Supplemental figure legends

Figure S1. Modified Ups1 and Ups2 variants harboring C-terminal tags restore normal phospholipid levels upon expression in Δ*ups1* and Δ*ups2* cells, respectively. The mitochondrial phospholipid profile was determined in wild type cells (WT, CG214), Δ*ups1* cells (PD49), Δ*ups2* cells (CG233), or cells expressing genomically modified *UPS1* (CG593, CG598, CG630) or *UPS2* (CG591, CG597, CG626) by TLC. The asterisk (*) indicates an unidentified lipid species.

Figure S2. Ups1 and Ups2 are present in independent protein complexes in the intermembrane space. Mitochondria (1 mg of protein) isolated from wild type cells (WT) and Δ*ups1* cells harboring $Ups2^{HA}$ expressed genomically (PD17, PD94) were resuspended at a protein concentration of 5 mg/ml in 1% (v/v) Triton X-100, 30 mM Tris/HCl pH 7.4, 150 mM K-acetate pH 7.4, 4 mM Mg-acetate, 1 mM PMSF and solubilized for 30 min at 4°C. After a clarifying spin for 20 min at 18,000 rpm, the supernatant was subjected to size exclusion chromatography using a Superose-12 column (GE Healthcare), equilibrated with 0.05% (v/v) Triton X-100, 30 mM Tris/HCl pH 7.4, 150 mM K-acetate pH 7.4, 4 mM Mg-acetate, 1 mM PMSF. Eluate fractions were TCA-precipitated and analyzed by SDS-PAGE and immunoblotting using HA-specific antibodies.

Figure S3. Specificity of Ups1 and Ups2 antibodies. Mitochondria isolated from wild type cells (WT, CG214) or cells lacking Yme1, Ups1, or Ups2 as indicated (CG113, PY132, PY153) were analyzed by SDS-PAGE and immunoblotting using Yme1-, Tom40- and affinity purified Ups1- and Ups2-antibodies.

Figure S4. (**A**) Import of Ups1, Ups2 and Su9-DHFR in Δ*mdm35* and Δ*mdm35*Δ*yme1* mitochondria. ^{35}S-labeled Ups1, Ups2 and Su9-DHFR were imported into wild type (WT,

CG1), Δ*mdm35* (CG323) and Δ*mdm35*Δ*yme1* (CW414) mitochondria for the indicated time. Samples were analyzed by SDS-PAGE and autoradiography. (**B**) Membrane potential of mitochondria isolated from the indicated strains was measured using the potential sensitive dye DiSC(3). Data represent ± standard deviation of three independent measurements. *p<0.05, *** p<0.0005

Fig. S1

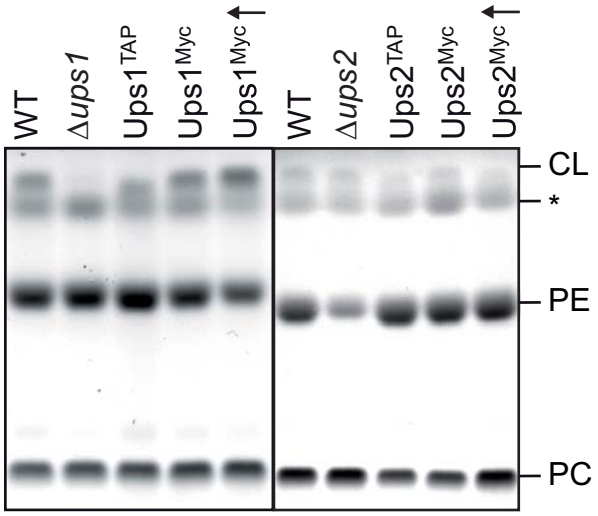

Fig. S2

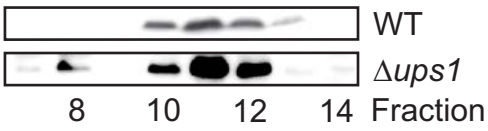

Fig. S3

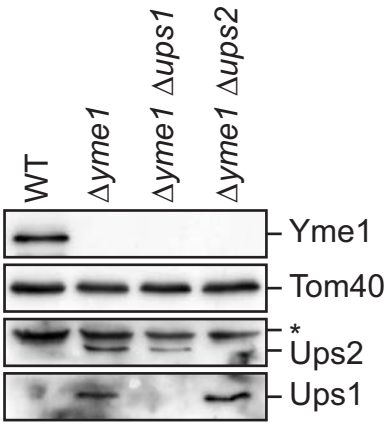

Fig. S4

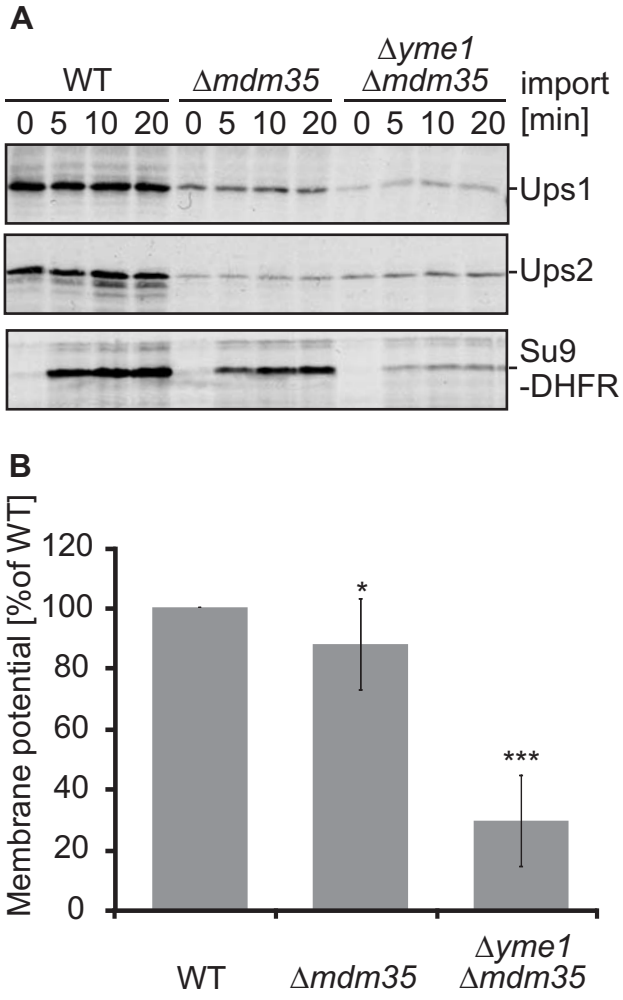

Table S1 Yeast strains used in this study

Strain Name	Alternative strain name	Back-ground	Mat	Genotype
CG1	WT	W303	a	*can1Δ100 his3Δ11,15 leu2Δ3,112 ura3Δ1 ade2Δ1 trp1Δ1*
CG113 (Euroscarf 7144)	Δ*yme1*	S288c	a	*his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 yme1::kanMX*
CG214	WT	S288c	a	*his3Δ1 leu2Δ0 met15Δ0 lys2Δ0 ura3Δ0*
CG233	Δ*ups2*	S288c	a	*his3Δ1 leu2Δ0 met15Δ0 lys2Δ0 ura3Δ0 Δups2::NAT Δyme1::kanMX*
CG278	Δ*phb1* [*PHB1*]	W303	a	*can1Δ100 his3Δ11,15 leu2Δ3,112 ura3Δ1 ade2Δ1 trp1Δ1 Δphb1::NAT pCM189-PHB1*
CG287	Δ*phb1* Δ*mdm35* [*PHB1*]	W303	a	*can1Δ100 his3Δ11,15 leu2Δ3,112 ura3Δ1 ade2Δ1 trp1Δ1 Δmdm35::kanMX Δphb1::NAT pCM189-PHB1*
CG323	Δ*mdm35*	W303	a	*can1Δ100 his3Δ11,15 leu2Δ3,112 ura3Δ1 ade2Δ1 trp1Δ1 Δmdm35::kanMX*
CG324	Δ*mdm35*	W303	α	*can1Δ100 his3Δ11,15 leu2Δ3,112 ura3Δ1 ade2Δ1 trp1Δ1 Δmdm35::kanMX*
CG524	Δ*mdm35*	S288c	a	*his3Δ1 leu2Δ0 met15Δ0 lys2Δ0 ura3Δ0 Δmdm35::kanMX*
CG560	Δ*mdm35*	S288c	α	*can1Δ0 his3Δ0 ura3Δ0 lys3Δ0 Δmfa1::MFA1prHIS3 Δmdm35::NAT*
CG591	$Ups2^{TAP}$	S288c	a	*his3Δ1leu2Δ0 met15Δ0 lys2Δ0 ura3Δ0 UPS2-TAP (kanMX)*
CG593	$Ups1^{TAP}$	S288c	a	*his3Δ1leu2Δ0 met15Δ0 lys2Δ0 ura3Δ0 UPS1-TAP (kanMX)*
CG597	$Ups2^{MYC}$	S288c	a	*his3Δ1 leu2Δ0 met15Δ0 lys2Δ0 ura3Δ0 UPS2-Myc-7His (kanMX)*
CG598	$Ups1^{MYC}$	S288c	a	*his3Δ1 leu2Δ0 met15Δ0 lys2Δ0 ura3Δ0 UPS1-Myc-7His (kanMX)*
CG626	$Ups2^{MYC}$↑	S288c	a	*his3Δ1leu2Δ0 met15Δ0 lys2Δ0 ura3Δ0 P_{Gal}(NAT)-UPS2-Myc-7His (kanMX)*
CG630	$Ups1^{MYC}$↑	S288c	a	*his3Δ1leu2Δ0 met15Δ0 lys2Δ0 ura3Δ0 P_{Gal}(NAT)-UPS1-Myc-7His (kanMX)*
CW1	Δ*atp23*	S288c	a	*his3Δ1 leu2Δ0 met15Δ0 lys2Δ0 ura3Δ0 Δatp23::HIS3MX6*
CW128	Δ*mdm35* Δ*ups2*	W303	a	*can1Δ100 his3Δ11,15 leu2Δ3,112 ura3Δ1 ade2Δ1 trp1Δ1 Δmdm35::HIS3 Δups2::NAT*
CW130	Δ*ups2*	W303	a	*can1Δ100 his3Δ11,15 leu2Δ3,112 ura3Δ1 ade2Δ1 trp1Δ1 Δups2::NAT*
CW143	Δ*ups1*	W303	a	*can1Δ100 his3Δ11,15 leu2Δ3,112 ura3Δ1 ade2Δ1 trp1Δ1 Δups1::NAT*
CW144	Δ*mdm35* Δ*ups1*	W303	a	*can1Δ100 his3Δ11,15 leu2Δ3,112 ura3Δ1 ade2Δ1 trp1Δ1 Δmdm35::HIS3MX6 Δups1::NAT*
CW3	Δ*atp23*	W303	a	*can1Δ100 leu2Δ3,112 ura3Δ1 ade2Δ1, trp1Δ1, Δatp23::HIS3MX6*
CW343	Mdm35↑	W303	a	*can1Δ100 his3Δ11,15 leu2Δ3,112 ura3Δ1 ade2Δ1 trp1Δ1 P_{Gal}(NAT)-MDM35*
CW385	Δ*mdm35*	S288c	a	*his3Δ1 leu2Δ0 met15Δ0 lys2Δ0 ura3Δ0 UPS1-9myc (HIS3) Δmdm35::NAT*
CW394	WT	S288c	a	*his3Δ1 leu2Δ0 met15Δ0 lys2Δ0 ura3Δ0 UPS1-9myc (HIS3) Ycplac111::ADH*
CW396	Δ*mdm35* [*MDM35*]	S288c	a	*his3Δ1 leu2Δ0 met15Δ0 lys2Δ0 ura3Δ0 UPS1-9myc (HIS3) Δmdm35::NAT Ycplac111::ADH-Mdm35-500bp3'*
CW398	Δ*mdm35*	S288c	a	*his3Δ1 leu2Δ0 met15Δ0 lys2Δ0 ura3Δ0 UPS1-9myc (HIS3) Δmdm35::NAT Ycplac111::ADH*
CW414	Δ*mdm35* Δ*yme1*	W303	a	*can1Δ100 his3Δ11,15 leu2Δ3,112 ura3Δ1 ade2Δ1 trp1Δ1 Δyme1::ADE2 Δmdm35::kanMX*
CW79	Δ*atp23* Δ*yme1*	W303	a	*can1Δ100 his3Δ11,15 Δatp23::HIS3 leu2Δ3,112 ura3Δ1 ade2Δ1 Δyme1::ADE2 trp1Δ1*

Euroscarf 3451	Δ*mgr1*	S288c	a	*his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 Δmgr1::kanMX*
Euroscarf 3464	Δ*prd1*	S288c	a	*his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 Δprd1::kanMX*
Euroscarf 4266	Δ*cym1*	S288c	a	*his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 Δcym1::kanMX*
Euroscarf 4731	Δ*pcp1*	S288c	a	*his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 Δpcp1::kanMX*
Euroscarf 6003	Δ*oma1*	S288c	a	*his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 Δoma1::kanMX*
Euroscarf 6555	Δ*mgr3*	S288c	a	*his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 Δmgr1::kanMX*
Euroscarf 732	Δ*imp1*	S288c	a	*his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 Δimp1::kanMX*
PD17	Ups2^{HA}	S288c	a	*his3Δ1leu2Δ0 (met15Δ0/MET15?) lys2Δ0 ura3Δ0 UPS2-6xHA (kanMX)*
PD49	Δ*ups1*	S288c	α	*his3Δ1leu2Δ0 ura3Δ0 Δups1::HYG*
PD94	Ups2^{HA} Δ*ups1*	S288c	a	*his3Δ1leu2Δ0 (met15Δ0/MET15?) lys2Δ0 ura3Δ0 UPS2-6xHA (kanMX) Δups1::HYG*
PY102	WT	S288c	a	*his3Δ1 leu2Δ0 met15Δ0 lys2Δ0 ura3Δ0 UPS2-9myc (HIS3)*
PY103	WT	S288c	a	*his3Δ1 leu2Δ0 met15Δ0 lys2Δ0 ura3Δ0 UPS1-9myc (HIS3)*
PY117	Δ*mdm35*	S288c	a	*his3Δ1 leu2Δ0 met15Δ0 lys2Δ0 ura3Δ0 UPS2-9myc (HIS3) Δmdm35::NAT*
PY127	WT	S288c	a	*his3Δ1 leu2Δ0 met15Δ0 lys2Δ0 ura3Δ0 UPS2-9myc (HIS3) Ycplac111::ADH*
PY128	WT [*MDM35*]	S288c	a	*his3Δ1 leu2Δ0 met15Δ0 lys2Δ0 ura3Δ0 UPS2-9myc (HIS3) Ycplac111::ADH-Mdm35-500bp3'*
PY129	Δ*mdm35*	S288c	a	*his3Δ1 leu2Δ0 met15Δ0 lys2Δ0 ura3Δ0 UPS2-9myc (HIS3) Δmdm35::NAT Ycplac111::ADH*
PY130	Δ*mdm35* [*MDM35*]	S288c	a	*his3Δ1 leu2Δ0 met15Δ0 lys2Δ0 ura3Δ0 UPS2-9myc (HIS3) Δmdm35::NAT Ycplac111::ADH-Mdm35-500bp3'*
PY132	Δ*ups2* Δ*yme1*	S288c	a	*his3Δ1 leu2Δ0 met15Δ0 lys2Δ0 ura3Δ0 Δups2::NAT Δyme1::kanMX*
PY153	Δ*ups1* Δ*yme1*	S288c	α	*his3Δ1leu2Δ0 ura3Δ0 Δups1::HYG Δyme1::kanMX*
PY64	Δ*mdm35 UPS2^{MYC}↑*	S288c	a	*his3Δ1leu2Δ0 met15Δ0 lys2Δ0 ura3Δ0 PGal(NAT)-UPS2-Myc-7His (kanMX) Δmdm35::HIS3MX6*
VI A4	Δ*yme1*	W303	α	*can1Δ100 his3Δ11,15 leu2Δ3,112 ura3Δ1 ade2Δ1 trp1Δ1 Δyme1::HIS3MX6*
VI A5	Δ*yme1*	W303	a	*can1Δ100 his3Δ11,15 leu2Δ3,112 ura3Δ1 ade2Δ1 trp1Δ1 Δyme1::ADE2*
YTE26	Yme1^{E541Q}	W303	α	*ade2Δ1 his3Δ11,15 leu2Δ112 trp1Δ1 ura3Δ52 can1Δ100 Δyme1::HIS3 pRS314-Yme1E541Q*
YTE89	His-Yme1^{E541Q}	W303	α	*ade2Δ1 his3Δ11,15 leu2Δ112 trp1Δ1 ura3Δ52 can1Δ100 Δyme1::HIS3 pRS314-Yme1(1-50)-6His-Yme1(51-747)-E541Q*