

# Supplementary File S1

## Exploring Metabolic Signatures of Ovarian Cancer Ex Vivo Models to Predict Drug Efficacy

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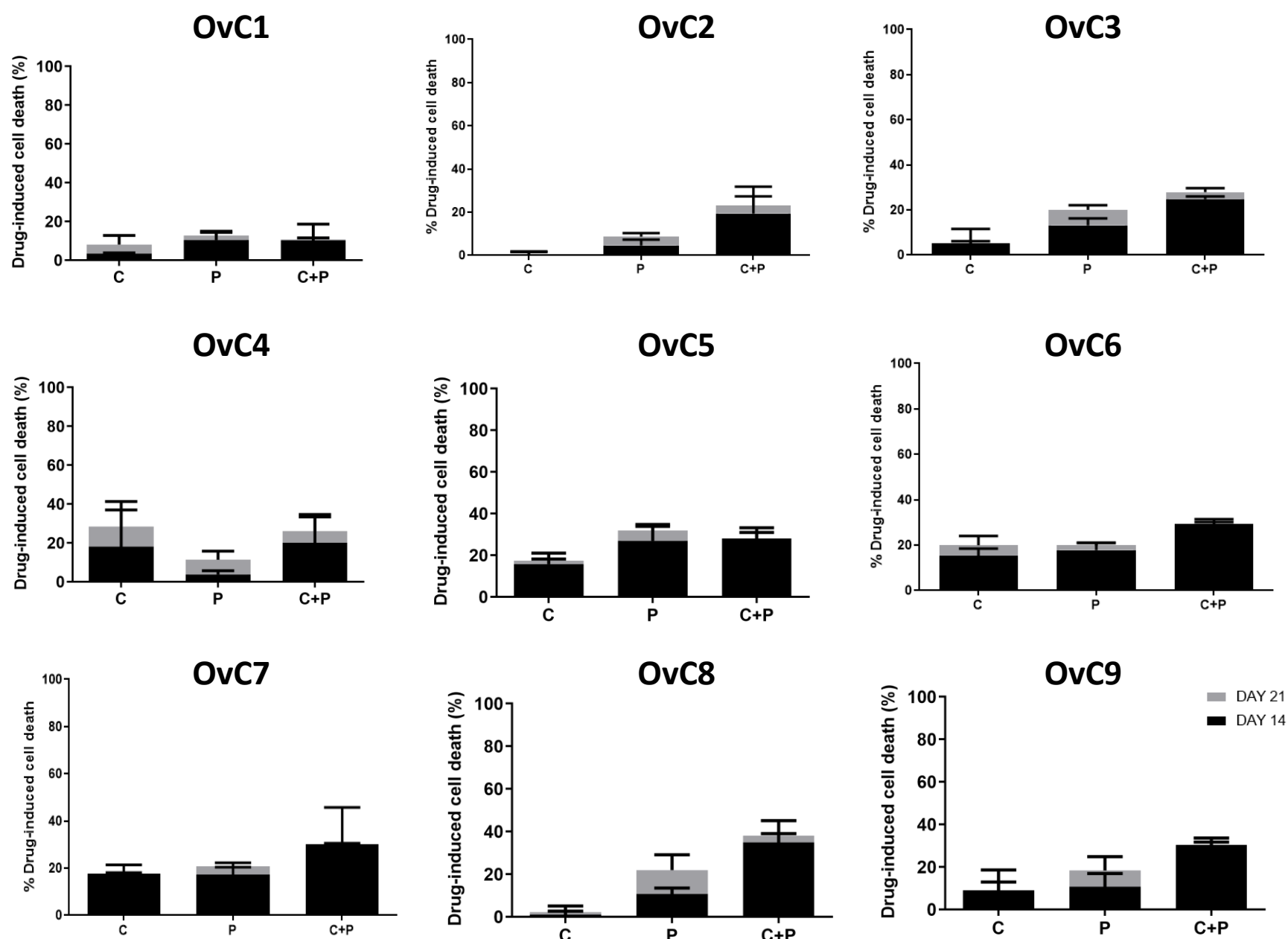
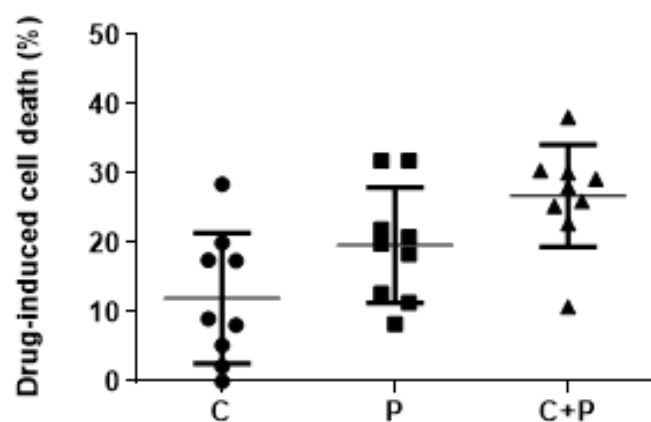
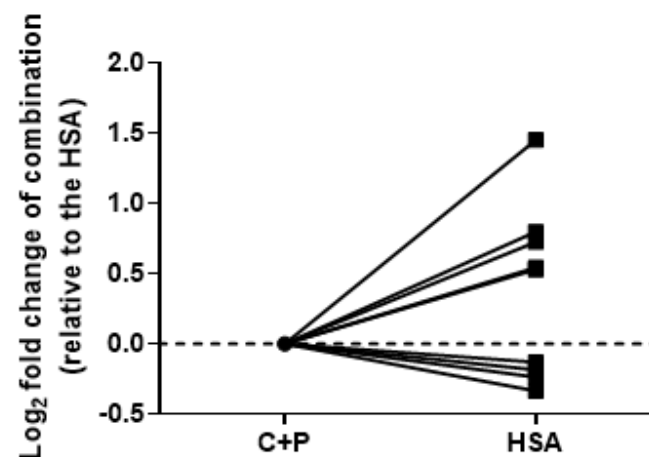
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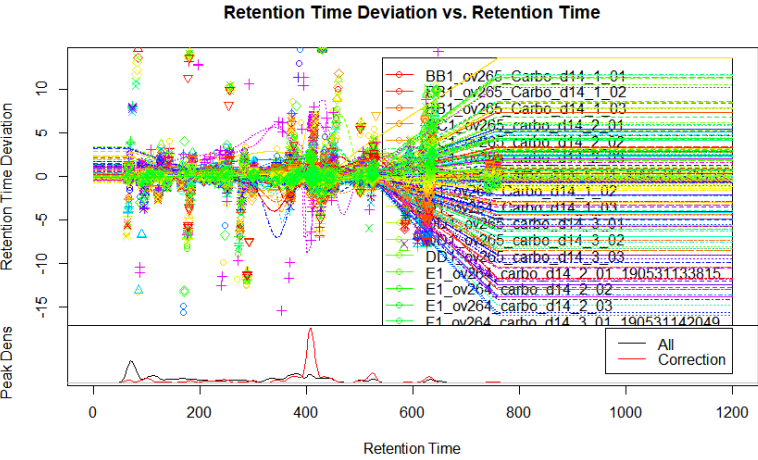
<sup>7</sup> Pathology Department, Instituto Português de Oncologia de Lisboa Francisco Gentil (IPOLFG), Rua Prof. Lima Basto, 1099-023 Lisboa, Portugal

\* Correspondence: [iaisidro@ibet.pt](mailto:iaisidro@ibet.pt)

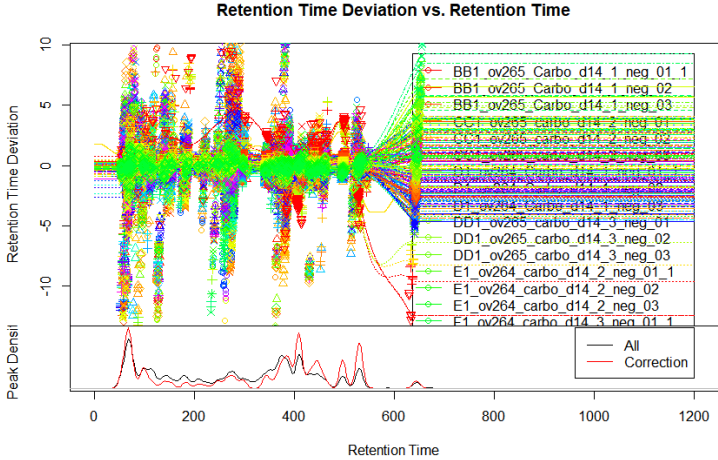
**A****B****C**

**Supplementary Figure S1: Sample-specific response to combination (C+P) and single drugs cyclic drug exposure in serous OvC patient cohort.** (A) Drug-induced cell death evaluation of OvC-PDE using LDH assay after 2 drug cycles. Data is presented as mean  $\pm$  SD of three technical replicates per OvC-PDE case. (B) Drug-induced cell death of OvC-PDE along two drug cycles, evaluated by the LDH assay. (C) Log<sub>2</sub> fold change of the combination drug-induced cell death relative to the drug-induced cell death of the highest single agent (HSA). (N=9, C: carboplatin, P: paclitaxel; LGSC: low-grade serous carcinoma).

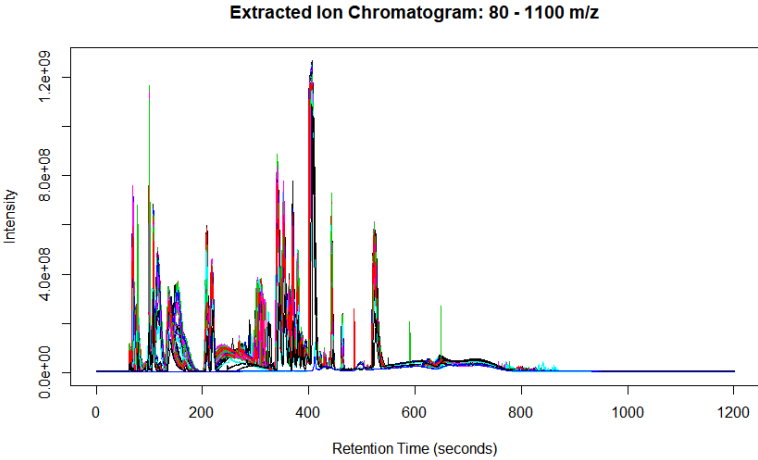
Ai



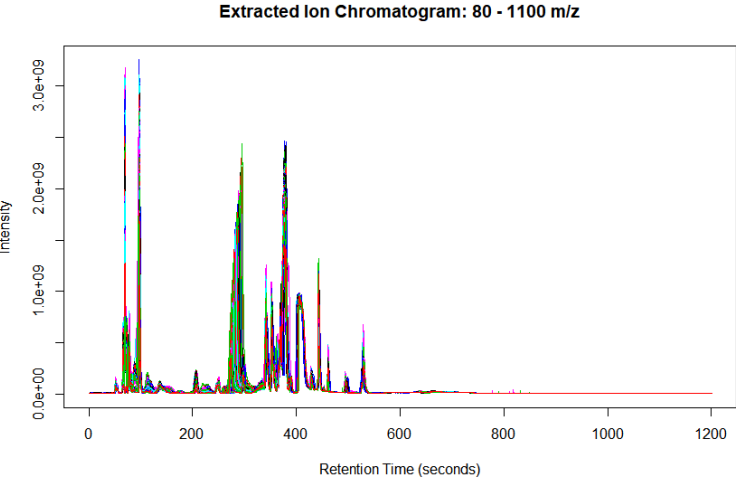
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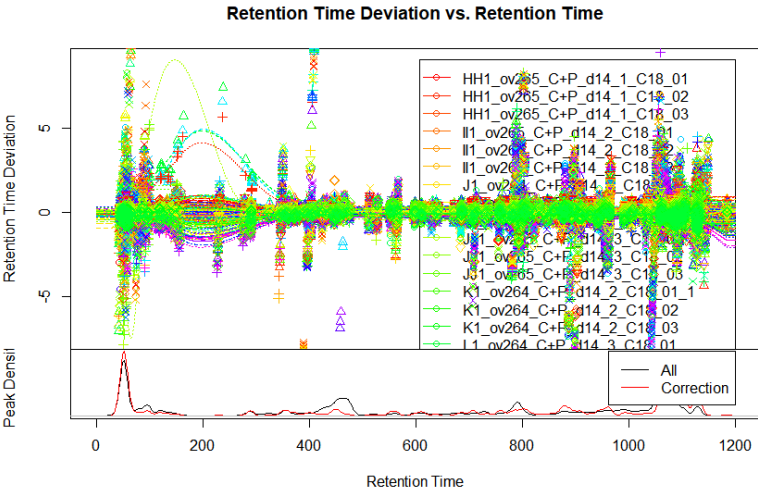
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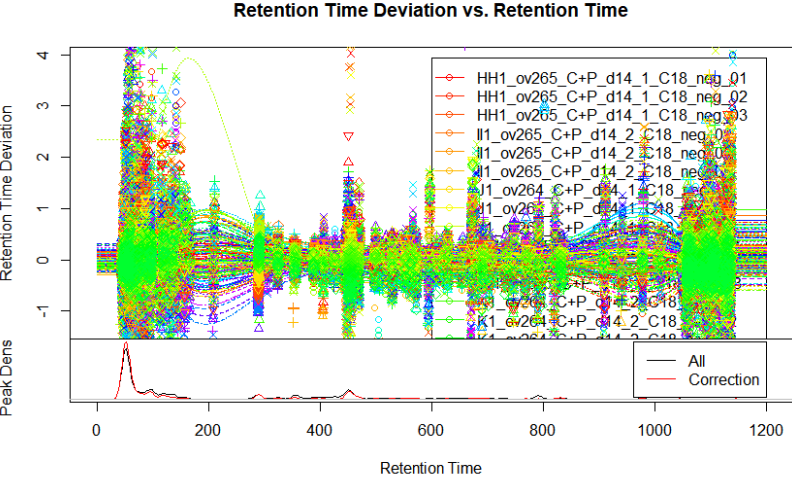
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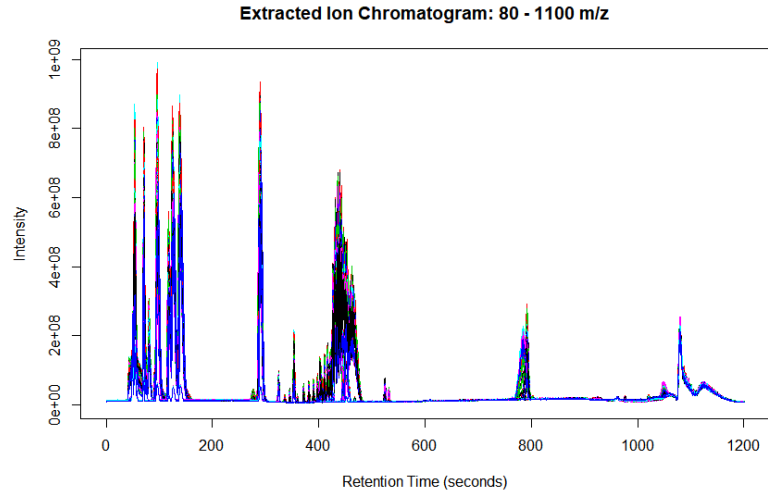
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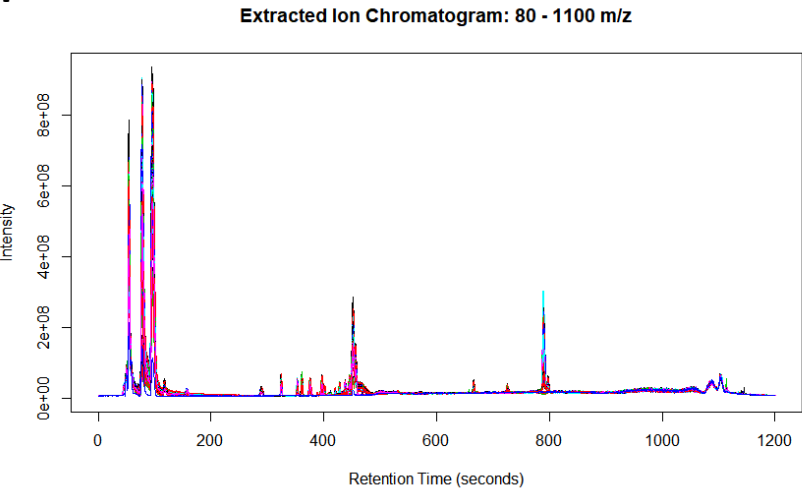
Di



Cii

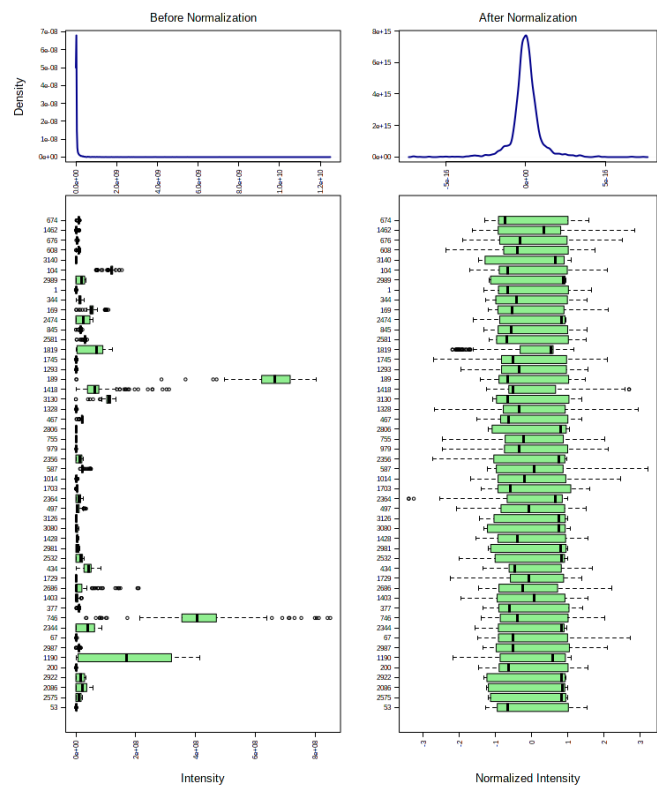


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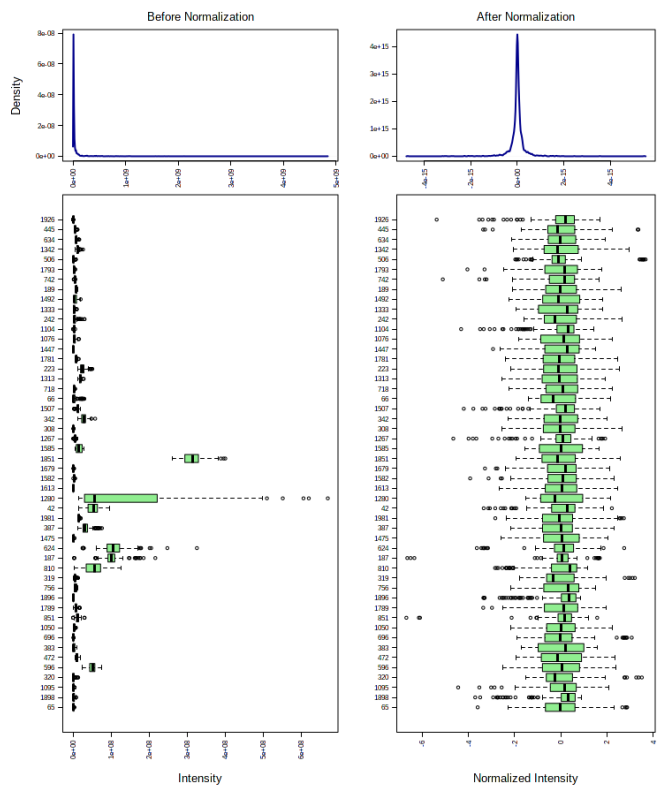


**Supplementary Figure S2: Metabolomics data preprocessing: Peak alignment (i) and extracted ion chromatogram (EIC) (ii) using XCMS software applied in the four untargeted metabolomics datasets. (A) HILIC(+); (B) HILIC(-); (C) RPLC(+); and (D) RPLC(-). (HILIC: hydrophilic interaction liquid chromatography; RPLC: reversed phase liquid chromatography;(+):positive mode; (-):negative mode)**

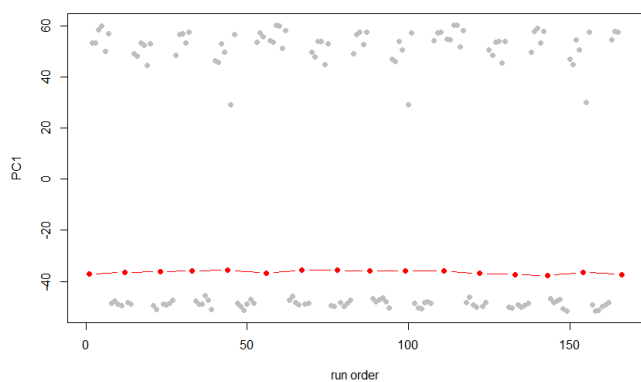
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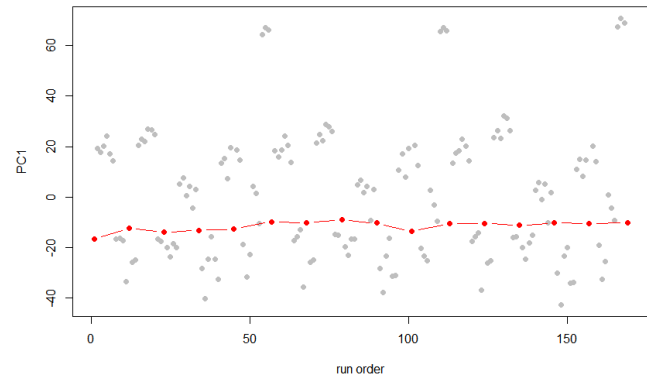
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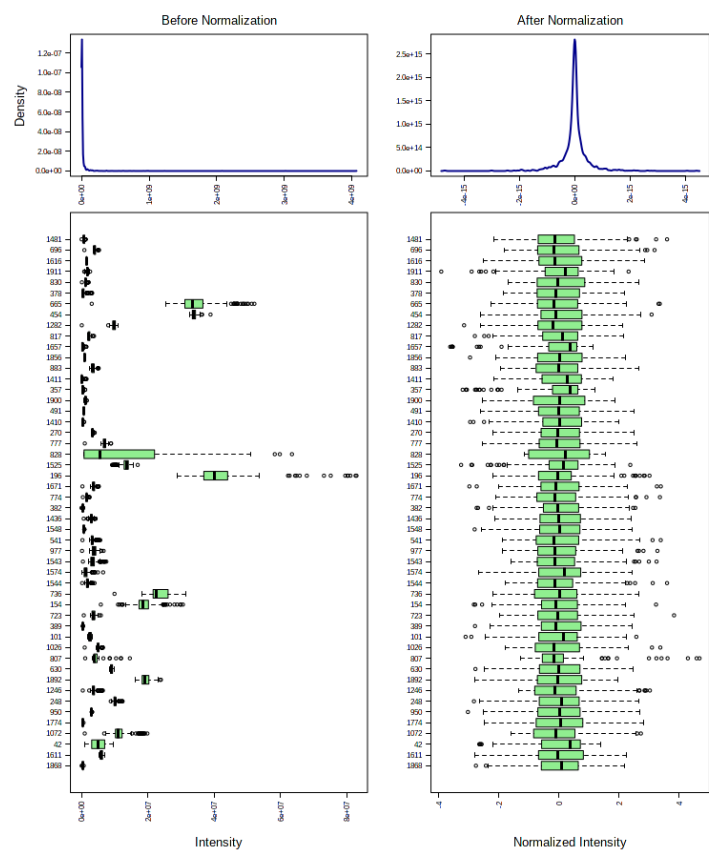
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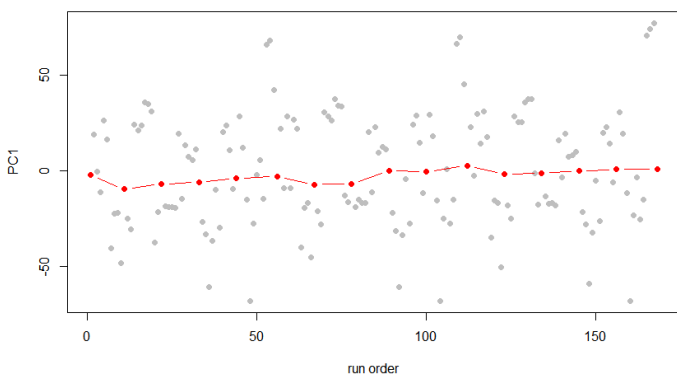
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Ci

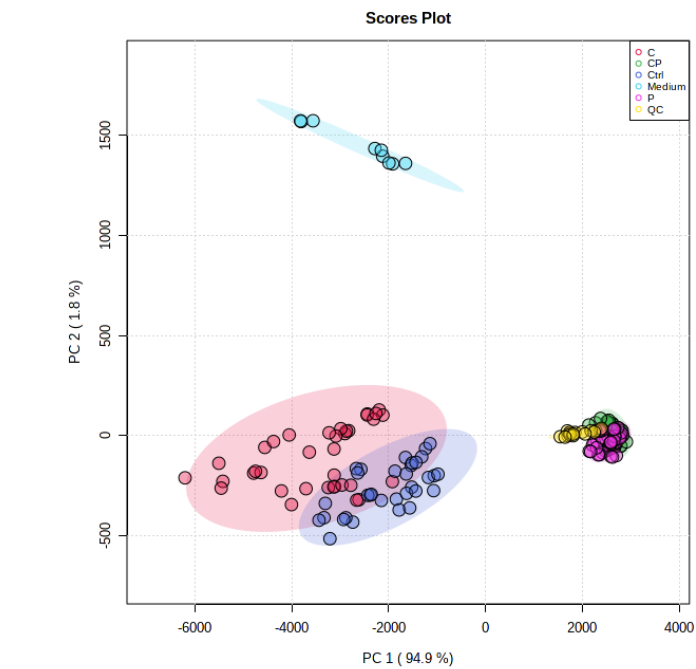


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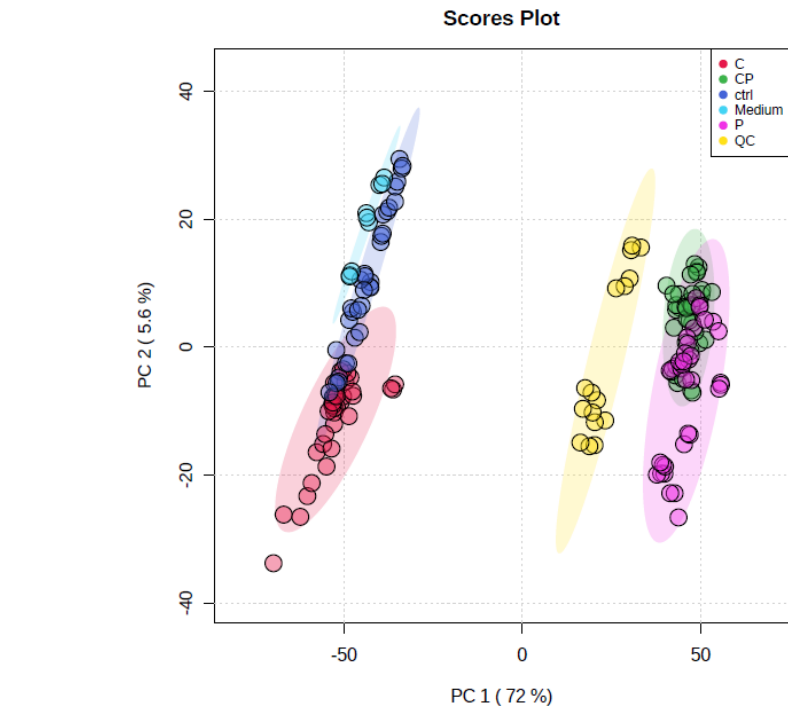


**Supplementary Figure S3: Metabolomics data pretreatment in untargeted metabolomics datasets. (i) Normalization, including scaling and transformation, of peak intensities across features and (ii) stability of QCs over injection run. QCs in red; samples in grey. (A) HILIC(+); (B) HILIC(-); (C) RPLC(+); and (D) RPLC(-). (HILIC: hydrophilic interaction liquid chromatography; RPLC: reversed phase liquid chromatography; (+):positive mode; (-):negative mode, QC: quality control samples).**

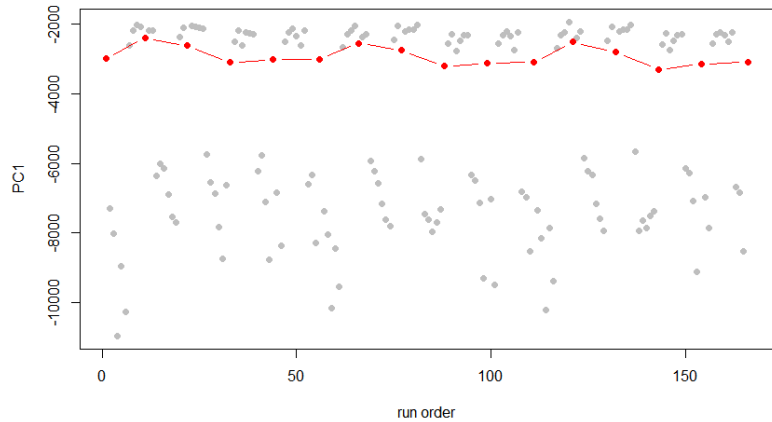
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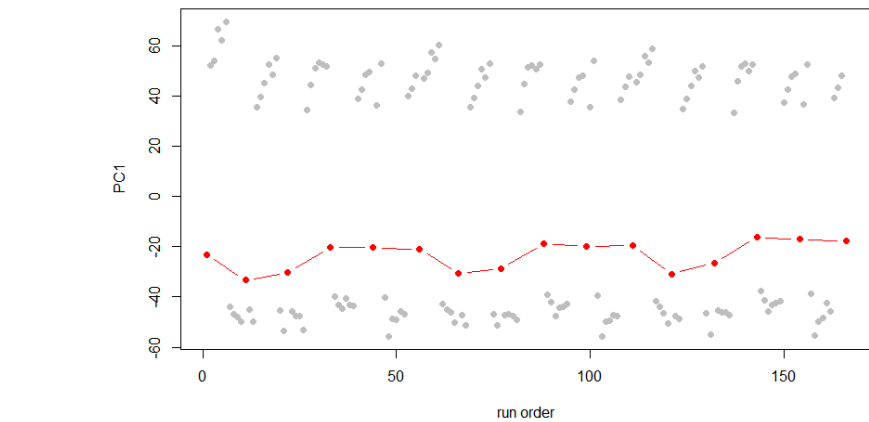
Bi



Aii

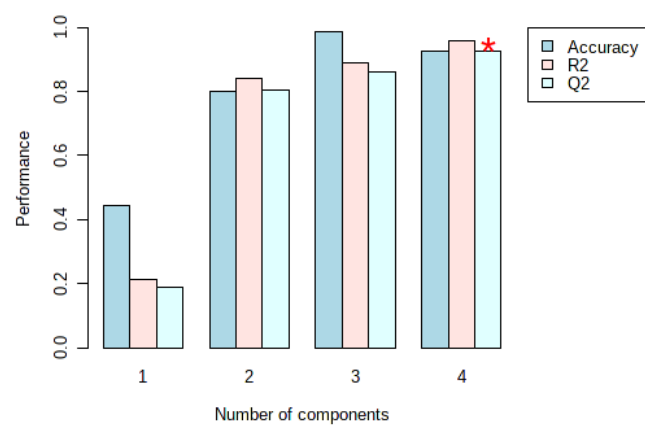


Bii



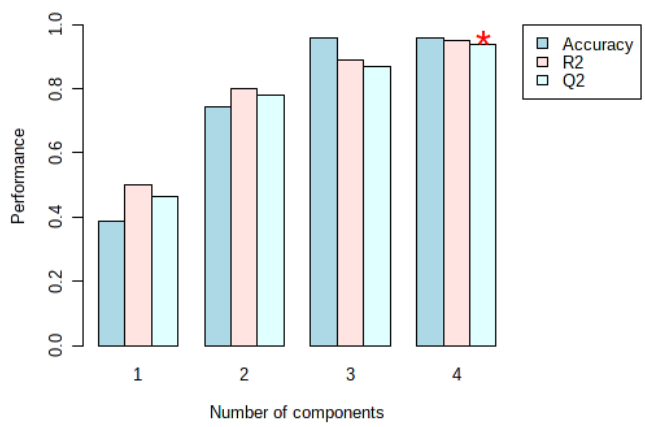
**Supplementary Figure S4: Untargeted metabolic footprints distinct treatment groups of 2 HGSC OvC-PDE samples using RPLC in the positive mode.** Multivariate data analysis performed using **(i)** principal component analysis (PCA) **(A)** when samples were normalized by the median or **(B)** normalized as other columns/modes and **(ii)** stability of QCs over injection run (ii: QCs in red; samples in grey) (C: carboplatin, P: paclitaxel; ctrl: untreated control; Medium: culture media blanks; QC: quality control sample; RPLC: reversed phase liquid chromatography)

Ai



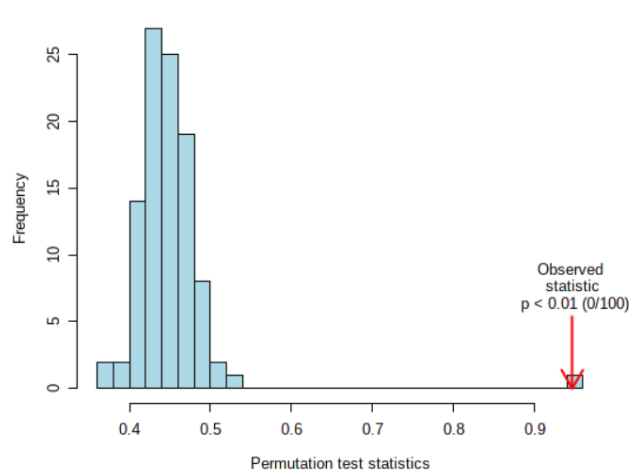
Empirical p value:  $p < 0.01$  (0/100)

Bi

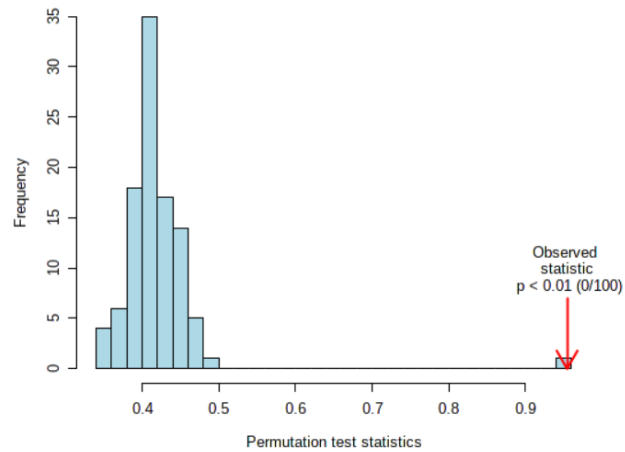


Empirical p value:  $p < 0.01$  (0/100)

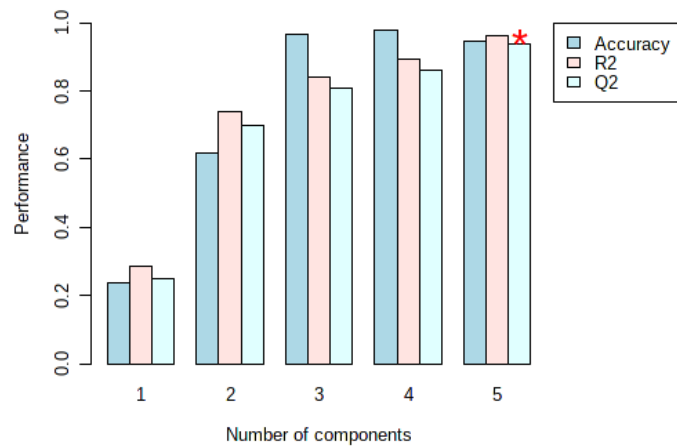
Aii



Bii

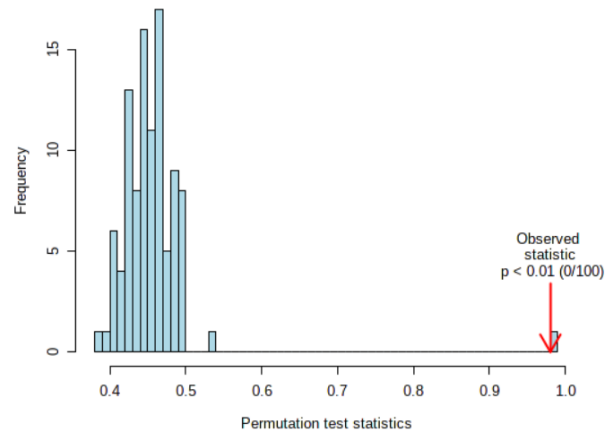


Ci



Empirical p value:  $p < 0.01$  (0/100)

Cii



**Supplementary Figure S5: Cross-validation (i) and 100 permutation tests (ii) for PLS-DA classification using (A) HILIC(+); (B) HILIC(-); and (C) RPLC(-) datasets. ((HILIC: hydrophilic interaction liquid chromatography; RPLC: reversed phase liquid chromatography; (+):positive mode; (-):negative mode)**

A

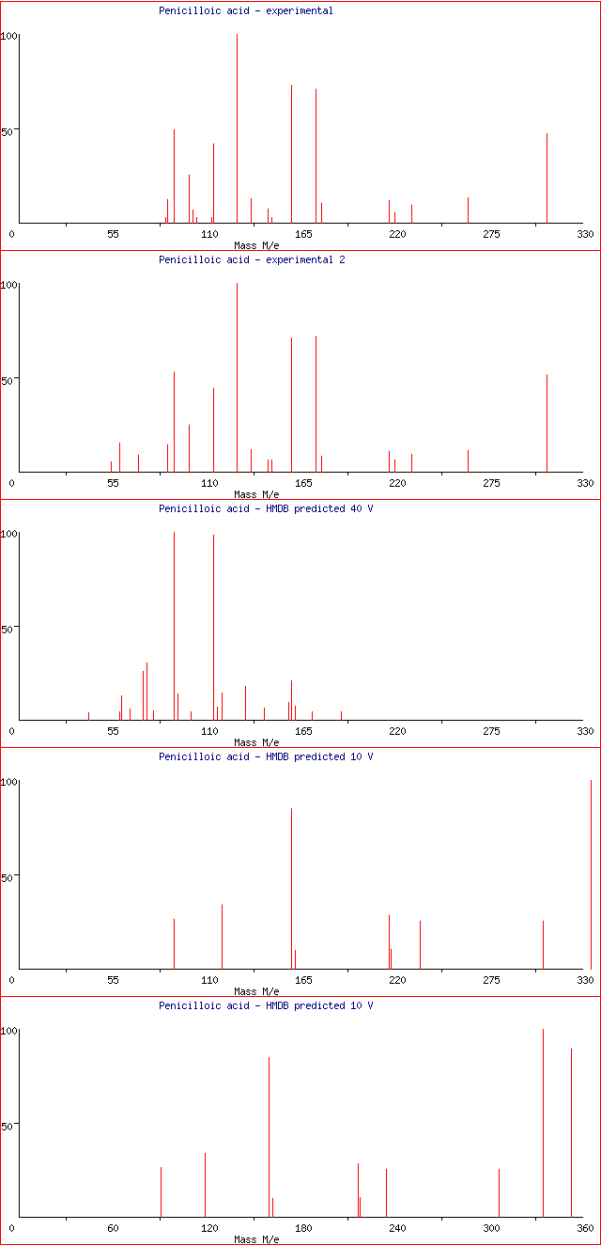
Feature 1597

B

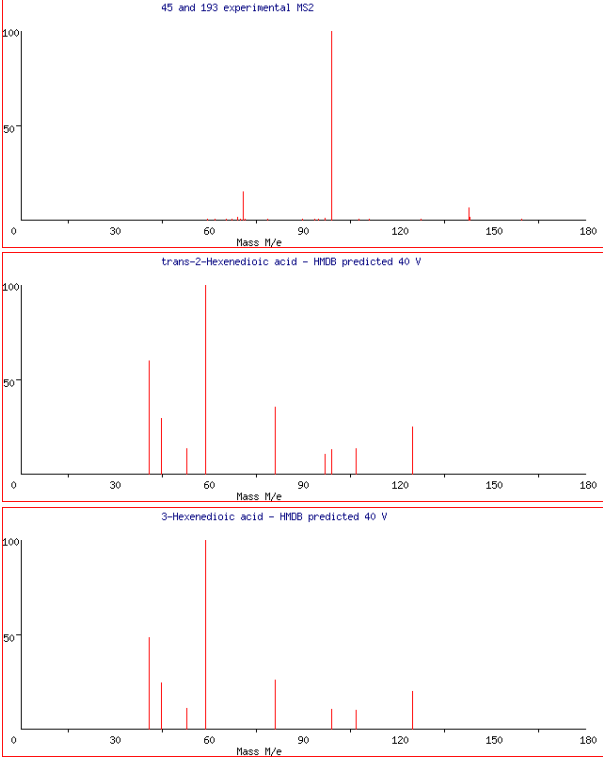
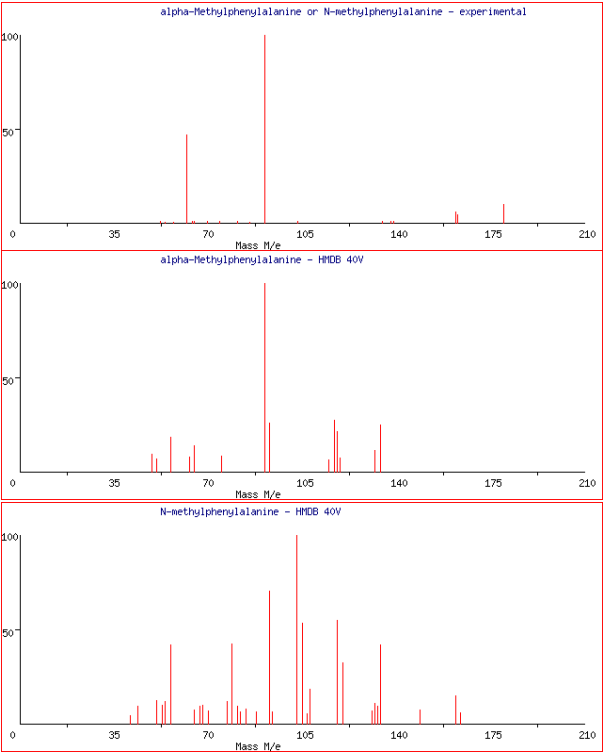
Features 45/193

C

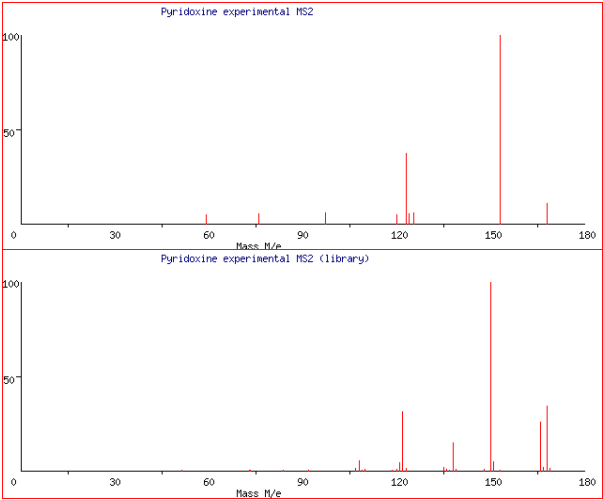
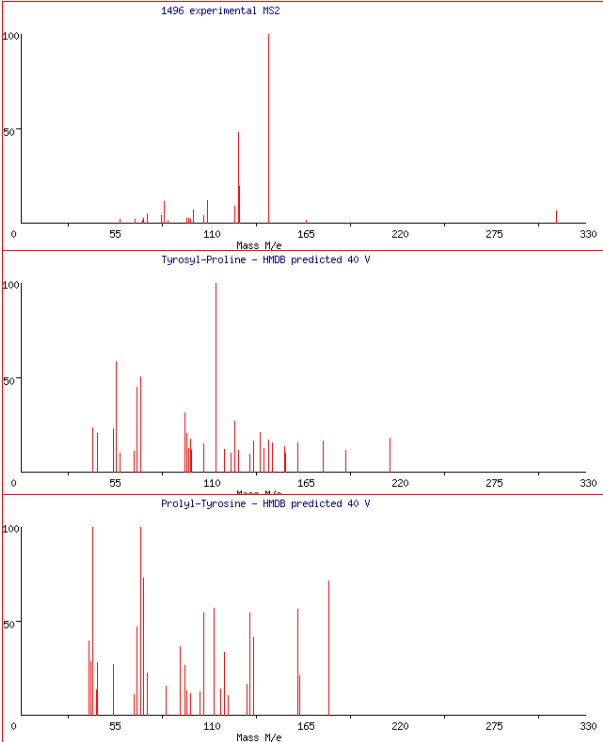
Features 1228/1291/1387/1385



Feature 376

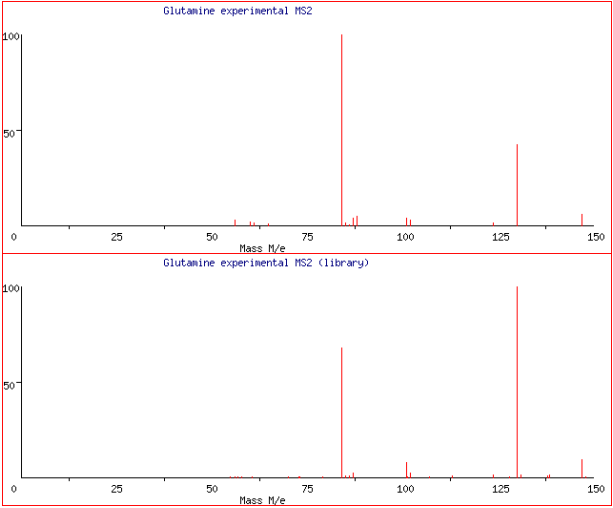


Feature 1496

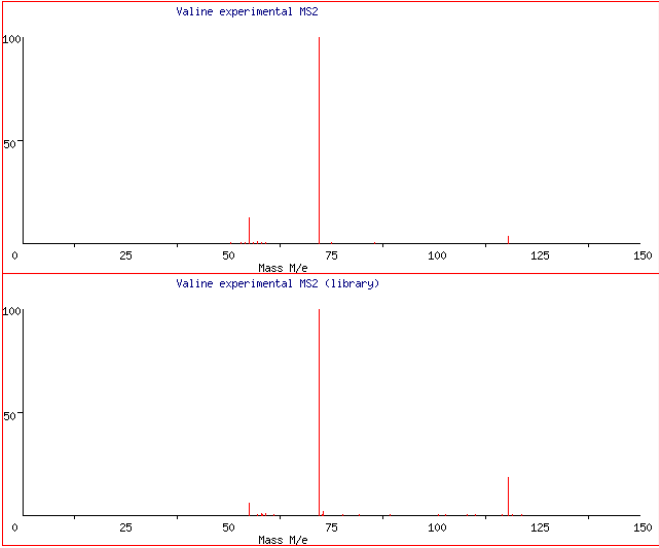


**Supplementary Figure S6: Spectra of top discriminating features uncovered by untargeted metabolomics in datasets (A) HILIC(+), (B) HILIC(-) and (C) RPLC(-).** (upper: experimental MS2 and lower: MS2 spectra from library) (HILIC: hydrophilic interaction liquid chromatography; RPLC: reversed phase liquid chromatography; (+):positive mode; (-):negative mode)

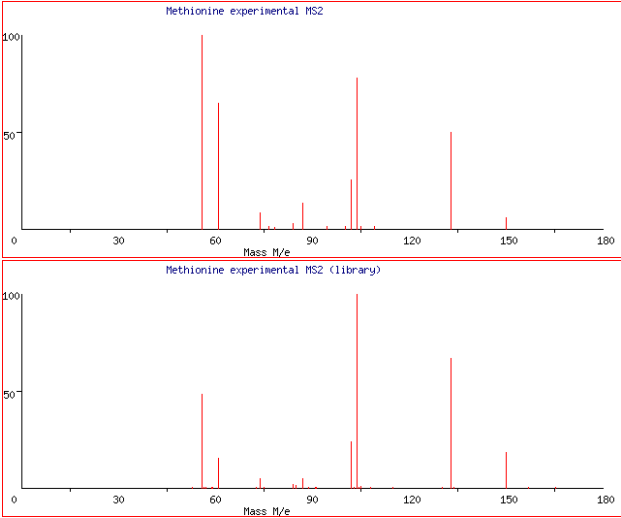
L-Glutamine



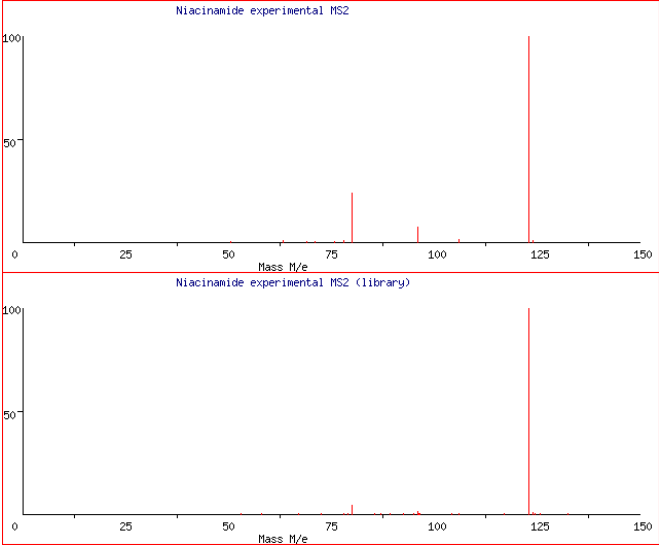
L-Valine



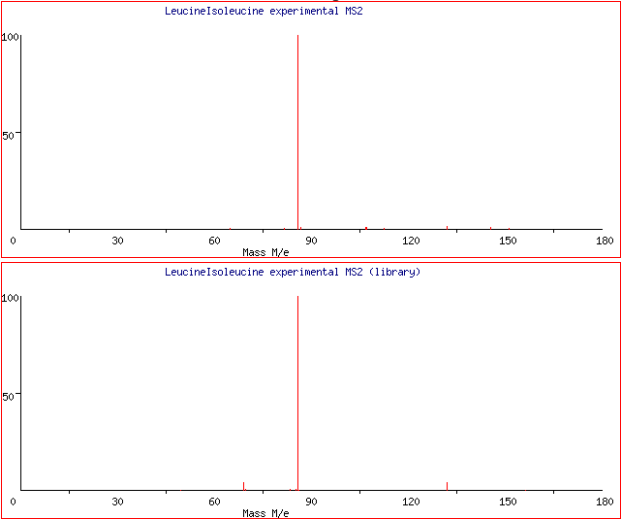
L-Methionine



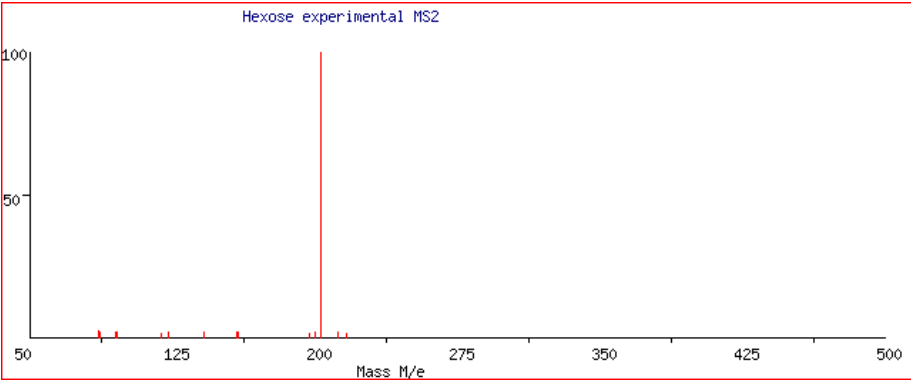
Niacinamide



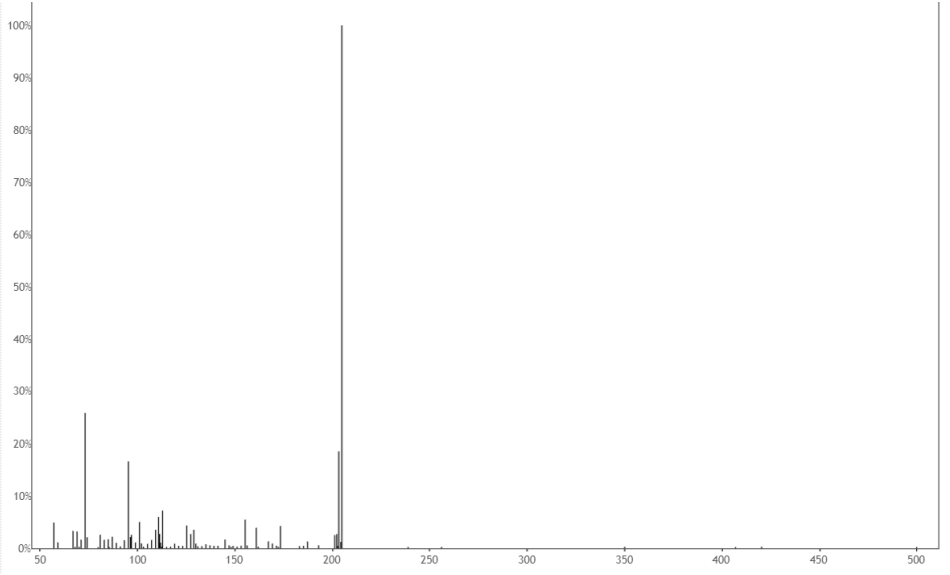
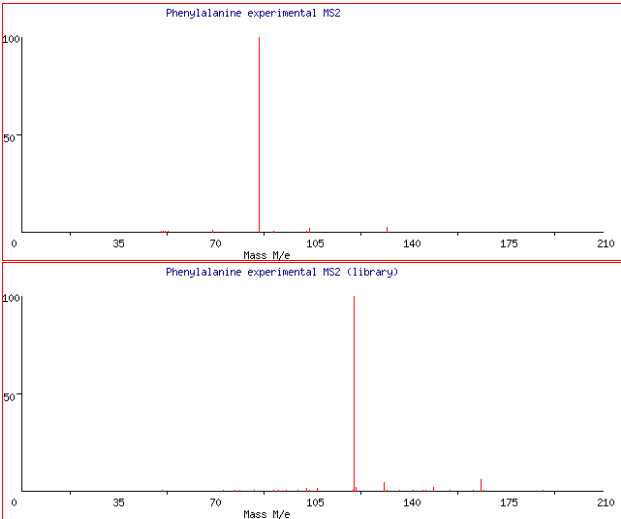
L-Isoleucine/L-Leucine



Hexose



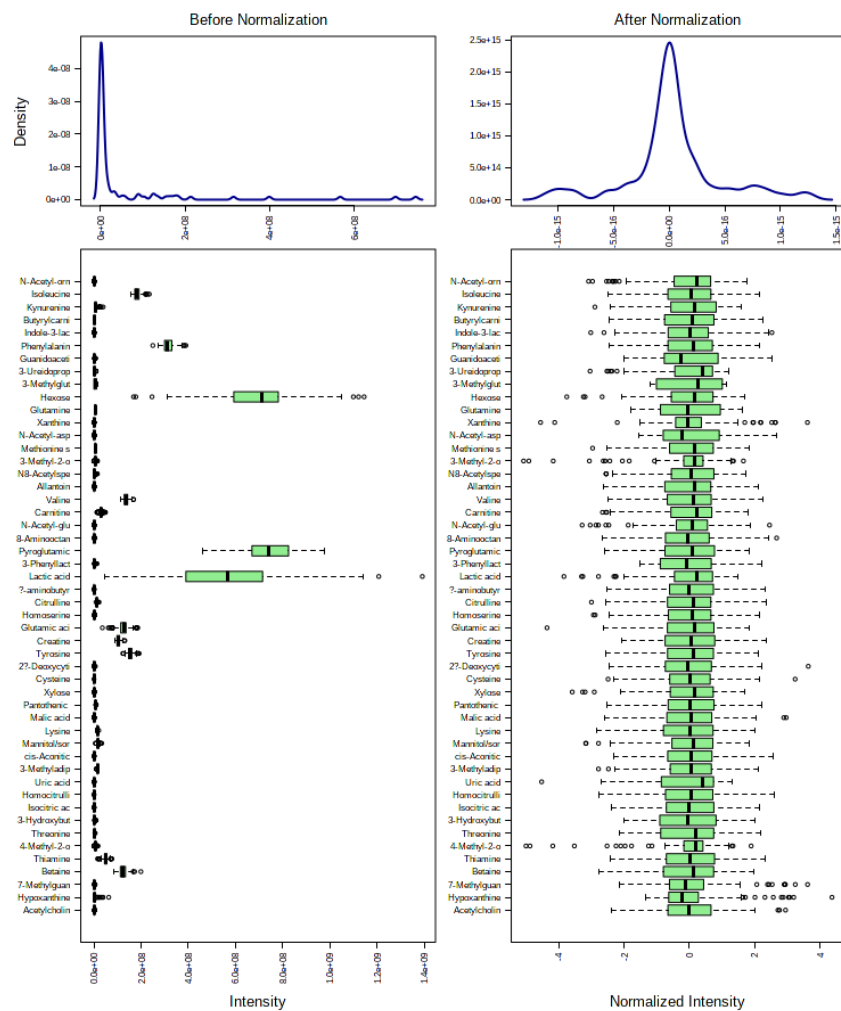
L-Phenylalanine



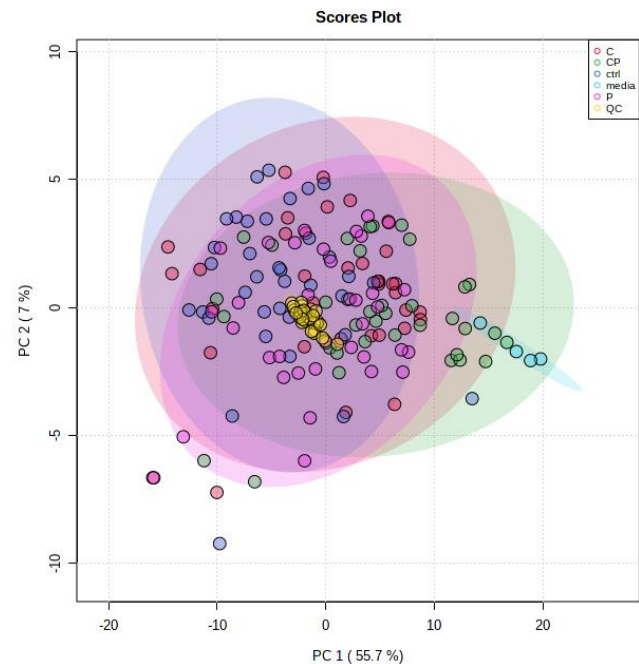
Supplementary Figure S7: Spectra of the metabolites present in the basal medium (DMEM) used to culture OvC-PDE.(upper: experimental MS2 and lower: MS2 spectra from library)



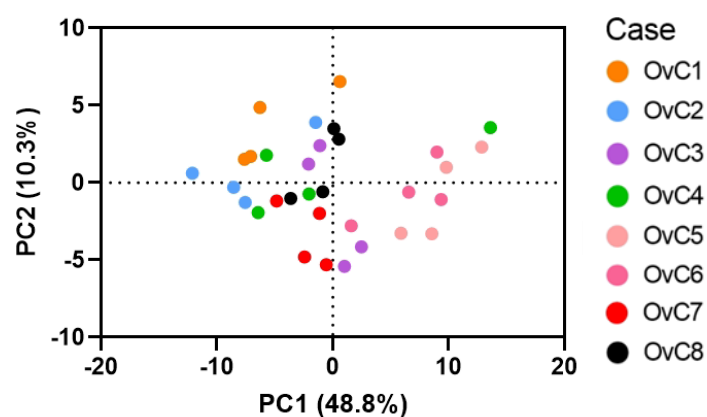
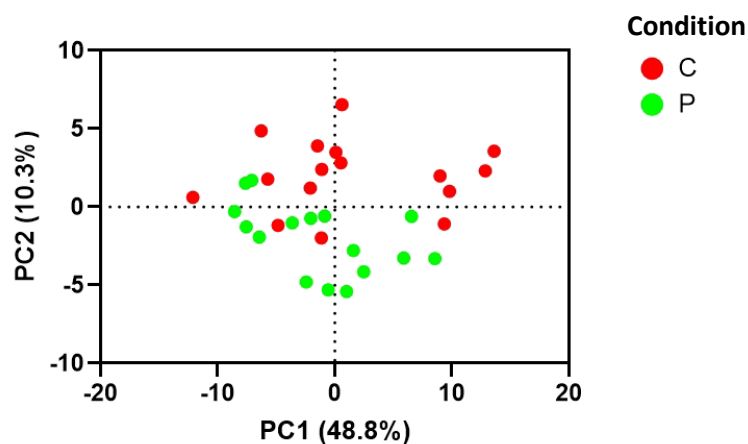
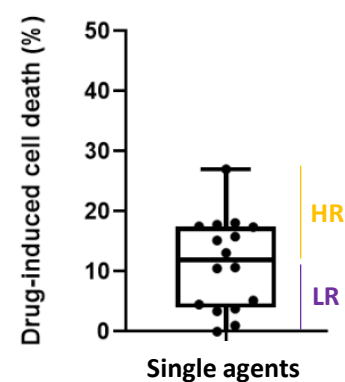
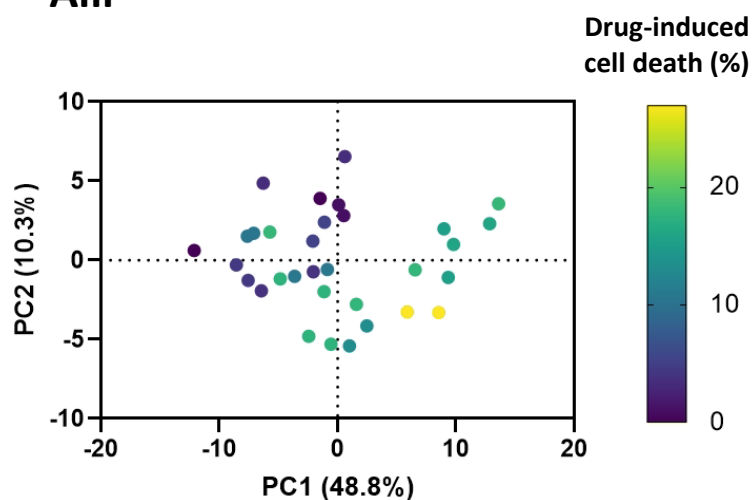
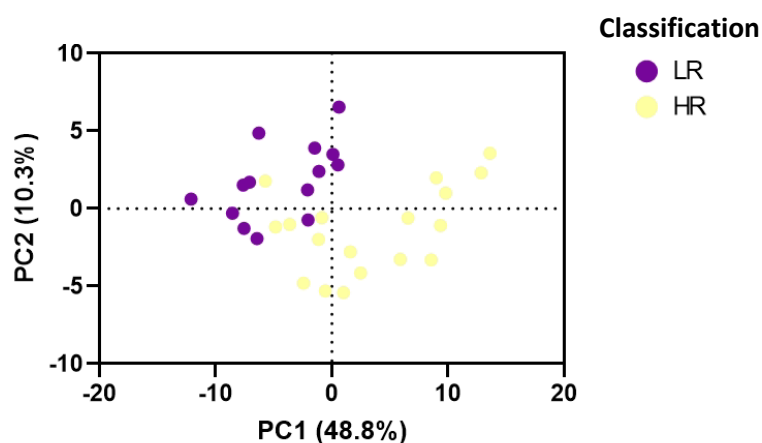
A



B



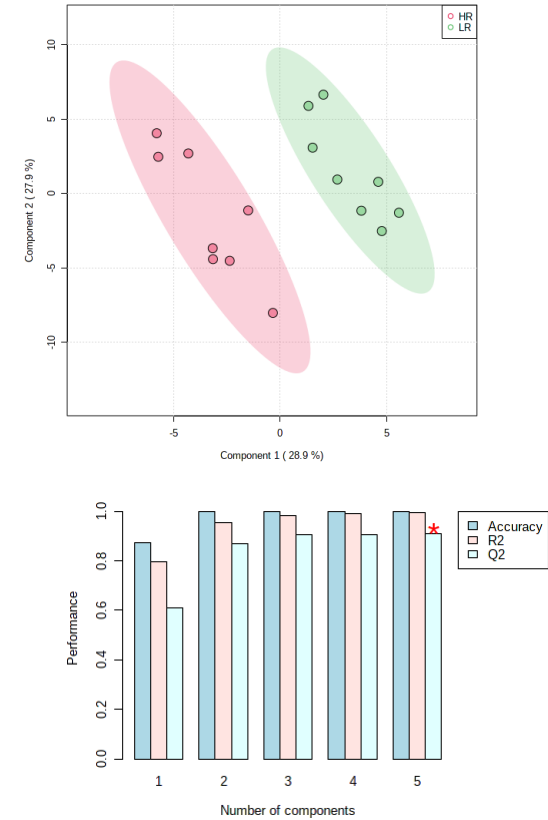
**Supplementary Figure S8: Semi-targeted analysis all samples (OvC1-9).** (A) Normalization, including scaling and transformation, of peak intensities across features. (B) Multivariate data analysis performed using principal component analysis. (the dataset used comprised all samples: untreated controls and treated conditions at day 14 and 21) (C: carboplatin, P: paclitaxel; Ctrl: untreated control; Medium: culture media blanks; QC: quality control sample)

**Ai****Aii****B****Aiii****Aiv**

**Supplementary Figure S9: Multivariate data analysis using dataset comprising single-agents treated samples at day 14.** Unsupervised analysis using **(A)** PCA by **(Ai)** OvC-PDE case; **(ii)** single-agent treatment (C vs P); drug response trends by the **(iii)** drug-induced cell death after one drug cycle (day 14) and **(iv)** based on high-responder (HR) and low-responder (LR) classification. **(B)** Cut-off between HR and LR based on the median drug-induced cell death for single drug-treated conditions. (C: carboplatin; P: paclitaxel; HR: high-responder; LR: low-responder; PCA: principal component analysis)

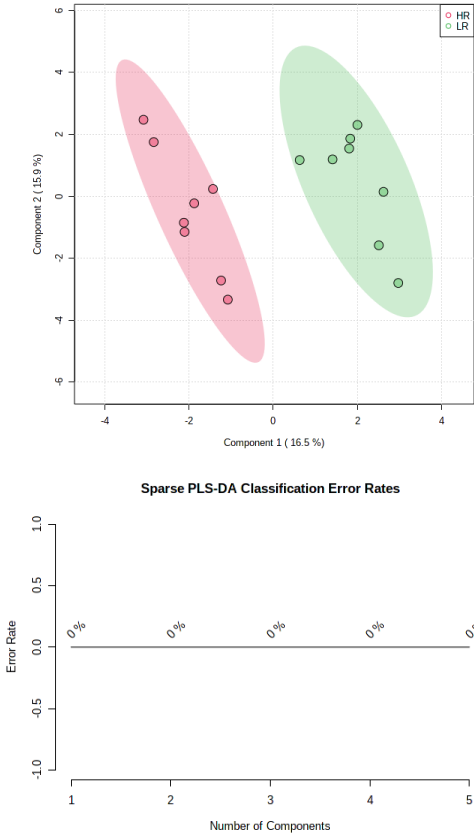
A

PLS-DA



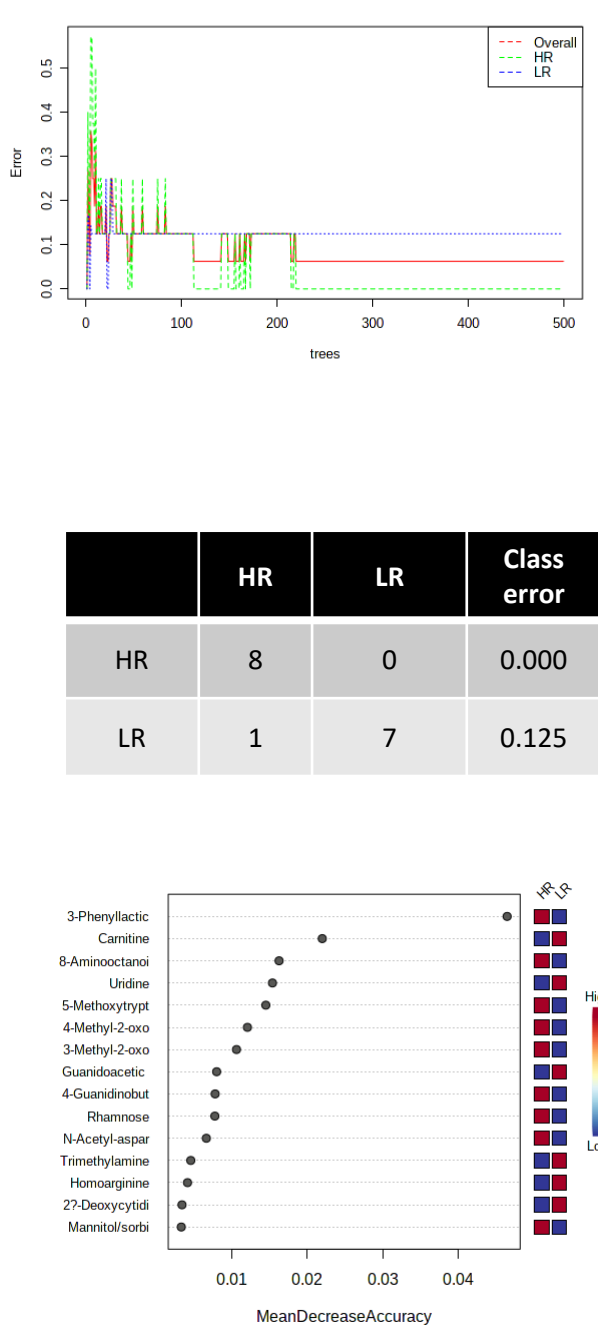
B

sPLS-DA



C

RF



**Supplementary Figure S10: Supervised analysis using dataset comprising untreated control PDE cultures at day 14. (A)** PLS-DA; **(B)** sPLS-DA and **(C)** RF models, with respective plots (upper panel), performance (middle panel) and top discriminating features (lower panel) separating HR vs LR. (HR: high-responder; LR: low-responder; PLS-DA: partial least-squares discriminant analysis; sPLS-DA: sparse partial least-squares discriminant analysis; RF: random forests)

A PCA plot showing the first two principal components. The x-axis is PC1 (53.3%) and the y-axis is PC2 (11.9%). Data points are colored by group: black (top left), pink (left), red (center), purple (top right), blue (bottom right), and orange (bottom right). Dotted lines represent the PC axes.

Case

- OvC1
- OvC2
- OvC3
- OvC4
- OvC5
- OvC6
- OvC7
- OvC8

PC1 (53.3%)

PC2 (11.9%)

Chemotherapy

- Adjuvant
- Neoadjuvant

Drug-Induced cell death (%)

C+P

HR

LR

**PLS-DA**

Component 2 (16.2 %)

Component 1 (49.6 %)

HR  
LR

**sPLS-DA**

Component 1 (35.2 %)

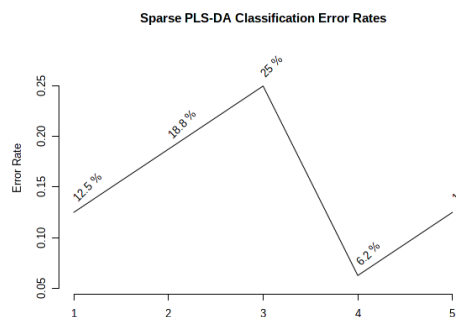
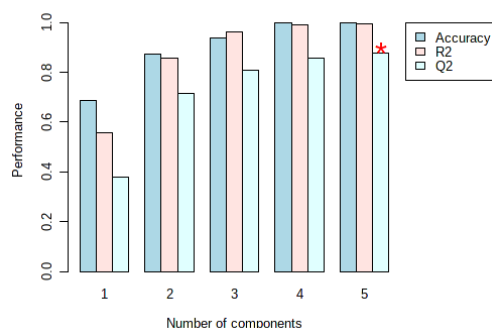
Component 2 (13.2 %)

Legend: HR (yellow), LR (purple)

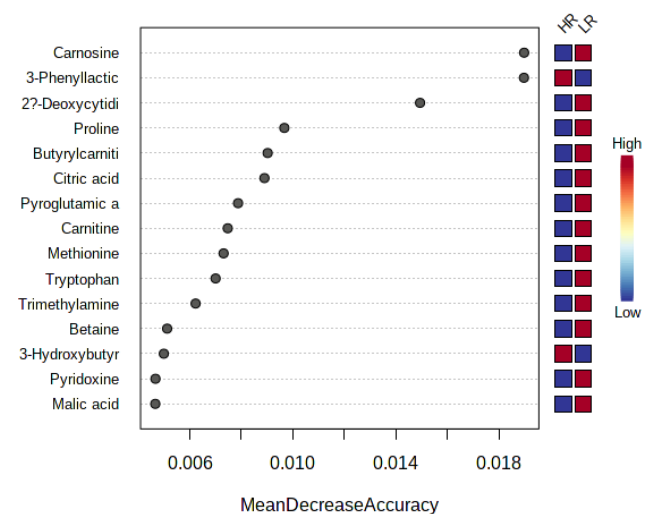
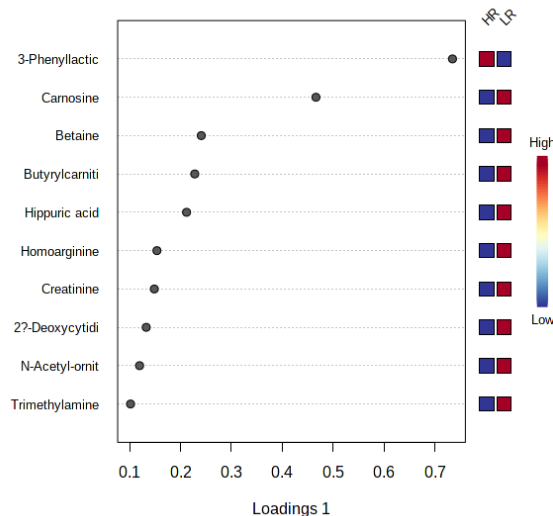
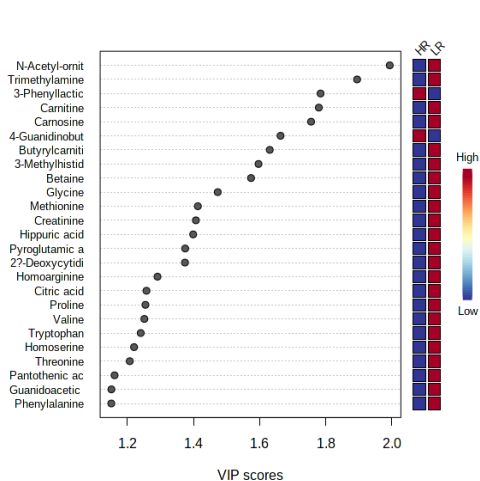
The plot shows two distinct clusters of data points. The HR group (yellow) is centered around Component 1 = -2.5 and Component 2 = 0. The LR group (purple) is centered around Component 1 = 2.5 and Component 2 = 0. The two groups are well-separated along the x-axis.

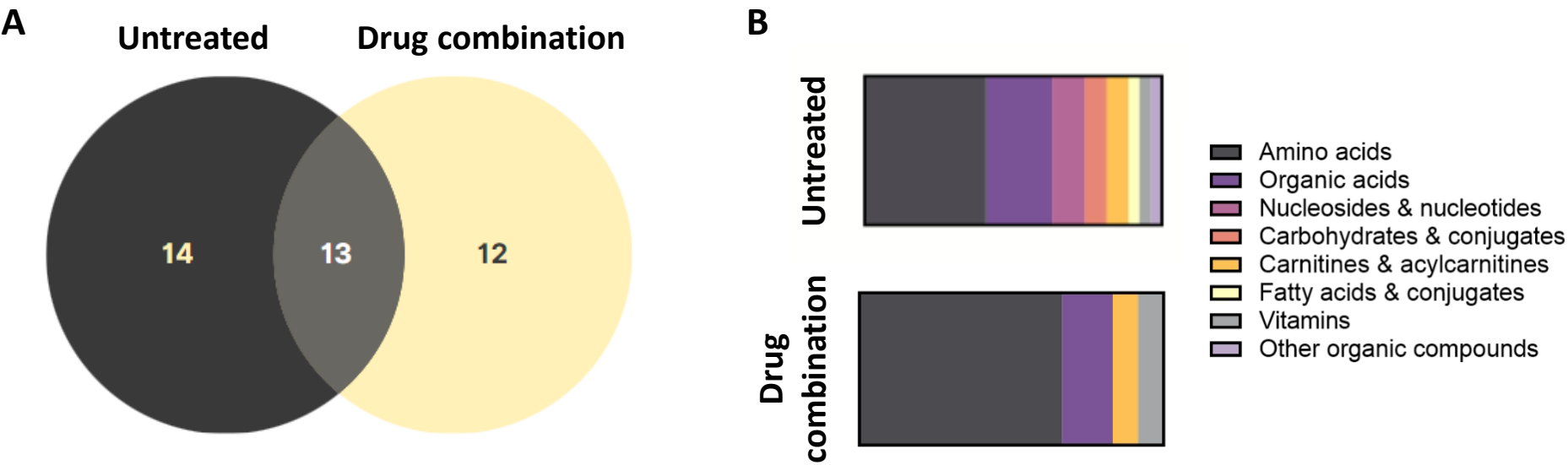
The graph, titled "RF", plots Error (y-axis, 0.0 to 1.0) against the number of trees (x-axis, 0 to 500). Three models are compared: Overall (red dashed line), HR (green dashed line), and LR (blue dashed line). All models show a sharp initial drop in error as the number of trees increases from 0 to approximately 20. The Overall model stabilizes at an error rate of about 0.25, with a slight step-down to 0.15 at 270 trees and another to 0.12 at 460 trees. The HR model stabilizes at a higher error rate of approximately 0.38. The LR model stabilizes at the lowest error rate of about 0.12, with a step-down to 0.08 at 270 trees and another to 0.05 at 460 trees.

trees	Overall	HR	LR
0	1.0	1.0	1.0
20	0.35	0.38	0.25
50	0.25	0.38	0.12
100	0.25	0.38	0.12
270	0.25	0.38	0.12
270	0.30	0.38	0.08
460	0.25	0.38	0.08
460	0.30	0.38	0.05
500	0.30	0.38	0.05

**RF**

	HR	LR	Class error
HR	5	3	0.38
LR	2	6	0.25

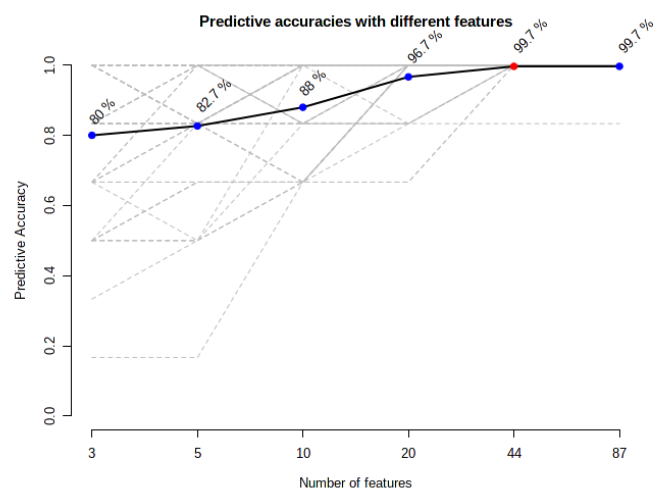




**Supplementary Figure S12: Untreated and drug combination challenged PDE cultures metabolic traits determine ex vivo drug response. (A)** Top discriminating metabolic features for drug response (high-responders, HR versus low-responders, LR) in untreated and combination-treated PDE datasets and **(B)** respective metabolite classes.

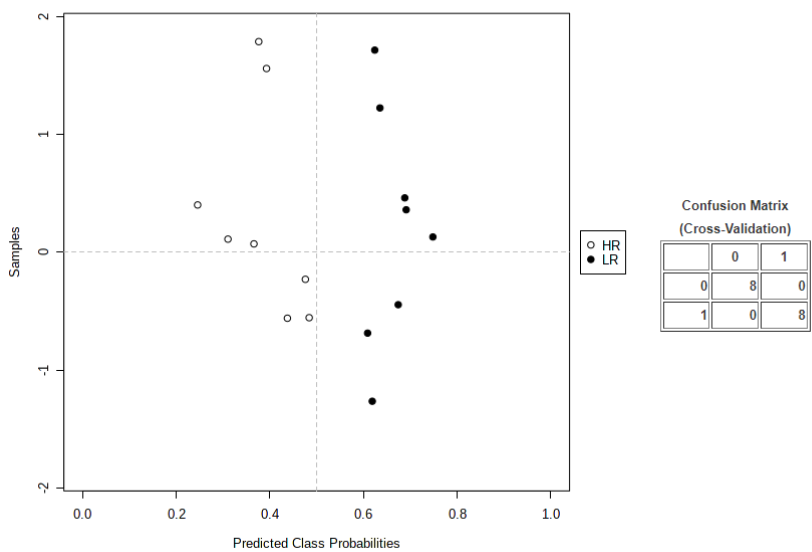
Ai

ROC



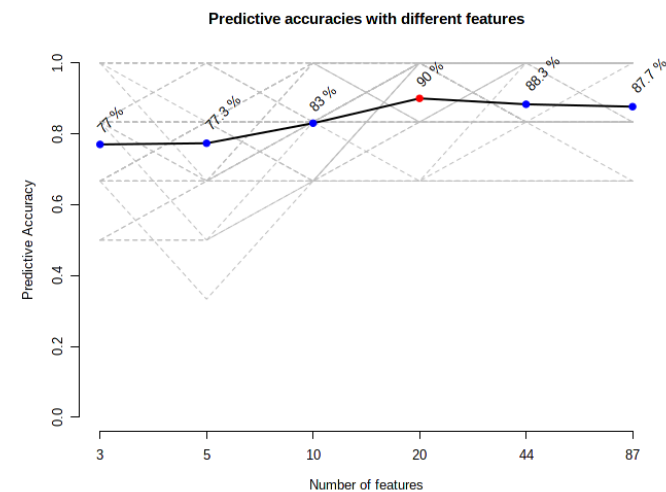
Aii

Model 3 – 10 features



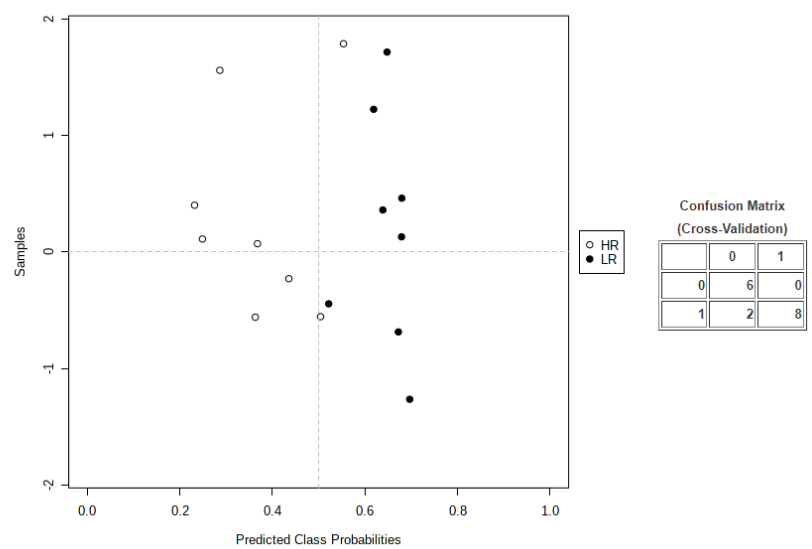
Bi

ROC



Bii

Model 4 – 20 features



**Supplementary Figure S13: PLS-DA ROC curve analysis using dataset comprising (A) untreated control PDE cultures and (B) combination (C+P) treated samples at day 14. (i) Accuracy using different number of features. (ii) No class error was observed for a model based on (Aii) 10 features (or more features) or (Bii) 20 features (optimal accuracy - red dot. (C: carboplatin; P: paclitaxel; HR: high-responder; LR: low-responder; PLS-DA: partial least-squares discriminant analysis; ROC: receiver operating curve)**