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Supporting Online Material for

An ER-Mitochondria Tethering Complex Revealed by a Synthetic Biology Screen

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Database S1 as a zipped archive: 1175088s2.zip

Correction: In the original version of the SOM, some images and fonts were slightly corrupted due to the file conversion process. Those errors have been fixed here.

Materials and Methods

Yeast Manipulations

All yeast transformation were done using standard methods. Loci replacements were achieved using the Pringle PCR toolbox (S1).

Construction of ChiMERA and ChiMERA-ra

A monomeric (A206R), yeast codon adapted version of GFP, derived from pKT127 (S2) was amplified using the BamHI-Akap1-GFP and XhoI-UBC6-GFP primers for ChiMERA-ra and BamHI-TOM70-GFP and XhoI-UBC6-GFP primers primers for ChiMERA. The resulting product was cloned using BamHI and XhoI into p415GPD (S*3*). The *ADE3* ORF was amplified from yeast genomic DNA using SacI-ADE3-F and SacI-ADE3-R and cloned into the SacI site of p415GPD-ChiMERA. This p415-GPD-ChiMERA/ADE3 plasmid was introduced into an *ade3Δ* strain.

The BamHI/XhoI ChiMERA fragment was also cloned into p406GAL (S*4*) generating the p406GAL-ChiMERA plasmid.

Mutagenesis Screen

Saturated cultures of ByK9 cells were mutagenized with 20 µl/ml EMS for 90 minutes and spread onto SC -URA -ADE +3% glycerol +3% ethanol +16 mg/l Adenine. Candidate uniformly red colonies were isolated. As a secondary screen, these candidates were plated on the same medium supplemented with 2% galactose and assessed for restored sectoring by the expression of the GAL induced copy of the ChiMERA. After loss of the p406GAL-ChiMERA plasmid by crossing, mutations were complemented using the Rose URA CEN/ARS genomic library (S*5*).

Microscopy

Exponentially growing cells were deposited on Concanavalin A coated slides and imaged using a Yokogawa CSU-22 spinning disc confocal on a Nikon TE2000 microscope. GFP was excited with the 488nm Ar-ion laser line and dsRed with the 568 nm Ar-Kr laser line. Images were recorded with a 100x/1.4 NA Plan Apo objective on a Cascade II EMCCD. The sample magnification at the camera was 60 nm/pixel. The microscope was controlled with the µManager and ImageJ softwares.

Phospholipid analysis

Phospholipid extraction was performed using the Folch procedure (S*6*). Briefly, cells were pelleted and resuspended in 20 µl of the original medium. 330 µl of methanol and 100 µl of glass beads were added and the samples were vortexed for 10 min. 660 µl of chloroform was added and the cell debris were spun down at 10,000 g for 10 minutes. The supernatant was kept and the aqueous phase was extracted with 200 µl of 0.9% NaCl in water. Samples were dried completely in a speedvac then resuspended in 50 µl methanol:chloroform (1:2).

One dimensional thin-layer chromatography (TLC) was performed using the Vaden procedure (S*7*). TLC plates (Whatman partisil LK6D) were wetted in a solution of 2.3% (w/v) boric acid in ethanol and allowed to dry. Development was performed in chloroform/ethanol/water/triethylamine (30/35/7/35, v/v).

For steady-state levels, dry TLC plate were dipped for 20 second in a solution of 10% cupric sulfate

(w/v) and 8% phosphoric acid and charred in a 180°C oven for 15 to 25 minutes.

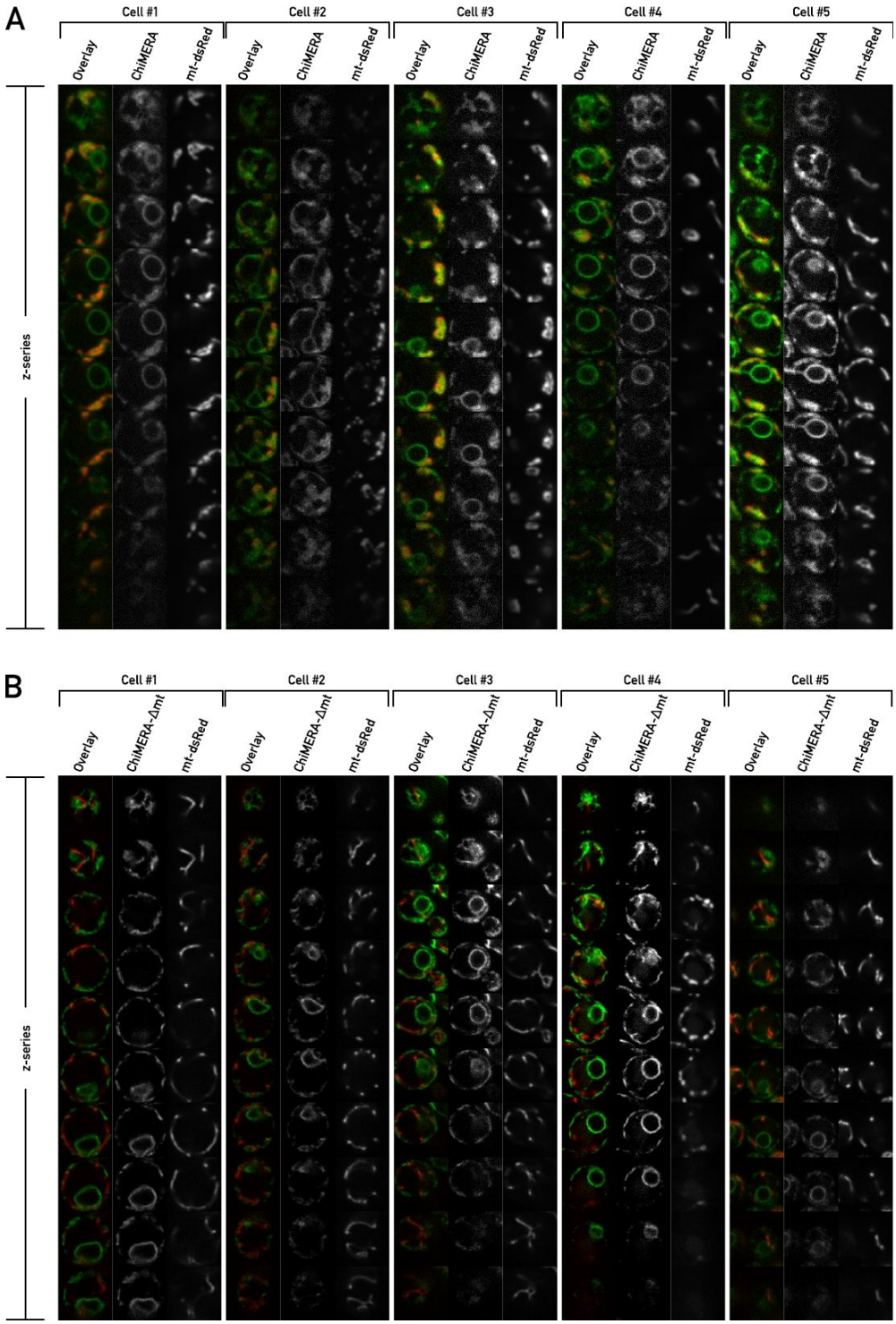
For incorporation analysis, exponentially growing yeast were cultured in SC medium supplemented with 2mM ethanolamine. They were diluted to OD 0.2 in the same medium containing 2 µCi/ml ^{14}C-serine (129.4 mCi/mmole) and labeled for 2 hours. Chase was performed by the addition of 1 mM cold serine. 1 ml aliquots were taken at different times and processed for phospholipid extraction and TLC. Incorporations were quantified by phosphorimaging using the imagej software.

Protein analysis

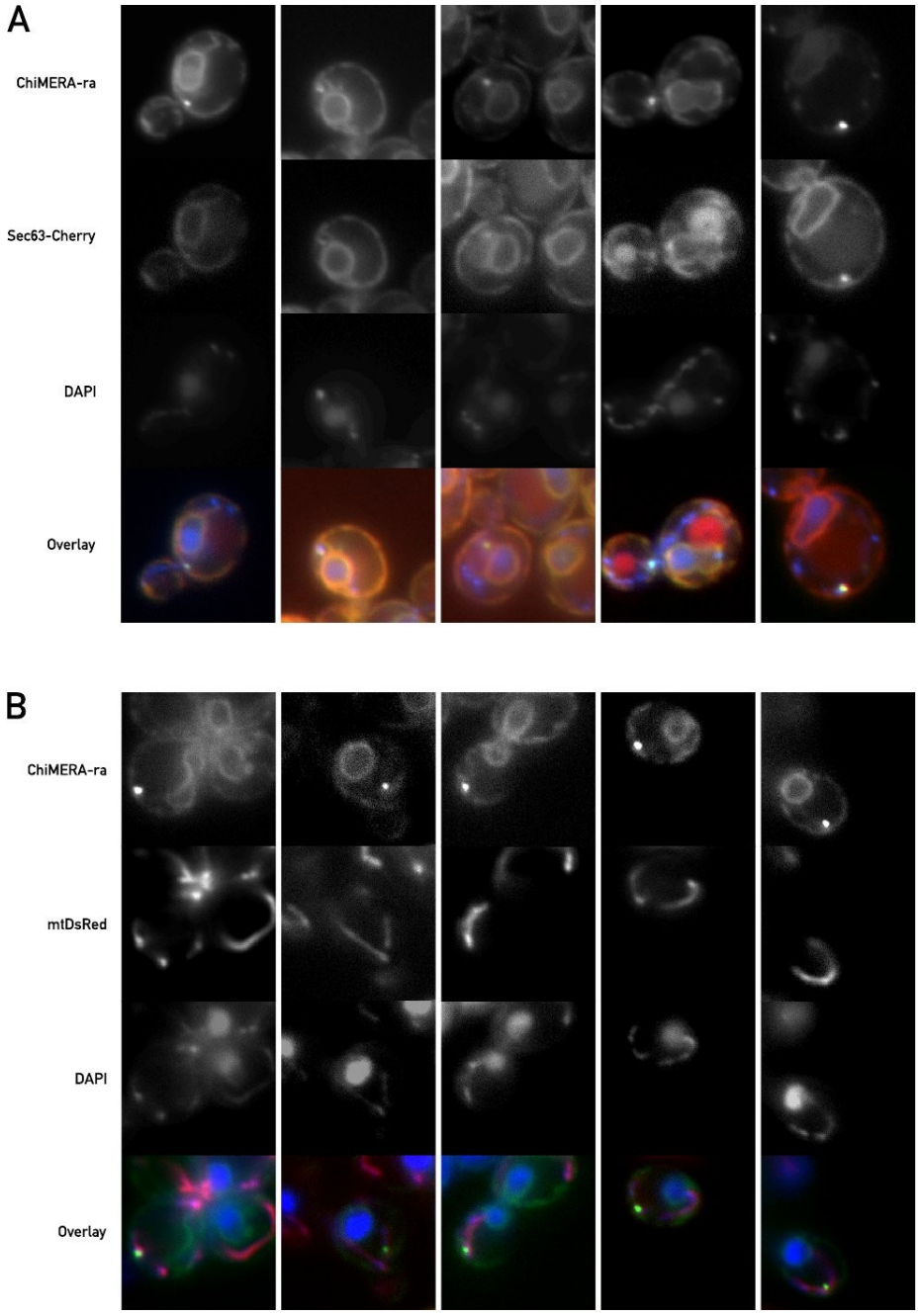
For EndoH treatment, 20 OD of exponentially growing cells in YP+ 3% Ethanol and 3% glycerol were harvested and bead beaten for 10 minutes in 400 µl lysis buffer (20 mM Hepes-KOH pH7.4, 150 mM potassium acetate, 2 mM magnesium acetate, 0.1 mM PMSF) at 4ºC. SDS and β-mercaptoethanol were added to 0.5% and 1% respectively. The extract was heated at 100ºC for 10 minutes then cellular debris were spun down. Extracts were then diluted two-fold in sodium citrate pH5.5 (final concentration 50 mM) with 1000 units EndoH_f. Deglycosylation took place for 3 hours at 37ºC.

For carbonate treatment, 20 OD of exponentially growing cells in YP + 3% Ethanol and 3% glycerol were harvested and bead beaten in 600 µl of the same lysis buffer 6 time 45 second with 1 minute on ice in between each stroke. Cell debris and nuclei were spun down 3 time 4 minutes at 400g. Total membranes were isolated from the supernatant by ultracentrifugation 40 minutes at 100,000g. Pellet were resuspended in 0.2M Na_2CO_3 (pH11.2) or in the original lysis buffer and allowed to sit for 90 minutes on ice followed by 40 minute centrifugation at 100,000g. Pellet and supernatant were kept for SDS-Page analysis. Supernatant proteins were TCA-precipitated to remove the carbonate.

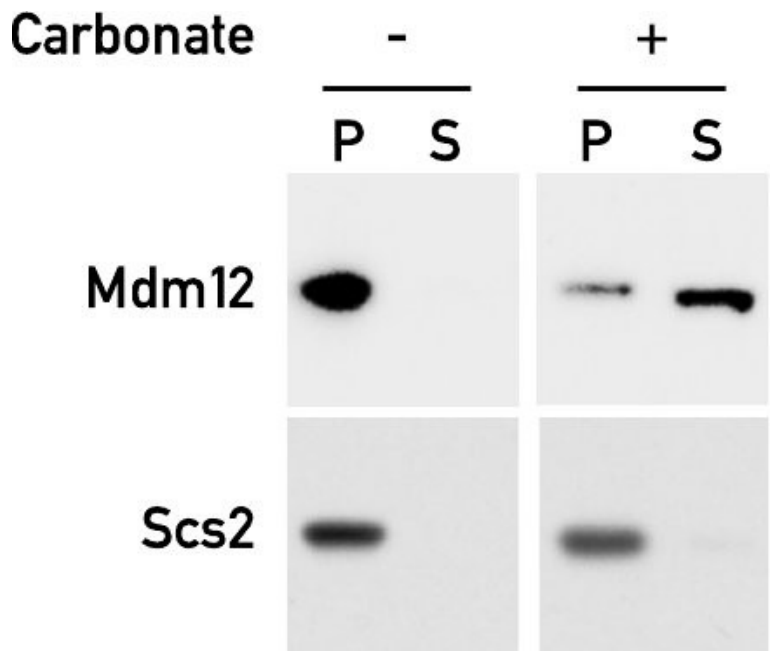
TEV cleavage was performed essentially as described (20). Briefly, Saturated yeast cultures grown in SC +Dextrose -Ura -Leu medium were washed 3 time and resuspended in SC +Dextrose -Ura -Leu or SC +Galactose -Ura -Leu at a OD of ~0.4 to repress or induce expression of the TEV protease. After 4h incubation at 30ºC, total proteins were isolated and used for SDS-Page analysis.



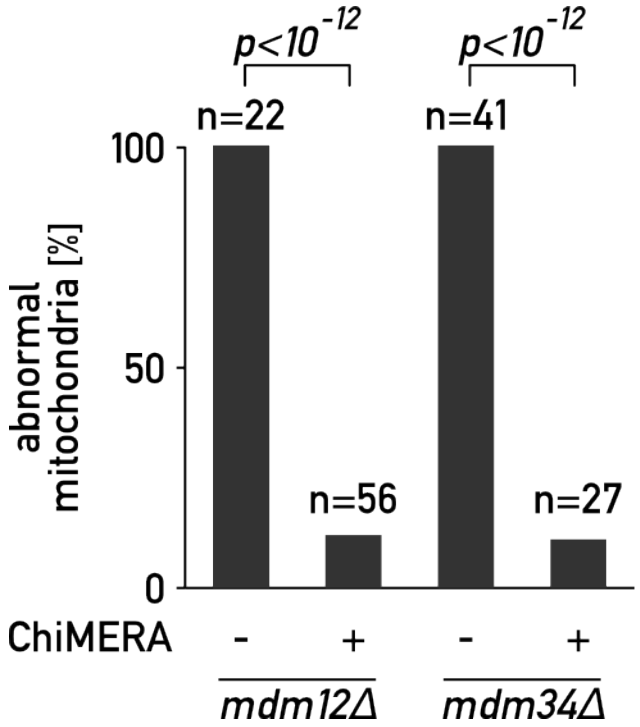
Supplemental Figure S1: ChiMERA expression distorts mitochondrial shape.
(A) Z confocal series of five representative cells expressing ChiMERA and the mitochondrial marker mtDsRed. ChiMERA-induced ER-mitochondria tethering distorts mitochondrial shape and creates contact patches where the mitochondria departs from its usual tubular morphology.
(B) Z confocal series of five representative cells expressing a version of ChiMERA in which the mitochondrial targeting signal has been removed (ChiMERA-Δmt). This synthetic protein is incapable of performing artificial organelle tethering and does not distort mitochondrial morphology.



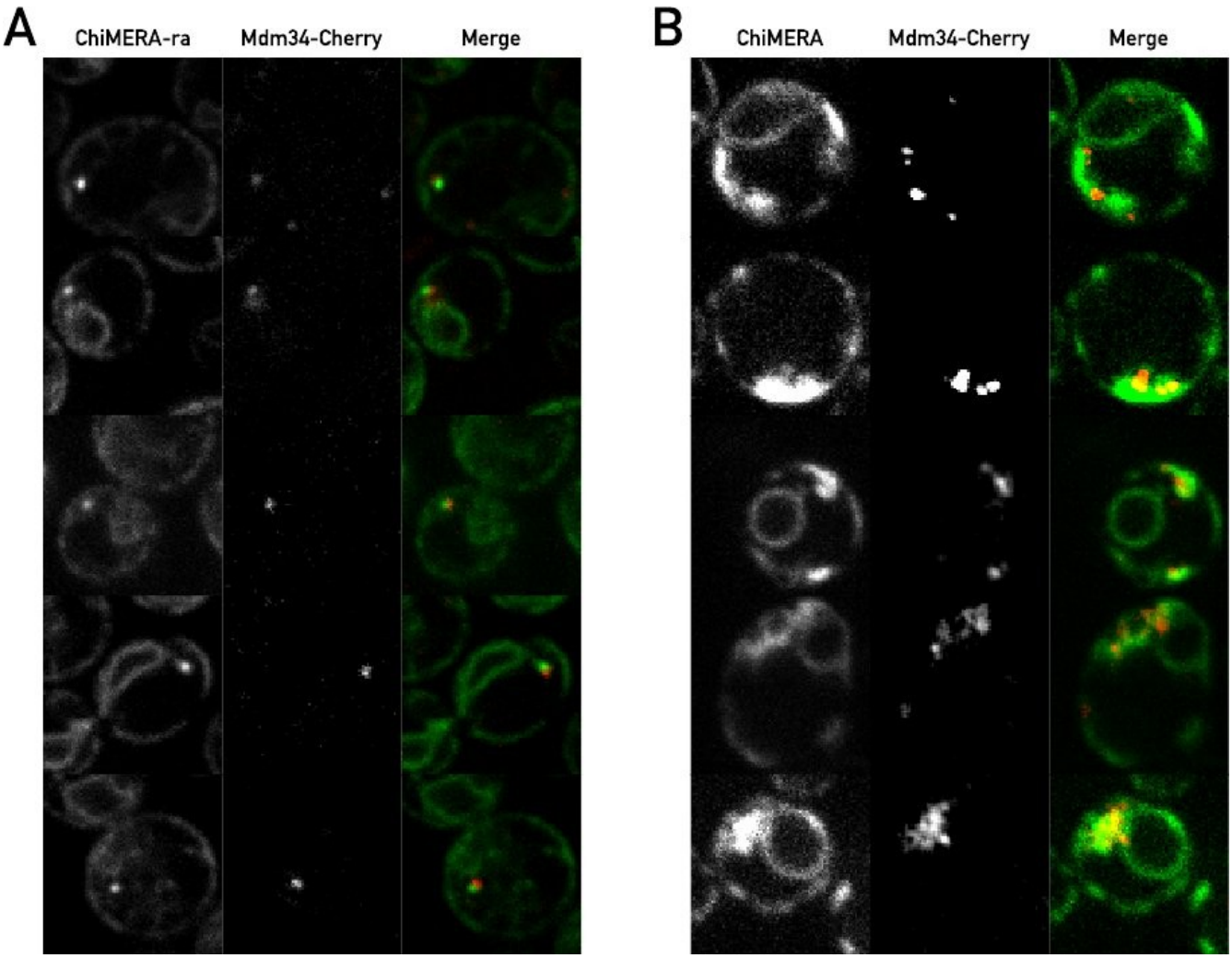
Supplemental Figure S2: Colocalization of ChiMERA-ra with mitochondria and ER.
(A) Epifluorescence micrographs of five representative yeast cells expressing ChiMERA-ra and a Sec63-mCherry fusion protein expressed from the endogenous *SEC63* locus and stained with DAPI that labels both nuclear and mitochondrial DNA. ChiMERA-ra labeling coincides with Sec63-mCherry labeled ER membranes. Puncta where ChiMERA-ra staining is enriched over Sec63-mCherry are shown (arrowheads).
(B) Epifluorescence micrographs of five representative yeast cells expressing ChiMERA-ra and the mitochondrial marker mtDsRed. The puncta identified in (arrowheads) colocalize with mitochondria as determined by coexpression of mtDsRed and by DAPI staining.
These data indicate that ChiMERA-ra is located in the ER and is enriched at ER-mitochondria contact sites.



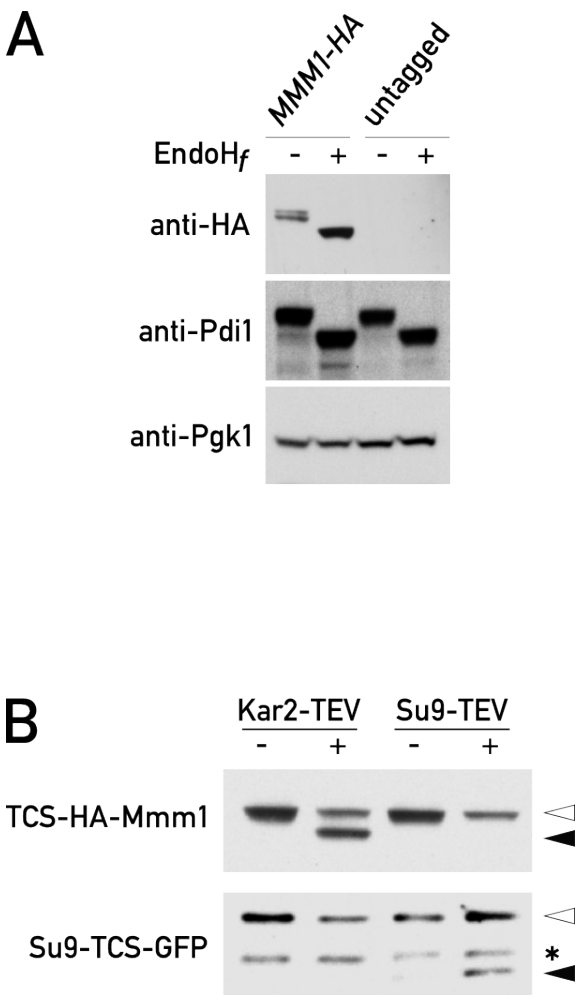
Supplemental Figure S3: Mdm12 is a membrane peripheral protein. Whole cell membrane extracts from a strain expressing a functional TAP-tagged Mdm12 from its endogenous locus were isolated and treated with 0.2M sodium carbonate (+) or original isolation buffer (-). After ultracentrifugation, the contents of the pellet (P) and supernatant (S) fractions were assessed by SDS-PAGE and western blotting. Upper panel: upon carbonate treatment, most of Mdm12 is extracted and is found in the supernatant fraction. Lower panel: the single pass transmembrane protein Scs2 is not extracted and is therefore found in the pellet fraction with or without carbonate treatment.
These data indicate that Mdm12 is a membrane peripheral protein. We do not exclude that Mdm12 makes some direct contacts with the membrane, for example via amphipathic patches, that might explain the partial carbonate extraction.



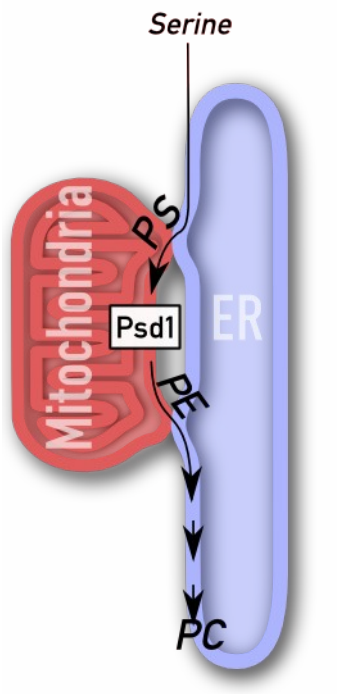
Supplemental Figure S4: Quantification of mitochondrial shape rescue by ChiMERA (see Fig. 2B). p-values are from a two-sided χ^2-test. *n* represents the number of cells analyzed.



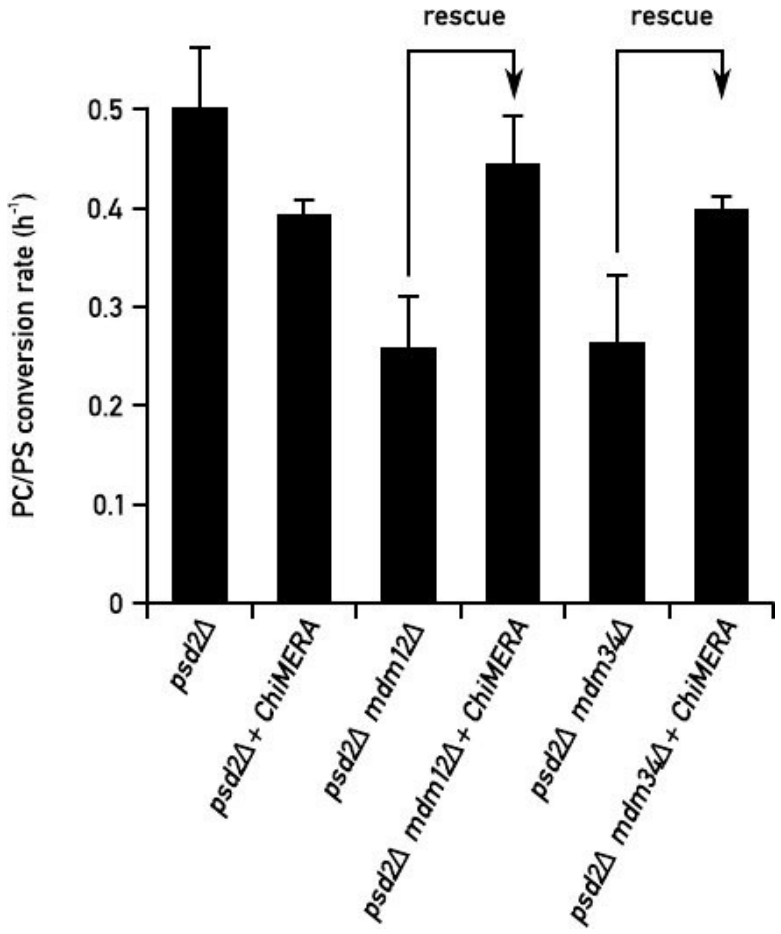
Supplemental Figure S5: ERMES is localized at the ER-mitochondria interface.
(A) Confocal images of five representative yeast cells expressing ChiMERA-ra and mCherry-fused Mdm34 from its endogenous locus. The arrowheads indicate the position of ERMES foci that colocalize with bright ChiMERA-ra signal.
(B) Confocal images of five representative yeast cells expressing ChiMERA and Mdm34-mCherry. Mdm34 not only colocalizes with ChiMERA but also spreads on the surface of the artificially created ER-mitochondria contact area.
Together, these data indicate that ERMES is located at the ER-mitochondria interface, either labeled by ChiMERA-ra or broadened by ChiMERA.



Supplemental Figure S6: Mmm1 is inserted in the ER membrane.
(A) Total protein extracts from cells expressing a functional Mmm1-HA fusion protein from the endogenous *MMM1* locus were subjected to deglycosylation by the glycosydase EndoH as in Fig 3. Upper panel: Western blotting using and anti-HA antibody shows that Mmm1-HA electrophoretic mobility is affected by EndoH treatment, indicative of Mmm1 glycosylation. Middle panel: The ER resident glycoprotein Pdi1 serves as a positive control for EndoH treatment and shows an electrophoretic mobility shift upon deglycosylation. Lower panel: The cytoplasmic phosphoglycerate kinase Pgk1 is not affected by EndoH treatment and serves as a negative control.
(B) Mmm1 N-terminus is exposed to site-specific TEV cleavage in the ER lumen. TEV cleavage assay was performed as in (20). Upper Panel: A construct encoding a HA-Mmm1 fusion protein engineered to contain a TEV cleavage site (TCS) in the N-terminus was introduced into cells that bore an additional plasmid encoding the TEV protease either targeted to the ER by the Kar2 cleavable signal sequence (Kar2-TEV) or targeted to the mitochondrial matrix using the mitochondrial targeting presequence from the ATPase subunit 9 of *N. Crassa* (Su9-TEV). Extracts collected from cells either expressing (+) or repressing (-) the TEV construct, were subjected to SDS-Page and western blotting. TCS-Mmm1-HA (open arrowhead) is accessible to protease digestion (plain arrowhead) when TEV is targeted to the ER lumen. Lower panel: a control TEV substrate was targeted to the mitochondrial matrix (Su9-TCS-TEV). The asterisk indicates a form of Su9-TCS-GFP generated by the mitochondrial processing peptidase (20).



Supplemental Figure S7: Pathway of aminoglycerophospholipid biosynthesis in yeast. Phosphatidyl-serine (PS) is synthesized in the ER. PS is decarboxylated to phosphatidyl-ethanolamine (PE) by the mitochondrial enzyme Psd1. PE is then transported back to the ER where it is converted to phosphatidyl-choline (PC).



Supplemental Figure S8: ChiMERA expression partially rescues the phospholipid transport defect of *mdm12Δ* and *mdm34Δ* strains. 14C-Serine pulse-chase was performed as for Fig. 7 in strains of the indicated genotype, and expressing or not the ChiMERA. Presented are the slopes of the linear regression of the change in PC/PS ratio. Error bars represent the standard error of the linear regression.

Supplemental Table 1: Primers used in this study

BamHI-TOM70-GFP	5'TAGGATCCAAAAATGAAGAGCTTCATTACAAGGAACAAGACAGCCATTTTGGCAACCGTTGCTGCTACAGGTACTGCCATCGGTGCCTACTATTATTACAACGAAGCTTCGTACGCTGCAG3'
BamHI-Akap1-GFP	5'AGGATCCAAAAATGGCTATTCAATTGAGATCTTTGTTTCCATTGGCTTTGCCAGGTATGTTGGCTTTGTTGGGTTGGTGGTGGTTTTTTTCTAGAAAAAAAGAAGCTTCGTACGCTGCAG3'
XhoI-UBC6-GFP	5'CTACTCGAGTCATTTCATAAAAAGGCCAACCAAAAACAAAAAAATA3'GCGATACCAATATAAACCATAACTCTAGAATTAGATTTGTACAATTCATCCATAC3'
SacI-ADE3-F	5'ATGGAGCTCATCGTTGGATGCAAGAAGGAC3'
SacI-ADE3-R	5'GTAGAGCTCGTGTAGTCCAATACCGT3'

Supplemental Table 2: Yeast strains used in this study. Only relevant genotype information is provided.

Strain	**Original Background**	**Genotype**
ByK9	W303	*Ade3Δ* p406GAL-ChiMERA p415-GPD-ChiMERA/ADE3
ByK3	W303	*SEC63-mCherry::His3* p415-GPD-ChiMERA-ra
ByK25	W303	*Ade3Δ mdm10Δ::His3* p406GAL-ChiMERA
ByK26	W303	*Ade3Δ mdm10Δ::His3* p406GAL-ChiMERA p415-GPD-ChiMERA/ADE3
ByK27	W303	*Ade3Δ mdm12Δ::His3* p406GAL-ChiMERA
ByK28	W303	*Ade3Δ mdm12Δ::His3* p406GAL-ChiMERA p415-GPD-ChiMERA/ADE3
Byk29	W303	*Ade3Δ mdm34Δ::His3* p406GAL-ChiMERA
ByK30	W303	*Ade3Δ mdm34Δ::His3* p406GAL-ChiMERA p415-GPD-ChiMERA/ADE3
ByK43	W303	*Ade3Δ MMM1-3xHA::HIS3*
ByK60	By4741	*MMM1-GFP::HIS3*
ByK61	By4741	*MMM1-GFP::HIS3 mdm12Δ::KanMX*
ByK62	By4741	*MMM1-GFP::HIS3 mdm34Δ::KanMX*
ByK63	By4741	*MDM10-GFP::HIS3*
ByK64	By4741	*MDM10-GFP::HIS3 mdm12Δ::KanMX*
ByK65	By4741	*MDM12-GFP::HIS3*
ByK66	By4741	*MDM12-GFP::HIS3 mmm1Δ::KanMX*
ByK67	By4741	*MDM12-GFP::HIS3 mdm34Δ::KanMX*
ByK68	By4741	*MDM34-GFP::HIS3*
ByK69	By4741	*MDM34-GFP::HIS3 mdm12Δ::KanMX*
ByK70	By4741	*MDM10-GFP::HIS3 mdm34Δ::KanMX*
ByK82	By4741	*MMM1-GFP::HIS3 mdm10Δ::KanMX*
ByK83	By4741	*MDM12-GFP::HIS3 mdm10Δ::KanMX*
ByK84	By4741	*MDM34-GFP::HIS3 mdm10Δ::KanMX*
ByK85	By4741	*psd1Δ::KanMX psd2Δ::KanMX*
ByK77	By4741	*psd2Δ::KanMX*
ByK78	By4741	*psd2Δ::KanMX mmm1Δ::KanMX*
ByK79	By4741	*psd2Δ::KanMX mdm10Δ::KanMX*
ByK80	By4741	*psd2Δ::KanMX mdm12Δ::KanMX*
ByK81	By4741	*psd2Δ::KanMX mdm34Δ::KanMX*
ByK87	By4741	*psd2Δ::KanMX* p415-GPD-ChiMERA/ADE3
ByK88	By4741	*psd2Δ::KanMX mdm12Δ::KanMX* p415-GPD-ChiMERA/ADE3
ByK89	By4741	*psd2Δ::KanMX mdm12Δ::KanMX* p415-GPD-ChiMERA/ADE3
ByK91	By4741	*MDM12-TAP::HIS3*

Supplemental References

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