Supplementary Information

Carbon metabolism-mediated myogenic differentiation

Abigail L. Bracha, Arvind Ramanathan, Sui Huang, Donald E. Ingber, Stuart L. Schreiber
Supplementary Materials

Cell line, Lysates, and Antibodies

HEK293T, and mouse C2C12 myoblast cells were maintained in DMEM (GIBCO, Carlsbad, CA) +10% FBS (Gemini Bio-Products, West Sacramento, CA). For differentiation of C2C12 cells, cells were grown in 2% FBS or 2% HS as indicated. For stable knockdown and measurement of gene expression, cells were infected with lentivirus and 72 hours post-infection, cells were washed twice in PBS and lysed in ice cold PBS +0.5% NP-40 (Sigma Aldrich, St. Louis, MO) + Complete Mini EDTA free protease inhibitor tablet (Roche, Basel, Switzerland) + 1:100 phosphatase inhibitor cocktail 1 (Sigma Aldrich, St. Louis, MO). Following normalization of protein by Bradford assay (Bio-Rad, Hercules, CA), samples were resolved by SDS-PAGE and western blotted with the indicated antibodies. Antibody for Acl was obtained from Epitomics (Burlingame, CA). Polyclonal H6pd antibody and monoclonal actin were obtained from Sigma Aldrich (St. Louis, MO). Monoclonal Myosin heavy chain was obtained from Upstate (Lake Placid, NY). Polyclonal antibody for Pgk1 was obtained from Abcam (Cambridge, MA). For measurement of histone acetylation positive control cells were treated overnight with TSA, DM cells were cultured for three days in DM, and hairpin infected cells were harvested 48 hours post-infection. Whole cell lysates were resolved by SDS-PAGE and western blotted with antibody against total histone H3 obtained from Cell Signaling (Danvers, MA). Blots were stripped and reprobed using antibody against acetylated lysine obtained from Cell Signaling (Danvers, MA).
shRNAs and viral production

Lentiviral based shRNAs were obtained from The RNAi Consortium (TRC, Broad Institute of Harvard and MIT), as described in Moffat J. et al. (1). Official clone names for hairpins were: Acl, NM_134037.2-3242s1c1 NM_134037.2-706s1c1, and NM_134037.2-3359s1c1, denoted as Acl-3242, Acl-706, and Acl-3359. Hairpins for Pgk1 were: NM_008828.1-761s1c1, NM_008828.1-1013s1c1 and NM_008828.1-703s1c1, denoted as Pgk1-761, Pgk1-1013 and Pgk1-703. Hairpins for H6pd were: NM_173371.2-1469s1c1 and NM_173371.2-2624s1c1, denoted as H6pd-1469 and H6pd-2624. Control hairpins were: rfp_283s1c1, rfp_401s1c1, shlacz lacZ_1935s1c1, clonetechGfp_437s1c1 and clonetechGfp_197s1c1. Lentivirus was generated by transient transfection of HEK293T cells with packaging plasmids and the target plasmid using polyethyleneimine (PEI). Virus was harvested 48 and 72 hours post-transfection.

Generation of stable cell lines

Plasmids for transient transfection and stable infection were generated by cloning the gene either, Pgk1 or H6pd with an N-terminal flag sequence into the pLOE_GC03 lentiviral expression vector (Broad Institute TRC). Pgk1 and H6pd human genes were purchased from Open Biosystems. Primer for H6pd overexpression with N-terminal Flag-tag and NheI restriction site -

GCCCGCTAGCATGGATTATAAAGATGATGATGATAAATGGAATATGCTCATA GTGGGCGA

H6pd reverse primer with MluI restriction site

GCGCAGCTGCTAGTTCCATCCAGGAAGGCCTCAGT
Primer for Pgk1 N-terminal Flag-tag with Nhel restriction site

GCCCCTAGCATGGATTATAAAGATGATGATGATTTTTCGCTTTCTAACAAG

Pgk1 reverse primer with MluI restriction site

GCGCACGCGTAGTCTAAATATTGCTGAGA.

C2C12 stable cell lines were generated by maintaining infected cells in 6µg/ml blasticidin (Invitrogen). The endogenous mouse gene was then knocked down using the respective hairpin either shH6pd2624 or shPGK761 and selected with 0.6µg/ml puromycin (Sigma-Aldrich, St. Louis, MO).

**Differentiation Assay RNAi Screen**

Cells were seeded in 96-well plates at a density of 5000 cells per well. The following day cells were infected with lentivirus containing 8µg/ml polybrene. 5 hairpins per gene were tested. Cells were spin-infected for 30 minutes at 2200 rpm. Plates were incubated overnight and then selected for using 0.6 µg/ml puromycin (Sigma-Aldrich, St. Louis, MO). C2C12 cells were assayed for differentiation two days post-selection. Differentiation was determined by fixing cells in 4% formaldehyde and staining cells using monoclonal anti-myosin heavy chain antibody Millipore-Upstate (Temecula, CA). Cells were visualized on a high-content ImageXpress microscope (Molecular Devices) with a 10X objective, 2-images were captured per well. MHC and DAPI intensity were quantitated using ImageXpress software.

**Data Analysis RNAi Screen**
Each well of the 96-well plate had a different shRNA. For each well of the plate the MHC intensity was normalized to the DAPI, nuclear intensity. The mean integrated intensity of MHC/DAPI was then determined for the entire plate. Each well was then compared to the average MHC/DAPI score of the plate. A z-score was determined for each well of the plate, which represents the number of standard deviations the hairpin scored above or below the mean of the entire plate. The general formula for the z-score was:

\[ z(x) = \frac{x - \mu}{\sigma} \]

where:
- \( x \) = the 'raw' well measurement
- \( \mu \) = the mean of all wells for the plate, and
- \( \sigma \) = the standard deviation of the wells for the plate.

**Growth on Soft Agar**

Rhabdomyosarcoma cells were plated in a 96-well plate. Individual wells were prepared with 0.8% soft agar. Cell suspensions containing 0.4% soft agar were plated on-top of the pretreated wells and growth media containing statins was placed above the cell suspension. Media was changed every three days for two weeks. Images were captured two weeks post-plating to assess colony formation on soft agar. Images were quantitated for colony size using ImageJ software.

**Quantitative RT-PCR**
Cells were plated in 96 well plates and infected with shRNA. 48 hours post-selection cells were lysed following the Qiagen RNeasy protocol (Cat #: 74182) for high-throughput qRT-PCR. Isolation of total RNA was performed according to the Qiagen RNeasy 96 protocol, with the following modification: media was removed prior to lysis. 150 μl of lysis buffer was added and the plate was shaken for 30 seconds. The centrifugation protocol was used rather than the vacuum manifold protocol.

DNA synthesis was performed immediately following RNA extraction. Synthesis of cDNA was performed according to the SuperScript II protocol. 13 μL total RNA was added to the dNTP/primer master mix per well. The plate was incubated at 75 degrees C for 5 minutes to denature RNA, and then immediately transferred to wet ice. The Superscript II Reverse Transcriptase protocol was used for the production of cDNA. The qPCR reactions were set-up in 384-well optically clear PCR plates using a robotic liquid handling robot MultiProbe (Perkin Elmer). For each assay both target gene and endogenous control gene were tested. 3 replicate qPCR reactions for each cDNA sample for each assay was run using a real-time PCR machine: 7900HT real-time PCR instrument (ABI). Analysis of target gene knockdown was performed by ΔΔCt analysis (see ABI User Bulletin #2 and/or Current Protocols in Molecular Biology, Unit 15.8: High-Throughput Real-Time Quantitative Reverse Transcription PCR). Sigma M-MLV Reverse Transcriptase (Cat #: M1302-40KU) was used for the production of cDNA along with the Stratagene Deoxynucleotide Mix 100 mM dNTP (mix Cat #200415). Sigma SYBR Green JumpStart Taq ReadyMix (Cat # S9194-
400RXN) was used for the PCR reaction. Values represent means and standard deviations of RNA levels quantitated in triplicate.

**Statin treatment**

Cells were plated in triplicate in a 96 well plate and fixed 3 days post-treatment. Fluvastatin, Pravastatin and Atorvastatin were purchased from Sigma Aldrich, (St. Louis, MO). Negative control wells contained 10% FBS non-differentiation medium, and positive control wells contained 2% FBS. Cells were fixed and stained three days post-treatment for myosin heavy chain antibody intensity. A p-value <0.05 was determined using a two-sided t-test.

**Metabolic profiling and data analysis**

C2C12 cells were seeded in 10 cm dishes at 80% confluence on day -2 in DMEM growth medium with 10% FBS. On day 0, cells were switched to 2% FBS to induce differentiation. Fresh media was added after aspiration. Metabolites from six biological replicates were extracted on days -2, 0, 3 and 6. 24 hours prior to extraction, fresh growth media with either with 10% FBS or 2% FBS was added to cells. Cells were washed twice with 50 ml of ice cold PBS, in order to remove metabolites from residual cell media. 2 mls of 80% Ethanol maintained on dry ice was added to the washed plates for 5 minutes and dried under nitrogen. Reconstituted extracts were analyzed using an LC-MS/MS based metabolic profiling platform, as described in Lewis et al. (2). Total signal of mass-spectral peaks of the intracellular metabolites were measured on day -2, day 0, day 3 and
day 6 of differentiation. Relative levels of each metabolite during differentiation were determined with respect to mass spectral peak areas from day -2. Fold change of each of the peaks, relative to that from day 0 was determined. Metabolites that displayed an increase or decrease of greater than 50% (p<0.05) were used for the hierarchical clustering analysis. The clustering analysis was performed using Spotfire data visualization software.
**Supplementary Results**

**Supplementary Figure 1a-f.** Validation of knockdown and differentiation caused by shPgk1, shH6pd and shAcl using quantitative RT-PCR. (a) Relative fold change in myosin heavy chain expression normalized to actin mRNA in response to Pgk1 knockdown. (b) Relative fold change in myogenin expression normalized to actin mRNA in response to Pgk1 knockdown. (c) Relative fold change in myosin heavy chain expression normalized to actin mRNA in response to Acl knockdown. (d) Relative fold change in myogenin expression normalized to actin mRNA in response to Acl knockdown. (e) Relative fold change in myosin heavy chain expression normalized to actin mRNA in response to H6pd knockdown. (f) Relative fold change in myogenin expression normalized to actin mRNA in response to H6pd knockdown.

**Supplementary Figure 1g.** Western Blot analysis of gene knockdown probed for levels of Pgk1, H6pd and Acl. shPgk1, shH6pd and shAcl infected cells were cultured in 10% FBS. Positive control cells (Pos c) were grown in 2% FBS, and allowed to differentiate. Negative control cells were infected with shGFP and grown in 10% FBS.

**Supplementary Figure 1h.** Rescue with Pgk1 and H6pd enzymes. Rescue with the human ortholog of H6PD and PGK1 reverses hairpin-induced differentiation. Stable cell lines expressing the human ortholog of H6PD and PGK1 were
infected with shH6pd2624 and shPgk1761, respectively. Cells were plated in quadruplicate and assayed three independent times for myosin heavy chain expression. Error bars represent standard error of the mean, p value < 0.05.

**Supplementary Figure 1i.** Metabolic profiling of C2C12 cell differentiation. Hierarchical clustering of fold changes in levels of metabolites that changed significantly (P-value < 0.05) during myogenic differentiation induced by culturing C2C12 cells in reduced serum. Red color indicates an increase and green color indicates a decrease in levels with respect to day -2. The unchanged levels are indicated in grey.

**Supplementary Figure 2a-d.** Cholesterol metabolism mediates differentiation of C2C12 myoblasts. (a) Treatment with either 1μM atorvastatin or 1μM fluvastatin causes differentiation of mouse C2C12 myoblasts. Mouse C2C12 myoblasts were plated in triplicate and treated with statins in non-differentiating conditions (10% FBS), positive control cells were plated in differentiating (2% HS) conditions and negative controls were plated in non-differentiating (10%FBS) conditions. (b) Increasing doses of pravastatin 1, 5 and 10 μM in non-differentiating conditions (10%FBS) causes C2C12 differentiation. Significance was tested with respect to pravastatin treated cells and 0 μM negative controls (10% FBS) (*P-value < 0.01, t-test). (c) 10 μM Pravastatin in the presence of 250 μM cholesterol abrogates differentiation, comparison between combined pravastatin cholesterol treatment and positive control cells, 2% FBS, (*P-value <
0.01, t-test). (d) 250µM cholesterol and 250nM trichostatin, TSA, prevent differentiation of myoblasts cultured in differentiation medium (DM) (*P-value < 0.01, t-test).

**Supplementary Figure 2e.** Effect of fluvastatin on colony size of RD cells grown on soft agar. At least 20 colonies were quantitated for each treatment using image J software, p value < 0.05.

**Supplementary Table 1.** List of genes targeted in the RNAi screen.

**Supplementary Table 2.** Complete list of measurements from the metabolic profile.

**References**

1. Moffat, J. *et al.* *Cell* **124**, 1283-1298 (2006).

2. Lewis, G.D. *et al.* *J. Clin. Invest.* **118**, 3503-12 (2008).
**S1a.**

Bar graph showing fold change in Myosin heavy chain for different conditions:
- Pgk-703
- Pgk-761
- Pgk-1013
- shRFP-283
- shLacz-1935
- Differentiated

**S1b.**

Bar graph showing fold change in Myogenin for different conditions:
- Pgk-703
- Pgk-761
- Pgk-1013
- shRFP-283
- shLacz-1935
- Differentiated
S1c. Myosin heavy chain (fold change)

S1d. Myogenin (fold change)
Myosin heavy chain (fold change)

- H6pd-1469
- H6pd-2624
- shRFP-283
- shLacz-1935
- Differentiated
Myogenin (fold change)

- H6pd-1469
- H6pd-2624
- shRFP-283
- shLacz-1935
- differentiated
S1g.

- **Pgk1**
  
  Pos c shGFP  1013  761  703

- **Actin**
  
- **Acl**
  
  Pos c shGFP 3242  706  3359

- **H6pd**
  
  Pos c shGFP  1469  2624
Myosin heavy chain (normalized fluorescence)

H6PD+shH6pd  PGK+shPgk  Differentiated
S2c.

![Bar chart showing Myosin heavy chain (normalized fluorescence) for Pravastatin + cholesterol, Pos Control, and Neg Control.]

S2d.

![Bar chart showing Myosin heavy chain (normalized fluorescence) for Cholesterol 250uM (DM), 250nM TSA (DM), and DM.]

* denotes significance at a 0.05 level.
S2e.
Supplementary Table 1.

| Gene                                      | NCBI gene ID |
|-------------------------------------------|--------------|
| **Glycolysis:**                           |              |
| Glucose phosphate isomerase (Gpi)         | NM_008155    |
| Phosphofructokinase muscle (Pfkm)         | NM_021514    |
| 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase (Pfkfb2) | NM_008825 |
| 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3 (Pfkfb3) | NM_133232 |
| Fructose-bisphosphate aldolase A (Aldoa) | NM_007438    |
| Triosephosphate isomerase (Tpi1)          | NM_009415    |
| Glyceraldehyde-3-phosphate dehydrogenase (Gapdh) | NM_008084 |
| Phosphoglycerate kinase 1 (Pgk1)          | NM_008828    |
| Phosphoglycerate mutase 2 Muscle-specific (Pgam2) | NM_018870 |
| Beta-enolase Muscle-specific (Eno3)       | NM_007933    |
| Pyruvate kinase isozymes M1/M2 (Pkm2)     | NM_011099    |
| Lactate dehydrogenase (Ldha)              | NM_010699    |
| **Pentose phosphate shunt:**              |              |
| Glucose-6-phosphate dehydrogenase (G6pd2) | NM_019468    |
| Hexose-6-phosphate dehydrogenase (H6pd)   | NM_173371    |
| Transketolase (Tkt)                       | NM_009388    |
| Transaldolase (Taldo1)                    | NM_011528    |
| **Glutaminolysis:**                       |              |
| Oxoglutarate (alpha-ketoglutarate) dehydrogenase (Ogdh) | NM_010956 |
| N-acetylglutamate synthase (Nags)         | NM_145829    |
| Glutamate dehydrogenase (Glud1)           | NM_008133    |
| Glutamate dehydrogenase (Glud2)           | NM_008133    |
| Glutamate oxaloacetate transaminase 2, mitochondrial (Got2) | NM_010325 |
| **Lipid metabolism:**                     |              |
| Glycerol kinase (Gyk)                     | NM_008194    |
| Fatty Acid Synthase (Fasn)                | NM_007988    |
| Acyl-CoA oxidase (Acox)                   | BC021339     |
| Malonyl-CoA decarboxylase (Mlycd)         | NM_019966    |
| Malonyl CoA:ACP acyltransferase (mitochondrial) (Mcat) | NM_001030014 |
| Acyl-Coenzyme A dehydrogenase, medium chain (Acadm) | NM_007382 |
| Carnitine acyl transferase (Crat)         | NM_007760    |
carnitine palmitoyltransferase 1b, muscle (Cpt1b)  NM_009948

**Mitochondrial metabolism/TCA cycle:**
ATP synthase, H+ transporting, mitochondrial F1 complex, gamma polypeptide 1 (Atp5c1)  NM_020615
Isocitrate dehydrogenase (Idh1)  NM_010497
Citrate synthase (Cs)  NM_026444
ATP synthase beta-subunit (beta-F1 ATPase), H+ transporting, mitochondrial F1 complex, beta polypeptide (Atp5b)  NM_016774
Malate NADP oxidoreductase  J02652
fumarate hydratase 1 (Fh1)  NM_010209
Succinate dehydrogenase complex, subunit B, iron sulfur (lp) (Sdhb)  NM_023374
Succinate dehydrogenase complex, subunit A, flavoprotein (Sdha)  NM_023281
Succinate-CoA ligase, GDP-forming, alpha subunit (Suclg1)  NM_019879
malic enzyme 2, NAD(+)-dependent, mitochondrial (Me2)  NM_145494, BC004709
Malate dehydrogenase 1, NAD (Mdh1)  NM_008618
Aconitase/aconitate hydratase (Aco1)  NM_007386
Pyruvate dehydrogenase [lipoamide] kinase isozyme 1, mitochondrial [Precursor] (Pdk1)  NM_172665
ATP citrate lyase (Acl)  NM_134037
dihydrolipoamide S-acetyltransferase (E2 component of pyruvate dehydrogenase complex) (Dlat)  NM_145614
Pyruvate dehydrogenase b (Pdhb)  NM_024221

**Reactive oxygen species:**
NADPH oxidase 1 (Nox1)  NM_172203
Glutathione reductase (Gsr)  NM_010344
NADPH oxidase activator 1 (Noxa1)  NM_172204
NADPH oxidase organizer 1 (Noxo1)  NM_027988
### Supplementary Table 2.

| Metabolite Name                  | fold change compared to day-2 | pvalues       |
|----------------------------------|-------------------------------|---------------|
|                                  | d0   | d3   | d6   | ttest | d0   | d3   | d6   |
| Creatine                         | 0.950832 | 0.905699 | 1.497057 | 4.82E-09 | 0.005647 | 0.000839 |
| Carnosine                        | 3.367642 | 5.868294 | 0      | 1.56E-08 | 0.135075 | 0.002368 |
| CMP                              | 0.652231 | 0.661112 | 0      | 6.93E-08 | 0.279262 | 0.000975 |
| Proline                          | 1.940354 | 1.813379 | 2.887517 | 9.04E-08 | 0.113562 | 0.000233 |
| UDP-Glucose/UDP-Galactose        | 1.010161 | 0.731924 | 0      | 1.96E-08 | 0.021362 | 0.000233 |
| M0009_Allantoin                  | 0.983780 | -2.17974 | -1.78837 | 3.57E-07 | 2.47E-06 | 2.55E-06 |
| GMP                              | 1.136654 | 0.649441 | 1.391801 | 6.46E-07 | 0.006761 | 0.000531 |
| Acetylcholine                    | 2.517966 | 1.725364 | 2.596768 | 9.84E-07 | 0.006761 | 0.000531 |
| Alpha-Keto-Glutарат/AdipicAcid   | 1.102566 | 0.723195 | 1.086058 | 1.23E-06 | 0.002294 | 0.000275 |
| UDP-Glucose/UDP-Galactose        | 1.229079 | 0.775377 | 0.953895 | 1.31E-06 | 0.002294 | 0.000275 |
| ClopidogrelCarboxylicAcid        | 2.843022 | 2.059676 | 3.0463   | 1.49E-06 | 0.006761 | 0.000531 |
| GDP                              | 0.934353 | 0.532535 | 0.900691 | 1.74E-06 | 0.006761 | 0.000531 |
| Inositol_p1                      | 2.883899 | 1.359574 | 1.22734  | 1.8E-06 | 0.002294 | 0.000275 |
| AdenylosuccinicAcid              | 0.871781 | 0      | 1.313151 | 2.12E-06 | 0.006761 | 0.000531 |
| DCMP                             | 0.738001 | 0      | 0.830289 | 2.55E-06 | 0.006761 | 0.000531 |
| ATP                              | 0.958861 | 0.475785 | 0.809664 | 3.61E-06 | 0.006761 | 0.000531 |
| Hydroxyproline                   | 1.237047 | 0.625321 | 1.401695 | 6.94E-06 | 0.042009 | 0.000371 |
| CitricAcid/IsocitricAcid         | 2.155345 | 1.098016 | 1.971255 | 7.86E-06 | 0.014622 | 0.000403 |
| Glutaronate                      | 2.339534 | 1.294568 | 1.955136 | 8.11E-06 | 0.000593 | 0.002724 |
| GlutathioneOxidized              | 0.746868 | 0.943002 | 0.758378 | 1.24E-05 | 0.006761 | 0.000531 |
| Oxaloacetate                     | 0.7672 | 0      | 1.468584 | 1.25E-05 | 0.006761 | 0.000531 |
| Lysine                           | 0.816882 | 0.92056 | 1.213497 | 1.32E-05 | 0.006761 | 0.000531 |
| GlutathioneReduced               | 3.392406 | 2.338397 | 3.340575 | 1.4E-05 | 0.006761 | 0.000531 |
| Pyridoxal-5-phosphate            | 0.390086 | 0.625323 | 0      | 1.42E-05 | 0.006761 | 0.000531 |
| ThiaminePyrophosphate            | 3.262642 | 2.669855 | 3.11800  | 1.43E-05 | 0.006761 | 0.000531 |
| GTP                              | 0.871744 | 0.586942 | 0.957303 | 1.73E-05 | 0.006761 | 0.000531 |
| Glycerol-3-P                     | 1.918188 | 0      | 5.872978 | 2.39E-05 | 0.006761 | 0.000531 |
| Betaine                          | 0.348238 | -1.80029 | -1.4235  | 2.62E-05 | 0.006761 | 0.000531 |
| Pyruvate                         | 0.948486 | 1.060558 | 1.981104 | 3.36E-05 | 0.006761 | 0.000531 |
| Malonyl-CoA                      | 1.013898 | 0.46806  | 1.095928 | 4.24E-05 | 0.006761 | 0.000531 |
| lsinopri1                        | 2.324822 | 1.224559 | 1.844598 | 4.7E-05 | 0.006761 | 0.000531 |
| Niacinamide                      | 0.831671 | 0.481145 | 1.094707 | 4.93E-05 | 0.006761 | 0.000531 |
| DUMP                             | 0.457518 | 0      | 0.627687 | 6.28E-05 | 0.006761 | 0.000531 |
| Uracil                           | 1.505587 | 0      | 0.915353 | 8.24E-05 | 0.006761 | 0.000531 |
| Homocysteine                     | 1.751458 | 1.275922 | 1.745184 | 0.000121 | 0.001339 | 0.000253 |
| NAD                              | 1.758952 | 1.252685 | 1.914347 | 0.000144 | 0.001339 | 0.000253 |
| PhosphoTyrosine                  | 0.977485 | 0      | 1.09795  | 0.000160 | 0.052281 | 0.000365 |
| Choline                          | 1.567816 | 0      | 0.713684 | 0.000180 | 0.088390 | 0.000561 |
| DTTP                             | 0.921396 | 1.89725 | 1.7662   | 0.000187 | 1.22E-06 | 6.6E-06 |
| UMP                              | 0.441581 | 0      | 1.02306 | 0.000201 | 0.373188 | 0.000210 |
| Compound                  | Value1 | Value2 | Value3 | Value4 | Value5 | Value6 |
|---------------------------|--------|--------|--------|--------|--------|--------|
| ADMA/SDMA                 | 1.274764 | 0 | 0.659703 | 0.0002260.3957840.024647 |
| Asparagine                | 2.465739 | 1.94115 | 1.745494 | 0.0002740.0003366.27E-05 |
| LacticAcid                | 0.841967 | 1.217315 | 2.193864 | 0.0002810.0146140.000754 |
| LacticAcid                | 0.929783 | 1.228154 | 2.186818 | 0.0002930.0110290.000279 |
| Tyrosine                  | 0.363737 | 0.286825 | 0.584346 | 0.0003230.138710.001019 |
| UDP-GlucuronicAcid        | 1.577688 | 0.558745 | 0.961924 | 0.0003560.162670.000254 |
| L-NMMA                    | 1.162303 | 0 | 2.727897 | 0.0005130.095250.001942 |
| NADP                      | 1.64977 | 1.038923 | 2.00076 | 0.0005280.02550.000753 |
| Inosine                   | 1.709662 | 0 | 2.51093 | 0.0006460.1963050.00576 |
| UricAcid                  | 0.896121 | -1.72641 | -1.56542 | 0.0007140.0007480.000467 |
| PantothenicAcid           | 0.892178 | 0 | 4.999293 | 0.0009550.0986890.00153 |
| Arginine                  | 0.933234 | 0.877226 | 1.174043 | 0.0010570.3298330.002638 |
| Sorbitol                  | -0.30662 | 0 | 1.467771 | 0.0015760.0773050.00011 |
| FumaricAcid/MaleicAcid    | 0.354638 | 0 | 3.402177 | 0.0015760.1659250.001817 |
| SuccinicAcid              | -0.45799 | -2.03581 | -1.53083 | 0.0016341.67E-066.09E-06 |
| 2-AminodipicAcid          | 0.926016 | 2.172327 | 3.17356 | 0.0018780.0398950.001322 |
| Alanine                   | 1.572233 | 0.594586 | 1.213526 | 0.001910.0346150.029246 |
| F1P/F6P/G1P/G6P_D97       | 0.2961 | 0 | 1.214274 | 0.0020690.2974270.003275 |
| OroticAcid                | 0.554959 | 0.367051 | 0.787753 | 0.0022430.011530.000878 |
| cGMP                      | 0.87168 | 0 | 1.218829 | 0.0026850.0773050.00011 |
| Histidine                 | 0.853472 | 0 | 0.922024 | 0.0042290.2257590.005293 |
| Xanthine                  | 0.745865 | -1.75949 | -1.9112 | 0.0052481.97E-051.87E-05 |
| Xanthosine-5'-monophosphate | 0.404653 | 0 | 0.861384 | 0.0085670.2949980.000428 |
| Glutamine                 | 0.505116 | 0.770061 | 0.810674 | 0.0173160.0006830.002636 |
| PPA                       | 1.017519 | 1.410496 | 3.815982 | 0.0260970.067540.038411 |
| Argininosuccinate         | 0.539731 | 0 | 3.604020 | 0.0320080.614030.000697 |
| Melatonin                 | -0.29549 | -1.17919 | -1.61574 | 0.0328862.83E-055.25E-06 |
| 3-PhosphoglycericAcid     | 0.346798 | 0 | -1.76252 | 0.0349640.1160130.002544 |
| GlutamicAcid_p2           | 0.339222 | 0 | 0.597533 | 0.0408070.1695260.005461 |
| Glycerate-2-P             | 0 | 0 | -1.54605 | 0.0511230.1072440.004303 |
| PEP                       | 0 | -0.45973 | -2.1583 | 0.0660770.442340.000649 |
| TaurochenodeoxycholicAcid | 0 | -2.96526 | -2.97841 | 0.0675790.0001920.000129 |
| Metanephrine              | 0 | 0 | 1.944166 | 0.1192650.26280.005595 |
| Lactose                   | 0 | -1.36666 | -1.34099 | 0.1305310.271810.028587 |
| NADH                      | 0 | 0 | 2.392461 | 0.141150.1249350.00329 |
| Glycerol                  | 0 | 0 | 0.718995 | 0.1514310.1831740.022065 |
| FumaricAcid/MaleicAcid    | 0 | 0 | 2.498515 | 0.1752190.1245640.001266 |
| AnthranilicAcid           | 0 | -0.9303 | -1.24249 | 0.202028.19E-075.13E-05 |
| MalicAcid                 | 0 | 0 | 2.14388 | 0.3504920.0988910.001077 |
| HomogentisicAcid          | 0 | 0 | 1.359471 | 0.4114800.0875920.000559 |
| GlycochenodeoxycholicAcid | 0.587073 | -1.5409 | -5 | 0.005624 | 0 | 0 |