Supplementary Material

Construction of Global Acyl Lipid Metabolic Map by Comparative Genomics and Subcellular Localization Analysis in the Red Alga Cyanidioschyzon merolae

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1 Supplementary Data

1.1 Supplementary Data 1. Results of Subcellular Localization Analysis of Other Enzymes Related to Acyl Lipid Metabolism in C. merolae

1.1.1 Lipase

By comparative genomic analysis, 20 enzymes involved in lipid degradation were detected in the genomic data of C. merolae (Supplementary Table 3). Eight enzymes were putative triacylglycerol lipases (TAGL). Six enzymes were also found as hydrolases that might act as lipases. Most of putative TAGLs and hydrolases were targeted to the cytosol or ER, but two TAGLs (CMP157C and CMR088C) and a hydrolase (CMT308C) were localized in the plastid (Supplementary Figure 5A), suggesting that these enzymes might be involved in the degradation of plastid membrane lipids. Additionally, C. merolae has three phospholipases A1 (PLA1; CMP267C, CMH204C and CMQ413C) and two phospholipases A2 (PLA2; CMR500C and CMT312C). In PLA1, CMP267C and CMH204C were targeted to the cytosol, whereas the fluorescence of GFP-fused CMQ413C was observed in both the cytoplasmic membrane and vesicle (Supplementary Figure 5A). C. merolae PLA1s are similar to the putative PA-preferring PLA1 in A. thaliana (Kato et al., 2002). In PLA2, CMR500C showed dual localization to the plastid and the nucleus, whereas CMT312C was localized in both the plastid and the ER (Supplementary Figure 5A). It seems that these enzymes are components of the acyl editing cycle (Lager et al., 2013; Pan et al., 2015) with lysophospholipid acyltransferase (LPLAT) encoded by CMI139C and CMR130C (Supplementary Figure 3). The existence of acyl editing cycle in C. merolae is supported by the finding that radioactive carbons were quickly incorporated into PC (Sato and Moriyama, 2007).

1.1.2 Lipid Trafficking

It is thought that TGD1-3 complex and TGD4 function as lipid transporters in the plastid envelopes (Hurlock et al., 2014). C. merolae has putative TGD1 and TGD2 homologs encoded in the plastid genome, and four putative TGD3 homologs (CMR180C, CMH235C, CMJ039C, and CMR388C), whereas no TGD4 homologs were detected (Supplementary Table 3). By subcellular localization analysis, CMR180C, CMJ039C and CMH235C were targeted to the ER, but CMR388C was
localized in the plastid (Supplementary Figure 5B). It is likely that CMR388C is the TGD3 ortholog in *C. merolae*.

Acyl-CoA binding proteins (ACBP) are thought to transfer fatty acids from the plastid to the ER in plants (Xiao and Chye, 2011). In *A. thaliana*, six isoforms, ACBP1-6, have been identified. *C. merolae* has a gene encoding ACBP6 ortholog (CMP278C, Supplementary Table 3). *A. thaliana* ACBP6 is a cytosolic, smallest ACBP (Xiao and Chye, 2011). *C. merolae* ACBP was also localized in the cytosol (Supplementary Figure 5B).

Flippase catalyzes the movement of polar lipids between the two membrane leaflets that does not happen spontaneously, which finally results in asymmetric distribution of lipids between the two leaflets of a membrane. In *A. thaliana*, the P4 subfamily of ATPases, ALA1-12, are believed to act as flippases (Gomès et al., 2000). Among them, ALA1-3 already have been characterized (López-Marqués et al., 2010, 2012; Poulsen et al., 2008). Additionally, ALA-interacting subunit (ALIS) proteins are involved in the determination of subcellular localization of ALA enzymes (López-Marqués et al., 2010, 2012; Poulsen et al., 2008). In *C. merolae*, two flippases (ALA1; CMR306C and ALA2; CMS375C) and an ALIS protein (CMT246C) were detected by the Gelust analysis (Supplementary Table 3). Both ALA1 and ALIS proteins were dually localized to the ER and cytoplasmic membrane (Figure 1D and Supplementary Figure 5B). ALA2 was targeted to the nucleus and cytosol (Supplementary Figure 5B).

### 1.1.3 Fatty Acid Activation, PI Signaling and Biotin-Dependent Carboxylation

Two genes encoding acyl-CoA thioesterases (ACT; CMJ263C and CMR113C) and five genes encoding long-chain acyl-CoA synthetases (LACS; CME186C, CMG147C, CMO037C, CML197C and CMT459C) were found in the genomic data of *C. merolae* (Supplementary Table 3). Both ACT enzymes were targeted to the mitochondrion (Supplementary Figure 6A). In LACSs, CME186C, CMG147C, CMO037C were localized in the cytosol, but CML197C showed dual localization to the cytosol and plastid (Supplementary Figure 6A). GFP-fused CMT459C was localized in the plastid (Supplementary Figure 6B).

*C. merolae* has two each genes encoding phosphatidylinositol-4-kinases (PI4K; CM1125C and CMS267C) and phosphatidylinositol-4-phosphate 5-kinases (PIP5K; CMN333C and CME153C), which are involved in the PI signaling (Supplementary Table 3). CM1125C and CMN333C were localized in the ER, whereas CMS267C and CME157C were targeted to both cytosol and cytoplasmic membrane (Supplementary Figure 6B).

Biotin is attached to biotin-dependent enzymes, such as carboxylases or decarboxylases, by posttranslational modification catalyzed by holocarboxylase synthetase (HCS). In *C. merolae*, HCS (CMC080C) was dually localized in the plastid and cytosol, as in plants (Supplementary Figure 6C). Additionally, we analyzed subcellular localization of typical biotin-dependent enzymes, namely methylcrotonyl-CoA carboxylase (MCC), propionyl-CoA carboxylase (PCC) and carbamoylphosphate synthase (CAR). Individual subunits of MCC (MCCA; CMT073C and MCCB; CMT071C) and PCC (PCCA; CMN243C and PCCB; CMM132C) were localized in the mitochondrion (Supplementary Figure 6C). *C. merolae* has two types of CARs involved in pyrimidine synthesis and arginine synthesis. They are multifunctional type and multisubunit type, respectively. Subcellular localization of a multifunctional type CAR (CAR1; CMQ255C) was not examined yet. In multisubunit type CAR, the small subunit (CarA) is encoded in the plastid genome,
and was not analyzed. A product of nuclear CarB gene (CML055C) encoding the large subunit of CAR was targeted in the plastid as expected (Supplementary Figure 6C).

1.1.4 References

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2 Supplementary Figures and Tables

2.1 Supplementary Figures

Supplementary Figure 1. A schematic diagram of lipid metabolism in plants (Li-Beisson et al., 2013).
Supplementary Figure 2. Subcellular localization of enzymes related to fatty acid synthesis, fatty acid elongation, desaturation and plastidic lipid synthesis in *C. merolae*. These fluorescence micrographs show *C. merolae* cells transiently expressing GFP- or HA-fused protein related to fatty
acid synthesis (A), desaturation (B), fatty acid elongation (C), and synthesis of glycolipids and PG (D). Abbreviation of enzyme names is indicated to Table 1. Subcellular localization of CMM311C was not analyzed, because amino acid sequence of this protein is identical to CMS056C. GPAT (CMJ027C) and mtKAS (CML329C) were examined subcellular localization using HA-tag construct. Asterisked enzymes of subcellular localization were detected by immunostained with anti-GFP antibody. DIC; Nomarski differential interference contrast, Chlorophyll; phycobilin and chlorophyll autofluorescences, GFP; GFP fluorescence or immunofluorescence using anti-GFP or anti-HA tag antibody, Merge; merged images of phycobilin and chlorophyll autofluorescences as well as green fluorescence. Bar = 2 µm.
Supplementary Figure 3. Subcellular localization of enzymes related to synthesis of phospholipids and TAG in *C. merolae*. These fluorescence micrographs show *C. merolae* cells transiently expressing GFP-fused protein related to synthesis of phospholipids and TAG. Abbreviation of enzyme names is indicated to Table 1. Asterisked enzymes of subcellular localization were detected by immunostained with anti-GFP antibody. DIC; Nomarski differential interference contrast, Chlorophyll; phycobilin and chlorophyll autofluorescences, GFP; GFP fluorescence or immunofluorescence using anti-GFP antibody, Merge; merged images of phycobilin and chlorophyll autofluorescences as well as green fluorescence. Bar = 2 µm.
**Supplementary Figure 4. Subcellular localization of enzymes related to β-oxidation.**

Fluorescence micrographs show *C. merolae* cells transiently expressing GFP-fused protein related to β-oxidation (A). Three β-oxidation enzymes were examined subcellular localization using constructs of GFP-fused C-terminal peptide (B). Abbreviation of enzyme names is indicated to Table 1. DIC; Nomarski differential interference contrast, Chlorophyll; phycobilin and chlorophyll autofluorescences, GFP; GFP fluorescence, Merge; merged images of phycobilin and chlorophyll autofluorescences as well as green fluorescence. Bar = 2 µm.
Supplementary Figure 5. Subcellular localization of enzymes related to lipid degradation, lipid trafficking in *C. merolae*. Fluorescence micrographs show *C. merolae* cells transiently expressing GFP-fused protein related to lipid degradation (A) and lipid trafficking (B). Abbreviation of enzyme names is indicated to Supplementary Table 3. Asterisked enzymes of subcellular localization were detected by immunostained with anti-GFP antibody. DIC; Nomarski differential interference contrast, Chlorophyll; phycobilin and chlorophyll autofluorescences, GFP; GFP fluorescence or immunofluorescence using anti-GFP antibody, Merge; merged images of phycobilin and chlorophyll autofluorescences as well as green fluorescence. Bar = 2 µm.

| Enzyme Name | DIC | Chlorophyll | GFP | Merge |
|-------------|-----|-------------|-----|-------|
| TAGL* (CMT151C) | ![Image] | ![Image] | ![Image] | ![Image]  |
| TAGL (CMP157C) | ![Image] | ![Image] | ![Image] | ![Image]  |
| TAGL* (CMS254C) | ![Image] | ![Image] | ![Image] | ![Image]  |
| TAGL (CMH156C) | ![Image] | ![Image] | ![Image] | ![Image]  |
| TAGL* (CMA056C) | ![Image] | ![Image] | ![Image] | ![Image]  |
| TAGL (CMR088C) | ![Image] | ![Image] | ![Image] | ![Image]  |
| TAGL (CMJ049C) | ![Image] | ![Image] | ![Image] | ![Image]  |
| TAGL (CMF053C) | ![Image] | ![Image] | ![Image] | ![Image]  |
| PLA1* (CMP267C) | ![Image] | ![Image] | ![Image] | ![Image]  |
| PLA1* (CMH204C) | ![Image] | ![Image] | ![Image] | ![Image]  |
| PLA1 (CMQ413C) | ![Image] | ![Image] | ![Image] | ![Image]  |
| PLA2* (CMR500C) | ![Image] | ![Image] | ![Image] | ![Image]  |
| PLA2 (CMT312C) | ![Image] | ![Image] | ![Image] | ![Image]  |
| PLAP (CMC146C) | ![Image] | ![Image] | ![Image] | ![Image]  |
| Hydrolase* (CMT274C) | ![Image] | ![Image] | ![Image] | ![Image]  |
| Hydrolase (CMP032C) | ![Image] | ![Image] | ![Image] | ![Image]  |
| Hydrolase (CMT308C) | ![Image] | ![Image] | ![Image] | ![Image]  |
| Hydrolase* (CML191C) | ![Image] | ![Image] | ![Image] | ![Image]  |
| Hydrolase* (CML191C) | ![Image] | ![Image] | ![Image] | ![Image]  |
| Hydrolase* (CMS228C) | ![Image] | ![Image] | ![Image] | ![Image]  |
| TGD3 (CMR180C) | ![Image] | ![Image] | ![Image] | ![Image]  |
| TGD3 (CMH235C) | ![Image] | ![Image] | ![Image] | ![Image]  |
| TGD3* (CMJ039C) | ![Image] | ![Image] | ![Image] | ![Image]  |
| TGD3 (CMR388C) | ![Image] | ![Image] | ![Image] | ![Image]  |
| ACBP (CMP278C) | ![Image] | ![Image] | ![Image] | ![Image]  |
| ALA2 (CMS375C) | ![Image] | ![Image] | ![Image] | ![Image]  |
| ALIS (CMT248C) | ![Image] | ![Image] | ![Image] | ![Image]  |
Supplementary Figure 6. Subcellular localization of enzymes related to fatty acid activation, PI signaling and biotin-dependent carboxylation in C. merolae. These fluorescence micrographs show C. merolae cells transiently expressing GFP-fused protein related to fatty acid activation (A), PI signaling (B) and biotin-dependent carboxylation (C). Abbreviation of enzyme names is indicated to Supplementary Table 3. Asterisked enzymes of subcellular localization were detected by immunostained with anti-GFP antibody. DIC; Nomarski differential interference contrast, Chlorophyll; phycobilin and chlorophyll autofluorescences, GFP; GFP fluorescence or immunofluorescence using anti-GFP antibody, Merge; merged images of phycobilin and chlorophyll autofluorescences as well as green fluorescence. Bar = 2 µm.
2.2 Supplementary Tables

**Supplementary Table 1. List of primers used for making of EGFP or HA tag constructs.**

A part of uppercase letters of sequence of primers indicates common sequences of pCEG1 or pBSHA-β′ vector required for the cloning using the In-Fusion Cloning Kit (Clontech laboratories, Mountain View, CA, USA). Because a peptide sequence of CMS056C and CMM311C is identical, the same primer is used for subcellular localization analysis of these proteins.

| Locus tag | Sequence of forward primer (5’ to 3’) | Sequence of reverse primer (5’ to 3’) | Cloned length (aa) | Full length of enzyme (aa) |
|-----------|---------------------------------------|---------------------------------------|--------------------|---------------------------|
| CMK217C   | TTCGTTGACCTCTAGAatgctttttgtaecaaectg | CCATGGATCCTCTAGAgtgcaegcecgagagacttc | 103                | 673                       |
| CMA017C   | TTCGTTGACCTCTAGAatggcgagcaacaacgecaaa | CCATGGATCCTCTAGAegtagataaactctggtggtca | 110                | 556                       |
| CMJ021C   | TTCGTTGACCTCTTAGAattgatctggatcatacagcg | CCATGGATCCTCTAGAagcagctgatgaataccag | 32                 | 202                       |
| CMF185C   | TTCGTTGACCTCTTAGAattgacagtcatgctggggtc | CCATGGATCCTCTAGAegcgagtctgagcctgca | 91                 | 373                       |
| CMS008C   | TTCGTTGACCTCTAGAatgaaacagcacctctact  | CCATGGATCCTCTAGAaaacgggttccgaccagtt | 103                | 430                       |
| CMR054C   | TTCGTTGACCTCTAGAatggcagcgaccaccgccaa | CCATGGATCCTCTAGAacgggttccgagcactcnggat | 69                 | 294                       |
| CMR488C   | TTCGTTGACCTCTAGAatggcagcgaccaccgccaa | CCATGGATCCTCTAGAacgggttccgagcactcnggat | 60                 | 302                       |
| CMT106C   | TTCGTTGACCTCTAGAattgatctggatcatacagcg | CCATGGATCCTCTAGAagcagctgatgaataccag | 74                 | 363                       |
| CMT239C   | TTCGTTGACCTCTAGAattgatctggatcatacagcg | CCATGGATCCTCTAGAagcagctgatgaataccag | 142                | 532                       |
| CMN061C   | TTCGTTGACCTCTAGAattgatctggatcatacagcg | CCATGGATCCTCTAGAagcagctgatgaataccag | 75                 | 944                       |
| CMT267C   | TTCGTTGACCTCTAGAattgatctggatcatacagcg | CCATGGATCCTCTAGAagcagctgatgaataccag | 73                 | 450                       |
| CMI271C   | TTCGTTGACCTCTAGAatgatctggatcatacagcg | CCATGGATCCTCTAGAagcagctgatgaataccag | 66                 | 683                       |
| CMR012C   | TTCGTTGACCTCTAGAatgatctggatcatacagcg | CCATGGATCCTCTAGAagcagctgatgaataccag | 80                 | 511                       |
| CMR015C   | TTCGTTGACCTCTAGAatgatctggatcatacagcg | CCATGGATCCTCTAGAagcagctgatgaataccag | 100                | 517                       |
| CMN215C   | TTCGTTGACCTCTAGAatgatctggatcatacagcg | CCATGGATCCTCTAGAagcagctgatgaataccag | 90                 | 542                       |
| CMS056C, CMM311C | TTCGTTGACCTCTAGAatgatctggatcatacagcg | CCATGGATCCTCTAGAagcagctgatgaataccag | 133                | 439                       |
| CMJ134C   | TTCGTTGACCTCTAGAatgatctggatcatacagcg | CCATGGATCCTCTAGAagcagctgatgaataccag | 248                | 429                       |
| CMN196C   | TTCGTTGACCTCTAGAatgatctggatcatacagcg | CCATGGATCCTCTAGAagcagctgatgaataccag | 33                 | 208                       |
| CMM125C   | TTCGTTGACCTCTAGAatgatctggatcatacagcg | CCATGGATCCTCTAGAagcagctgatgaataccag | 33                 | 237                       |
| CMR011C   | TTCGTTGACCTCTAGAatgatctggatcatacagcg | CCATGGATCCTCTAGAagcagctgatgaataccag | 84                 | 385                       |
| CMS052C   | TTCGTTGACCTCTAGAatgatctggatcatacagcg | CCATGGATCCTCTAGAagcagctgatgaataccag | 92                 | 442                       |
CMF133C TTCGTTGACCTCTAGAatgtcactctggtgttaa CCATGGATCCTCTAGAaagctcttcagttgacaaat 78 554
CMF090C TTCGTTGACCTCTAGAatggagttaccgacggttcggaaat CCATGGATCCTCTAGAaagctcttcagttgacaaat 102 749
CMI139C TTCGTTGACCTCTAGAatgatacgttaggagctcg CCATGGATCCTCTAGAaagctcttcagttgacaaat 66 264
CMA134C TTCGTTGACCTCTAGAatgttggcacttgatcgctg CCATGGATCCTCTAGAagctcttcagttgacaaat 99 311
CMQ199C TTCGTTGACCTCTAGAatgcgtgcttgtgtgcgctg CCATGGATCCTCTAGAagctcttcagttgacaaat 130 572
CMJ039C TTCGTTGACCTCTAGAatgggactggtctcttttcg CCATGGATCCTCTAGAagctcttcagttgacaaat 67 1055
CMJ162C TTCGTTGACCTCTAGAatgcctctacggacctacat CCATGGATCCTCTAGAagctcttcagttgacaaat 88 310
CMM188C TTCGTTGACCTCTAGAatgggggatttgggtgaaca CCATGGATCCTCTAGAagctcttcagttgacaaat 38 1316
CMS299C TTCGTTGACCTCTAGAatgcggcctttcggttccgc CCATGGATCCTCTAGAagctcttcagttgacaaat 33 590
CMS375C TTCGTTGACCTCTAGAatggacccacagcgacgtac CCATGGATCCTCTAGAagctcttcagttgacaaat 168 1157
CML055C TTCGTTGACCTCTAGAatgcaaacgatcaactgcag CCATGGATCCTCTAGAagctcttcagttgacaaat 146 1316
CMJ039C TTCGTTGACCTCTAGAatgggactggtctcttttcg CCATGGATCCTCTAGAagctcttcagttgacaaat 88 506
CMJ162C TTCGTTGACCTCTAGAatgcctctacggacctacat CCATGGATCCTCTAGAagctcttcagttgacaaat 88 310
| Name    | Sequence 1 | Sequence 2 | Sequence 3 | Sequence 4 | Sequence 5 | Sequence 6 |
|---------|------------|------------|------------|------------|------------|------------|
| CMM286C | TTCGTTGACCTCTAGAatggtt cacactgt | CCATGGATCCTCTAGAatggtt cacactgt | 80 | 490 |
| CMD118C | TTCGTTGACCTCTAGAatggtt cacactgt | CCATGGATCCTCTAGAatggtt cacactgt | 138 | 549 |
| CMT420C | TTCGTTGACCTCTAGAatggtt cacactgt | CCATGGATCCTCTAGAatggtt cacactgt | 81 | 398 |
| CMK172C | TTCGTTGACCTCTAGAatggtt cacactgt | CCATGGATCCTCTAGAatggtt cacactgt | 69 | 327 |
| CMS393C | TTCGTTGACCTCTAGAatggtt cacactgt | CCATGGATCCTCTAGAatggtt cacactgt | 88 | 321 |
| CMR006C | TTCGTTGACCTCTAGAatggtt cacactgt | CCATGGATCCTCTAGAatggtt cacactgt | 65 | 303 |
| CMT381C | TTCGTTGACCTCTAGAatggtt cacactgt | CCATGGATCCTCTAGAatggtt cacactgt | 84 | 332 |
| CMD146C | TTCGTTGACCTCTAGAatggtt cacactgt | CCATGGATCCTCTAGAatggtt cacactgt | 97 | 311 |
| CMH111C | TTCGTTGACCTCTAGAatggtt cacactgt | CCATGGATCCTCTAGAatggtt cacactgt | 100 | 240 |
| CMI240C | TTCGTTGACCTCTAGAatggtt cacactgt | CCATGGATCCTCTAGAatggtt cacactgt | 59 | 656 |
| CMG147C | TTCGTTGACCTCTAGAatggtt cacactgt | CCATGGATCCTCTAGAatggtt cacactgt | 95 | 230 |
| CMD146C | TTCGTTGACCTCTAGAatggtt cacactgt | CCATGGATCCTCTAGAatggtt cacactgt | 103 | 499 |
| CMK291C | TTCGTTGACCTCTAGAatggtt cacactgt | CCATGGATCCTCTAGAatggtt cacactgt | 72 | 499 |
| CMK115C | TTCGTTGACCTCTAGAatggtt cacactgt | CCATGGATCCTCTAGAatggtt cacactgt | 87 | 773 |
| CML080C | TTCGTTGACCTCTAGAatggtt cacactgt | CCATGGATCCTCTAGAatggtt cacactgt | 58 | 450 |
| CMT072C | TTCGTTGACCTCTAGAatggtt cacactgt | CCATGGATCCTCTAGAatggtt cacactgt | 151 | 632 |
| CMK139C | TTCGTTGACCTCTAGAatggtt cacactgt | CCATGGATCCTCTAGAatggtt cacactgt | 68 | 300 |
| Code   | Sequence 1 | Sequence 2 | Length 1 | Length 2 |
|--------|------------|------------|----------|----------|
| CMT074C| TTCGTTGACCTCTAGAatgtcagagctgtcattctg | CCATGGATCCTCTAGAAtgctcgttccggccggttca | 98       | 347      |
| CMC137C| TTCGTTGACCTCTAGAatgcaggttcctaggattgt | CCATGGATCCTCTAGAatgcaggttcctaggattgt | 52       | 256      |
| CMR380C| TTCGTTGACCTCTAGAatgtcagagctgtcattctg | CCATGGATCCTCTAGAAtgctcgttccggccggttca | 115      | 1145     |
| CMA042C| TTCGTTGACCTCTAGAatgacgggagagagtgtgag | CCATGGATCCTCTAGAatgcaggttcctaggattgt | 46       | 438      |
| CME087C| TTCGTTGACCTCTAGAatgctgtgttctcgtggcac | CCATGGATCCTCTAGAatgacgggagagagtgtgag | 96       | 584      |
| CMS254C| TTCGTTGACCTCTAGAatggtggcggtaggcaagtt | CCATGGATCCTCTAGAatgctgtgttctcgtggcac | 144      | 778      |
| CMJ049C| TTCGTTGACCTCTAGAatgtccacaaaacgtgcagg | CCATGGATCCTCTAGAatggtggcggtaggcaagtt | 150      | 1117     |
| CML191C| TTCGTTGACCTCTAGAatggaagatatgggaagttt | CCATGGATCCTCTAGAatggtggcggtaggcaagtt | 150      | 780      |
| CMP032C| TTCGTTGACCTCTAGAatgcaggttcctaggattgt | CCATGGATCCTCTAGAatggtggcggtaggcaagtt | 150      | 547      |
| CMT312C| TTCGTTGACCTCTAGAatgttcgtgcagagaacgtt | CCATGGATCCTCTAGAatggtggcggtaggcaagtt | 150      | 1035     |
| CMR500C| TTCGTTGACCTCTAGAatggtggcggtaggcaagtt | CCATGGATCCTCTAGAatggtggcggtaggcaagtt | 150      | 1284     |
| CMP278C| TTCGTTGACCTCTAGAatggtggcggtaggcaagtt | CCATGGATCCTCTAGAatggtggcggtaggcaagtt | 150      | 1284     |
| CMS372C| TTCGTTGACCTCTAGAatgacgctcgttacagtagc | CCATGGATCCTCTAGAatggtggcggtaggcaagtt | 150      | 292      |
| CMT151C| TTCGTTGACCTCTAGAatgcaggttcctaggattgt | CCATGGATCCTCTAGAatggtggcggtaggcaagtt | 150      | 1035     |
| CMC146C| TTCGTTGACCTCTAGAatgcaggttcctaggattgt | CCATGGATCCTCTAGAatggtggcggtaggcaagtt | 150      | 1284     |
| CMS228C| TTCGTTGACCTCTAGAatggtggcggtaggcaagtt | CCATGGATCCTCTAGAatggtggcggtaggcaagtt | 150      | 1284     |
| CMO177C| TTCGTTGACCTCTAGAatggtggcggtaggcaagtt | CCATGGATCCTCTAGAatggtggcggtaggcaagtt | 150      | 292      |
| CMR088C| TTCGTTGACCTCTAGAatgcaggttcctaggattgt | CCATGGATCCTCTAGAatggtggcggtaggcaagtt | 150      | 1035     |
| CMF053C| TTCGTTGACCTCTAGAatgcaggttcctaggattgt | CCATGGATCCTCTAGAatggtggcggtaggcaagtt | 150      | 1284     |

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| Construct | Sequence 1 | Sequence 2 | Length 1 | Length 2 |
|-----------|------------|------------|----------|----------|
| CMB069C   | TTCGTTGACCTCTAGAatggctttttgtgattttag | CATGGATCCCTCTAGAgtctgtgtaatgcatactca | 188      | 1040     |
| CMP267C   | TCGTTGACCTCTAGAatgctttttgcgatttgaga | CATGGATCCCTCTAGAaecgtctacggacaa | 180      | 944      |
| CMH204C   | TCGTTGACCTCTAGAatggcgcgcttttttgacca | CATGGATCCCTCTAGAaeseaaacacacaatctct | 192      | 889      |
| CMQ413C   | TCGTTGACCTCTAGAatgggttgtacttgcgggga | CATGGATCCCTCTAGAataacacgcatgggagca | 112      | 388      |

**HA tag construct**

| Construct | Sequence 1 | Sequence 2 | Length 1 | Length 2 |
|-----------|------------|------------|----------|----------|
| CMJ027C   | CTGCAGTTAATTAATAgatgtgggtgttcctttttt | TGGGTAATTAATTAAtttgcgcgcttcgtaaccca | 452      | 452      |
| CML329C   | CTGCAGTTAATTAATAgatgctgacgaacttgtcca | TGGGTAATTAATTAAGttgatagcgctcct | 477      | 477      |

**EGFP-C-terminal peptide construct**

| Construct | Sequence 1 | Sequence 2 | Length 1 | Length 2 |
|-----------|------------|------------|----------|----------|
| CMK115C   | GACGAGCTGTACAGAGcgcacatttccaaataa | CGGCGCGCGCGCTTTAaagataegctcgctcgccgaa | 67       | 773      |
| CMR380C   | GACGAGCTGTACAAGatgtgcccgcagcttgcgaa | CGGCGCGCGCGCTTTAaagataegctcgctcgccgaa | 67       | 1145     |
| CMA042C   | GACGAGCTGTACAGAGggtggtcctcggggtcct | CGGCGCGCGCGCTTTAaagataegctcgctcgccgaa | 67       | 438      |
**Supplementary Table 3. A summary of other enzymes related to acyl lipid metabolism in C. merolae.** This table is a list of acyl lipid metabolic enzymes in *C. merolae*. Column 4 indicates the results of subcellular localization analysis in this study. Column 5 is a summary of the results of prediction of subcellular localization using three different programs. TargetP and the PredAlgo can predict plastidic, mitochondrial and secretory pathway proteins. Proteins predicted as targeted to other subcellular compartments were indicated “Other”. WoLF PSORT can predict various subcellular localizations of proteins. Results of prediction using the WoLF PSORT indicated subcellular localization(s) having the highest score. Abbreviations: CM; cytoplasmic membrane, Cyt; cytosol, ER; endoplasmic reticulum, Ext; extracellular, Mt; mitochondrion, Nuc; nucleus, Per; peroxisome, PM; Plasma membrane, Pt; plastid, Pt-genome; genes encoded in the plastid genome, SP; secretary pathway, Ves; vesicle.

| 1. Enzyme name | 2. Abbreviation of enzyme name and/or gene name | 3. Locus tag | 4. Subcellular localization | 5. Result of prediction of subcellular localization |
|----------------|-----------------------------------------------|-------------|-----------------------------|-----------------------------------------------|
| Lipase         |                                               |             |                             | TargetP | WoLF PSORT | PredAlgo |
| Triacylglycerol lipase | TAGL  | CMT151C | ER | Pt | Nuc, PM | Pt |
| Triacylglycerol lipase |              | CMP157C | Pt | Mt | Pt | Pt |
| Triacylglycerol lipase |              | CMS254C | ER | SP | PM | Pt |
| Triacylglycerol lipase |              | CMH156C | Cyt | Other | Cyt | Other |
| Triacylglycerol lipase |              | CMA056C | ER | Mt | Pt | SP |
| Triacylglycerol lipase |              | CMR088C | Pt | Mt | Mt | Pt |
| Triacylglycerol lipase |              | CMJ049C | ER | Mt | ER | Other |
| Triacylglycerol lipase |              | CMF053C | ER, CM | Mt | Nuc | Pt |
| Phospholipase A1 (PA-prefering phospholipase A1?) | PLA1 |             | Cyt | Other | Nuc | Other |
| Phospholipase A1 (PA-prefering phospholipase A1?) |              | CMH204C | Cyt | Other | PM | Other |
| Phospholipase A1 (PA-prefering phospholipase A1?) |              | CMQ413C | Ves, CM | Other | Nuc | Pt |
| Phospholipase A2 | PLA2 |             | Nuc, Pt | Pt | Nuc | Pt |
| Phospholipase A2 |              | CMR500C | Nuc | Pt | Nuc | Pt |
| Phospholipase A2 activating protein | PLAP |             | ER, Pt | Other | Pt | Other |
| Phospholipase C | PLC |             | Not detected | Other | Pt | Other |
| Phospholipase D | PLD |             | Not detected | Other | Pt | Other |
| Hydrolase (Lipase?) | - |             | Cyt | Other | Cyt | Other |
| Lipid trafficking |                   |             | Cyt | Other | Ext | Other |
| Phosphatidic acid transporter (TGD1-3 complex) | TGD1 | CMV212C | Pt-genome |             |             |             |
| Phosphatidic acid transporter (TGD1-3 complex) | TGD2 | CMV057C | Pt-genome |             |             |             |
| Phosphatidic acid transporter (TGD1-3 complex) | TGD3 | CMR180C | ER | Mt | Pt | Mt |
| Phosphatidic acid transporter (TGD1-3 complex) | TGD3 | CMH235C | ER | Other | PM | Other |
| Phosphatidic acid transporter (TGD1-3 complex) | TGD3 | CMJ039C | ER | Mt | PM | SP |
| Phosphatidic acid transporter (TGD1-3 complex) | TGD3 | CMR388C | Pt | Pt | Pt | Pt |
| Phosphatidic acid transporter (TGD4) | TGD4 | Not detected |             |             |             |             |
| Acyl-CoA binding protein | ACBP |             | Cyt | Other | Pt | Other |
| Flippase (P4 type-ATPase) | ALA1 | CMR306C | ER, CM | Other | PM | Other |
| Category                          | Protein | Location | Function |
|----------------------------------|---------|----------|----------|
| **ALA-interacting subunit**      | ALA2    | Cyt, Nuc | Mt, Cyt  |
| ALIS                             | CMS375C | ER, CM   | Other, Nuc | Other |
| **Fatty acid activation**        | ACT     | Mt       | Mt, Pt   |
| Acyl-CoA thioesterase            | CMJ263C | Mt       | Mt, Pt   |
| CMR113C                          | Mt      | Pt       | Pt       |
| Long-chain acyl-CoA synthetase   | LACS    | Cyt, Pt  | Cyt, Other | Other |
| CME186C                          | Cyt     | Other    | Pt       |
| CMG147C                          | Cyt     | Other    | Cyt      |
| CML197C                          | Cyt, Pt | Other    | Cyt, Other | Other |
| CMO037C                          | Cyt     | Other    | Pt       |
| CMT459C                          | Pt      | Pt       | Pt       |
| **PI signaling**                 | PI4K    | ER       | Other, Pt |
| Phosphatidylinositol-4-kinase    | CM1125C | Cyt, CM  | Other, Nuc | Other |
| CMS267C                          | Cyt     | Other    | Nuc, Other |
| **Biotin-dependent carboxylase** | HCS     | Pt, Cyt  | Mt       |
| Holocarboxylase synthetase       | CMC080C | Mt       | Mt       |
| MCC (MCCA) (MCCB)                | CMT073C | Pt       | Pt       |
| MCC (MCCA) (MCCB)                | CMT071C | Mt       | Cyt      |
| Methylcrotonyl-CoA carboxylase   | CMN243C | Mt       | Mt       |
| Propionyl-CoA carboxylase        | CMM132C | Mt       | Pt, Nuc, Pt |
| Carboxylase synthase (Multifunctional type) | CAR (CAR1) | CMQ255C | Not analyzed |
| Carboxylase synthase (Multisubunit type) | CAR (CarA) | CMV036C | Pt-genome |
| Carbamoylphosphate synthase (Multifunctional type) | (CarB) | CML055C | Pt |
| Biotin carboxyl carrier protein  | BCCP    | Pt       | Cyt, Pt  |
| CMO037C                          | Pt      | Mt       | Pt       |
| CMC015C                          | Pt      | Pt       | Pt       |
| CME153C                          | Cyt, CM | Mt       | Pt       |
| CMM132C                          | Mt      | Mt       | Pt       |
| CML055C                          | Pt      | Pt       | Pt       |
| CMC015C                          | Pt      | Mt       | Pt       |