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Figure S1. One-dimensional ¹H NMR PROJECT spectra of untreated (a) HEK-293, (b) A2780, and (c) A2780cisR cells.



Figure S2. Aromatic region of PROJECT spectra of the three untreated cells lines: (a) HEK-293, (b) A2780, and (c) A2780cisR.

Table S1. The 52 buckets in the aliphatic region of the 1-D ¹H NMR spectra (0.79–4.40 ppm) and the corresponding metabolites. (The metabolites in brackets can be present partially.) Ac = acetate; Ala = alanine; Chol = choline; Cre = creatine; Cys = cysteine; Glu = glutamate; Gln = glutamine; GSH = glutathione; Lac = lactate; Leu = leucine; myo-Ino = myo-inositol; = phosphocholine; Tyr = tyrosine.

Bucket	Range (ppm)	Metabolites	
1	0.791–0.991	lipid ω-CH3, Leu	
2	0.991-1.029	not assigned	
3	1.029–1.069	Val	
4	1.227–1.318	lipid (–CH2)n	
5	1.318–1.361	lipid (–CH2)n, Lac	
6	1.361-1.465	not assigned	
7	1.465–1.502	Ala	
8	1.545–1.635	lipid β-CH2	
9	1.635–1.756	Leu	
10	1.756–1.815	not assigned	
11	1.880–1.946	Ac (+ other)	
12	1.982-2.143	lipid –CH2–CH=, Gln/Glu	
13	2.153-2.198	GSH, Glu, Gln	
14	2.219–2.287	lipid α-CH ₂ (+ other)	
15	2.291-2.321	not assigned	
16	2.321-2.381	Glu	
17	2.381-2.404	Glu	
18	2.404-2.422	Glu , Gln	
19	2.422-2.484	Gln	
20	2.484-2.618	GSH	
21	2.618-2.674	not assigned	
22	2.674-2.731	not assigned	
23	2.731-2.748	not assigned	
24	2.748-2.764	not assigned	
25	2.909–2.938	(GSH)	
26	2.938-2.976	GSH	
27	2.976-3.020	not assigned	
28	3.022-3.051	Cre (+ possibly Cys)	
29	3.075-3.203	Tyr, Cys	
30	3.203–3.218	Chol	
31	3.218-3.246	PC	
32	3.246-3.304	myo-ino, Tyr	
33	3.341-3.354	not assigned	
34	3.354-3.368	not assigned	
35	3.383–3.457	not assigned	
36	3.471-3.519	not assigned	
37	3.519–3.537	Chol, myo-ino	
38	3.537-3.557	myo-ino (+ possibly Chol)	
39	3.557-3.572	not assigned	
40	3.572-3.587	not assigned	
41	3.587-3.632	PC, myo-ino	
42	3.732-3.816	GSH, Glu, Ala, (Gln)	

43	3.816–3.871	not assigned	
44	3.871-3.925	not assigned	
45	3.925–3.951	Cre (+ Tyr)	
46	3.951–3.987	Cys (+ Tyr)	
47	3.987-4.044	Cys	
48	4.044-4.077	Chol, myo-ino	
49	4.093-4.148	Lac	
50	4.148-4.200	PC	
51	4.218-4.365	not assigned	
52	4.365-4.400	not assigned	

Table S2. The 44 buckets in the aromatic region of the 1-D 1H NMR spectra (5.78–8.95 ppm) and the corresponding metabolites. (The metabolites in brackets can be present partially.) AMP = adenosine monophosphate; Cyd = cytidine; Fum = fumarate; NADH = Nicotinamide adenine dinucleotide; Phe = phenylalanine; Tyr = tyrosine, UDP = uridine diphosphate; Ura = uracil; Urd = uridine; UTP = uridine triphosphate.

Bucket	Range (ppm)	Metabolites
1	5.786-5.818	Ura
2	5.86-5.898	not assigned
3	5.898-5.927	Cyt/Urd
4	5.927-5.946	not assigned
5	5.946-5.968	Ura/UDP/UTP
6	5.968-5.983	Ura/UDP/UTP
7	5.983-6.008	Ura/UDP/UTP
8	6.008-6.024	Ura/UDP/UTP
9	6.042-6.057	Cyt/Urd
10	6.057–6.076	not assigned
11	6.082–6.123	not assigned
12	6.123–6.17	NADH*
13	6.513–6.532	Fum*
14	6.875–6.893	Tyr
15	6.893–6.911	Tyr
16	7.085–7.125	not assigned
17	7.125–7.17	not assigned
18	7.17–7.208	Tyr
19	7.284–7.302	not assigned
20	7.302–7.346	Phe
21	7.346–7.399	not assigned
22	7.399–7.451	Phe
23	7.52–7.536	Ura
24	7.536–7.554	Ura
25	7.554–7.612	not assigned
26	7.846–7.864	not assigned
27	7.864–7.882	not assigned
28	7.886–7.903	not assigned
29	7.903–7.922	not assigned
30	7.922–7.957	not assigned

31	7.957–7.996	UDP/UTP	
32	7.996–8.027	UDP	
33	8.081-8.1	not assigned	
34	8.1-8.179	not assigned	
35	8.182-8.201	NADH*	
36	8.201-8.245	NADH*	
37	8.245-8.275	AMP	
38	8.354-8.371	not assigned	
39	8.371-8.426	not assigned	
40	8.446-8.462	NADH*	
41	8.529-8.558	not assigned	
42	8.594-8.637	AMP	
43	8.691-8.725	not assigned	
44	8.923-8.952	not assigned	

*tentative assignment



Figure S3. PCA score plots of the aromatic region of (**a**) HEK-293, (**b**,**c**) A2780, and (**d**) A2780cisR cells treated with 0.03 µM of DiRu-1 (blue), 0.015 of µM DiRu-1 (green), or the control (dark red).



Figure S4. PLS-DA score plots of the aliphatic region of (**a**) HEK-293, (**b**) A2780, and (**c**) A2780cisR cells treated with 0.03 μM of DiRu-1 (blue) and the untreated control (dark red).

Table S3: Summary	of	parameters ar	nd st	tatistical	results of	ΡI	LS-DA	shown	in	Figure S	S6.
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Groups	HEK-293	A2780	A2780cisR		
X-block	20 x 52	20 x 52	19 x 52		
Number of LVs	4	5	3		
Included samples	(1–20) (1–52)	(1–20) (1–52)	(1–13 15–20) (1–52)		
Preprocessing	mean center, UVS	mean center, UVS	mean center, UVS		
Algorithm	PLS-DA	PLS-DA	PLS-DA		
Cross validation	Venetian blinds 4	Venetian blinds 4	Venetian blinds 4		
	splits, 1 sample/split	splits, 1 sample/split	splits, 1 sample/split		
RMSEC	0.088	0.079	0.100		
RMSECV	0.318	0.542	0.231		
R ² Cal	0.969	0.975	0.960		
R ² CV	0.601	0.055	0.788		
Wilcoxon (self-	0.025	0.026	0.010		
pred.)* y-column 1					
Wilcoxon (self-	0.025	0.026	0.010		
pred.)* y-column 2					
Wilcoxon (cross-	0.021	0.162	0.002		
val.)* y-column 1					
Wilcoxon (cross-	0.021	0.162	0.002		
val.)* y-column 2					
Sign test (self-pred.)*	0.081	0.089	0.036		
y-column 1					

Sign test (self-pred.)*	0.081	0.089	0.036		
y-column 2					
Sign test (cross-val.)*	0.082	0.263	0.011		
y-column 1					
Sign test (cross-val.)*	0.082	0.263	0.011		
y-column 2					
Rand t-test (self-	0.043	0.056	0.060		
pred.)* y-column 1					
Rand t-test (self-	0.043	0.052	0.061		
pred.)* y-column 2					
Rand t-test (cross-	0.021	0.207	0.007		
val.)* column 1					
Rand t-test (cross-	0.020	0.212	0.008		
val.)* column 2					
Total variance	50.90%	66.50%	52.04%		
captured					
LV 1	18.12%	26.43%	36.43%		
LV 2	20.37%	10.51%	8.78%		
LV 3	6.73%	17.83%	6.83%		
LV 4	5.68%	5.86%			
LV 5		5.88%			
* Values bellow 0.05 indicate significance at 95% confidence level.					



Figure S5. PLS analysis score plots of the aromatic region of (**a**) HEK-293, (**b**) A2780, and (**c**) A2780cisR cells treated with 0.03 μ M of DiRu-1 (blue), 0.015 μ M of DiRu-1 (green), or the untreated control (dark red).



Figure S6. PCA loading plots for the PCA shown in Figure 3 (aliphatic region) of (**a**–**c**) HEK-293, (**d**–**f**) A2780, and (**g**,**h**) A2780cisR cells. Control, low dose (0.015 μ M), and full dose (0.03 μ M) were analysed.



Figure S7. PLS loading plots for the latent variables (LV1–3) of the PLS plots shown in Figure 3 (aliphatic region) of (**a**,**b**) HEK-293, (**c**–**e**) A2780, and (**f**,**g**) A2780cisR cells: Control, low dose (0.015 μ M), and full dose (0.03 μ M) were analysed.



Figure S8. PLS-DA loading plots for the latent variables of the PLS-DA plots shown in Figure S6 (aliphatic region) of (**a**,**b**) HEK-293, (**c**–**e**) A2780, and (**f**–**h**) A2780cisR cells: Only control and full dose (0.03 µM) were analysed.



Figure S9. PCA loading plots of the PCA shown in S5 (aromatic region) of (**a**–**c**) HEK-293, (**d**,**e**) A2780, and (**f**–**h**) A2780cisR cells: Control, low dose (0.015 μ M), and full dose (0.03 μ M) were analysed.



Figure S10. PLS loading plots for the latent variables of the PLS plots shown in Figure S8 (aromatic region) of (**a**–**c**) HEK-293, (**d**,**e**) A2780, and (**f**–**h**) A2780cisR cells: Control, low dose (0.015 μ M), and full dose (0.03 μ M) were analysed.



Figure S11. TOCSY spectra of HEK-293 cells treated with a low dose (0.015 $\mu M)$ of DiRu-1.



Figure S12. TOCSY spectra of A2780 cells untreated.



Figure S13. TOCSY spectra of A2780cisR cells treated with a high dose (0.03 $\mu M)$ of DiRu-1.

A2780cisR



Figure S14. Percental change in the aliphatic buckets in A2780cisR cells: The increasing/decreasing level of each bucket compared to the control in cells treated with 0.015 μ M DiRu-1 (blue) and 0.03 μ M DiRu-1 (red) are expressed in percentage. The buckets marked with * show *p*-values < 0.05. The buckets marked with # show Benjamini–Hochberg corrected *p*-values < 0.05.



Figure S15. Percental change in the aliphatic buckets in A2780 cells: The increasing/decreasing level of each bucket compared to the control in cells treated with 0.015 μ M DiRu-1 (blue) and 0.03 μ M DiRu-1 (red) are expressed in percentage. The buckets marked with * show *p*-values < 0.05. The buckets marked with # show Benjamini–Hochberg corrected *p*-values < 0.05.

A2780





Figure S16. Percental change in the aliphatic buckets in HEK-293 cells: The increasing/decreasing level of each bucket compared to the control in cells treated with 0.015 μ M DiRu-1 (blue) and 0.03 μ M DiRu-1 (red) are expressed in percentage. The buckets marked with * show *p*-values < 0.05. The buckets marked with # show Benjamini–Hochberg corrected *p*-values < 0.05.

A2780cisR - aromatic region



Figure S17. Percental change in the aromatic buckets in A2780cisR cells: The increasing/decreasing level of each bucket compared to the control in cells treated with 0.015 μ M DiRu-1 (turquoise) and 0.03 μ M DiRu-1 (red) are expressed in percentage. * *p*-value < 0.05



A2780 - aromatic region

Figure S18. Percental change in the aromatic buckets in A2780 cells: The increasing/decreasing level of each bucket compared to the control in cells treated with 0.015 μ M DiRu-1 (red) and 0.03 μ M DiRu-1 (turquoise) are expressed in percentage. * *p*-value < 0.05

% change upon treatment

HEK-293 - aromatic region



Figure S19. Percental change in the aromatic buckets in HEK-293 cells: The increasing/decreasing level of each bucket compared to the control in cells treated with 0.015 μ M DiRu-1 (turquoise) and 0.03 μ M DiRu-1 (red) are expressed in percentage. * *p*-value < 0.05

A2780cisR



Figure S20. Comparison of bucket means for aliphatic region of A2780cisR: Control (blue), treated with 0.015 μ M DiRu-1 (yellow), and 0.03 μ M DiRu-1 (red). Error bars show standard error.



Figure S21. Comparison of bucket means for aliphatic region of A2780: Control (blue), treated with 0.015 µM DiRu-1 (yellow), and 0.03 µM DiRu-1 (red). Error bars show standard error.

A2780

HEK-293



Figure S22. Comparison of bucket means for aliphatic region of HEK-293: Control (blue), treated with 0.015 µM DiRu-1 (yellow), and 0.03 µM DiRu-1 (red). Error bars show standard error.

A2780cisR aromatic



Figure S23. Comparison of bucket means for aromatic region of A2780cisR: Control (blue), treated with 0.015 μM DiRu-1 (yellow), and 0.03 μM DiRu-1 (red). Error bars show standard error.

A2780 aromatic



Figure S24. Comparison of bucket means for aromatic region of A2780: Control (blue), treated with 0.015 μ M DiRu-1 (yellow), and 0.03 μ M DiRu-1 (red). Error bars show standard error.



HEK-293 aromatic

Figure S25. Comparison of bucket means for aromatic region of HEK-293: Control (blue), treated with 0.015 μM DiRu-1 (yellow), and 0.03 μM DiRu-1 (red). Error bars show standard error.