**Supplementary Information**

**LC-MS Low Energy**

![LC-MS Low Energy graph](image)

**LC-MS High Energy**

![LC-MS High Energy graph](image)

**Mixed QC sample**

![Mixed QC sample graph](image)

**Standard Proline (10 µM)**

![Standard Proline graph](image)

**Fig. S1** LC-MS/MS analysis of m/z 116.0714. LC-MS low (A) and high (B) energy data showing the calculated elemental composition (C₅H₉NO₂) and proposed metabolite (proline) (A) and high energy (MS²) data interrogated using online database search results and MassFragment analysis (B). The structures in red boxes indicate high confidence in the mass differences although the fragmentation event is deemed unlikely to occur, LC-MS/MS sample (C) and proline (D) data showing unambiguous confirmation of metabolite ID by comparative LC-MS/MS of a sample exact mass retention time (EMRT) pair and an authenticated reference standard.
Fig. S2 2-way PCA scores plot (A) and S-plot (B) showing the anti-viral effect on C33A E6 transfected cells exposed to two different doses of indinavir (0 mM vs. 0.15 mM). (A) PC1 (69.78 %) vs. PC2 (12.51 %); first two letters indicate the type of cells and the name of the drug, third number indicates the number of biological replicates, last number represents the drug concentrations (mM); red: 0 mM; blue: 0.15 mM, (B) each point represents an EMRT pair. The EMRT pairs observed to show positive or negative values represent the ions contributing to the separation of each sample group.

Fig. S3 Block loadings plots of E6 cells exposed to indinavir (A) and lopinavir (B) with significant variables marked by red. Significant variables were identified by Friedman test.