Electronic Supplementary Material (ESI) for Metallomics.

Preclinical studies on metal based anticancer drugs as enabled by integrated metallomics and metabolomics

Luis Galvez[a] • Mate Rusz[b] • Michaela Schwaiger-Haber[a,c,d] • Yasin El Abiead[a,c,d] • Gerrit Hermann[a,e] • Ute Jungwirth[f] • Walter Berger[g,h] • Bernhard K. Keppler[b,g] • Michael A. Jakupec[b,g,h] • Gunda Koellensperger[a,c,d]

[a]Institute of Analytical Chemistry, Faculty of Chemistry, University of Vienna, Waehringer Strasse 38, 1090 Vienna (Austria).

[b]Institute of Inorganic Chemistry, Faculty of Chemistry, University of Vienna, Althanstrasse 14 (UZA II), 1090 Vienna (Austria).

[c]Vienna Metabolomics Center (VIME), University of Vienna, Althanstrasse 14, 1090 Vienna (Austria).

[d]Research Network "Chemistry Meets Microbiology", Althanstrasse 14, 1090 Vienna (Austria).

[e]ISOtopic solutions, Waehringer Strasse 38, 1090 Vienna (Austria).

[f]Department of Pharmacy & Pharmacology, Centre for Therapeutic Innovation, University of Bath, Claverton Down, BA2 7AY, Bath (UK).

[g]Medical University of Vienna, Department of Medicine I, Institute of Cancer Research, Vienna (Austria).

[h]Research Cluster "Translational Cancer Therapy Research", University of Vienna and Medical University of Vienna, Vienna (Austria).

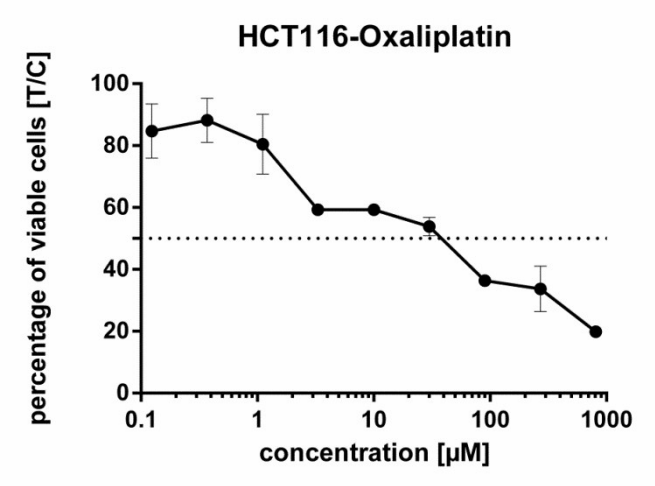

Figure S1 Concentration–effect curves for oxaliplatin in the colorectal cancer line HCT116. An average IC_{50} value of 35 ± 6 µM ensured that a concentration of 5 µM oxaliplatin was adequate (sub-cytotoxic) for the following preparations.

Table S1 Chromatographic conditions and ICP-MS operation parameters for the quantification of the platinum uptake with FI-ICP-MS.

Agilent 1260 Infinity Bio-inert chromatographic conditions	
Eluent	H_2O
Flow rate	400 µL/min
Injection volume	10 µL
Autosampler temperature	2°C
ICP-MS operation parameters	
Nebulizer	MicroMist
Spray chamber	Scott double-pass
Nebulizer gas flow	1.05 L/min
Plasma gas	15 L/min
ICP RF Power	1550 W
m/z measured	195

Table S2 Parameters of the full mass scan (Full-MS), ESI-source (ESI) and untargeted approach (ddMS2) for the metabolomic studies with HILIC-HR-MS.

ESI	
Sheath gas	38
Auxiliary gas	3
Sweep gas	0
Spray voltage	3.5 kV (pos), 2.8 kV (neg)
Capillary temperature	280°C
S-Lens RF	30
Auxiliary gas heater	320°C
Full-MS	
Scan	70-1000 m/z
Polarity	Positive and Negative
Resolution	60000
Automatic Gain Control (AGC) target	$1x10^6$
Maximum Injection Time (IT)	200 ms
ddMS2	
TopN	5
Polarity	Positive and Negative
AGC target	$1x10^5$
IT	50 ms
Isolation	1 m/z
Fragmentation with HCD energy	NCE 30
Resolution	30000

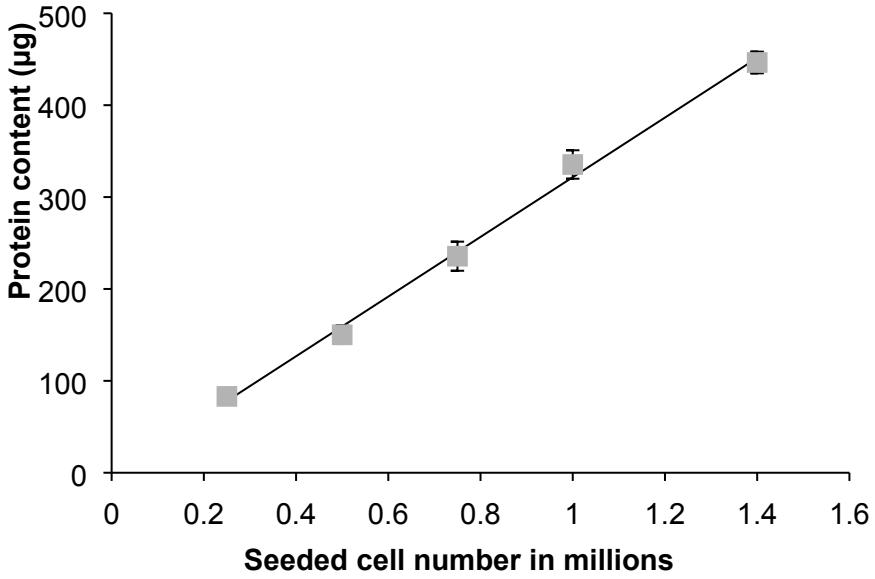

Figure S2 Protein content of different numbers of seeded cells (melanoma cell line FTSLA) after 24 h of incubation (N=3 biological replicates) measured with the 2D-Quant Kit.

Table S3 Analytical figures of merit for the protein content determination with the micro BCA protein kit.

Sensitivity (µg/mL)	Linear working range (µg/mL)	Biological Repeatability (%)	Instrumental Repeatability (%)
0.5	0.5 - 20	< 11 (N=6)	< 7 (N=3)

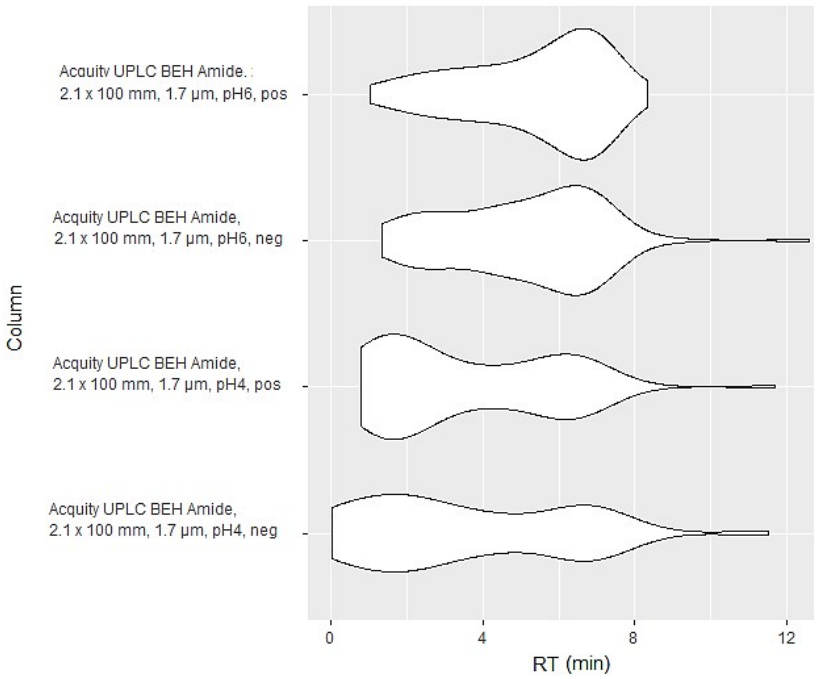

Figure S3 Retention time distribution of a 10 µM multicomponent standard measured on the UPLC BEH Amide column at pH 4 and pH 6 in positive and negative mode. A better separation was observed at pH 4.

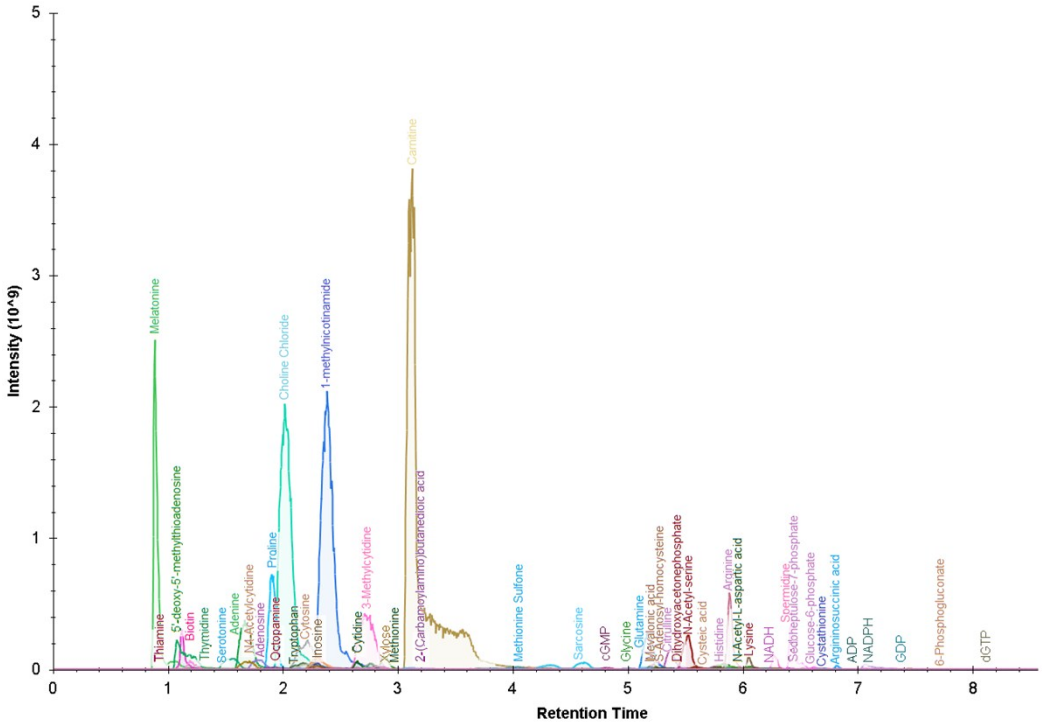

Figure S4 Chromatogram of a 10 µM multicomponent standard measured on the UPLC BEH Amide column at pH 4 in positive mode.

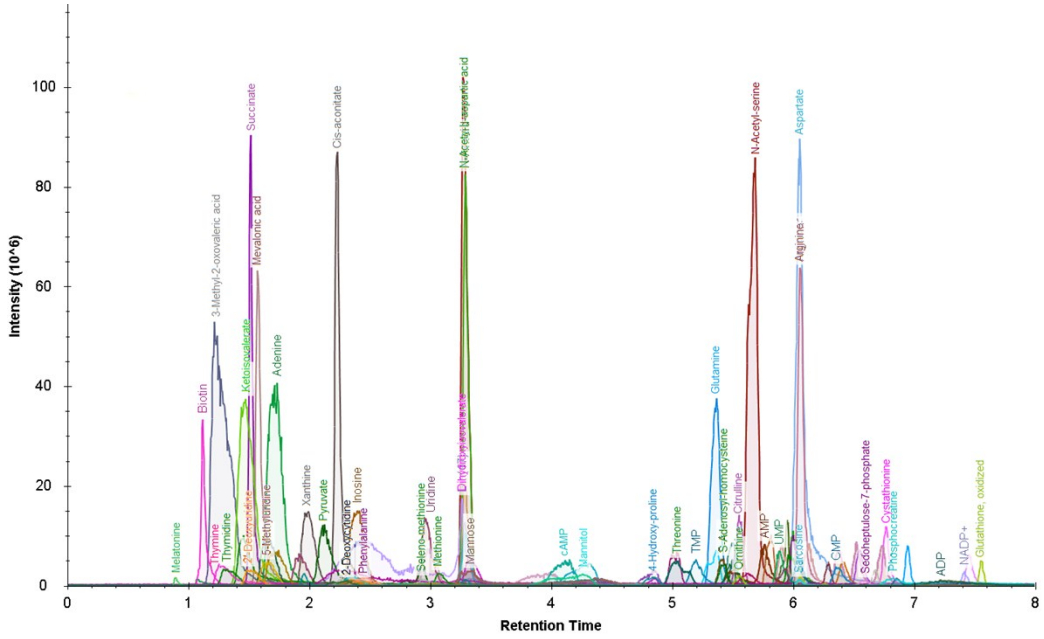

Figure S5 Chromatogram of a 10 µM multicomponent standard measured on the UPLC BEH Amide column at pH 4 in negative mode.

Table S4 Chromatographic conditions and gradients for the HILIC analysis of a 10 µM multicomponent standard.

Chromatographic conditions								
Eluent*						A 50 mM $HCOONH_4$, pH 4 B 80% ACN 50 mM HCOONH4, pH 4		
Flow rate						250 µL/min		
Injection volume						5 µL		
Column temperature**						30°C		
Autosampler temperature						6°C		
Chromatographic gradient								
Acclaim Trinity P2***, 2.1x100 mm, 3 µm			Acclaim Trinity P1***, 2.1x150 mm, 3 µm			Acquity UPLC BEH Amide, 2.1x100 mm, 1.7 µm		
Time (min)	A (%)	B (%)	Time (min)	A (%)	B (%)	Time (min)	A (%)	B (%)
0	0	100	0	0	100	0	0	100
2	0	100	2	0	100	2	0	100
8	100	0	13	100	0	8	50	50
10	100	0	15	100	0	10	50	50
10	0	100	15	0	100	10	0	100
15	0	100	20	0	100	15	0	100

*50 mM CH_3COONH_4 for pH6.

**40°C for the column Acquity UPLC BEH Amide.

***The mixed mode columns Trinity P1 and Trinity P2 required 1 h pre-equilibration prior to the analysis.

Table S5 Multicomponent standard 10 µM in positive mode and retention times measured on the UPLC BEH Amide column at pH 4. The uniformly ^{13}C labeled metabolites present in the samples were used for internal standardization.

Positive					
Compound	**RT [min]**	**^{12}C metabolite**		**^{13}C labeled internal standard**	
		Formula	**$[M+H]^+$**	**ISTD formula**	**ISTD $[M+H]^+$**
melatonine	0.88	$C_{13}H_{16}N_2O_2$	233.1285		
5'-Deoxy-5'-Methylthioadenosine	1.08	$C_{11}H_{15}N_5O_3S$	298.0968	$^{13}C_{11}H_{15}N_5O_3S$	309.1337
Biotin	1.13	$C_{10}H_{16}N_2O_3S$	245.0954		
thymidine	1.3	$C_{10}H_{14}N_2O_5$	243.0976		
2'-Deoxyuridine	1.47	$C_9H_{12}N_2O_5$	229.0819		
Uracil	1.48	$C_4H_4N_2O_2$	113.0346		
Mevalonic acid	1.55	$C_6H_{12}O_4$	149.0808		
serotonine	1.58	$C_{10}H_{12}N_2O$	177.1022		
5-methyluridine	1.59	$C_{10}H_{14}N_2O6$	259.0925		
Adenine	1.69	$C_5H_5N_5$	136.0618	$^{13}C_5H_5N_5$	141.0785
N4-acetylcytidine	1.71	$C_{11}H_{15}N_3O_6$	286.1034		
Adenosine	1.81	$C_{10}H_{13}N_5O_4$	268.104	$^{13}C_{10}H_{13}N_5O_4$	278.1376
propionyl-L-carnitine	1.94	$C_{10}H_{19}NO_4$	218.1387		
Octopamine	1.97	$C_8H_{11}NO_2$	154.0863		
Choline chloride	2.05	$C_5H_{13}NO$	104.107	$^{13}C_5H_{13}NO$	109.1238
2-deoxycytidine	2.21	$C_9H_{13}N_3O_4$	228.0979		
Tryptophan	2.22	$C_{11}H_{12}N_2O_2$	205.0972	$^{13}C_{11}H_{12}N_2O_2$	216.1341
cytosine	2.23	$C_4H_5N_3O$	112.0505		
Phenylalanine	2.29	$C_9H_{11}NO_2$	166.0863	$^{13}C_9H_{11}NO_2$	175.1164
Inosine	2.32	$C_{10}H_{12}N_4O_5$	269.088	$^{13}C_{10}H_{12}N_4O_5$	279.1216
Kynurenine	2.32	$C_{10}H_{12}N_2O_3$	209.0921	$^{13}C_{10}H_{12}N_2O_3$	219.1256
Guanine	2.34	$C_5H_5N_5O$	152.0567	$^{13}C_5H_5N_5O$	157.0735
1-Methylnicotinamide	2.39	$C_7H_8N_2O$	137.0709	$^{13}C_7H_8N_2O$	144.0944
3-methylcytidine	2.71	$C_{10}H_{15}N_3O_5$	258.1085		
Pseudouridine	1.90/2.85*	$C_9H_{12}N_2O_6$	245.0768		
Cytidine	2.84	$C_9H_{13}N_3O_5$	244.0928	$^{13}C_9H_{13}N_3O_5$	253.123
Uridine	1.90/2.85*	$C_9H_{12}N_2O_6$	245.0768		
seleno-methionine	2.86	$C_5H_{11}NO_2Se$	198.0028		
Methionine	2.89	$C_5H_{11}NO_2S$	150.0583	$^{13}C_5H_{11}NO_2S$	155.0751
Guanosine	2.91/3.79*	$C_{10}H_{13}N_5O_5$	284.0989	$^{13}C_{10}H_{13}N_5O_5$	294.1325
Isoguanosine	2.91/3.79*	$C_{10}H_{13}N_5O_5$	284.0989	$^{13}C_{10}H_{13}N_5O_5$	294.1325
Homocysteine	3.14	$C_4H_9NO_2S$	136.0427		
N-acetyl-L-aspartic acid	3.15	$C_6H_9NO_5$	176.0554		
Tyrosine	3.15	$C_9H_{11}NO_3$	182.0812	$^{13}C_9H_{11}NO_3$	191.1114
Carnitine	3.2	$C_7H_{15}NO_3$	162.1125	$^{13}C_7H_{15}NO_3$	169.136

2-(carbamoylamino)butanedioic acid	3.25	$C_5H_8N_2O_5$	177.0506		
cAMP	3.83	$C_{10}H_{12}N_5O_6P$	330.0598		
Mannitol	3.99	$C_6H_{14}O_6$	183.0863		
methionine sulfone	4.11	$C_5H_{11}NO_4S$	182.0482		
sarcosine	4.66	$C_3H_7NO_2$	90.055	$^{13}C_3H_7NO_2$	93.065
Alanine	4.70	$C_3H_7NO_2$	90.055	$^{13}C_3H_7NO_2$	93.065
cGMP	4.80	$C_{10}H_{12}N_5O_7P$	346.0547	$^{13}C_{10}H_{12}N_5O_7P$	356.0883
Threonine	5.00	$C_4H_9NO_3$	120.0655	$^{13}C_4H_9NO_3$	122.0789
Homoserine	5.00	$C_4H_9NO_3$	120.0655	$^{13}C_4H_9NO_3$	122.0789
Glycine	5.01	$C_2H_5NO_2$	76.0393	$^{13}C_2H_5NO_2$	78.046
TMP	5.10	$C_{10}H_{15}N_2O_8P$	323.0639		
alpha-aminoadipic acid	5.20	$C_6H_{11}NO_4$	162.0761	$^{13}C_6H_{11}NO_4$	168.0962
Glutamine	5.22	$C_5H_{10}N_2O_3$	147.0764	$^{13}C_5H_{10}N_2O_3$	152.0932
S-Adenosyl-homocysteine	5.30	$C_{14}H_{20}N_6O_5S$	385.1289		
Serine	5.30	$C_3H_7NO_3$	106.0499	$^{13}C_3H_7NO_3$	109.0599
dAMP	5.34	$C_{10}H_{14}N_5O_6P$	332.0755		
Asparagine	5.42	$C_4H_8N_2O_3$	133.0608	$^{13}C_4H_8N_2O_3$	137.0742
Dihydroxyacetonephosphate	5.43	$C_3H_7O_6P$	171.0053		
Citrulline	5.45	$C_6H_{13}N_3O_3$	176.103	$^{13}C_6H_{13}N_3O_3$	182.1231
Ornithine	5.53	$C_5H_{12}N_2O_2$	133.0972	$^{13}C_5H_{12}N_2O_2$	138.1139
Glutamate	5.56	$C_5H_9NO_4$	148.0604	$^{13}C_5H_9NO_4$	153.0772
5AMP	5.62	$C_{10}H_{14}N_5O_7P$	348.0704	$^{13}C_{10}H_{14}N_5O_7P$	358.1039
3AMP	5.62	$C_{10}H_{14}N_5O_7P$	348.0704	$^{13}C_{10}H_{14}N_5O_7P$	358.1039
Cysteic acid	5.73	$C_3H_7NO_5S$	170.0178		
UMP	5.80	$C_9H_{13}N_2O_9P$	325.0431	$^{13}C_9H_{13}N_2O_9P$	334.0733
Ribose-5-phosphate	5.81	$C_5H_{11}O_8P$	231.0264	$^{13}C_5H_{11}O_8P$	236.0432
Ribulose-5-phosphate	5.81	$C_5H_{11}O_8P$	231.0264	$^{13}C_5H_{11}O_8P$	236.0432
Cysteinyl-glycine	5.84	$C_5H_{10}N_2O_3S$	179.0485		
Glutamyl-cysteine	5.85	$C_8H_{14}N_2O_5S$	251.0696		
Histidine	5.86	$C_6H_9N_3O_2$	156.0768	$^{13}C_6H_9N_3O_2$	162.0969
Glutathione reduced	5.87	$C_{10}H_{17}N_3O_6S$	308.0911	$^{13}C_{10}H_{17}N_3O_6S$	318.1246
Aspartic acid	5.90	$C_4H_7NO_4$	134.0448	$^{13}C_4H_7NO_4$	138.0582
dCMP	5.90	$C_9H_{14}N_3O_7P$	308.0642		
IMP	5.92	$C_{10}H_{13}N_4O_8P$	349.0544	$^{13}C_{10}H_{13}N_4O_8P$	359.0879
Arginine	5.94	$C_6H_{14}N_4O_2$	175.119	$^{13}C_6H_{14}N_4O_2$	181.1391
erythrose-4-phosphate	6.02	$C_4H_9O_7P$	201.0159		
Lysine	6.10	$C_6H_{14}N_2O_2$	147.1128	$^{13}C_6H_{14}N_2O_2$	153.1329
Flavinadenin dinucleotide	6.14	$C_{27}H_{33}N_9O_{15}P_2$	786.1644	$^{13}C_{27}H_{33}N_9O_{15}P_2$	813.255
CMP	6.24	$C_9H_{14}N_3O_8P$	324.0591	$^{13}C_9H_{14}N_3O_8P$	333.0893
GMP	6.30	$C_{10}H_{14}N_5O_8P$	364.0653	$^{13}C_{10}H_{14}N_5O_8P$	374.0988
NADH	6.31	$C_{21}H_{29}N_7O_{14}P_2$	666.1321	$^{13}C_{21}H_{29}N_7O_{14}P_2$	687.2025
S-(adenosyl)-methionine	6.35	$C_{15}H_{22}N_6O_5S$	399.1445		
Fructose-6-Phosphate	6.42	$C_6H_{13}O_9P$	261.037	$^{13}C_6H_{13}O_9P$	267.0571
Glucose-6-Phosphate	6.42	$C_6H_{13}O_9P$	261.037	$^{13}C_6H_{13}O_9P$	267.0571
Glucose-1-Phosphate	6.42	$C_6H_{13}O_9P$	261.037	$^{13}C_6H_{13}O_9P$	267.0571
Mannitol-1-phosphate	6.44	$C_6H_{15}O_9P$	263.0526		
spermidine	6.50	$C_7H_{19}N_3$	146.1652		
Sedoheptulose-7-phosphate	6.50	$C_7H_{15}O_{10}P$	291.0476		

NAD+	6.55	$C_{21}H_{27}N_7O_{14}P_2$	663.1091	$^{13}C_{21}H_{27}N_7O_{14}P_2$	684.1796
Cystathionine	6.64	$C_7H_{14}N_2O_4S$	223.0747	$^{13}C_7H_{14}N_2O_4S$	230.0982
Cystine	6.70	$C_6H_{12}N_2O_4S_2$	241.0311		
phosphocreatine	6.75	$C_4H_{10}N_3O_5P$	212.0431		
Argininosuccinic acid	6.81	$C_{10}H_{18}N_4O_6$	291.1299	$^{13}C_{10}H_{18}N_4O_6$	301.1635
ADP	7.08	$C_{10}H_{15}N_5O_{10}P_2$	428.0367	$^{13}C_{10}H_{15}N_5O_{10}P_2$	438.0702
UDP	7.12	$C_9H_{14}N_2O_{12}P_2$	405.0095		
NADPH	7.15	$C_{21}H_{30}N_7O_{17}P_3$	746.0984	$^{13}C_{21}H_{30}N_7O_{17}P_3$	767.1688
spermine	7.15	$C_{10}H_{26}N_4$	203.223		
NADP+	7.31	$C_{21}H_{29}N_7O_{17}P_3$	744.0833	$^{13}C_{21}H_{29}N_7O_{17}P_3$	765.1537
Fructose-1,6-bisphosphate	7.36	$C_6H_{14}O_{12}P$	341.0033	$^{13}C_6H_{14}O_{12}P$	347.0235
GDP	7.42	$C_{10}H_{15}N_5O_{11}P_2$	444.0316	$^{13}C_{10}H_{15}N_5O_{11}P_2$	454.0652
Glutathione oxidized	7.50	$C_{20}H_{32}N_6O_{12}S_2$	613.1592	$^{13}C_{20}H_{32}N_6O_{12}S_2$	633.2263
3-Phosphoglycerate	7.64	$C_3H_7O_7P$	187.0002	$^{13}C_3H_7O_7P$	190.0103
6-Phosphogluconate	7.78	$C_6H_{13}O_{10}P$	277.0319	$^{13}C_6H_{13}O_{10}P$	283.052
TTP	7.81	$C_{10}H_{18}N_2O_{14}P_3$	482.9965		
dGTP	8.02	$C_{10}H_{16}N_5O_{13}P_3$	508.003	$^{13}C_{10}H_{16}N_5O_{13}P_3$	518.0366
dATP	8.06	$C_{10}H_{17}N_5O_{12}P_3$	492.0081		
ATP	8.11	$C_{10}H_{16}N_5O_{13}P_3$	508.003	$^{13}C_{10}H_{16}N_5O_{13}P_3$	518.0366
GTP	8.14	$C_{10}H_{16}N_5O_{14}P_3$	523.9979		
UTP	8.21	$C_9H_{16}N_2O_{15}P_3$	484.9758		
CTP	8.43	$C_9H_{16}N_3O_{14}P_3$	483.9918		
dCTP	8.46	$C_9H_{17}N_3O_{13}P_3$	467.9969		

*Isomers which could be separated on the UPLC BEH Amide column at pH 4 in positive mode. For further identification an analysis of the single standard is necessary.

Table S6 Multicomponent standard 10 µM in negative mode and retention times measured on the UPLC BEH Amide column at pH 4. The uniformly ^{13}C labeled metabolites present in the samples were used for internal standardization.

Negative					
Compound	**RT [min]**	**^{12}C metabolite**		**^{13}C labeled internal standard**	
		Formula	**$[M-H]^-$**	**ISTD formula**	**ISTD $[M-H]^-$**
melatonine	0.90	$C_{13}H_{16}N_2O_2$	231.1139		
5'-Deoxy-5'-Methylthioadenosine	1.08	$C_{11}H_{15}N_5O_3S$	296.0823		
biotin	1.12	$C_{10}H_{16}N_2O_3S$	243.0809		
3-Methyl-2-oxovaleric acid	1.22	$C_6H_{10}O_3$	129.0557	$^{13}C_6H_{10}O_3$	135.0759
thymine	1.26	$C_5H_6N_2O_2$	125.0357		
thymidine	1.32	$C1_0H_{14}N_2O_5$	241.0830		
Uracil	1.45	$C_4H_4N_2O_2$	111.0200		
Ketoisovalerate	1.47	$C_5H_8O_3$	115.0401	$^{13}C_5H_8O_3$	120.0568
2'-deoxyuridine	1.50	$C_9H_{12}N_2O_5$	227.0673		
Succinate	1.52	$C_4H_6O_4$	117.0193	$^{13}C_4H_6O_4$	121.0328
Mevalonic acid	1.57	$C_6H_{12}O_4$	147.0663	$^{13}C_6H_{12}O_4$	153.0864
5-methyluridine	1.65	$C_{10}H_{14}N_2O$~~6~~	257.0779		
Adenine	1.72	$C_5H_5N_5$	134.0472	$^{13}C_5H_5N_5$	139.0640
N4-acetylcytidine	1.75	$C_{11}H_{15}N_3O_6$	284.0888		

Adenosine	1.84	$C_{10}H_{13}N_5O_4$	266.0895		
Pseudouridine	1.92/2.91*	$C_9H_{12}N_2O_6$	243.0623		
Uridine	1.92/2.91*	$C_9H_{12}N_2O_6$	243.0623		
Xanthine	1.99	$C_5H_4N_4O_2$	151.0262		
Lactate	2.23	$C_3H_6O_3$	89.0244	$^{13}C_3H_6O_3$	92.0345
cis-Aconitate	2.24	$C_6H_6O_6$	173.0092	$^{13}C_6H_6O_6$	179.0293
2-deoxycytidine	2.30	$C_9H_{13}N_3O_4$	226.0833		
Tryptophan	2.33	$C_{11}H_{12}N_2O_2$	203.0826		
gluconate	2.35	$C_6H_{12}O_7$	195.0510		
Ribose	2.35/3.02*	$C_5H_{10}O_5$	149.0456		
Xylose	2.35/3.02*	$C_5H_{10}O_5$	149.0456		
Guanine	2.39	$C_5H_5N_5O$	150.0421		
Pyruvate	2.40	$C_3H_4O_3$	87.0088	$^{13}C_3H_4O_3$	90.0188
Inosine	2.42	$C_{10}H_{12}N_4O_5$	267.0735		
Kynurenine	2.43	$C_{10}H_{12}N_2O_3$	207.0775		
Phenylalanine	2.44	$C_9H_{11}NO_2$	164.0717		
seleno-methionine	2.82	$C_5H_{11}NO_2Se$	195.9882		
Cytidine	2.90	$C_9H_{13}N_3O_5$	242.0782		
Methionine	2.94	$C_5H_{11}NO_2S$	148.0438		
Guanosine	2.98/3.89*	$C_{10}H_{13}N_5O_5$	282.0844		
Isoguanosine	2.98/3.89*	$C_{10}H_{13}N_5O_5$	282.0844		
Tyrosine	3.23	$C_9H_{11}NO_3$	180.0666		
Dihydroxyisovalerate	3.23	$C_5H_{10}O_4$	133.0506	$^{13}C_5H_{10}O_4$	138.0674
Fructose	3.26	$C_6H_{12}O_6$	179.0561	$^{13}C_{10}H_{13}N_5O_4$	185.0762
Galactose	3.26	$C_6H_{12}O_6$	179.0561	$^{13}C_{10}H_{13}N_5O_4$	185.0762
Glucose	3.26	$C_6H_{12}O_6$	179.0561	$^{13}C_{10}H_{13}N_5O_4$	185.0762
Inositol	3.26	$C_6H_{12}O_6$	179.0561	$^{13}C_{10}H_{13}N_5O_4$	185.0762
Mannose	3.26	$C_6H_{12}O_6$	179.0561	$^{13}C_{10}H_{13}N_5O_4$	185.0762
N-Acetyl-serine	3.26	$C_5H_9NO_4$	146.0459		
N-acetyl-L-aspartic acid	3.29	$C_6H_9NO_5$	174.0408		
alpha-Ketoglutarate	3.29	$C_5H_6O_5$	145.0142	$^{13}C_5H_6O_5$	150.0310
Fumarate	3.29	$C_4H_4O_4$	115.0037	$^{13}C_4H_4O_4$	119.0171
Hydroxyglutaric acid	3.29	$C_5H_8O_5$	147.0299	$^{13}C_5H_8O_5$	152.0467
2-(carbamoylamino)butanedioic acid	3.32	$C_5H_8N_2O_5$	175.0360		
cAMP	4.15	$C_{10}H_{12}N_5O_6P$	328.0452		
Mannitol	4.24	$C_6H_{14}O_6$	181.0718		
methionine sulfone	4.31	$C_5H_{11}NO_4S$	180.0336		
Alanine	4.66	$C_3H_7NO_2$	88.0404		
sarcosine	4.66	$C_3H_7NO_2$	88.0404		
guanidineacetic acid	4.83	$C_3H_7N_3O_2$	116.0466		
Glycine	4.85	$C_2H_5NO_2$	74.0248		
cGMP	5.00	$C_{10}H_{12}N_5O_7P$	344.0402		
Threonine	5.05	$C_4H_9NO_3$	118.0510	$^{13}C_4H_9NO_3$	122.0642
Homoserine	5.05	$C_4H_9NO_3$	118.0510	$^{13}C_4H_9NO_3$	122.0642
TMP	5.19	$C_{10}H_{15}N_2O_8P$	321.0493		
alpha-aminoadipic acid	5.34	$C_6H_{11}NO_4$	160.0615	$^{13}C_6H_{11}NO_4$	166.0817
Glutamine	5.36	$C_5H_{10}N_2O_3$	145.0619	$^{13}C_5H_{10}N_2O_3$	150.0786
S-Adenosyl-homocysteine	5.42	$C_{14}H_{20}N_6O_5S$	383.1143		
Serine	5.42	$C_3H_7NO_3$	104.0353	$^{13}C_3H_7NO_3$	107.0454
Asparagine	5.42	$C_4H_8N_2O_3$	131.0462	$^{13}C_4H_8N_2O_3$	135.0596
dAMP	5.45	$C_{10}H_{14}N_5O_6P$	330.0609		
Citrulline	5.49	$C_6H_{13}N_3O_3$	174.0884	$^{13}C_6H_{13}N_3O_3$	180.1085
Ornithine	5.53	$C_5H_{12}N_2O_2$	131.0826	$^{13}C_5H_{12}N_2O_2$	136.0994
Dihydroxyacetonephosphate	5.62	$C_3H_7O_6P$	168.9908	$^{13}C_3H_7O_6P$	172.0008
3AMP	5.62	$C_{10}H_{14}N_5O_7P$	346.0558	$^{13}C_{10}H_{14}N_5O_7P$	356.0894
Glutamate	5.64	$C_5H_9NO_4$	146.0459	$^{13}C_5H_9NO_4$	151.0627
5AMP	5.72	$C_{10}H_{14}N_5O_7P$	346.0558	$^{13}C_{10}H_{14}N_5O_7P$	356.0894
Cysteic acid	5.78	$C_3H_7NO_5S$	167.9972	$^{13}C_3H_7NO_5S$	171.0073
UMP	5.88	$C_9H_{13}N_2O_9P$	323.0286	$^{13}C_9H_{13}N_2O_9P$	332.0588

Glutamyl-cysteine	5.94	$C_8H_{14}N_2O_5S$	249.0551		
Glutathione reduced	5.95	$C_{10}H_{17}N_3O_6S$	306.0765	$^{13}C_{10}H_{17}N_3O_6S$	316.1101
Histidine	5.96	$C_6H_9N_3O_2$	154.0622	$^{13}C_6H_9N_3O_2$	160.0823
dCMP	5.99	$C_9H_{14}N_3O_7P$	306.0497		
Arginine	6.00	$C_6H_{14}N_4O_2$	173.1044	$^{13}C_6H_{14}N_4O_2$	179.1245
IMP	6.04	$C_{10}H_{13}N_4O_8P$	347.0398		
Aspartate	6.05	$C_4H_7NO_4$	132.0302	$^{13}C_4H_7NO_4$	136.0437
erythrose-4-phosphate	6.09	$C_4H_9O_7P$	199.0013		
Ribose-5-phosphate	6.13	$C_5H_{11}O_8P$	229.0119	$^{13}C_5H_{11}O_8P$	234.0287
Ribulose-5-phosphate	6.13	$C_5H_{11}O_8P$	229.0119	$^{13}C_5H_{11}O_8P$	234.0287
Lysine	6.18	$C_6H_{14}N_2O_2$	145.0983		
Flavinadenin dinucleotide	6.29	$C_{27}H_{33}N_9O_{15}P_2$	784.1499		
CMP	6.35	$C_9H_{14}N_3O_8P$	322.0446		
NADH	6.40	$C_{21}H_{29}N_7O_{14}P_2$	664.1175		
GMP	6.41	$C_{10}H_{14}N_5O_8P$	362.0507	$^{13}C_{10}H_{14}N_5O_8P$	372.0843
Mannitol-1-phosphate	6.55	$C_6H_{15}O_9P$	261.0381		
Fructose-6-Phosphate	6.52	$C_6H_{13}O_9P$	259.0224	$^{13}C_6H_{13}O_9P$	265.0426
Glucose-6-Phosphate	6.52	$C_6H_{13}O_9P$	259.0224	$^{13}C_6H_{13}O_9P$	265.0426
Glucose-1-Phosphate	6.52	$C_6H_{13}O_9P$	259.0224	$^{13}C_6H_{13}O_9P$	265.0426
Sedoheptulose-7-phosphate	6.52	$C_7H_{15}O_{10}P$	289.033		
NAD+	6.68	$C_{21}H_{27}N_7O_{14}P_2$	662.1018	$^{13}C_{21}H_{27}N_7O_{14}P_2$	683.1723
Cystathionine	6.72	$C_7H_{14}N_2O_4S$	221.0602	$^{13}C_7H_{14}N_2O_4S$	228.0836
Cystine	6.80	$C_6H_{12}N_2O_4$	239.0166		
phosphocreatine	6.82	$C_4H_{10}N_3O_5P$	210.0285		
Argininosuccinic acid	6.89	$C_{10}H_{18}N_4O_6$	289.1154	$^{13}C_{10}H_{18}N_4O_6$	299.1489
ADP	7.13	$C_{10}H_{15}N_5O_{10}P_2$	426.0221	$^{13}C_{10}H_{15}N_5O_{10}P_2$	436.0557
NADPH	7.21	$C_{21}H_{30}N_7O_{17}P_3$	744.0838		
UDP	7.28	$C_9H_{14}N_2O_{12}P_2$	402.9949		
NADP+	7.41	$C_{21}H_{29}N_7O_{17}P_3$	742.0682		
Fructose-1,6-bisphosphate	7.46	$C_6H_{14}O_{12}P$	338.9888	$^{13}C_6H_{14}O_{12}P$	345.0089
GDP	7.51	$C_{10}H_{15}N_5O_{11}P_2$	442.0171		
Citric acid	7.53	$C_6H_8O_7$	193.0343		
Isocitric acid	7.53	$C_6H_8O_7$	193.0343		
Glutathione oxidized	7.55	$C_{20}H_{32}N_6O_{12}S_2$	611.1447		
Malate	7.55	$C_4H_6O_5$	133.0142	$^{13}C_4H_6O_5$	137.0277
6-Phosphogluconate	7.83	$C_6H_{13}O_{10}P$	275.0174		
3-Phosphoglycerate	7.87	$C_3H_7O_7P$	184.9857		
ATP	8.13	$C_{10}H_{16}N_5O_{13}P_3$	505.9885	$^{13}C_{10}H_{16}N_5O_{13}P_3$	516.022
dATP	8.21	$C_{10}H_{17}N_5O_{12}P_3$	491.0013		
dGTP	8.25	$C_{10}H_{16}N_5O_{13}P_3$	505.9885	$^{13}C_{10}H_{16}N_5O_{13}P_3$	516.022
UTP	8.27	$C_9H_{16}N_2O_{15}P_3$	483.9691		
GTP	8.36	$C_{10}H_{16}N_5O_{14}P_3$	521.9834		
dCTP	8.66	$C_9H_{17}N_3O_{13}P_3$	466.9901		
TTP	8.78	$C_{10}H_{18}N_2O_{14}P_3$	481.9898		
CTP	9.14	$C_9H_{16}N_3O_{14}P_3$	481.9772		

*Isomers which could be separated on the UPLC BEH Amide column at pH 4 in negative mode. For further identification an analysis of the single standard is necessary.

Table S7 Isomers present in the 10 µM multicomponent standard and measured on the UPLC BEH Amide column at pH 4 in positive and negative mode. The isomers Pseudouridine/Uridine, Gultamate/N-acetyl-serine, Ribose/Xylose, Guanosine/Isoguanosine, could be separated with this method. The rest of the isomers were partially separated and integrated as one single peak with the exception of Homoserine/Threonine, 2-phosphoglycerate/3-phosphoglycerate, Citrate/isocitrate, 3-methylcytidine/4-methylcytidine and ATP/dGTP which could not be separated.

Isomers	Separation
Hexose phosphates	partial
Glutamate/N-Acetyl-serine	separated
3AMP/5AMP	partial
Hexoses	partial
Ribose-5-phosphate/Ribulose-5-phosphate	partial
Alanine/sarcosine	partial
Pseudouridine/Uridine	separated
Guanosine/isoguanosine	separated
Homoserine/Threonine	not separated
2-phosphoglycerate/3- phosphoglycerate	not separated
Citrate/isocitrate	not separated
3-methylcytidine/4-methylcytidine	not separated
Ribose/xylose	separated
ATP/dGTP	not separated

Table S8 Repeatability over >25 h of the UPLC BEH Amide column at pH 4 in positive and negative mode regarding a 10 µM multicomponent standard (N=3 technical replicates).

positive		negative	
# of metabol.	RSD (%)	# of metabol.	RSD (%)
19	0-5	98	0-5
39	6-15	18	6-15
31	16-30	2	16-30
16	31-60	0	31-60
1*	>60	0	>60

*corresponding to UTP.

Table S9 Full width at half maximum (FWHM) observed with the UPLC BEH Amide column at pH 4 in positive and negative mode regarding a multicomponent standard 10 µM.

positive		negative	
# of metabol.	FWHM	# of metabol.	FWHM
16	1.0-3.0	18	1.0-3.0
34	3.1-5.0	34	3.1-5.0
40	5.1-10.0	44	5.1-10.0
4	10.1-20.0	3	10.1-20.0
3	20.0-40.0	7	20.0-40.0
1	40.1-60.0	3	40.1-60.0
8*	>60	9*	>60

*corresponding mainly to nucleotides. Their peak width increased with the number of phosphates.

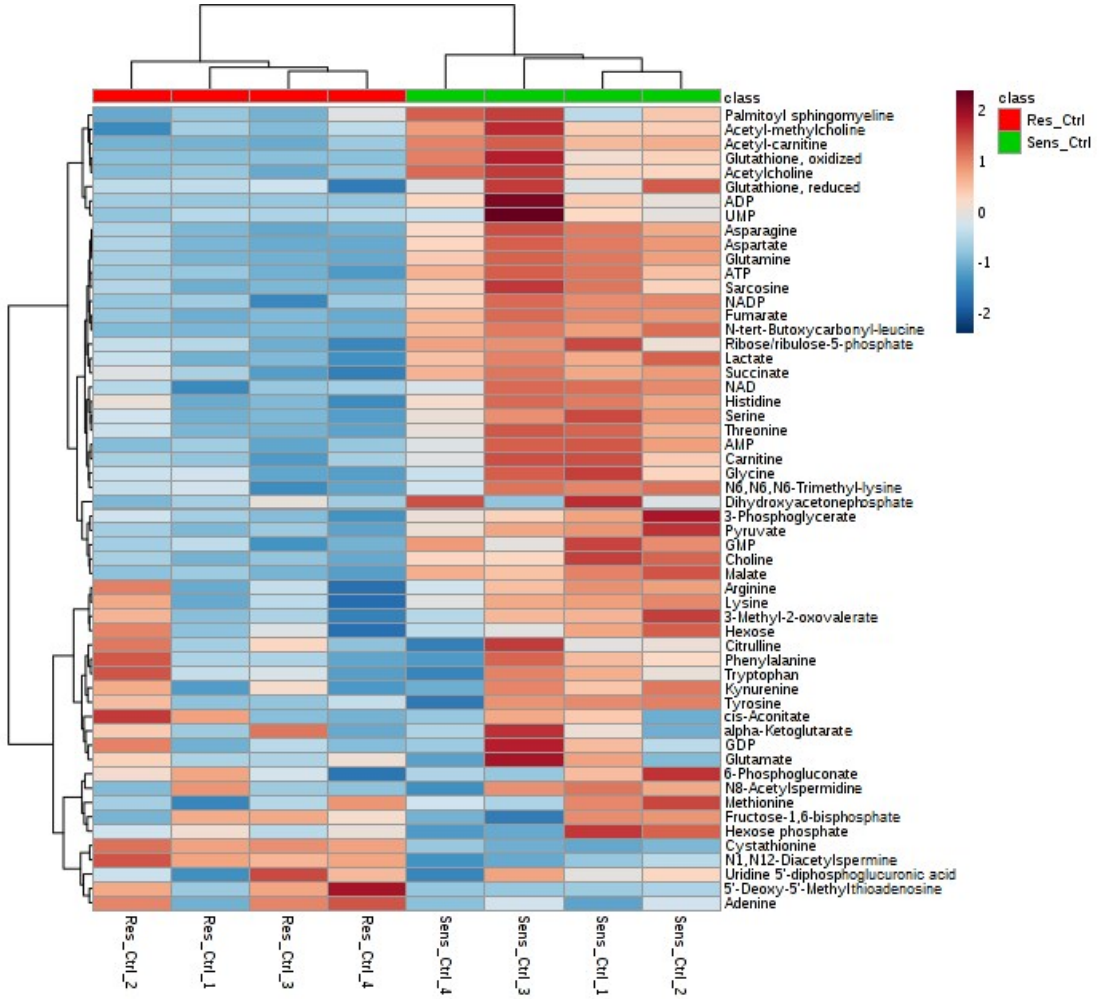

Figure S6 Heatmap of the different compounds found in sensitive and resistant HCT116 cell control for 24 h. Method: UPLC BEH Amide at pH 4 in positive and negative mode combining targeted (internal standardization) and untargeted metabolomics. Hierarchical clustering separated the two investigated groups. Sensitive HCT116 cells showed overall higher metabolite amounts as compared to the resistant counterpart, with the exception of N1,N12-diacetylspermine, cystathionine, uridine 5´-diphosphoglucuronic acid and 5´-deoxy-5´-methylthioadenosine.

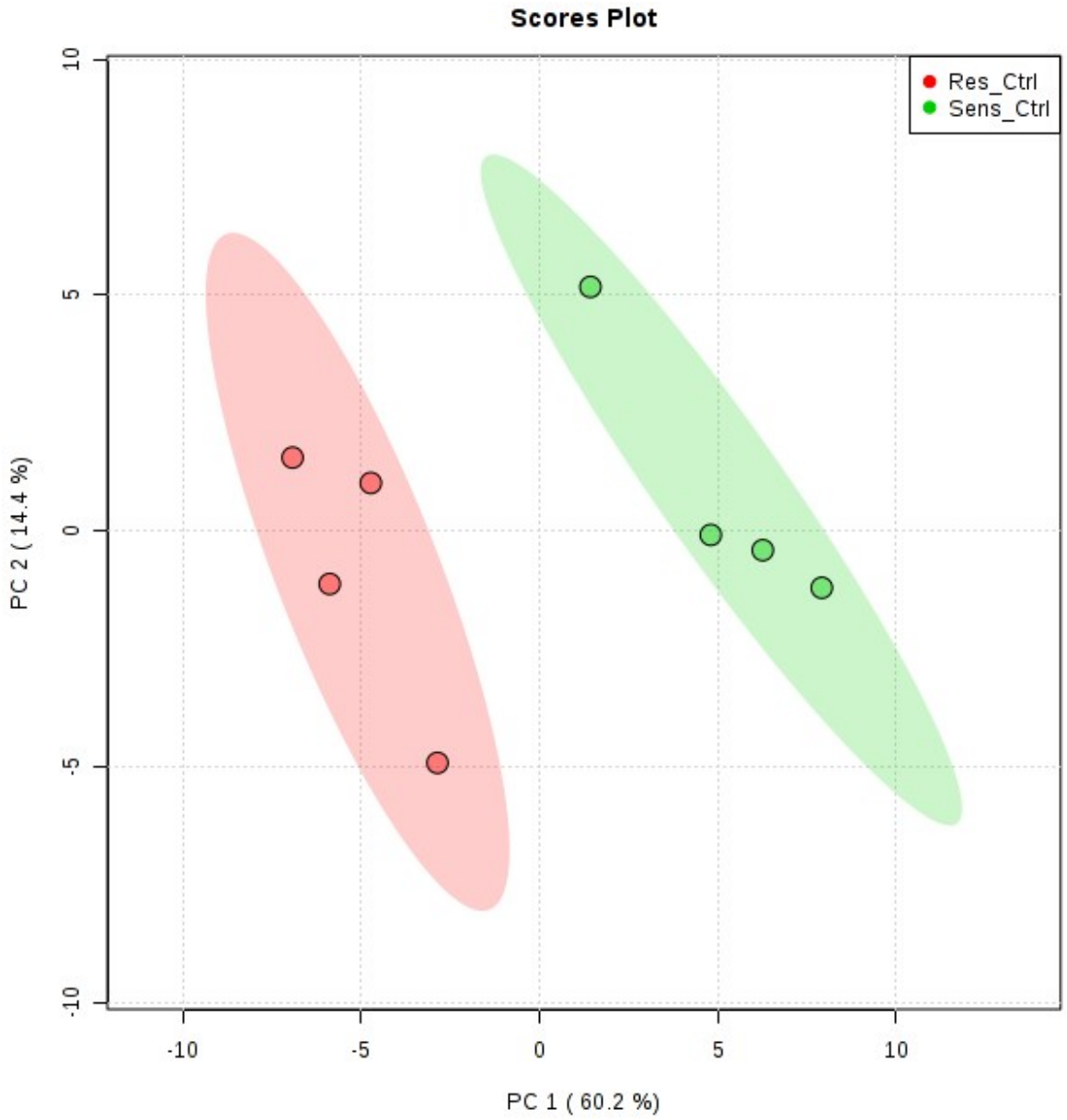

Figure S7 Principal component analysis of sensitive HCT116 cell control vs resistant HCT116 cell control for 24 h. Method: UPLC BEH Amide at pH 4 in positive and negative mode combining targeted (internal standardization) and untargeted metabolomics. A clear separation could be observed between both groups.

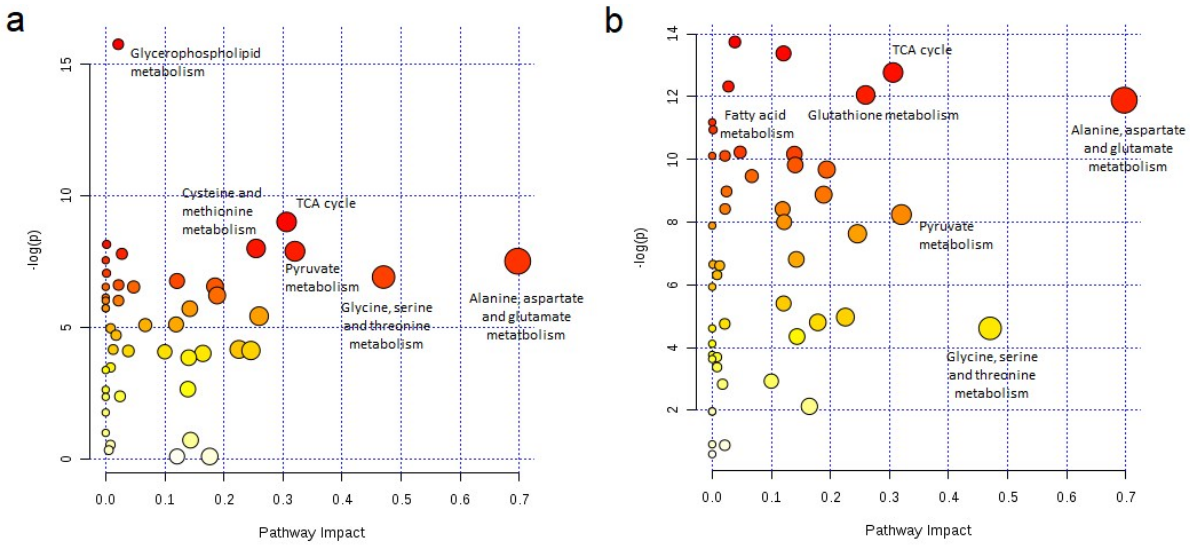

Figure S8 Main metabotype differences found after 24 h in **(a)** sensitive vs resistant HCT116 cell control and in **(b)** sensitive HCT116 cell control vs sensitive HCT116 cell incubated with oxaliplatin 5 μM. Method: UPLC BEH Amide at pH 4 in positive and negative mode combining targeted (internal standardization) and untargeted metabolomics. Source: Metaboanalyst 4.0 (autoscaled, pathway enrichment analysis global test, pathway topology analysis with relative betweeness centrality, pathway library Homo sapiens KEGG).

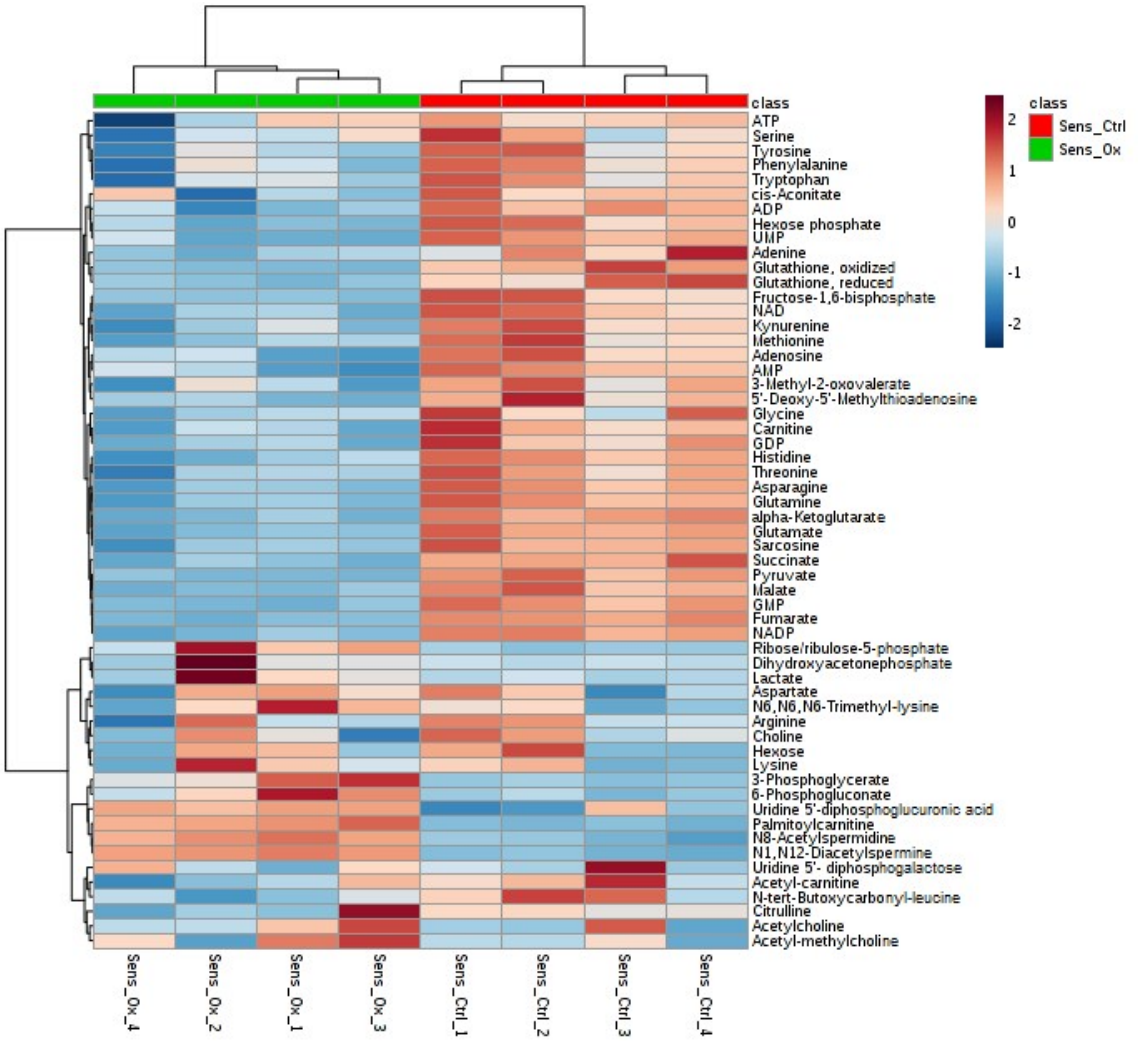

Figure S9 Heatmap of the different compounds found in sensitive HCT116 cell control and in sensitive HCT116 cell incubated with oxaliplatin 5 μM for 24 h. Method: UPLC BEH Amide at pH 4 in positive and negative mode combining targeted (internal standardization) and untargeted metabolomics. A clear separation could be observed between sensitive cells with and without drug (denoted as sensitive control). Overall the metabolite levels were higher in the sensitive line control experiment, with the exception of 3-phosphoglycerate, 6-phosphogluconate, uridine 5´-diphosphoglucuronic acid palmitoylcarnitine, N8-acetylspermine and N1,N12-diacetylspermine.

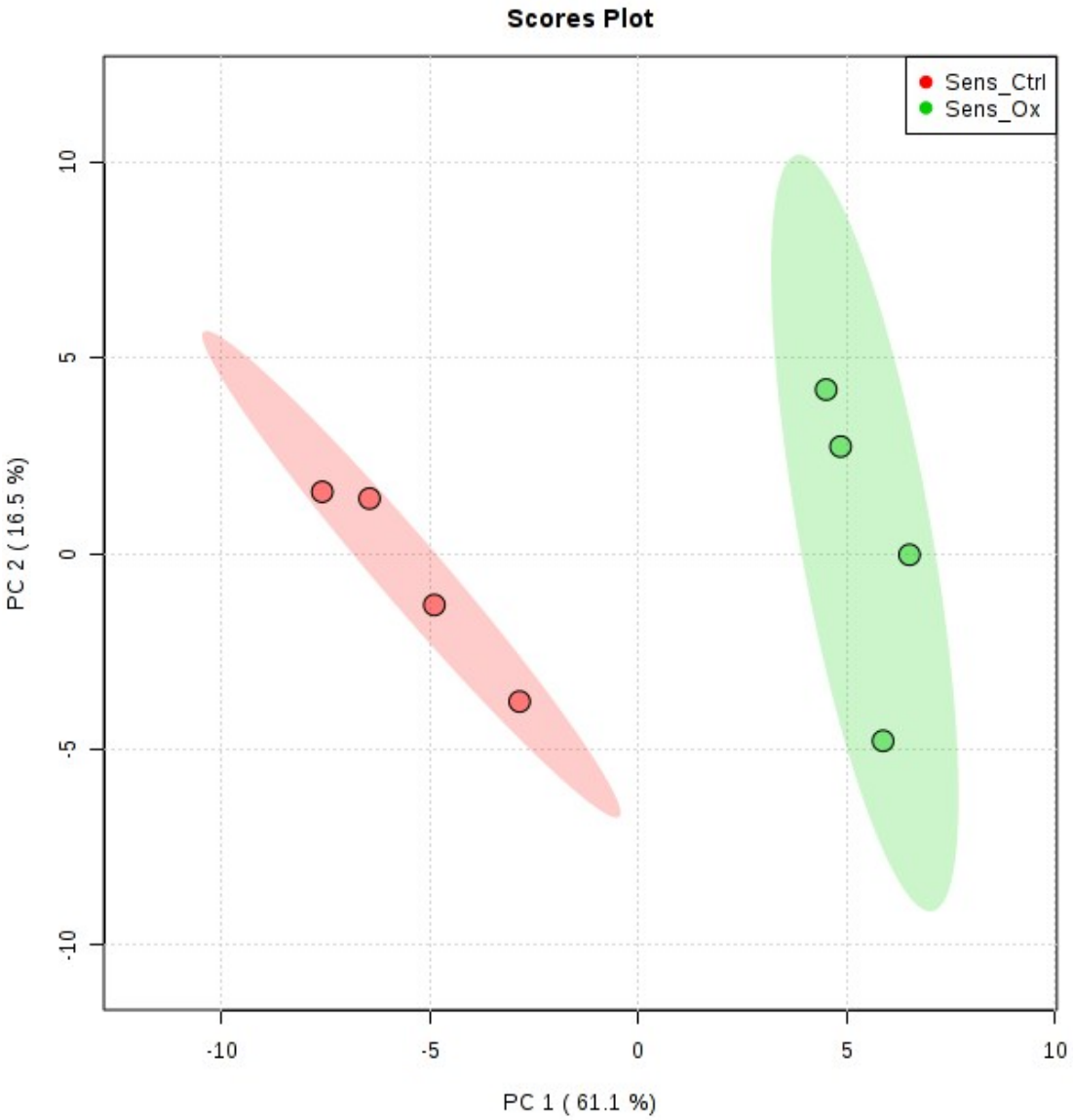

Figure S10 Principal component analysis of sensitive HCT116 cell control vs sensitive HCT116 incubated with oxaliplatin 5 µM for 24 h. Method: UPLC BEH Amide at pH 4 in positive and negative mode combining targeted (internal standardization) and untargeted metabolomics. A clear separation could be observed between both groups.

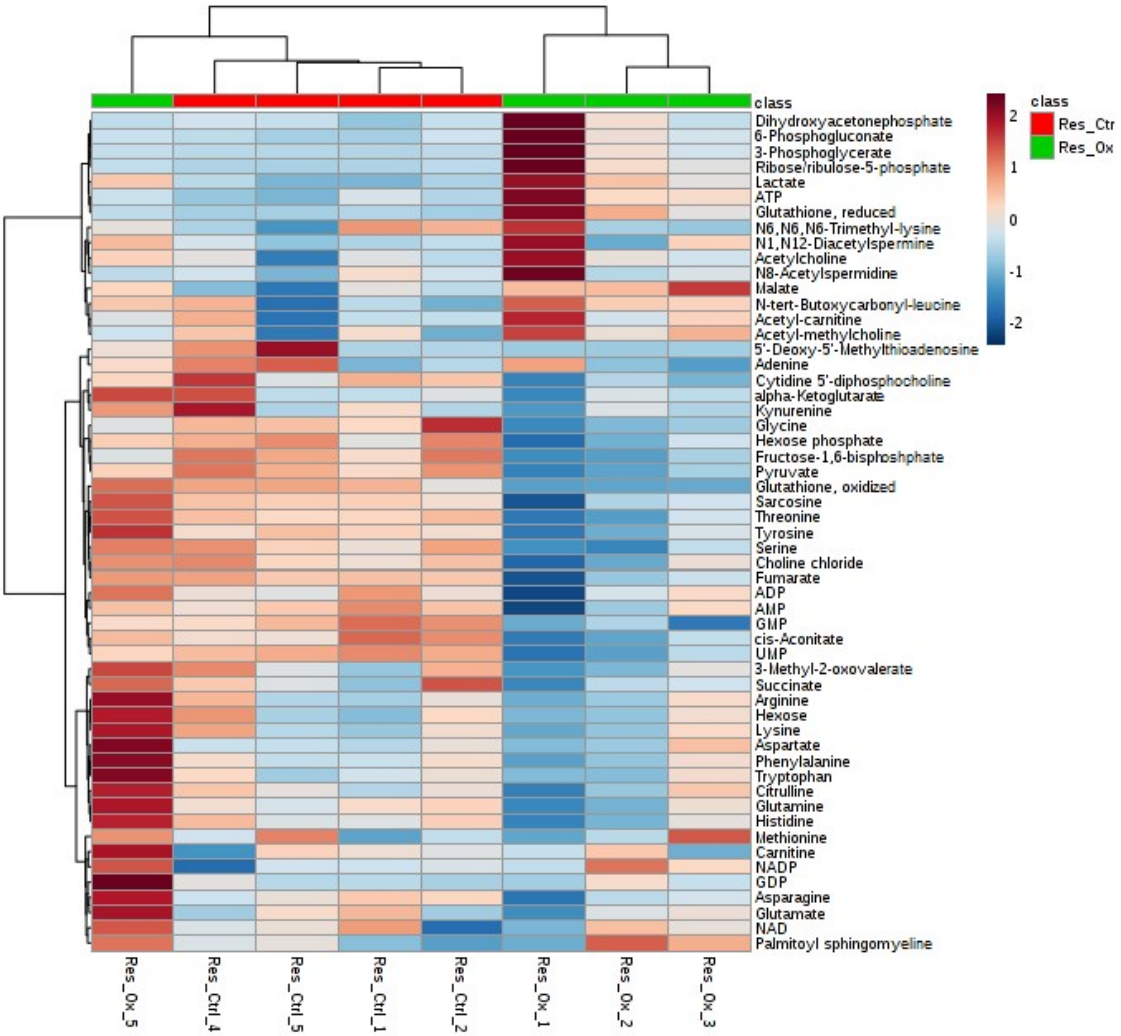

Figure S11 Heatmap of the different compounds found in resistant HCT116 cell control and in resistant HCT116 cell incubated with oxaliplatin 5 µM for 24 h. Method: UPLC BEH Amide at pH 4 in positive and negative mode combining targeted (internal standardization) and untargeted metabolomics. Only subtle changes could be observed.

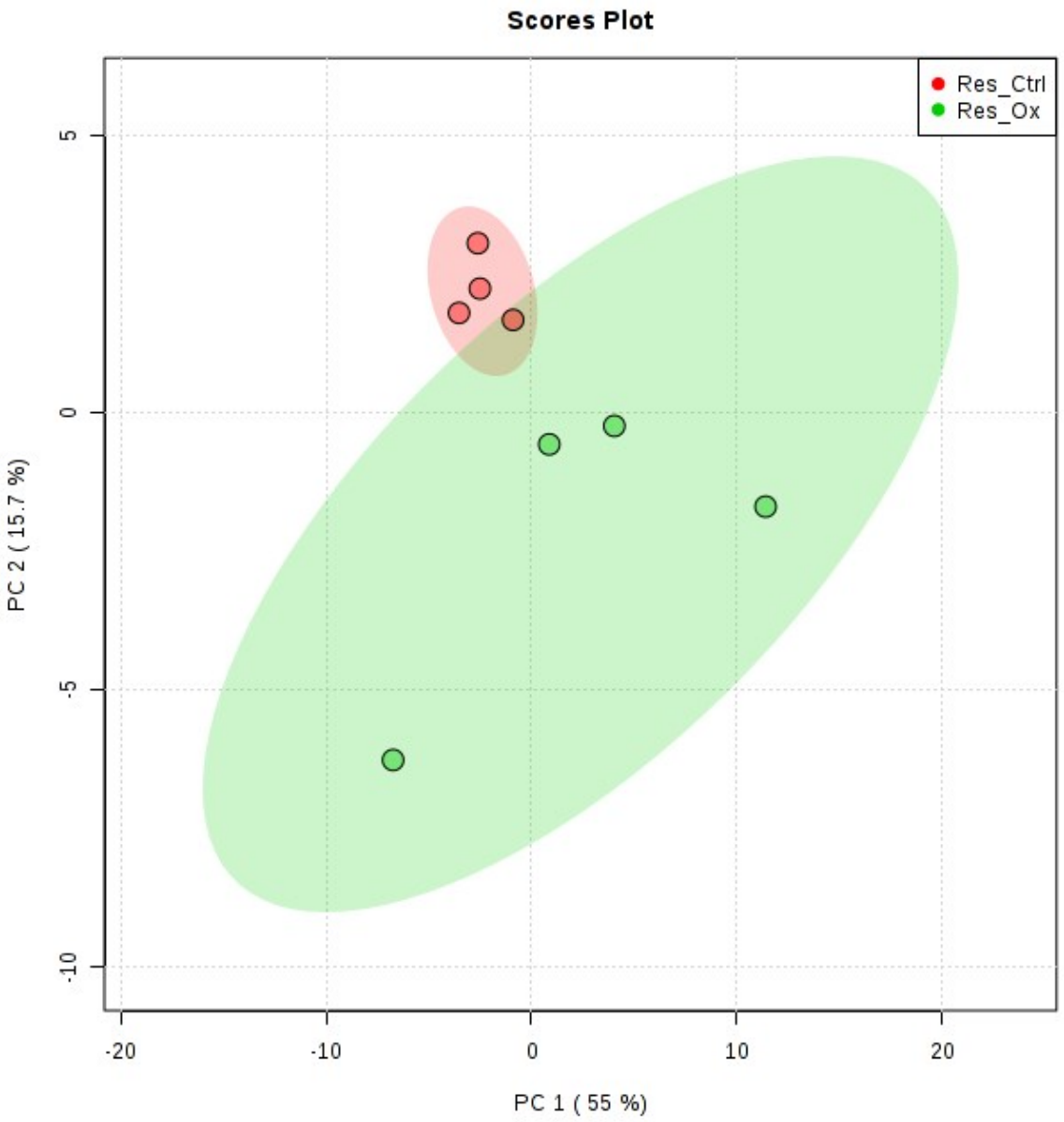

Figure S12 Principal component analysis of resistant HCT116 cell control vs resistant HCT116 incubated with oxaliplatin 5 μM for 24 h. Method: UPLC BEH Amide at pH 4 in positive and negative mode combining targeted (internal standardization) and untargeted metabolomics. The separation between both groups was not pronounced.

Table S10 Pearson correlation of the different metabolites (and their associated pathways) with the incubation time (4, 8, 24 h) in targeted metabolomics. Samples: sensitive HCT116 cell control and sensitive HCT116 cell incubated with 5 µM oxaliplatin. Method: UPLC BEH Amide at pH 4 in positive and negative mode with internal standardization and protein normalization. Differences in Pearson correlation between experiments without (sensitive control) and with drug incubation (sensitive oxaliplatin) were interpreted as metabolic perturbation exerted by the drug. The highest differences in the Pearson correlation are found for dihydroxyacetonephosphate, 2/3-Phosphoglycerate, glutamine, fructose-1,6-bisphosphate, serine, adenine, GMP and pyruvate.

Metabolite	Sens Ctrl	Sens Oxali	Pathway
NADP+	0.912	0.953	Glutathione metabolism. Nicotinate and nicotinamide metabolism
Glutathione, oxidized	0.810	0.778	Glutathione metabolism
Glutathione, reduced	-0.457	-0.921	Cysteine and methionine metabolism. Glutathione metabolism.
Methionine	-0.971	-0.935	Cysteine and methionine metabolism
Hexose phosphate	0.466	0.438	Glycolysis / Gluconeogenesis. Pentose phosphate pathway
Hexose	-0.900	-0.395	Glycolysis / Gluconeogenesis. Pentose phosphate pathway
Lactate	-0.819	-0.829	Glycolysis / Gluconeogenesis
Dihydroxyacetonephosphate	0.554	-0.502	Glycolysis / Gluconeogenesis
2/3-Phosphoglycerate	-0.026	-0.985	glycolysis and calvin cycle
Glutamate	0.957	0.971	Arginine biosynthesis. Alanine, aspartate and glutamate metabolism. Arginine and proline metabolism. D-Glutamine and D-glutamate metabolism.
Glutamine	0.218	-0.230	Arginine biosynthesis. Alanine, aspartate and glutamate metabolism. Purine metabolism. Pyrimidine metabolism. D-Glutamine and D-glutamate metabolism.
Alanine	0.752	0.872	Alanine, aspartate and glutamate metabolism
Citrulline	0.926	0.344	Arginine biosynthesis
Arginine	-0.849	-0.518	Arginine and proline metabolism
Fructose-1,6-bisphosphate	0.778	-0.743	Phenylalanine, tyrosine and tryptophan biosynthesis
Phenylalanine	-0.869	-0.733	Phenylalanine, tyrosine and tryptophan biosynthesis. Phenylalanine metabolism
Tyrosine	-0.868	-0.485	Phenylalanine, tyrosine and tryptophan biosynthesis. Tyrosine metabolism
Threonine	0.921	0.973	Glycine, serine and threonine metabolism. Valine, leucine and isoleucine biosynthesis
Serine	-0.019	-0.440	Glycine, serine and threonine metabolism. Cysteine and methionine metabolism
Tryptophan	-0.829	-0.748	Glycine, serine and threonine metabolism. Tryptophan metabolism
NAD+	0.975	0.900	Oxidative phosphorylation
Carnitine	0.935	0.788	Lysine degradation ABC transporters
Choline	-0.962	-0.982	Glycine, serine and threonine metabolism
Lysine	-0.889	-0.538	Lysine biosynthesis
Histidine	-0.785	-0.254	Histidine metabolism
Ribose-/ribulose-5-phosphate	-0.916	-0.968	Pentose phosphate pathway. Purine metabolism
Glycine	0.966	0.948	Purine and pyrimidine metabolism

Adenine	0.677	-0.971	Purine and pyrimidine metabolism
GMP	0.243	-0.998	Purine and pyrimidine metabolism
GDP	0.922	0.999	Purine and pyrimidine metabolism
ADP	0.989	1.000	Purine and pyrimidine metabolism
AMP	0.845	0.982	Purine and pyrimidine metabolism
ATP	0.996	1.000	Purine and pyrimidine metabolism
alpha-Ketoglutarate	0.711	1.000	TCA cycle
Fumarate	0.864	0.790	TCA cycle
Malate	0.735	0.990	TCA cycle
Pyruvate	0.826	-0.226	TCA cycle. Glycolysis / Gluconeogenesis.
Succinate	-0.558	-0.998	TCA cycle. Oxidative phosphorylation
3-Methyl-2-oxovaleric acid	0.310	0.990	Valine, leucine and isoleucine biosynthesis/degradation
Asparagine	0.711	0.896	Alanine, aspartate and glutamate metabolism
Aspartate	0.992	0.995	Alanine, aspartate and glutamate metabolism. Glycine, serine and threonine metabolism

Table S11 Pearson correlation of the different metabolites (and their associated pathways) with the incubation time (4, 8, 24h) in untargeted metabolomics. Samples: sensitive HCT116 cell control and sensitive HCT116 cell incubated with 5 µM oxaliplatin. Method: UPLC BEH Amide at pH 4 in positive and negative mode with protein normalization. Differences in Pearson correlation between experiments without (sensitive control) and with drug incubation (sensitive oxaliplatin) were interpreted as metabolic perturbation exerted by the drug. A difference was observed in palmitoylcarnitine upon drug incubation.

Metabolite	Sens Ctrl	Sens Oxali	Pathway
N-(tert-Butoxycarbonyl)-L-leucine	0.662	0.811	N/A
N1,N12-Diacetylspermine	0.692	0.675	N/A
N8-Acetylspermidine	0.704	0.741	N/A
Palmitoylcarnitine	-0.949	0.820	Fatty acid degradation. Fatty acid metabolism

Table S12 Pearson correlation of the different metabolites (and their associated pathways) with the incubation time (4, 8, 24 h) in targeted metabolomics. Samples: resistant HCT116 cell control and resistant HCT116 cell incubated with 5 µM oxaliplatin. Method: UPLC BEH Amide at pH 4 in positive and negative mode with internal standardization and protein normalization. Differences in Pearson correlation between experiments without (resistant control) and with drug incubation (resistant oxaliplatin) were interpreted as metabolic perturbation exerted by the drug. Only, small changes of metabolite levels could be observed upon drug incubation even after 24 h. High differences in the Pearson correlation can be observed for glutathione reduced, choline, adenine and 3-Methyl-2-oxovaleric acid.

Metabolite	Res Ctrl	Res Oxali	Pathway
NADP+	0.999	0.990	Glutathione metabolism. Nicotinate and nicotinamide metabolism
Glutathione, oxidized	0.983	0.976	Glutathione metabolism

Glutathione, reduced	-0.983	0.924	Cysteine and methionine metabolism. Glutathione metabolism.
Methionine	-0.116	-0.612	Cysteine and methionine metabolism
Hexose phosphate	-0.742	0.062	Glycolysis / Gluconeogenesis
Hexose	-0.900	-0.395	Glycolysis / Gluconeogenesis. Pentose phosphate pathway
Lactate	-0.874	-0.934	Glycolysis / Gluconeogenesis
Dihydroxyacetonephosphate	0.614	-0.057	Glycolysis / Gluconeogenesis
2/3-Phosphoglycerate	-0.427	-0.935	Glycolysis and calvin cycle
Glutamate	0.997	0.999	Arginine biosynthesis. Alanine, aspartate and glutamate metabolism. Arginine and proline metabolism. D-Glutamine and D-glutamate metabolism.
Glutamine	-0.107	-0.142	Arginine biosynthesis. Alanine, aspartate and glutamate metabolism. Purine metabolism. Pyrimidine metabolism. D-Glutamine and D-glutamate metabolism.
Alanine	0.981	0.983	Alanine, aspartate and glutamate metabolism
Citrulline	-0.535	-0.310	Arginine biosynthesis
Arginine	-0.820	-0.760	Arginine and proline metabolism
Fructose-1,6-bisphosphate	0.867	0.988	Phenylalanine, tyrosine and tryptophan biosynthesis
Phenylalanine	-0.497	-0.320	Phenylalanine, tyrosine and tryptophan biosynthesis. Phenylalanine metabolism
Tyrosine	0.031	-0.228	Phenylalanine, tyrosine and tryptophan biosynthesis. Tyrosine metabolism
Threonine	0.013	0.089	Glycine, serine and threonine metabolism. Valine, leucine and isoleucine biosynthesis
Serine	-0.681	-0.681	Glycine, serine and threonine metabolism. Cysteine and methionine metabolism
Tryptophan	-0.544	-0.368	Glycine, serine and threonine metabolism. Tryptophan metabolism
NAD+	0.996	1.000	Oxidative phosphorylation
Carnitine	0.991	0.989	Lysine degradation ABC transporters
Choline	0.979	-0.829	Glycine, serine and threonine metabolism
Lysine	-0.825	-0.776	Lysine biosynthesis
Histidine	-0.433	-0.287	Histidine metabolism
Ribose-/ribulose-5-phosphate	-0.544	-0.754	Pentose phosphate pathway. Purine metabolism
Glycine	0.989	1.000	Purine and pyrimidine metabolism
Adenine	-0.629	0.450	Purine and pyrimidine metabolism
GMP	0.803	0.628	Purine and pyrimidine metabolism
GDP	1.000	0.993	Purine and pyrimidine metabolism
ADP	1.000	1.000	Purine and pyrimidine metabolism
AMP	0.985	0.966	Purine and pyrimidine metabolism
ATP	0.997	0.984	Purine and pyrimidine metabolism
alpha-Ketoglutarate	0.999	0.945	TCA cycle
Fumarate	0.999	0.993	TCA cycle
Malate	0.997	0.997	TCA cycle
Pyruvate	-0.041	0.934	TCA cycle. Glycolysis / Gluconeogenesis.
Succinate	-0.748	-0.949	TCA cycle. Oxidative phosphorylation
3-Methyl-2-oxovaleric acid	-0.654	0.777	Valine, leucine and isoleucine biosynthesis/degradation
Asparagine	0.726	0.615	Alanine, aspartate and glutamate metabolism

Aspartate	0.548	0.883	Alanine, aspartate and glutamate metabolism. Glycine, serine and threonine metabolism

Table S13 Pearson correlation of the different metabolites (and their associated pathways) with the incubation time (4, 8, 24 h) in untargeted metabolomics. Samples: resistant HCT116 cell control and resistant HCT116 cell incubated with 5 µM oxaliplatin. Method: UPLC BEH Amide at pH 4 in positive and negative mode with protein normalization. No changes could be observed upon drug incubation.

Metabolite	Res Ctrl	Res Oxali	Pathway
N-(tert-Butoxycarbonyl)-L-leucine	0.969	0.990	N/A
N1,N12-Diacetylspermine	-0.999	-0.690	N/A
N8-Acetylspermidine	0.208	0.665	N/A
Palmitoylcarnitine	-0.996	-0.920	Fatty acid degradation. Fatty acid metabolism