Supplementary Data

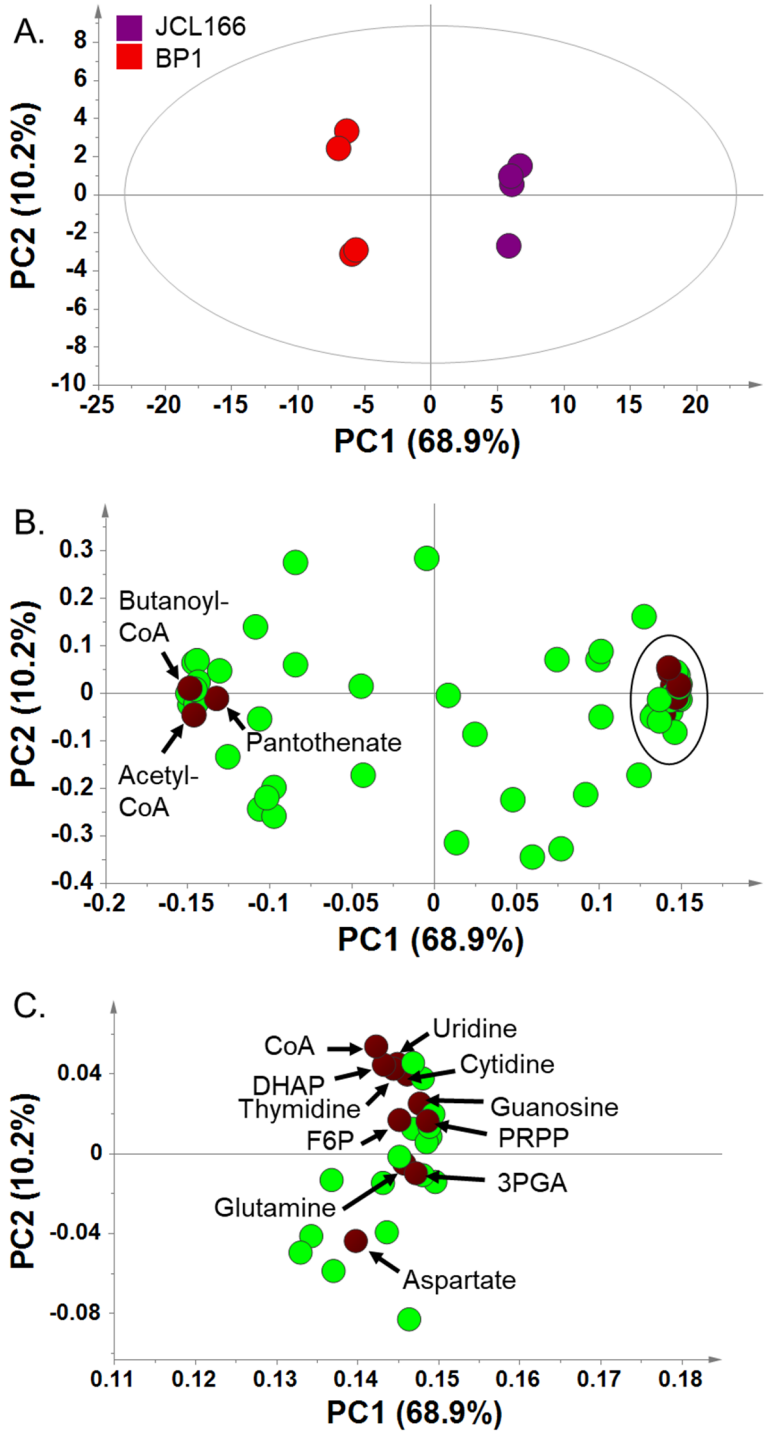

Fig. S1 (A) PCA score plot for metabolic profiling of JCL166 (purple) and BP1 (red) at stationary phase (24 h). The ellipse indicates 95% confidence border based on Hotelling's T^2. **(B)** Corresponding PCA loading plot showing metabolites (green and brown circles) that contributed to the separation of the two strains. **(C)** Enlarged PCA loading plot showing encircled metabolites in the positive region of PC1 in (**B**). Brown circles indicate the metabolites that were further discussed in the manuscript. Dataset derived from *Metab Eng*. *49*, 153–163, Pontrelli, S.; Fricke, R. C. B.; Sakurai, S. S. M.; Putri, S. P.; Fitz-Gibbon, S.; Chung, M.; Wu, H. Y.; Chen, Y. J.; Pellegrini, M.; Fukusaki, E.; Liao, J. C Directed strain evolution restructures metabolism for 1-butanol production in minimal media, 2018, with permission from Elsevier.

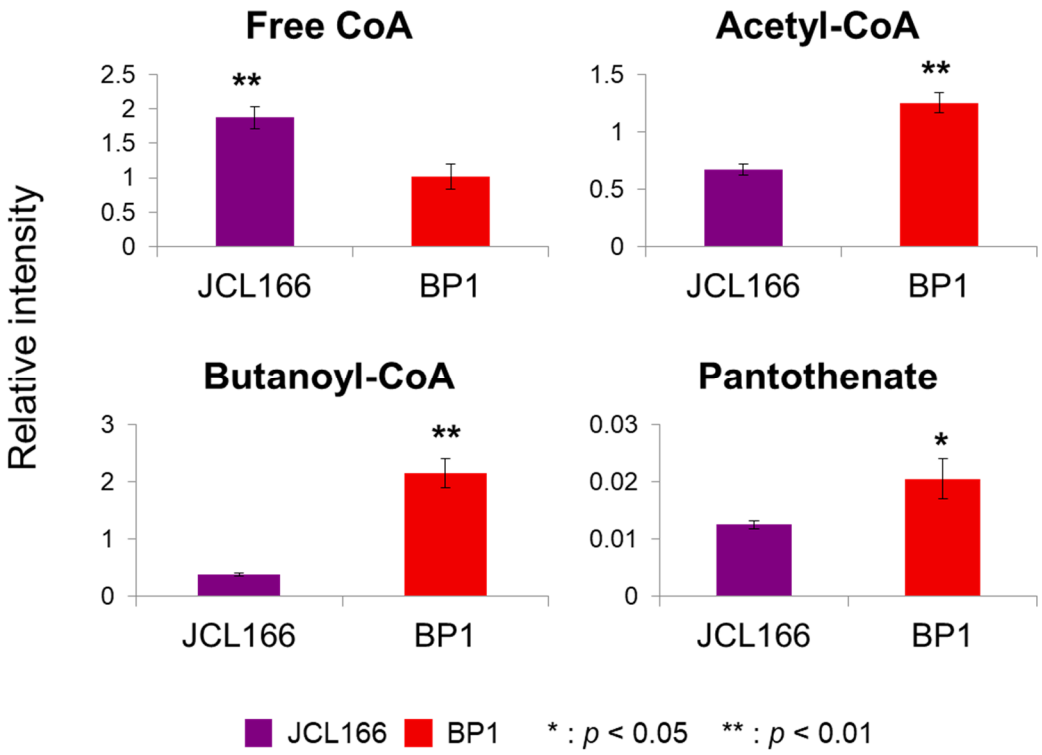

Fig. S2 Metabolite intensities of CoA-related compounds. Bar graphs show relative intensities on y-axis obtained by normalization of peak area with the internal standard. Asterisks indicate significant difference between the two strains (*: $p < 0.05$, **: $p < 0.01$). Error bars indicate standard deviation obtained from 4 replicates. Dataset derived from Metab Eng. 49, 153–163, Pontrelli, S.; Fricke, R. C. B.; Sakurai, S. S. M.; Putri, S. P.; Fitz-Gibbon, S.; Chung, M.; Wu, H. Y.; Chen, Y. J.; Pellegrini, M.; Fukusaki, E.; Liao, J. C Directed strain evolution restructures metabolism for 1-butanol production in minimal media, 2018, with permission from Elsevier.

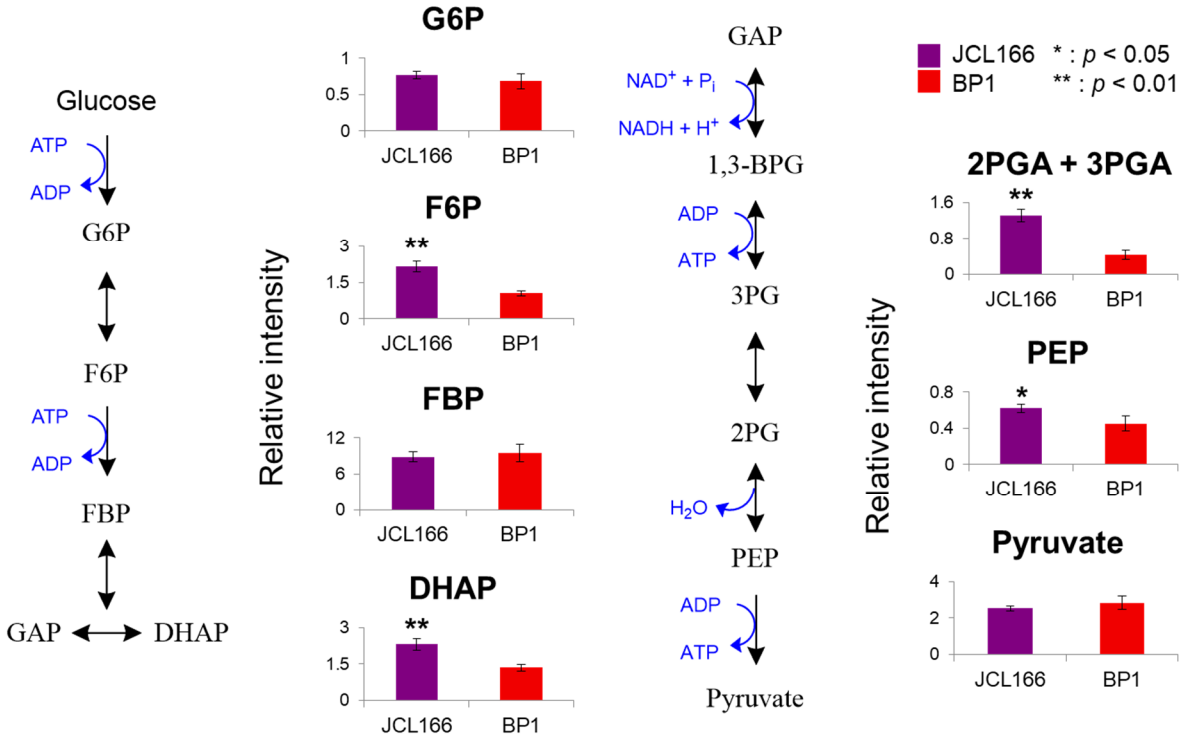

Fig. S3 Metabolite intensities of glycolysis pathway intermediates. Bar graphs show relative intensity on y-axis obtained by normalization of peak area with the internal standard. Asterisks indicate significant difference between the two strains (*: $p < 0.05$, **: $p < 0.01$). Error bars indicate standard deviation obtained from 4 replicates. Dataset derived from Metab Eng. 49, 153–163, Pontrelli, S.; Fricke, R. C. B.; Sakurai, S. S. M.; Putri, S. P.; Fitz-Gibbon, S.; Chung, M.; Wu, H. Y.; Chen, Y. J.; Pellegrini, M.; Fukusaki, E.; Liao, J. C Directed strain evolution restructures metabolism for 1-butanol production in minimal media, 2018, with permission from Elsevier.

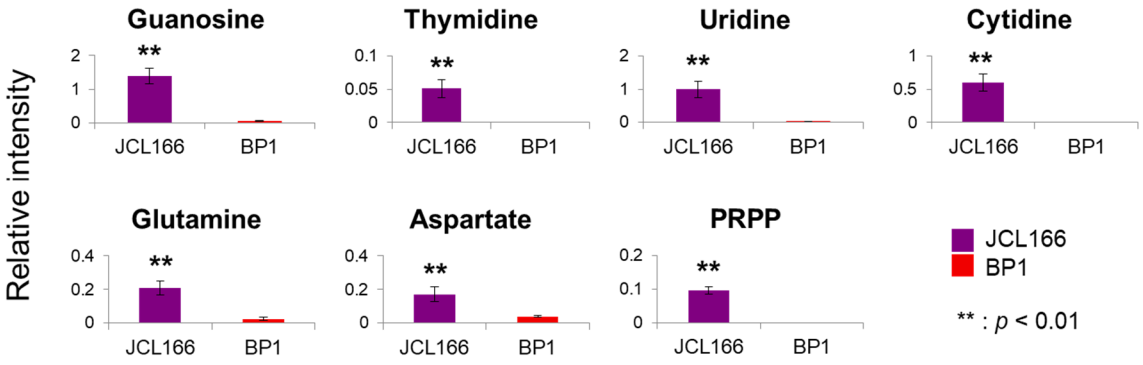

Fig. S4 Metabolite intensities of nucleotide biosynthesis-related compounds. Bar graphs show relative intensities on the y-axis obtained by normalization of peak area with the internal standard. Asterisks indicate significant difference between the two strains (**: p < 0.01). Error bars indicate standard deviation obtained from 4 replicates. Dataset derived from Metab Eng. 49, 153–163, Pontrelli, S.; Fricke, R. C. B.; Sakurai, S. S. M.; Putri, S. P.; Fitz-Gibbon, S.; Chung, M.; Wu, H. Y.; Chen, Y. J.; Pellegrini, M.; Fukusaki, E.; Liao, J. C Directed strain evolution restructures metabolism for 1-butanol production in minimal media, 2018, with permission from Elsevier.

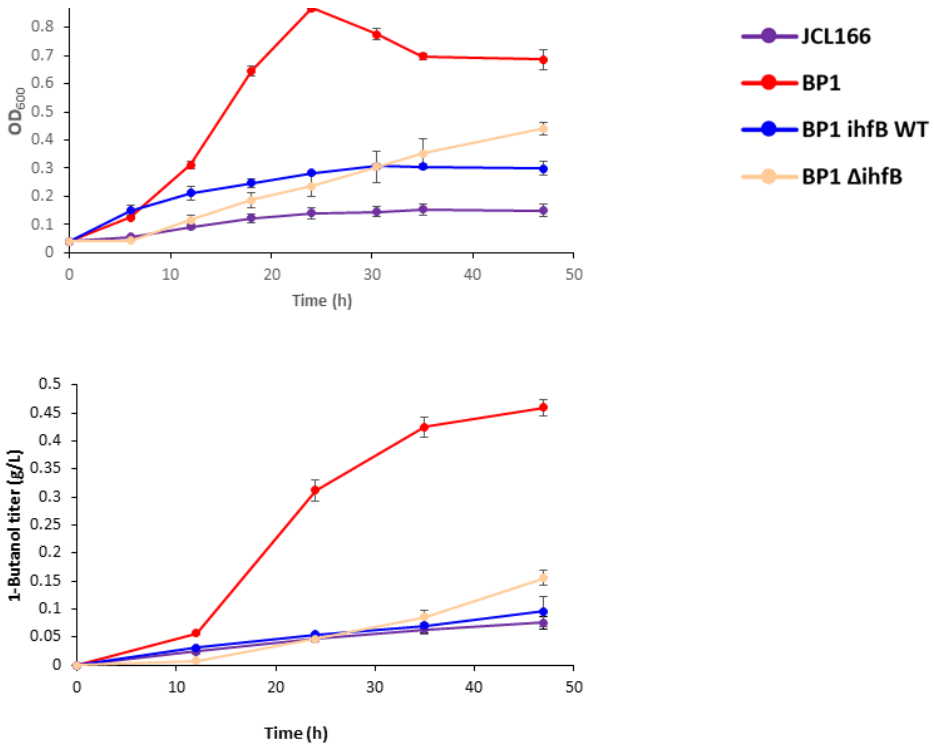

Fig. S5. A) Growth curve of JCL166, BP1, BP1 ihfB WT and BP1 *ΔihfB* strain in 4 g/L glucose minimal medium under anaerobic conditions. (B) 1-Butanol titer (g/L) of JCL166, BP1, BP1 *ihfB* WT and BP1 *ΔihfB* strain from anaerobic fermentation in 4 g/L minimal medium. Error bars indicate standard deviation obtained from 3 biological replicates.

Table. S1 Data matrix subjected to PCA of BP1 and BP1 *ihfB* WT

	BP1-1	**BP1-2**	**BP1-3**	**BP1 ihfB WT-1**	**BP1 ihfB WT-2**	**BP1 ihfB WT-3**
Glutamine	0.011415	0.015097	0.014592	0.093205	0.087108	0.127386
Threonine	0.031697	0.025992	0.03442	0.029334	0.033382	0.033569
Trehalose	0.639859	0.745842	0.624517	0.099901	0.114561	0.037066
Cytidine	0.029385	0.023774	0.038411	0.011868	0.042605	0.012037
Methionine	0.019481	0.018964	0.018823	0.009962	0.010608	0.009399
Tyrosine	0.019723	0.019138	0.02493	0.09738	0.103322	0.089647
Xanthine	0.001302	0.001125	0.001034	0.005578	0.008701	2.01E-05
Glutamate	0.417558	0.474426	0.459906	3.632352	3.622189	3.311581
Uridine	0.151941	0.122965	0.188447	0.053068	0.135013	0.062424
Aspartate	0.075072	0.074835	0.091359	0.107821	0.111435	0.10755
Thymine	0.002608	0.002327	0.002323	0	0	0
Guanosine	0.187952	0.154594	0.222186	0.052509	0.16883	0.060489
Adenosine	0.006349	0.005643	0.008656	0.002639	0.010743	0.003034
Phenylalanine	0.017214	0.017539	0.022789	0.074263	0.077232	0.078757
G6P	0.365081	0.450731	0.3243	1.045729	0.955416	1.07244
Sor6P	1.845134	1.931761	1.689705	1.932076	2.289274	2.050239
Tryptophan	0.025733	0.03174	0.021883	0.01003	0.007927	0.009471
R5P	0.072851	0.08062	0.072935	0.286491	0.263343	0.307214
Lactate	0.046347	0.037577	0.073839	-1E-04	-0.00104	-0.0107
F6P	0.503675	0.523952	0.404531	2.744823	2.000335	2.309836
G1P	0.458885	0.573183	0.571291	1.234093	1.441638	1.187907
a-GP	0.539839	0.617233	0.568093	0.686825	0.880521	0.633635
NAD	0.972445	0.938175	0.955549	0.799174	0.852416	0.89983
Orotate	7.450467	6.601834	6.713027	14.16377	13.70175	12.33199
Ru5P+Xu5P	0.478489	0.618823	0.451125	1.929128	2.160991	2.230475
CMP	0.079758	0.074551	0.082908	0.165169	0.217903	0.162784
Pyruvate	0.61319	0.548166	0.54433	1.598509	1.562846	1.386458
R1P	0.117788	0.096708	0.16779	0.13415	0.233655	0.12611
UMP	0.254697	0.250938	0.337378	0.351355	0.427584	0.284544
AICAR	0.126201	0.125124	0.132756	0.223528	0.210611	0.243252
GMP	0.088299	0.09199	0.125823	0.122402	0.166652	0.080409
IMP	0.052649	0.064323	0.062451	0.082421	0.084012	0.086397
DHAP	2.240666	2.733841	2.596046	4.14188	5.174906	5.083663
TMP	0.164723	0.127206	0.157776	0.197575	0.17669	0.192771
AMP	0.847916	0.87703	1.112971	1.112288	2.000808	0.940613

Pantothenate	0.026509	0.023808	0.027788	0.302301	0.302048	0.345557
Nicotinate	0.053194	0.047007	0.056386	0.057667	0.054691	0.053059
Succinate	0.869114	0.731885	0.798384	0.471794	0.510606	0.385084
Malate	1.221566	1.141971	1.373237	1.734686	1.986808	1.662247
UDP-Glc	2.065808	1.930819	2.023611	3.500157	3.398332	3.838185
XMP	0.024917	0.032207	0.029272	0.015733	0.019882	0.019424
CDP	0.147474	0.129107	0.159531	0.151969	0.207305	0.131024
Acetyl-P	0.100199	0.182934	0.126369	0.141259	0.064364	0.038319
ADP-Glc	0.008999	0.008919	0.008691	0.029148	0.022721	0.015708
GDP	0.099026	0.084387	0.10115	0.214735	0.256511	0.207023
6PGA	0.538831	0.632335	0.560526	0.909202	0.935955	0.971294
2PGA+3PGA	0.968114	1.156164	0.964917	1.842556	1.831351	1.986348
NADP	0.252748	0.275205	0.276938	0.342415	0.362266	0.372036
ADP	0.099633	0.099713	0.103874	0.241188	0.325915	0.206756
SBP	0.133009	0.137151	0.134011	0.188249	0.202839	0.259061
FBP	5.71904	5.861737	5.505976	3.59836	3.75076	4.819577
PEP	0.679177	0.677917	0.899807	0.805611	1.308833	0.818204
Iso-/citrate	4.2306	4.648352	2.9252	9.715756	8.822293	9.079553
2-Isopropylmalate	0.76358	0.737427	0.734253	0.842359	0.91805	0.92632
GTP	0.486168	0.489364	0.405974	1.265197	1.222387	1.336522
CTP	0.420632	0.397884	0.36163	0.596392	0.577656	0.52436
UTP	2.461343	2.437211	2.175919	1.764168	1.855995	1.454997
ATP	0.840911	0.805222	0.662249	2.127732	1.863682	2.002572
FAD	0.051862	0.038811	0.041675	0.038248	0.031503	0.028898
PRPP	0.25919	0.256899	0.264702	0.627935	0.549981	0.864633
CoA	1.437538	1.301405	1.239883	1.911884	2.316226	1.858471
3HB-CoA	0.007985	0.007177	0.011098	0.035834	0.030752	0.042835
IPP,DMAPP	0.032551	0.034553	0.031134	0.07052	0.078928	0.068909
Malonyl-CoA	0.008486	0.004875	0.010104	0.033497	0.027845	0.044771
Acetyl-CoA	1.476448	1.456737	1.445948	1.790139	1.558383	1.876677
Butanoyl-CoA	1.559542	1.530566	1.525437	0.2649	0.245996	0.333833

Table. S2 Optimized Multiple Reaction Monitoring (MRM) parameters for 124 metabolites targeted using IP-LC/QqQ-MS. *m/z* (1): Precursor ion *m/z*; *m/z* (2): Product ion *m/z*; Ret. Time: Retention Time

Metabolite	***m/z* (1)**	***m/z* (2)**	**Ret. Time**	**Target Q1 Pre Bias (V)**	**Target Collision Energy (V)**	**Target Q3 Pre Bias (V)**
Arginine	173.05	131.05	1.272	13	15	25
Lysine	145.1	97.05	1.275	10	13	18
Histidine	154.05	93	1.277	12	21	16
4-Aminobutanoate	162.05	102	1.465	11	8	18
Serine	104.05	74.1	1.653	12	16	13
Asparagine	131.05	113.05	1.659	10	15	21
Glutamine	145.1	127.05	1.691	12	18	18

Threonine	118.05	74.05	1.702	21	16	26
Hydroxyproline	190.05	130.05	1.709	13	10	23
Hexose	179.05	89	1.743	13	19	15
2-Aminobutanoate	162.05	102	1.817	11	8	18
Cysteine	239.05	120.1	1.825	11	13	21
Trehalose	341.05	89.1	1.885	27	23	16
Proline	174.05	114	1.898	11	10	20
Sucrose	341.05	89.1	2.074	27	23	16
Valine	176.05	116.05	2.201	12	10	20
Cytidine	302.05	242	2.337	24	10	19
Pyridoxamine-5P	247.05	230	2.453	17	11	23
Methionine	148.05	47.05	2.767	11	14	16
Guanine	150.05	133.05	3.126	11	21	23
Hypoxanthine	135.05	92	3.546	13	28	14
Tyrosine	180.05	163.05	3.554	12	18	18
Adenine	134.1	107.05	3.589	14	22	19
Isoleucine	190.05	130.05	3.638	13	10	23
Leucine	190.05	130.05	3.963	13	10	23
Xanthine	151.05	108.05	3.978	10	18	18
Glutamate	146.05	102.05	4.284	11	15	18
Uridine	243.05	110.05	4.325	19	17	20
Aspartate	132.05	88.05	4.426	10	14	15
Inosine	267.05	135.05	4.566	21	23	25
Thymine	125.05	42	4.575	10	18	14
Guanosine	282.1	150.05	4.605	23	21	29
Urate	167.1	124.05	4.699	11	15	21
Shimikate	173.05	93	4.854	11	17	16
Adenosine	266.1	134.05	4.975	18	25	23
Glycerate	105.05	75.05	5.097	12	13	26
Thymidine	301.1	241	5.151	23	10	18
Phenylalanine	164.05	147.05	5.208	13	18	27
Glycolate	75.05	47.05	5.335	16	13	16
Glyoxylate	73	73	5.754	15	5	15
G6P	259.05	97	6.303	20	17	17
Disaccharide-P	421.1	79.05	6.372	29	40	27
Mn6P	259.05	97	6.535	18	17	17
Sor6P	261.05	97	6.593	19	23	16
Pyroglutamate	188.05	128	6.618	22	12	20
Tryptophan	203.1	116.05	6.675	13	18	19
R5P	229.05	97	6.691	18	13	18
SSA	101.05	57	6.697	20	13	19
Lactate	89.05	43	6.779	20	14	15
S7P	289.1	97	6.803	20	21	16
F6P	259.05	97	6.823	20	17	17
Ara5P	229.05	97	6.982	16	13	17

G1P	259.05	79.05	7.062	20	28	27
a-GP	171.05	79.05	7.063	13	18	13
TPP	424.1	302.05	7.103	30	16	20
NAD	662.1	540.1	7.188	26	18	26
GAP	169.05	97	7.288	13	12	17
Orotate	155.05	111.05	7.301	12	14	20
Ru5P	229.05	97	7.459	18	13	18
CMP	322.1	79.05	7.488	25	28	14
b-GP	171.05	79.05	7.597	13	18	13
MEP	215.05	79.05	7.623	24	27	29
F1P	259.05	97	7.651	20	17	17
Pyruvate	87.05	43	7.782	10	11	14
R1P	229.05	79.05	7.812	16	25	27
UMP	323.1	79.05	7.951	26	36	13
AICAR	337.1	79.05	8.001	12	37	26
GMP	362.1	79.05	8.031	27	26	13
IMP	347.05	79.05	8.049	25	40	28
DHAP	169.05	97	8.085	13	12	17
TMP	321.1	195.05	8.572	25	20	22
AMP	346.1	79.05	8.618	14	38	13
Pantothenate	218.05	88	8.814	17	17	15
Nicotinate	122.05	78	8.818	13	16	13
cAMP	328.1	134.05	9.143	26	27	25
Succinate	117.05	73	9.568	13	15	12
Carbamoyl-P	140.05	79.05	9.569	10	22	26
Glutathione	306.05	143.05	9.571	16	20	26
Malate	133.05	115	9.848	10	17	21
UDP-Glc	565.05	323.05	9.878	22	27	15
XMP	363.1	211.05	9.961	27	20	21
CDP	402.1	79.05	10.021	16	42	14
Acetyl-P	139	79.05	10.045	27	14	30
2OG	145.1	101.05	10.046	15	10	17
ADP-Glc	588.05	346.05	10.069	24	23	24
Fumarate	115.05	71	10.128	13	10	12
GDP	442.1	79.05	10.131	18	45	13
6PGA	275.05	177.05	10.154	19	16	30
UDP	403.1	159	10.176	16	28	29
3PGA	185.05	97	10.181	14	16	17
KDPG	257.05	97	10.215	10	18	17
Shikimate-3P	253.05	97	10.224	18	13	17
NADH	664.1	79.05	10.238	24	57	13
NADP	742.1	620.1	10.329	26	18	30
ADP	426.1	79.05	10.353	17	46	13
SBP	369.1	97	10.374	14	27	17
Citrate	191.05	87	10.399	13	18	14

FBP	339.05	97	10.423	26	18	17
PEP	167.05	79.05	10.477	17	15	27
RuBP	309.05	97	10.482	24	18	17
HMBPP	261.05	79.05	10.518	19	23	28
Isocitrate	191.05	73	10.568	13	22	26
FMN	455.1	97	10.593	18	27	17
2-Isopropylmalate	175.05	115.05	10.603	13	18	21
GTP	522.1	159	10.706	20	33	29
CTP	482.1	159	10.729	19	36	29
UTP	483.1	159	10.759	19	36	29
ATP	506.1	159	10.801	20	40	29
BPG	265.05	167.05	10.845	20	18	29
Indol-Ac	174.05	130.05	10.848	11	13	24
FAD	784.1	346.1	10.863	20	37	23
PRPP	389.1	177.05	10.948	28	21	30
NADPH	744.1	159.05	10.976	26	49	30
PQQ	329.1	241.05	11.051	12	15	24
(+)-10-Camphorsulfonic acid	231.1	80	11.055	10	32	30
CoA	766.1	408.1	11.105	20	30	27
3HB-CoA	852.1	772.1	11.109	32	41	34
IPP, DMAPP	245.05	79.05	11.123	17	27	27
Malonyl-CoA	852.1	808.1	11.125	26	27	40
Acetyl-CoA	808.1	408.1	11.147	20	37	28
HMG-CoA	910.1	408.1	11.148	34	48	26
Succinyl-CoA	866.1	408.1	11.149	30	44	25
Crotonyl-CoA	834.1	408.1	11.299	30	36	26
Butanoyl-CoA	836.1	408.1	11.373	31	37	26