Supporting Information

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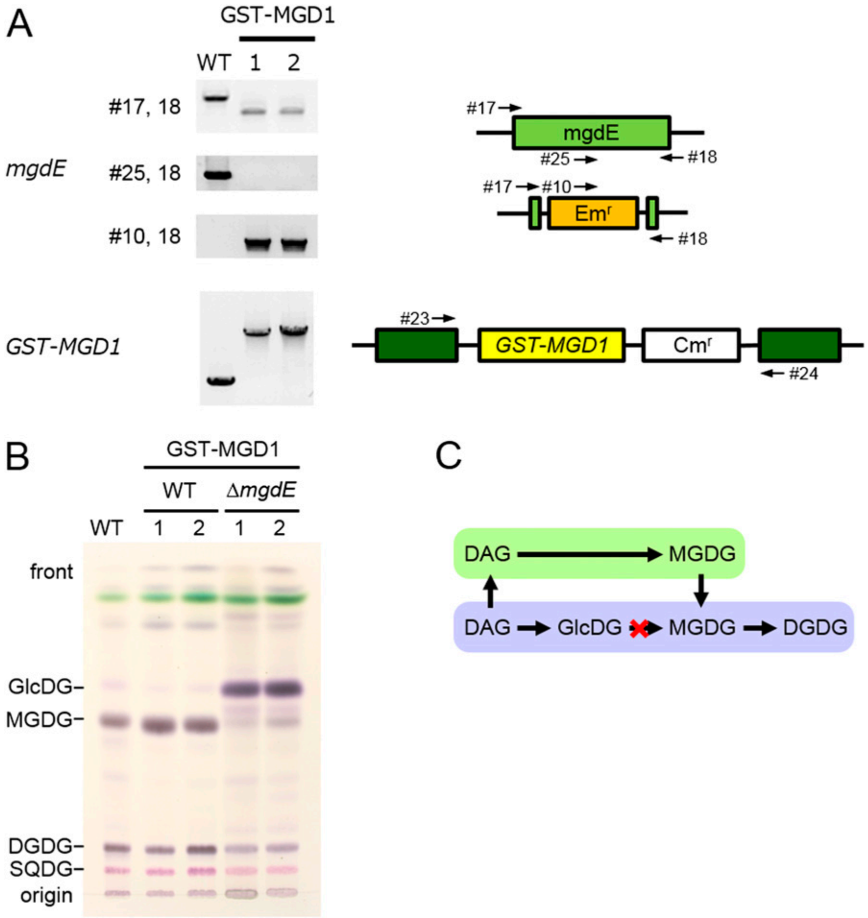

Fig. S1. Disruption of the gene for the glucolipid epimerase (*mgdE*) in the monogalactosyldiacylglycerol (MGDG) synthase gene 1 (*MGD1*) background. (*A*) The genomic *mgdE* region and *GST-MGD1* inserted in the neutral site of the *Synechocystis* sp. PCC 6803 genome (shown schematically at the right) were amplified from the wild-type and Δ*mgdE* strains by PCR with the indicated primer pairs (numbers and arrows) and were separated by agarose gel electrophoresis. (*B*) TLC analysis of lipids in the wild-type and Δ*mgdE* cells of *Synechocystis* sp. PCC 6803 expressing *GST-MGD1*. (*C*) Galactolipid synthetic pathways of the Δ*mgdE* in the *MGD1* background. DAG, diacylglycerol.

Fig. S2. Alignment of MdgE proteins. Translated amino acid sequence alignment of *mgdE* orthologs from 36 cyanobacteria strains (from top to bottom): *Nostoc azollae* strain 0708, *Nostoc punctiforme* PCC 73102, *Anabaena* PCC 7120, *Anabaena variabilis* ATCC 29413, *Synechococcus* sp. JA-3–3Ab, *Synechococcus* sp. JA-2–3B'a(2-13), *Synechococcus elongatus* PCC 6301, *Synechococcus elongatus* PCC 7942, *Synechocystis* sp. PCC 6803, *Trichodesmium erythraeum* IMS101, *Cyanobacterium* UCYN-A, *Synechococcus* sp. PCC 7002, *Microcystis aeruginosa* NIES-843, *Cyanothece* sp. PCC 7424, *Cyanothece* sp. PCC 7822, *Cyanothece* sp. PCC 8801, *Cyanothece* sp. PCC 8802, *Cyanothece* sp. ATCC 51142, *Synechococcus* sp. RCC307, *Prochlorococcus marinus* str. NATL2A, *P. marinus* str. NATL1A, *P. marinus* str. MIT 9312, *P. marinus* str. MIT 9215, *P. marinus* str. MIT 9301, *P. marinus* str. AS9601, *P. marinus* subsp. *pastoris* str. CCMP1986, *P. marinus* str. MIT 9515, *P. marinus* str. CCMP1375, *Synechococcus* sp. WH 7803, *Synechococcus* sp. CC9311, *P. marinus* str. MIT 9211, *P. marinus* str. MIT 9313, *P. marinus* str. MIT 9303, *Synechococcus* sp. CC9902, *Synechococcus* sp. WH8102, *Synechococcus* sp. CC9605. The different amino acids are color-coded.

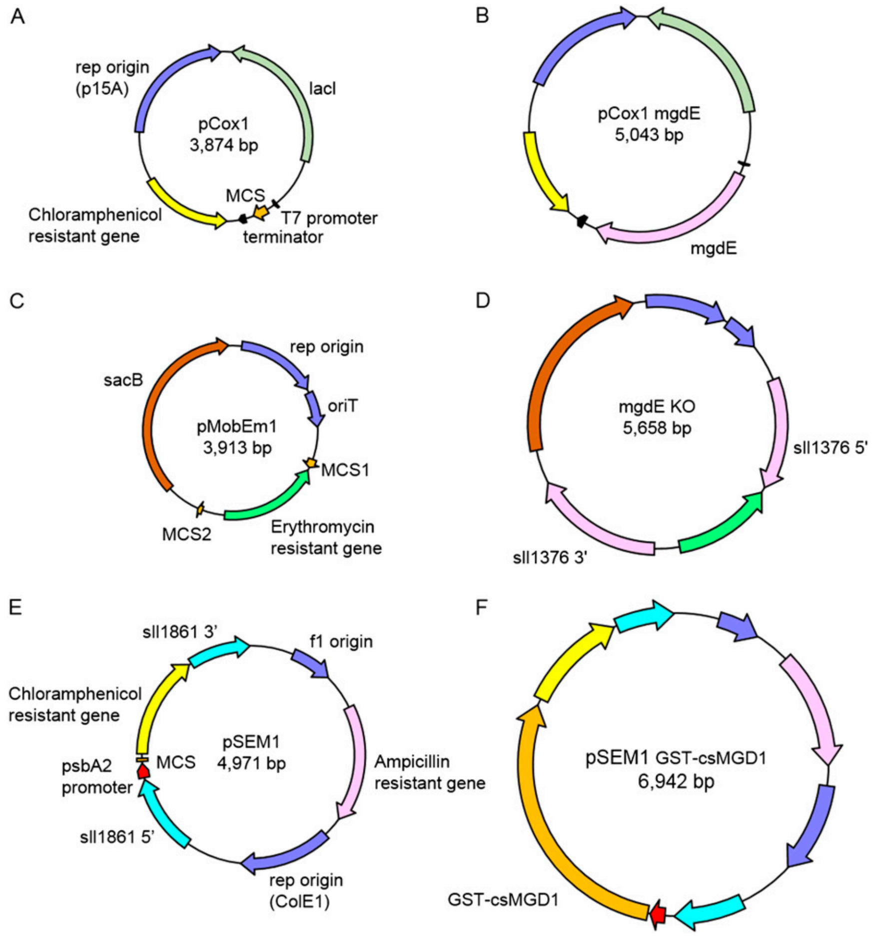

Fig. S3. DNA constructs for transformation of *Escherichia coli* and *Synechocystis* cells. (*A*) *E. coli* coexpression vector pCox1. The chloramphenicol-resistance gene and replication origin (p15A) were amplified from pACYC184, and the *lacI* gene, LacI-repressible T7 promoter, and terminator were from pET24a. (*B*) *mgdE* of *Synechocystis* sp. PCC 6803 (*sll1376*) was inserted into the SmaI site of pCox1 to make pCox1-mgdE. (*C*) Transformation vector pMobEm1. The *sacB* was from pK18mobSacB, the replication origin (*oriV*), transfer origin (*oriT*), and erythromycin-resistance gene were from pRL271, and multiple cloning sites (MCS) were from pBluscriptII SK+. (*D*) The 5′ region of *mgdE* (*sll1376*) was inserted in MCS1, and the 3′ region was inserted in MCS2 of pMobEm1 for the *mgdE* knockout vector. (*E*) *Synechocystis* expression vector pSEM1. (*F*) For expression of the plant-type MGDG synthase gene in *Synechocystis*, GST-fused cucumber *MGD1* (*CsMGD1*) was inserted downstream of the light-inducible *psbA* promoter in pSEM1.

Table S1. Synteny of *mgdA* and *mgdE*

Strain	melB	mgdE	DUF95	mgdA
Synechocystis sp. PCC 6803	sll1374	sll1376	slr1478	sll1377
Synechococcus sp. WH8102	SYNW0583	SYNW1821		SYNW0060
Synechococcus elongatus PCC 6301	syc0951_d	syc0679_d		syc0466_c
Synechococcus elongatus PCC 7942	Synpcc7942_0571	Synpcc7942_0861		Synpcc7942_1083
Synechococcus sp. CC9605	Syncc9605_2095	Syncc9605_0646		Syncc9605_0060
Synechococcus sp. CC9902	Syncc9902_0576	Syncc9902_1713		Syncc9902_0057
Synechococcus sp. CC9311	sync_2195	sync_2171		sync_0061
Synechococcus sp. RCC307	SynRCC307_0478	SynRCC307_1661		SynRCC307_0062
Synechococcus sp. WH 7803	SynWH7803_1931 SynWH7803_1136	SynWH7803_1831		SynWH7803_0061
Synechococcus sp. PCC 7002	SYNPCC7002_A2845 SYNPCC7002_A0460	SYNPCC7002_A2519		SYNPCC7002_A0533
Synechococcus sp. JA-3–3Ab	CYA_2141	CYA_0235		CYA_0245
Synechococcus sp. JA-2–3B'a(2-13)	CYB_0528	CYB_1779		CYB_1788
Thermosynechococcus elongates BP 1	tlr1521		tll1058	tlr1854
Microcystis aeruginosa NIES-843	MAE_37730	MAE_58610		MAE_58600
Cyanothece sp. PCC 8801	PCC8801_4415 PCC8801_2906	PCC8801_0598		PCC8801_0599
Cyanothece sp. ATCC 51142	cce_1874 cce_2499	cce_1989		cce_3237
Cyanothece sp. PCC 8802	Cyan8802_3190 Cyan8802_4479	Cyan8802_0613		Cyan8802_0614
Cyanothece sp. PCC 7424	PCC7424_0200 PCC7424_3387	PCC7424_3384		PCC7424_4425
Cyanothece sp. PCC 7822	Cyan7822_2100 Cyan7822_1101 Cyan7822_5851	Cyan7822_1105		Cyan7822_1106
Cyanobacterium UCYN-A		UCYN_01810		
Gloeobacter violaceus PCC 7421	gll4250 glr3959			glr2559
Anabaena sp. PCC 7120	alr3705	alr1909	alr1808	all4933
Nostoc punctiforme PCC 73102	Npun_F1762	Npun_F1048	Npun_R4113	Npun_R4124 Npun_R4706
Anabaena variabilis ATCC 29413	Ava_3583	Ava_3742	Ava_4811	Ava_2217
Nostoc azollae str. 0708		Aazo_4290		Aazo_3301
Prochlorococcus marinus subsp. pastoris str. CCMP1986	PMM1323	PMM0478		PMM1659
Prochlorococcus marinus str. MIT 9313	PMT1398	PMT1304		PMT0058
Prochlorococcus marinus str. CCMP1375	Pro1404	Pro0476		Pro1819
Prochlorococcus marinus str. MIT 9312	PMT9312_1421	PMT9312_0478		PMT9312_1751
Prochlorococcus marinus str. NATL2A	PMN2A_0894	PMN2A_1810		PMN2A_1257
Prochlorococcus marinus str. MIT 9301	P9301_15091	P9301_05031		P9301_18491
Prochlorococcus marinus str. MIT 9303	P9303_05681	P9303_06851		P9303_00631
Prochlorococcus marinus str. MIT 9515	P9515_14841	P9515_05411		P9515_18491
Prochlorococcus marinus str. NATL1A	NATL1_17501	NATL1_05341		NATL1_21281
Prochlorococcus marinus str. AS9601	A9601_15231	A9601_05331		A9601_18681
Prochlorococcus marinus str. MIT 9211	P9211_13771	P9211_04781		P9211_17851
Prochlorococcus marinus str. MIT 9215	P9215_15521	P9215_05591		P9215_19321
Trichodesmium erythraeum IMS101	Tery_3858 Tery_2105	Tery_1743		Tery_2749
Acaryochloris marina MBIC11017	AM1_3342		AM1_3172	AM1_4576

The genes *melB*, *mgdE*, *DUF95*, and *mgdA* are aligned as a gene cluster in the genome of *Synechocystis* sp. PCC 6803. Orthologs of these genes in various cyanobacterial genomes are listed. The orthologs that are present at the same positions of the corresponding genes in the gene cluster of *Synechocystis* sp. PCC 6803 are highlighted in green. A blank column indicates there is no significant ortholog in the corresponding genomes. Pink highlighting indicates that *Thermosynechococcus elongates* BP 1, *Gloeobacter violaceus* PCC 7421, and *Acaryochloris marina* MBIC11017 do not have significant orthologs of mgdE, even though these bacteria have orthologs of mgdA.

Table S2. Primers used in this study

	Primer name	Sequence
1	lacI-MCS Fw	CTTATCATCGGTCGAGATCCCG
2	lacI-MCS Rv	ATAAGCGGATATAGTTCCTCCTTTCAG
3	Cm ori Fw	ACTATATCCGCTTATTATCACTTATTCAGG
4	Cm ori Rv	TCGACCGATGATAAGCTGTCAAACATGAG
5	oriTV Fw	CGATGGGCCAAAATCCCTTAACGTGAG
6	oriTV Rv	CATAGCTGTTTCCTGCCTCGCGCGTTTC
7	SacB Fw	GGCCGTCGTTTTACAAGTAAATCGCGCGGGTTTG
8	SacB Rv	GATTTTGGCCCATCGGCATTTTCTTTTG
9	Em-Fw	AAGCTTCGCGTGCTATAATTATACTAA
10	Em-Rv	AAGCTTGACCTGCATCCCTTAAC
11	M13 forward	TGTAAAACGACGGCCAG
12	M13 reverse	CAGGAAACAGCTATGAC
13	MCS2Fw	GGAATTATAACCATATGTCGCGACTAGTGCATGCC
14	MCS2Rv	GATCTGCTAGCCATACCGCGGCATGCACTAGTCGCG
15	GST-Fw1	CCCGGGATGTCCCCTATACTAGGTTATTG
16	CsMGD1Rv	CCCGGGTCACGATGAATTCCCGCC
17	sll1376 Fw	AAGGAGATATACATATGGCAATGGCTTGGTTAATG
18	sll1376 Rv	GTGCGGCCGCAAGCTAGGACTTTTTGGTGAAGC
19	sll1376 KO 5′ Fw	ACTAGTGGATCCCCCGCATTCCTGGGGA
20	sll1376 KO 5′ Rv	GAATTCCTGCAGCCCACAGCACCGAAGC
21	sll1376 KO 3′ Fw	TCGACCTCGAGGGGGTAAAACGGGATAGC
22	sll1376 KO 3′ Rv	GCGAATTGGGTACCGGGCTACTTCCGC
23	pSEM1 Fw	CCCCTTCCTGATTTACAGG
24	pSEM1 Rv	GGGAGTAACGCTTGTCAC
25	sll1376 mid	CCATTACGTCCTGTGGTC