method for normal and cancer human colon cells. For perfect performance has an AUC=1. The lower value of AUC for normal cells is expected compared to cancer one taking into account that the analyzed set of data contains Raman spectra of cancer cells upon mevastatin supplementation, the highest AUC value for cancer cells confirm that spectral signatures of this type of cells allow perfectly distinguish them from normal cells and cancer cells after statin treatment.

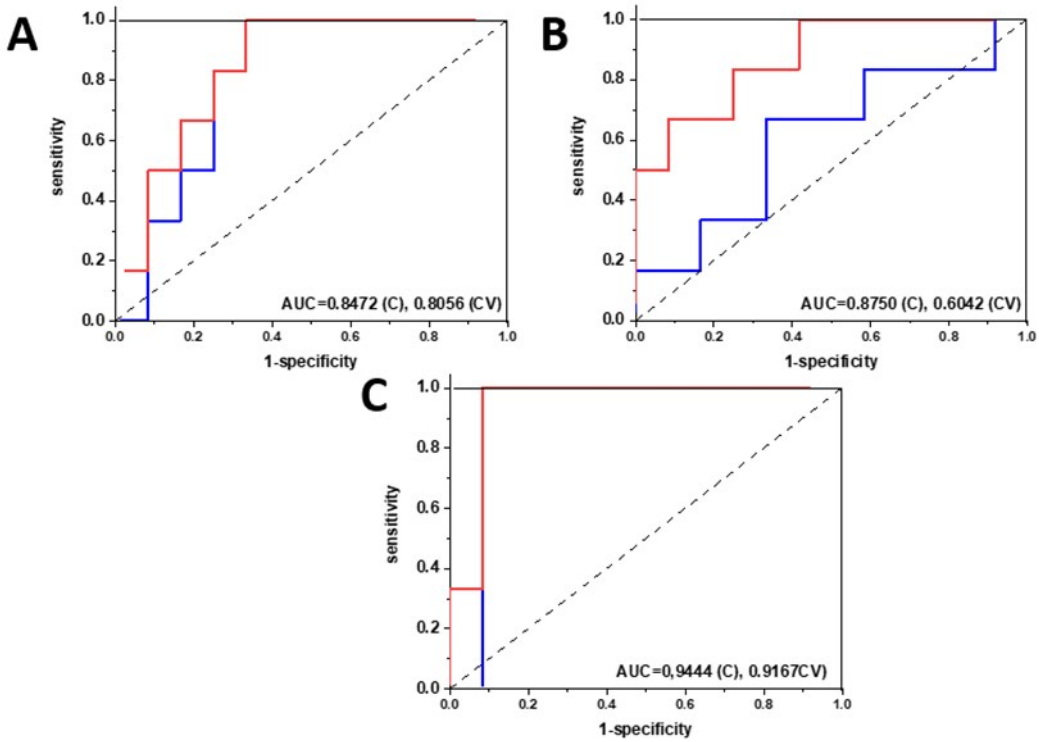
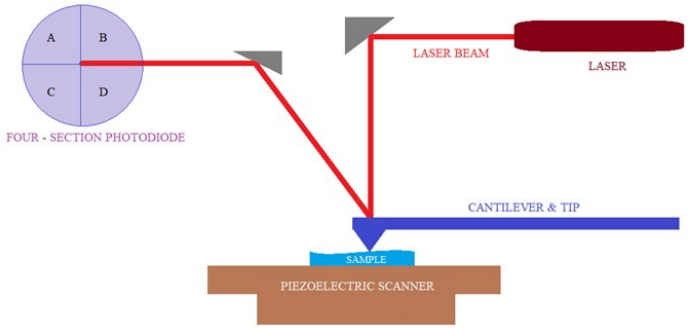



**Figure S1:** *Receiver operating characteristic (ROC) curves for the classification (C) and cross-validation (CV) for normal, cancer and cancer upon mevastatin supplementation (50µM, 48h)human colon cells.*

**AFM**

Atomic Force Microscopy (AFM) is type of scanning probe microscopy, which allow to analyze samples with resolution on the order of fractions of a nanometer, more than 103 times better than typical for optical methods. The information is obtained based on the interaction of sample and mechanical probe. AFM operation can be described as one of three modes: contact mode, non-contact mode or tapping mode. Tapping mode is very often used for biological sample analysis. AFM force spectroscopy mode is usually used to measure the nanomechanical properties of samples including living material (tissues or cells). Scheme 3 shows the principles of AFM technique.




**Scheme S3:** *Principles of AFM technique.*

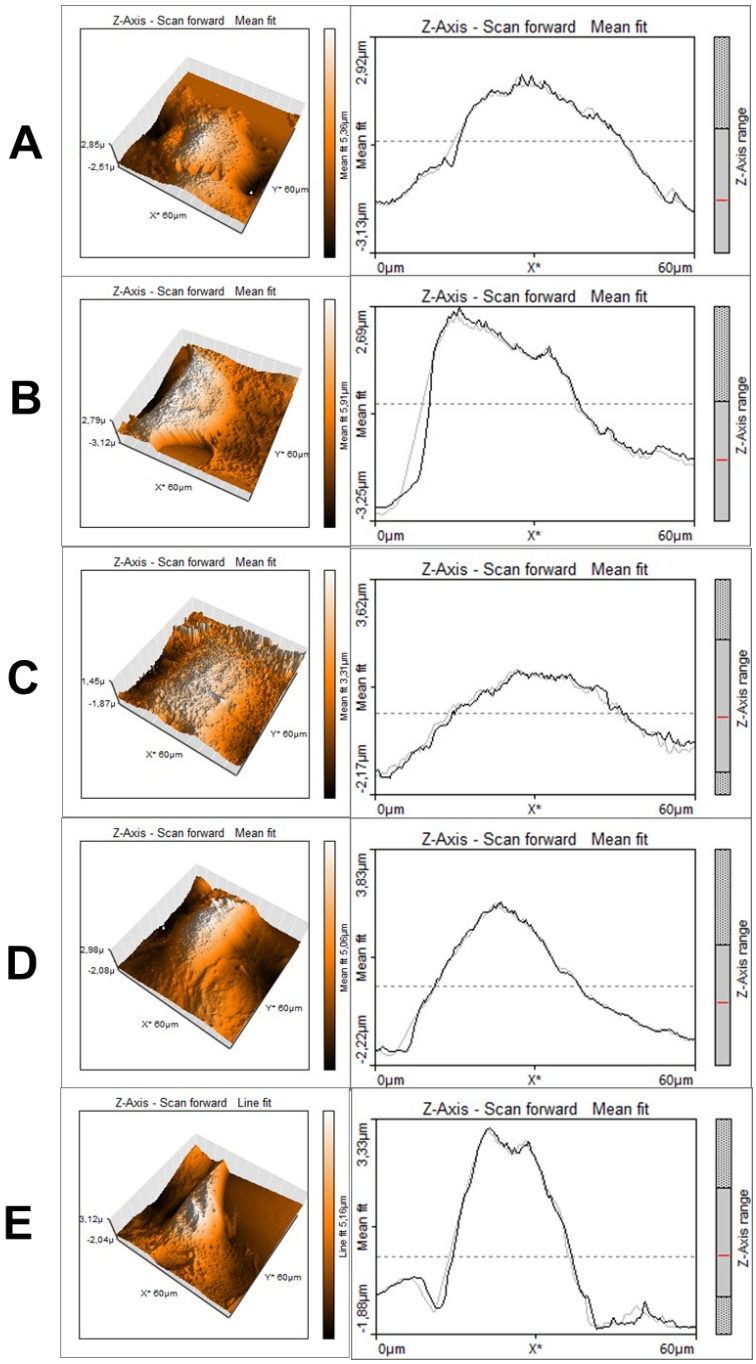



**Figure S2:** *AFM 3D topography maps of CaCo-2 (A) and CaCo-2 supplemented by mevastatin: 10 µM, 24 h (B), 10 µM, 48 h (C), 50 µM, 24 h (D), and 50 µM, 48 h (E).*

Based on force-distance curves recorded by using AFM technique we calculated YM for all analyzed types of samples, Figure 11C*(See Main text)* shows the obtained results.