

Supplemental Material

Fig S1. Gene targeting strategy for Mpc1 hypomorphic allele in mouse. Related to **Fig 3** and **Fig 4**. (A) Exon diagram of *Mpc1* locus and schematic of Mpc1 knock-in allele. Mpc1 is encoded by a five exon gene, and the knock-in reporter construct was targeted to the last three exons of the *Mpc1* locus. The Mpc1 deletion allele was generated by germline expression of a Cre recombinase transgene and subsequent inheritance of the deletion allele without Cre. (B) Body weights (mean \pm SD, in mg) of e17.5 embryos from all crosses (n=10-64).

Fig S2. Changes in lipid abundance and acyl composition reflect tissue-specific alterations in Mpc1-deficient liver. Related to **Fig 7**. Total acyl content is decreased in Mpc1 KI e17.5 liver. Acyl composition from total lipid extract was measured by GC-MS as pentafluorobenzyl bromide esters and is shown as $\mu\text{g FA/mg protein}$ (mean \pm SD, n=5). (*p<0.05 by Student's t-test)

Fig S3. Transcriptional regulation of glucose metabolism-related genes in Mpc1-deficient embryonic tissues. Related to **Fig 7**. (A) mRNA expression of genes involved in glucose metabolism in e17.5 Mpc1 KI/KI cerebral cortex, heart (B), and liver (C) by qRT-PCR array. Data is presented as fold-change relative to WT (mean \pm SEM, n=3). Asterisks indicate significant differences by Student's t-test (p<0.05).

Fig S4. Generation of GPT KO HEK293T cells. Related to **Fig 9**. (A) Dose-dependent impairment of [2-¹⁴C]pyruvate incorporation into the total lipid fraction in HEK293T cells treated with UK-5099, an inhibitor of the MPC (mean \pm SEM, n=3). (B) Genomic mutations identified in *GPT1*, *GPT2*, and pan GPT knockout (KO) HEK293T clones used for flux experiments in **Fig 9**. The guide RNA (gRNA) target site is shown in blue with the protospacer-adjacent motif in red. Predicted mutations at the protein level are listed at right. (C) Alanine aminotransferase activity assay in select GPT KO HEK293T clones (mean \pm SEM, n=4). Significant differences as determined by Tukey post-hoc test (p<0.05) after one-way ANOVA are indicated by different letters.

Table S1. List of metabolites significantly regulated in Mpc1 KI/KI brain or liver. Values shown are fold-change over WT and p-value as determined by Welch's two-sample t-test (n=8).

Figure S1

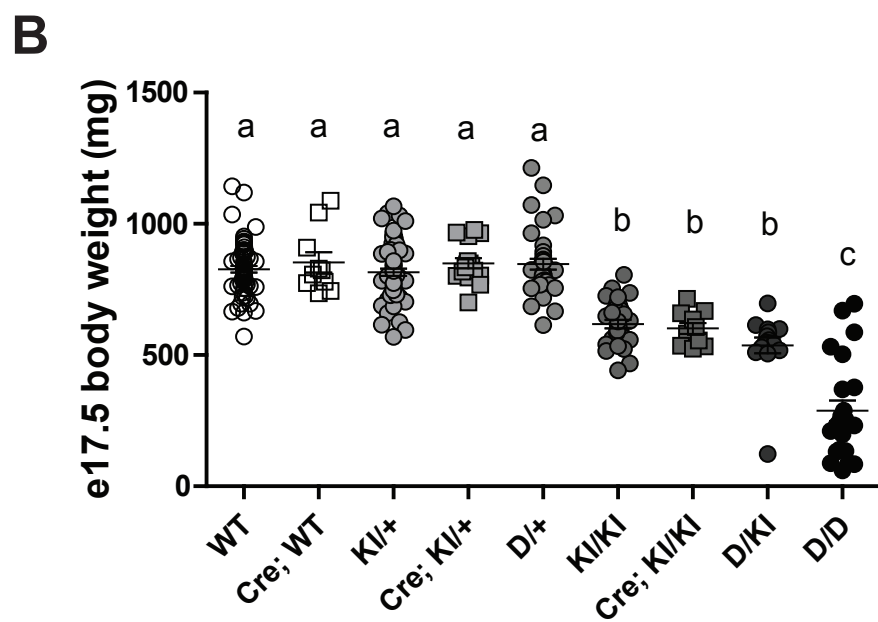
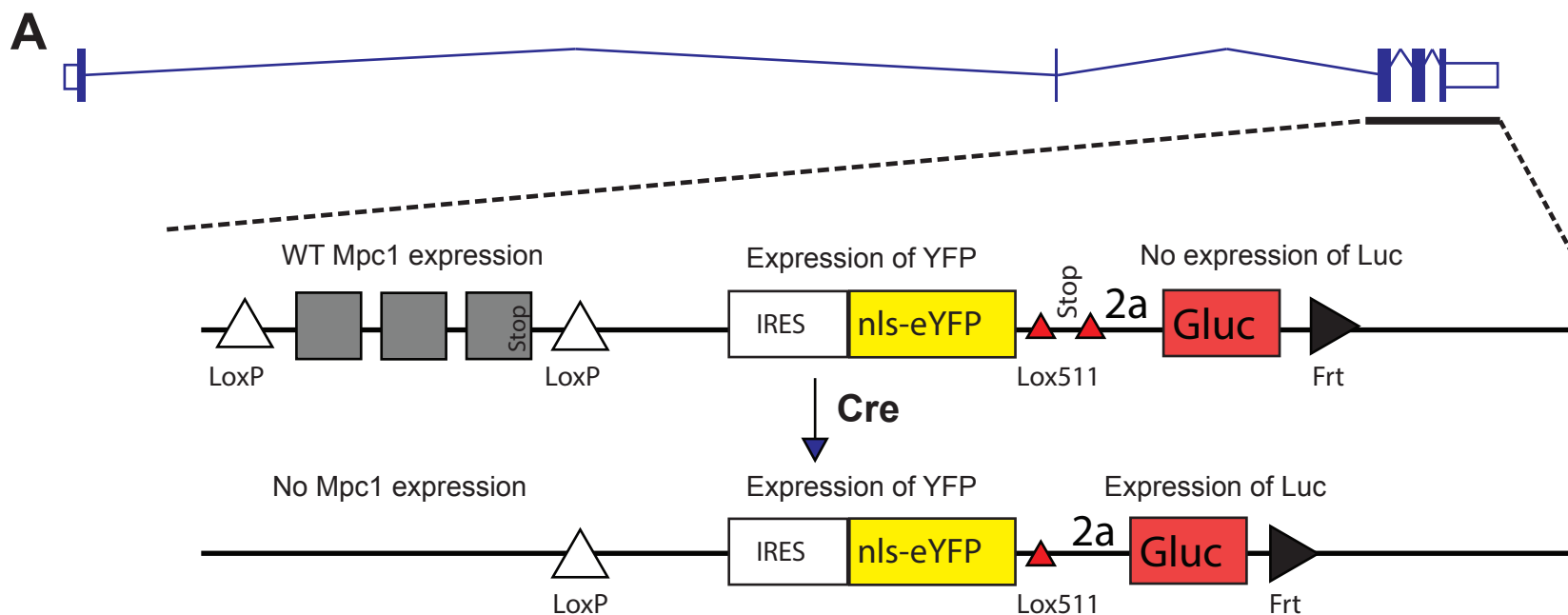


Figure S2

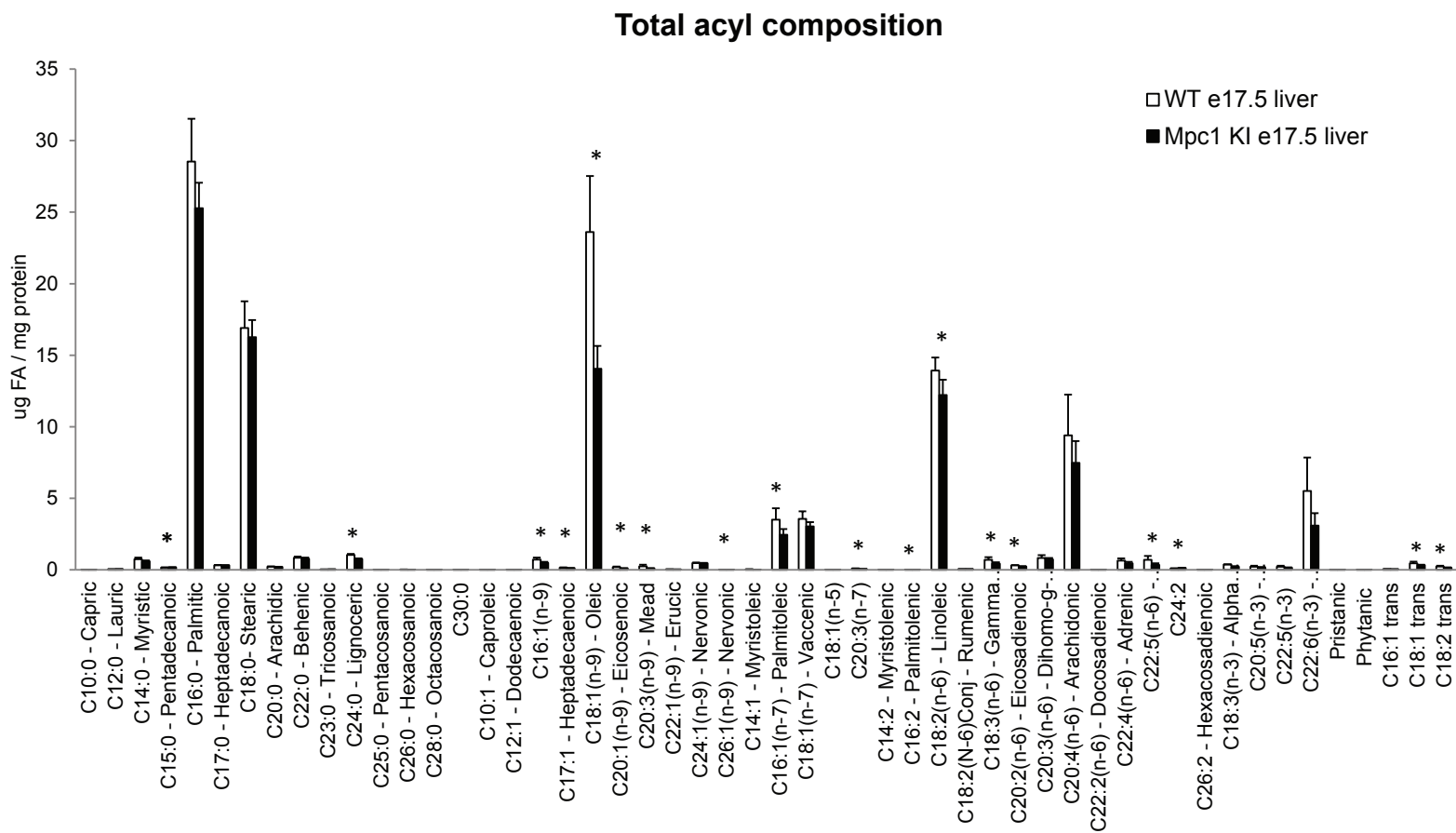


Figure S3

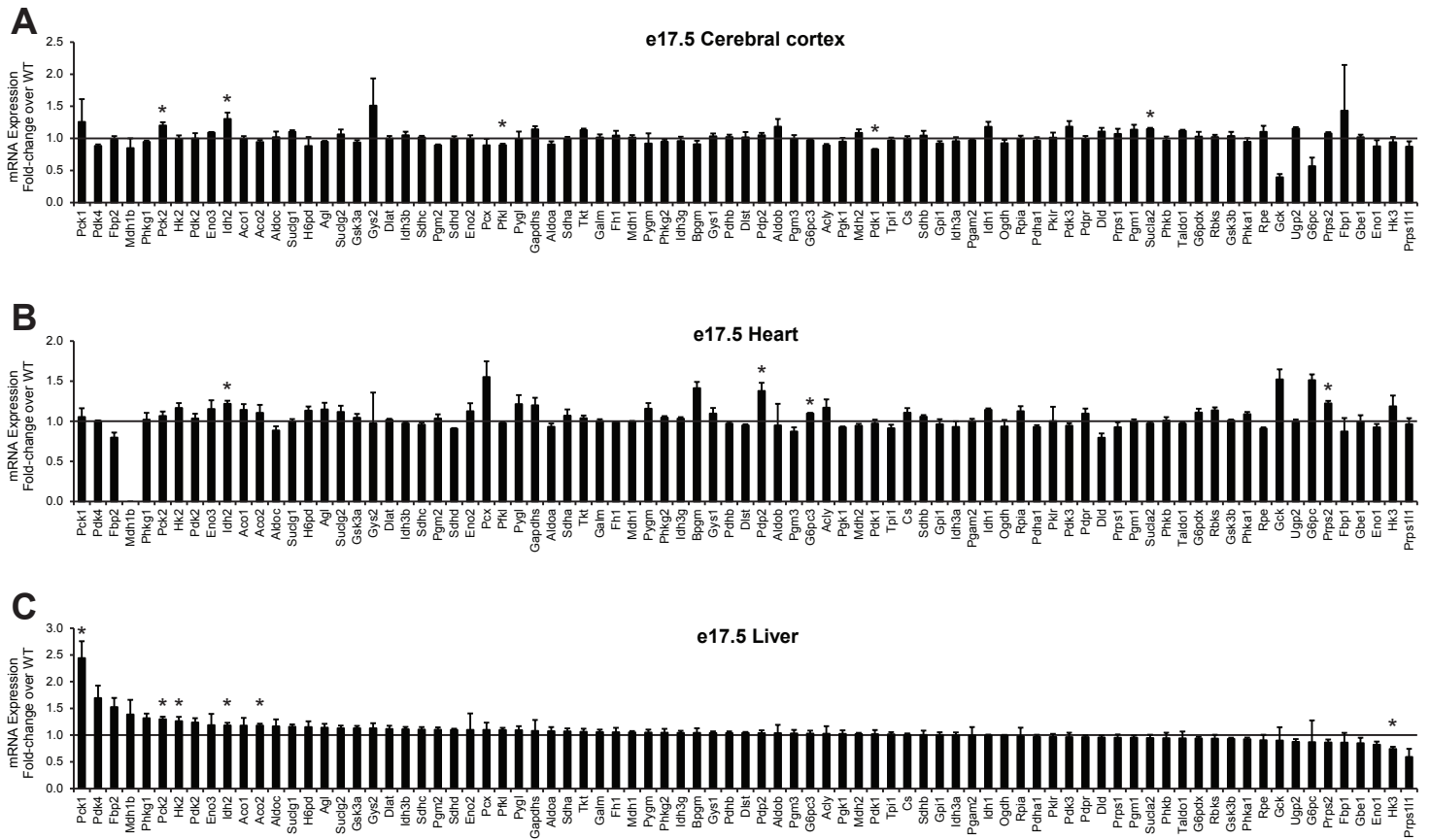


Figure S4

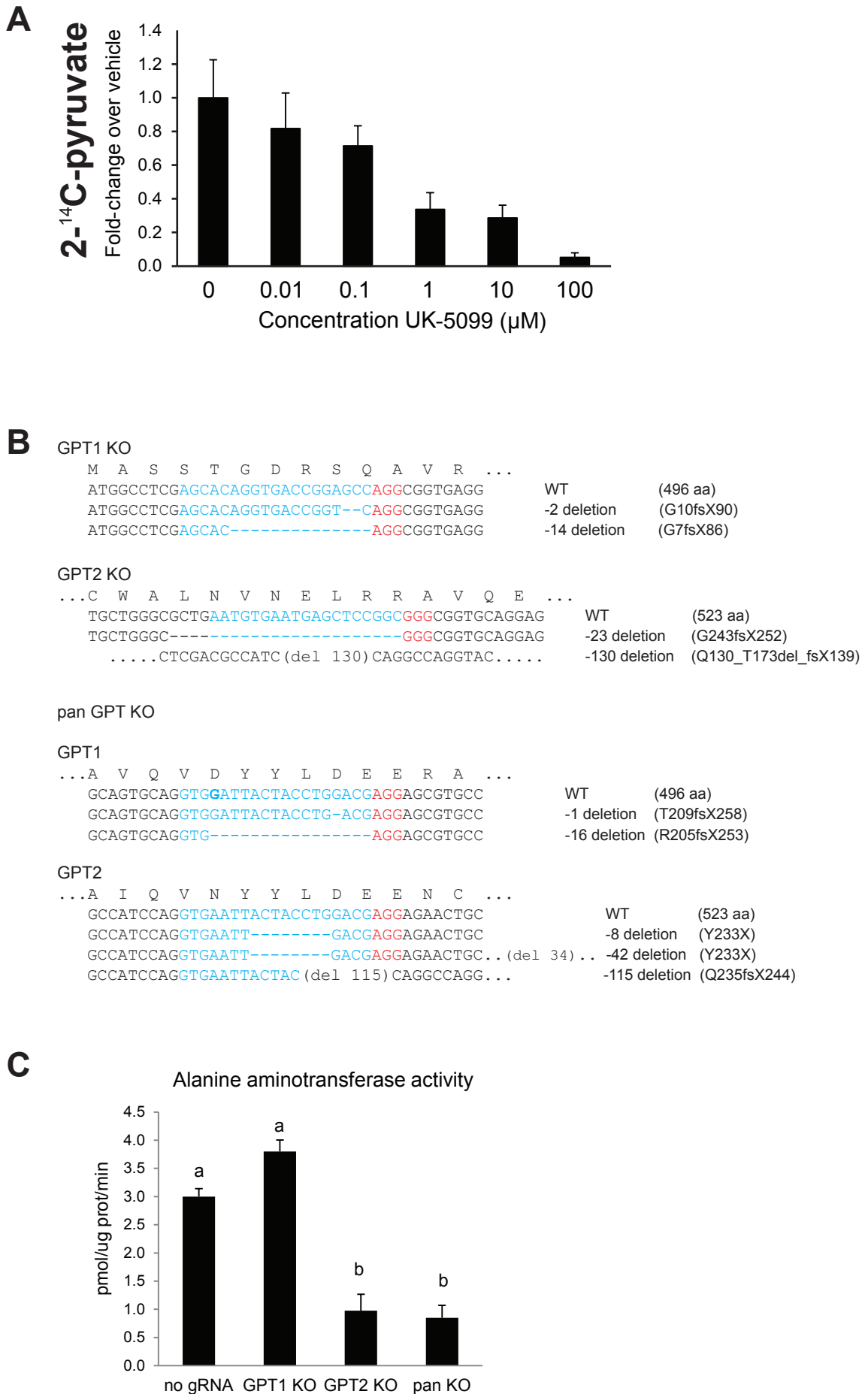


Table S1**Metabolites significantly regulated by Mpc1 deficiency in e17.5 brain**

Biochemical Name	Mpc1 KI/WT	p-value
hypotaurine	15.51	0.0000
pyruvate	5.04	0.0000
4-imidazoleacetate	3.16	0.0000
phenyllactate (PLA)	2.98	0.0001
histamine	2.77	0.0113
sphingomyelin (d18:1/15:0, d16:1/17:0)	2.35	0.0025
N-acetylglycine	2.34	0.0000
N-acetylhistidine	2.30	0.0000
sarcosine (N-Methylglycine)	2.24	0.0000
serine	2.19	0.0000
sphingomyelin (d18:1/14:0, d16:1/16:0)	2.06	0.0007
4-vinylphenol sulfate	2.00	0.0210
proline	1.98	0.0000
glycine	1.94	0.0000
guanidinoacetate	1.93	0.0003
lactate	1.89	0.0000
phenylacetylglycine	1.88	0.0009
3-indoxyl sulfate	1.88	0.0033
N-acetylserine	1.84	0.0000
imidazole propionate	1.84	0.0013
aspartate	1.83	0.0000
anserine	1.83	0.0000
1-palmitoyl-2-linoleoyl-GPE (16:0/18:2)	1.81	0.0025
catechol sulfate	1.80	0.0028
beta-alanine	1.78	0.0000
hippurate	1.76	0.0396
N6-acetyllysine	1.75	0.0219
phenol sulfate	1.72	0.0150
4-acetamidobutanoate	1.71	0.0000
cysteine	1.68	0.0000
cysteine sulfinic acid	1.67	0.0413
2-aminophenol sulfate	1.63	0.0137
methylsuccinate	1.61	0.0000
carnosine	1.61	0.0081
isovalerylcarnitine	1.60	0.0036
ethylmalonate	1.59	0.0000
2-methylcitrate/homocitrate	1.59	0.0001
N-acetyltaurine	1.55	0.0002
gamma-glutamyl-epsilon-lysine	1.55	0.0009
orotate	1.55	0.0001
1-methylimidazoleacetate	1.54	0.0004
beta-hydroxyisovalerate	1.54	0.0001
palmitoyl dihydrosphingomyelin (d18:0/16:0)	1.54	0.0211
beta-hydroxyisovalerylcarnitine	1.52	0.0001

3-ureidopropionate	1.52	0.0037
4-hydroxyphenylpyruvate	1.49	0.0091
N-acetylalanine	1.48	0.0010
N-formylmethionine	1.48	0.0001
N-acetylvaline	1.46	0.0003
N-acetylmethionine	1.46	0.0001
1,2-dioleoyl-GPS (18:1/18:1)	1.46	0.0087
N-acetylthreonine	1.45	0.0001
betaine	1.42	0.0011
methylmalonate (MMA)	1.42	0.0013
N-acetylphenylalanine	1.41	0.0001
N-methylproline	1.41	0.0071
pyrraline	1.41	0.0027
N-acetylputrescine	1.38	0.0015
1-stearoyl-2-linoleoyl-GPE (18:0/18:2)	1.38	0.0116
N-acetylarginine	1.36	0.0040
trigonelline (N'-methylnicotinate)	1.35	0.0032
N-glycolylneuraminate	1.35	0.0360
1-oleoyl-GPE (18:1)	1.34	0.0434
sphinganine	1.34	0.0352
succinylcarnitine	1.33	0.0275
arginine	1.32	0.0102
3-hydroxyisobutyrate	1.29	0.0150
2-hydroxyglutarate	1.26	0.0006
N-acetyltyrosine	1.25	0.0488
argininosuccinate	1.25	0.0243
1,2-dioleoyl-GPC (18:1/18:1)	1.25	0.0411
carnitine	1.20	0.0373
1-palmitoyl-2-linoleoyl-GPC (16:0/18:2)	1.20	0.0403
lysine	1.18	0.0038
prolylglycine	1.18	0.0001
acetylcarnitine	1.18	0.0353
1-palmitoleoyl-GPC (16:1)	1.17	0.0403
N-acetyl-aspartyl-glutamate (NAAG)	1.16	0.0177
N-acetylglucosaminylasparagine	1.09	0.0027
glycerophosphoglycerol	0.93	0.0174
ribitol	0.90	0.0107
glycerate	0.89	0.0076
gulonic acid	0.88	0.0157
allantoin	0.87	0.0138
tartronate (hydroxymalonate)	0.87	0.0363
myo-inositol	0.86	0.0227
oxalate (ethanedioate)	0.86	0.0023
trans-4-hydroxyproline	0.84	0.0054
glucosamine-6-phosphate	0.84	0.0469
adenosine 5'-diphosphate (ADP)	0.84	0.0295
1-methylnicotinamide	0.84	0.0462

gamma-aminobutyrate (GABA)	0.82	0.0064
erythronate	0.82	0.0001
leucine	0.81	0.0105
oleoyl ethanolamide	0.81	0.0005
palmitoyl ethanolamide	0.80	0.0001
2'-deoxycytidine	0.79	0.0087
threonate	0.79	0.0003
valine	0.78	0.0149
gamma-glutamylisoleucine	0.78	0.0228
glycerophosphoethanolamine	0.78	0.0001
hexanoylcarnitine	0.77	0.0170
citrate	0.76	0.0077
pseudouridine	0.76	0.0000
N-palmitoyltaurine	0.75	0.0134
spermidine	0.74	0.0002
mannitol/sorbitol	0.73	0.0017
palmitoylcarnitine	0.73	0.0030
glutamate	0.72	0.0000
glutamate, gamma-methyl ester	0.72	0.0012
gamma-glutamylvaline	0.72	0.0034
isocitrate	0.71	0.0012
docosapentaenoate (n6 DPA; 22:5n6)	0.71	0.0275
stearoylcarnitine	0.71	0.0061
glutathione, oxidized (GSSG)	0.70	0.0001
5-oxoproline	0.70	0.0314
galactose 1-phosphate	0.70	0.0188
galactonate	0.70	0.0036
laurylcarnitine	0.70	0.0014
citrulline	0.69	0.0063
2-aminoheptanoate	0.69	0.0003
octanoylcarnitine	0.69	0.0033
glycerophosphorylcholine (GPC)	0.69	0.0000
glycerol	0.69	0.0045
2-aminoadipate	0.68	0.0207
kynurenine	0.67	0.0033
S-adenosylhomocysteine (SAH)	0.67	0.0078
ascorbate (Vitamin C)	0.67	0.0061
1-methylhistidine	0.66	0.0000
cystathionine	0.66	0.0024
ophthalmate	0.66	0.0202
myristoleoylcarnitine	0.66	0.0003
2-aminobutyrate	0.65	0.0039
gamma-glutamylmethionine	0.65	0.0376
succinate	0.65	0.0012
myristoylcarnitine	0.65	0.0000
gamma-glutamylleucine	0.62	0.0006
tryptophan	0.60	0.0000

isoleucine	0.60	0.0015
3-methyl-2-oxovalerate	0.60	0.0059
methionine sulfone	0.60	0.0008
S-methylglutathione	0.60	0.0170
homocitrulline	0.59	0.0005
glutathione, reduced (GSH)	0.58	0.0013
gamma-glutamylthreonine	0.56	0.0004
gamma-glutamylglutamine	0.54	0.0001
glycerol 3-phosphate	0.54	0.0001
S-methylcysteine	0.53	0.0018
N-delta-acetylornithine	0.53	0.0000
3-methylhistidine	0.47	0.0000
3-methoxytyrosine	0.45	0.0000
nicotinamide adenine dinucleotide reduced (NADH)	0.45	0.0001
2-hydroxyadipate	0.42	0.0003
glutamine	0.39	0.0000
alanine	0.32	0.0000

Metabolites significantly regulated by Mpc1 deficiency in e17.5 liver

Biochemical Name	Mpc1 KI/WT	p-value
3-methylglutaryl carnitine (2)	3.68	0.0003
heme	2.99	0.0005
pyruvate	2.61	0.0017
4-imidazoleacetate	2.41	0.0021
sphingomyelin (d18:1/14:0, d16:1/16:0)	2.41	0.0000
1-linoleoyl-GPE (18:2)	2.35	0.0024
1-arachidonylglycerol (20:4)	2.35	0.0079
palmitoyl dihydrosphingomyelin (d18:0/16:0)	2.30	0.0000
histamine	2.19	0.0002
1-palmitoyl-2-linoleoyl-GPG (16:0/18:2)	2.16	0.0000
1-arachidonoyl-GPE (20:4)	2.10	0.0069
biliverdin	2.03	0.0283
sphingomyelin (d18:1/15:0, d16:1/17:0)	2.00	0.0000
docosahexaenoate (DHA; 22:6n3)	1.96	0.0066
sphingomyelin (d18:1/17:0, d17:1/18:0, d19:1/16:0)	1.96	0.0000
2-arachidonoylglycerol (20:4)	1.95	0.0424
indolepropionate	1.94	0.0371
sphinganine	1.92	0.0004
1-stearoyl-GPS (18:0)	1.90	0.0090
stearoyl sphingomyelin (d18:1/18:0)	1.90	0.0000
adenosine 3',5'-diphosphate	1.84	0.0230
1-docosahexaenoyl-GPE (22:6)	1.80	0.0267
eicosapentaenoate (EPA; 20:5n3)	1.77	0.0023
N-acetylhistidine	1.73	0.0049
methylsuccinate	1.72	0.0035
1-palmitoyl-2-linoleoyl-GPE (16:0/18:2)	1.69	0.0003
1-stearoyl-2-arachidonoyl-GPS (18:0/20:4)	1.67	0.0003

sphingomyelin (d18:1/20:0, d16:1/22:0)	1.66	0.0000
N-acetyltaurine	1.64	0.0012
gamma-carboxyglutamate	1.62	0.0005
1-stearoyl-2-linoleoyl-GPE (18:0/18:2)	1.62	0.0005
1-palmitoyl-2-arachidonoyl-GPE (16:0/20:4)	1.60	0.0000
p-cresol-glucuronide	1.56	0.0479
1-oleoyl-GPE (18:1)	1.55	0.0164
N-acetylmethionine sulfoxide	1.53	0.0211
gamma-glutamylglycine	1.53	0.0350
1-palmitoyl-GPE (16:0)	1.53	0.0058
2-palmitoleoyl-GPC (16:1)	1.51	0.0377
sphingomyelin (d18:1/18:1, d18:2/18:0)	1.50	0.0004
palmitoyl sphingomyelin (d18:1/16:0)	1.49	0.0000
N-acetylphenylalanine	1.48	0.0009
phenylacetylglycine	1.48	0.0300
ethylmalonate	1.48	0.0000
1-oleoyl-2-linoleoyl-GPE (18:1/18:2)	1.45	0.0008
flavin adenine dinucleotide (FAD)	1.45	0.0185
N-acetylglycine	1.44	0.0040
guanosine 5'- monophosphate (5'-GMP)	1.43	0.0007
1-stearoyl-GPE (18:0)	1.42	0.0020
histidine	1.41	0.0498
glycine	1.40	0.0015
1-stearoyl-2-arachidonoyl-GPE (18:0/20:4)	1.40	0.0014
1,2-dilinoeoyl-GPC (18:2/18:2)	1.40	0.0003
N-palmitoyl-sphingosine (d18:1/16:0)	1.40	0.0175
sphingomyelin (d18:1/22:1, d18:2/22:0, d16:1/24:1)	1.40	0.0016
1-stearoyl-2-oleoyl-GPS (18:0/18:1)	1.39	0.0153
N-acetyltyrosine	1.38	0.0445
1-stearoyl-2-arachidonoyl-GPI (18:0/20:4)	1.38	0.0104
sulfate	1.38	0.0106
phosphoethanolamine	1.37	0.0061
1-stearoyl-2-linoleoyl-GPC (18:0/18:2)	1.36	0.0084
serine	1.35	0.0022
3-indoxyl sulfate	1.35	0.0453
1-methylhistamine	1.34	0.0241
1-stearoyl-2-arachidonoyl-GPC (18:0/20:4)	1.34	0.0228
1-(1-enyl-palmitoyl)-2-arachidonoyl-GPE (P-16:0/20:4)	1.33	0.0046
1-palmitoleoyl-2-linoleoyl-GPC (16:1/18:2)	1.32	0.0028
1-(1-enyl-palmitoyl)-2-linoleoyl-GPE (P-16:0/18:2)	1.32	0.0124
sphingomyelin (d18:2/24:1, d18:1/24:2)	1.31	0.0082
1-linoleoyl-2-arachidonoyl-GPC (18:2/20:4)	1.30	0.0006
anserine	1.29	0.0244
adenosine 5'-diphosphate (ADP)	1.29	0.0116
1-oleoyl-2-linoleoyl-GPC (18:1/18:2)	1.25	0.0010
1,2-dipalmitoyl-GPC (16:0/16:0)	1.24	0.0034
1-palmitoyl-2-linoleoyl-GPC (16:0/18:2)	1.23	0.0024

1-palmitoyl-2-oleoyl-GPE (16:0/18:1)	1.23	0.0362
sphingomyelin (d18:2/16:0, d18:1/16:1)	1.23	0.0013
pyridoxamine phosphate	1.23	0.0131
proline	1.22	0.0366
lactate	1.21	0.0015
palmitoyl ethanolamide	1.21	0.0360
beta-hydroxyisovaleroylcarnitine	1.18	0.0185
adenosine 5'-monophosphate (AMP)	1.18	0.0207
1-palmitoyl-2-arachidonoyl-GPC (16:0/20:4)	1.16	0.0154
N-acetylglucosaminylasparagine	1.12	0.0104
carnitine	0.87	0.0149
methionine sulfoxide	0.86	0.0373
threonate	0.85	0.0158
2'-deoxyuridine	0.84	0.0390
pro-hydroxy-pro	0.81	0.0451
2-aminoheptanoate	0.81	0.0030
glutamate	0.79	0.0304
thiamin diphosphate	0.79	0.0091
erythronate	0.78	0.0008
tryptophan	0.77	0.0073
trans-4-hydroxyproline	0.77	0.0003
ribitol	0.77	0.0022
erythritol	0.77	0.0029
alanine	0.76	0.0005
1-methylhistidine	0.76	0.0022
citrulline	0.76	0.0003
5-methylthioadenosine (MTA)	0.75	0.0012
mannitol/sorbitol	0.75	0.0244
glycerol	0.75	0.0115
oxalate (ethanedioate)	0.75	0.0000
N6,N6,N6-trimethyllysine	0.74	0.0023
3-methoxytyrosine	0.74	0.0496
putrescine	0.74	0.0249
gamma-glutamylisoleucine	0.74	0.0006
gamma-glutamylleucine	0.73	0.0076
orotate	0.73	0.0499
2'-deoxycytidine	0.73	0.0236
glycerol 3-phosphate	0.72	0.0279
thymidine	0.72	0.0363
orotidine	0.71	0.0003
pseudouridine	0.71	0.0000
S-adenosylhomocysteine (SAH)	0.70	0.0226
5-methyl-2'-deoxycytidine	0.70	0.0118
ergothioneine	0.70	0.0004
homocitrulline	0.69	0.0017
gamma-glutamylvaline	0.69	0.0002
N6-succinyladenosine	0.69	0.0403

N4-acetylcytidine	0.68	0.0010
2-aminobutyrate	0.67	0.0475
creatine	0.67	0.0000
N-acetylglucosamine 6-phosphate	0.67	0.0344
maleate	0.67	0.0001
nicotinamide adenine dinucleotide (NAD+)	0.67	0.0156
dimethylglycine	0.66	0.0000
N-delta-acetylornithine	0.66	0.0001
arabitol/xylitol	0.66	0.0085
methionine sulfone	0.65	0.0005
cysteine-glutathione disulfide	0.64	0.0182
gamma-glutamylthreonine	0.64	0.0085
gamma-glutamyltryptophan	0.64	0.0119
alpha-ketoglutarate	0.62	0.0001
2-hydroxyglutarate	0.62	0.0091
S-methylglutathione	0.61	0.0139
gamma-glutamylalanine	0.61	0.0010
fumarate	0.61	0.0000
3-methylhistidine	0.60	0.0014
alpha-hydroxyisocaproate	0.59	0.0376
malate	0.59	0.0001
gulonic acid	0.59	0.0000
ophthalmate	0.57	0.0398
argininosuccinate	0.56	0.0007
N-acetyl-glucosamine 1-phosphate	0.55	0.0102
5-ketogluconate	0.55	0.0000
creatinine	0.54	0.0000
pipecolate	0.53	0.0006
glutamine	0.51	0.0152
isovalerylcarnitine	0.50	0.0414
glutarylcarnitine (C5)	0.49	0.0104
3-methylglutarylcarnitine (1)	0.43	0.0003
pregn steroid monosulfate	0.43	0.0000
N-carbamoylaspartate	0.37	0.0013
creatine phosphate	0.33	0.0011
isobutyrylcarnitine	0.31	0.0015