Mass Spectrometry Imaging of Lipids in a Gut Epithelial Cell Model

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Supplementary Information

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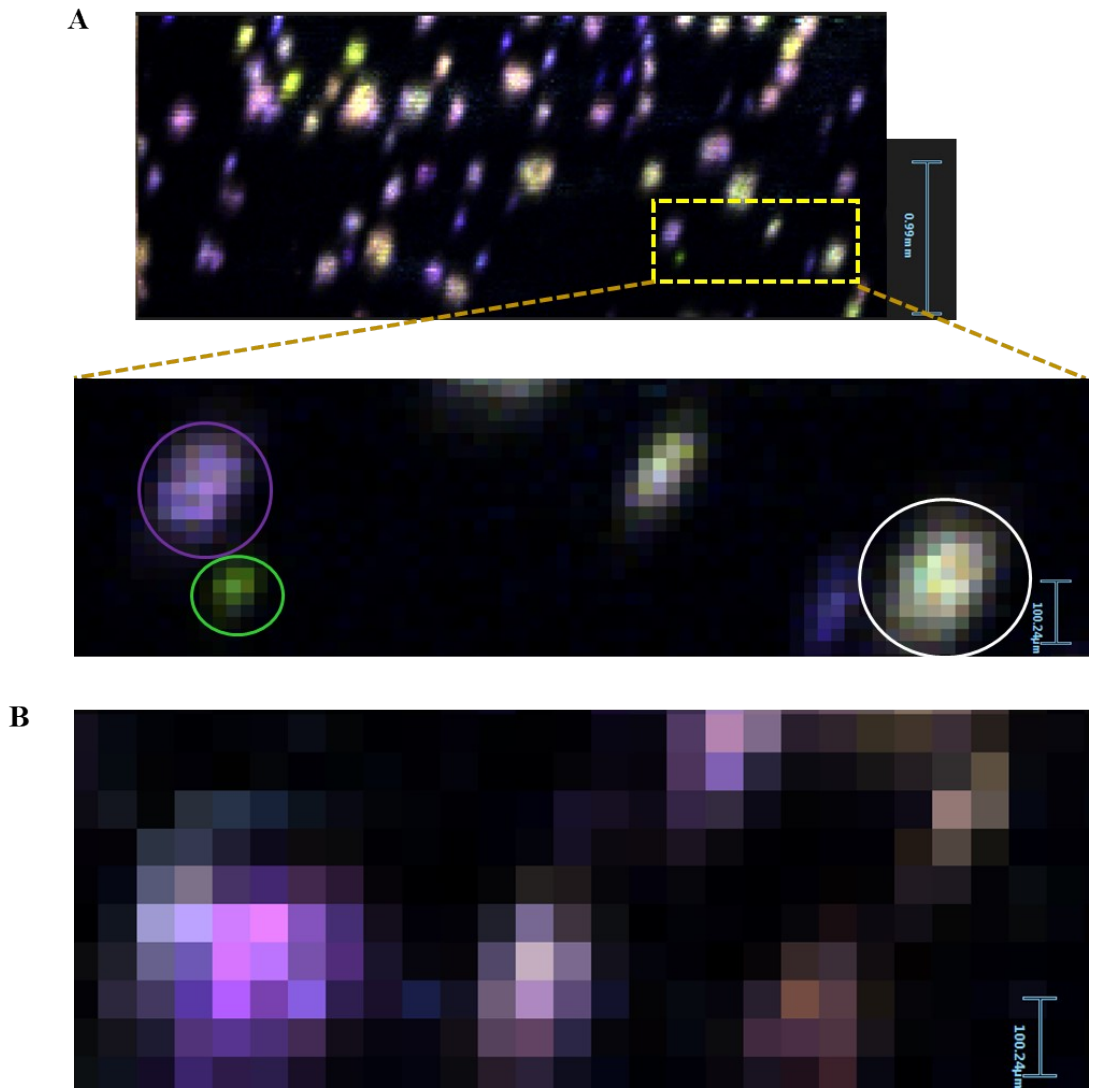


Fig S1: Mass spectrometry imaging of individual HT29-MTX cells.
Cells were imaged in positive ionization mode using a pixel size of either (A) 20 µm or (b) 50 µm for three phosphatidylcholine (PC) lipids as follows: m/z 798.55 (red; PC (16:0; 18:1) K), 770.51 (green; PC (16:0_ 16:1) K+) and 848.56 (blue; PC (38:4)+K) to provide a red-green-blue (RGB) overlay. The white zone (white circle) represents the presence of the three lipids in the cluster of HT29-MTX cells, the purple zone (purple circle) represents two lipids (m/z 798.55 and 848.56 in small cluster of HT29-MTX cells, while the green zone (green circle) represents one lipid m/z 770.52 in individual HT29-MTX cell. The scale bar=100.2 µm.

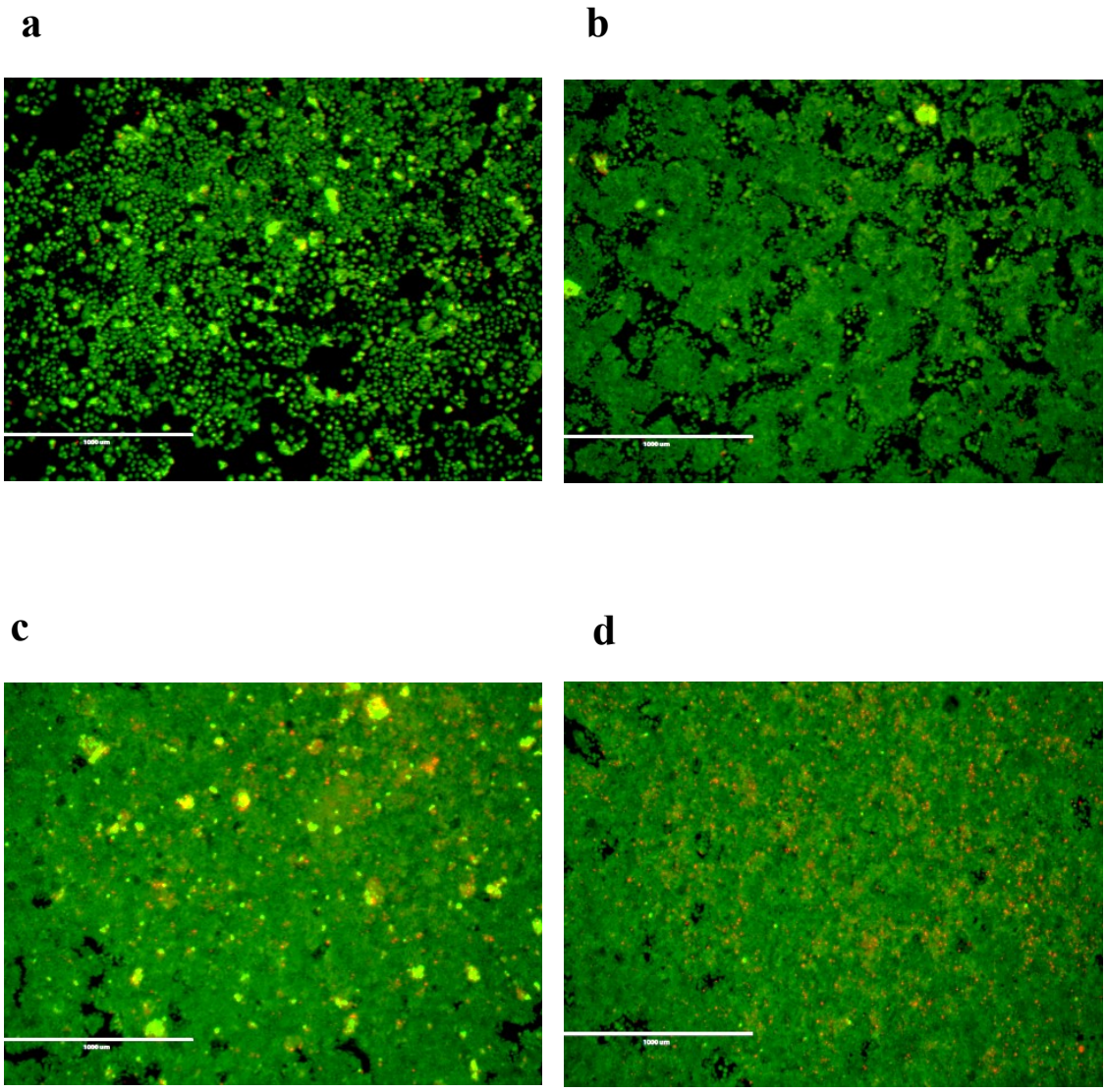


Fig S2 Live/dead staining assay of Caco2 and HT29-MTX cell cultures on day 21 days after seeding.

Cells cultured on coverslips either singly (100% culture) (**a** and **d**) or in the co-culture system (**b** and **c**). Panels are as follows; **a-** Caco2 cells only (100%), **b** and **c-** Caco2: HT29-MTX cells co-cultures seeded at cell densities of 75:25 (**b**) or 50: 50 (**c**), **d-** HT29-MTX cells (100%). Live cells (green) and dead cells (red). Cells were incubated for 35-40 min with Live/Dead viability kit then cells were imaged using a fluorescent microscope using 10x objective with scale bar=1000µm. Filter emission 494 nm (green) and filter emission 535 nm (red).

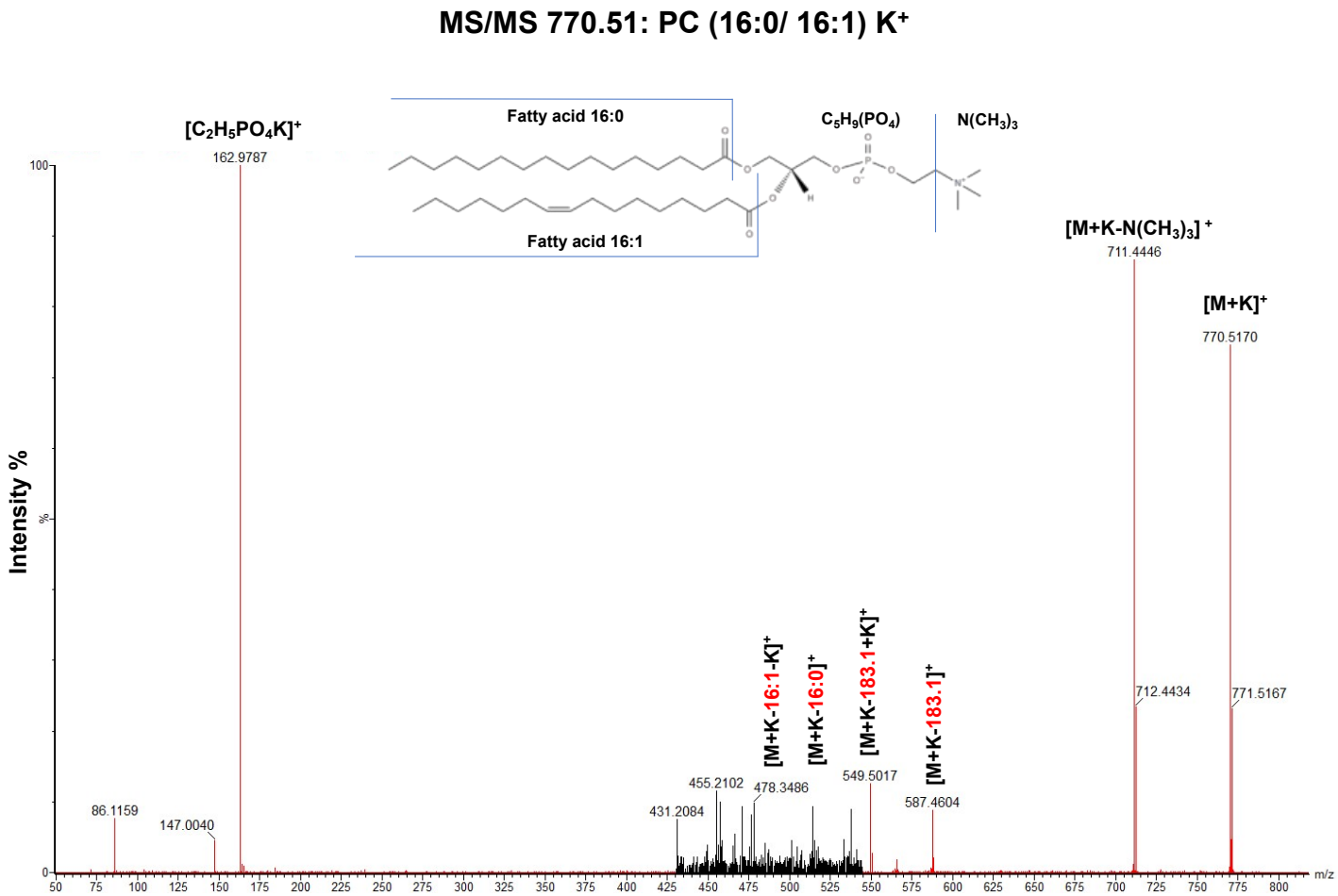


Fig S3. DESI MS/MS spectra and structural identification of mass ion 770.51 analysed in positive ion mode in Caco2 cells. The positively charged K adducted lipid was identified as PC (16:0/ 16:1). A choline head group N $(CH_3)_3$ loss was observed at 711.44 (M+K-$N(CH_3)_3$). The ions at m/z 587.46 and 549.50 correspond to a loss of phosphocholine group (183.1) and K, respectively. The ion at m/z 478.34 corresponds to a loss of 16:1 fatty acid chain. PC- phosphatidylcholine, K- potassium, $N(CH_3)_3$- choline group, and $C_5H_{15}NO_4P$-phosphocholine group. Identification with red colour indicates the lost that occur during the fragmentation.

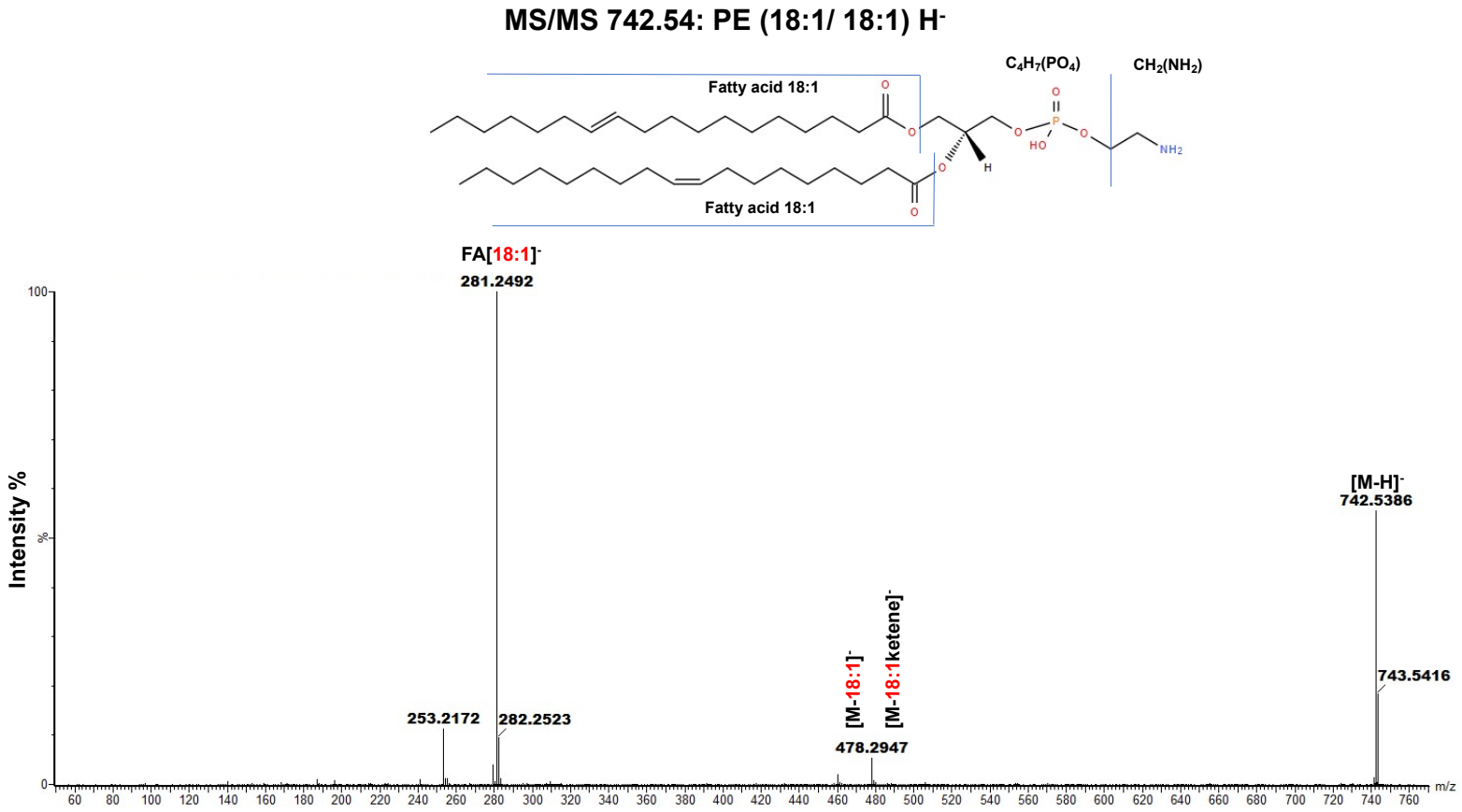


Fig S4. DESI MS/MS spectra and structural identification of mass ion 742.54 analysed in negative ion mode in Caco2 cells.
The negatively charged H adducted lipid was identified as PE (18:1/18:1). The ion at m/z 478.29 corresponds to a loss of 18:1 fatty acid chain and ketene. PE- phosphatidylethanolamine, H- hydrogen, FA- fatty acid. Identification with red colour indicates the lost that occur during the fragmentation.

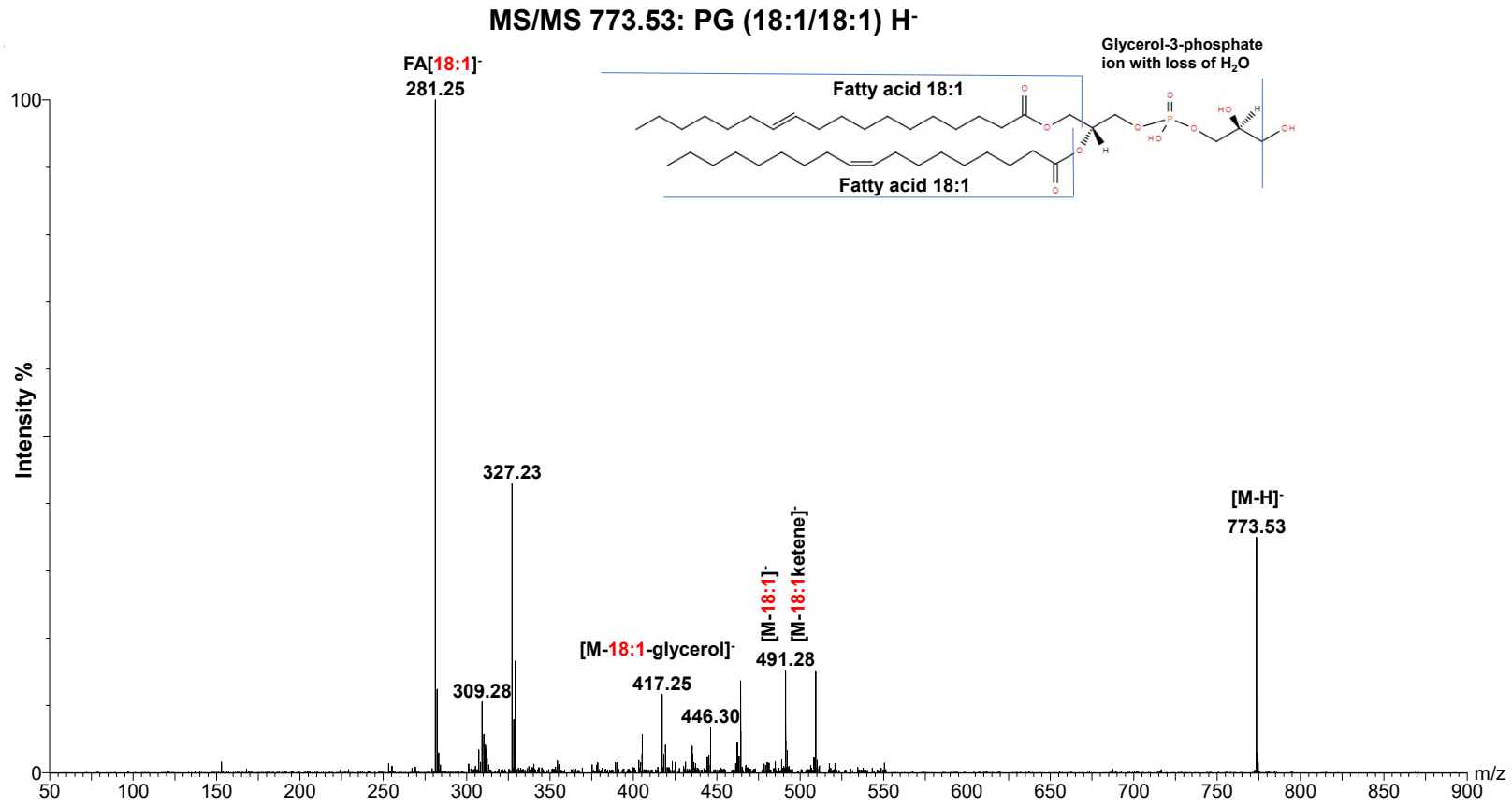


Fig S5. DESI MS/MS spectra and structural identification of mass ion 773.53 analysed in negative ion mode in Caco2 cells.
The negatively charged H adducted lipid was identified as PG (18:1/18:1). A loss of hydrogen ion was observed at 773.53 $[M-H]^-$. The ions at m/z 491.28 correspond to a loss of ketene and 18:1 fatty acid chain. The ion at m/z 417.25 corresponds to a loss of 18:1 fatty acid chain and glycerol. The ion at m/z 281.25 corresponds to a loss of 18:1 fatty acid chain. Identification with red colour indicates the lost that occur during the fragmentation.

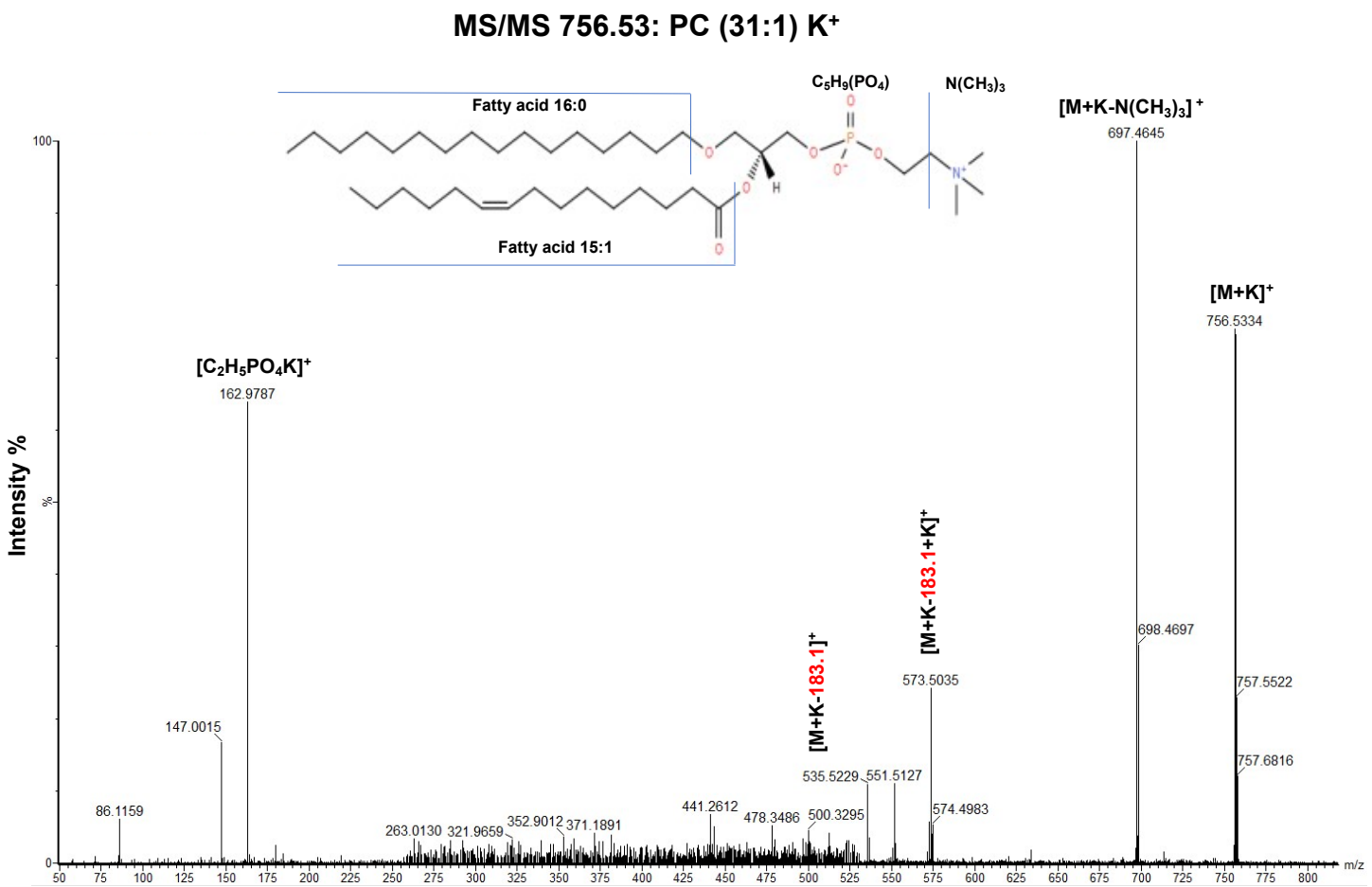


Fig S6. DESI MS/MS spectra and structural identification of mass ion 756.53 analysed in positive ion mode in HT29-MTX cells. The positively charged K adducted lipid was identified as PC (31:1). A choline head group N (CH3)3 loss was observed at 697.46 (M+K-N(CH3) 3). The ions at m/z 573.50 and 535.52 correspond to a loss of phosphocholine group (183.1) and K, respectively. PC-phosphatidylcholine, K- potassium, N(CH3)3- choline group, and C5H15NO4P-phosphocholine group. Identification with red colour indicates the lost that occur during the fragmentation.

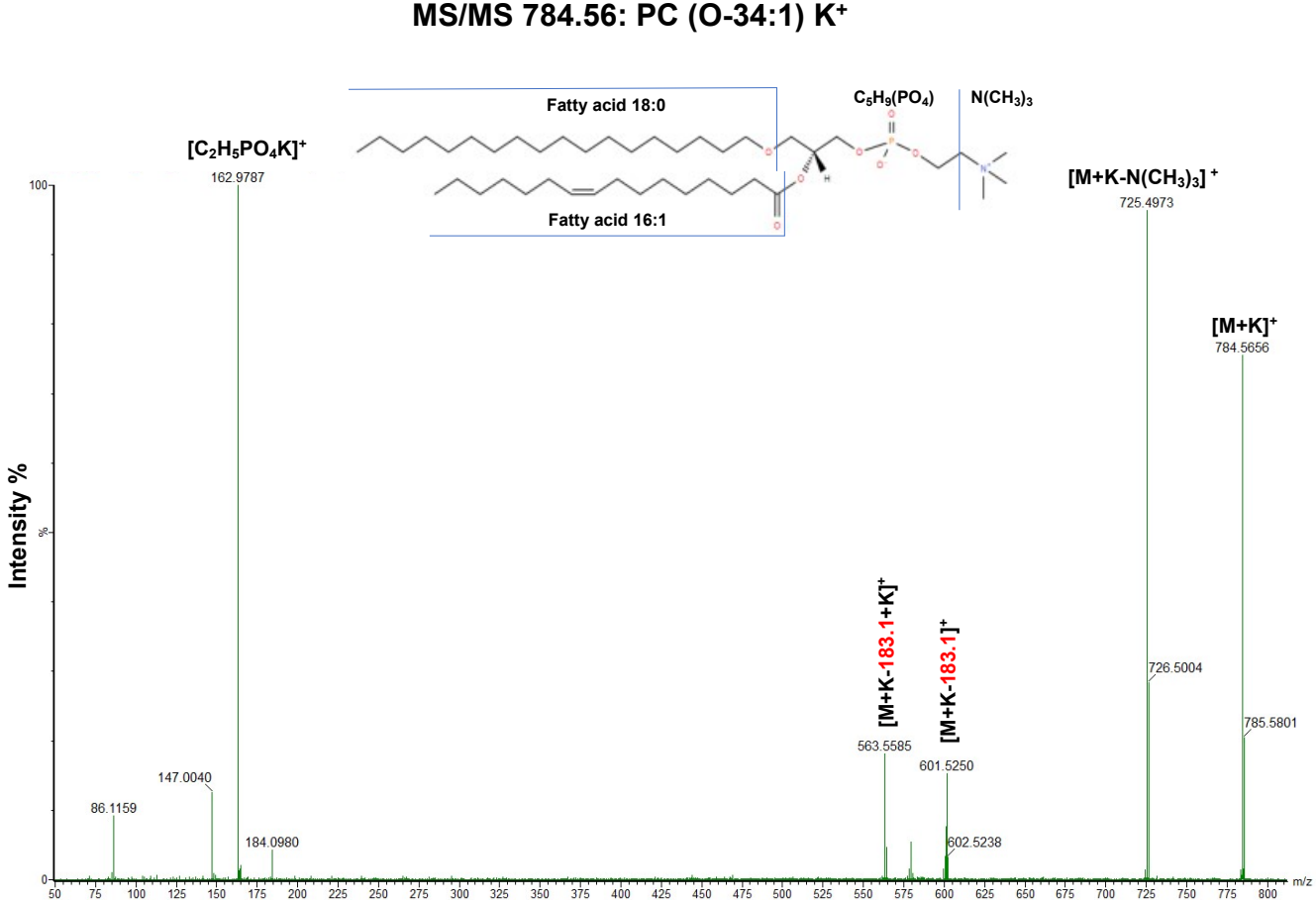


Fig S7. DESI MS/MS spectra and structural identification of mass ion 784.56 analysed in positive ion mode in HT29-MTX cells. The positively charged K adducted lipid was identified as PC (O-34:1). A choline head group N (CH3)3 loss was observed at 725.49 (M+K-N(CH3) 3). The ions at m/z 601.52 and 563.55 correspond to a loss of phosphocholine group (183.1) and K, respectively. PC-phosphatidylcholine, K- potassium, N(CH3)3- choline group, and C5H15NO4P-phosphocholine group. Identification with red colour indicates the lost that occur during the fragmentation.

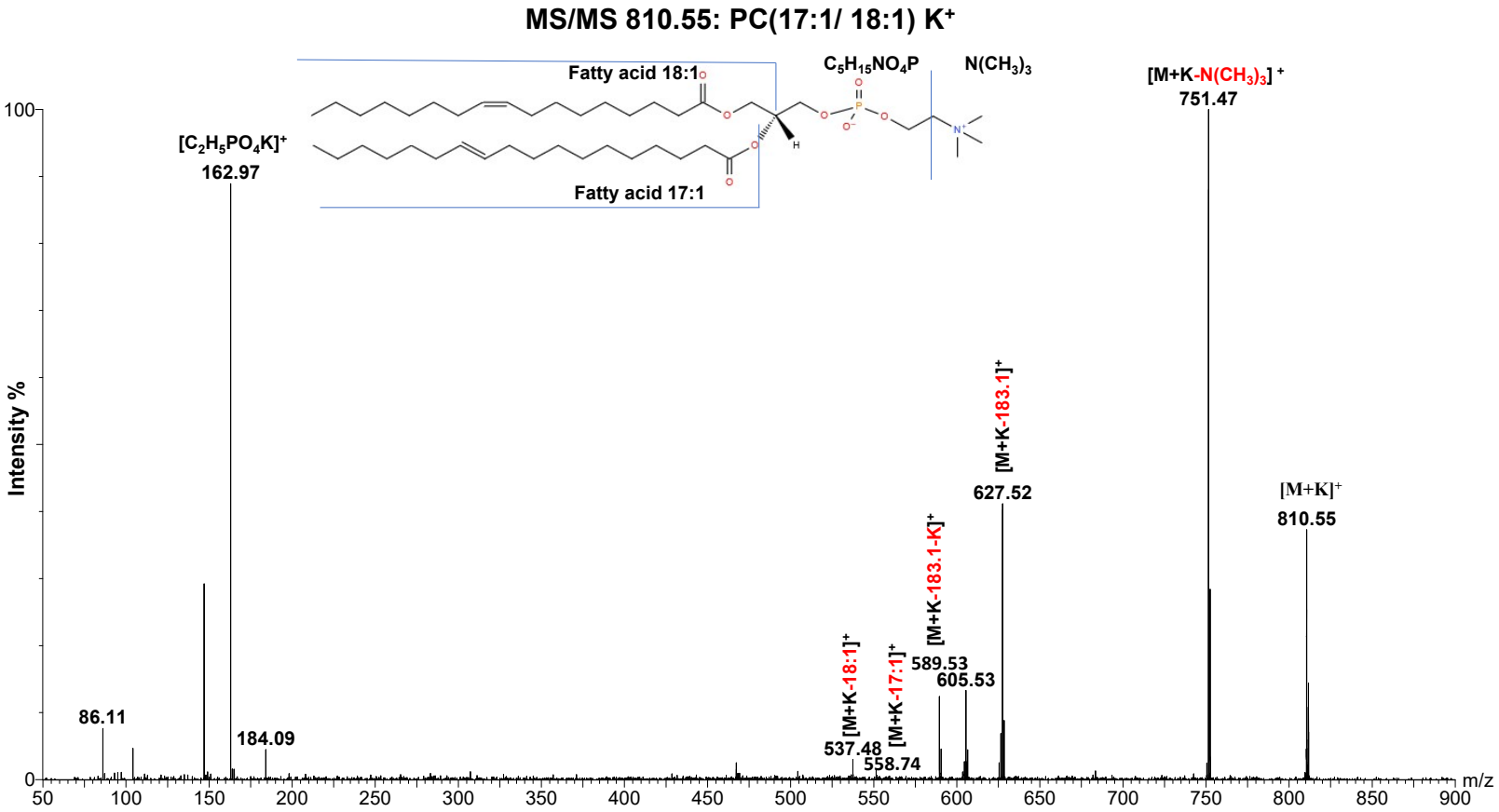


Fig S8. DESI MS/MS spectra and structural identification of mass ion 810.55 analysed in positive ion mode in HT29-MTX cells. The positively charged K adducted lipid was identified as PC (17:1/18:1). A choline head group N (CH3)3 loss was observed at 751.47 (M+K-N(CH3) 3). The ions at m/z 627.52 and 589.53 correspond to a loss of phosphocholine group (183.1) and K, respectively. The ion at m/z 558.74 corresponds to a loss of 17:1 fatty acid chain. The ion at m/z 537.48 corresponds to a loss of 18:1 fatty acid chain. PC-phosphatidylcholine, K- potassium, N(CH3)3- choline group, and C5H15NO4P-phosphocholine group. Identification with red colour indicates the lost that occur during the fragmentation.

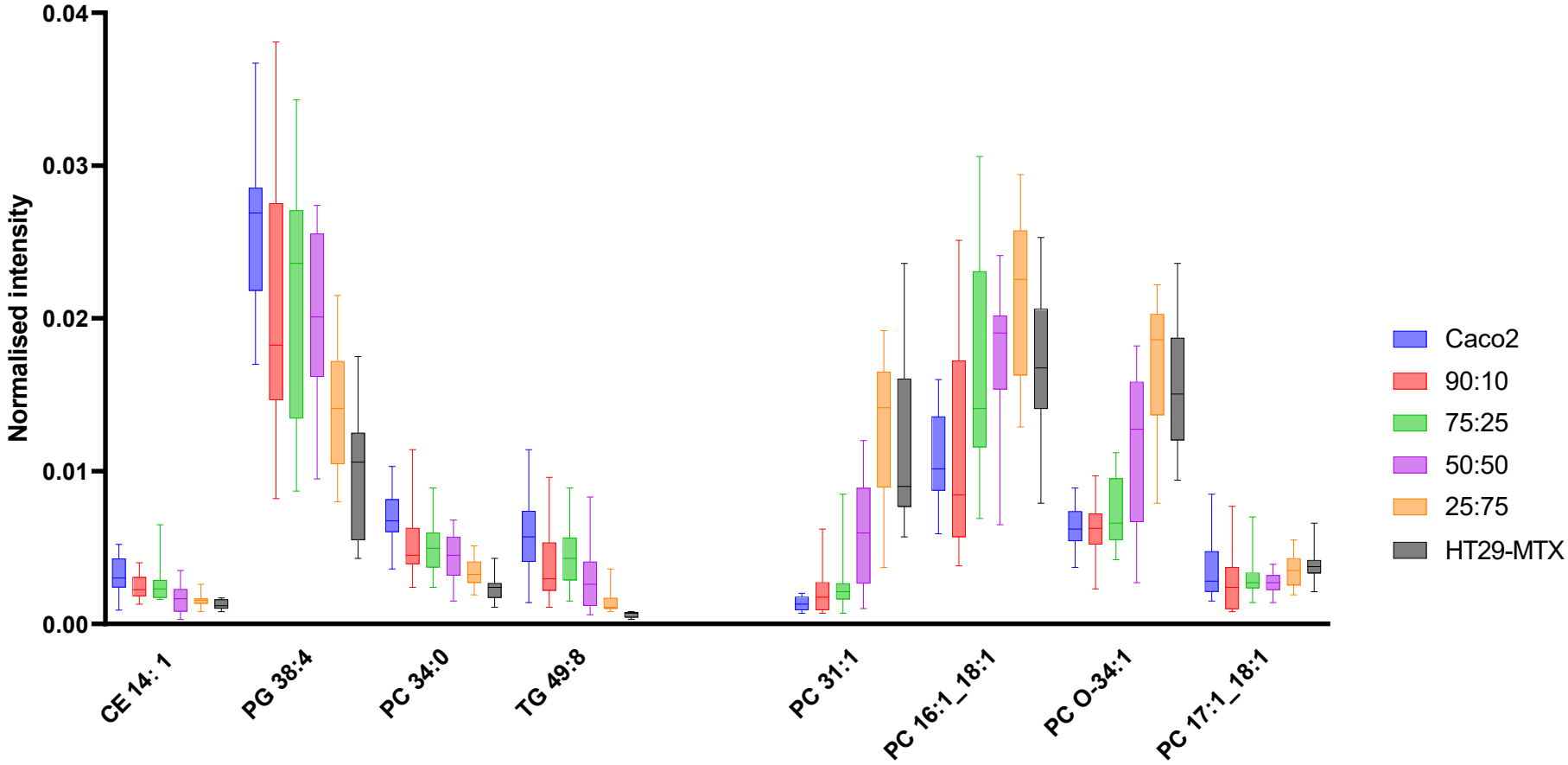


Fig S9. PCA loading plot of the selected lipid species was performed for PCA loading plot of the selected lipid species was performed for Caco2 and HT29-MTX in co-culture system in positive ionisation mode.

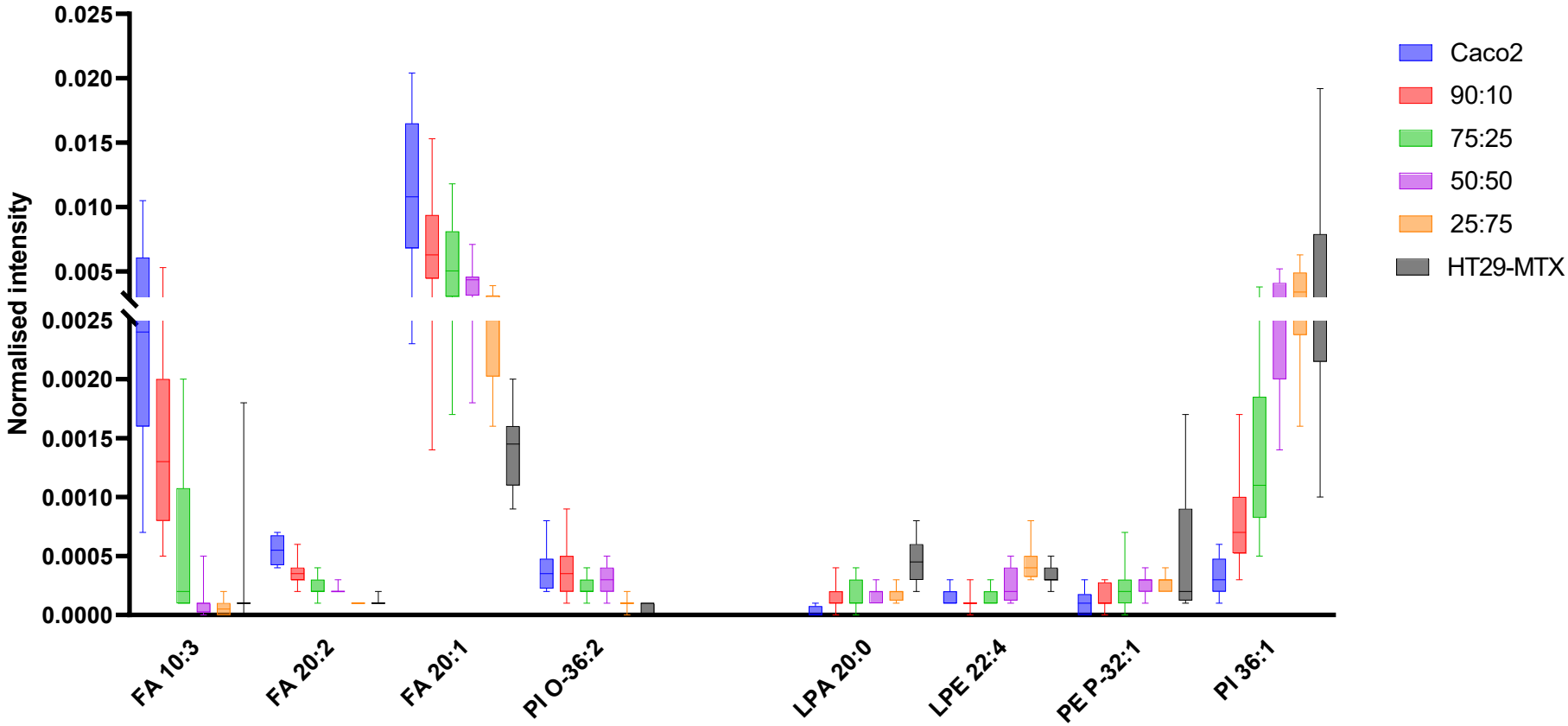
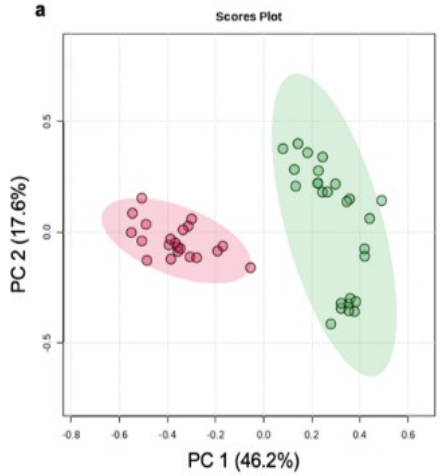
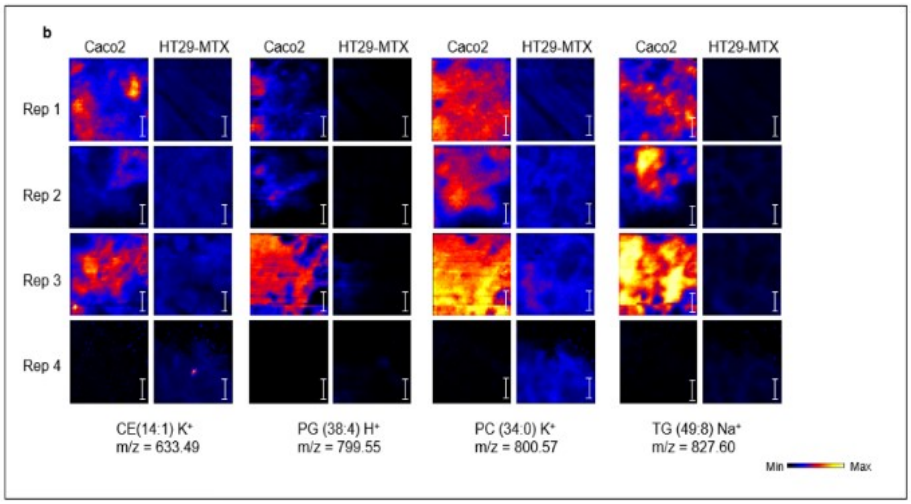


Fig S10. PCA loading plot of the selected lipid species was performed for PCA loading plot of the selected lipid species was performed for Caco2 and HT29-MTX in co-culture system in negative ionisation mode

Figure S11. Comparison of the spatial distribution of lipid in Caco2 and HT29-MTX cell cultures imaged using DESI in positive ionisation mode.
(a) ROIs of 0.05 mm^2 were selected from each replicate MS image and principal analysis (PCA) used to identify lipids that were differentially expressed between two cell lines. ROIs from Caco2 cells are shown by red circles and HT29-MTX cells by green circles. Loadings from PCA plots were used to identify lipids predominantly found in either Caco2 cells (b) or HT29-MTX cells (c). The scale bar = 0.05 mm. Lipid identifications are summarised in Table 1.





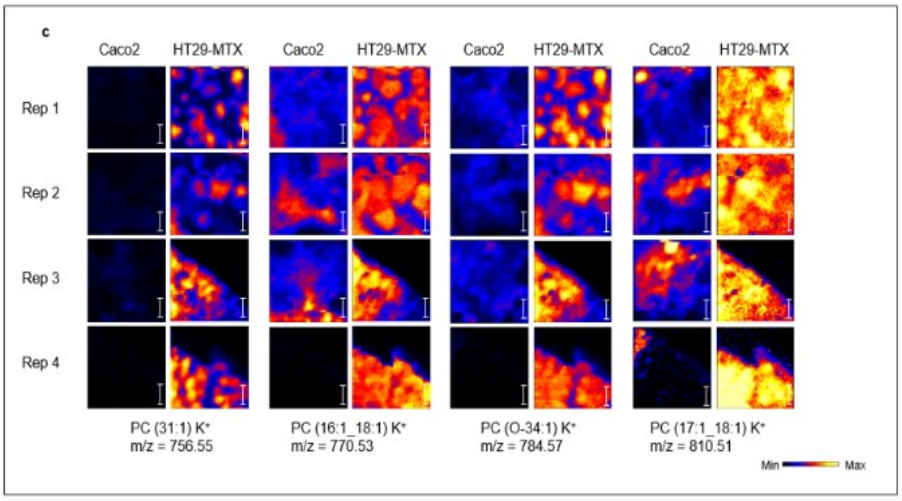
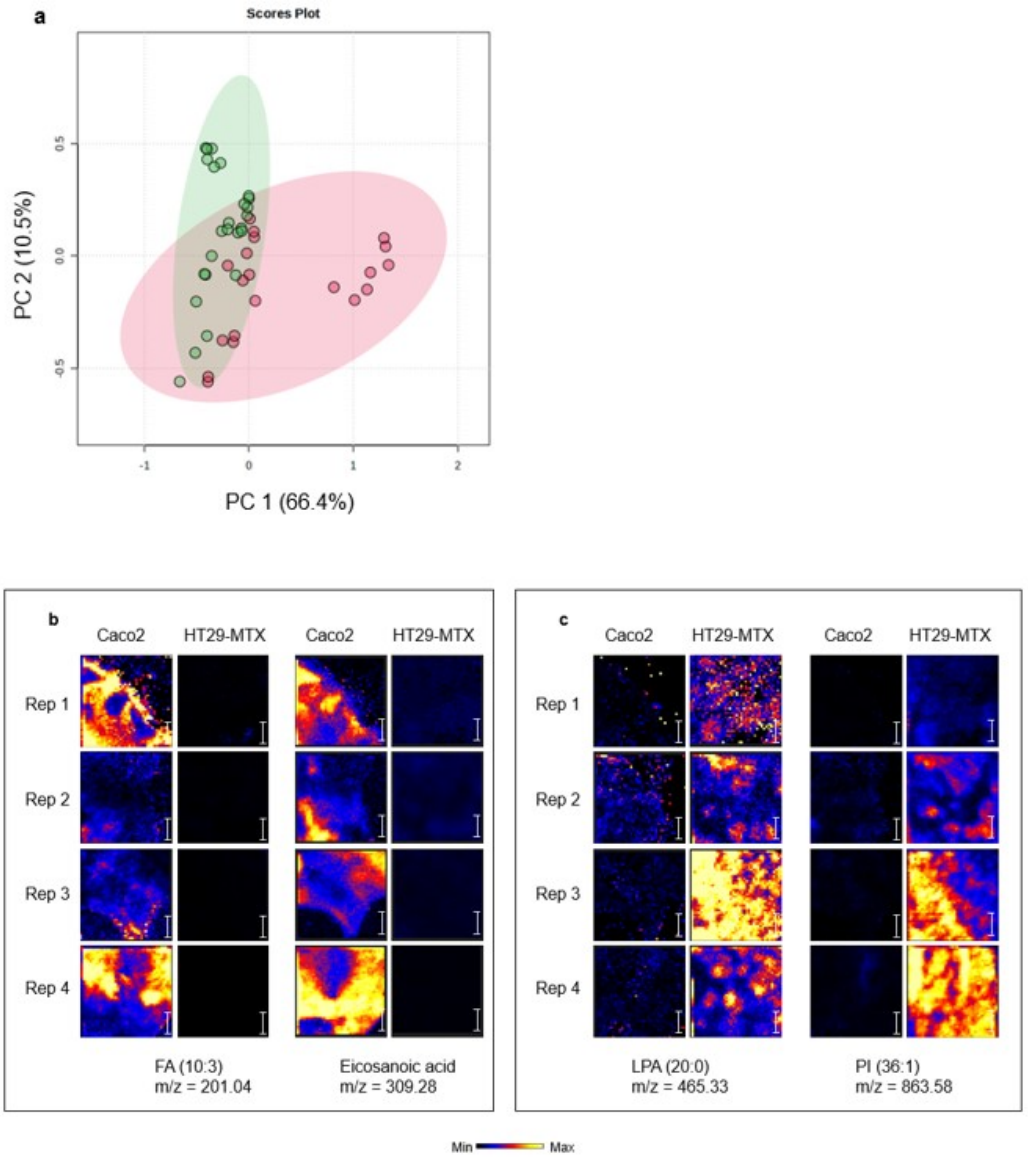


Figure S12. Comparison of the spatial distribution of lipids in Caco2 and HT29-MTX cell cultures imaged in negative ionisation mode.
(a) ROIs of 0.05 mm^2 were selected from each replicate MS image and principal analysis (PCA) used to identify lipids that were differentially expressed between two cell lines. ROIs from Caco2 cells are shown by red circles and HT29-MTX cells by green circles. Loadings from PCA plots were used to identify lipids predominantly found in either Caco2 cells (b) or HT29-MTX cells (c). The scale bar = 0.05 mm. Lipid identifications are summarised in Table 1.



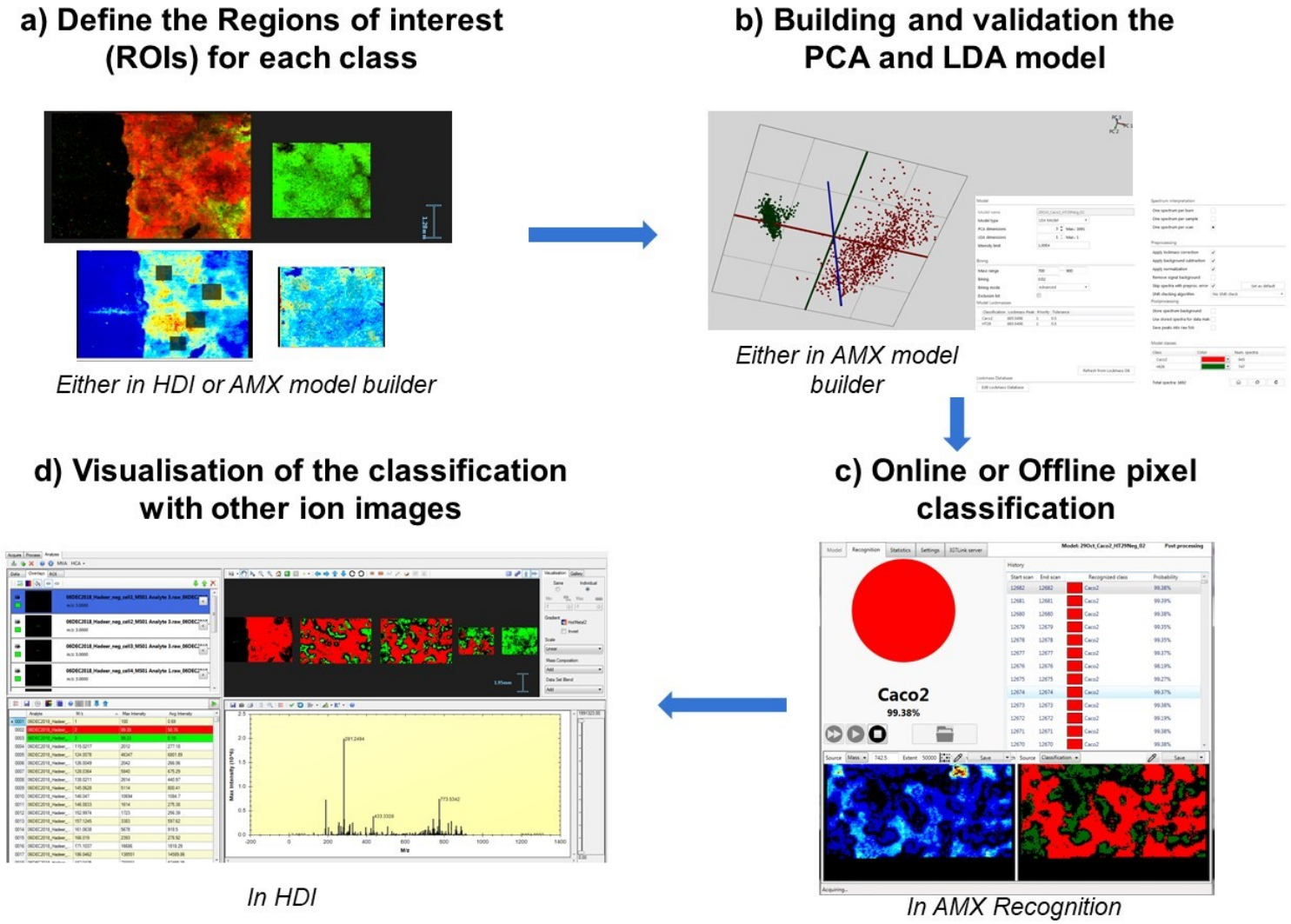


Fig S13. Workflow for pixel classification to localise Caco2 and HT29-MTX cells in the co-culture system.