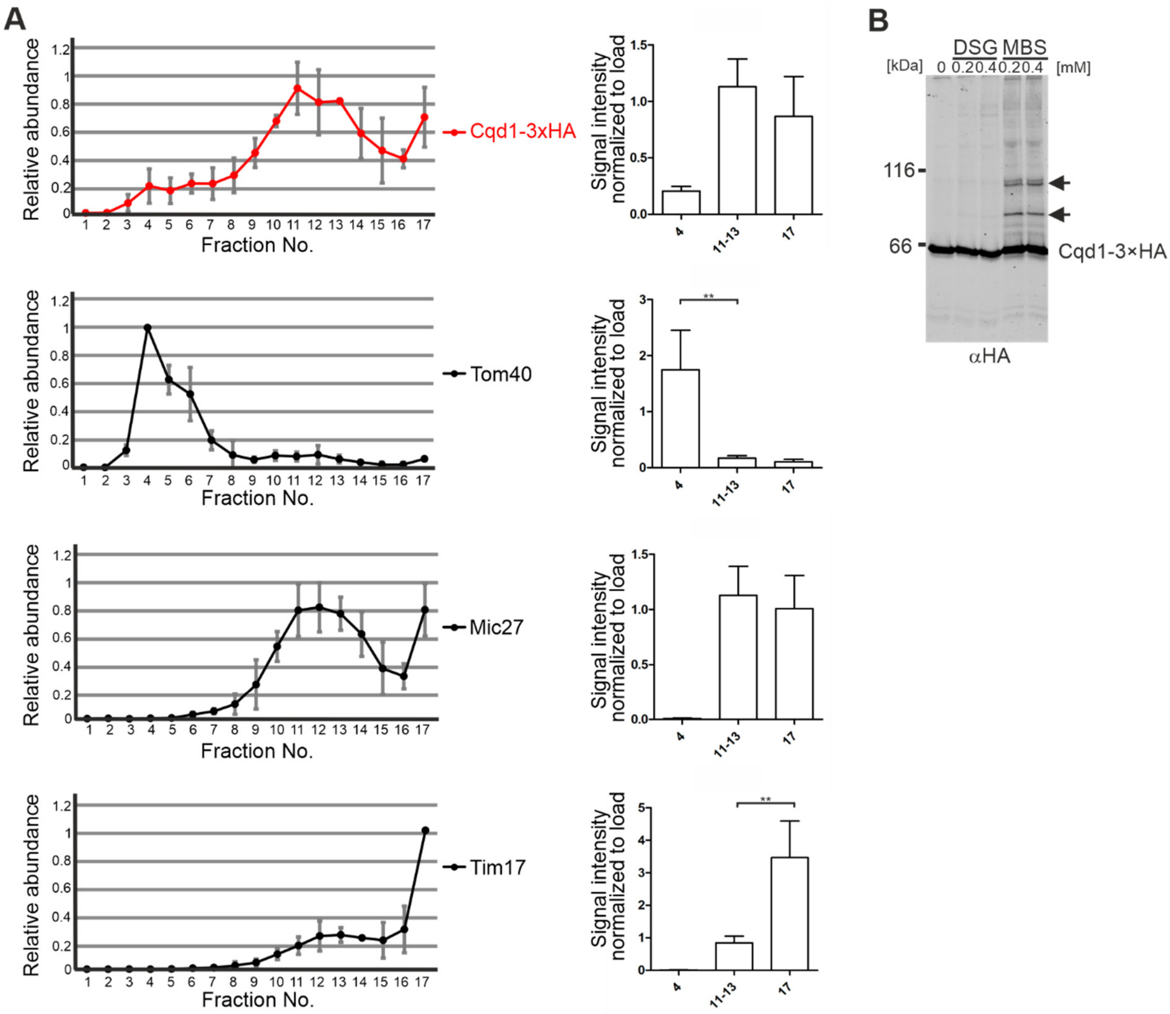

Fig. S1. Cqd1 is enriched in fractions containing contact site proteins.

(A) (Left) The average of relative protein abundance in three independent experiments described in Fig. 6A is shown. The graph shows mean values of the distribution of Cqd1-3xHA and the marker proteins for the outer membrane (Tom40), the inner membrane (Tim17) and contact sites (Mic27). Error bars indicate standard deviation. (Right) The quantification of the amount of the respective proteins present in low density fractions (No. 4), intermediate density fractions (No. 11-13) and high density fractions (No. 17) is shown as mean from three independent experiments. Quantification was done using Image Studio software. Error bars indicate standard deviation. Asterisks represent p-values obtained by unpaired Student's t test (**p≤0.01). **(B)** Cqd1 interacts with proteins other than itself. Isolated mitochondria of a Cqd1-3xHA expressing strain were exposed to DMSO or the chemical crosslinkers DSG and MBS at the indicated concentrations. Samples were analyzed by immunoblotting with an anti-HA antibody. Arrows indicate Cqd1-containing crosslinks.

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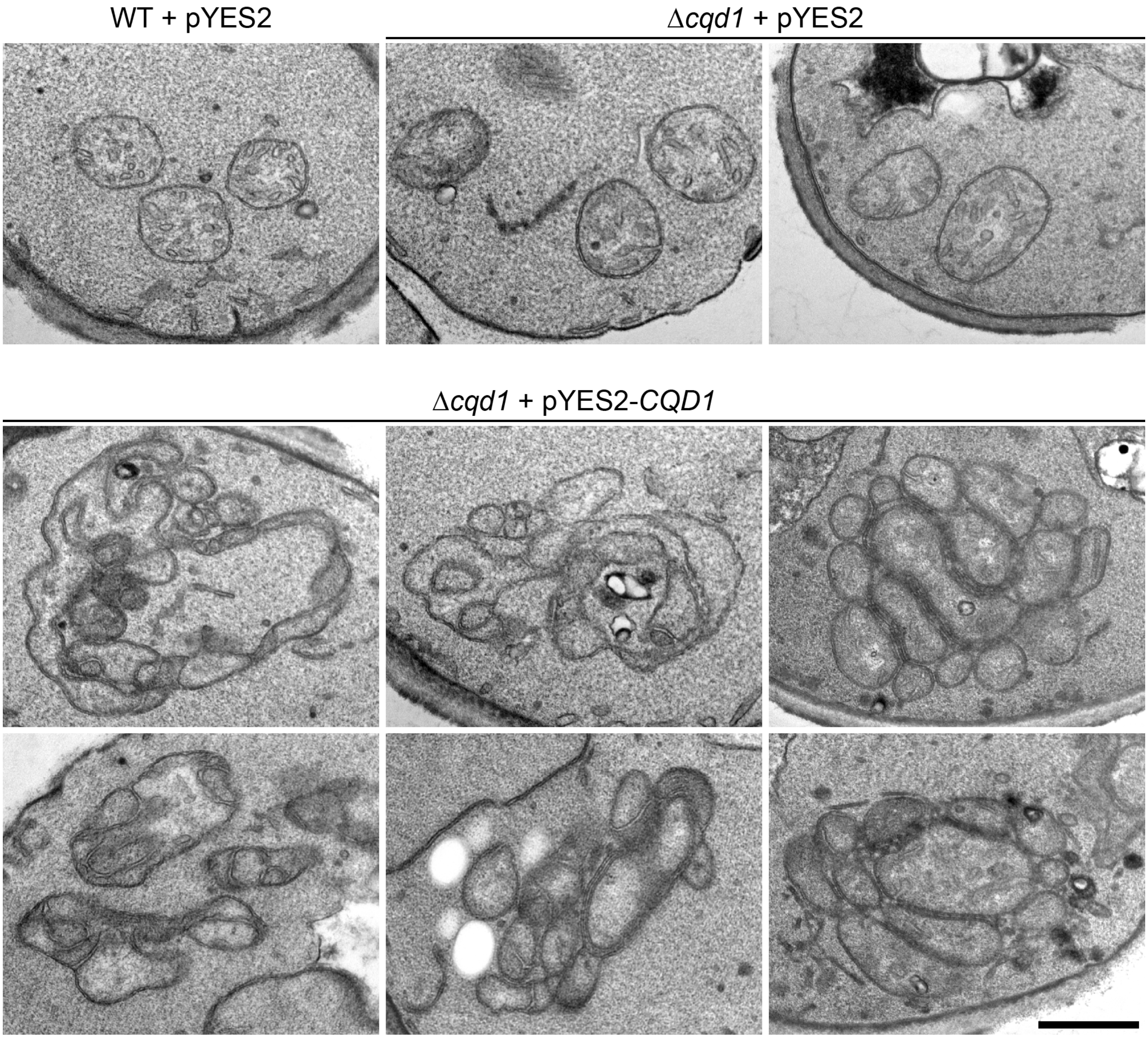

Fig. S2. *CQD1* overexpression is associated with altered mitochondrial architecture. WT and Δ*cqd1* cells carrying the indicated plasmids were grown and analyzed by electron microscopy as described in Fig. 7D. Scale bar, 500 nm.

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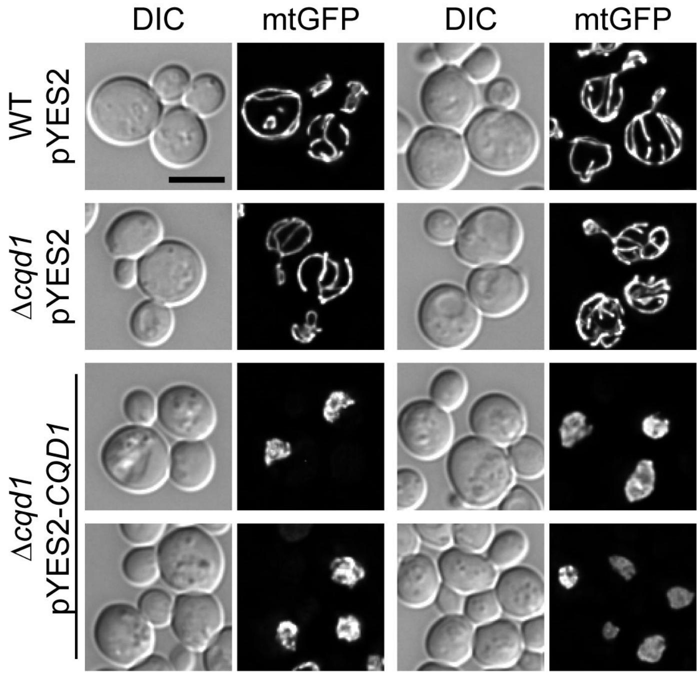

Fig. S3. Overexpression of *CQD1* causes changes in mitochondrial morphology.

WT and Δ*cqd1* cells carrying the indicated plasmids and expressing mitochondria-targeted GFP (mtGFP) were grown and analyzed as described in Fig. 7E. Shown are DIC (differential interference contrast) images and maximum intensity projections of deconvolved z stacks (mtGFP) from representative cells. Scale bar, 5 µm.

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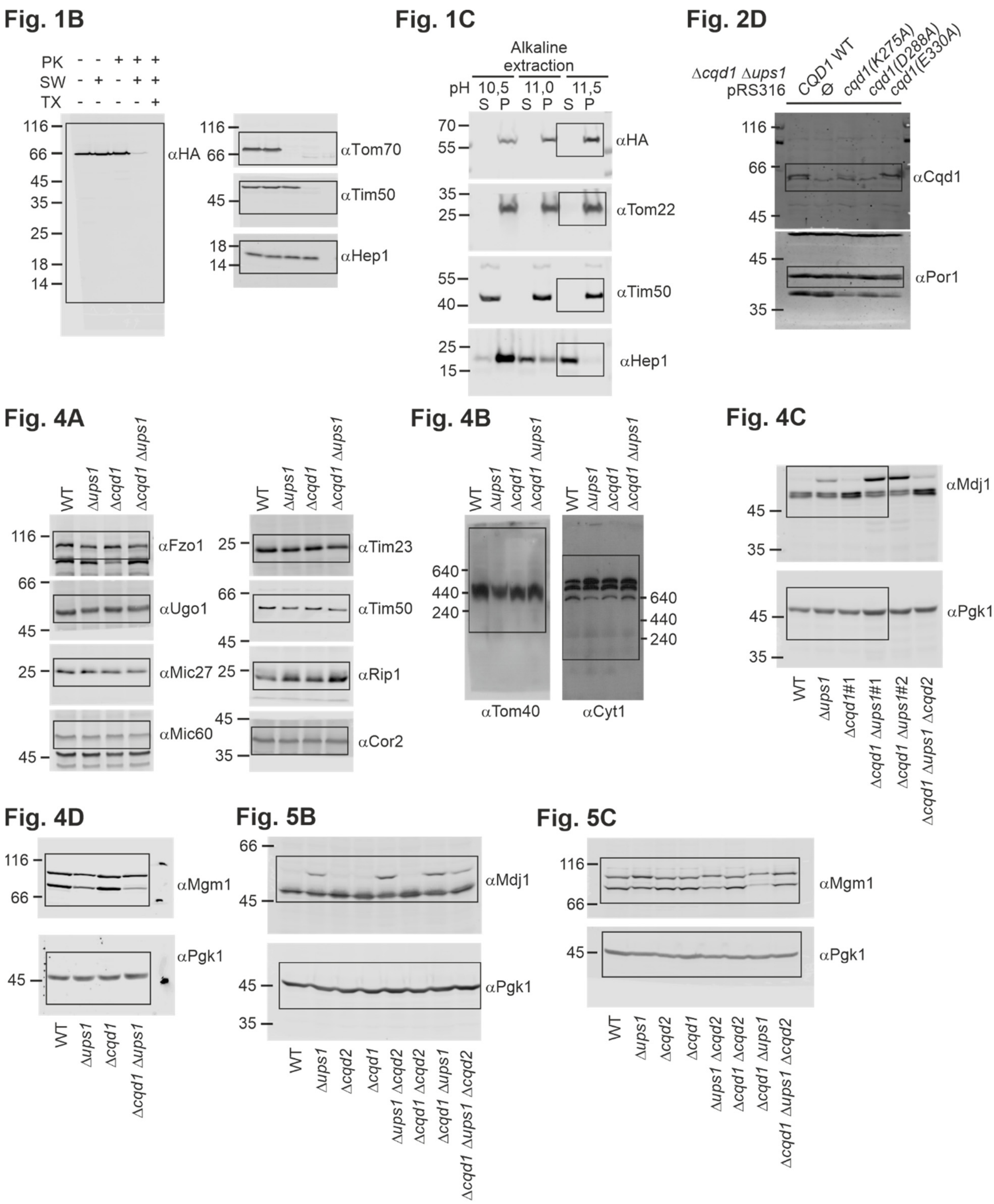

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Fig. 6A

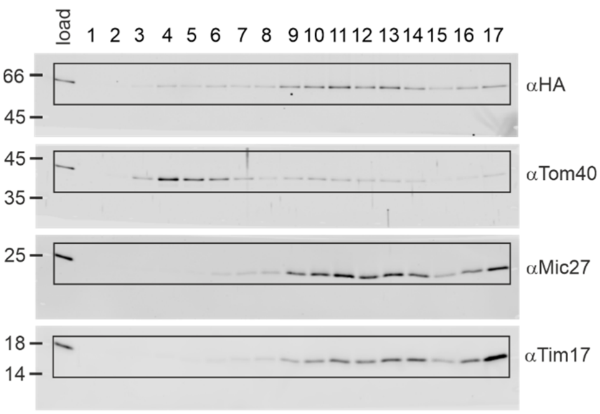

Fig. 6B

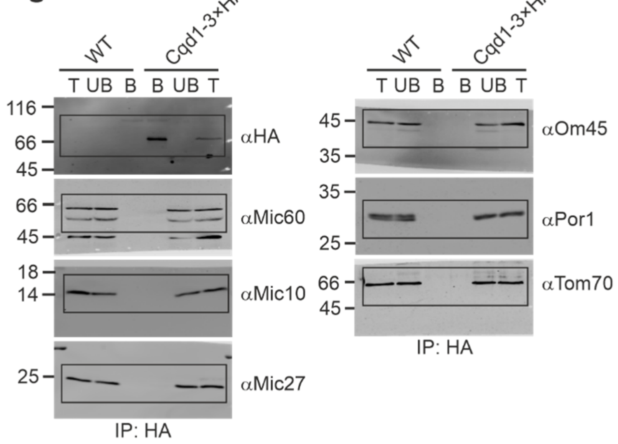

Fig. 6C

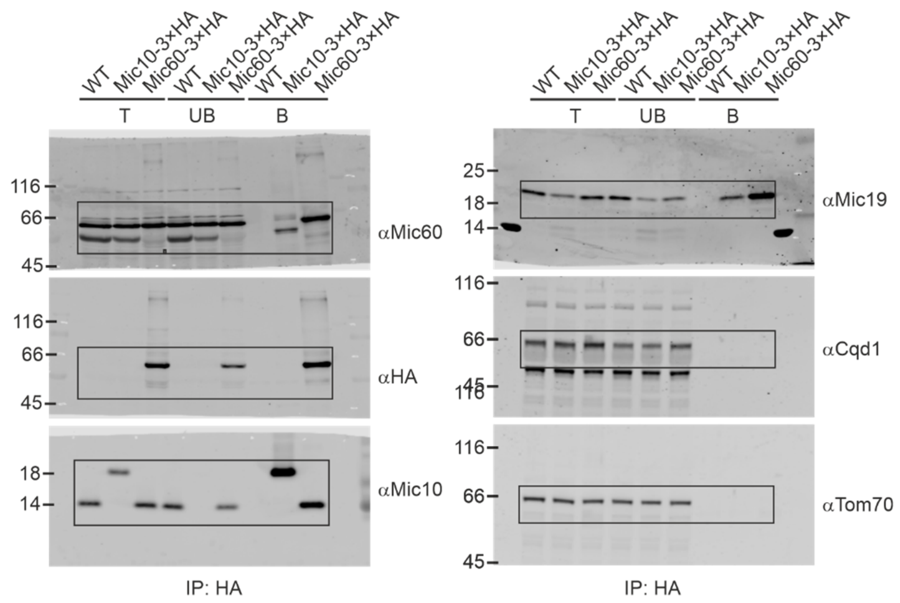

Fig. 6D

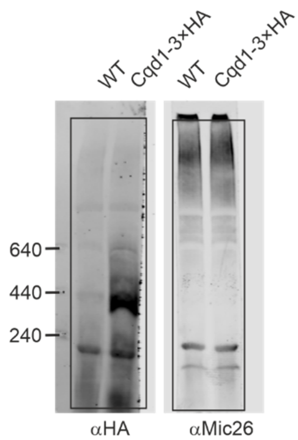

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Fig. 6E

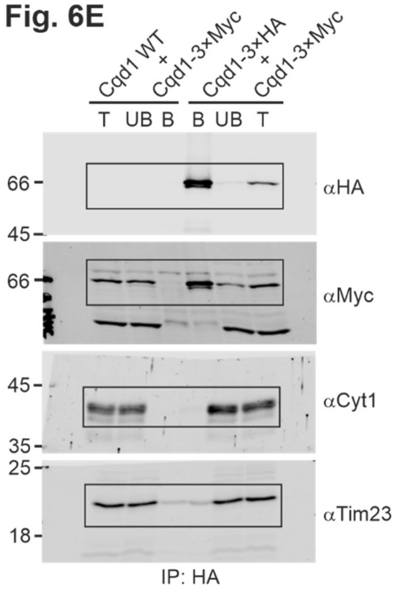

Fig. 6F

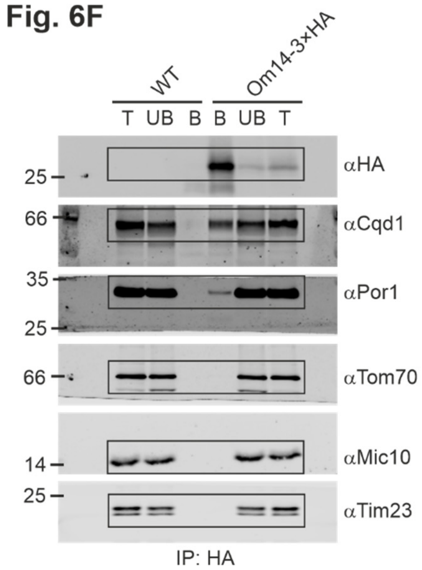

Fig. 6G

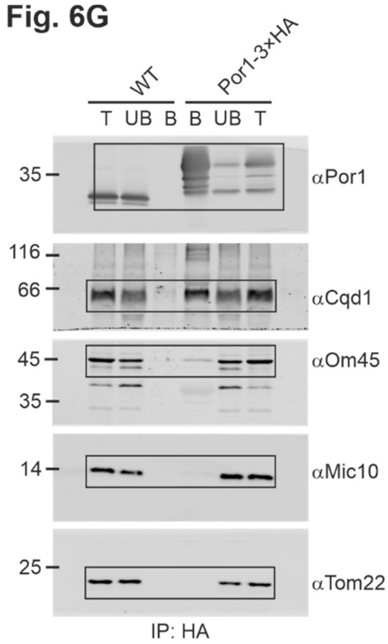

Fig. 6H

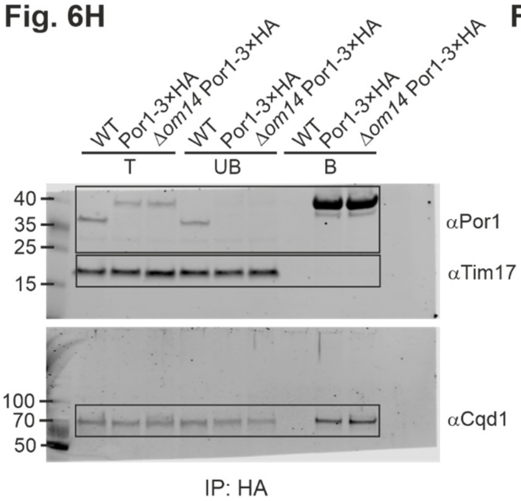

Fig. 6I

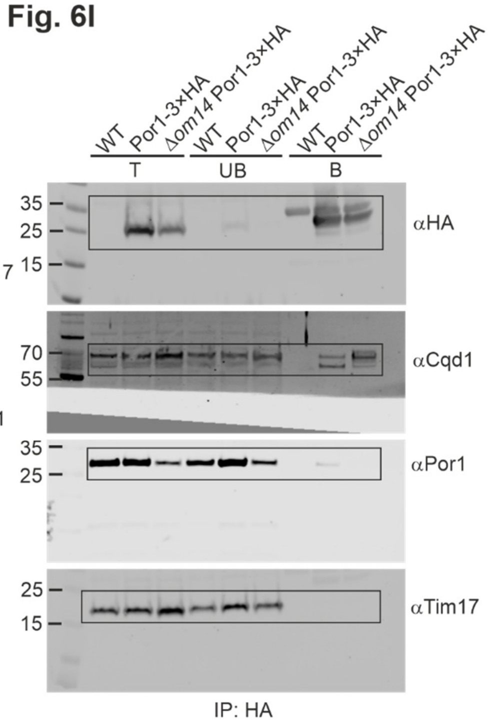

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Fig. 6J

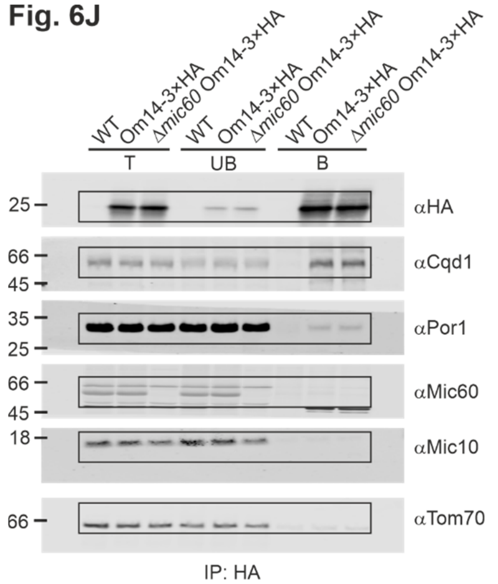


Fig. 7B

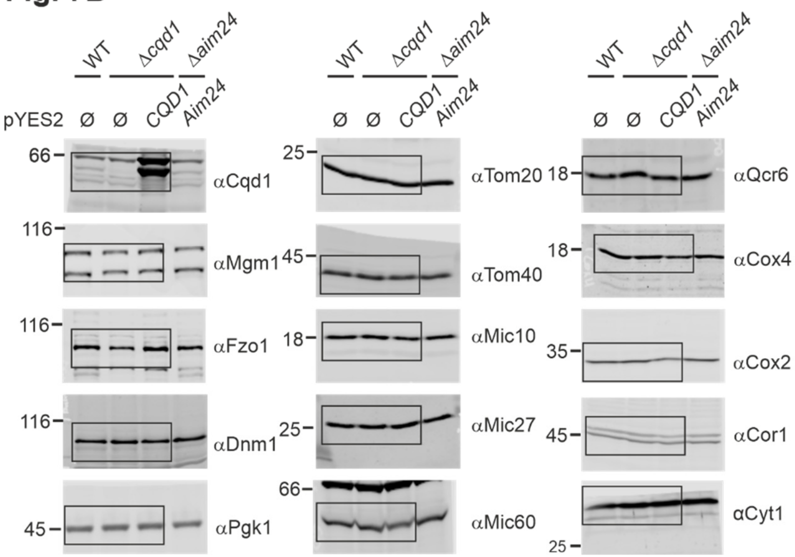
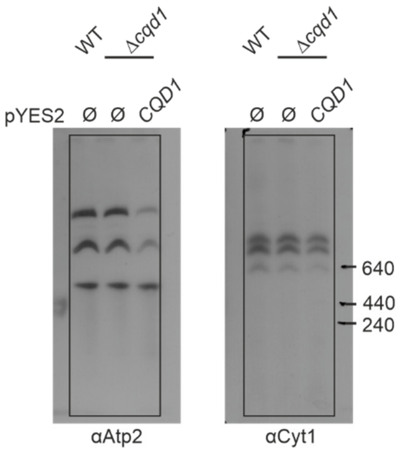


Fig. 7C



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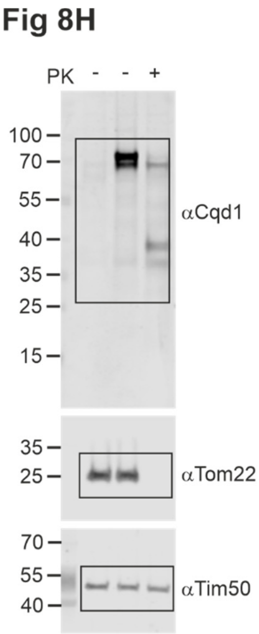

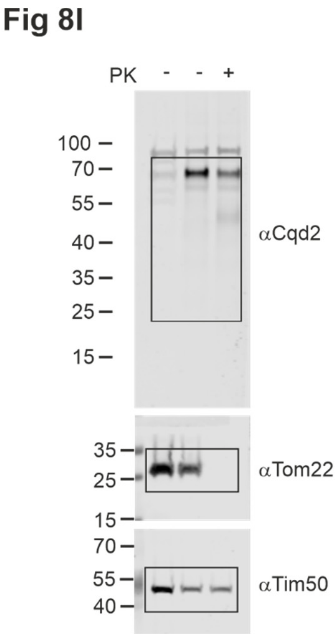

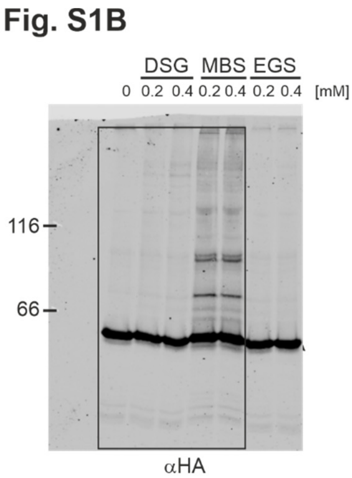

Fig. S4. Blot transparency

Table S1. Relative abundance of mitochondrial phospholipids.

Values of the graph shown in Figure 3. PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine; PI, phosphatidylinositol; CL, cardiolipin; PG, phosphatidylglycerol; PA, phosphatidic acid; MLCL, monolysocardiolipin.

average mol % lipid								
Sample	**PC**	**PE**	**PS**	**PI**	**PG**	**PA**	**CL**	**MLCL**
WT	36.17	27.30	5.05	16.48	0.15	4.17	10.45	0.22
Δ*ups1*	39.34	30.59	5.18	15.55	0.13	3.03	6.15	0.04
Δ*cqd1*	40.28	25.68	4.85	15.01	0.18	3.19	10.65	0.16
Δ*ups1* Δ*cqd1*	41.17	27.69	6.94	14.12	0.12	3.41	6.49	0.07
Standard deviation								
Sample	**PC**	**PE**	**PS**	**PI**	**PG**	**PA**	**CL**	**MLCL**
WT	5.29	3.20	0.66	4.15	0.04	0.48	1.41	0.11
Δ*ups1*	3.12	3.80	1.22	1.81	0.10	0.16	0.61	0.03
Δ*cqd1*	1.50	2.30	0.62	1.49	0.06	0.46	1.04	0.04
Δ*ups1* Δ*cqd1*	2.36	2.17	2.15	2.27	0.04	0.80	2.13	0.07

Table S2. Relative amount of different species of single phospholipids

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Table S3. *S. cerevisiae* strains used in this study.

Strain name	Genotype	Reference
YPH499 (ρ$^+$)	*MATa ade2-101 his3-Δ200 leu2- trp1-Δ63 ura3-52 lys2-801*	(Sikorski and Hieter, 1989)
YPH499 (ρ^0)	*MATa ade2-101 his3-Δ200 leu2- trp1-Δ63 ura3-52 lys2-801*	Harner et al., 2014
Δcqd1	YPH499 *cqd1Δ::HIS3MX6*	This study
Δcqd2	YPH499 *cqd2Δ::HIS3MX6*	This study
Δups1	YPH499 *ups1Δ::kanMX4*	This study
Δups1	YPH499 *ups1Δ::LEU2 (K. lactis)*	This study
Δtam41	YPH499 *tam41Δ::LEU2 (K. lactis)*	This study
Δpgs1	YPH499 *pgs1Δ::LEU2 (K. lactis)*	This study
Δgep4	YPH499 *gep4Δ::LEU2 (K. lactis)*	This study
Δcrd1	YPH499 *crdΔ::LEU2 (K. lactis)*	This study
Δtaz1	YPH499 *taz1Δ::LEU2 (K. lactis)*	This study
Δcld1	YPH499 *cld1Δ::LEU2 (K. lactis)*	This study
Δups2	YPH499 *ups2Δ::LEU2 (K. lactis)*	This study
Δmdm35	YPH499 *mdm35Δ::LEU2 (K. lactis)*	This study
Δpsd1	YPH499 *psd1Δ::LEU2 (K. lactis)*	This study

Δpor1	YPH499 *por1Δ::kanMX4*	This study
Δom14	YPH499 *om14Δ::LEU2 (K. lactis)*	This study
Δmgm1	YPH499 *mgm1Δ:: kanMX4*	Harner et al., 2014
Δfzo1	YPH499 *fzo1Δ::HIS3MX6*	This study
Δups1 Δcqd1	YPH499 *cqd1Δ::HIS3MX6 ups1Δ::LEU2 (K. lactis)*	This study
Δups1 Δcqd1	YPH499 *cqd1Δ::HIS3MX6 ups1Δ::kanMX4*	This study
Δtam41 Δcqd1	YPH499 *cqd1Δ::HIS3MX6 tam41Δ::LEU2 (K. lactis)*	This study
Δpgs1 Δcqd1	YPH499 *cqd1Δ::HIS3MX6 pgs1Δ::LEU2 (K. lactis)*	This study
Δgep4 Δcqd1	YPH499 *cqd1Δ::HIS3MX6 gep4Δ::LEU2 (K. lactis)*	This study
Δcrd1 Δcqd1	YPH499 *cqd1Δ::HIS3MX6 crd1Δ::LEU2 (K. lactis)*	This study
Δtaz1 Δcqd1	YPH499 *cqd1Δ::HIS3MX6 taz1Δ::LEU2 (K. lactis)*	This study
Δcld1 Δcqd1	YPH499 *cqd1Δ::HIS3MX6 cld1Δ::LEU2 (K. lactis)*	This study
Δups2 Δcqd1	YPH499 *cqd1Δ::HIS3MX6 ups2Δ::LEU2 (K. lactis)*	This study
Δmdm35 Δcqd1	YPH499 *cqd1Δ::HIS3MX6 mdm35Δ::LEU2 (K. lactis)*	This study
Δpsd1 Δcqd1	YPH499 *cqd1Δ::HIS3MX6 psd1Δ::LEU2 (K. lactis)*	This study
Δcqd1 Δcqd2	YPH499 *cqd2Δ::HIS3MX6 cqd1Δ::kanMX4*	This study
Δups1 Δcqd2	YPH499 *ups1Δ::kanMX6 cqd2Δ::hphNT1*	This study
Δups1 Δcqd2 Δcqd1	YPH499 *cqd2Δ::HIS3MX6 cqd1Δ::kanMX4 ups1Δ::LEU2 (K. lactis)*	This study
CQD1-3xHA	YPH499 *CQD1-3xHA::HIS3MX6*	This study
CQD1-3xMyc	YPH499 *CQD1::3xMyc::TRP1 (K. lactis)*	This study
POR1-3xHA	YPH499 *POR1-3xHA::HIS3MX6*	This study
OM14-3xHA	YPH499 *OM14-3xHA::HIS3MX6*	This study
OM14-3xHA Δpor1	YPH499 *OM14-3xHA::HIS3MX6 por1Δ:: LEU2 (K. lactis)*	This study
POR1-3xHA Δom14	YPH499 *POR1-3xHA::TRP1 (K. lactis) HIS3MX6om14Δ::LEU2 (K. lactis)*	This study
Δmic60 OM14-3xHA	YPH499 *mic60Δ::HIS3MX6 OM14-3xHA::TRP1 (K. lactis)*	This study
WT mKate	YPH499 *ho-PGK1pr-su9-KATE2::kanMX4*	This study
Δcqd1 mKate	YPH499 *cqd1Δ::HIS3MX6 ho-PGK1pr-su9-KATE2::kanMX4*	This study
Δups1 mKate	YPH499 *ups1Δ::LEU2 (K. lactis) ho-PGK1pr-su9-KATE2::kanMX4*	This study
Δups1 Δcqd1 mKate	YPH499 *ups1Δ::LEU2 (K. lactis) cqd1Δ::HIS3MX6 ho-PGK1pr-su9-KATE2::kanMX4*	This study
Δups1 CQD1-3xHA	YPH499 *cqd1Δ::CQD1-3xHA::HIS3MX6 ups1::LEU2 (K. lactis)*	This study
MIC10-3xHA	YPH499 *MIC10-3xHA::HIS3MX6*	(Harner et al., 2011)
MIC60-3xHA	YPH499 *MIC60-3xHA::kanMX4*	(Harner et al., 2011)

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Table S4. Primers used in this study.

Construct	Primer name	Sequence
pYES2-*CQD1*	*CQD1*(SacI)_for	CCCGAGCTCATGTCATTTTTAAAGTTCGC
	CQD1-int(EcoRI)_rev	CCCGAATTCACGTAATATAGGCAGCGAACTTTCA AATAAGTCCAAG
	CQD1-int(EcoRI)_for	CGTGAATTCGGGTTTAAGC
	CQD1(NotI)_rev	CCCGCGGCCGCTTAATAATTAGGACACAATTG
pRS316-*CQD1*	F HindIII *CQD1* (gib)	GCAGGAATTCGATATCAAGCTTGGTACTGGAAAG ATCGCGTTC
	R XhoI *CQD1*	CTTACCGGGCCCCCCCTCGACGTACCGTTGCCT TATTGTTC
pRS316-*cqd1(K275A)*	Cqd1_K275A_for	GCGATCTTGCATCCAAATGTAAG
	Cqd1_K275A_rev	GATGGCACACCAACGATTTC
pRS316-*cqd1(D288A)*	Cqd1_D288A_for	GCTTTGAAAATAATGAAATTCTG
	Cqd1_D288A_rev	TCTCCGGATCTGAGATCTTAC
pRS316- *cqd1(E330A)*	Cqd1_E330A_for	GCGGCGTTAAACCTGGAAAG
pYES2-*cqd1(E330A)*	Cqd1_E330A_rev	AATTCTTAGATCCAACTGAATA
pRS316-*CQD1-3xHA*	F HindIII *CQD1* (gib)	GCAGGAATTCGATATCAAGCTTGGTACTGGAAAG ATCGCGTTC
	Gib(XhoI)*ADH1*terR1	ACCGGGCCCCCCCTCGAGGTAGAGGTGTGGTCA ATAAG
pYES2-*CQD2*	*CQD2*(SacI)_for	CCCGAGCTCATGATGACCAAAGCTTTTTTTAAC
	CQD2(XhoI)_rev	CCCCTCGAGTTAAGATGACAACCAAGTCTTCG
pYX233-*CQD2*	pYXCQD2 (EcoRI)_for	CTACAAAAAACACATACAGGAATTCATGATGACC AAAGCTTTTTT
	pYXCQD2(NheI)_rev	ATTGTTCCTTATTCAGTTAGCTAGCTTAAGATGAC AACCAAGTC

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Table S5. Antibodies used in this study.

Antibody	Source	Identifier/Reference	Dilution
Mouse monoclonal anti-HA	Santa Cruz	Cat. #sc-7392	1:250
Mouse monoclonal anti-Pgk1	Life Technologies	Cat. #459250	1:250
Mouse monoclonal anti-Pgk1	MPI of Biochemistry	(Izawa et al., 2017)	1:250
Rabbit polyclonal anti-Cqd1	LMU Munich	This study	1:25
Rabbit polyclonal anti-Cqd2	LMU Munich	This study	1:25
Rabbit polyclonal anti-Tim17	LMU Munich	(Moro et al., 1999)	1:500
Rabbit polyclonal anti-Tim23	LMU Munich	(Moro et al., 1999)	1:500
Rabbit polyclonal anti-Tim50	LMU Munich	(Mokranjac et al., 2003)	1:500
Rabbit polyclonal anti-Tom20	LMU Munich	(Krimmer et al., 2001)	1:500
Rabbit polyclonal anti-Tom22	LMU Munich	(Krimmer et al., 2001)	1:500
Rabbit polyclonal anti-Tom40	LMU Munich	(Kiebler et al., 1990)	1:1,000
Rabbit polyclonal anti-Tom70	LMU Munich	(Schlossmann et al., 1996)	1:500
Rabbit polyclonal anti-Hep1	LMU Munich	(Sichting et al., 2005)	1:250
Rabbit polyclonal anti-Atp2	LMU Munich	(Izawa et al., 2017)	1:1,000
Rabbit polyclonal anti-Fzo1	LMU Munich	(Basch et al., 2020)	1:250
Rabbit polyclonal anti-Ugo1	LMU Munich	(Basch et al., 2020)	1:250
Rabbit polyclonal anti-Mgm1	LMU Munich	(Harner et al., 2016)	1:250
Rabbit polyclonal anti-Dnm1	LMU Munich	(Harner et al., 2016)	1:250
Rabbit polyclonal anti-Mdj1	LMU Munich	(Rowley et al., 1994)	1:250
Rabbit polyclonal anti-Mic10	LMU Munich	(Harner et al., 2011)	1:250
Rabbit polyclonal anti-Mic19	LMU Munich	(Harner et al., 2011)	1:250
Rabbit polyclonal anti-Mic26	LMU Munich	(Harner et al., 2011)	1:250
Rabbit polyclonal anti-Mic27	LMU Munich	(Harner et al., 2011)	1:250
Rabbit polyclonal anti-Mic60	LMU Munich	(Harner et al., 2011)	1:250
Rabbit polyclonal anti-Por1	LMU Munich	(Krimmer et al., 2001)	1:1,000
Rabbit polyclonal anti-Om45	LMU Munich	(Waizenegger et al., 2003)	1:250
Rabbit polyclonal anti-Rip1	LMU Munich	(Cruciat et al., 1999)	1:250
Rabbit polyclonal anti-Cor1	LMU Munich	(Cruciat et al., 2000)	1:250
Rabbit polyclonal anti-Cor2	LMU Munich	(Vogel et al., 2006)	1:250
Rabbit polyclonal anti-Cyt1	LMU Munich	(Izawa et al., 2017)	1:250
Rabbit polyclonal anti-Cox2	LMU Munich	(Izawa et al., 2017)	1:250
Rabbit polyclonal anti-Cox4	LMU Munich	(Cruciat et al., 2000)	1:250
Rabbit polyclonal anti-Qcr6	LMU Munich	(Cruciat et al., 1999)	1:250
IRDye 680RD Goat anti-Mouse IgG	LI-COR	Cat. #962-68070	1:10,000
IRDye 800CW Goat anti-Rabbit IgG	LI-COR	Cat. #926-32211	1:10,000
Goat Anti-Rabbit IgG-HRP Conjugate	BIO-RAD	Cat. #170-6515	1:10,000

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