Supplementary Material

Mitochondrial morphogenesis is regulated by inner membrane proteins PMI/TMEM11 independently of the DRP1-MFN fission/fusion pathways

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Supplementary figure legends

Figure S1: Genetic organisation of the *PMI_PGRP-LD* bi-cistronic locus

a. *PMI_PGRP-LD* bi-cistronic locus organization. The locus contains non-overlapping open reading frames for both PMI (orange boxes) and PGRP-LD (grey boxes). A unique transcription start site (arrow) drives the transcription of a bi-cistronic messenger RNA (mRNA) (*EST GH14535*). The 5'UTR contains a Kozak (kz) box (CAAA) for PMI translation. A complete deletion of the *PMI_PGRP-LD* locus was generated by targeted homologous recombination using 5' and 3' homology regions. In knock-out flies, the *white* cDNA replaced the *PMI_PGRP-LD* locus. Exons (box), untranslated regions (empty box), coding sequence (colored rectangle), introns (line). In the *Pac[PMI*_*PGRP-LD]* and *Pac[PMI_PGRP-LD]* genomic transgenes, a frame shift mutation was introduced in either *PMI* (red asterisk) or *PGRRP-LD* (green asterisk) coding sequence to create a premature stop codon. A second frame shift mutation (grey asterisk) was introduced in the first intron of *PGRP-LD* that possibly contains a cryptic translation start according to computational analysis (for details see methods).

b. PCR and RT-PCR performed on genomic DNA and cDNA from control (wt) and *PMI_PGRP-LD* homozygous mutant (-/-) flies. The *PGRP-LF* locus and the *actin5c* mRNA were used as positive control.

Figure S2. Mitochondria of non-neuronal tissue of *PMI_PGRP-LD* mutant

Mitochondria from various larval and adult fly tissue labelled with an anti-mitochondrial ATP synthase antibody (green) or a *UAS-mito::GFP* reporter (*actin-GAL4* driver). In all tissues, the size of mitochondria is increased in *PMI_PGRP-LD* mutant condition whereas
mitochondria are less numerous (*PMI_PGRP-LD*). Normal mitochondrial morphogenesis is restored by ubiquitous ectopic expression of PMI (*PMI_PGRP-LD ubi-PMI*).

**Figure S3. In PMI mutant myocytes mitochondria size is increased but their number is reduced.**

a. TEM analysis of adult flight muscle (DLM). Mitochondria of *PMI* (*PMI_PGRP-LD*) and *drp1* (*drp1^1/drp1^2*) mutants are enlarged and round-shape but contain intact internal structures. In contrast, *pink1* mutant mitochondria are rather elongated and have disorganized cristae likely reflecting degeneration of the organelle. Mitochondria (arrow), muscle fibers (fm).

b. Size distribution of mitochondria in flight muscles of *PMI, drp1* and *pink1* mutant flies. Mitochondria from *PMI, drp1* and *pink1* mutant were significantly larger (Student t-test results: control versus *PMI* mutant, P<0.001; control versus *drp1*, P<0.01; control versus *pink1*, P<0.001). n>30 mitochondria. 3 independent samples.

c. Number of mitochondria in adult flight muscles (determined on TEM pictures of adult flight muscle). *PMI, drp1* and *pink1* mutant have significantly less mitochondria per μm^2^ than control animals. Student t test results: ** P< 0.001, *** P<0.001. n>10 views, 100μm^2^ in average.

**Figure S4. Condensation of the mitochondrial network in PMI mutant neurons.**

a. A *UAS-mito::GFP* reporter was targeted to dopaminergic neurons (*TH-GAL4* driver) of control and *PMI_PGRP-LD* mutant flies to visualise mitochondria. To obtain high-resolution images of the mitochondrial network, giant dopaminergic neurons (PAL) of adult fly brains were imaged. Z-stacks of confocal images were submitted to tri-dimensional reconstruction (Volocity software). In control neuron, the mitochondrial network is branched and
interconnected, whereas it appears condensed into well individualised blocks in the
PMI_PGRP-LD mutant flies.

b. Adult flight muscles were immunolabelled for mitochondria (anti ATP synthase antibody),
and Z-stack images were submitted to tri-dimensional reconstruction (Volocity software). In
PMI_PGRP-LD mutant, mitochondria appear of bigger size but are less numerous.

c. Histogramm shows the total volume occupied by mitochondria within a given volume of
muscles (expressed as μm³ of mitochondria per μm³ of muscle). Consistent with the
observation made in (b), the overall volume of mitochondria in muscles is not significantly
increased in flies lacking both PMI and PGRP-LD (PMI_PGRP-LD) or PMI only
(PMI_PGRP-LD Pac[PMI*_PGRP-LD]) compared to control flies. Histogramm showing the
mean value (+/- standard error) obtained from at least 6 independent Z-stacks of muscle
stained with an ATP synthase antibody. Each Z-stack represents at least 6000 μm³ of muscle.
Volumes ratio was determined using the Volume3D application (Matlab computing
environment).

Figure S5. PMI does not genetically interact with known components of the
fusion/fission machineries in adult myocytes.

Immunolabelling of mitochondria in adult flight muscles (anti ATP synthase antibody).
Whereas adding one copy of the drp1 gene is sufficient to increase mitochondrial fission in a
wild-type background (d, Pac[drp1] versus a, control), over-expression of PMI by the mean
of a ubiquitin promoter (c, ubi-PMI) or UAS activation sequences (b, tubulin-GAL4 UAS-
PMI) has no effect on the morphology of mitochondria. Over-expression of PMI using the
same transgene rescues the enlarged mitochondria phenotype of PMI_PGRL-LD mutant (g,
PMI_PGRL-LD ubi-PMI versus e, PMI_PGRL-LD) as efficiently as a PMI genomic construct
(f, PMI_PGRL-LD Pac[PMI_PGRL-LD*]). On the contrary, adding one copy of the drp1
gene and removing one copy of \( \text{opai} \) does not rescue mitochondrial morphology in \( \text{PMI}_\text{PGRP-LD} \) mutant (h, \( \text{PMI}_\text{PGRP-LD} \text{ Pac[drpl]} \text{ opal/+} \)) whereas these genetic manipulations abolish the hyperfusion of the \( \text{pinkl} \) mutant mitochondria (k, \( \text{pinkl} \text{ Pac[drpl]} \)) and l, \( \text{pinkl} \text{ opal/+ versus i, pinkl} \)). Finally, over-expression of PMI has no effect on \( \text{pinkl} \) mutant mitochondria (j, \( \text{pinkl} \text{ ubi-PMI} \)).

m,n,o. Analysis of genotypes distribution in the adult fly progeny born from the following crosses: \( \text{drpl}/\text{CyO ; PMI}_\text{PGRP-LD}/\text{TM6 x drpl}/\text{CyO ; PMI}_\text{PGRP-LD}/\text{TM6} \) (m), \( \text{drpl}/\text{CyO ; drpl}/\text{CyO} \) (n), \( \text{PMI}_\text{PGRP-LD}/\text{TM6 x PMI}_\text{PGRP-LD}/\text{TM6} \) (o). Black bars represent the expected mendelian distribution of genotypes. White bars show the percentage of each genotypes obtained. Whereas the percentage of \( \text{drpl}/\text{drpl} \) (n) and \( \text{PMI}_\text{PGRP-LD} \) (o) mutants hatching as viable adults is close to the expected mendelian distribution, no \( \text{drpl}/\text{drpl} ; \text{PMI}_\text{PGRP-LD} \) double mutant adults were obtained (m, Ø).

**Figure S6. PMI defines a novel family of proteins**

a. PMI orthologs were searched using tBLASTn analysis with *Drosophila* PMI as a probe. BLAST searches were performed independently for each of the indicated taxa. The indicated taxa names were typed in the “search set / organism” entry of the NCBI BLAST webpage to restrict the search. A “+” indicated that at least one PMI ortholog was identified in the taxa (species in which orthologs were retrieved are mentioned in brackets), whereas a “−” indicated that no PMI ortholog was found.

b. Sequence alignment (Muscle) of *Drosophila* PMI sequence with PMI ortholog of different species identifies blocks of highly conserved amino-acid residues. Colour code indicates level of amino-acid conservation between species ranging from high (dark blue) to low (light blue). Three conserved transmembrane helix (blue boxes) are detected by TMHMM, Scampi and Jpred.
a

Genomic DNA

PMI

PGRP-LD

PGRP-LF

mRNA

wt -/-

PGRP-LD

actin5C

Rival et al., Figure S1

b

Genomic DNA

PMI

PGRP-LD

PGRP-LF

wt -/-

PMI

PGRP-LD

actin5C

Messenger RNA
Control  PMI_PGRP-LD  PMI_PGRP-LD ubi-PMI

**adult flight muscles**

**adult proventriculus**

**ovaries (folicular cells)**

**larval fat body**

**larval salivary gland**

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Rival et al., Figure S2
a muscles

control

PMI_PGRP-LD

drp11/drp12

pink1

1 μm

Rival et al., Figure S3
Rival et al., Figure S4

**Figure S4**

(a) Control PMI_PGRP-LD vs. PMI_PGRP-LD

(b) Control PMI_PGRP-LD vs. PMI_PGRP-LD

![Graph showing mitochondrial density](image)

μm³ of mitochondria / 100 μm³ of muscle

- control
- PMI_PGRP-LD
- PMI_PGRP-LD Pac[PMI*_PGRP-LD]
a

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<td>Urochordata (Ciona)</td>
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<tr>
<td>Echinodermata (Stronglylocentrotus)</td>
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<tr>
<td>Insects (Anopheles, Apis, Culex, Drosophila, Tribolium...)</td>
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<td>Platelminthes (Shistosoma)</td>
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b

Rival et al., Figure S6
Supplementary material and methods

DNA constructs

UAS-PMI and ubi-PMI: A 1138 bp DNA fragment containing the complete PMI coding sequence preceded by 38bp of 5’UTR region and followed by 580 bp of 3’UTR was PCR amplified from a PMI_PGRP-LD bicistronic cDNA (EST GH14535 from BDGP). Forward and reverse primers (Fw primer 5’-ATGAACACATAGAAAACACAA, Rv primer 5’-TCAACCACGTGGCGAGAATG) included either EcoRI and a XhoI sites to directional cloning downstream of the UAS sequence of a pUAST-attB vector, or Gateway recombination sequences for specific insertion downstream of ubiquitin promotor sequences of a pUpH27 vector. A minimal UAS transgene (UAS-PMI_min) which contained only the coding sequence of PMI preceded by a CAAA kozac sequence (see Fig. S1) was obtained in a similar manner (Fw primer: ATGTTAGCAGAAATAGAA ; Rv primer TTACTTGTAGGACCTCCAAATC).

UAS-PGRP-LD: Similarly, the coding sequence of PGRP-LD was PCR amplified (Fw primer 5’-ATGGACTCCAGCCACATAGC, Rv primer 5’-TCACTTGGACGTGATGCTG) and inserted into pUAST-attB by directional cloning following EcoRI XhoI digestion.

UAS-PMI::mCherry: The coding sequence of PMI was PCR amplified with the 3’ primer designed to delete the TGA stop codon. In frame cloning with the mCherry sequence was performed using Gateway technology into the pUAST-attB vector. This construct allows the expression of a C-terminal tagged version of PMI under the control of UAS sequences.
Genomic rescue construct: The PMI_PGRP-LD bicistronic locus (a 5353 bp genomic fragment starting 494 bp before PMI ATG and ending 282 bp after PGRP-LD stop codon) was recovered by recombineering from a BAC (BAC 29P05, Drosophila BAC library, BACPAC Resource Center) (Venken et al., 2009, Venkel et al., 2006) into a Pacman-AmpR-attB vector to give rise to the Pac[PMI_PGRP-LD] rescue transgene. In the derived Pac[PMI*_PGRP-LD] construct, a Thymidin was added just after the second codon of PMI (see below on Fig. S0a, b asterisk) to create a premature TAG stop codon. To generate the Pac[PMI_PGRP-LD*] construct two frame shift mutations were added (see below Fig. S0a, b asterisks). Indeed, Flybase annotation predicts two types of PGRP-LD mRNA. The first isoform is predicted from EST analysis (EST GH14535, see below Fig. S0b), and is scored by Flybase as “strongly supported”. The second isoform is predicted from computational analysis, and is scored by Flybase as “moderately supported” (see below Fig. S0a). Therefore a Thymidin was added just after the ATG of the first PGRP-LD isoform to create a premature TGA stop codon, and another Thymidin was added 12 nucleotides after the ATG of the second putative isoform to create a TAG stop codon (see below Fig. S0a, b asterisks). BAC mutagenesis was essentially realized as described by (Warming et al., 2005).

Pac[CFP::PMI] and Pac[PMI::CFP] reporter transgene: In the PMI_PGRP-LD locus isolated as described above, the CFP ‘caerulean’ coding sequence was fused to PMI ATG (CFP::PMI) or cloned in frame to the 3’ end of PMI coding sequence (PMI::CFP) by recombineering (Venkel 2009). These CFP reporters were therefore placed under the control of the endogenous regulatory sequences of PMI, and allows the expression of a N-terminal or C-terminal CFP-tagged version of PMI protein.
**Figure S0. Annotated sequences of the PMI_PGRP-LD genomic (A) and PMI_PGRP-LD mRNA (B).**

**A)** Italic: introns. Yellow: coding sequence of PMI. Dark grey: coding sequence of PGRP-LD (isoform1). Underlined: coding sequence of PGRP-LD isoform-2. Green: Kozac sequence. The asterisks shows the positions of the frame shift mutations introduced in PMI and PGRP-LD coding sequence to generate respectively the Pac[PMI*|PGRP-LD] and Pac[PMI_PGRP-LD*] genomic transgene.

> PMI_PGRP-LD genomic sequence

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> PMI_PGRP-LD genomic sequence
GATAATACGACGCTATGACTCATAGGGATCATG
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> PMI_PGRP-LD mRNA sequence

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GATAATACGACGCTATGACTCATAGGGATCATG
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B) Italic: 3 prime and 5 prime UTR. Yellow: coding sequence of PMI. Dark grey : coding sequence of PGRP-LD (isoform1). Green: Kozac sequence. The asterisks shows the positions of the frame shift mutations introduced in PMI and PGRP-LD coding sequence to generate respectively the Pac[PMI*_PGRP-LD] and Pac[PMI_PGRP-LD*] genomic transgene.

> PMI_PGRP-LD mRNA (EST GH14535)

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AAACAACATAGAAAGAACAATAATTTATTAGTAACACGCGCTGCAGCTGCAGCTGACATGAGTTTATGAACAACATAGAAAACAACAAATTTATTAGCAGAA
ATG
GTT*AGCAGAAATATAGAAAGCACGAGTAAGGTGCCCACTTTCCATGTGCACTGCCGACACGCTGACATTTGGCACTTTCCATGTGCACTGCCGACACGCTGC
AGACATGGCTTATAGCAGAAATATAGAAAGCACGAGTAAGGTGCCCACTTTCCATGTGCACTGCCGACACGCTGACATTTGGCACTTTCCATGTGCACTGC
CTGACACGCTGACATTTGGCACTTTCCATGTGCACTGCCGACACGCTGACATTTGGCACTTTCCATGTGCACTGCCGACACGCTGACATTTGGCACTTTCC
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Knock out of the *PMI_PGRP-LD* locus

Knock out of the *PMI_PGRP-LD* locus was realized by ends-out gene targeting as described by Gong WJ and Golic KG (Gong and Golic, 2003). The resulting deletion covers a genomic region starting from 140 bp upstream the ATG of *PMI*, up to 77 bp after *PGRP-LD* stop codon.

*pw25-PMI_PGRP-LD-KO DNA construct:* 5’ and 3’ homology regions (5’HR and 3’HR) were PCR amplified from genomic DNA (*Oregon* background). 5’HR and 3’ HR were respectively 2790bp and 2885bp long. Primers used for 5’HR amplification (Fw primer 5’-CTGCGGGATTTGGATG, Rv primer 5’-prime TTTAAGAGCGGCCT) contain *KpnI* sites, whereas primers for 3’HR (Fw primer AGGATGAGTAAAGCG, Rev primer ACGAACC GGCCCTCCT) contain *Asci*. HRs were cloned into the polylinkers of the *pw25* vector. 5’HR was inserted on the left of *miniwhite* 5’ region, and 3’HR on the right of *miniwhite* 3’. Positive clones were selected for correct orientation such that 5’HR, 3’HR and *miniwhite* were in a parallel orientation, and the HRs were fully sequenced. This gave rise to the *pw25-PMI_PGRP-LD-KO* construct.

*Donor fly lines:* The *pw25-PMI_PGRP-LD-KO* construct was transformed into *y,w* embryos using standard procedure for P-element (Rubin and Spradling, 1982).

*Knock out:* Male donor fly were crossed with *y,w ; P{ry+, hsp70-FLP}11 P{v+, hsp70-I-Scel}2B*, *Sco* virgin females (Bloomington stock center). At day 2 and day 3 after egg laying the culture vials were heat-shocked at 37°C for 1,5h twice a day (morning and evening). Mosaic and white eyes virgin females were collected from the progeny and crossed to males carrying a *P{w+, eyeless-FLP}* transgene on X chromosome. In the resulting progeny red eye males were screened. Out of 10,000 males, 6 red eye males were isolated, and 3 of them were fertile. Genetic analysis showed that as expected the *white* marker was inserted on the 3rd chromosome in those 3 lines. Genomic deletion of the *PMI_PGRP-LD* locus was confirmed
by PCR, and we verified by sequencing that the 5’ and 3’ flanking regions were intact. One of our 3 knock out lines (namely “N3”) was submitted to 4 rounds of back crosses with y, w virgin flies to rule out the possible selection of mutations in our individual red eyed male. The deletion was balanced over *TM6b* and this strain was exclusively used in all described experiments.

**Drosophila lines**

*Culture of PMI_PGRP-LD mutant flies:* Mutant flies are neurologically impaired from eclosion. If no cautions are taken emerging flies fall down into the media and die. We therefore collected pupae and placed them into a clean fresh culture vial which was kept horizontally until the adults hatched.

*UAS-PMI, UAS-PGRP-LD and UAS-PMI::mCherry, Pac[PMI_PGRP-LD], Pac[CFP::PMI], Pac[PMI::CFP] transgenic lines:* These constructs were transformed using phiC31-mediated transgenesis (Bischof J, 2006). *attP* (25C7) or *attP* (68A4) landing sites (*P{CarryP y^+}*)) were used to generate independent insertions on the 2nd and 3rd chromosomes respectively.

*ubi-PMI transgenic line:* This construct was transformed into y,w embryos using standard P-element transposase-mediated transgenesis (Rubin and Spradling, 1982). *PM8* line was used in the described experiments.

*Fly stocks: UAS-mito::GFP line* (import mitochondrial sequence of human cCoxVIII fused to GFP, Pilling et al., 2006), *drp1* and *drp1* alleles and *Pac[drp1]*, were from Dr Patrick Verstreken (Verstreken et al., 2005), *UAS-mfn2-IR, UAS-opa1-IR, UAS-drp1* from Dr Ming Guo (Deng. et al., 2008), *opa1, pink1* from Dr Jongkyeong Chung (Park J et al., 2006), *elav-GAL4, act5c-GAL4* from the Bloomington stock center. Fly stocks were maintained at 25°C on a standard cornmeal ager diet.
Electron Microscopy

Adult brain and thorax sections were prepared for electron microscopy essentially as described by (Renfranz and Benzer, 1989). Adult fly brains and thoraxes were dissected rapidly in saline and fixed immediately in 2% paraformaldehyde, 2.5% glutaraldehyde for 24 hr at 4°C. Ultrathin Epon plastic sections were poststained with 2% uranyl acetate, followed by Reynolds’ lead citrate, and stabilized for transmission electron microscopy by carbon coating. Examination was done with a Leo 912 microscope at 100 kV.

Immunogold labeling

Adult fly thoraxes were dissected rapidly in saline, fixed immediately in 3% paraformaldehyde in PBS for 2 hr at 4°C and incubated in sucrose solution of increased concentrations to finally reach 2.3M sucrose. Samples were frozen in liquid nitrogen and submitted to freeze substitution in a Leica AFS automat in the presence of methanol and 1% uranyl acetate. Lowicryl HM20 resin was used for embedding. Ultrathin sections were washed in TBS, saturated in 0.5% BSA in TBS (TBSB). Primary were diluted in TBSB and incubated over-night at 4°C. Secondary antibodies were incubated for 2 hours at room temperature. 0.1% BSA in TBS was used for the washing steps. Before examination, the stained sections were post-fixed in 2.5% glutaraldehyde. Examination was done with a Leo 912 microscope at 100 kV.

In situ labeling and imaging

For in situ detection of mito::GFP, tissues were rapidly dissected in PBS, fixed for 15 min in 4% formaldehyde, and washed 5 min in PBS. For co-imaging of CFP::hPMI and mitochondria, live transfected C2C12 cells were incubated for 45 min in MitoTracker CMXROS (Molecular Probe) (1/5000e in DMEM culture medium), washed 10 min twice in
PBS and fixed for 10 min in 4% formaldehyde. For immunolabelling of mitochondria, tissues were dissected in PBS, fixed for 30 min in 4% formaldehyde, washed 5 min in PBS, permeabilized 2 hours in 0.5% Triton in PBS, and saturated for 1 hour in 0.5% BSA, 0.1% Tween20 in PBS (PBTB). Primary antibody diluted in PBTB was incubated over-night at 4°C, and later washed for 1 hour in 0.1% Tween20 in PBS (PBTw). Secondary antibody was incubated for 2 hours and washed for 1 hour in PBTw. Preparations were mounted in Vectashield containing DAPI (Vector). A LSM510-Meta confocal microscope (Zeiss) was used for imaging. We used the Volocity software to create 3D reconstructions of mitochondria from Z-stak image. Total volume of mitochondria was determined using the Volume3D application developed by F. Daian (IBDML) on the computing environment Matlab. Each image from a Z-stack was binarized by applying a threshold determined as described in (Otsu N, 1979) to discriminate between stained (anti-mitochondrial ATP synthase immunostaining) and unstained area. The ratio between stained and unstained area was then calculated for the whole Z-stack to give the estimated overall volume occupied by mitochondria within the analyzed volume of muscle.

**Cells Culture, transfection and fluorescence microscopy**

C2C12 mouse cells were culture in DMEM medium. Transitory transfection were performed using Cell Line Nucleofector Kit V (Amaxa). CFP::PMI constructs were obtained from Dr Stefan Wiemann (Human Protein Localization Project, EMBL, http://gfp-cdna.embl.de/).

HeLa cells were cultured in DMEM, 10% FCS. Amaxa Nucleofector kits (Amaxa Biosystems) were used for transfections of plasmids and siRNA (1 ug and 3 ug for 10^6 cells respectively). pDsRed2-Mito plasmid was purchased from Clontech. siRNA for Luciferase (luc_si) and TMEM11 (si1 : J-00540-09 and si2 : J-005440-10) were purchased from Dharmacon Research.
Cell fractionation

Cells were suspended in mitochondrial buffer (MB, 210 mM mannitol, 70 mM sucrose, 1 mM EDTA and 10 mM Hepes pH 7.5) supplemented with a cocktail of protease inhibitors (Boehringer) and broken by 15-20 strokes of a tight-fitting Dounce homogenizer. The suspension was centrifuged (800 x g, 4°C, 10 min) and the supernatant spun (12,000 x g, 4°C, 15 min) to separate the mitochondrial pellet from the cytosol plus light membrane fraction.

To determine the sub-compartmentalization, mitochondria were suspended in MB or MB/10 (MB with 1/10 concentrations of mannitol and sucrose) containing trypsin and the indicated concentrations of digitonin or Triton X-100. After 30 min on ice, proteolysis was terminated by adding 5 mM PMSF and Laemli's sample buffer, and heating at 90°C for 10 min.

To assess membrane association, mitochondria in hypo-osmotic buffer (MB/10) were fragilized by 3 freeze-thaw cycles and sonicated 3 times for 15 sec on ice. Residual intact mitochondria were removed (12,000 x g, 4°C, 10 min) and the supernatant made either 1 M NaCl or 0.1 M Na₂CO₃, pH 11 and incubated for 30 min on ice with frequent vortexing. The solution was centrifuged (150,000 x g, 4°C, 45 min) to separate the supernatant from the membrane pellet. Samples (100-200 µg of mitochondrial proteins) were analyzed by SDS-PAGE and Western blotting using the antibodies indicated.

Antibodies

Primary antibody for microscopy analysis: mouse anti-ATP Synthase subunit-alpha monoclonal antibody MS507 (MitoSciences) (1/300 for IF and 1/150 for EM) was used to detect mitochondria; chicken anti-VDAC polyclonal antibody (generous gift from Brett H. Graham, Graham BH et al., 2005) (1/200); chicken anti-GFP antibody (Aves Labs) (1/500
for IF and 1/250 for EM) and anti-RFP monoclonal antibody (Cliniscience) (1/500) were used to amplify CFP::PMI and PMI::mCherry signal respectively.

*Primary antibodies for cell fractionation*: anti-HSP60 (1/200) (Sigma), anti-VDAC (1/1000) (Calbiochem), anti-Smac/Diablo (1/1000) (Sigma), anti-COX4 (1/500) (Cell Signaling), anti-TMEM11 (1/500) (Proteintech Group Inc), anti-DRP1 (1/1000) (BD Biosciences), anti-cytoC (1/500) (BD Pharmingen), anti-rabbit IgG-HRP and anti-mouse IgG-HRP (Abcam).

*Secondary antibody for IF*: Alexa488 goat anti-mouse IgG antibody, Alexa546 goat anti-mouse IgG antibody, Alexa488 goat anti-mouse IgG (Interchim Jackson) (1/500), Alexa488 goat anti-chicken IgY antibody (Molecular Probe) (1/500).

*Secondary antibody for EM*: 10 nm colloidal gold particle-conjugated goat anti-chicken IgY antibody and 15nm colloidal gold particle-conjugated goat anti-mouse IgG antibody.

*Secondary antibodies for cell fractionation*: anti-rabbit IgG-HRP or anti-mouse IgG-HRP (1/10 000), (Abcam).

**Supplementary references**


