

# Identification of novel lipid modifications and inter-membrane dynamics in *Corynebacterium glutamicum* using high resolution mass spectrometry

Stephan Klatt, Rajini Brammananth, Sean O'Callaghan, Konstantinos Andreas Kouremenos,  
Dedreia Tull, Paul K. Crellin, Ross L. Coppel, Malcolm J. McConville

## **Supplementary Data**

### **Supplementary Table 1**

Established lipid libraries (1-37) and libraries from LipidBlast (38-44) for lipidome analysis of *C. glutamicum*.

	<b>Lipid (Sub)Class</b>	<b>Libraries (MS-DIAL)</b>	<b>Number of Spectra</b>	<b>Origin of Library</b>
1	hTMCM	hTMCM+NH4pos	115	this study
2	ketoTMCM	AcTMCM+NH4pos	115	this study
3	AcTMCM	hTMCM+NH4pos	115	this study
4	Acyl(16:0)-hTMCM	Acyl160hTMCM+NH4pos	115	this study
5	Acyl(18:1)-hTMCM	Acyl181hTMCM+NH4pos	115	this study
6	Acyl(16:0)-AcTMCM	Acyl160AcTMCM+NH4pos	115	this study
7	h2TDCM	hTDCM+NH4pos	13225	this study
8	Ac1-hTDCM	Ac1hTDCM+NH4pos	13225	this study
9	hGMM	hGMM+NH4pos	115	this study
10	AcGMM	AcGMM+NH4pos	115	this study
11	Acyl(16:0)hGMM	Acyl160hGMM+NH4pos	115	this study
12	hGroMM	hGroMM+NH4pos	115	this study
13	DAG	DAG+NH4pos	3025	this study
14	Ala-DAG	AlaDAG+Hpos	3025	this study
15	Lys-DAG	LysDAG+Hpos	3025	this study
16	CDP-DAG	CDPDAG+Hpos	3025	this study
17	PG	PG+NH4pos	3025	this study
18	PGP	PGP+NH4pos	3025	this study
19	Ala-PG	AlaPG+Hpos	3025	this study
20	Lys-PG	LysPG+Hpos	3025	this study
21	Acyl-PG	Acyl181PGtest2-Hneg	3025	this study
22	PG-like	PGlike+NH4pos (+H)	3025	this study
23	PI	PI+NH4pos/PI+Hpos	3025	this study
24	PIP	PIP+NH4pos	3025	this study
25	PIM1	PIM1+NH4pos	3025	this study
26	PIM2	PIM2+NH4pos	3025	this study
27	PIM3	PIM3+NH4pos	3025	this study
28	PIM4	PIM4+NH4pos	3025	this study
29	AcPIM2	AcPIM2+NH4pos	540	this study
30	AcPIM3	AcPIM3+NH4pos	540	this study
31	AcPIM4	AcPIM4+NH4pos	540	this study
32	diAcPIM2	diAcPIM2+NH4pos	540	this study
33	GI-A	GI-A+NH4pos	3025	this study
34	GI-X	GI-X+NH4pos	3025	this study
35	GI-Y	GI-Y+NH4pos	3025	this study
36	GI-Z	GI-Z+NH4pos	3025	this study
37	CL	CLownLib+Hpos	665500	this study
38	MAG	MAG+NH4pos	80	LipidBlast
39	TAG	TAG+NH4pos	2304	LipidBlast
40	PA	PA+NH4pos	5476	LipidBlast
41	PC	PC+Hpos	5476	LipidBlast
42	PE	PE+Hpos	5746	LipidBlast
43	PS	PS+Hpos	5123	LipidBlast
44	SM	SM+Hpos	168	LipidBlast

**Supplementary Table 2**

Summary of LC-MS/MS ESI TOF (positive ionisation mode) identified hTMCM, ketoTMCM, AcTMCM and hGMM glycolipid (sub)classes in *C. glutamicum* wildtype (TL, IM, OM), TmaT KO mutant (TL, IM, OM) and PBQC (TL, IM, OM).

The mycolic acids are sorted according to their size (Da) and degree of saturation. Saturated, mono-, di- and tri-unsaturated mycolic acid chains were identified, with decreasing absolute numbers for increasing saturation levels. **(A)** 33 hTMCM species were identified within the size range of 726.5 Da (MA<sub>24:0</sub>) to 916.7 Da (MA<sub>38:3</sub>). 30% more species were detected in the IM of the TmaT KO mutant, compared to the IM of the WT, whereas 25% less species were detected when comparing the OMs. They are equally distributed in the WT, and highly increased in the IM of the KO. **(B)** A total of 11 ketoTMCM species were identified, with a higher number represented in both the IM and OM of the TmaT KO mutant. In addition, no ketoTMCM species were identified in the OM of the WT, and a highly-increased number in the IM of the KO compared to its OM and the IM of the WT. **(C)** For AcTMCM species (8 species), more species were identified in both the IM and OM of the WT. They are completely absent in the KO. **(D)** hGMM species possess only one glucose unit compared to hTMCM. Here, 12 species were identified which are equally represented in the IM and OM of the KO. For the WT, 55% more species were detected in the OM. However, hGMM species are highly increased in the IM of the KO compared to the IM of the WT. Isomeric lipid species are annotated in brackets as numbers.

A	trehalose monohydroxycorynomycolate (hTMCM)										Total (33):
	hTMCM species		WT		TmaT KO		PBQC				
	Mass (NH4+)	TL	IM	TL	IM	OM	TL	IM	OM		
1	hTMCM (24:0)	726.499	yes								
2	hTMCM (25:0)	740.515	no								
3	hTMCM (26:0)	754.5312	yes								
4	hTMCM (28:0)	782.562	yes								
5	hTMCM (30:0)	810.593	yes								
6	hTMCM (31:0)	824.6095	no	no	yes	yes	yes	yes	yes		
7	hTMCM (32:0)	838.627	yes								
8	hTMCM (33:0)	852.64	yes								
9	hTMCM (34:0)	866.6583	yes								
10	hTMCM (35:0)	880.6716	no	no	no	yes	yes	yes	yes		
11	hTMCM (36:0)	894.688	yes								
12	hTMCM (24:1)	724.485	yes	no	no	no	no	no	no		
13	hTMCM (26:1)	752.5159	yes								
14	hTMCM (27:1)	766.5327	no								
15	hTMCM (28:1)	780.546	yes								
16	hTMCM (30:1)	808.5798	no	yes	yes	no	no	yes	yes		
17	hTMCM (32:1)	836.6095	yes								
18	hTMCM (34:1)	864.6433	yes								
19	hTMCM (35:1)	878.6559	no	no	no	yes	yes	yes	yes		
20	hTMCM (36:1)	892.673	yes								
21	hTMCM (38:1)	920.7036	no	no	no	yes	yes	yes	yes		
22	hTMCM (26:2)	750.504	(yes)	no	no	yes	no	no	no		
23	hTMCM (28:2)	778.5307	yes								
24	hTMCM (30:2)	806.5723	no	no	yes	yes	yes	yes	yes		
25	hTMCM (32:2)	834.5939	no	no	yes	yes	yes	yes	yes		
26	hTMCM (34:2)	862.6259	yes								
27	hTMCM (36:2)	890.658	yes								
28	hTMCM (38:2)	918.6877	no	no	no	yes	yes	yes	yes		
29	hTMCM (30:3)	804.5468	no	no	yes	yes	yes	yes	yes		
30	hTMCM (32:3)	832.5805	no	no	yes	yes	yes	yes	yes		
31	hTMCM (34:3)	860.6093	no	no	yes	yes	yes	yes	yes		
32	hTMCM (36:3)	888.6413	yes								
33	hTMCM (38:3)	916.6728	no	no	no	yes	yes	yes	yes		
			19	18	20	26	26	15	26	22	19
<b>Total (33):</b>			<b>19</b>	<b>18</b>	<b>20</b>	<b>26</b>	<b>26</b>	<b>15</b>	<b>26</b>	<b>22</b>	<b>19</b>

  

B	trehalose monoketocorynomycolate (ketoTMCM)										Total (11):
	ketoTMCM species		WT		TmaT KO		PBQC				
	Mass (NH4+)	TL	IM	TL	IM	OM	TL	IM	OM		
1	ketoTMCM (24:0)	724.4845	no	no	no	yes	yes	no	yes	no	
2	ketoTMCM (26:0)	752.5158	no	no	no	yes	yes	no	yes	no	
3	ketoTMCM (32:0)	836.6088	(yes)	yes	no	yes	yes	yes	yes	yes	
4	ketoTMCM (34:0)	864.6409	no	no	no	yes	yes	yes	yes	no	
5	ketoTMCM (28:1)	750.501	no	no	no	yes	yes	no	yes	no	
6	ketoTMCM (28:1)	778.5308	yes	no	no	yes	yes	no	yes	yes	
7	ketoTMCM (34:1)	862.6244	yes	yes	no	yes	yes	no	yes	yes	
8	ketoTMCM (28:2)	776.5169	no	no	no	(yes)	no	no	(yes)	no	
9	ketoTMCM (34:2)	860.6108	no	no	no	yes	no	no	yes	no	
10	ketoTMCM (36:2)	888.6403	yes	yes	no	yes	yes	yes	yes	yes	
11	ketoTMCM (36:3)	886.6256	no	no	no	(yes)	yes	no	(yes)	yes	
			4	3	0	10	10	3	9	7	2
<b>Total (11):</b>			<b>4</b>	<b>3</b>	<b>0</b>	<b>10</b>	<b>10</b>	<b>3</b>	<b>9</b>	<b>7</b>	<b>2</b>

  

C	trehalose monoacetylcorynomycolate (AcTMCM)										Total (8):
	AcTMCM species		WT		TmaT KO		PBQC				
	Mass (NH4+)	TL	IM	TL	IM	OM	TL	IM	OM		
1	AcTMCM (28:0)	824.5728	no	no	yes	no	no	no	yes	yes	
2	AcTMCM (30:0)	852.6056	no	no	yes	no	no	no	no	no	
3	AcTMCM (32:0)	880.6368	yes								
4	AcTMCM (34:0)	908.6689	yes								
5	AcTMCM (32:1)	878.6223	no	no	yes	no	no	no	yes	yes	
6	AcTMCM (34:1)	906.6536	yes								
7	AcTMCM (34:2)	904.6373	yes								
8	AcTMCM (36:2)	932.668	no	no	yes	no	no	no	no	yes	
			4	3	8	0	0	0	6	5	7
<b>Total (8):</b>			<b>4</b>	<b>3</b>	<b>8</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>6</b>	<b>5</b>	<b>7</b>

  

D	glucose monohydroxycorynomycolate (hGMM)										Total (12):
	hGMM species		WT		TmaT KO		PBQC				
	Mass (NH4+)	TL	IM	TL	IM	OM	TL	IM	OM		
1	hGMM (24:0)	564.4478	yes	no	yes	yes	no	yes	no	yes	
2	hGMM (26:0)	592.4853	yes	yes	yes	yes	no	yes	yes	yes	
3	hGMM (28:0)	620.5108	no	no	yes	no	no	no	no	no	
4	hGMM (30:0)	648.5416	no	no	yes	no	no	yes	no	no	
5	hGMM (32:0)	676.5734	yes	yes	yes	yes	yes	yes	yes	yes	
6	hGMM (34:0)	704.6046	yes	yes	yes	yes	yes	yes	yes	yes	
7	hGMM (36:0)	732.6359	yes	no	yes	no	no	yes	no	no	
8	hGMM (28:1)	618.4972	yes	yes	no	yes	no	yes	no	yes	
9	hGMM (32:1)	674.5562	yes	yes	no	yes	no	yes	no	yes	
10	hGMM (34:1)	702.5885	yes	yes	yes	yes	yes	yes	yes	yes	
11	hGMM (36:1)	730.6204	no	no	yes	yes	yes	yes	no	yes	
12	hGMM (36:2)	728.6052	yes	no	yes	yes	yes	yes	yes	yes	
			9	5	11	6	8	7	8	7	7
<b>Total (12):</b>			<b>9</b>	<b>5</b>	<b>11</b>	<b>6</b>	<b>8</b>	<b>7</b>	<b>8</b>	<b>7</b>	<b>7</b>

**Supplementary Table 3:**

Summary of LC-MS/MS ESI TOF (pos. mode) identified h2TDCM glycolipid species (cord factor) in *C. glutamicum* wildtype (TL, IM, OM), TmaT KO mutant (TL, IM, OM) and PBQC (TL, IM, OM).

Altogether, 43 h2TDCM species were identified within the size range of 1174.9 Da (MA<sub>26:0</sub> + MA<sub>28:1</sub>) to 1427.2 Da (MA<sub>36:0</sub> + MA<sub>36:1</sub>) containing 21 different mycolic acid (MA) moieties. h2TDCM species carry two MA moieties. The five most identified mycolic acid moieties were MA<sub>32:0</sub> (15x), MA<sub>34:1</sub> (10x), MA<sub>34:0</sub> (8x), MA<sub>36:2</sub> (6x) and MA<sub>36:1</sub>/MA<sub>26:0</sub> (5x each). For 10 h2TDCM species, the two MA moieties could not be clearly identified. Moreover, three isobaric h2TDCMs were identified. In the IM and OM of the WT, h2TDCM species were equally present, whereas there were more species identified in the IM of the TmaT KO compared to its OM. Isomeric lipid species are annotated in brackets as numbers.

	h2TDCM species	1st MA	2nd MA	Mass (NH4+)	trehalose di-hydroxycorynomycolate (h2TDCM)								
					WT			TmaT KO			PBQC		
					TL	IM	OM	TL	IM	OM	TL	IM	OM
1	h2TDCM	(26:0)	(28:1)	1174.927	yes	yes	no	no	yes	no	no	yes	no
2	h2TDCM	(26:0)	(28:0)	1176.943	yes	yes	no	no	no	no	yes	yes	no
		(22:0)	(32:0)										
3	h2TDCM	(24:1)	(32:0)	1202.96	yes	no	no	yes	yes	yes	yes	no	no
4	h2TDCM	(24:0)	(32:0)	1204.975	yes	no	no	no	no	yes	yes	no	yes
5	h2TDCM		(58:2)	1228.974	no	yes	yes	no	yes	no	no	no	yes
6	h2TDCM	(26:1)	(32:0)	1230.989	yes	yes	yes	yes	yes	yes	yes	yes	yes
7	h2TDCM	(26:0)	(32:0)	1233.006	yes	yes	yes	yes	yes	yes	yes	yes	yes
8	h2TDCM		(60:3)	1254.991	no	no	no	no	yes	no	no	no	no
9	h2TDCM	(26:1)	(34:1)	1257.006	yes	yes	yes	yes	yes	yes	yes	no	yes
10	h2TDCM	(26:0)	(34:1)	1259.022	yes	yes	yes	yes	yes	yes	yes	yes	yes
		(28:1)	(32:0)										
11	h2TDCM	(28:0)	(32:0)	1261.037	yes	yes	yes	yes	yes	yes	yes	yes	yes
		(26:0)	(34:0)										
12	h2TDCM	(28:2)	(34:1)	1283.023	no	yes	yes	yes	yes	yes	no	yes	yes
13	h2TDCM	(28:1)	(34:1)	1285.038	no	yes	no	yes	yes	no	no	no	yes
14	h2TDCM	(28:0)	(34:1)	1287.053	yes	yes	yes	yes	yes	yes	yes	yes	yes
15	h2TDCM	(30:0)	(32:0)	1289.069	yes	yes	yes	yes	yes	yes	yes	yes	yes
16	h2TDCM	(31:0)	(32:1)	1301.068	yes	no	no	no	no	no	no	yes	no
17	h2TDCM		(64:4)	1309.033	no	no	yes	no	yes	yes	no	no	no
18	h2TDCM		(64:2)	1313.069	yes	no	no	yes	no	yes	yes	no	no
19	h2TDCM	(32:0)	(32:1)	1315.083	yes	yes	yes	yes	yes	yes	yes	yes	yes
20	h2TDCM	(32:0)	(32:0)	1317.099	yes	yes	yes	yes	yes	yes	yes	yes	yes
21	h2TDCM		(65:1)	1329.099	yes	yes	yes	no	no	no	yes	yes	no
22	h2TDCM	(32:0)	(33:0)	1331.115	no	no	no	no	no	no	no	yes	no
23	h2TDCM		(66:3)	1339.083	no	yes	no	no	no	no	no	yes	no
24	h2TDCM	(32:0)	(34:2)	1341.099	yes	yes	yes	yes	yes	yes	yes	yes	yes
25	h2TDCM	(32:0)	(34:1)	1343.113	yes (2)	yes							
26	h2TDCM	(32:0)	(34:0)	1345.13	yes	yes	yes	yes	yes	yes	yes	no	yes
27	h2TDCM	(33:1)	(34:0)	1357.141	no	no	yes	no	yes	no	yes	yes	yes
28	h2TDCM		(67:0)	1359.144	no	no	no	no	no	no	no	yes	no
29	h2TDCM		(68:4)	1365.1	yes	yes	no	no	no	yes	yes	no	no
30	h2TDCM		(68:3)	1367.116	no	yes	yes	no	yes	yes	yes	no	yes
31	h2TDCM	(34:1)	(34:1)	1369.129	yes	yes	yes	yes	yes	no	yes	yes	yes
32	h2TDCM	(34:0)	(34:1)	1371.146	yes	no	yes	no	yes	yes	yes	yes	no
33	h2TDCM	(34:0)	(34:0)	1373.162	yes	yes	no	no	no	no	yes	no	no
34	h2TDCM	(34:2)	(36:2)	1393.131	no	no	yes	no	yes	no	no	yes	yes
35	h2TDCM	(34:1)	(36:2)	1395.145	yes	yes	yes	yes	yes	yes	yes	yes	yes
36	h2TDCM		(70:2)	1397.162	no	yes	yes	yes	yes	yes	no	yes	no
37	h2TDCM	(34:0)	(36:1)	1399.172	yes	no	yes	yes	yes	no	yes	yes	yes
38	h2TDCM	(34:0)	(36:0)	1401.192	yes	yes	no	no	no	no	yes	no	no
39	h2TDCM	(36:2)	(36:3)	1419.144	no	no	yes	no	yes	no	no	no	no
40	h2TDCM	(36:2)	(36:2)	1421.16	yes	yes	yes	yes	yes	yes	yes	yes	yes
41	h2TDCM	(36:1)	(36:2)	1423.176	yes	no	yes	no	no	yes	yes	yes	yes
42	h2TDCM	(36:1)	(36:1)	1425.192	yes	no	no	yes	yes	yes	no	no	no
43	h2TDCM	(36:0)	(36:1)	1427.209	yes	yes	yes	no	no	no	yes	yes	no
				<b>Total (43):</b>	<b>30</b>	<b>28</b>	<b>28</b>	<b>22</b>	<b>30</b>	<b>25</b>	<b>29</b>	<b>29</b>	<b>23</b>

**Supplementary Table 4:**

Summary of LC-MS/MS ESI TOF (pos. mode) identified Ac1-hTDCM, Acyl-hTMCM and Acyl-AcTMCM glycolipid species in *C. glutamicum* wildtype (TL, IM, OM), TmaT KO mutant (TL, IM, OM) and PBQC (TL, IM, OM).

All three mycolic acid subclasses have not been described before. **(A)** Ac1-hTDCM species are closely related to h2TDCM species with the only difference that one of the two mycolic acid moieties carries an acetyl group instead of a hydroxyl group. This new species was identified in the WT as well as in the TmaT KO mutant, and also in both the IM and OM fraction. **(B)** Acyl-hTMCM species are based on hTMCM species, but carry an additional fatty acid or fatty alcohol (16:0 or 18:1) on their terminal glucose unit of the trehalose. More species were identified in the TmaT KO mutant compared to the WT, and they also were more abundant in the IM compared to the OM. **(C)** Acyl-AcTMCM species are based on AcTMCM, but carry the same additional group on their trehalose unit as Acyl-hTMCM species. Only one species was identified in the IM of the WT. Isomeric lipid species are annotated in brackets as numbers.

**A**

	Ac1-hTDCM species	1st MA	2nd MA	Mass (NH4+)	trehalose acetyl-hydroxycorynomycolate (Ac1-hTDCM)								
					WT			TmaT KO			PBQC		
					TL	IM	OM	TL	IM	OM	TL	IM	OM
1	Ac1-hTDCM	(32:0)	(32:0)	1359.111	yes	yes	yes	yes	yes	no	yes	yes	yes
2	Ac1-hTDCM	(32:0)	(34:1)	1385.126	yes	yes	yes	yes	yes	yes	yes	yes	yes
3	Ac1-hTDCM	(32:0)	(34:0)	1387.137	yes	yes	yes	no	yes	no	yes	yes	yes
<b>Total (3):</b>					<b>3</b>	<b>3</b>	<b>3</b>	<b>2</b>	<b>3</b>	<b>1</b>	<b>3</b>	<b>3</b>	<b>3</b>

**B**

	Acyl-hTMCM species	Mass (NH4+)	acyl-trehalose monohydroxycorynomycolate (Acyl-hTMCM)								
			WT			TmaT KO			PBQC		
			TL	IM	OM	TL	IM	OM	TL	IM	OM
1	Acyl(16:0)-hTMCM (30:0)	1048.824	yes	no	no	yes	yes	no	yes	yes	no
2	Acyl(16:0)-hTMCM (32:0)	1076.853	yes (2)	yes	yes	yes (2x)	yes	yes	yes	yes	yes
3	Acyl(16:0)-hTMCM (34:0)	1104.886	yes	yes	yes (2)	yes	yes	no	yes	yes	yes
4	Acyl(16:0)-hTMCM (32:1)	1074.839	yes	yes	no	yes (2x)	yes	no	yes (2x)	yes (2)	no
5	Acyl(16:0)-hTMCM (34:1)	1102.87	yes	no	no	yes	yes (2)	no	yes	yes	no
6	Acyl(16:0)-hTMCM (36:1)	1130.903	no	no	no	no	yes	no	(yes)	no	no
7	Acyl(16:0)-hTMCM (34:2)	1100.854	yes	no	no	yes (2x)	yes (2)	no	yes (2x)	yes (2)	no
8	Acyl(16:0)-hTMCM (36:3)	1126.869	(yes)	no	no	yes (2x)	(yes 2)	no	(yes)	yes	no
9	Acyl(18:1)-hTMCM (32:0)	1102.868	no	yes	yes	yes	no	yes	no	no	yes
10	Acyl(18:1)-hTMCM (34:0)	1130.901	yes	yes	yes	no	no	yes	no	yes	yes
11	Acyl(18:1)-hTMCM (34:1)	1128.887	yes	yes	yes	yes	yes	yes	yes	yes	yes
12	Acyl(18:1)-hTMCM (36:1)	1156.918	no	no	no	yes	yes	no	yes	yes	no
13	Acyl(18:1)-hTMCM (36:2)	1154.901	yes	yes	no	yes	yes	yes	yes	yes	yes
14	Acyl(18:1)-hTMCM (36:3)	1152.885	no	no	no	yes	yes	no	no	no	no
<b>Total (14):</b>			<b>10</b>	<b>7</b>	<b>5</b>	<b>12</b>	<b>12</b>	<b>5</b>	<b>11</b>	<b>11</b>	<b>6</b>

**C**

	Acyl-AcTMCM species	Mass (NH4+)	acyl-trehalose monoacetylcorynomycolate (Acyl-AcTMCM)								
			WT			TmaT KO			PBQC		
			TL	IM	OM	TL	IM	OM	TL	IM	OM
1	Acyl(16:0)-AcTMCM (32:0)	1118.8646	yes	yes	no	no	no	no	yes	yes	no
<b>Total (1):</b>			<b>1</b>	<b>1</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>1</b>	<b>1</b>	<b>0</b>

**Supplementary Table 5**

Summary of LC-MS/MS ESI TOF (pos. mode) identified DAG, Ala-DAG, CDP-DAG, PA and TAG lipid species in *C. glutamicum* wildtype (TL, IM, OM), TmaT KO mutant (TL, IM, OM) and PBQC (TL, IM, OM).

The lipids are sorted according to their size (Da). Saturated, mono-, di- and one tri-unsaturated fatty acid chains were identified, with FA<sub>16:0</sub>, FA<sub>16:1</sub>, FA<sub>18:0</sub> and FA<sub>18:1</sub> being the most abundant ones. All identified DAGs were also found to be alanylated (13 species). CDP-DAG is an intermediate in the biosynthesis of PI and PG. Only one CDP-DAG was identified in the IM, carrying the highly abundant DAG<sub>16:0/18:1</sub>. Altogether, 13 TAGs were identified. They were equally identified in the IM and OM of the WT and KO. Isomeric lipid species are annotated in brackets as numbers.

DAG species	1st FA	2nd FA	Mass (NH4+)	diacylglycerol (DAG)						Total (8):		
				TL	IM	OM	WT	TmaT KO	PBQC			
				TL	IM	OM	TL	IM	OM	TL	IM	OM
1 DAG	(14:0)	(18:1)	584.5245	yes	yes	no	yes	yes	no	yes	yes	no
2 DAG	(16:2)	(18:1)	608.5252	no	yes	no	yes	no	no	yes	no	no
3 DAG	(16:1)	(18:1)	610.5417	yes	yes	yes	yes	yes	yes	yes	yes	yes
4 DAG	(16:0)	(18:1)	612.5586	yes	yes	yes(2)	yes	yes(2)	yes	yes	yes	yes
5 DAG	(17:0)	(18:1)	626.5725	yes	no	no	yes	no	yes	no	yes	no
6 DAG	(18:1)	(18:2)	636.5558	yes	yes	no	yes(3)	yes(2)	no	yes	yes	no
7 DAG	(18:1)	(18:1)	638.5742	yes	yes	yes	yes	yes	yes	yes	yes	yes
8 DAG	(18:0)	(18:1)	640.5872	yes	yes	yes	yes	yes	yes	yes	yes	yes
<b>Total (8):</b>				<b>7</b>	<b>7</b>	<b>4</b>	<b>8</b>	<b>8</b>	<b>4</b>	<b>7</b>	<b>8</b>	<b>4</b>

  

Ala-DAG species	1st FA	2nd FA	Mass (H+)	alanylated diacylglycerol (Ala-DAG)						Total (13):		
				TL	IM	OM	WT	TmaT KO	PBQC			
				TL	IM	OM	TL	IM	OM	TL	IM	OM
1 Ala-DAG	(14:0)	(18:1)	638.538	yes	yes	no	yes	yes	no	yes	yes	no
2 Ala-DAG	(16:0)	(16:0)	640.5514	yes	no	no	yes	no	no	yes	no	no
3 Ala-DAG	(15:0)	(18:1)	652.5512	yes	yes	no	yes	yes(2)	no	yes	yes(2)	no
4 Ala-DAG	(16:2)	(18:1)	662.5353	yes	yes(2)	no	yes	no	yes	yes	yes	no
5 Ala-DAG	(16:1)	(18:1)	664.554	yes(3)	yes(2)	no	yes	yes(3)	yes(3)	yes	yes(3)	no
6 Ala-DAG	(16:0)	(18:1)	666.5667	yes	yes(3)	yes	yes	yes	yes	yes	yes(2)	yes
7 Ala-DAG	(16:0)	(18:0)	668.5825	yes	no	no	yes	no	yes	yes(2)	no	no
8 Ala-DAG	(17:0)	(18:1)	680.5821	yes	yes	no	yes	no	yes	yes	yes	no
9 Ala-DAG	(36:4)		688.5492	yes	yes	yes	yes	yes	yes	yes	yes(2)	yes
10 Ala-DAG	(18:1)	(18:2)	690.5671	yes	yes	no	yes	yes	no	yes	yes	no
11 Ala-DAG	(18:1)	(18:1)	692.5893	yes	yes	yes	yes	yes	yes	yes	yes	yes
12 Ala-DAG	(18:0)	(18:1)	694.5999	yes	yes	yes	yes	yes	yes	yes	yes	yes
13 Ala-DAG	(38:5)		714.5643	yes	yes	yes	yes	yes	yes	yes	yes	no
<b>Total (13):</b>				<b>13</b>	<b>11</b>	<b>5</b>	<b>9</b>	<b>9</b>	<b>2</b>	<b>12</b>	<b>12</b>	<b>4</b>

  

CDP-DAG species	1st FA	2nd FA	Mass (H+)	cytidine diphosphate diacylglycerol (C)						Total (1):		
				TL	IM	OM	WT	TmaT KO	PI			
				TL	IM	OM	TL	IM	OM	TL	IM	OM
1 CDP-DAG	(34:1)		980.54	yes	yes	no	no	yes	no	no	no	no
<b>Total (1):</b>				<b>1</b>	<b>1</b>	<b>0</b>	<b>0</b>	<b>1</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>

  

PA species	1st FA	2nd FA	Mass (NH4+)	phosphatidic acid (PA)						Total (2):		
				TL	IM	OM	WT	TmaT KO	OM			
				TL	IM	OM	TL	IM	OM	TL	IM	OM
1 PA	(16:0)	(18:1)	692.5227	yes	yes(2)	no	yes	no	no	yes	no	no
2 PA	(18:1)	(18:1)	718.5381	yes	yes	no	yes	yes	no	no	no	no
<b>Total (2):</b>				<b>2</b>	<b>2</b>	<b>0</b>	<b>2</b>	<b>0</b>	<b>1</b>	<b>0</b>	<b>0</b>	<b>0</b>

  

TAG species	1st FA	2nd FA	3rd FA	Mass (NH4+)	triacylglycerol (TAG)						Total (13):		
					TL	IM	OM	WT	TmaT KO	OM			
					TL	IM	OM	TL	IM	OM	TL	IM	OM
1 TAG	(14:0)	(16:0)	(16:0)	796.739	no	no	no	no	no	no	no	no	no
2 TAG	(14:0)	(16:0)	(18:1)	822.755	no	yes	yes(2)	no	no	yes	no	yes	no
3 TAG	(16:0)	(16:0)	(16:0)	824.77	yes	yes	yes	yes	yes	yes	yes	yes	yes
4 TAG	(16:0)	(16:0)	(18:2)	848.77	yes	yes	yes	yes	yes	yes	yes	yes	yes
5 TAG	(16:0)	(16:0)	(18:1)	850.786	no	yes	yes	yes	yes	yes	yes	yes	yes
6 TAG	(16:0)	(18:1)	(18:2)	874.786	yes	yes	yes	yes	yes	yes	yes	yes	yes
7 TAG	(16:0)	(18:1)	(18:1)	876.801	yes	yes	yes	yes	yes	yes	yes	yes	yes
8 TAG	(16:0)	(18:0)	(18:1)	878.817	yes	yes	yes	yes	yes	yes	yes	yes	yes
9 TAG	(18:1)	(18:1)	(18:2)	900.802	yes	no	no	yes	yes(2)	yes	yes	yes	yes
10 TAG	(18:1)	(18:1)	(18:1)	902.817	yes	yes	yes	yes	yes	yes	yes	yes	yes
11 TAG	(18:0)	(18:1)	(18:1)	904.833	yes	yes	yes	yes	yes	yes	yes	yes	yes
12 TAG	(18:0)	(18:0)	(18:1)	906.848	yes	yes	yes	yes	yes	yes	yes	yes	yes
13 TAG	(18:0)	(18:0)	(18:0)	908.865	no	no	no	no	no	no	no	no	no
<b>Total (13):</b>				<b>9</b>	<b>10</b>	<b>10</b>	<b>10</b>	<b>10</b>	<b>10</b>	<b>10</b>	<b>11</b>	<b>11</b>	<b>11</b>

**Supplementary Table 6**

Summary of LC-MS/MS ESI TOF (pos. mode) identified PG, Ala-PG, Acyl-PG, and Acyl-PG like lipid species in *C. glutamicum* wildtype (TL, IM, OM), TmaT KO mutant (TL, IM, OM) and PBQC (TL, IM, OM).

The lipids are sorted according to their size (Da). Saturated, mono- and di-unsaturated fatty acid chains were identified. **(A)** In total, 11 PG species were identified. They were more abundant in the IM compared to the OM. In addition, 55-60% less PG species were identified in the IM as well as the OM of the KO compared to the WT. **(B)** Two alanylated PG species were identified. They were found in the IM and the OM. **(C)** Three Acyl-PG species were identified in the IM. They were absent in the OM. **(D)** Four Acyl-PG like species were identified in the IM and OM of the WT. These species are completely absent in the KO. Isomeric lipid species are annotated in brackets as numbers.

<b>A</b>														
PG species	phosphatidylglycerol (PG)						acyl-phosphatidylglycerol (Acyl-PG)							
	1st FA	2nd FA	Mass (NH4+)	TL	IM	OM	TL	IM	OM	TL	IM	OM	PBQC	
1	PG (12:0)	(18:1)	710.497	yes	yes	no	no	no	no	no	no	no	no	
2	PG (16:0)	(16:2)	736.513	yes	yes (2)	no	no	no	no	yes	yes	no	no	
3	PG (14:0)	(18:1)	738.528	yes	yes	yes	yes	yes	no	yes	yes	yes	yes	
4	PG (15:0)	(18:1)	752.543	yes	yes	no	no	no	no	yes	yes	no	no	
5	PG (16:2)	(18:1)	762.528	yes	yes	no	no	no	no	yes	yes	no	no	
6	PG (16:1)	(18:1)	764.544	yes	yes	yes	yes	yes	no	yes	yes	yes	yes	
7	PG (16:0)	(18:1)	766.56	yes	yes (2)	yes	yes	yes	yes	yes	yes	yes	yes	
8	PG (17:0)	(18:1)	780.574	yes	yes	no	no	no	no	yes	yes	no	no	
9	PG (18:1)	(18:2)	790.559	yes	yes	no	no	no	no	yes	yes	no	no	
10	PG (18:1)	(18:1)	792.576	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	
11	PG (18:0)	(18:1)	794.59	yes	yes	yes	yes	yes	no	yes	yes	yes	yes	
<b>Total (11):</b>				11	11	5	5	5	2	10	10	5		
<b>B</b>														
Ala-PG species	alanylated phosphatidylglycerol (Ala-PG)						acyl-phosphatidylglycerol like (Acyl-PG like)							
	1st FA	2nd FA	Mass (H+)	TL	IM	OM	TL	IM	OM	TL	IM	OM	PBQC	
1	Ala-PG (16:0)	(18:1)	820.5737	yes	yes (2)	yes	yes	yes	no	yes	yes	yes	yes (2)	
2	Ala-PG (18:1)	(18:1)	846.5849	yes	yes	no	no	no	no	yes	yes	yes	no	
<b>Total (2):</b>				2	2	1	1	1	0	2	2	2	1	
<b>C</b>														
Acyl-PG species	acyl-phosphatidylglycerol (Acyl-PG)						acyl-phosphatidylglycerol like (Acyl-PG like)							
	1st FA	2nd FA	3rd FA	Mass (NH4+)	TL	IM	OM	TL	IM	OM	TL	IM	OM	PBQC
1	Acyl-PG (16:0)	(16:0)	(18:1)	1004.7898	yes	yes	no	no	no	no	no	no	yes	yes
2	Acyl-PG (16:0)	(18:1)	(18:1)	1030.805	yes	yes	no	yes	yes	no	yes	no	yes	yes
3	Acyl-PG (18:1)	(18:1)	(18:1)	1056.823	yes	yes	no	no	no	no	no	no	no	no
<b>Total (3):</b>				3	3	0	1	1	0	2	2			
<b>D</b>														
Acyl-PG like species	acyl-phosphatidylglycerol like (Acyl-PG like)						acyl-phosphatidylglycerol like (Acyl-PG like)							
	1st FA	2nd FA	3rd FA	Mass (H+)	TL	IM	OM	TL	IM	OM	TL	IM	OM	PBQC
1	Acyl-PG like (16:0)	(16:0)	240.24 Da	963.7625	yes	yes	yes	no	no	no	no	yes	yes	
2	Acyl-PG like (16:0)	(16:0)	266.26 Da	989.7786	yes	yes	yes	no	no	no	no	yes	yes	
3	Acyl-PG like (16:0)	(16:0)	269.27 Da	991.7939	yes	yes	yes	no	no	no	no	yes	yes	
4	Acyl-PG like (16:0)	(16:0)	269.27 Da	1034.835	yes	yes	no	no	no	no	no	no	no	
<b>Total (4):</b>				4	4	3	0	0	0	3	3			

**Supplementary Table 7**

Summary of LC-MS/MS ESI TOF (positive ionisation mode) identified cardiolipin (CL) lipid species in *C. glutamicum* wildtype (TL, IM, OM), TmaT KO mutant (TL, IM, OM) and PBQC (TL, IM, OM).  
 Altogether, 18 cardiolipin species were identified within the size range of 1349.9 Da (DAG<sub>30:1</sub> + DAG<sub>34:1</sub> and DAG<sub>32:1</sub> + DAG<sub>32:1</sub>) to 1460.1 Da (DAG<sub>36:1</sub> + DAG<sub>36:2</sub>), based on 12 different diacylglycerol moieties. Isomeric lipid species are annotated in brackets as numbers.

	CL species	1st DAG	2nd DAG	Mass (H+)	cardiolipin (CL)								
					WT			TmaT KO			PBQC		
					TL	IM	OM	TL	IM	OM	TL	IM	OM
1	CL	(30:1) (32:1)	(34:1) (32:1)	1349.947	yes	yes	no	yes	yes	no	yes	yes	no
2	CL	(32:2) (30:1)	(34:1) (34:1)	1375.963	yes	yes	no	yes	yes	no	yes	yes	no
3	CL	(32:1)	(34:1)	1377.98	yes	yes	yes	yes	yes	yes	yes	yes	yes
4	CL	(33:1)	(34:1)	1391.996	yes	yes	no	yes	yes	no	yes	yes	no
5	CL	(34:1)	(34:3)	1401.98	yes	yes	no	yes (2)	yes (2)	no	yes (2)	yes	no
6	CL	(34:1)	(34:2)	1403.996	yes	yes	no	yes	yes	no	yes	yes	no
7	CL	(34:1)	(34:1)	1406.012	yes (3)	yes	yes	yes (5)	yes (2)	yes	yes (3)	yes (3)	yes
8	CL	(33:1) (34:1)	(36:2) (35:2)	1418.01	yes	no	no	no	no	no	no	no	no
9	CL	(34:1)	(35:1)	1420.02	yes (2)	yes	no	yes (2)	yes	no	yes (2)	yes	no
10	CL	(34:1) (34:0)	(36:3) (36:2)	1430.01	yes	yes	no	yes	yes	no	yes	yes	no
11	CL	(34:1)	(36:2)	1432.027	yes	yes	yes	yes	yes	yes	yes	yes	yes
12	CL	(34:1)	(36:1)	1434.041	yes	yes	no	yes	yes	yes	yes	yes	no
13	CL	(36:2)	(36:2)	1458.042	no	yes	no	no	no	no	no	no	no
14	CL	(36:1)	(36:2)	1460.056	no	yes	no	no	no	no	no	no	no
<b>Total (18):</b>					<b>16</b>	<b>16</b>	<b>3</b>	<b>14</b>	<b>14</b>	<b>4</b>	<b>14</b>	<b>14</b>	<b>3</b>



**Supplementary Table 9**

Summary of 185 identified lipids in *C. glutamicum*, representing 28 lipid (sub)classes. The relative abundance (n=6) of each lipid is given, as well as the raw p values and fold-changes when the inner and outer membranes are compared between the *C. glutamicum* wildtype and the TmaT mutant. The letters A-F refer to the same letters as in Figure 4. The slash signs mean that the p value or the fold change are outside of significance (p value 0.05, fold change <1). The fold change values also refer to Figure 5. The n/a signs mean that this lipid wasn't identified.

		abundance/peak area (mean, n=6)				TmaT (IM)/WT (IM)		TmaT (OM)/WT (OM)	
		WT (IM)	WT (OM)	TmaTKO (IM)	TmaTKO (OM)	raw p-value	fold change	raw p-value	fold change
1	DAG 14:0 18:1	7.67E+05	0.00E+00	7.08E+05	0.00E+00	1.71E-02	/	n/a	n/a
2	DAG 16:2 18:1	5.23E+05	0.00E+00	5.89E+05	0.00E+00	9.74E-04	/	n/a	n/a
3	DAG 16:1 18:1	3.26E+06	0.00E+00	3.61E+06	0.00E+00	5.98E-05	/	n/a	n/a
4	DAG 16:0 18:1	1.29E+07	1.29E+06	1.28E+07	2.90E+06	/	/	6.38E-08	3.4156
5	DAG 18:1 18:2	2.49E+06	0.00E+00	2.24E+06	0.00E+00	/	/	n/a	n/a
6	DAG 18:1 18:1	1.05E+07	4.30E+05	6.34E+06	1.97E+05	/	/	/	/
7	DAG 18:0 18:1	1.84E+06	0.00E+00	1.38E+06	0.00E+00	/	/	n/a	n/a
8	Ala-DAG 14:0 18:1	3.62E+06	0.00E+00	4.53E+05	0.00E+00	1.98E-08	0.1492	n/a	n/a
9	Ala-DAG 15:0 18:1	8.99E+05	0.00E+00	3.00E+05	0.00E+00	4.12E-07	0.40278	n/a	n/a
10	Ala-DAG 16:2 18:1	3.41E+05	0.00E+00	7.77E+04	0.00E+00	6.00E-07	0.27191	n/a	n/a
11	Ala-DAG 16:1 18:1	4.84E+06	0.00E+00	5.48E+05	0.00E+00	1.34E-09	0.13594	n/a	n/a
12	Ala-DAG 16:0 18:1	1.19E+07	1.77E+06	1.04E+07	3.89E+05	/	/	1.68E-06	0.31627
13	Ala-DAG 16:0 18:0	1.01E+05	0.00E+00	7.33E+04	0.00E+00	/	/	n/a	n/a
14	Ala-DAG 17:0 18:1	4.54E+05	0.00E+00	1.10E+05	0.00E+00	6.05E-07	/	n/a	n/a
A 15	Ala-DAG 36:4	0.00E+00	3.43E+05	0.00E+00	7.67E+04	n/a	n/a	1.16E-07	0.32835
16	Ala-DAG 18:1 18:2	1.23E+06	0.00E+00	1.97E+05	0.00E+00	1.77E-07	0.18997	n/a	n/a
17	Ala-DAG 18:1 18:1	1.22E+07	0.00E+00	1.31E+06	0.00E+00	9.03E-12	0.12906	n/a	n/a
18	Ala-DAG 18:0 18:1	2.72E+06	0.00E+00	2.55E+05	0.00E+00	4.32E-09	0.1117	n/a	n/a
19	Ala-DAG (38:5)	5.78E+05	0.00E+00	6.31E+04	0.00E+00	3.23E-12	0.13348	n/a	n/a
20	PA 18:1 18:1	3.51E+05	0.00E+00	8.04E+04	0.00E+00	4.34E-07	0.28253	n/a	n/a
21	CDP-DAG 34:1	1.75E+05	0.00E+00	7.36E+04	0.00E+00	3.03E-07	/	n/a	n/a
22	TAG 16:0 16:0 18:0	1.37E+05	1.06E+05	1.87E+05	1.18E+05	/	/	/	/
23	TAG 16:0 16:0 18:2	1.99E+05	1.12E+05	2.62E+05	1.30E+05	1.75E-06	/	/	/
24	TAG 16:0 16:0 18:1	6.43E+05	4.65E+05	8.94E+05	5.46E+05	1.70E-06	/	1.26E-02	/
25	TAG 16:0 18:1 18:2	1.95E+05	1.14E+05	2.96E+05	1.36E+05	3.91E-07	/	/	/
26	TAG 16:0 18:1 18:1	6.35E+05	2.90E+05	2.45E+06	4.69E+05	1.96E-12	4.6924	6.53E-04	2.5586
27	TAG 16:0 18:0 18:1	0.00E+00	7.06E+04	0.00E+00	8.03E+04	1.39E-12	4.8479	/	/
28	TAG 18:1 18:1 18:1	1.98E+05	9.00E+04	2.11E+05	1.04E+05	/	/	/	/
29	TAG 18:0 18:0 18:1	1.11E+05	7.85E+04	1.55E+05	9.26E+04	6.90E-07	/	/	/
30	PG 14:0 18:1	4.34E+06	1.62E+05	6.06E+05	1.76E+04	4.20E-08	0.16965	7.44E-12	0.16199
31	PG 15:0 18:1	8.70E+05	0.00E+00	1.80E+05	0.00E+00	8.56E-08	0.25186	n/a	n/a
32	PG 16:2 18:1	2.00E+05	0.00E+00	2.79E+04	0.00E+00	1.73E-12	0.16871	n/a	n/a
33	PG 16:1 18:1	4.19E+06	1.07E+05	6.07E+05	8.26E+03	1.02E-07	0.17659	8.97E-11	0.1176
34	PG 16:0 18:1	5.83E+06	6.60E+06	5.75E+06	4.75E+06	/	/	/	/
35	PG 17:0 18:1	4.48E+05	0.00E+00	1.46E+05	0.00E+00	1.66E-06	0.40197	n/a	n/a
36	PG 18:1 18:2	1.03E+06	0.00E+00	1.66E+05	0.00E+00	3.67E-08	0.1961	n/a	n/a
37	PG 18:1 18:1	3.34E+06	1.16E+06	9.67E+05	3.95E+04	3.69E-05	0.35683	5.93E-12	0.052255
38	PG 18:0 18:1	3.45E+06	1.19E+05	7.44E+05	1.38E+04	1.48E-04	0.26962	1.00E-08	0.17871
39	Ala-PG 16:0 18:1	6.37E+06	3.21E+05	8.77E+05	1.75E+04	8.22E-13	0.16649	1.59E-11	0.081972
40	Ala-PG 18:1 18:1	4.04E+05	0.00E+00	1.42E+04	0.00E+00	1.07E-13	0.042677	n/a	n/a
41	Acyl-PG (49:1)	1.50E+05	0.00E+00	3.57E+04	0.00E+00	/	/	n/a	n/a
42	Acyl-PG 18:1 16:0 16:0	1.59E+05	0.00E+00	2.70E+04	0.00E+00	2.34E-10	0.204	n/a	n/a
43	Acyl-PG 18:1 18:1 16:0	1.56E+06	0.00E+00	3.73E+05	0.00E+00	2.12E-09	0.28777	n/a	n/a
44	Acyl-PG like (m/z 963.763)	1.37E+06	6.16E+05	1.57E+04	5.98E+03	1.61E-12	0.013746	6.41E-12	0.014804
B 45	Acyl-PG like (m/z 989.778)	4.42E+05	1.44E+05	8.78E+03	3.26E+03	5.26E-11	0.023691	1.73E-10	0.034763
46	Acyl-PG like (m/z 991.794)	4.12E+05	0.00E+00	6.91E+03	0.00E+00	2.07E-13	0.02054	n/a	n/a
47	Acyl-PG like (m/z 1008.82)	4.63E+05	1.04E+05	2.88E+03	1.25E+03	1.70E-13	0.0074487	1.06E-11	0.01859
48	CL 30:1 34:1 / 32:1 32:1	2.35E+05	0.00E+00	4.67E+04	0.00E+00	4.87E-08	0.23938	n/a	n/a
49	CL 32:2 34:1 / 30:1 36:2	2.09E+05	0.00E+00	1.89E+04	0.00E+00	3.40E-11	0.10845	n/a	n/a
50	CL 32:1 34:1	3.83E+06	0.00E+00	2.38E+06	0.00E+00	1.89E-05	/	n/a	n/a
51	CL 33:1 34:1	9.64E+05	0.00E+00	4.87E+05	0.00E+00	4.34E-08	/	n/a	n/a
52	CL 34:1 34:3	1.48E+05	0.00E+00	6.99E+04	0.00E+00	2.68E-06	/	n/a	n/a
53	CL 34:1 34:2	2.90E+06	0.00E+00	1.40E+06	0.00E+00	2.28E-07	/	n/a	n/a
54	CL 34:1 34:1	5.99E+06	2.93E+06	6.06E+06	3.56E+06	5.10E-03	/	2.25E-03	/
55	CL 34:1 35:1	4.13E+05	0.00E+00	4.22E+05	0.00E+00	/	/	n/a	n/a
56	CL 34:1 36:3 / 34:0 36:2	3.79E+05	0.00E+00	1.78E+05	0.00E+00	1.44E-06	/	n/a	n/a
57	CL 34:1 36:2	2.52E+06	3.87E+05	7.34E+05	1.04E+05	5.96E-08	0.35941	6.24E-05	0.42468
58	CL 34:1 36:1	1.81E+06	0.00E+00	1.49E+06	0.00E+00	/	/	n/a	n/a
59	CL 36:2 36:2	2.49E+05	0.00E+00	1.26E+04	0.00E+00	4.39E-12	0.061953	n/a	n/a
60	CL 36:1 36:2	1.47E+05	0.00E+00	2.81E+04	0.00E+00	2.14E-10	0.23305	n/a	n/a

Supplementary Table 9, continued

61	PI 14:0 18:1	9.68E+05	0.00E+00	5.34E+05	0.00E+00	2.98E-06	/	n/a	n/a
62	PI 16:1 18:1	7.73E+05	0.00E+00	4.87E+05	0.00E+00	/	/	n/a	n/a
63	PI 16:0 18:1	4.40E+06	5.80E+06	3.85E+06	5.53E+06	/	/	/	/
64	PI 18:0 18:1	6.06E+05	0.00E+00	3.49E+05	0.00E+00	1.67E-02	/	n/a	n/a
65	PI 18:1 18:1	0.00E+00	3.13E+05	0.00E+00	4.79E+04	n/a	n/a	1.21E-08	0.23352
66	PIM1 16:0 18:1	1.23E+06	0.00E+00	5.12E+05	0.00E+00	4.24E-05	/	n/a	n/a
67	PIM2 16:0 18:1	1.46E+06	0.00E+00	1.07E+06	0.00E+00	8.44E-03	/	n/a	n/a
68	AcPIM2 32:1 16:0	6.84E+05	0.00E+00	3.06E+05	0.00E+00	1.32E-04	/	n/a	n/a
69	AcPIM2 34:2 16:0	1.96E+05	0.00E+00	6.10E+04	0.00E+00	4.05E-09	0.37716	n/a	n/a
70	AcPIM2 34:1 16:0	5.57E+06	5.35E+05	4.41E+06	5.62E+05	/	/	3.28E-03	/
71	AcPIM2 36:2 16:0	1.07E+06	0.00E+00	3.10E+05	0.00E+00	1.23E-10	0.35274	n/a	n/a
72	AcPIM2 36:1 16:0	1.47E+06	0.00E+00	1.06E+06	0.00E+00	0.0013707	/	n/a	n/a
73	AcPIM3 34:1 16:0	7.17E+05	0.00E+00	9.11E+05	0.00E+00	1.02E-02	/	n/a	n/a
74	AcPIM4 34:1 16:0	6.85E+05	0.00E+00	5.32E+05	0.00E+00	/	/	n/a	n/a
75	GI-A 14:0 18:1	1.56E+06	0.00E+00	6.41E+05	0.00E+00	1.08E-07	0.49591	n/a	n/a
76	GI-A 15:0 18:1	1.88E+05	0.00E+00	1.52E+05	0.00E+00	/	/	n/a	n/a
77	GI-A 16:1 18:1	6.95E+05	0.00E+00	3.83E+05	0.00E+00	1.89E-05	/	n/a	n/a
78	GI-A 16:0 18:1	8.13E+06	8.66E+06	1.19E+07	3.20E+06	1.08E-03	/	1.03E-05	/
79	GI-A 18:1 18:1	2.11E+05	0.00E+00	1.07E+05	0.00E+00	1.21E-02	/	n/a	n/a
80	GI-A 18:0 18:1	7.85E+05	0.00E+00	2.22E+05	0.00E+00	2.71E-07	0.34949	n/a	n/a
81	GI-X 14:0 18:1	6.26E+05	0.00E+00	3.95E+05	0.00E+00	4.86E-03	/	n/a	n/a
82	GI-X 16:0 18:2 / 16:1 18:1	4.19E+05	0.00E+00	2.97E+05	0.00E+00	7.41E-04	/	n/a	n/a
83	GI-X 16:0 18:1	5.66E+06	3.79E+06	7.86E+06	2.46E+06	9.79E-03	/	/	/
84	GI-X 18:0 18:1	6.96E+05	0.00E+00	1.51E+05	0.00E+00	3.17E-11	0.26507	n/a	n/a
85	GI-Y 16:0 18:1	1.25E+06	0.00E+00	5.09E+05	0.00E+00	2.81E-05	/	n/a	n/a
86	GI-Z 16:0 18:1	2.19E+06	0.00E+00	1.50E+06	0.00E+00	5.22E-03	/	n/a	n/a
87	hTMC 24:0	2.87E+05	1.79E+05	2.83E+05	4.58E+04	/	/	9.80E-06	0.40114
88	hTMC 26:1	3.56E+05	2.09E+05	5.70E+05	8.29E+04	3.20E-07	/	3.59E-03	/
89	hTMC 26:0	5.29E+05	2.97E+05	9.39E+05	7.14E+04	1.19E-06	2.1679	4.93E-06	0.37291
90	hTMC 27:1	2.07E+05	0.00E+00	4.57E+04	0.00E+00	0.26822	4.68E-11	n/a	n/a
91	hTMC 28:2	1.40E+05	0.00E+00	2.67E+05	0.00E+00	1.21E-03	2.3019	n/a	n/a
92	hTMC 28:1	4.74E+05	2.38E+05	1.23E+06	7.49E+04	6.71E-08	3.1449	1.24E-04	0.4934
93	hTMC 28:0	5.80E+05	2.22E+05	1.60E+06	3.94E+04	1.05E-08	3.3461	2.55E-08	0.27406
94	hTMC 30:3	1.47E+05	1.24E+05	1.73E+05	1.46E+05	/	/	1.05E-02	/
95	hTMC 30:2	4.85E+04	0.00E+00	7.57E+05	0.00E+00	1.92E-11	19.021	n/a	n/a
96	hTMC 30:1	4.72E+05	1.50E+05	1.20E+06	2.37E+04	1.02E-08	3.0808	2.26E-07	0.24843
97	hTMC 30:0	2.18E+05	1.36E+06	1.87E+06	1.35E+05	7.20E-08	10.676	4.32E-07	0.15617
98	hTMC 31:0	1.01E+05	7.02E+04	3.25E+05	1.12E+04	1.63E-06	3.9734	5.26E-10	0.2353
99	hTMC 32:3	2.86E+04	0.00E+00	4.72E+05	0.00E+00	4.37E-14	20.113	n/a	n/a
100	hTMC 32:2	2.25E+05	0.00E+00	2.35E+05	0.00E+00	/	/	n/a	n/a
101	hTMC 32:1	1.77E+06	1.40E+06	5.04E+06	1.59E+05	3.06E-10	3.4847	1.29E-10	0.17204
102	hTMC 32:0	1.44E+05	8.60E+06	6.25E+05	5.21E+06	1.14E-08	5.3786	/	/
103	hTMC 32:0	7.66E+06	0.00E+00	9.24E+06	0.00E+00	2.11E-03	/	n/a	n/a
104	hTMC 33:0	2.45E+05	6.24E+04	8.41E+05	1.39E+04	2.21E-08	4.2193	1.49E-08	0.3336
105	hTMC 34:3	0.00E+00	1.07E+05	0.00E+00	2.61E+04	n/a	n/a	2.12E-05	0.3855
106	hTMC 34:2	2.90E+06	1.32E+06	1.19E+07	2.57E+05	1.19E-09	5.0778	7.55E-10	0.28692
107	hTMC 34:1	7.71E+06	8.47E+06	8.66E+06	5.60E+06	2.57E-02	/	/	/
108	hTMC 34:0	6.93E+06	6.21E+06	7.59E+06	1.32E+06	1.59E-03	/	7.92E-08	0.31501
109	hTMC 35:1	1.21E+05	0.00E+00	4.27E+05	0.00E+00	7.75E-07	4.2954	n/a	n/a
110	hTMC 35:0	3.52E+04	0.00E+00	1.69E+05	0.00E+00	1.59E-10	5.8738	n/a	n/a
111	hTMC 36:3	2.23E+06	8.75E+05	5.93E+06	2.15E+05	2.01E-07	3.2915	1.69E-09	0.36463
112	hTMC 36:2	0.00E+00	6.52E+06	0.00E+00	3.99E+06	n/a	n/a	/	/
113	hTMC 36:1	4.90E+06	3.96E+06	6.87E+06	6.67E+05	2.07E-04	/	3.14E-10	0.24393
114	hTMC 36:0	7.61E+05	4.78E+05	3.05E+06	2.94E+04	9.66E-09	4.923	8.79E-11	0.094379
115	hTMC 38:3	7.23E+04	0.00E+00	4.26E+05	0.00E+00	1.85E-09	7.2154	n/a	n/a
116	hTMC 38:2	2.01E+05	1.28E+05	5.19E+05	2.54E+04	1.42E-06	3.1621	6.92E-10	0.29319
117	hTMC 38:1	4.07E+04	0.00E+00	2.38E+05	0.00E+00	9.64E-12	7.1515	n/a	n/a

Supplementary Table 9, continued

118	hGMM 26:0	2.97E+05	7.16E+04	1.77E+05	3.02E+04	2.07E-03	/	5.45E-05	/
119	hGMM 28:1	1.49E+05	0.00E+00	1.11E+05	0.00E+00	/	/	n/a	n/a
120	hGMM 30:0	5.94E+04	0.00E+00	2.65E+05	0.00E+00	5.89E-07	5.3258	n/a	n/a
121	hGMM 32:1	7.47E+04	0.00E+00	1.39E+05	0.00E+00	1.83E-06	2.2585	n/a	n/a
122	hGMM 32:0	2.58E+06	1.09E+06	1.34E+06	2.53E+06	3.90E-04	/	1.13E-07	3.4376
123	hGMM 34:1	1.48E+06	7.05E+05	2.20E+06	3.23E+06	3.76E-04	/	4.05E-10	6.9371
124	hGMM 34:0	1.26E+06	3.12E+05	6.39E+05	3.15E+05	3.73E-05	/	1.09E-03	/
125	hGMM 36:2	3.48E+05	1.85E+05	1.35E+06	1.44E+06	1.36E-09	4.6693	/	/
126	hGMM 36:1	1.96E+05	7.44E+04	3.14E+05	1.41E+05	1.16E-05	/	7.61E-10	2.7762
127	hGMM 36:0	4.07E+04	0.00E+00	2.02E+05	0.00E+00	1.90E-07	5.9716	6.18E-13	11.451
128	AcTMCM 28:0	7.45E+04	0.00E+00	9.25E+03	0.00E+00	1.48E-09	0.15112	n/a	n/a
129	AcTMCM 32:1	1.26E+05	1.71E+05	6.19E+04	6.05E+04	2.74E-04	/	3.86E-05	/
130	AcTMCM 32:0	1.49E+06	1.61E+06	2.57E+04	2.30E+05	1.20E-15	0.020997	3.01E-09	0.21325
131	AcTMCM 34:2	1.12E+05	7.42E+04	4.66E+04	6.07E+03	7.55E-05	/	8.38E-12	0.12064
132	AcTMCM 34:1	6.41E+05	7.57E+05	1.41E+04	1.28E+05	5.98E-14	0.026541	5.80E-08	0.25321
133	AcTMCM 34:0	5.17E+05	4.61E+05	9.54E+03	1.96E+04	1.89E-16	0.022482	1.27E-12	0.061733
E 134	AcTMCM 36:2	1.82E+05	1.90E+05	9.55E+03	2.53E+04	3.20E-14	0.064055	6.33E-10	0.19718
135	ketoTMCM 32:0	5.81E+05	1.06E+05	8.44E+06	2.35E+05	2.17E-10	17.985	7.33E-09	3.3205
136	ketoTMCM 34:2	1.10E+05	0.00E+00	9.82E+05	0.00E+00	1.62E-09	10.992	n/a	n/a
137	ketoTMCM 34:0	6.40E+05	0.00E+00	3.82E+06	0.00E+00	1.69E-10	7.3251	n/a	n/a
138	ketoTMCM 36:3	3.31E+05	0.00E+00	1.74E+06	0.00E+00	1.72E-09	6.473	n/a	n/a
139	ketoTMCM 36:2	1.91E+06	0.00E+00	1.18E+07	0.00E+00	3.00E-09	7.5841	n/a	n/a
140	ketoTMCM 36:1	3.83E+05	0.00E+00	3.65E+06	0.00E+00	/	/	n/a	n/a
141	Acyl-hTMCM 16:0 32:1	4.28E+04	0.00E+00	2.53E+05	0.00E+00	2.09E-10	7.1772	n/a	n/a
142	Acyl-hTMCM 16:0 32:0	8.35E+05	1.67E+05	1.85E+06	5.68E+04	2.75E-07	2.6787	1.14E-06	0.49993
143	Acyl-hTMCM 16:0 34:1	1.25E+06	2.22E+05	2.44E+06	3.13E+05	1.34E-07	2.3492	1.34E-06	2.02
144	Acyl-hTMCM 16:0 34:0	5.58E+05	0.00E+00	5.93E+05	0.00E+00	1.08E-03	/	n/a	n/a
145	Acyl-hTMCM 18:1 34:1	8.69E+05	1.07E+05	1.56E+06	3.41E+05	4.41E-07	2.1591	1.27E-09	4.549
146	Acyl-hTMCM 18:1 34:0	3.96E+05	0.00E+00	4.52E+05	0.00E+00	5.66E-03	/	n/a	n/a
147	Acyl-hTMCM 18:1 36:2	1.67E+05	0.00E+00	5.37E+05	0.00E+00	2.06E-07	3.8421	n/a	n/a
148	Ac1-hTDCM 32:0 32:0	3.98E+05	2.55E+05	2.09E+05	1.69E+05	9.30E-08	/	/	/
149	Ac1-hTDCM 32:0 34:1	0.00E+00	2.68E+05	0.00E+00	2.55E+05	n/a	n/a	2.17E-03	/
150	Ac1-hTDCM 32:0 34:0	1.66E+05	1.14E+05	5.61E+04	4.56E+04	3.58E-10	0.41042	1.82E-04	/
151	h2TDCM 24:0 32:0	0.00E+00	2.33E+05	0.00E+00	3.62E+05	n/a	n/a	1.31E-06	2.3306
152	h2TDCM 58:2	5.80E+04	0.00E+00	1.78E+05	0.00E+00	7.58E-09	3.7198	n/a	n/a
153	h2TDCM 26:1 32:0	1.07E+05	4.75E+05	1.85E+05	5.15E+05	7.37E-07	2.1051	1.93E-03	/
154	h2TDCM 26:0 32:0	8.96E+05	1.18E+06	8.04E+05	1.07E+06	/	/	7.48E-04	/
155	h2TDCM 26:1 34:1	2.91E+05	3.98E+05	4.72E+05	5.83E+05	5.30E-08	/	1.60E-06	2.2307
156	h2TDCM 26:0 34:1 / 28:1 32:0	1.12E+06	1.52E+06	1.20E+06	1.76E+06	2.12E-02	/	5.41E-06	/
157	h2TDCM 28:0 32:0 / 26:0 34:0	1.90E+06	1.15E+06	8.85E+05	6.23E+05	1.34E-07	/	9.42E-03	/
158	h2TDCM 28:2 34:1	2.64E+05	2.05E+05	4.77E+05	3.82E+05	4.06E-08	2.1881	4.55E-08	2.7956
159	h2TDCM 28:1 34:1	8.62E+05	8.26E+05	1.13E+06	1.18E+06	9.87E-06	/	2.52E-07	2.1544
160	h2TDCM 28:0 34:1	2.08E+06	1.26E+06	1.36E+06	9.70E+05	3.54E-05	/	/	/
161	h2TDCM 30:0 32:0	1.83E+06	1.21E+06	9.43E+05	7.15E+05	3.14E-08	/	/	/
162	h2TDCM 64:2	1.23E+06	7.01E+05	1.04E+06	7.22E+05	/	/	8.91E-05	/
163	h2TDCM 32:0 32:1	2.94E+06	2.19E+06	2.03E+06	1.79E+06	6.51E-04	/	3.29E-02	/
164	h2TDCM 32:0 32:0	4.38E+06	3.60E+06	3.08E+06	2.89E+06	/	/	/	/
165	h2TDCM 65:1	1.16E+05	8.15E+04	6.35E+04	5.56E+04	9.41E-06	/	/	/
166	h2TDCM 66:3	8.69E+05	0.00E+00	8.31E+05	0.00E+00	3.74E-03	/	n/a	n/a
167	h2TDCM 32:0 34:2	2.98E+06	2.44E+06	2.76E+06	2.56E+06	/	/	1.35E-03	/
F 168	h2TDCM 32:0 34:1	4.16E+06	3.71E+06	4.04E+06	3.74E+06	/	/	1.69E-02	/
169	h2TDCM 32:0 34:0	4.18E+06	3.31E+06	2.11E+06	1.89E+06	2.09E-06	/	/	/
170	h2TDCM 33:1 34:0	6.78E+04	1.57E+05	5.70E+04	3.60E+04	/	/	2.32E-08	0.34424
171	h2TDCM 68:4	6.72E+05	3.44E+05	8.40E+05	5.49E+05	8.09E-06	/	4.19E-08	2.4173
172	h2TDCM 68:3	2.07E+06	1.69E+06	2.59E+06	2.43E+06	7.32E-06	/	1.91E-05	2.2173
173	h2TDCM 34:1 34:1	0.00E+00	2.70E+06	0.00E+00	3.19E+06	n/a	n/a	2.64E-03	/
174	h2TDCM 34:0 34:1	3.81E+06	3.01E+06	2.89E+06	2.66E+06	/	/	/	/
175	h2TDCM 34:0 34:0	2.91E+06	1.93E+06	8.12E+05	5.99E+05	2.92E-12	0.33833	3.01E-04	0.49538
176	h2TDCM 34:2 36:2	8.97E+05	6.63E+05	1.51E+06	1.36E+06	9.66E-10	2.0522	6.58E-07	3.1937
177	h2TDCM 34:1 36:2	1.55E+06	1.22E+06	2.07E+06	1.89E+06	1.94E-07	/	1.70E-04	2.4533
178	h2TDCM 70:2	1.83E+06	1.55E+06	2.03E+06	1.76E+06	1.02E-04	/	1.18E-03	/
179	h2TDCM 34:0 36:1	2.04E+06	8.51E+05	8.20E+05	7.80E+05	1.29E-10	0.4865	4.29E-05	/
180	h2TDCM 34:0 36:0	6.59E+05	0.00E+00	9.86E+04	0.00E+00	1.18E-10	0.1803	n/a	n/a
181	h2TDCM 36:2 36:3	1.36E+05	9.61E+04	3.49E+05	3.14E+05	1.94E-11	3.114	2.04E-08	5.0821
182	h2TDCM 36:2 36:2	3.08E+05	2.49E+05	5.76E+05	5.35E+05	1.64E-08	2.2687	1.42E-05	3.3997
183	h2TDCM 36:1 36:2	4.17E+05	3.40E+05	6.17E+05	5.12E+05	6.75E-08	/	6.06E-05	2.3435
184	h2TDCM 36:1 36:1	4.94E+05	3.16E+05	2.90E+05	1.81E+05	1.18E-05	/	/	/
185	h2TDCM 36:0 36:1	2.48E+05	9.95E+04	3.71E+04	1.94E+04	8.18E-10	0.1792	2.06E-07	0.30275

Supplementary Figure 1:

**High-performance thin-layer chromatography (HPTLC) analysis of lipid extracts from *C. glutamicum* wildtype, TmaT mutant and TmaT complementation strain.** Six biological replicates were analyzed for each lipid fraction and representative lanes shown in Figure 6A. A = Total Lipid, B = Inner Membrane (IM), C = Outer Membrane (OM) and sample set (Wildtype, TmaT mutant, TmaT Complementation Strain,. Some of the most common lipids are highlighted on the left-hand side of the figure. The asterisks mark the replicates which were selected for figure 6A.

