

#### Supplementary table 1

Comparison of relative abundances of metabolites detected by GC/MS (white) and GCxGC-TOFMS (grey) of the WT and mutant *Stm6Glc4* before and during peak H<sub>2</sub> production. Relative masses used for detection and/or identification of each compound are indicated.

Different ratios of O<sub>2</sub> and H<sub>2</sub> peaks for adenosine and alanine were obtained by using the two different techniques.

#### Supplementary table 2

Statistical analyses (student's s t-test) for selected metabolites presented in the figures 2-7. Time points, which were used for student's s t-test are listed. Significance is shown by p-values, where p < 0.05 indicates statistically significance (\*), p < 0.01 indicates high significance (\*\*), and p < 0.001 indicated very high significance (\*\*\*) of the data.

#### Supplementary table 3-6

Compounds of two different sample groups with Fisher ratios higher than 1000, as determined with the '*statistical compare*' feature of the LECO ChromaTOF® software v.4.22.

compound	relative masses	WT Peak O <sub>2</sub>	WT Peak H <sub>2</sub>	<i>Stm6Glc4</i> Peak O <sub>2</sub>	<i>Stm6Glc4</i> Peak H <sub>2</sub>
adenine	264	0.99	0.54	1.22	0.77
adenosine	236	183.44	38.67	146.34	23.35
adenosine	236	0.25	0.22	0.29	0.30
alanine	116	11553.76	6875.92	8004.28	22256.66
alanine	116	4.43	4.29	3.99	7.66
alpha-tocopherol	502	1.15	2.20	0.98	0.88
asparagine	115	85.26	8.99	15.83	6.43
aspartate	232	274.59	605.31	494.55	672.47
aspartate	232	0.50	0.92	0.33	0.78
benzoate	119	35.18	29.59	43.52	38.59
beta-alanine	248	0.00	18.57	0.00	27.98
beta-alanine	248	0.20	0.33	0.08	0.28
calystegine	216	2.79	5.32	2.54	3.09
cholesterol	377	6.72	6.19	5.52	2.95
citrate	257	0.22	0.25	0.08	0.10
citrulline	142	36.15	25.52	21.27	28.35
cysteine	220	36.18	n.d.	31.55	n.d.
cysteine	220	0.30	0.35	0.32	0.21
dehydroergosterol	251	254.21	185.70	354.95	222.51
dihydroxyacetone-p	400	0.01	0.04	0.01	0.01
disulfide	172	89.05	n.d.	185.06	n.d.
ergosterol	363	10.55	9.38	8.75	4.39
ethanolamine	174	10.62	n.d.	25.49	n.d.
ethanolamine	174	2.37	2.40	1.63	0.85
ethanolaminophosphate	174	5668.06	455.30	6559.70	995.37
fructose-6-p	315	1.35	4.02	1.96	3.84
fumarate	245	1.35	0.37	0.54	0.36
galactose	319	4.97	7.80	7.82	18.72
galactose	319	1.95	2.42	2.03	4.62
galactosylglycerol	204	3.48	4.03	38.34	24.47
glucose	319	20061.46	21860.72	24253.16	45148.46
glucose	319	0.58	3.53	1.30	5.76
glucose-6-p	387	2.32	6.24	2.35	5.13
glucuronolactone	73	486.04	140.63	542.46	0.00
glutamate	246	5834.15	2196.91	4048.32	4097.35
glutamate	246	13.30	4.76	3.65	4.88
glycerate	189	0.38	0.45	2.06	0.98
glycerate-2-p	315	0.85	1.13	0.64	0.73
glycerol-2-p	243	0.93	1.40	0.55	1.27
glycerol-3-p	357	28.84	38.64	15.80	18.54
glycine	174	81.39	27.99	7.50	16.37
glycine	174	1.73	1.38	1.38	1.66
guanine	352	1.57	7.48	2.44	3.95
guanine	352	n.d.	3775.99	n.d.	3332.08
guanosine	324	27.84	44.12	35.73	46.32

compound	relative masses	WT Peak O <sub>2</sub>	WT Peak H <sub>2</sub>	<i>Stm6Glc4</i> Peak O <sub>2</sub>	<i>Stm6Glc4</i> Peak H <sub>2</sub>
homocysteine	234	7.91	11.89	4.22	3.06
homoserine	218	47.74	n.d.	n.d.	n.d.
homoserine	218	0.55	0.17	0.26	0.17
inositol	305	0.75	0.68	1.39	1.74
inositol-2-p	318	6.61	8.39	5.77	7.57
isoleucine	158	266.02	699.41	309.31	1785.97
isoleucine	158	0.89	2.17	0.48	4.03
leucine	158	269.22	598.51	424.16	1296.76
linoleic acid	337	14.60	62.50	12.93	34.04
linolenic acid	79	7.88	37.46	12.96	21.76
lycopene	119	37.20	9.26	33.81	11.06
lysine	156	2.33	2.81	1.13	3.97
malate	245	7527.13	2210.30	5867.42	3235.13
malate	245	2.29	0.60	1.23	0.67
methionine	176	87.12	11.65	97.18	17.70
oleic acid	339	28.68	113.44	59.72	71.75
ornithine	174	n.d.	n.d.	0.00	17.61
oxalate	147	15.96	n.d.	33.71	n.d.
p-enolpyruvate	369	0.05	0.06	0.01	0.02
palmitelaidic acid	311	2.01	17.79	12.75	22.05
palmitic acid	129	378.90	455.33	73.04	151.62
palmitic acid	129	22.62	118.79	30.18	68.07
pantothenate	237	81.53	0.00	96.26	55.10
phenylalanine	192	781.05	1426.69	933.08	5402.33
phenylalanine	192	0.46	0.98	0.21	1.42
phenylethanolamine	174	25.88	n.d.	24.19	n.d.
phytol	143	9.57	3.03	5.27	1.54
phytol	143	1.96	n.d.	5.52	n.d.
proline	142	0.67	1.06	0.63	1.86
purin-2-amine	324	459.58	332.51	237.01	620.81
putrescine	174	15.24	1.37	2.47	0.56
pyrimidine	255	366.08	213.48	376.96	173.82
pyroglutamate	156	3.76	0.77	2.22	1.61
ribonate	217	36.81	36.17	77.03	14.48
ribose	315	46.90	10.86	45.46	10.72
serine	204	3.12	3.20	5.13	4.13
stearic acid	110	249.46	380.17	507.00	1316.12
stearic acid	110	13.60	42.50	17.23	25.19
succinate	247	85.39	8.36	96.14	18.32
succinate	247	1.24	0.42	0.73	0.40
threonine	101	477.07	579.79	575.84	2861.10
threonine	101	1.00	1.10	1.28	2.63
tyrosine	218	0.70	2.41	0.48	2.21
uracil	241	627.00	218.83	435.34	393.39
uridine	217	1718.63	166.25	3708.69	1097.95
valine	144	70.19	90.02	988.96	4732.28
valine	144	1.30	2.53	0.64	3.20

		cysteine	serine	glutamate	threonine	aspartate	lysine	tyrosine
WT	time points	a & e	a & b	a & b	a & e	a & e	a & e	a & e
	p value	0.694	0.007824**	0.4816	0.2719	0.07105	0.2498	0.04441*
<i>Stm6Glc4</i>	time points	a & e	a & b	a & b	a & e	a & e	a & e	a & e
	p value	0.3435	0.000681***	0.0008552***	0.0003947***	2.969e-05***	0.001323**	7.423e-05***
		glycine	$\beta$ -alanine	alanine	proline	valine	phenylalanine	isoleucine
WT	time points	a & b	a & e	a & b; a & e	a & e	a & e	a & e	a & e
	p value	0.07865	0.00579**	0.2513; 0.5578	0.066	0.06054	0.04964*	0.05531
<i>Stm6Glc4</i>	time points	a & b	a & e	a & b; a & e	a & e	a & e	a & e	a & e
	p value	0.01093*	0.01772*	0.003019**; 0.002931**	0.01085*	9.455e-05***	7.49e-05***	0.00002872***
		glucose	glucose-6-p	fructose-6-p	2-p-glycerate	glycerate	citrate	succinate
WT	time points	a & e	a & e	a & e	a & e	a & b	a & b; b & e	a & b; b & e
	p value	0.005112**	0.0778	0.03377*	0.06996	0.246	0.08363; 0.09743	0.6839; 0.004021**
<i>Stm6Glc4</i>	time points	a & e	a & e	a & e	a & e	a & b	a & b; b & e	a & b; b & e
	p value	0.05234	0.02132*	0.01195*	0.9227	0.004842**	0.002696**; 0.00399**	0.0001878***; 3.645e-07***
		fumarate	malate	myristic acid	palmitic acid	palmitoleic acid	stearic acid	oleic acid
WT	time points	a & b; b & e	a & b; b & e	a & e	a & e	a & e	a & e	a & e
	p value	0.2129; 0.0008277***	0.5796; 0.01881*	6.785e-05***	0.001646**	9.317e-09***	0.001388**	0.001598**
<i>Stm6Glc4</i>	time points	a & b; b & e	a & b; b & e	a & e	a & e	a & e	a & e	a & e
	p value	4.452e-05***; 3.972e-06***	0.0001718***; 7.193e-06***	0.004134**	0.008143**	0.08523	0.05955	0.5679
		linoleic acid	linolenic acid	lipids	formate	ethanol	acetate	starch
WT	time points	a & e	a & e	a & e	a & e	a & e	a & c; c & e	a & c; c & e
	p value	0.001061**	6.416e-05***	0.002263***	0.0006122***	0.04579*	2.195e-07***; 0.1856	0.0977; 0.01400*
<i>Stm6Glc4</i>	time points	a & e	a & e	a & e	a & e	a & e	a & c; c & e	a & c; c & e
	p value	0.005336**	0.1141	0.0002314***	0.00192**	0.006015**	0.002488**; 0.1912	0.01860**; 0.1636

<b>compounds WT Peak O<sub>2</sub> vs. Peak H<sub>2</sub></b>				
<b>compound</b>	<b>retention time (1st dim)</b>	<b>relative mass</b>	<b>average area</b>	<b>Fisher ratio</b>
glucose	3461.67	73	48254418.00	8245.2
succinate	1634.17	247	70300.89	7537.1
malate	1660.00	147	7604718.96	4272.00
alpha tocopherol	4455.00	237	2014090.54	3483.2
panthothenate	2671.67	103	105694743.00	3320.7
adenosine	4110.00	236	172880241.00	2276.7
5-oxo-proline	1725.83	156	15048263.00	2237.2
valine	1644.17	144	5398967.86	2189.3
serine	1234.17	116	1007140.24	1548.7
isoleucine	1300.00	158	1619566.89	1510.1
ethanolaminophosphate	2216.67	73	8475081.28	1381.1
uridine	3651.67	217	1631691.92	1195.1
uracil	1389.17	99	661900902.00	1144.9
alanine	937.5	116	14526345.7	1069.4

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<b>compounds <i>Stm6Glc4</i></b>				
<b>Peak O<sub>2</sub> vs. Peak H<sub>2</sub></b>				
<b>compound</b>	<b>retention time (1st dim)</b>	<b>relative mass</b>	<b>average area</b>	<b>Fisher ratio</b>
valine	1644.17	144	4403594.76	8224.60
isoleucine	1300.00	158	3236383.42	5981.80
succinate	1635.00	247	69761.59	5820.30
guanine	3000.83	73	6080253.20	5327.10
alpha tocopherol	4450.00	237	3867248.50	3688.40
ethanolaminephosphate	2217.50	174	1083760.34	2582.10
phenylalanine	1779.17	120	4112024.10	2133.00
alanine	936.67	116	19298620.50	1974.50
adenosine	4110.00	236	116556.40	1747.80
oleic acid	3707.50	98	161770.42	1408.40
lycopene	4540.00	73	682505.30	1324.90
proline	930.83	142	161294.53	1294.40

<b>compounds WT vs. <i>Stm6Glc4</i> Peak O<sub>2</sub></b>				
<b>compound</b>	<b>retention time (1st dim)</b>	<b>relative mass</b>	<b>average area</b>	<b>Fisher ratio</b>
malate	1660.00	147	9866932.90	3978.30
alpha tocopherol	4450.00	237	359363.61	2954.10
isoleucine	1300.00	158	736315.93	1963.80
5-oxo-proline	1727.50	156	18913489.20	1742.20
succinate	1635.00	247	132462.82	1555.30
valine	1644.17	144	3501257.66	1507.30
uridine	3651.67	73	3897341.15	1424.90
panthothenate	2672.50	103	156717.31	1372.00
linoleic acid	1742.50	110	228237.06	1129.30
glutarate	1731.00	129	158108.07	1117.00

compounds WT vs. <i>Stm6Glc4</i> Peak H <sub>2</sub>				
compound	retention time (1st dim)	relative mass	average area	Fisher ratio
alpha tocopherol	4455.00	237	3042447.11	22365.00
palmitelaidic acid	2691.67	117	6763813.94	18932.00
mannose	2484.17	205	2878628.60	10628.00
ethanolaminephosphate	2217.50	174	1011275.86	5864.30
serine	1235.00	116	1266010.33	4538.50
linoleic acid	3337.50	67	157069.45	3752.80
pantothenate	2672.00	103	81658.07	3346.10
myo-inositol	2917.50	73	7800011.07	2997.70
stearic acid	4515.00	55	19901.76	2695.30
adenosine	4111.67	236	46649.33	2517.60
uracil	1389.17	99	431532.19	1990.20
uridine	3652.50	73	1012846.84	1873.10
alanine	935.83	116	19905225.40	1851.90
phenylalanine	1775.83	120	4633075.55	1715.10
oleic acid	3707.50	103	887364.68	1686.50
glucose	3462.50	73	61764171.10	1621.90
glyoxylate	866.67	100	125028.53	1522.10
malate	1660.00	147	3885276.12	1334.40
isoleucine	1300.00	158	3702956.46	1291.10
proline	1825.83	142	238568.14	1187.00
palmitic acid	3067.50	56	533773.78	1039.60
5-oxo-proline	1725.83	156	13872675.10	1008.00