Metabolic characterization of directly reprogrammed renal tubular epithelial cells (iRECs) – Supplementary Data

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Supplementary table S1: Adjusted extraction volume for the different tissue weights

Kidney sample number	Kidney weight [mg]	Adjusted volume for extraction [ml]
1	240	1.50
2	220	1.38
3	200	1.25
4	210	1.31
5	190	1.19
6	190	1.19

Supplementary table S2: Randomized injection order for untargeted GC/MS profiling and targeted LC/MS validation

Injection order	GC/MS analysis	LC/MS analysis
1	n-hexane blank	Equilibration pool 1
2	C10-C40 alkane standard	Equilibration pool 2
3	Equilibration pool 1	Equilibration pool 3
4	Equilibration pool 2	Equilibration pool 4
5	Equilibration pool 3	Equilibration pool 5
6	Equilibration pool 4	Equilibration pool 6
7	Equilibration pool 5	MEF_CP3
8	Equilibration pool 6	Pool_1
9	MEF_NT_3	MEF_NT_1
10	iREC_CP_2	iREC_CP2
11	iREC_CP_3	IMCD_NT_3
12	Kidney_4	iREC_CP1
13	MEF_CP_3	iREC_NT_2
14	Pool_differentiation_1	iREC_CP3
15	iREC_CP_1	Pool_2
16	MEF_CP_1	MEF_CP1
17	Kidney_2	IMCD_CP1
18	Equilibration pool 9	IMCD_NT_2
19	Blank plate_3	blank1
20	MEF_NT_2	MEF_CP2
21	Kidney_3	iREC_NT_3
22	Kidney_6	IMCD_CP2
23	IMCD_NT_3	MEF_NT_2
24	iREC_NT_2	blank3
25	Blank plate_2	MEF_NT_3
26	IMCD_CP_1	IMCD_NT_1
27	Equilibration pool 7	blank2
28	IMCD_NT_2	iREC_NT_1
29	Kidney_1	Pool_3
30	IMCD_CP_3	IMCD_CP3
31	Pool_differentiation_3	
32	Kidney_5	

33	iREC_NT_1	
34	Pool_CP_1	
35	Pool_CP_2	
36	MEF_CP_2	
37	iREC_NT_3	
38	IMCD_CP_2	
39	MEF_NT_1	
40	Pool_CP_3	
41	Equilibration pool 8	
42	IMCD_NT_1	
43	Pool_differentiation_2	
44	Blank plate_1	

Supplementary table S3: Optimized mass spectrometry parameters and retention time of key metabolites changed upon cisplatin

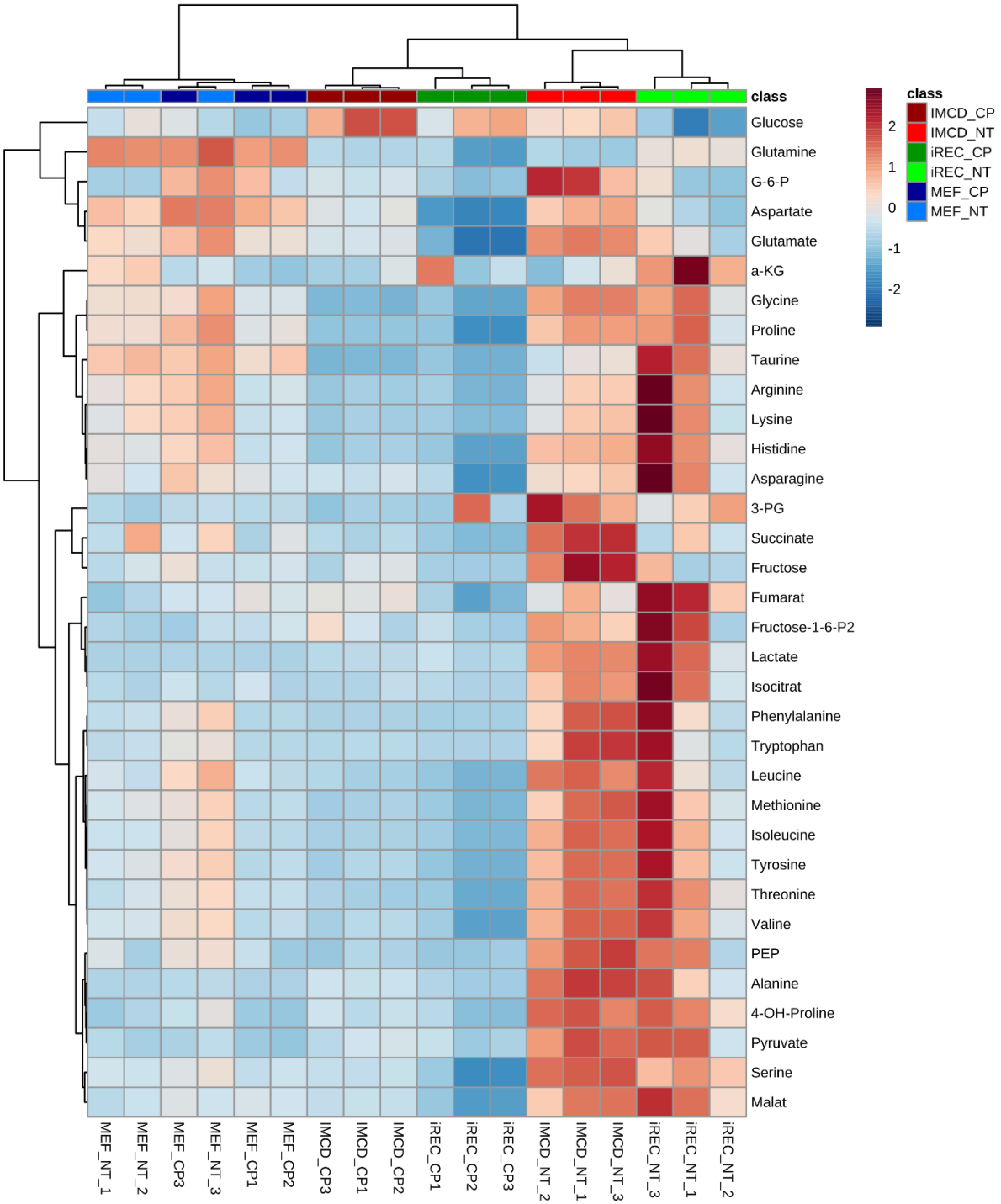
Compound name	Precursor ion [m/z]	Quantifier/qualifier ions [m/z]	Retention time [min]	Fragmentor Voltage [V]	Collision Energy [V]	Accelerator Voltage [V]	Polarity
$^{13}C_3$-Pyruvate	90.1	45.1	3.18	60	7	3	Negative
$^{13}C_4{}^{15}N$-Aspartate	139.1	92.2/77.2, 48.2, 45.2	3.88	60	8/12, 20, 24	4	Positive
3-Phosphoglycerate	185	97/79, 167	5.15	60	13/37, 5	4	Negative
4-OH-Proline	132.1	86.1/68.1, 58.2, 41.1	3.79	80	9/21, 25, 33	3	Positive
Alanine	90.1	44.1	3.74	45	9	3	Positive
alpha-Ketoglutarate	145.1	101/57.1	4.76	60	4/0	4	Negative
Arginine	175.1	70.1/130.1, 116.1, 60.1	7	100	25/9, 9, 13	3	Positive
Asparagine	133.1	74.1/87.1, 46.1, 44.1	3.95	50	13/5, 17, 17	3	Positive
Aspartate	134	88.1/74.1, 46.1, 43.1	3.88	50	5/9, 13, 21	3	Positive
Citrate	191	87.1/111.1, 85.1, 67.1	5.2	60	12/8, 12, 24	4	Negative
Cystine	241	74.2/152.2, 122.2, 120.2	4.5	84	28/8, 16, 20	4	Positive
d_4-Succinate	121	77.1/102.1	4.85	84	9/9	4	Negative
Dihydroxyacetone-phosphate	169	79/97	5.05	84	25/9	4	Negative
Fructose	179.1	89.2/113.2, 113.2, 71.2, 59.1, 43.2	4.47	60	4/0, 4, 12, 16, 28	4	Negative
Fructose-1-6-bisphosphate	339	97/241, 79	5.17	110	13/5, 40	3	Negative
Fructose-6-phosphate	259	97/169.1, 139, 79	5.15	84	13/9, 13, 49	4	Negative
Fumarate	115	71	4.79	60	0	4	Negative
Glucose	179.1	89.1/123.9, 119.1, 71.2, 59.1, 43.3	4.72	60	4/0, 0, 12, 16, 28	4	Negative
Glucose-6-phophate	259	97/199, 139, 79	5.25	84	13/5, 9, 49	4	Negative
Glutamate	148.1	84.1/130.1, 102.1, 56.1	3.8	70	13/5, 5, 29	3	Positive
Glutamine	147.1	130.1, 56.1, 41.1	3.9	70	5/33, 29	3	Positive
Glyceraldehyde-3-phosphate	169.1	79/151.1, 97	5.15	80	35/15, 15	3	Negative
Glycine	76	30.3	3.9	40	5	3	Positive

Histidine	156.1	110.1/93.1, 83.1, 56.1	6.1	80	9/25, 25, 37	3	Positive
Isocitrate	191	73.1/173.1, 111, 85.1	5.1	84	21/9, 9, 13	4	Negative
Isoleucine	132.1	69.1/86.1, 44.2	3.15	50	25/5, 25	3	Positive
Lactate	89	43.1	4.41	60	15	3	Negative
Leucine	132.1	43.2/86.1, 44.1, 30.1	3	60	25/5, 21, 13	3	Positive
Lysine	147.1	130.1/84.1, 67.1, 56.1	7.5	70	5/13, 25, 33	3	Positive
Malate	133	115.1/73, 71.1, 43.1	4.9	60	4/12, 12, 16	4	Negative
Methionine	150.1	56.1/133.1, 104.1, 61.1	3.2	60	13/5, 5, 21	3	Positive
o-Tyrosine	182.1	136.1/165.1, 123.1, 91	2.9	60	9/5, 13, 29	3	Positive
Phenylalanine	166.1	120.1/103.1, 91.1, 77.1	3	70	9/29, 37, 40	3	Positive
Phosphoenol-pyruvate	167	79	5.12	60	9	4	Negative
Proline	116.1	70.1/68.1, 43.1, 28.1	3.5	70	13/33, 33, 37	3	Positive
Pyruvate	87	43.1	3.18	60	5	3	Negative
Serine	106.1	60.1/88.1, 70.1, 42.1	4	50	5/5, 9, 5	3	Positive
Succinate	117	73.1/99.1	4.85	60	8/4	3	Negative
Taurine	126	44.2/108.1, 97.1, 65.1	3.4	108	20/8, 24, 44	4	Positive
Threonine	120.1	56.1/102.1, 74.1, 57	3.8	50	13/5, 5, 17	3	Positive
Tryptophan	205.1	188.1/146.1, 118.1, 91.1	2.9	70	5/13, 25, 40	3	Positive
Tyrosine	182.1	136.1/165.1, 123.1, 91	3.4	60	9/5, 13, 29	3	Positive
Valine	118.1	72.1/57.1, 55.1, 29.1	3.5	50	5/29, 17, 29	3	Positive

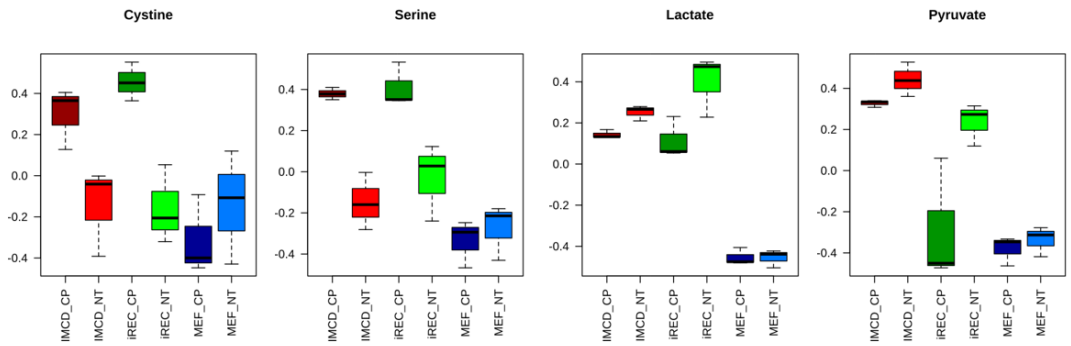
Supplementary table S4: Validation of methodology for key metabolites changed upon cisplatin

Metabolite	Abbreviation	Sum formula	R^2	Equation of regression	lowest tested concentration with Signal/Noise >= 5 [ng/µl]
3-Phosphoglyceric acid	3-PG	$C_3H_7O_7P$	0.966003	y = 7.6787x-6.1506	15
4-Hydroxyproline	4-OH-Proline	$C_5H_9NO_3$	0.996154	y = 0.3007x-3.1506	0.2
alpha-Ketoglutaric acid	a-KG	$C_5H_6O_5$	0.988594	y = 0.0037x-0.1506	15
Alanine	-	$C_3H_7NO_2$	0.983956	y = 0.0747x-0.1506	0.0416
Arginine	-	$C_6H_{14}N_4O_2$	0.843329	y = 0.0017x-0.1506	0.0416
Asparagine	-	$C_4H_8N_2O_3$	0.997408	y = 0.0177x-0.1506	0.2
Aspartate	-	$C_4H_7NO_4$	0.99756	y = 0.0037x-0.1506	0.2
Citrate	-	$C_6H_8O_7$	0.96894	y = 0.0027x-0.1506	15
Cystine	-	$C_6H_{12}N_2O_4S_2$	0.850668	y = 0.0017x-0.1506	20
Fructose-6-phosphate	F-6-P	$C_6H_{13}O_9P$	0.985592	y = 0.0007x-0.1506	15
Fructose	-	$C_6H_{12}O_6$	0.9675	y = 0.0007x-0.1506	15
Fructose-1,6-bisphosphate	Fructose-1-6-P2	$C_6H_{14}O_{12}P_2$	0.857609	y = 3.8027x-8.1506	3.12
Fumarat	-	$C_4H_4O_4$	0.959924	y = 0.0007x-0.1506	0.3
Glucose-6-phosphate	G-6-P	$C_6H_{13}O_9P$	0.995898	y = 0.0007x-0.1506	6.2
Glucose	-	$C_6H_{12}O_6$	0.966061	y = 7.6737x-6.1506	0.62
Glutamate	-	$C_5H_9NO_4$	0.985351	y = 0.0367x-0.1506	0.0416
Glutamine	-	$C_5H_{10}N_2O_3$	0.994367	y = 0.5657x-5.1506	0.0416
Glyceraldehyde-3-phosphate	Glyceraldehyde-3-P	$C_3H_7O_6P$	0.810612	y = 9.1977x-1.1506	15
Glycine	-	$C_2H_5NO_2$	0.992682	y = 0.0017x-0.1506	0.0416
Histidine	-	$C_6H_9N_3O_2$	0.587296	y = 0.0017x-0.1506	0.0416
Isocitrat	-	$C_6H_8O_7$	0.927715	y = 0.0017x-0.1506	6.2
Isoleucine	-	$C_6H_{13}NO_2$	0.986358	y = 0.0077x-0.1506	0.2
Lactate	-	$C_3H_6O_3$	0.988113	y = 0.0007x-0.1506	0.0626
Leucine	-	$C_6H_{13}NO_2$	0.972434	y = 0.0127x-0.1506	0.104
Lysine	-	$C_6H_{14}N_2O_2$	0.994367	y = 0.5657x-5.1506	0.0416
Malat	-	$C_4H_6O_5$	0.992823	y = 0.0127x-0.1506	3.12
Methionine	-	$C_5H_{11}NO_2S$	0.973717	y = 0.0167x-0.1506	0.2
ortho-Tyrosine	o-Tyrosine	$C_9H_{11}NO_3$	0.851967	y = 0.0907x-0.1506	0.104
Phosphoenolpyruvate	PEP	$C_3H_5O_6P$	0.999403	y = 0.0007x-0.1506	0.62

Phenylalanine	-	$C_9H_{11}NO_2$	0.982843	y = 0.1867x-1.1506	0.0416
Proline	-	$C_5H_9NO_2$	0.993064	y = 0.7247x-7.1506	0.0416
Pyruvate	-	$C_3H_4O_3$	0.999601	y = 0.0057x-0.1506	0.3
Serine	-	$C_3H_7NO_3$	0.980071	y = 0.0277x-0.1506	0.0416
Succinate	-	$C_4H_6O_4$	0.978143	y = 0.0037x-0.1506	0.0626
Taurine	-	$C_2H_7NO_3S$	0.981986	y = 0.0077x-0.1506	0.0416
Threonine	-	$C_4H_9NO_3$	0.992306	y = 0.0427x-0.1506	0.0416
Tryptophan	-	$C_{11}H_{12}N_2O_2$	0.948304	y = 0.0520x-0.4891	0.4
Tyrosine	-	$C_9H_{11}NO_3$	0.997303	y = 0.0457x-0.1506	0.104
Valine	-	$C_5H_{11}NO_2$	0.998356	y = 0.2137x-2.1506	0.0416



Supplementary figure S1: Heat map and cluster analysis of endometabolome discriminates cisplatin treatment only in renal epithelial cells. Range-scaled Z-scores of annotated features acquired by targeted multiple reaction monitoring (MRM). Displayed are metabolites with q-value < 0.05 according to ANOVA and FDR. No effect could be observed in MEFs after cisplatin application, while there was a strong influence on renal epithelial cells. Amino acids and intermediates of glycolysis and the TCA-cycle were concomitantly down-regulated by cisplatin in iRECs and mIMCD-3 cells, whereas glucose accumulated after cisplatin-treatment. Detection of phosphorylated glycolysis intermediates in cisplatin-treated samples were below the linear range. Therefore, these alterations should be judged qualitatively. Light color: untreated, dark color: Cisplatin-treated; blue: MEFs, red: mIMCD-3 cells, green: iRECs, each n=3



Supplementary figure S2: Box plots of significantly altered exometabolites after cisplatin-treatment. Range-scaled Z-scores of annotated features acquired by targeted MRM-acquisition. Exometabolites with a q-value < 0.05 according to ANOVA and FDR as well as a positive post-hoc test after treatment in the same cellular back-ground are shown. No alterations were observed in MEFs, serine was the sole metabolite significantly changed in mIMCD-3 cells and all displayed metabolites changed significantly in iRECs. Light color: untreated, dark color: Cisplatin-treated; blue: MEFs, red: mIMCD-3 cells, green: iRECs, each n=3