SUPPLEMENTAL FIGURES AND LEGENDS

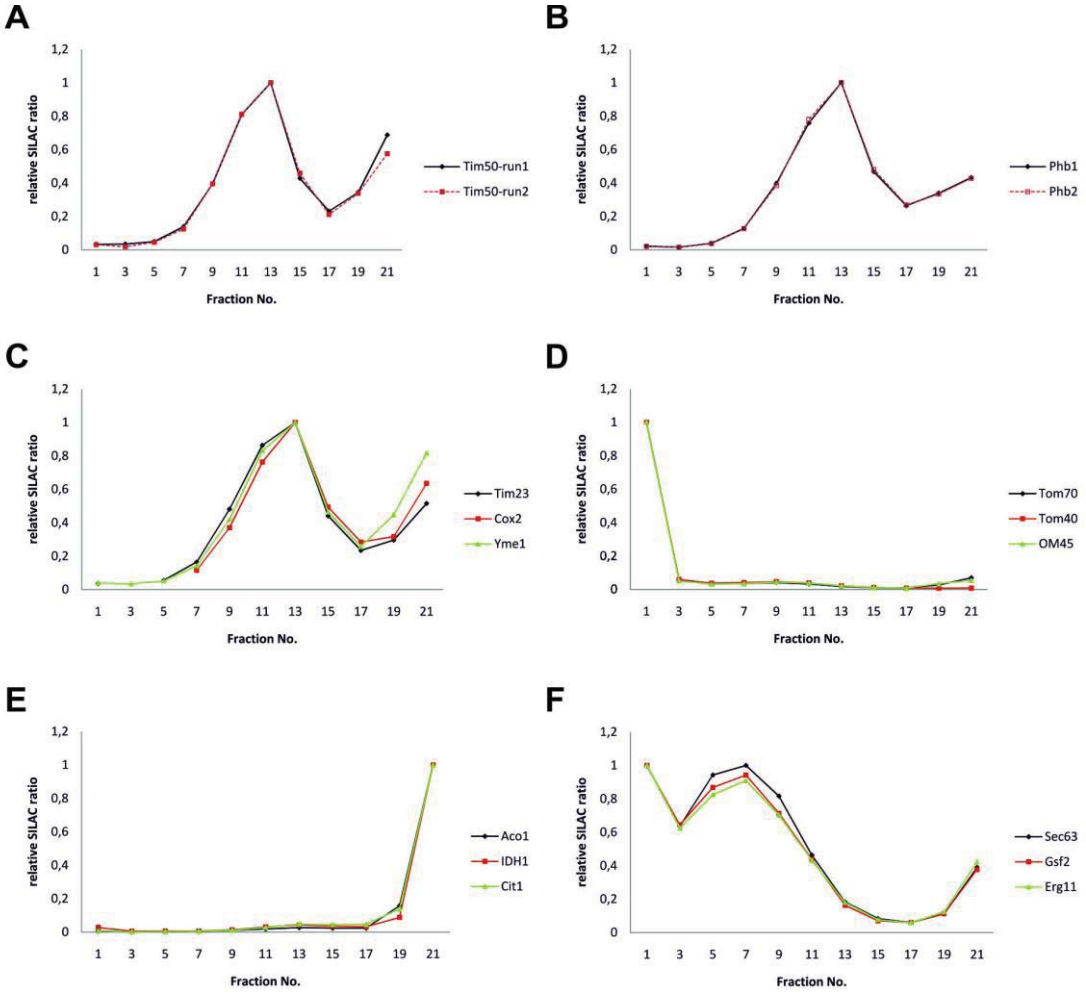
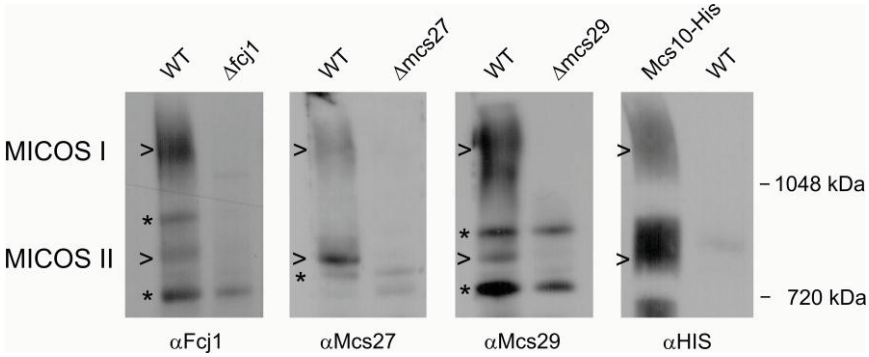
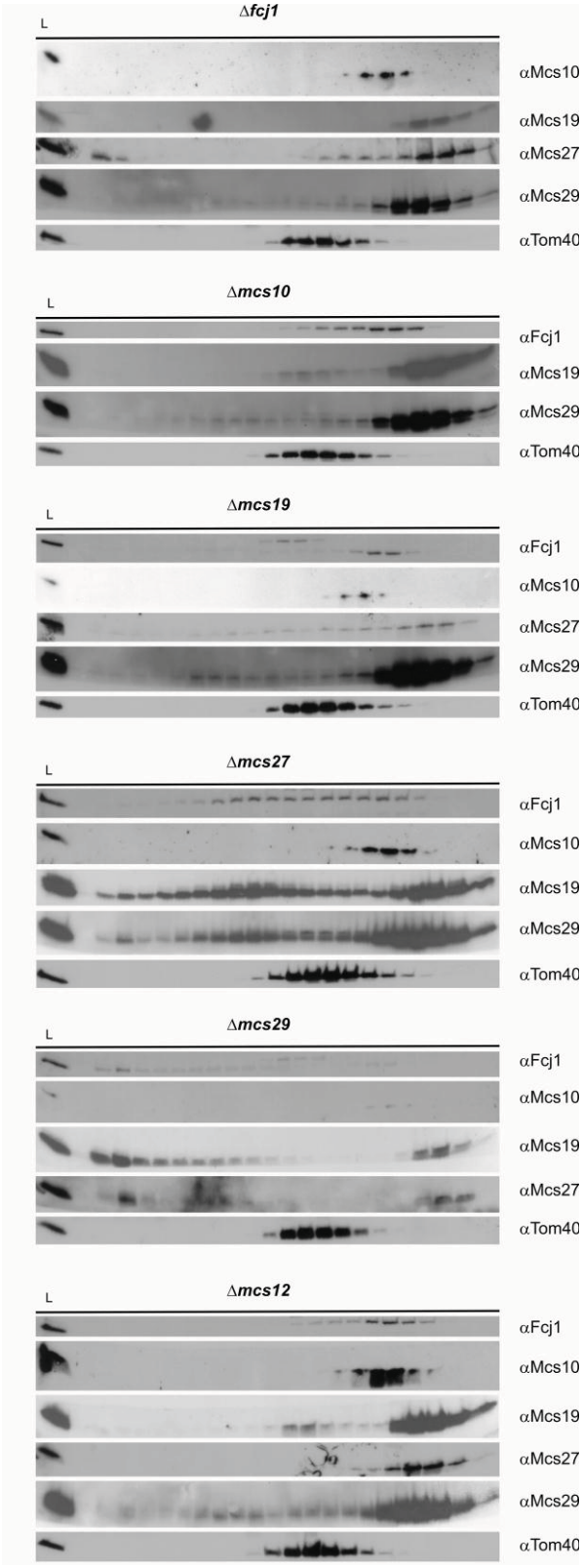


Figure S1, related to Figure 1. **Density gradient profiles of various mitochondrial proteins (see Figure 1C) determined by quantitative mass spectrometry. (A)** Two different analyses of the same gradient, Tim50. **(B)** Two subunits of one protein complex,prohibitin (Phb1 and Phb2). **(C)** Examples of inner membrane proteins. Tim23; Cox2, cytochrome oxidase subunit 2; Yme1, i-AAA-protease. **(D)** Examples of outer membrane proteins, Tom70, Tom40, OM45. **(E)** Examples of matrix proteins. Aco1, aconitase; Idh1, isocitrate dehydrogenase; Cit1, citrate synthase. **(F)** Examples of ER proteins. Sec63, Gsf2, Erg11.

A


WT
Δfcj1
WT
Δmcs27
WT
Δmcs29
Mcs10-His
WT
MICOS I
MICOS II
1048 kDa
720 kDa
αFcj1
αMcs27
αMcs29
αHIS

B


Δfcj1
L
αMcs10
αMcs19
αMcs27
αMcs29
αTom40
Δmcs10
L
αFcj1
αMcs19
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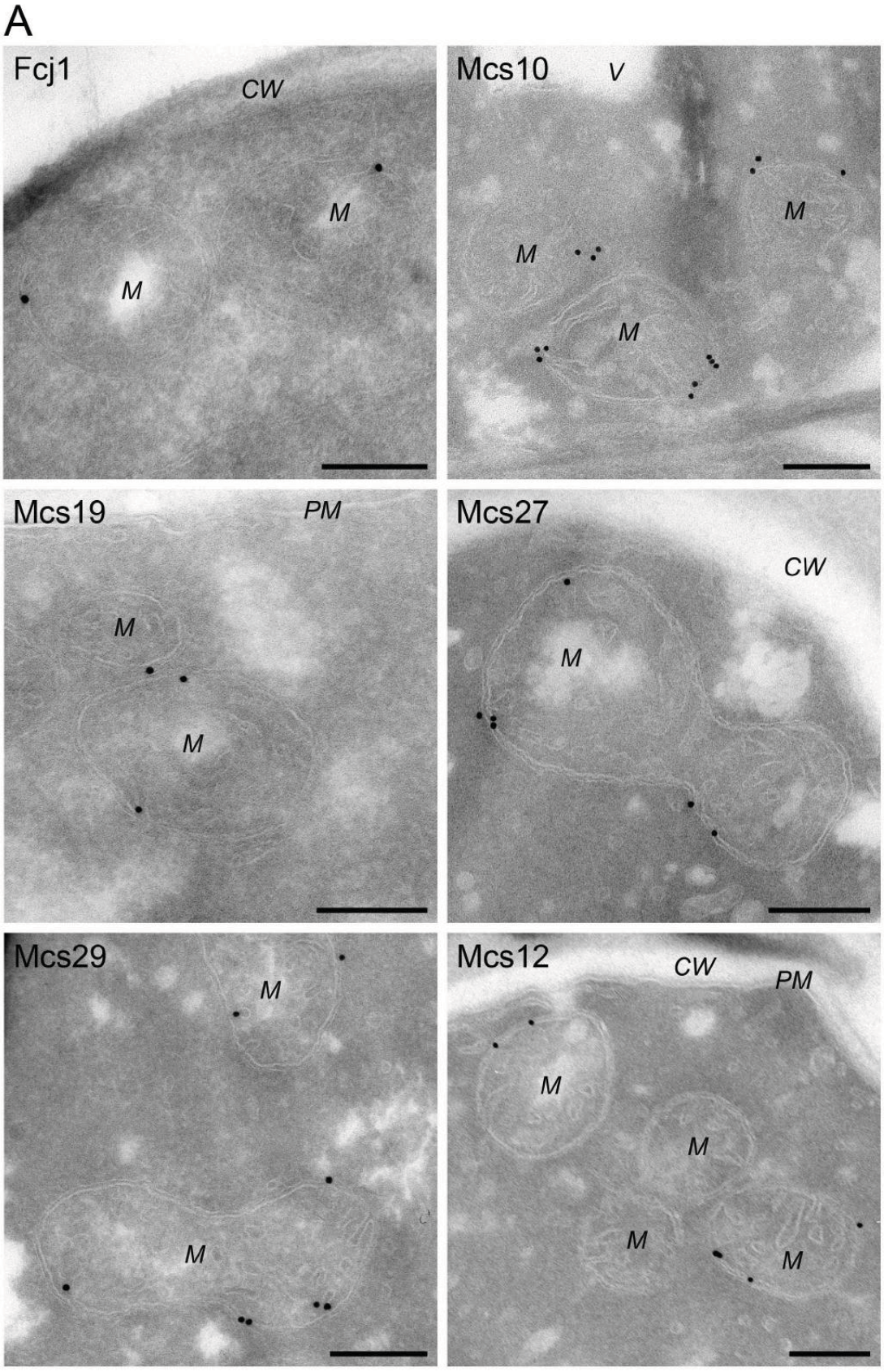
αTom40
Δmcs19
L
αFcj1
αMcs10
αMcs27
αMcs29
αTom40
Δmcs27
L
αFcj1
αMcs10
αMcs19
αMcs29
αTom40
Δmcs29
L
αFcj1
αMcs10
αMcs19
αMcs27
αTom40
Δmcs12
L
αFcj1
αMcs10
αMcs19
αMcs27
αMcs29
αTom40

Figure S2, related to Figure 2. Analysis of the MICOS complex. (A) Analysis by Blue native gel electrophoresis. Mitochondria isolated from wild type cells and cells lacking either Fcj1, Mcs27 or Mcs29 were lysed with digitonin, subjected to BN-PAGE followed by immunodecoration with antibodies against Fcj1, Mcs27 and Mcs29. In addition, mitochondria isolated from a strain containing a his-tagged version of Mcs10 were analyzed in the same way and then subjected to immunodecoration with antibodies against the his-tag. (B) Molecular sizing of the MICOS complex in mutants in which each one of the genes coding for the contact site proteins was deleted. The analysis was performed as described in Figure 2C. Arrow heads indicate positions of MICOS complexes I and II; Asterisks, unspecific cross reactions.


A
Fcj1
CW
M
M
Mcs10
V
M
M
M
Mcs19
PM
M
M
Mcs27
CW
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M
M
Mcs12
CW
PM
M
M
M
M

B

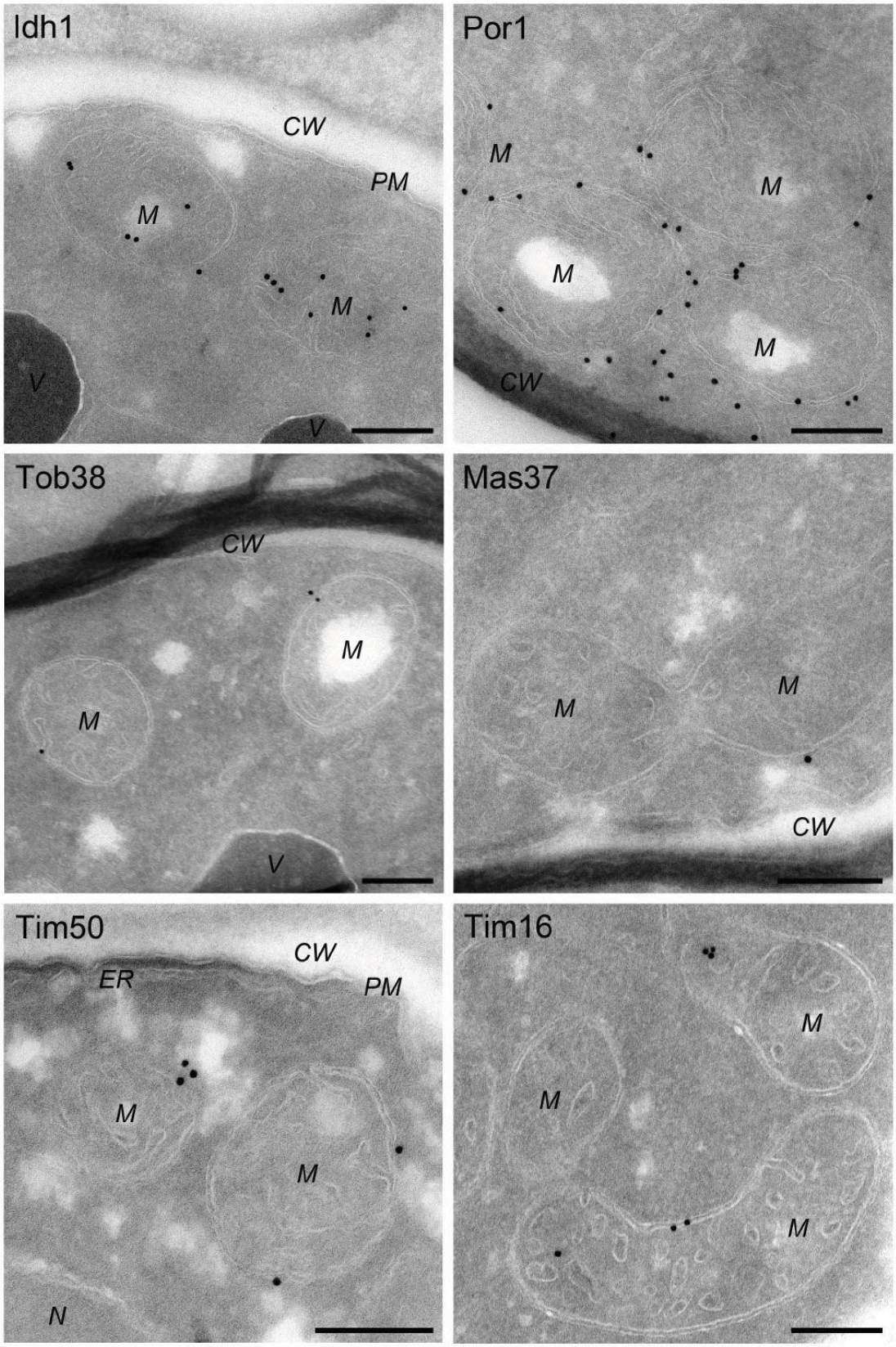
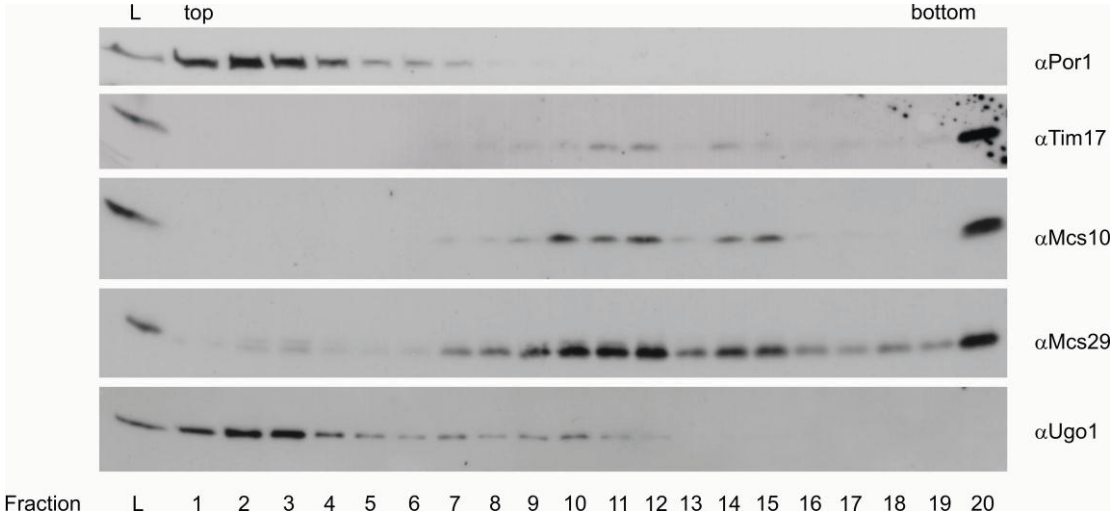

Idh1
CW
PM
M
M
V
V
Por1
M
M
M
M
CW
Tob38
CW
M
M
V
Mas37
M
M
CW
Tim50
CW
ER
PM
M
M
N
Tim16
M
M
M

Figure S3, related to Figure 3. **Additional examples of immuno-electron microscopy micrographs**. (A) Contact site proteins. (B) Proteins of various mitochondrial subcompartments in cells grown on lactate containing medium. CW, cell wall; ER, endoplasmic reticulum; M, mitochondrion; N, nucleus; PM, plasma membrane; V, vacuole. Bar size, 200 nm.

A


L
top
bottom
αPor1
αTim17
αMcs10
αMcs29
αUgo1
Fraction
L 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20

B

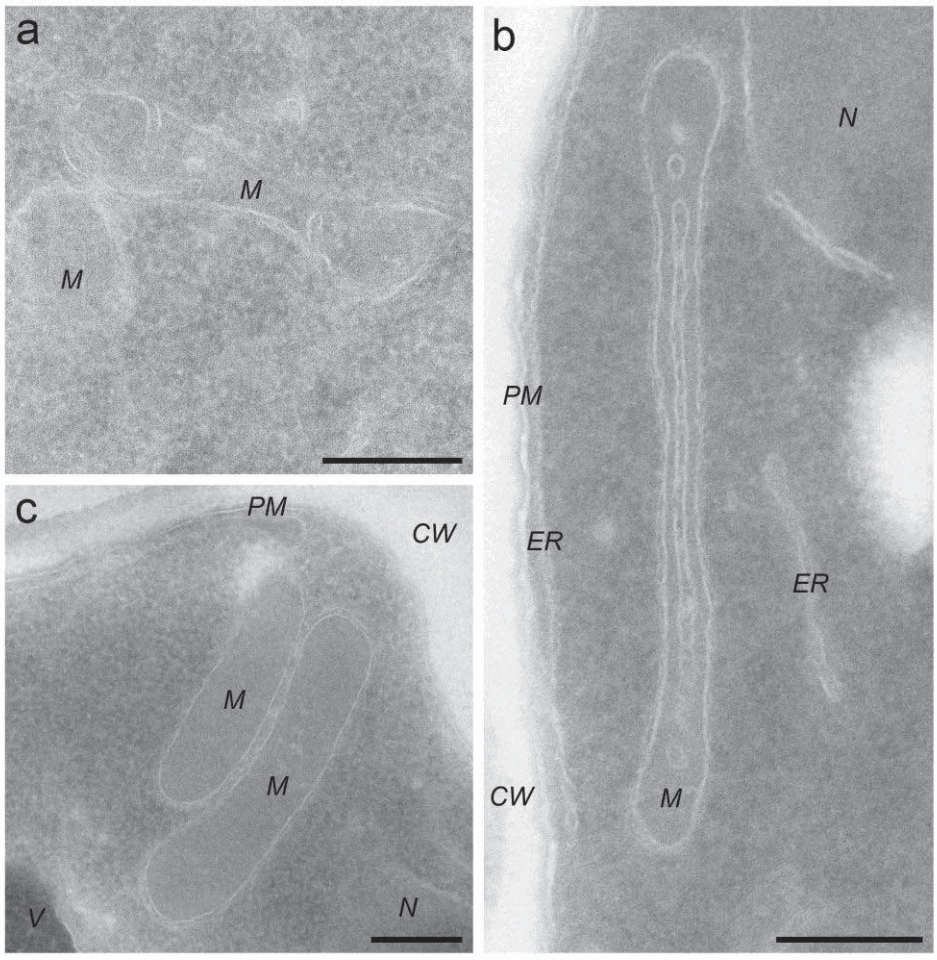

a
M
M
b
N
PM
ER
ER
CW
M
c
PM
CW
M
M
V
N

Figure S4, related to Figure 4. Requirement of Fcj1 for the presence of contact sites and of Ugo1 for the presence of cristae.

(A) Alteration of the submitochondrial distribution of Mcs proteins and Ugo1 upon deletion of Fcj1. Mitochondria from a strain deficient in Fcj1 were subfractionated and the vesicles separated by gradient centrifugation as described in Fig. 1. The distribution of Mcs10 and Mcs29 and of Ugo1 were analysed by SDS-PAGE and immunoblotting with the indicated antibodies.

(B) Ultrastructural analysis of Δ*ugo1* cells. (a) Wild type, (b) Δ*mcs10* and (c) Δ*ugo1* strains were grown in YPD before preparing ultrathin cryo-sections as described in Material and Methods. CW, cell wall; ER, endoplasmic reticulum; M, mitochondrion; N, nucleus; PM, plasma membrane; V, vacuole. Size bars, 200 nm.

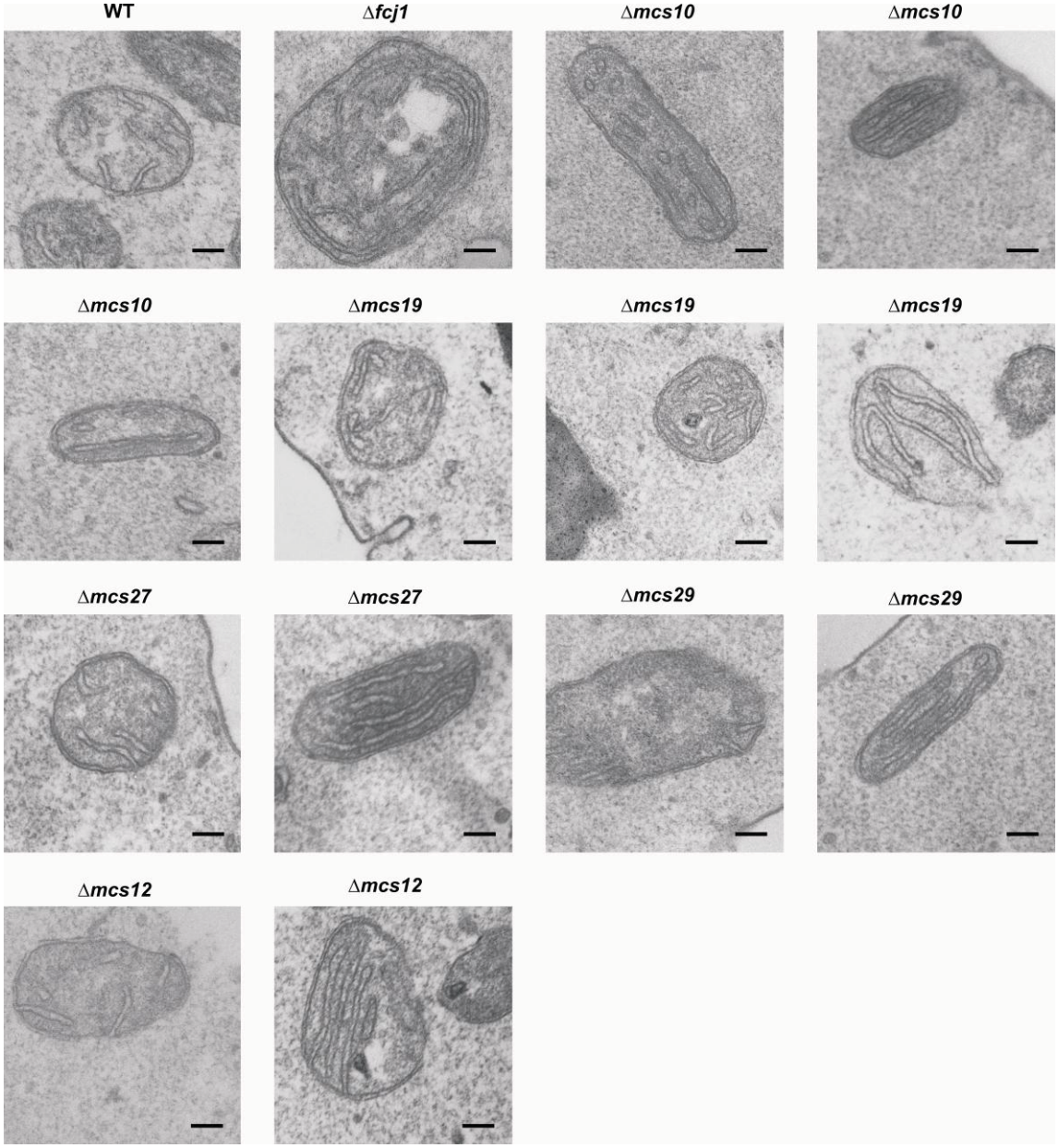


Figure S5, related to Figure 5. Electron microscopy images of mitochondria in wild type and in mutant cells lacking one of the contact site proteins. Additional EM images to those shown in Fig.5A. Scale bars, 100 nm.

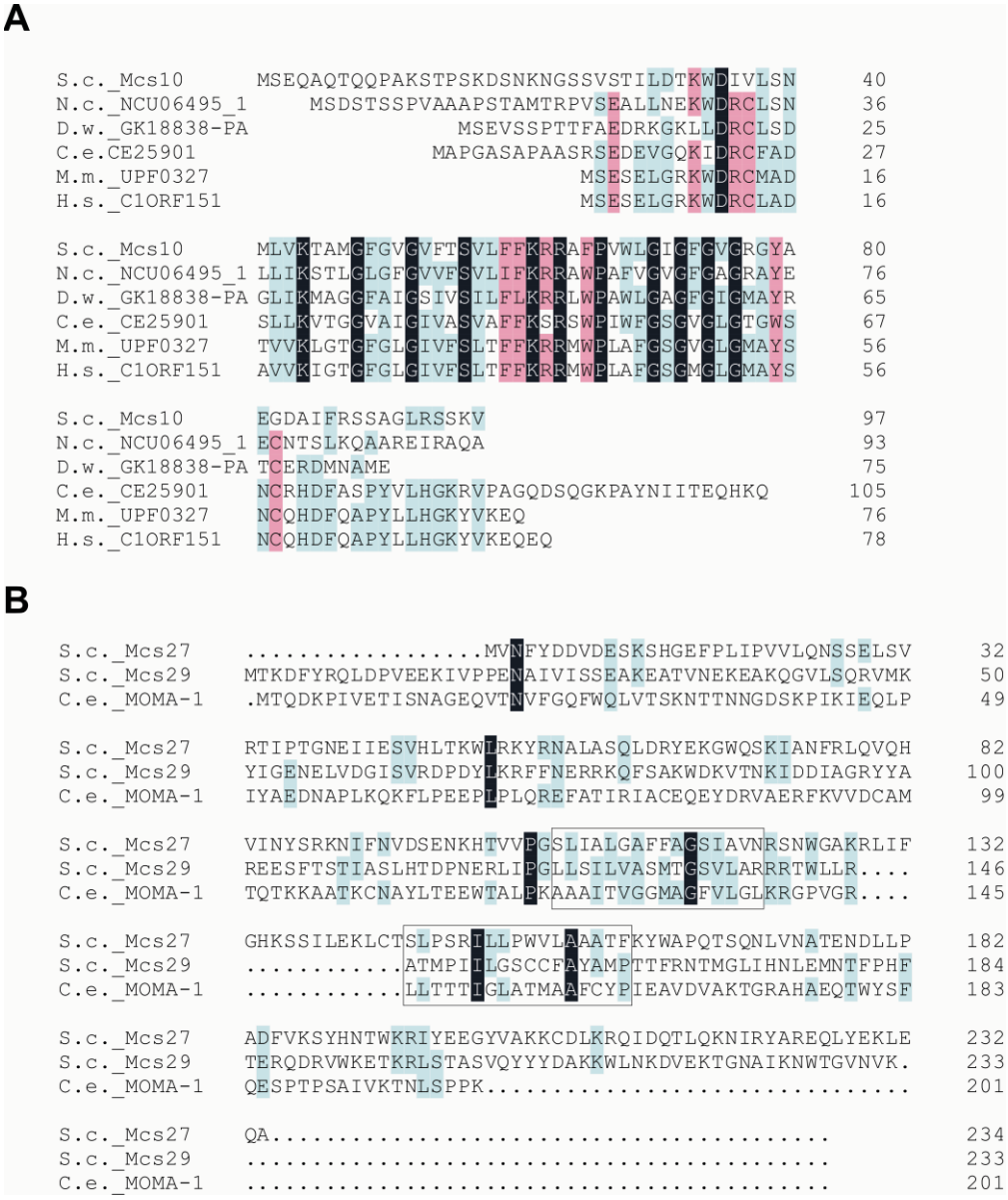


Figure S6, related to Figure 7. Amino acid sequence alignments of yeast Mcs proteins with possible homologs from higher eukaryotes. (A) Mcs10. Sc, *Saccharomyces cerevisiae*; Nc, *Neurospora crassa*; Dw, *Drosophila willistoni*; Ce, *Caenorhabditis elegans*; Mm, *Mus musculus*; Hs, *Homo sapiens*. (B) Amino acid sequence alignment of Mcs29 and Mcs27 from yeast and MOMA-1 from *Drosophila melanogaster*.

SUPPLEMENTAL TABLES AND LEGENDS

Protein	Counted gold particles	Present at sites where crista junctions meet the mitochondrial envelope	Present at mitochondrial envelope	Present in mitochondrial inner space (matrix and cristae)
Fcj1	157	41.40%	87.90%	12.10%
Mcs10	463	46.44%	92.33%	7.77%
Mcs19	171	40.94%	75.03%	24.97%
Mcs27	90	35.56%	88.89%	11.11%
Mcs29	65	36.93%	83.08%	16.92%
Mcs12	71	36.62%	91.55%	8.45%
Por1	354	7.91%	92.66%	7.34%
Tim16	96	6.25%	52.08%	47.92%
Tim50	188	7.98%	78.72%	21.28%
Idh1	494	4.66%	24.29%	75.71%

Table SI. Quantitative analysis of the immuno-EM labelling. The distribution of the gold particles (% of total) was determined as described in Materials and methods. The mitochondrial envelope comprises both outer membrane and inner boundary membrane. The weak labeling of Tob38 and Mas37 did not allow counting of a relevant number of gold particles and therefore these data are not statistically significant and not presented.

Strain	Number of mitochondrial sections	Number of CJ	Number of CJs per mitochondrial section
WT	24	41	1.71
Δ*fcj1*	30	0	0.00
Δ*mcs10*	28	0	0.00
Δ*mcs19*	48	23	0.48
Δ*mcs27*	28	20	0.71
Δ*mcs29*	27	37	1.37
Δ*mcs12*	39	46	1.18

Strain	Number of crista rims	Number of crista rims per mitochondrial section	Number of branches	Number of branches per mitochondrial section
WT	96	4.00	0	0.00
Δ*fcj1*	216	7.20	0	0.00
Δ*mcs10*	189	6.75	0	0.00
Δ*mcs19*	273	5.69	8	0.17
Δ*mcs27*	134	4.79	0	0.00
Δ*mcs29*	106	3.93	0	0.00
Δ*mcs12*	181	4.64	0	0.00

Table SII, related to Figure 5. Quantitation of the number of crista junctions (CJ), crista rims and crista branches in mitochondria of wild type cells and cells in which the invidual Mcs proteins were deleted.

SUPPLEMENTARY DATASET

Supplementary File F1 (Excel). Abundance profiles of a collection of proteins listed by SGD nomenclature. SILAC-based quantifications for gradient fraction samples of mitochondrial vesicles (odd numbers of 1-21) were normalized to the highest observed value for each of the proteins. Further information is provided on the number of identified unique peptide sequences, the subcellular and chromosomal localization and functional information. GOCC, GOMF, GOBP: Gene Ontology terms for the categories "Cellular Component", "Molecular Function" and "Biological Process", respectively.

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Isolation and Subfractionation of Mitochondria

Mitochondria were purified as described previously (Herrmann et al., 1994). They were resuspended in 0.6 M sorbitol, 20 mM MOPS, pH 7.4 and swelling buffer (20 mM MOPS, pH 7.4, 0.5 mM EDTA, 1 mM PMSF, 1x Roche complete protease inhibitor) was added dropwise under stirring for 30 min at 0°. 2.5 M sucrose was added to a final concentration of 0.5 M followed by incubation for 15 min at 0°C. Then the suspension was subjected to sonication (Branson Sonifier 250) using a microtip for 3x30 s with 30 s breaks in between at 0°C at 60% duty cycle and output control 0. Remaining intact mitochondria and large fragments were removed by centrifugation at 20,000 xg for 20 min at 4°C. The supernatant was centrifuged on a 2.5 M sucrose cushion at 120,000 xg for 100 min to concentrate the generated submitochondrial vesicles. The vesicle pellet was harvested, homogenized by pottering, sucrose concentration was measured and adjusted to 1.3 M sucrose. Separation of different kinds of vesicles was achieved by centrifugation of a continuous flotation sucrose gradient (13 ml, 0.8-1.25 M sucrose in 20 mM MOPS, pH 7.4, 0.5 mM EDTA) at 200,000 xg for 24 h at 4°C. The gradient was harvested and proteins were TCA precipitated. TCA precipitates were either subjected to SDS-PAGE followed by immunoblotting, or to protein mass spectrometry.

Analysis of yeast cell growth

Growth phenotypes were analyzed by drop dilution assays. Wild type YPH499 (WT) and strains YPH499Δ*fcj1*, YPH499Δ*mcs10*, YPH499Δ*mcs19*, YPH499Δ*mcs27*,

YPH499Δ*mcs29* and YPH499Δ*mcs12* were cultured at 30°C in YP liquid medium containing 2% glucose. Each culture was subcultured 3-4 times and kept in the logarithmic growth phase. Liquid cultures were diluted to an OD_{600} of 0.3 with sterile water. Dilution series (1:10, 1:100, 1:1000, and 1:10 000) were prepared, 3 µl of each dilution were spotted on either YPD, Lac, SD or SLac containing agar plates and incubated at 24°C, 30°C or 37°C.

Electron microscopy

Cells were prepared using a modification of a previously described procedure (Banta et al., 1988). Cells were grown on 40 ml lactate medium containing 0.1% glucose to an OD_{600} of 0.5. Cells were harvested by centrifugation (3,000 rpm), washed once in 2.5 % (w/v) glucose and fixed for 2 h at 30°C in 1 ml of fixation buffer (0.15 M sodium cacodylate, pH 6.8, 5 mM $MgCl_2$, 2.5% sucrose, 3% glutaraldehyde). After fixation cells were washed once in 1 ml of DTT-buffer (1.2 M sorbitol, 100 mM Tris-HCl, pH 8.0, 25 mM dithiothreitol, 5 mM EDTA) and incubated in the same buffer for 10 min at 30°C. Pellets were then washed once in 1 ml of phosphate buffer (1.2 M sorbitol, 0.1 M potassium phosphate, pH 5.8, adjusted with citric acid), resuspended in the same buffer containing glucuronidase (0.05 mg/ml) and zymolyase (5 mg/ml), incubated for 2 h at 30°C, washed once in 1 ml cacodylate buffer (2.5% sucrose, 0.15 M sodium cacodylate, pH 6.8, 5 mM $MgCl_2$,) and resuspended in the same buffer for storage.

Chemically fixed cells prepared as described above were centrifuged 3 min at 1,000 xg and incubated in 1% thiocarbohydrazide at room temperature. After a further centrifugation step cells were washed twice in water. Cells were stained with 1% OsO_4

and 1% potassium ferrocyanide for 6 min and washed 4 times in water. Samples were dehydrated with ethanol, washed twice with propylenoxide for 20 min and embedded in Araldit (Araldit-Kit CY-212 (Serva)). Thin sections of 45 nm were cut using Ultracut E (Fa. Reichert Jung), collected on 150-mesh copper grids and contrasted by consecutive incubations of 5 min in uranyl acetate and in $PbNO_3$ solutions. Cells were observed with a transmission electron microscope (CM 10, Philips and EM 900, Zeiss).

SUPPLEMENTAL REFERENCES

Banta, L.M., Robinson, J.S., Klionsky, D.J., and Emr, S.D. (1988). Organelle assembly in yeast: characterization of yeast mutants defective in vacuolar biogenesis and protein sorting. J Cell Biol *107*, 1369-1383.

Herrmann, J.M., Foelsch, H., Neupert, W., and Stuart, R.A. (1994). Isolation of yeast mitochondria and study of mitochondrial protein translation. In Cell Biology: A Laboratory Handbook, J.E. Celis, ed. (San Diego, Academic Press), pp. 538-544.