

# Supplemental Information

for

## **DNAJA1 dysregulates metabolism promoting an anti-apoptotic phenotype in pancreatic ductal adenocarcinoma**

Heidi Roth,<sup>1,†</sup> Fatema Bhinderwala,<sup>1,2,3,†</sup> Rodrigo Franco,<sup>4,5</sup> You Zhou,<sup>2,6</sup> Robert Powers<sup>1,2\*</sup>

<sup>1</sup>*Department of Chemistry, University of Nebraska-Lincoln, Lincoln NE 68588-0304*

<sup>2</sup>*Nebraska Center for Integrated Biomolecular Communication, University of Nebraska-Lincoln, Lincoln NE 68588-0304*

<sup>3</sup>*Current Affiliation - University of Pittsburgh School of Medicine, Department of Structural Biology, Pittsburgh, PA 15213*

<sup>4</sup>*School of Veterinary Medicine and Biomedical Sciences, University of Nebraska-Lincoln, Lincoln NE 68583-0905*

<sup>5</sup>*Redox Biology Center, University of Nebraska-Lincoln, Lincoln, NE 68588-0304*

<sup>6</sup>*Morrison Microscopy Core Research Facility, University of Nebraska-Lincoln, Lincoln NE 68588-0664*

‡ These authors contributed equally to the work

\*To whom correspondence should be addressed:

Robert Powers  
University of Nebraska-Lincoln  
Department of Chemistry  
722 Hamilton Hall  
Lincoln, NE 68588-0304  
Email: [rpowers3@unl.edu](mailto:rpowers3@unl.edu)  
Phone: (402) 472-3039  
Fax: (402) 472-9402

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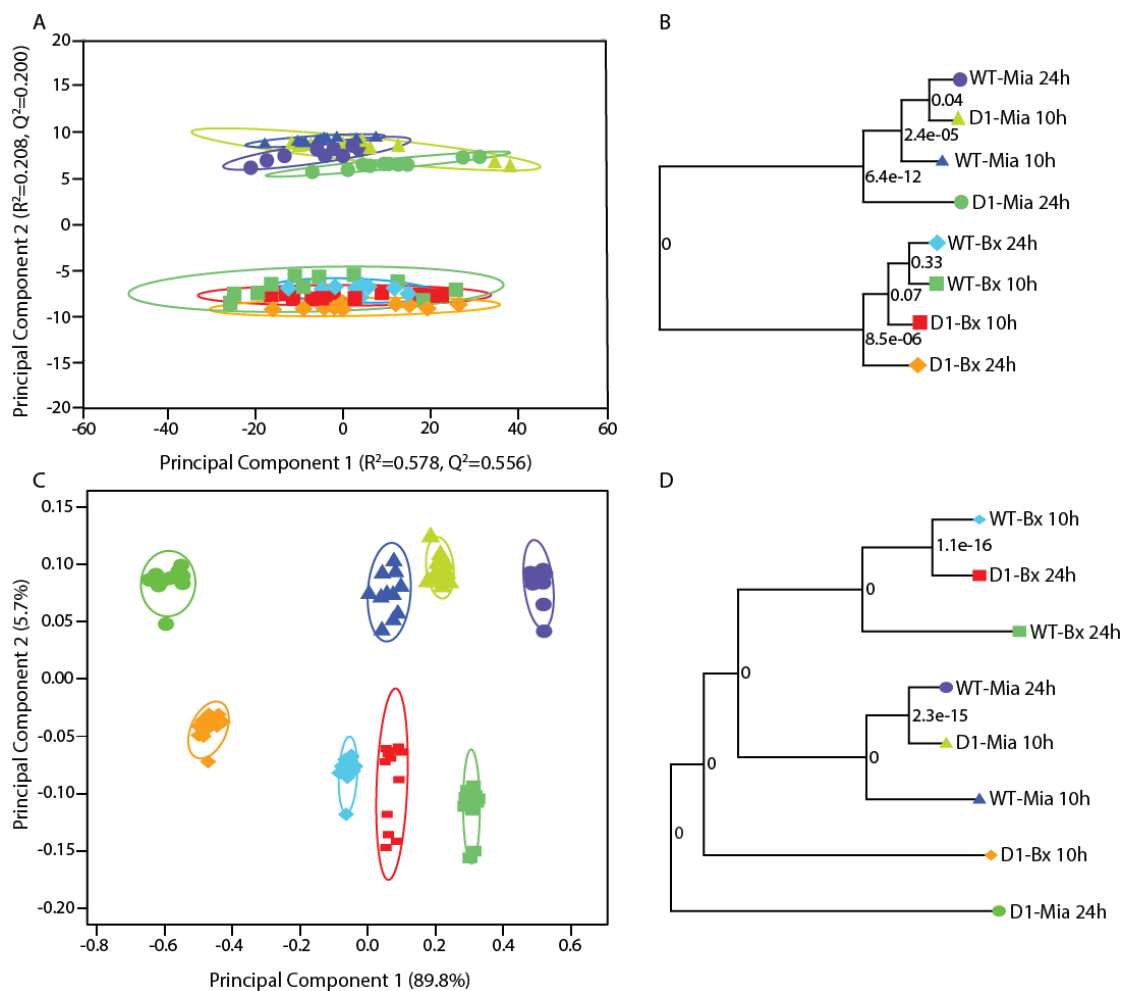
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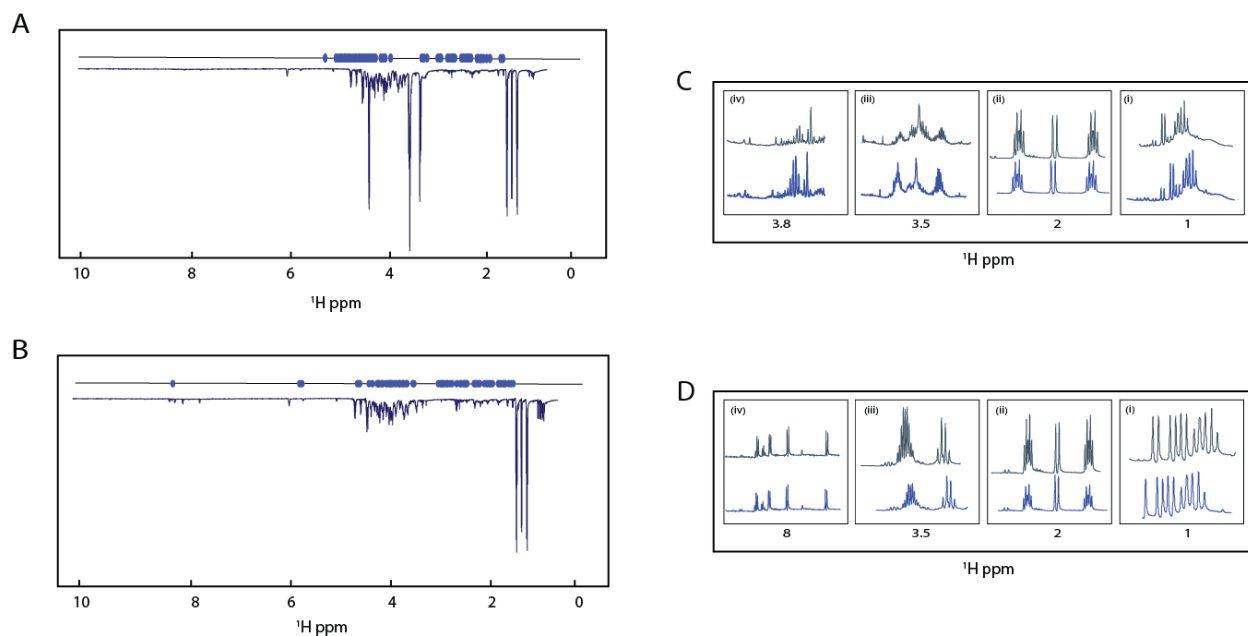
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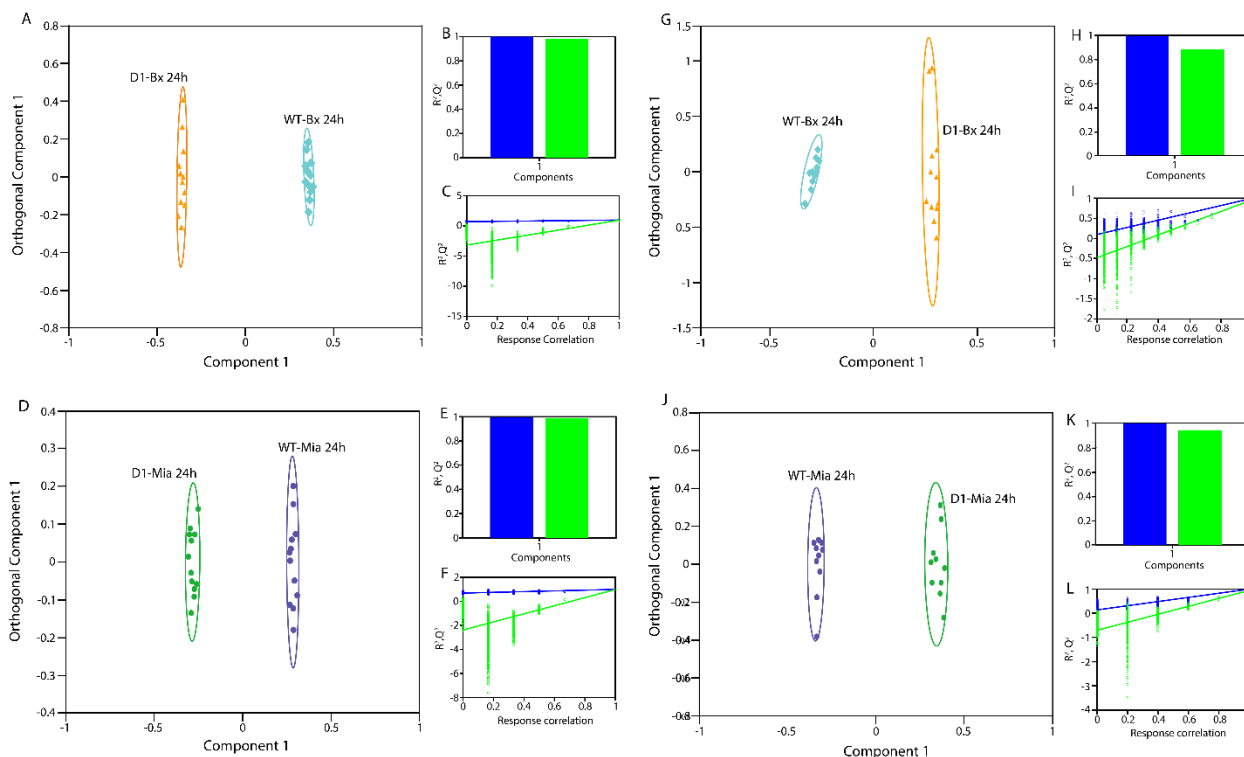
**Figure S1. Unique exo-metabolome of pancreatic cancer cells following DNAJA1 overexpression**

(A) PCA scores plot generated from 1D  $^1\text{H}$  NMR spectra of spent media of WT and D1 cell lines of BxPC-3 and MiaPaCa-2 ( $R^2$  0.762,  $Q^2$  0.730). The ellipses around each cluster corresponds to the 95% confidence interval for a normal distribution of data. Each cluster contains twelve replicates except WT-Mia at 24 hours where  $n=11$ . (B) Metabolic tree diagram generated from the PCA scores plot in A. The p-value denoted for each node is calculated from the Mahalanobis distance between each group. The coloring is identical between the PCA scores plot and the tree diagram. (C) PCA scores plot generated from 2D  $^1\text{H}$ - $^{13}\text{C}$  NMR spectra of spent media of WT and D1 cell lines of BxPC-3 and MiaPaCa-2 ( $R^2$  0.99,  $Q^2$  0.97). (D) Metabolic tree diagram generated from PCA scores plot in C. The color scheme is: WT-Bx cells at 10 hours (■) and at 24 hours (◆), and D1-BX cells at 10 hours (■) and at 24 hours (◆). Similarly, WT-Mia at 10 hours (▲) and at 24 hours (●), and D1-Mia at 10 hours (▲) and at 24 hours (●).



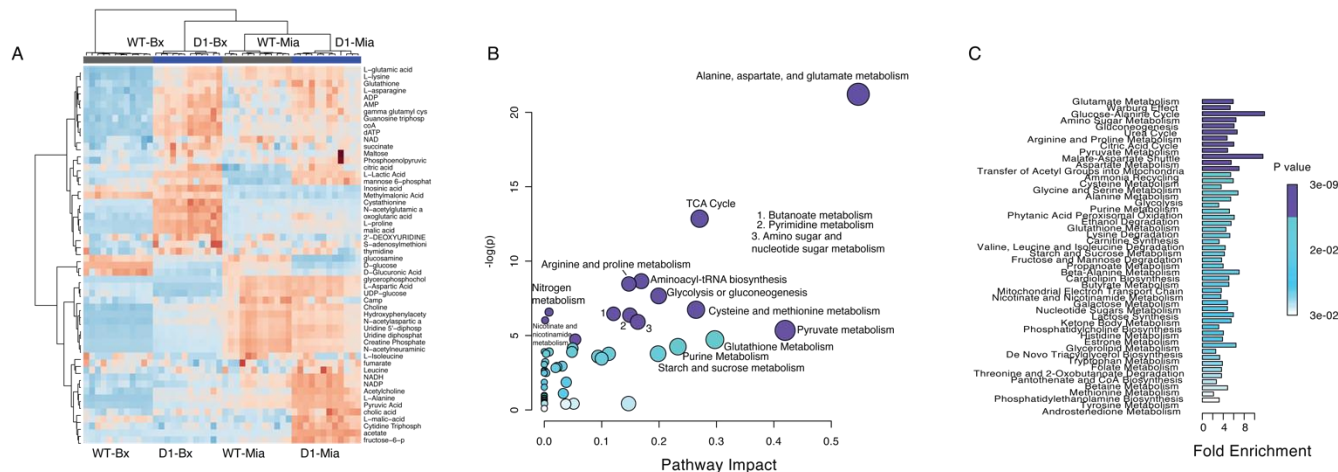
**Figure S2. Metabolic changes in the media following DNAJA1 overexpression**

Spectral variations between WT and D1 cell culture media. A mean 1D  $^1\text{H}$  NMR spectrum of media collected from wild-type (A) BxPC-3 and (C) MIA PaCa-2 cell cultures are marked with blue points indicating the significantly altered ( $p < 0.001$ ) NMR spectral bins in the corresponding 1D  $^1\text{H}$  NMR spectrum for D1 cell culture media. Expanded views of mean 1D  $^1\text{H}$  NMR spectra with WT shown in gray (*bottom*) and D1 shown in blue (*top*) for (C) WT-Bx and D1-Bx, and (D) WT-MIA and D1-Mia. The panels in C and D correspond to: (i) branched chain amino acids, (ii) glutamine and glutamate, (iii) glucose, and (iv) energy metabolites.



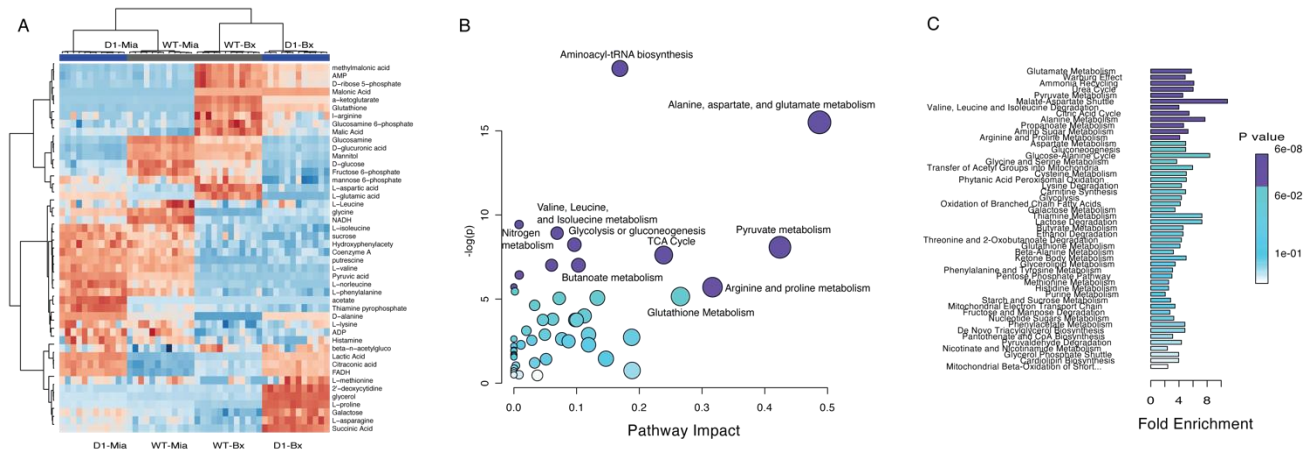
**Figure S3. OPLS models generated from cell lysate and media NMR data sets**

OPLS scores plots generated from 1D  $^1\text{H}$  NMR spectra of (A) BxPC-3 ( $R^2$  0.999,  $Q^2$  0.982, CV-ANOVA p-value  $1.2 \times 10^{-13}$ ) and (D) MIA PaCa-2 ( $R^2$  0.997,  $Q^2$  0.989, CV-ANOVA p-value  $7.27 \times 10^{-14}$ ) cell lysate (n=12) with their corresponding (B, E)  $R^2/Q^2$  and (C, F) and cross-permutation test (1,000 iterations, p-values < 0.05) plots demonstrating model reliability. OPLS scores plots generated from 1D  $^1\text{H}$  NMR spectra of (G) BxPC-3 ( $R^2$  0.996,  $Q^2$  0.882, CV-ANOVA p-value 1) and (J) MIA PaCa-2 ( $R^2$  0.996,  $Q^2$  0.942, CV-ANOVA p-value 1) cell culture media (n=12) with their corresponding (H, K)  $R^2/Q^2$  and (I, L) and cross-permutation test (1,000 iterations, p-values < 0.05) plots demonstrating the models may be of limited reliability.



**Figure S4: Summary of all  $^{13}\text{C}$  glucose derived intracellular metabolic changes resulting from DNAJA1 overexpression**

(A) Heat map and hierarchical clustering of all metabolites derived from  $^{13}\text{C}_6$  glucose from lysed WT and D1 cell. Heat map plots normalized peak intensities from 2D  $^1\text{H}$ - $^{13}\text{C}$  HSQC spectra. Each row displays the relative metabolite abundance across the four groups, where red identifies a relative metabolite accumulation and blue indicates metabolite depletion. (B) Pathway impact plot based on all metabolite occurrence from the analysis of the 2D  $^1\text{H}$ - $^{13}\text{C}$  HSQC spectra. (C) Pathway enrichment plot based on all metabolite concentrations from the analysis of the 2D  $^1\text{H}$ - $^{13}\text{C}$  HSQC spectra. Pathway fold enrichment is colored according to the corresponding p-value using the indicated purple to teal scale. The pathway impact and enrichment plots were generated using MetaboAnalyst 4.0 (<https://www.metaboanalyst.ca/>).



**Figure S5: Summary of all  $^{13}\text{C}$  glucose derived exometabolic metabolic changes resulting from DNAJA1 overexpression**

(A) Heat map and hierarchical clustering of all metabolites derived from  $^{13}\text{C}_6$  glucose from WT and D1 cell culture media. Heat map plots normalized peak intensities from 2D  $^1\text{H}$ - $^{13}\text{C}$  HSQC spectra. Each row displays the relative metabolite abundance across the four groups, where red identifies a relative metabolite accumulation and blue indicates metabolite depletion. (B) Pathway impact plot based on all metabolite occurrence from the analysis of the 2D  $^1\text{H}$ - $^{13}\text{C}$  HSQC spectra. (C) Pathway enrichment plot based on all metabolite concentrations from the analysis of the 2D  $^1\text{H}$ - $^{13}\text{C}$  HSQC spectra. Pathway fold enrichment is colored according to the corresponding p-value using the indicated purple to teal scale. The pathway impact and enrichment plots were generated using MetaboAnalyst 4.0 (<https://www.metaboanalyst.ca/>).

**Table S1.** Metabolites identified from cell lysate with statistical significance

Metabolite Identity	Univariate statistics comparing WT to D1 samples (p-values) <sup>a</sup>			
	D1-Bx	FC <sup>b</sup>	D1-MIA	FC
2'-deoxycytidine	ND <sup>c</sup>	--	ND	--
2'-deoxyuridine	0.00570856	200.94473	0.059331417	55.55536662
3-iodotyrosine	ND	--	ND	--
Acetate	9.5224E-07	303.80235	2.77005E-21	693.9945399
Acetylcholine	6.4564E-06	201.04313	1.03255E-10	178.0317973
Acetylphosphate	ND	--	ND	--
ADP	1.4416E-12	204.81925	0.01584079	123.1770047
AMP	4.4238E-12	200.46942	4.56763E-05	138.0759444
Arginosuccinic acid	ND	--	ND	--
ATP	2.4823E-13	206.77912	6.93558E-05	136.3662378
Biotin	ND	--	ND	--
cAMP	0.08573328	132.347	0.013439101	68.10961928
Cholic acid	0.00075727	132.31671	3.71912E-07	170.1515786
Choline	1.629E-14	472.69152	NS <sup>d</sup>	99.66770078
Citraconic acid	ND	--	ND	--
Citric acid	0.00149052	121.84648	1.97024E-06	152.3823436
Coenzyme A	5.2975E-15	493.21576	(NS) <sup>e</sup>	112.0516684
Creatine Phosphate	3.5345E-13	334.49172	0.042821498	109.5020972
Cystathionine	3.5345E-13	3862.8601	0.042821498	31.52002636
Cytidine Tri/monophosphate	NS	81.786037	1.72416E-07	217.3491259
D-alanine	ND	--	ND	--
dATP	2.1636E-15	492.48622	0.069093311	112.3884789
Deoxyadenosine monophosphate	ND	--	ND	--
D-Glucaronic acid	ND	--	ND	--
D-glucose	4.9041E-18	80.877011	6.26403E-12	80.10559238
D-ribose 5-phospahte	ND	--	ND	--
FADH	ND	--	ND	--
Fructose 6-phosphate	0.01146448	69.94147	4.05164E-09	181.5478365
Fumarate	0.00753454	60.156261	0.062350685	147.7971823
Galactose	ND	--	ND	--
Gamma glutamyl cysteine	6.2716E-12	415.04047	0.019791633	135.831602
Glucaronic Acid	3.4987E-10	64.785883	0.000797543	88.55987106
Glucosamine	1.9784E-09	85.254101	2.28876E-13	68.30852809
Glucosamine 6-sulfate	ND	--	ND	--
Glutathione	2.4881E-10	201.42466	0.000468158	124.2423941
Glycerophosphocholine	0.03398999	126.90768	NS	105.8335472
Glycine	ND	--	ND	--
Guanosine triphosphate	3.6958E-10	440.6368	0.008172087	137.0686064
Heparin	ND	--	ND	--
Histamine	ND	--	ND	--
Hydroxyphenylacetylglcine	4.0994E-11	304.24017	0.000459931	81.18437309
Inosinic acid	8.5194E-06	149.04446	0.049096929	87.82899356
L-alanine	2.5113E-18	480.86203	3.05405E-08	145.2377558
L-arginine	ND	--	ND	--
L-asparagine	6.9449E-12	461.98595	0.021745955	134.3214325
L-aspartic Acid	1.1339E-13	37.767204	NS	94.92949788
L-glutamic acid	2.6564E-11	208.40413	NS	103.191905
L-glutamine	3.5345E-13	42.798791	0.004869405	89.40802821
L-histidine	NS	--	NS	--
L-isoleucine	0.01894132	25.502167	0.032199431	49.3130407
L-lactic Acid	1.7894E-22	173.58778	2.23856E-20	213.7776716
L-leucine	NS	107.59508	0.031214079	175.4814999
L-lysine	NS	80.038509	NS	93.1479338



L-malic-acid	1.8879E-06	63.749019	1.00622E-10	230.1937641
L-methionine	ND	--	ND	--
L-norleucine	ND	--	ND	--
L-phenylalanine	ND	--	ND	--
L-proline	1.5967E-18	1162.7692	8.66451E-09	150.1851932
L-valine	ND	--	ND	--
Malic acid	3.5218E-07	52.651811	5.74632E-07	195.5204485
Malonic Acid	ND	--	ND	--
Maltose	6.4564E-06	298.12906	NS	207.0609749
Mannitol	ND	--	ND	--
Mannose 6-phosphate	0.0008184	131.28924	6.26403E-12	227.3473436
Methanol	ND	--	ND	--
Methylmalonic Acid	0.00907868	87.645317	NS	90.94507249
N-acetylaspartic acid	3.2712E-16	1441.6252	0.021773843	86.48927149
N-acetylglutamic acid	1.3488E-15	407.79386	0.000314578	90.18770759
N-acetylneuraminic acid	1.1729E-13	450.2449	0.001573464	80.21910527
NAD	0.03620937	107.00103	0.001681943	122.925212
NADH	5.5679E-06	131.42848	9.57787E-05	129.2518561
NADP	NS	101.57376	0.002301368	121.1078461
$\alpha$ -ketoglutarate	4.9409E-13	406.96535	0.056861438	129.6828372
Phosphoenolpyruvic acid	0.00404092	184.24955	NS	135.4915442
Putrescine	ND	--	ND	--
Pyruvic Acid	8.8445E-13	259.28624	1.34475E-09	136.7920174
S-adenosylhomocysteine	2.0011E-07	37.430838	0.027748322	112.2189561
S-adenosylmethionine	0.05878973	252.58068	NS	101.2526552
Serotonin	ND	--	ND	--
Succinate	1.5885E-05	272.5842	NS	120.5132115
Succinic acid	ND	--	ND	--
Sucrose	ND	--	ND	--
Thiamine pyrophosphate	ND	--	ND	--
Thymidine	0.00052734	296.13645	NS	130.3837384
Tyrosine	NS	70.838847	NS	142.8533908
UDP-glucose	4.3751E-07	53.523205	NS	105.7523241
Uridine 5'-diphosphate	4.2813E-14	260.42289	NS	100.2829848
Uridine diphosphategalactose	2.1636E-15	346.65526	0.042821498	88.28994032

<sup>a</sup>p-value from a pairwise (WT vs. D1) Student's t-test followed by a Benjamini- Hochberg multiple hypothesis correction.

<sup>b</sup>FC: fold change referenced to WT.

<sup>c</sup>ND: metabolite was not detected in lysate, but was detected in media.

<sup>e</sup>NS: metabolite change was not statistically significant based on a pairwise Student's t-test.

<sup>d</sup>(NS): metabolite change was not statistically significant based on a pairwise Student's t-test followed by a Benjamini-Hochberg multiple hypothesis correction.

**Table S2.** Metabolites identified from cell culture media with statistical significance

Metabolite Identity	Univariate statistics comparing WT to D1 samples (p-values) <sup>a</sup>			
	D1-Bx	FC <sup>b</sup>	D1-MIA	FC
2'-deoxycytidine	9.0059E-18	1750.33131	(NS) <sup>e</sup>	46.6302258
2'-deoxyuridine	ND <sup>c</sup>	--	ND	--
3-iodotyrosine	NS <sup>d</sup>	112.396343	0.06794465	99.0329275
Acetate	ND	--	ND	--
Acetylcholine	ND	--	ND	--
Acetylphosphate	0.03450657	95.1302018	1.8824E-19	606.325428
ADP	0.07864849	119.729891	NS	115.358731
AMP	2.161E-05	75.9377611	NS	92.4518118
Arginosuccinic acid	NS	75.3092542	0.00751061	180.327598
ATP	ND	--	ND	--
Biotin	1.3242E-06	6.95851167	NS	99.1426032
cAMP	ND	--	ND	--
Cholic acid	ND	--	ND	--
Choline	ND	--	ND	--
Citraconic acid	1.9742E-11	137.496449	1.9367E-18	210.094701
Citric acid	ND	--	ND	--
Coenzyme A	NS	106.107796	0.02963082	110.65018
Creatine Phosphate	ND	--	ND	--
Cystathionine	ND	--	ND	--
Cytidine Tri/monophosphate	ND	--	ND	--
D-alanine	7.3321E-35	434.732468	6.634E-22	154.231737
dATP	ND	--	ND	--
Deoxyadenosine monophosphate	NS	69.5631662	NS	104.593403
D-Glucaronic acid	3.2796E-17	90.1583721	4.3617E-21	82.9446912
D-glucose	1.6023E-12	89.8861325	3.0507E-13	84.3048573
D-ribose 5-phospahte	1.1479E-07	65.4705984	NS	98.391549
FADH	4.8178E-15	146.369019	1.8121E-21	222.979628
Fructose 6-phosphate	1.3591E-24	83.2898561	4.2656E-13	81.9775267
Fumarate	ND	--	ND	--
Galactose	1.1189E-13	168.2994	2.0278E-05	113.226878
Gamma glutamyl cysteine	ND	--	ND	--
Glucaronic Acid	ND	--	ND	--
Glucosamine	4.1423E-16	88.976695	1.8121E-21	83.0092613
Glucosamine 6-sulfate	3.6138E-08	68.0730158	NS	93.55534
Glutathione	2.5923E-11	76.1062291	NS	95.9934046
Glycerophosphocholine	ND	--	ND	--
Glycine	1.2335E-13	170.177506	5.753E-06	80.973239
Guanosine triphosphate	ND	--	ND	--
Heparin	3.6997E-07	81.4818366	1.2078E-07	99.4347399
Histamine	NS	100.702427	0.0805772	141.016464
Hydroxyphenylacetyl glycine	ND	--	ND	--
Inosinic acid	ND	--	ND	--
L-alanine	ND	--	ND	--
L-arginine	0.00025789	69.5703765	0.09391717	131.471408
L-asparagine	2.8013E-14	240.527996	4.9371E-05	162.278107
L-aspartic Acid	3.8857E-14	11.8302736	1.441E-05	146.144684
L-glutamic acid	5.207E-17	4.85927958	2.3396E-06	213.308001
L-glutamine	ND	--	ND	--
L-histidine	ND	--	ND	--
L-isoleucine	NS	98.5687542	NS	95.9624454
L-lactic Acid	6.7222E-23	149.317767	4.0289E-23	201.325121
L-leucine	NS	132.646383	0.02615413	73.1535101
L-lysine	NS	92.6148369	NS	98.4960528

L-malic-acid	ND	--	ND	--
L-methionine	0.00235309	54.2759656	NS	107.26345
L-norleucine	(NS)	41.7830683	1.0096E-06	171.661943
L-phenylalanine	NS	75.4633006	NS	102.486432
L-proline	1.8375E-27	1477.78566	5.6262E-05	106.396085
L-valine	NS	82.6899958	NS	103.001973
Malic acid	3.0873E-09	45.7459908	6.8533E-05	246.294027
Malonic Acid	6.715E-14	109.806352	NS	138.49748
Maltose	ND	--	ND	--
Mannitol	2.5509E-16	86.1654878	3.6578E-19	82.3250379
Mannose 6-phosphate	2.2122E-07	76.8998353	NS	97.3626759
Methanol	3.0946E-08	34.9280527	1.4877E-10	186.01572
Methylmalonic Acid	2.1318E-07	73.1138493	0.02963082	81.2052735
N-acetylaspartic acid	ND	--	ND	--
N-acetylglutamic acid	ND	--	ND	--
N-acetylneuraminic acid	ND	--	ND	--
NAD	ND	--	ND	--
NADH	NS	99.5013539	7.5426E-10	37.0062367
NADP	ND	--	ND	--
$\alpha$ -ketoglutarate	9.2739E-18	54.2109706	0.00010089	89.2908317
Phosphoenolpyruvic acid	ND	--	ND	--
Putrescine	1.8688E-06	195.562806	0.0032564	107.686555
Pyruvic Acid	0.00768675	128.50416	5.2465E-16	136.274477
S-adenosylhomocysteine	ND	--	ND	--
S-adenosylmethionine	ND	--	ND	--
Serotonin	0.0043824	62.764332	0.00128964	285.756992
Succinate	ND	--	ND	--
Succinic acid	5.7902E-18	268.309509	8.8721E-07	137.062348
Sucrose	0.00798885	26.5899283	0.0008592	129.432741
Thiamine pyrophosphate	0.00199568	69.8750012	1.2415E-10	192.852503
Thymidine	ND	--	ND	--
Tyrosine	ND	--	ND	--
UDP-glucose	ND	--	ND	--
Uridine 5'-diphosphate	ND	--	ND	--
Uridine diphosphategalactose	ND	--	ND	--

<sup>a</sup>p-value from a pairwise (WT vs. D1) Student's t-test followed by a Benjamini- Hochberg multiple hypothesis correction.

<sup>a</sup>FC: fold change relative WT.

<sup>b</sup>ND: metabolite was not detected in media, but was detected in lysate.

<sup>c</sup>NS: metabolite change was not statistically significant based on a pairwise Student's t-test

<sup>d</sup>(NS): metabolite change was not statistically significant based on a pairwise Student's t-test followed by a Benjamini-Hochberg multiple hypothesis correction.