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Supplemental Information

AtMic60 Is Involved in Plant Mitochondria Lipid Trafficking and Is Part of a Large Complex

Morgane Michaud, Valérie Gros, Marianne Tardif, Sabine Brugière, Myriam Ferro, William A. Prinz, Alexandre Toulmay, Jaideep Mathur, Michael Wozny, Denis Falconet, Eric Maréchal, Maryse A. Block, and Juliette Jouhet

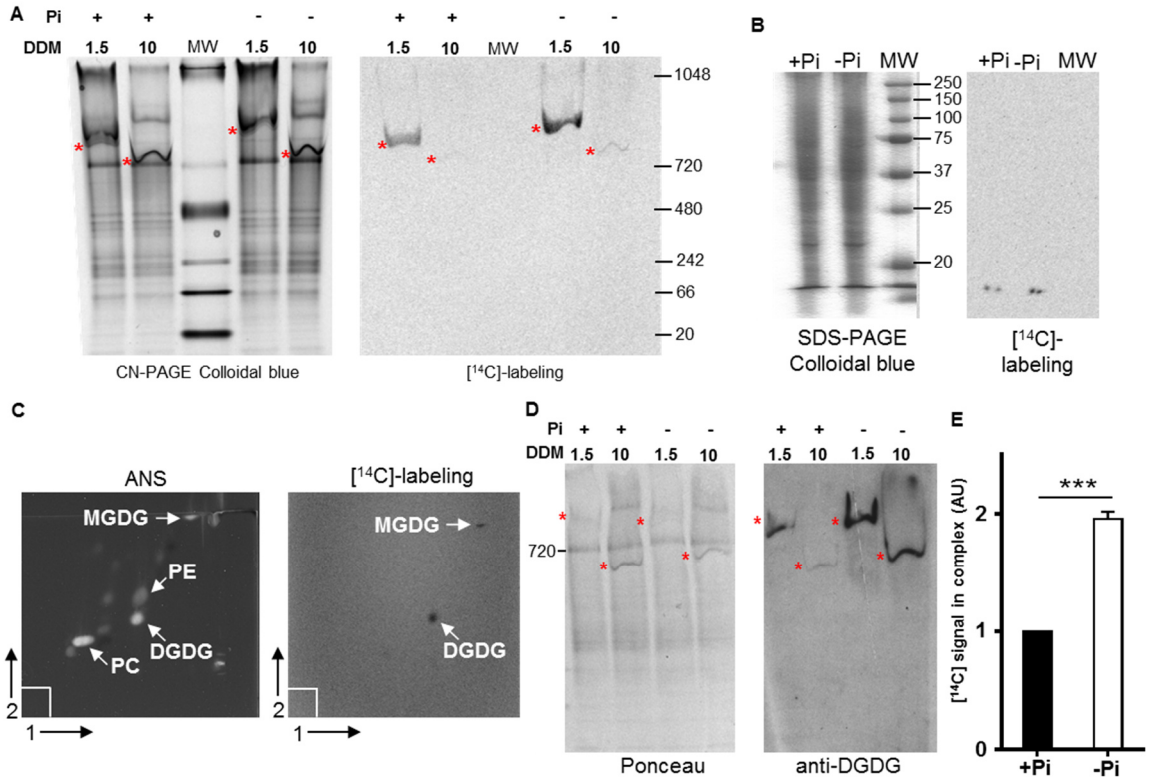

Figure S1. Identification of the MTL complex enriched in DGDG in –Pi in photosynthetic cell cultures. Related to Figure 1. (A) Identification of one complex (*) radiolabeled with DGDG in mitochondria isolated from cell cultures grown two days in presence (+Pi) or absence (-Pi) of Pi. Complexes were solubilized with 1.5 or 10 µg of DDM per µg of proteins and separated on CN-PAGE. MW: Molecular Weight (KDa). (B) After *in vitro* labeling with UDP-[^{14}C]-galactose, 20 µg of purified mitochondria from *A. thaliana* cells grown in + or –Pi were denatured and analyzed by SDS-PAGE. A radioactive signal was obtained at the front of the gel, consistent with a lipid molecule. The absence of another band in SDS-PAGE showed the absence of incorporation of UDP-[^{14}C]-galactose into proteins. MW: Molecular weight (KDa). (C) After *in vitro* labeling with UDP-[^{14}C]-galactose, 20 µg of purified mitochondria from *A. thaliana* cells grown in –Pi were solubilized with 1.5 µg of DDM per µg of proteins and separated on CN-PAGE. Lipids were extracted from the radiolabeled band detected in CN-PAGE. To visualize the main glycerolipids on HPTLC, extracted lipids were mix with non-labeled total lipids extract from *A. thaliana* cell cultures and then separated by a 2D-HPTLC. Glycerolipids were revealed at 360 nm by ANS. The radioactive signals on HPTLC were detected only on galactoglycerolipids MGDG and DGDG. (D) Western blot with antibodies raised against DGDG on CN-PAGE showing the presence of DGDG in the complex (*). (E) Quantification of the radioactive signals obtained in (A). Signals were normalized with +Pi. Data represent the mean value ±SEM, n=3.

A

Name	*S. cerevisiae*	Conserved domain	Putative homolog(s) in *A. thaliana*
Mic10	YCL057C-A	DUF543 (IPR007512)	At1g72170 At1g22520
Mic12	YBR262C	no	no
Mic19	YFR011C	DUF1690 (IPR012471)	no
Mic26	YGR235C	Apolipoprotein-O (IPR019166)	no
Mic27	YNL100W	no	no
Mic60	YKR016W	Mitofilin (IPR019133)	At4g39690

B

% of protein similarity	*Homo sapiens*	*Arabidopsis thaliana*
Saccharomyces cerevisiae	24,5	31,8
Homo sapiens	100	32,4

C

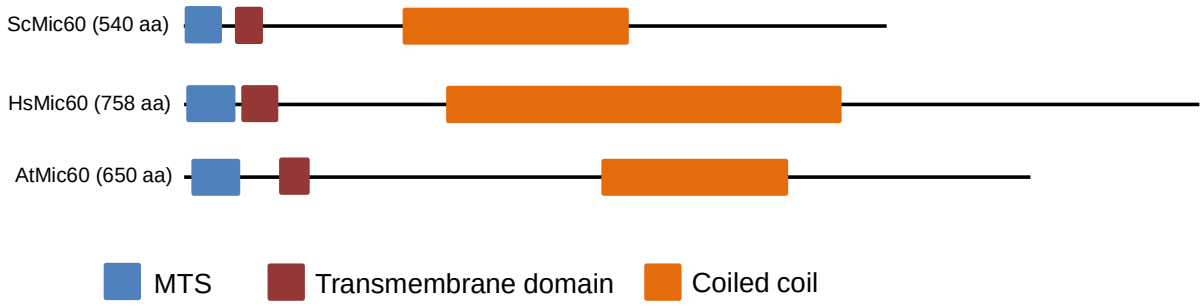

Figure S2. Research of homologous subunits of the yeast MICOS complex in *A. thaliana*. Related to Figure 3. (A) Putative homologs were found by Psi-Blast search or from domain conservation in Interpro database using yeast sequence as bait. Only two subunits of the MICOS complex (AtMic10 and AtMic60) seem to be conserved in *A. thaliana*. (B) and (C) Comparison of putative AtMic60 sequence with the well-described ScMic60 and HsMic60. Despite a weak sequence similarity (B), AtMic60 present the conserved Mitofilin signature (IPR019133) composed of a N-terminal mitochondrial targeting sequence (MTS) followed by a transmembrane domain and a coiled coil domain (C). The organization of ScMic60 is described in [S1] and the one of HsMic60 in [S2, S3]. For AtMic60, MTS was predicted using MITOPROT software [S4], transmembrane domain using TopPred [S5] and coiled coil domain using Multicoil [S6].

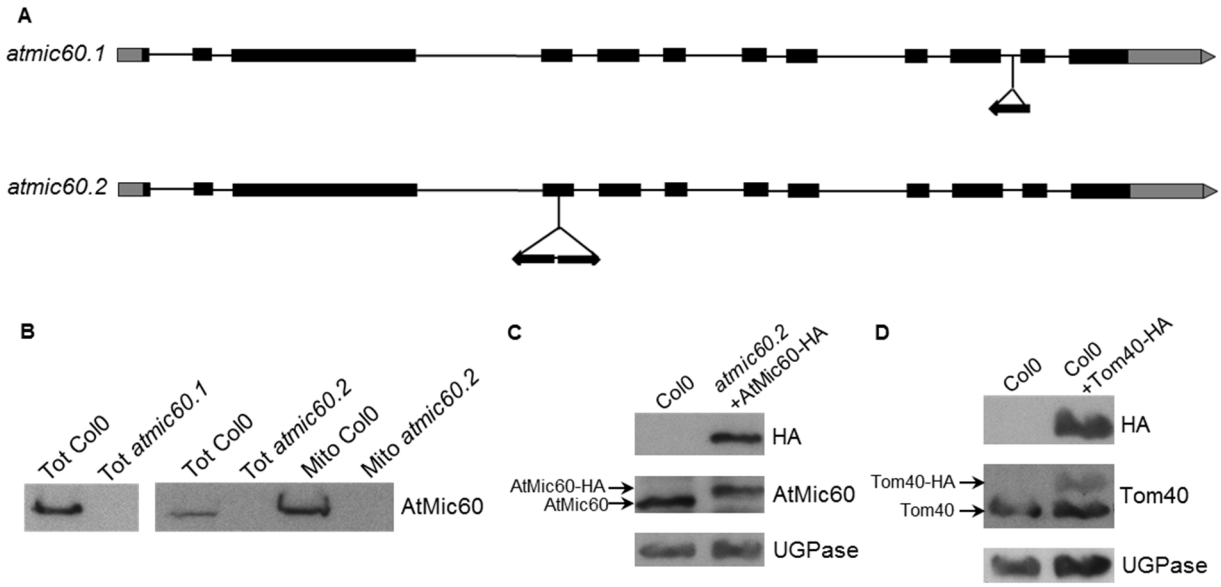

Figure S3. *A. thaliana* lines used in this study. Related to Figure 4 and 5. (A) Representation of the T-DNA insertion (black arrow) in AtMIC60 gene (At4g39690). *atmic60.1* and *atmic60.2* correspond to the SALK_007876c and SALK_087650c lines respectively [S7]. Exons are represented by boxes and introns by lines. Black boxes correspond to coding sequences and the grey ones to UTRs. (B) Western blots with total (Tot) or mitochondrial (Mito) fractions of Col0, *atmic60.1* and *atmic60.2* calli showing the absence of AtMic60 protein and confirming that both mutant lines are KO for AtMic60. (C) Western Blot with total fraction of Col0 or *atmic60.2*+AtMic60-HA calli showing the complemented line express only the AtMic60-HA protein. (D) Western Blot with total fraction of Col0 or Col0 calli stably transformed with TOM40-HA (Col0+Tom40-HA) showing the presence of both the endogenous and the Tom40-HA proteins in the transformed line.

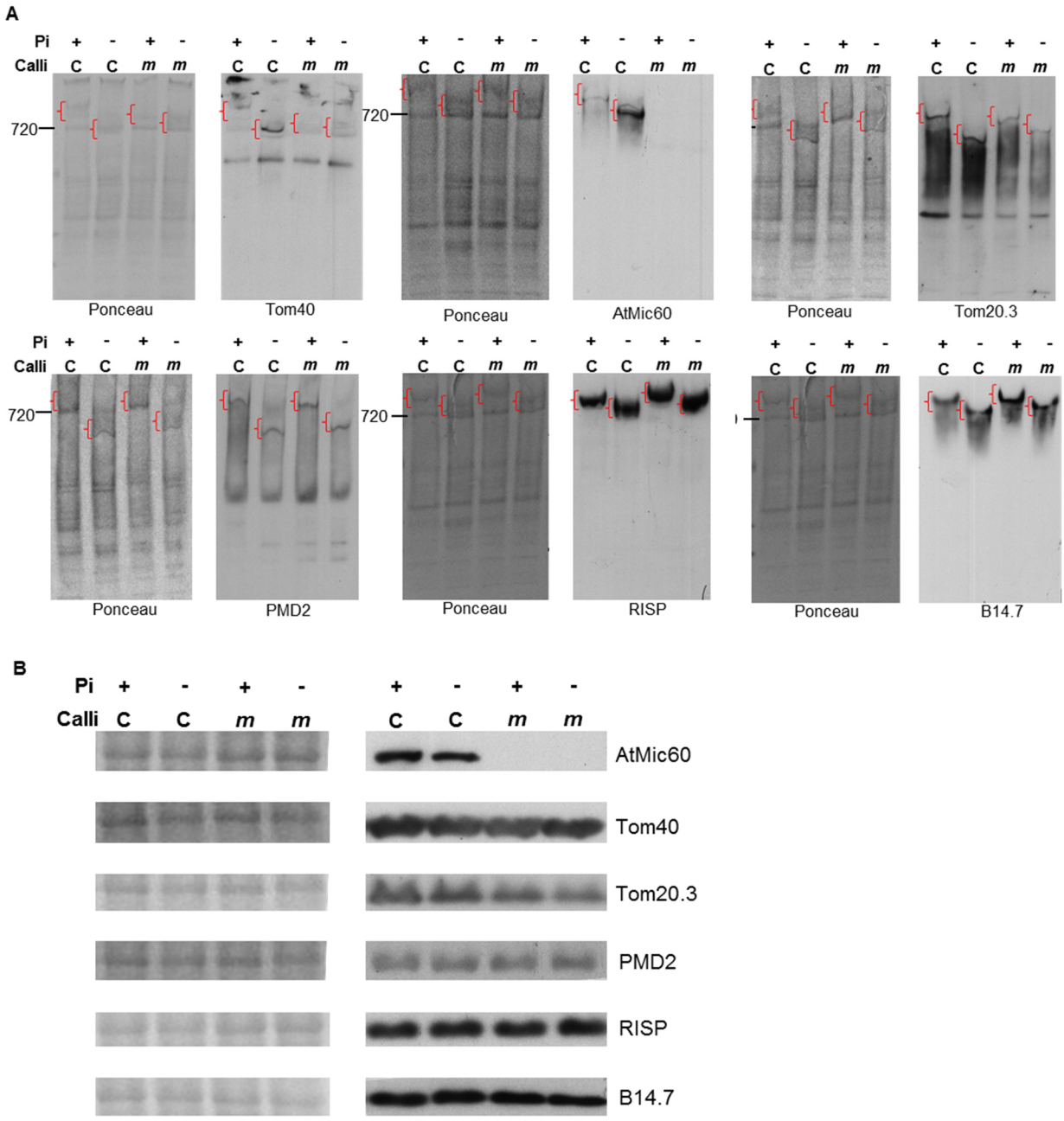

Figure S4. Western blot analyses of different proteins in the MTL complex (A) and in whole mitochondria (B) of Col0 (c) and *atmic60.2* (*m*) calli grown 4 days in + or in –Pi. Related to Figure 4. (A) 20 µg of mitochondria membrane proteins were solubilized with 1.5 µg of DDM per µg of proteins and separated on CN-PAGE. The MTL complex is indicated by curly brackets. (B) 5 µg of mitochondria membrane proteins were denatured and analyzed by SDS-PAGE.

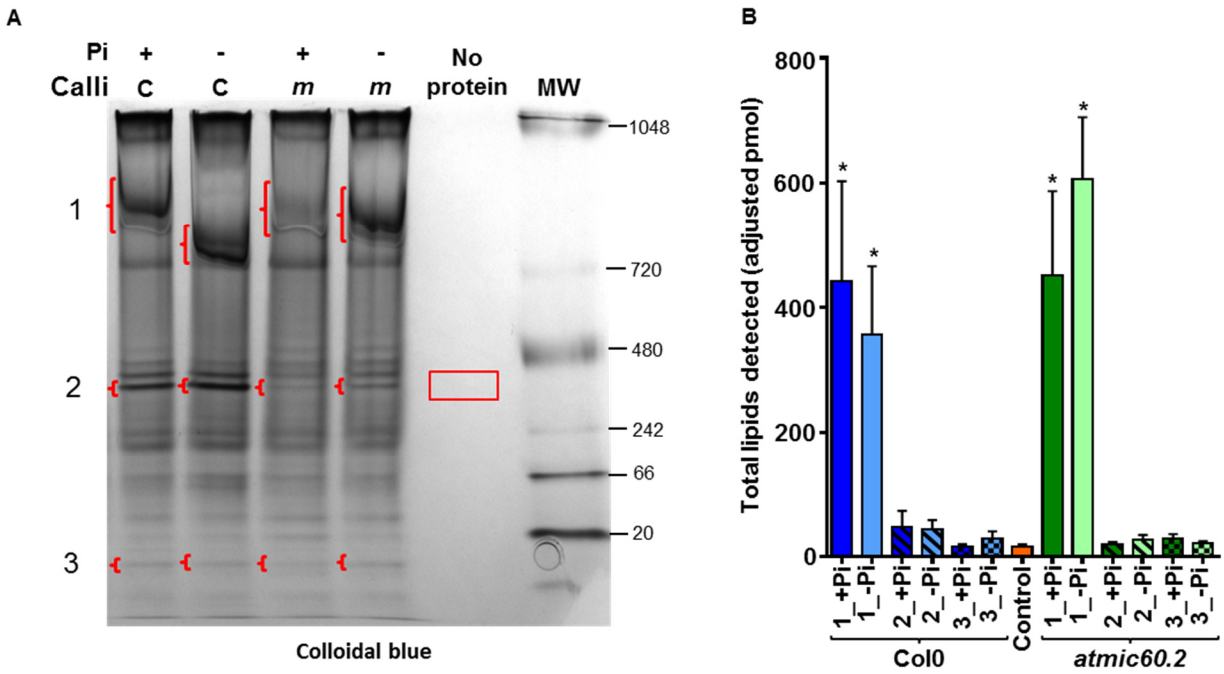

Figure S5. Lipid analyses of complexes present on CN-PAGE by mass spectrometry. Related to Figure 5. (A) Representative CN-PAGE showing the different complexes used for lipidomic analysis by mass spectrometry. N°1 corresponds to the MTL complex. N°2 and 3 are complexes present at different level of the CN-PAGE. To evaluate the background of lipids in acrylamide gels, a control band was used in a lane without proteins. MW: molecular weight in KDa. (B) Total amount of lipids detected in the different band analyzed. The only lipids detected are PC, PE and DGDG. An accumulation of lipids is observed only in the MTL complex (Band n°1) compared to control further supporting the enrichment of lipids in this complex. Data represent the mean value ±SEM, n=3.

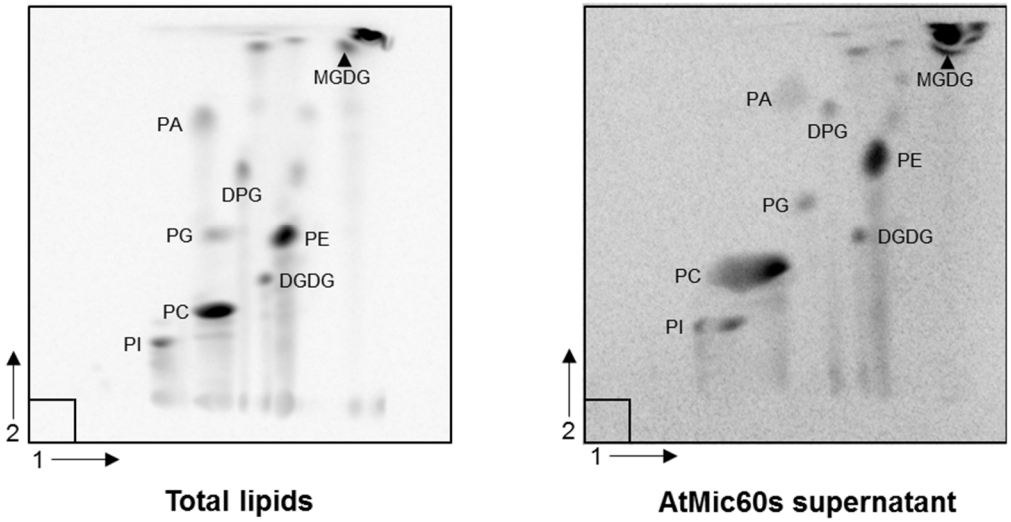

Figure S6. All the lipids are increased in the supernatant of liposomes incubated with AtMic60s. Related to Figure 6. After extraction assays with liposomes composed of 70% PC and 30% of [^{14}C]-labeled glycerolipids from *A. thaliana* cell cultures, the lipids present in the supernatant were extracted and separated by 2D-HPTLC. As a control, a 2D-HPTLC was done with total [^{14}C]-labeled glycerolipids from *A. thaliana* cell cultures.

Supplemental Experimental Procedures

Plant materials and growth conditions

Arabidopsis thaliana ecotype Col0 plants were used as wild type. Two T-DNA insertion lines in Col0 background (*atmic60.1*: SALK_007876c; *atmic60.2*: SALK_087650c) in ATMIC60 gene (At4g39690) were obtained from the SALK collection [S7]. The presence of a T-DNA was verified using primer LBa1 (TGGTTCACGTAGTGGGCCATCG) located on the T-DNA and primers located on the gene (for *atmic60.1*: GATAAAGGCACTGAGTATGGC; for *atmic60.2*: CCTGCCGCTCTAAAGACAC). The absence of AtMic60 protein expression was verified by western blot.

Calli were obtained from mesophyll tissue of two week old Arabidopsis leaves, and grown on agar plates containing Murashige and Skoog (MS) (MSP09, Caisson Laboratories, Inc, USA) supplemented with 3 % (w/v) sucrose, 1.2 $mg.L^{-1}$ 2,4-dichlorophenoxyacetic acid and 0.8 % (w/v) agar. After formation, calli were maintained in 200 mL of liquid MS (MSP09, Caisson Laboratories, Inc, USA) containing 1 mM of phosphate, 1.5 % (w/v) sucrose and 1.2 $mg.L^{-1}$ 2,4-dichlorophenoxyacetic acid. Calli were kept under continuous light (100 $\mu E.m^{-2}.s^{-1}$) at 22°C, agitated with rotary shaking at 125 rpm and subcultured every 7 days. For phosphate starvation experiments, calli were washed three times with 150 mL of MS medium with 4 mM Pi or without Pi and grown for 4 or 8 days in MS with 4 mM or without Pi.

Photosynthetic *Arabidopsis thaliana* cell cultures [S8] were maintained in 200 mL of MS medium (MSP09, Caisson Laboratories, Inc, USA) containing 4 mM of phosphate, 1.5 % (w/v) sucrose and 1.2 $mg.L^{-1}$ 2,4-dichlorophenoxyacetic acid. Cultures were kept under continuous light (100 $\mu E.m^{-2}.s^{-1}$) at 22°C, and agitated with rotary shaking at 125 rpm and subcultured every 7 days. For phosphate starvation experiments, cells were washed three times with 150 mL of MS medium with 4 mM Pi or without Pi and grown for 2 days in MS with 4 mM or without Pi.

Arabidopsis transgenic line construction

The full length ATMIC60 gene (At4g39690) was amplified from RIKEN plasmid PDA01616 with forward primer ATGCTTCGGAAGTCGGTTCTC and reverse primer AGTGAGGCTAACACAAGTTGC. The full length TOM40 gene was amplified from *A. thaliana* cDNA library with forward primer ATGGCGGATCTTTTACCACCTC and the reverse primer ACCAACTGTTAATCCGAAACC. Genes were cloned in the pFP108 [S9] plant expression vector in frame with 3xHA tags added in C-terminal (pFP108-3xHA).

Expression of genes from this vector is under the control of two 35S promotors and a NOS terminator. Plasmids were transferred in *Agrobacterium tumefaciens* GV3101 bacteria strain and used from stably transformation of *atmic60.2* or Col0 plants by floral dip [S10]. Transformed plants were selected using Basta® and leaves from F2 plants expressing the constructions were used for calli formation.

Mitochondria purification

Mitochondria were purified from photosynthetic cell cultures or from calli according to the protocol described in [S11] with a slight modification for the grinding of calli (30 mL of grinding buffer and 15 mL of sand for 64 g of material).

Mitoplasts preparation

Mitoplasts correspond to mitochondria with ruptured OM. After purification, mitochondria were carefully resuspended in 10 mM 3-(N-Morpholino)propanesulfonic acid (MOPS) pH 7.4 and incubated 20 min on ice to allow mitochondria membranes swelling and OM rupture. Mitoplasts were then pelleted and resuspended in washing buffer (0.3 M mannitol, MOPS 10 mM, 5 mM α-aminocaproic acid, 1 mM benzamidine, pH7.4).

Mitochondrial membrane isolation, complex solubilization and separation on native gels

After purification, mitochondria were resuspended in washing buffer without mannitol (MOPS 10 mM, 5 mM α-aminocaproic acid, 1 mM benzamidine, pH7.4) and vortexed. Membranes were retrieved by a centrifugation 20 min at 100 000g at 4°C and resuspended in membrane buffer (50 mM imidazole, 0.5 M α-aminocaproic acid, 1 mM EDTA, pH7). Complexes were solubilized by addition of β-dodecylmaltoside (DDM) at 1.5 to 10 µg per µg of mitochondrial proteins. After 5 min on ice, samples were centrifuged 20 min at 100 000g at 4°C and supernatant containing complexes were retrieved and mix with loading buffer (Tris-HCl 60 mM, glycerol 10 %, bromophenol blue 0,1 % (w/v), pH 6.8). Samples are loaded on CN-PAGE (stacking gel: 3% acrylamide/bisacrylamide 37.5/1 (w/v), 0.125 M Tris-HCl, pH6.8; separating gel: 3.5 to 12 % acrylamide/bisacrylamide (w/v) 37.5/1, 0.4 M Tris-HCl, pH 8.8) and separated at 80V in migration buffer (0.05 M Tris-HCl, 0.38 M Glycine, pH 8.3). After migration, gels were fixed (40 % (v/v) ethanol, 10 % (v/v) acetic acid) and colored (34 % (v/v) methanol, 17 % (w/v) ammonium sulfate, 0.5 % (v/v) acetic acid, 0.1 % (w/v) colloidal blue G250) or transferred on nitrocellulose membrane for western blot.

Protease treatment

For thermolysin treatment, mitochondria or mitoplasts are washed in washing buffer without protease inhibitors (0.3 M mannitol, MOPS 10 mM, pH 7.4). 100 µg of proteins were incubated 1h on ice with 0 to 10 µg of thermolysin (Sigma Aldrich) in washing buffer without protease inhibitors containing 1 mM of $CaCl_2$ in a final volume of 400 µL. A controlled sample was incubated with Triton X-100 1% (v/v) prior to treatment with 10 µg of thermolysin. After incubation, organelles are pelleted and resuspended in 50 µL of SDS-PAGE loading buffer (Tris-HCl 60 mM, SDS 2 % (w/v), β-mercaptoethanol 1 % (v/v), glycerol 10 %, bromophenol blue 0.1 % (w/v), pH 6.8). 10 µL are used for western blot analysis.

Western blot analyses

Antibodies against Cox2, UGPase, GDC-H, NDPK3, ATP-B and BIP2 were obtained from Agrisera and anti-HA-HRP from Miltenyi Biotec. Anti-Tom40, Tom20.3, Tim17.2 and RISP were kindly provided by James Whelan and Monika Murcha (University of Western Australia) and anti-VDAC by André Dietrich (IBMP, Strasbourg). Anti-DGDG antibodies were produced in our laboratory [S12]. Polyclonal antibodies against AtMic60, B14.7, PMD2 and Miro1 were produced in rabbit by Biotem. Peptides used for immunization are: AtMic60: EEAAILDKELKRERTKKA and GVEDSTEKDGKVETQP; B14.7: YPYTVEKRAEADS and YIGVEQLVQNFRSKRD; PMD2: EIVELQKQLDDAEKM and ITEKMVVEDSLKDSEKKVV; Miro1: KEAPYEDAAEKTALG and CFLGRSYTDNQESTTDER. Antibodies were affinity purified on peptides after production.

Proteomic analysis by mass spectrometry

Protein digestion

Samples from Arabidopsis cell cultures mitochondria solubilized with 1.5 and 10 µg of DDM per µg of proteins and from Col0 calli solubilized with 1.5 µg of DDM per µg of proteins, in + and –Pi, were prepared in triplicates. CN-PAGE bands corresponding to the MTL complexes were manually excised for in-gel digestion with trypsin using a Freedom EVO150 robotic platform (Tecan Traging AG, Switzerland) as follows. Gel bands were washed six times by successive incubations in 25 mM NH_4HCO_3 and then in 50% (v/v) CH_3CN, 25 mM NH_4HCO_3. After dehydration in pure CH_3CN, reduction was carried out with 10 mM DTT in 25 mM NH_4HCO_3 (45 min at 53°C) and alkylation with 55 mM iodoacetamide in 25 mM NH_4HCO_3 (35 min in the dark). Alkylation was stopped by the addition of 10 mM DTT in 25

mm NH_4HCO_3 (10-min incubation). Gel pieces were then washed again in 25 mM NH_4HCO_3 and dehydrated with pure acetonitrile. Modified trypsin (sequencing grade, Promega) in 25 mM NH_4HCO_3 was added to the dehydrated gel pieces for incubation at 37 °C overnight. Peptides were extracted from gel pieces in three sequential extraction steps (each 15 min) in 30 µl of 50% (v/v) CH_3CN, 30 µl of 5% (v/v) formic acid, and finally 30 µl of pure CH_3CN. The pooled supernatants were dried under vacuum.

Nano-LC-MS/MS analyses

The dried extracted peptides were resuspended in 5% acetonitrile and 0.1% trifluoroacetic acid and analyzed via online nano-LC-MS/MS (Ultimate 3000, Dionex, Germering, Germany; LTQ-Orbitrap Velos Pro, Thermo Fisher Scientific). Peptide mixtures were desalted on line using a reverse phase precolumn (Acclaim PepMap 100 C18, 5 µm bead size, 100 Å pore size, 5 mm × 300 µm, Dionex) and resolved on a C18 column (Acclaim PepMap 100 C18, 3 µm bead size, 100 Å pore size, 25 cm × 75 µm, Dionex)**.** Peptides were separated using 120-min gradients with aqueous solvent A (2% (v/v) CH_3CN, 0.1% (v/v) HCOOH) and solvent B (20% (v/v) CH_3CN, 0.08% (v/v) HCOOH) at a flow rate of 300 nL/min. For photosynthetic cells samples, the gradient was developed as follows: 4–45% B in 114 min, 45–90% B in 1 min, 90% B for 5 min. For calli samples, the gradient was: 4–12% B in 21 min, 12–30% B in 87 min, 30–40% B in 6 min, 40–90% B in 1 min, 90% B for 14 min. MS and MS/MS data were acquired using Xcalibur (Thermo Fisher Scientific). The spray voltage was set at 1.4 kV and the heated capillary was adjusted to 270 °C. Survey full-scan MS spectra (m/z = 400–1600) were obtained in the Orbitrap with a resolution of 60,000. The LTQ-Orbitrap mass spectrometer was operated in the data-dependent mode. The 20 most intense ions from the preview survey scan delivered by the Orbitrap were fragmented via collision-induced dissociation (collision energy: 35%) in the LTQ.

Database searches and results processing

RAW files from all analyses were processed in one unique search session using MaxQuant version 1.5.1.2 [S13]. Spectra were searched against a compilation of the *A. thaliana* protein database (nuclear, mitochondrial, and plastid genome; TAIR v10.0; December 14, 2010; 35,386 entries) and the frequently observed contaminant database embedded in MaxQuant. Trypsin/P was chosen as the enzyme and 2 missed cleavages were allowed. Precursor mass error tolerances were set respectively at 20 ppm and 4.5 ppm for first and main searches. Fragment mass error tolerance was set to 0.5 Da. Peptide modifications allowed during the

search were: carbamidomethylation (C, fixed), acetyl (Protein N-ter, variable), and oxidation (M, variable). Minimum peptide length was set to seven amino acids. Minimum number of peptides was set to 2, razor + unique peptides and unique peptides were set to 1. Maximum false discovery rates, calculated by employing a reverse database strategy, were set to 0.01 at peptide and protein levels. For quantification, the "Match between runs" option (with Matching time set to 0.7 min) was allowed only between cells samples or between calli samples. Identification and quantification data are provided for proteins in supplemental Table S1. The protein iBAQ was used for quantification [S14]. A ranking analysis approach was performed to evaluate the abundance of each protein in the MTL complexes. Individual ranks in each replicate were attributed according to decreasing iBAQs values and an arbitrary highest rank of "1550" was assigned in all replicates for proteins that were not quantified. Protein ranks were obtained using the median of individual ranks from the biological triplicates. To determine proteins present in the complex, 4 filters were applied in plus and in minus Pi conditions, independently: 1) protein rank <400 in the 3 conditions (Cells DDM1.5 and DDM10, Calli), 2) in cells: rank differences between DDM10 and DDM1.5<300, 3) number of total missing values ≤2 (in -Pi) or ≤1 (in +Pi), 4) peptide number ≥3. For annotations: descriptions of the proteins were obtained from TAIR and the localization annotations correspond to the consensus ones proposed by SUBA [S15].

Co-Immunoprecipitation (co-IP)

For AtMic60-HA co-IPs, 500 µg of mitochondrial membrane proteins purified from Col0 or *atmic60.2*+AtMic60-HA calli (4 days of growth in + or –Pi) were solubilized in membrane buffer with 50 mM of NaCl and 1% (v/v) Triton-X100 in a final volume of 750 µL. After 30 min on ice, samples were centrifuged 20 min at 100 000g and the supernatant was incubated for 30 min at 4°C with 50 µL of µMACS^{TM} HA-Tag Microbeads (Miltenyi Biotec). µColumns (Miltenyi Biotec) were placed in the magnetic field of the µMACS separator (Miltenyi Biotec) and equilibrated with membrane buffer containing 50 mM NaCl and 1% (v/v) Triton-X100. Samples were added to the column and were washed 5 times with membrane buffer containing 50 mM NaCl and 0.1% (v/v) Triton-X100. Elution was performed by denaturation according to the manufacturer instructions in a final volume of 70 µL. 20 µL were used for SDS-PAGE and western blots analysis.

For Tom40-HA co-IPs, 300 µg of mitochondrial membrane proteins purified from Col0 or Col0+Tom40-HA calli (4 days of growth in + or –Pi) were solubilized in membrane buffer with DDM at 1.5 µg per µg of mitochondrial proteins. After 30 min on ice, samples were

centrifuged 20 min at 100 000g and the supernatant was incubated for 30 min at 4°C with 50 µL of µMACS^{TM} HA-Tag Microbeads (Miltenyi Biotec). µColumns (Miltenyi Biotec) were placed in the magnetic field of the µMACS separator (Miltenyi Biotec) and equilibrated with membrane buffer containing 150 mM NaCl and 1% (w/v) DDM. Samples were added to the column and were washed 5 times with membrane buffer containing 150 mM NaCl and 1% (w/v) DDM. Elution was performed by denaturation according to the manufacturer instructions in a final volume of 70 µL. 20 µL were used for SDS-PAGE analysis and western blot.

Protein surexpression and purification in *Escherichia coli*

For AtMic60, primers forward GGGAAAGAACAGCAGAAA and reverse AGTGAGGCTAACACAAGTTG were used for gene amplification. For Tom20.3, primers forward GATACGGAAACTGAGTTCGA and reverse TTAATCACTACTTTTCTTATTC were used for gene amplification. Proteins overexpressions were performed overnight at 17°C for AtMic60s and 2h at 37°C for Tom20.3s. Protein purification was done on Ni Sepharose 6 Fast Flow resin (GE Healthcare). Proteins were desalted on PD-10 gel filtration column (GE healthcare) with protein buffer (20 mM Tris-HCl, NaCl 300 mM, glycerol 10 % (w/v)) and stored at -80°C.

Lipid extractions

Lipids from calli and mitochondria purified from 8 days grown calli were extracted by the Folch method [S16]. 500 mg of calli were lyophilized overnight prior to extraction.

Lipids from mitochondrial membranes purified from 4 days grown calli and from supernatants after liposome extraction assays were extracted by the Bligh and Dyer method [S17].

For lipid extraction from polyacrylamide gels, the bands of interest were cut from 4 lanes containing 20 µg of mitochondria proteins and were pooled. The lipid extraction method was adapted from the Bligh and Dyer method [S17]. 500 µL of H_2O and 1.875 µL of chloroform/methanol 1:2 (v/v) were added on the bands. After vortexing, samples were incubated 1h at room temperature under agitation. Formation of biphase was promoted by the addition of 625 µL of H_2O and 625 µL of chloroform and after centrifugation the organic phase was retrieved and a second extraction was done on the polar phase. Lipids were dried under argon and stored at -20°C for further analysis.

Lipid analysis by mass spectrometry

Lipids were extracted from mitochondria and from CN-PAGE bands cut as described in Figure S5. Lipid extracted from the band were resuspended in 40 µL of chloroform/methanol 2:1 (v/v) containing 50 pmol of each internal standard and 50 nmol of mitochondria lipid were resuspended in 100 µL of chloroform/methanol 2:1, (v/v) containing 125 pmol of each internal standard. Internal standards were obtained from Avanti Polar Lipids Inc. for PC 18:0-18:0, PE 18:0-18:0, PI 18:0-18:0, PS 18:0-18:0, PG 18:0-18:0, PA 18:0-18:0, and CL 14:0-14:0-14:0-14:0, or synthesized by D. Lafont [S18, S19] for MGDG 18:0-18:0 and DGDG 16:0-16:0. Lipids were then separated by HPLC and quantified by MS/MS.

The HPLC separation method was adapted from [S20]. Lipid classes were separated using an Agilent 1200 HPLC system using a 150 mm×3 mm (length × internal diameter) 5 µm diol column (Macherey-Nagel), at 40°C. The mobile phases consisted of hexane/isopropanol/water/ammonium acetate 1M, pH5.3 [625/350/24/1, (v/v/v/v)] (A) and isopropanol/water/ammonium acetate 1M, pH5.3 [850/149/1, (v/v/v)] (B). The injection volume was 20 µL. After 5 min, the percentage of B was increased linearly from 0% to 100% in 30 min and stayed at 100% for 15 min. This elution sequence was followed by a return to 100% A in 5 min and an equilibration for 20 min with 100% A before the next injection, leading to a total runtime of 70 min. The flow rate of the mobile phase was 200 µL/min. The distinct glycerolipid classes were eluted successively as a function of the polar head group.

Mass spectrometric analysis was done on a 6460 triple quadrupole mass spectrometer (Agilent) equipped with a Jet stream electrospray ion source under following settings: Drying gas heater: 260°C, Drying gas flow 13 L/min, Sheath gas heater: 300°C, Sheath gas flow: 11L/min, Nebulizer pressure: 25 psi, Capillary voltage: ± 5000 V, Nozzle voltage ± 1000. Nitrogen was used as collision gas. The quadrupoles Q1 and Q3 were operated at widest and unit resolution respectively. PC analysis was carried out in positive ion mode by scanning for precursors of m/z 184 at collision energy (CE) of 34 eV. PE, PI, PS, PG, PA, MGDG and DGDG measurements were performed in positive ion mode by scanning for neutral losses of 141 Da, 277 Da, 185 Da, 189 Da, 115 Da, 179 Da and 341 Da at CEs of 20 eV, 12 eV, 20 eV, 16 eV, 16 eV, 8 eV and 8 eV, respectively. Quantification was done by multiple reaction monitoring (MRM) with 25 ms dwell time. CL species were quantified by MRM as singly charged ions $[M-H]^-$ at a CE of -45 eV with 50 ms dwell time. Mass spectra were processed by MassHunter Workstation software (Agilent) for identification and quantification of lipids.

Lipid amounts (pmol) were corrected for response differences between internal standards and endogenous lipids.

Synthesis of total [^{14}C]-labeled lipids in *A. thaliana* cell cultures

30 mL of 7 week old *A. thaliana* cells were subcultured in 100 mL of MS medium containing 4 mM of phosphate, 1.5 % (w/v) sucrose and 1.2 mg.L^{-1} 2,4-dichlorophenoxyacetic acid and 0.05 mCi of [^{14}C]-acetate at 50 mCi/mmol. A control culture was done in the same condition but without [^{14}C]-acetate. After 4 days of growth, cells were harvested and lipids were extracted as described above. The quantity of lipids was estimated by analysis of the FAMEs from the non-labeled sample.

Galactoglycerolipid synthase activity

Calli grown 4 days in presence or absence of Pi were grinded with sand in grinding buffer (15 mM MOPS pH 8, 0.3 M mannitol, 0.6 % (p/v) polyvinylpyrrolidone 25, 2 mM EGTA, 10 mM DTT, 5 mM aminocaproic acid, 1 mM benzamidine). After a centrifugation at 500 g for 5 min at 4°C, the supernatant, corresponding to the total cellular extract, was rescued. 1 mg of total cellular protein extract was incubated in a final volume of 200 µL with 0.35 mM of diacylglycerol, 1 mM UDP-[^{14}C]-galactose, 1 mM $MgCl_2$ in grinding buffer at 22°C and 100 µL of the mix were retrieved after 30 and 60 min of incubation. Lipids were extracted by Bligh and Dyer [17] and radioactivity was measured using a β-counter.

Liposome binding, extraction and leakage assays

Liposome binding, extraction and leakage assays were adapted from [S21-S23]. Lipids were obtained from Avanti Polar Lipids Inc. For liposome formation, desired mix of lipids was dried at least 2 hour under argon and resuspended in heavy buffer (0.18 M sucrose, 20 mM Hepes-KOH, pH 7.3) at a final concentration of 1 mM. For leakage assays, liposomes were supplemented with 8-aminonaphthalene-1,3,6- trisulfonic acid (ANTS, Life Technology) at 12.5 mM and p-xylene-bis-pridinium bromide (DPX, Life Technology) at 45 mM. Membranes were then incubated at room temperature at least 1h and vigorously vortexed regularly. After eight cycles of liquid nitrogen freezing and defrosting in water bath at 50°C, liposomes were made by extrusion through a 0.4 µm filter in a Mini Extruder (Avanti Polar Lipids Inc.). Heavy liposomes are then washed and resuspended in light buffer (100 mM NaCl, 20 mM Hepes-KOH, pH 7.3) at a final concentration of 1 mM.

Liposome binding assays were performed in a final volume of 100 µL with 90 µL of liposomes and 10 µL of purified protein centrifuged at 100 000g 10 min prior to binding assays. After incubation 1h at 22°C, liposomes are pelleted at 16 000g for 10 min. Pellet were resuspended in 15 µL of SDS-PAGE loading buffer and 1/5 of supernatant volume was mix with 5X SDS-PAGE loading buffer. Samples were separated by SDS-PAGE, fixed and colored. Protein signals in pellet and supernatant were quantified using ImageJ software.

Liposome extraction assays were performed in a final volume of 100 µL with 90 µL of liposomes and 100 pmol of purified proteins centrifuged at 100 000g 10 min prior to extraction assays. After incubation 1h at 22°C, 5 µg of thermolysin (Sigma Aldrich) was added and the mix was incubated 15 min at 22°C. Liposomes are pelleted 20 min at 16 000g and the radioactivity present in the pellet and in the supernatant was quantified using a β-counter.

For liposome leakage assays, 90 µL of liposomes containing ANTS and DPX were incubated with 100 pmol of purified proteins in a final volume of 100 µL. Fluorescence increase in liposome mix was followed during time with excitation at 360 nm and emission at 520 nm in an Infinite® M1000 Pro microplate reader (Tecan).

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