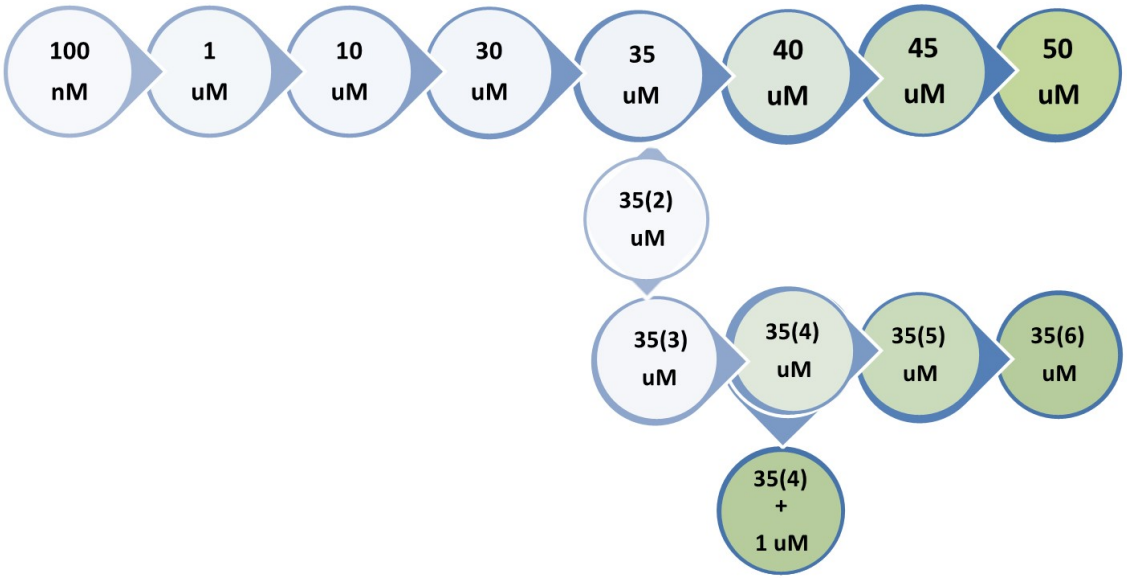
Supplementary figures:



Scheme 1. Concentrations of tamoxifen used to treat sensitive MCF-7 cells to develop tamoxifen resistance to produce the three resistant cell lines.

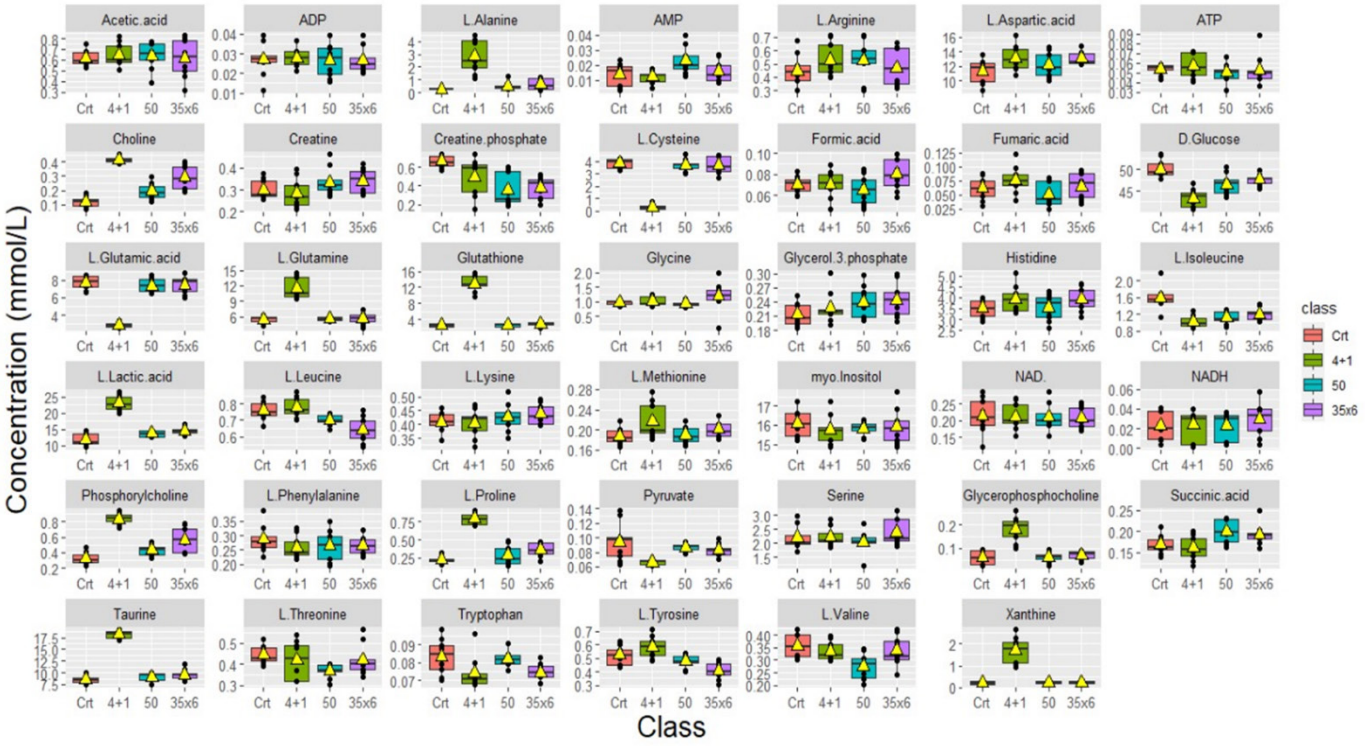


Figure S1. Metabolic changes in tamoxifen resistant MCF7 breast cancer cells. Boxplots showing the concentrations (y-axis) of the 41 metabolites via NMR-based profiling found in control (n = 9), 4+1 (n = 9), 50 (n = 9) and 35x6 (n = 9) groups. The black bars show the respective median of a distribution, while the yellow triangles show the respective average. Please note that the scale of the y-axis was adapted to the concentration range and is therefore different among the different metabolites.

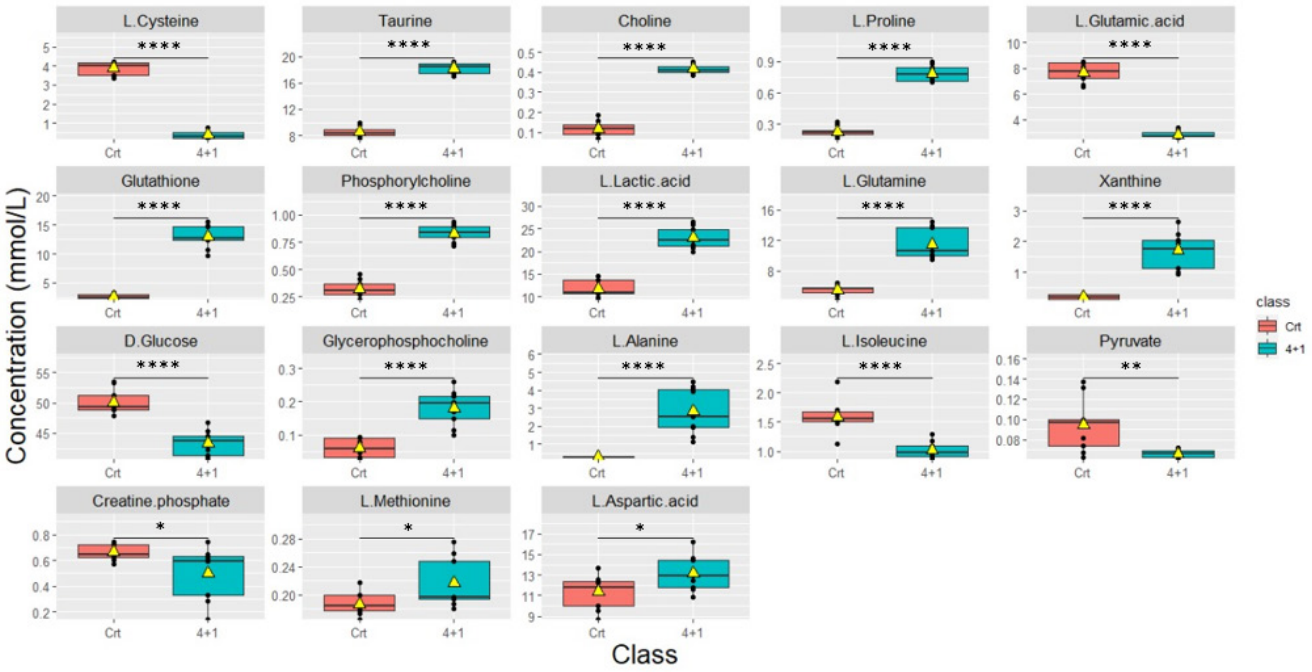


Figure S2. Metabolic changes in tamoxifen-resistant MCF7 breast cancer cells. Boxplots showing the concentrations (y-axis) of the significant metabolites found in control (n=9) vs 4+1 (n=9) groups. The black bars show the respective median of a distribution, while the yellow triangles show the respective average. Please note that the scale of the y-axis was adapted to the concentration range and is therefore different among the different metabolites. *p < 0.05, **p< 0.01, and ****p < 0.0001 calculated using the Students' t-test.

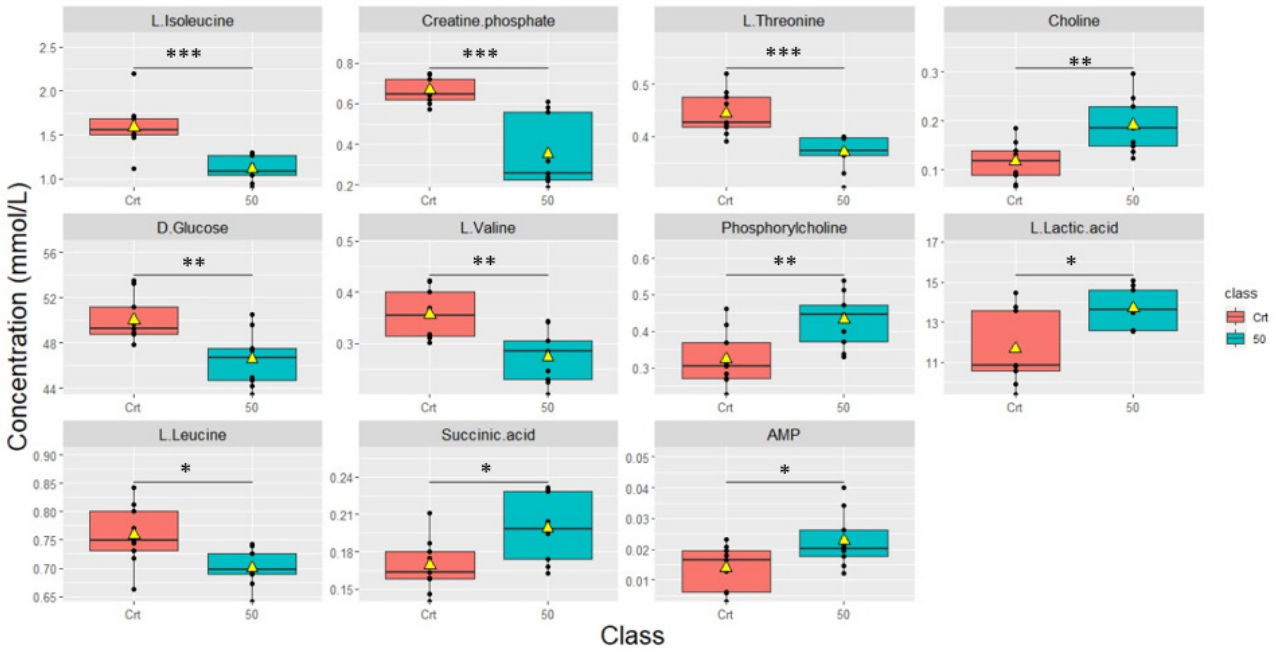


Figure S3. Metabolic changes in tamoxifen-resistant MCF7 breast cancer cells. Boxplots showing the concentrations (y-axis) of the significant metabolites found in control (n = 9) vs 50 (n = 9) groups. The black bars show the respective median of a distribution, while the yellow triangles show the respective average. Please note that the scale of the y-axis was adapted to the concentration range and is therefore different among the different metabolites. *p < 0.05, **p< 0.01, and ***p < 0.001 calculated using the Students' t-test.

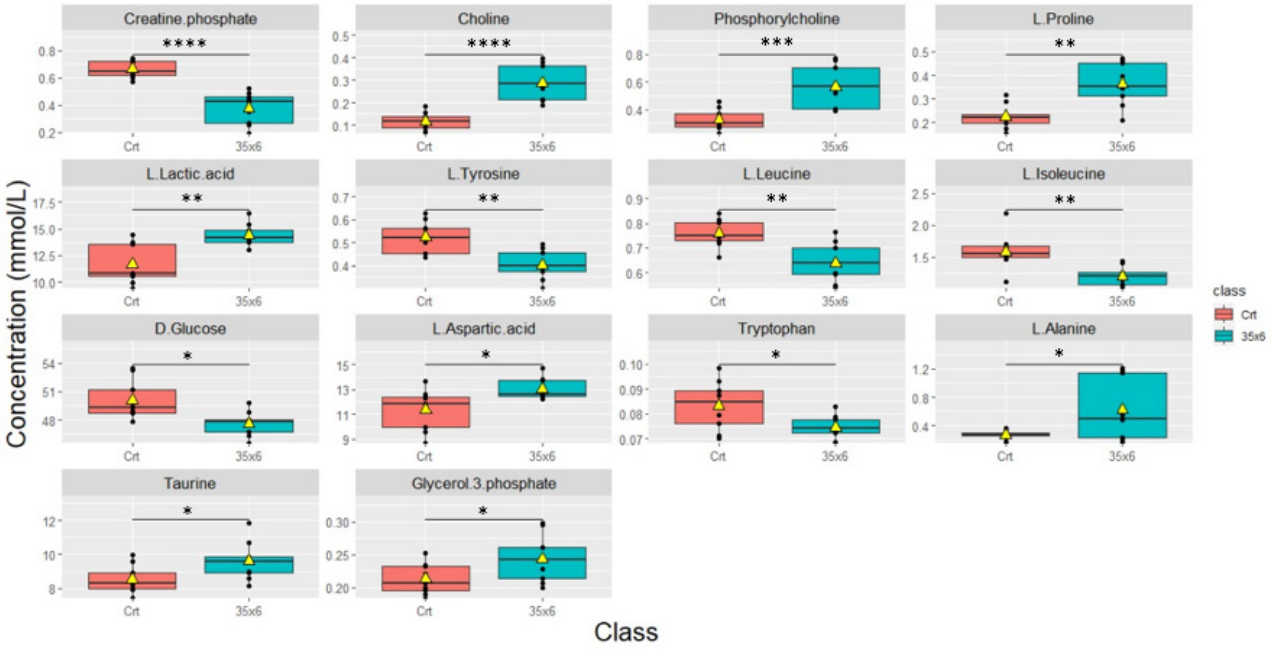
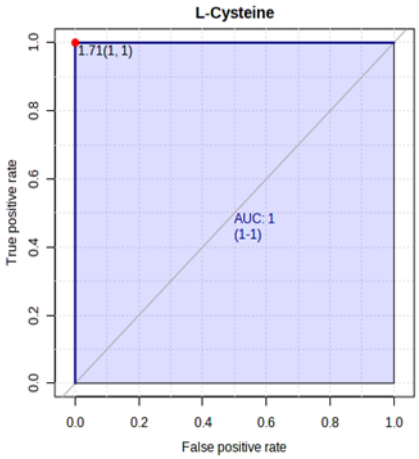
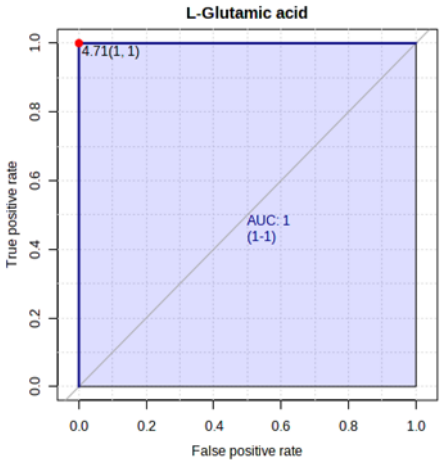
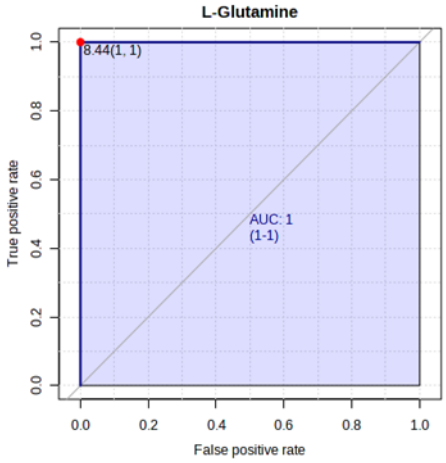
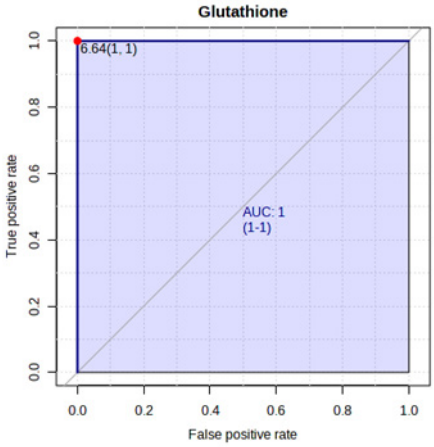


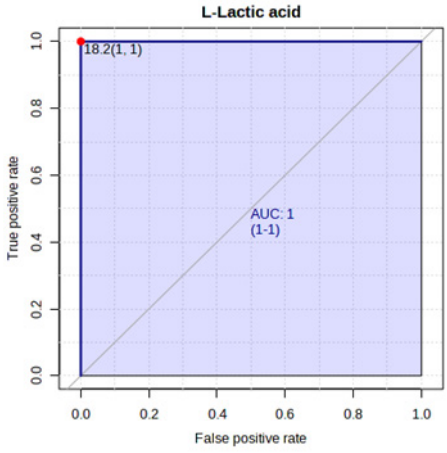
Figure S4. Metabolic changes in tamoxifen-resistant MCF7 breast cancer cells. Boxplots showing the concentrations (y-axis) of the significant metabolites found in control (n=9) vs 35x6 (n=9) groups. The black bars show the respective median of a distribution, while the yellow triangles show the respective average. Please note that the scale of the y-axis was adapted to the concentration range and is therefore different among the different metabolites. *p < 0.05, **p< 0.01, ***p < 0.001, and ****p < 0.0001 calculated using the Students' t-test.

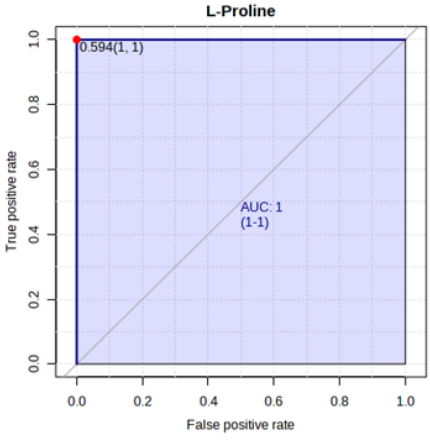

L-Cysteine
1.71(1, 1)
AUC: 1
(1-1)
True positive rate
False positive rate
0.0
0.2
0.4
0.6
0.8
1.0

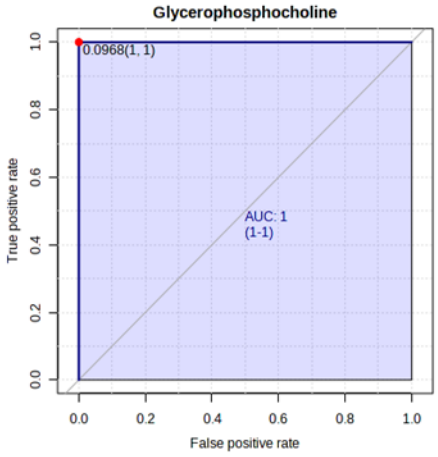

L-Glutamic acid
4.71(1, 1)
AUC: 1
(1-1)
True positive rate
False positive rate
0.0
0.2
0.4
0.6
0.8
1.0

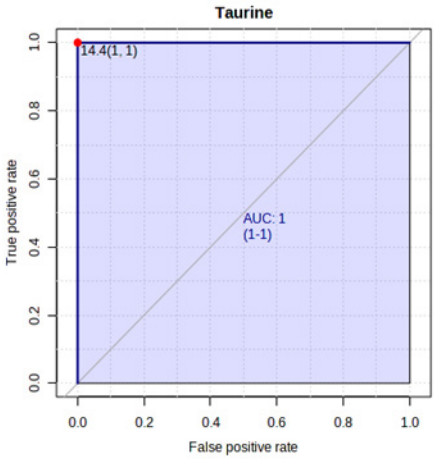

L-Glutamine
8.44(1, 1)
AUC: 1
(1-1)
True positive rate
False positive rate
0.0
0.2
0.4
0.6
0.8
1.0

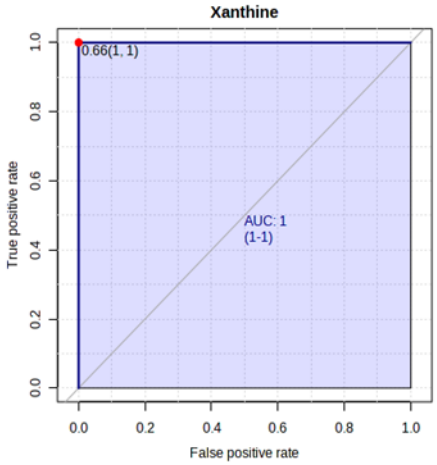

Glutathione
6.64(1, 1)
AUC: 1
(1-1)
True positive rate
False positive rate
0.0
0.2
0.4
0.6
0.8
1.0

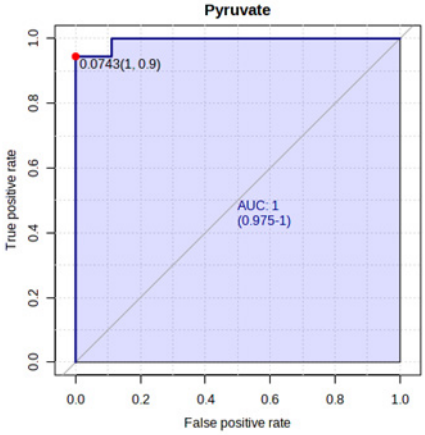

L-Lactic acid
18.2(1, 1)
AUC: 1
(1-1)
True positive rate
False positive rate
0.0
0.2
0.4
0.6
0.8
1.0

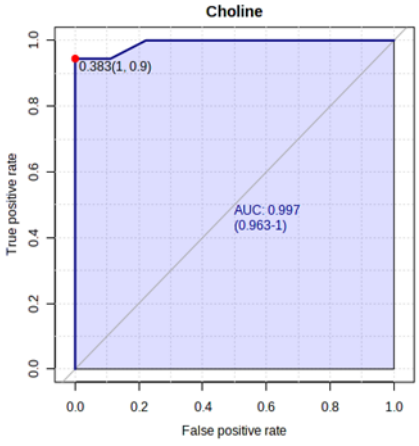

L-Proline
0.594(1, 1)
AUC: 1
(1-1)
True positive rate
False positive rate
0.0
0.2
0.4
0.6
0.8
1.0

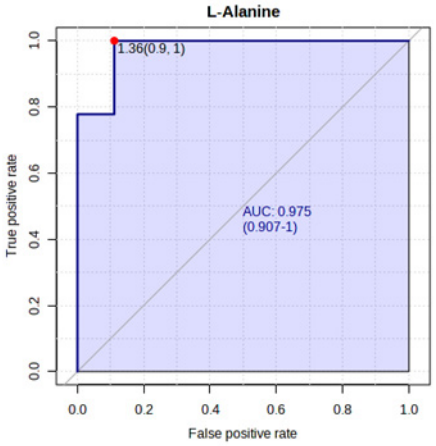

Glycerophosphocholine
0.0968(1, 1)
AUC: 1
(1-1)
True positive rate
False positive rate
0.0
0.2
0.4
0.6
0.8
1.0


Taurine
14.4(1, 1)
AUC: 1
(1-1)
True positive rate
False positive rate
0.0
0.2
0.4
0.6
0.8
1.0


Xanthine
0.66(1, 1)
AUC: 1
(1-1)
True positive rate
False positive rate
0.0
0.2
0.4
0.6
0.8
1.0


Pyruvate
0.0743(1, 0.9)
AUC: 1
(0.975-1)
True positive rate
False positive rate
0.0
0.2
0.4
0.6
0.8
1.0


Choline
0.383(1, 0.9)
AUC: 0.997
(0.963-1)
True positive rate
False positive rate
0.0
0.2
0.4
0.6
0.8
1.0


L-Alanine
1.36(0.9, 1)
AUC: 0.975
(0.907-1)
True positive rate
False positive rate
0.0
0.2
0.4
0.6
0.8
1.0

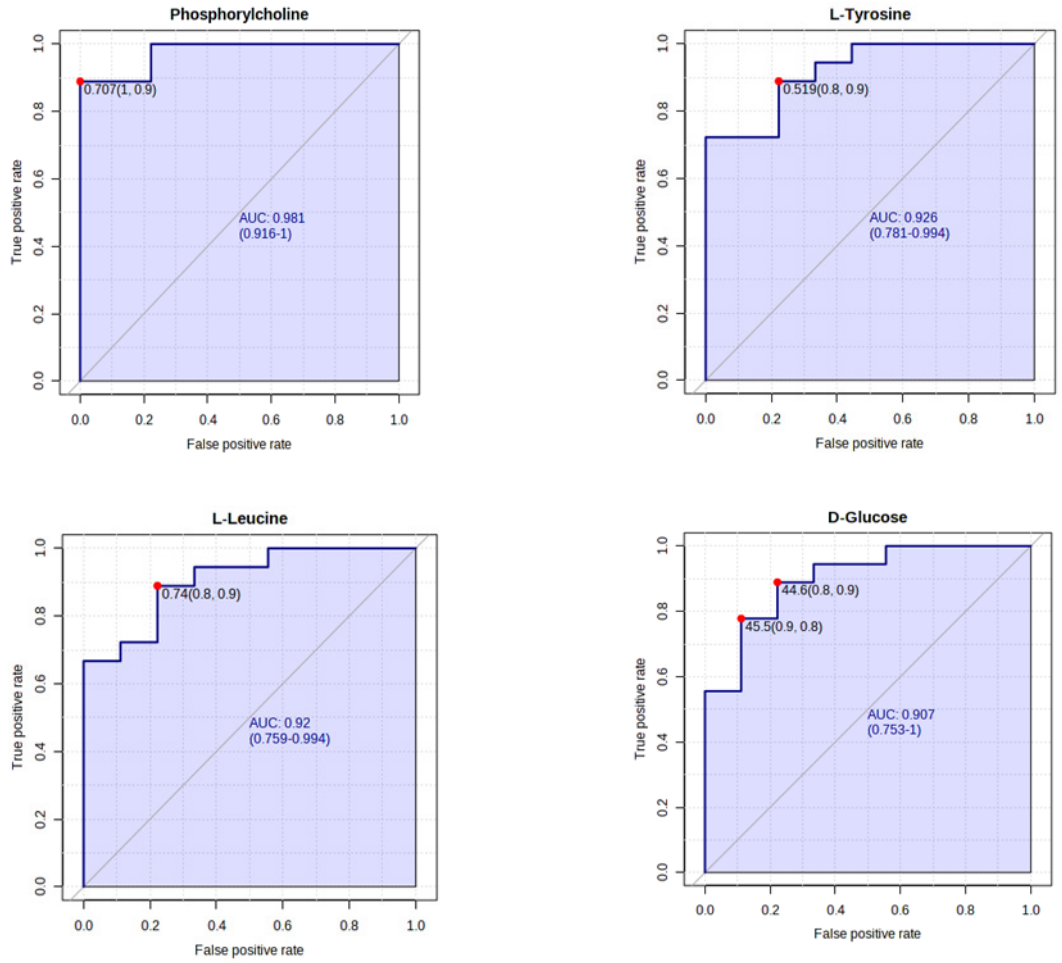


Figure S5. Receiver operating characteristic curves (ROC) are used to distinguish between groups 4+1 and 50, 35x6. The AUCs for 16 metabolites were above 0.9, indicating that they were of high diagnostic value.

Supplementary tables:

Table S1. Forward and reverse primers used in gene expression analysis.

Gene symbol	*Forward*	*Reverse*
G6PD	GGAAACGGTCGTACACTTCG	AGCCCACGATGAAGGTGTTT
GLS	CATCTTTGAAGGCCACTGCT	AGACCATGCTGAGAAAGCGT
XDH	ATGGGCCAAGGCCTTCATAC	TAGACGGCCTGTCCATTGAG
GSS	GAGAACCGTTCGCGGAGGAA	AGAGCGTGAATGGGGCATAG
ACTB	AGAGCTACGAGCTGCCTGAC	AGCACTGTGTTGGCGTACAG

Table S2. "Area under the curve" (AUC) values obtained from receiver operating characteristic (ROC) curves analysis in 4+1 compared to 50 and 35x6. based on the metabolite levels determined in the 27 treated breast cancer cells analyzed in this study. Nine of the 41 metabolites, namely L-cysteine, L-glutamic acid, L-glutamine, glutathione, L-lactic acid, L-proline, glycerophosphocholine, taurine, and xanthine, showed an AUC value equal to 1.

Metabolite	*AUC*	*Metabolite*	*AUC*	*Metabolite*	*AUC*
L-Cysteine	1	L-Leucine	0.90741	Myo-Inositol	0.58642
L-Glutamic acid	1	D-Glucose	0.90123	ADP	0.57716
L-Glutamine	1	Succinic acid	0.8179	Histidine	0.57407
Glutathione	1	AMP	0.75617	Glycine	0.5679
L-Lactic acid	1	Creatine	0.74691	L-Phenylalanine	0.5679
L-Proline	1	L-Isoleucine	0.74691	L-Aspartic acid	0.5679
Glycerophosphocholine	1	Tryptophan	0.74691	NADH	0.56173
Taurine	1	Creatine phosphate	0.73457	L-Arginine	0.55864
Xanthine	1	Fumaric acid	0.70062	L-Threonine	0.55556
Pyruvate	0.99383	L-Valine	0.67901	Formic acid	0.53704
Choline	0.99074	L-Methionine	0.66667	NAD+	0.51852
L-Alanine	0.97531	ATP	0.64198	Acetic acid	0.51235
Phosphorylcholine	0.97531	L-Lysine	0.6358	Serine	0.50617
L-Tyrosine	0.91975	Glycerol 3-phosphate	0.62346		

Table S3. Metabolites (n = 41) detected in breast cancer cells continuously treated samples with tamoxifen (A) vs 50, 35x6 (B) using cryogenic probe NMR spectroscopy. Given are p-values. Incidences represent the number of samples (continuously treated (n=9) and group 50, 35x6 (n=18)), in which the respective metabolite could be detected. As data processing for statistical evaluation described in methodological part, p-values study the comparison of metabolite levels in the group of continuously treated cells (A) and group 50, 35x6 (B). Metabolites (n = 21) with significantly different levels between the two groups are shown in bold. p-values were determined by t-test using Metaboanalyst.

Metabolites	***P-value***	***Metabolites***	***P-value***	***Metabolites***	***P-value***
Taurine	2.12E-17	**D-Glucose**	0.000101	L-Aspartic acid	0.37291
Glutathione	2.41E-17	**L-Leucine**	0.000336	L-Threonine	0.42763
L-Cysteine	4.69E-15	**Succinic acid**	0.00324	Histidine	0.46724
L-Glutamic acid	2.11E-14	**AMP**	0.029101	L-Arginine	0.57871
L-Lactic acid	3.64E-13	**L-Isoleucine**	0.035111	ADP	0.64457
L-Glutamine	3.40E-11	**L-Methionine**	0.040434	NADH	0.65593
Xanthine	7.65E-11	**Creatine**	0.040595	Glycine	0.67438
L-Proline	9.88E-11	Fumaric acid	0.059914	L-Phenylalanine	0.67818
Glycerophosphocholine	1.63E-08	Creatine phosphate	0.061416	Formic acid	0.71988
Pyruvate	2.27E-07	Tryptophan	0.1523	Acetic acid	0.73349
L-Alanine	2.60E-07	L-Lysine	0.16593	myo-Inositol	0.76999
Phosphorylcholine	4.31E-07	L-Valine	0.19795	NAD+	0.87593
Choline	2.00E-06	ATP	0.28395	Serine	0.98869
L-Tyrosine	6.69E-05	Glycerol 3-phosphate	0.31052		