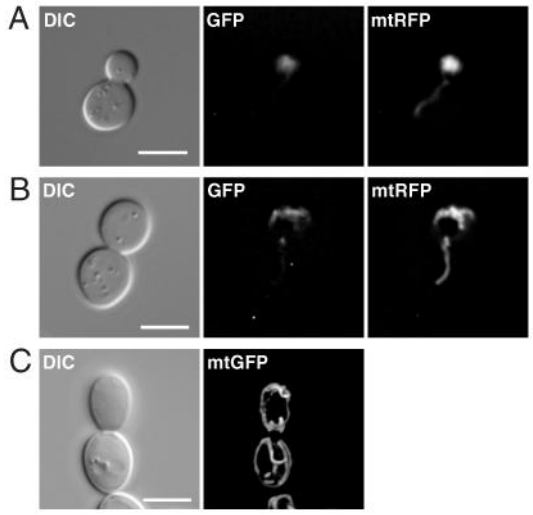
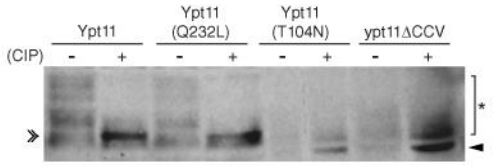
SUPPLEMENTAL MATERIALS

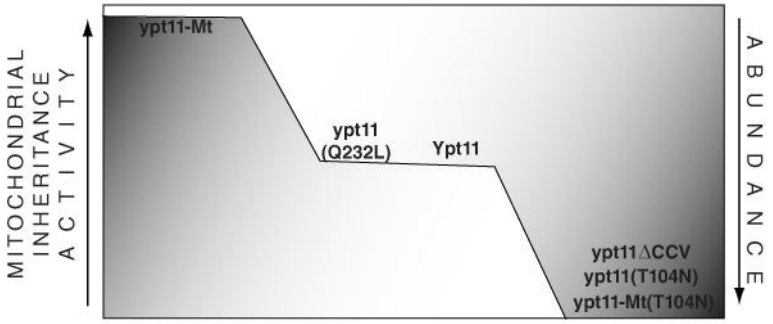
Supplemental Figure S1. Expression of Ypt11-Mt causes mitochondrial accumulation in small (A) and large buds (B), leading to a decrease in mitochondrial content of mother cells. Representative DIC and fluorescent images of *ypt11*Δ cells co-expressing GFP-tagged ypt11-Mt (*MET25* promoter) and mtRFP are shown. (C) WT distribution of the mitochondrial network in a large budded cell. Bar, 5 µm.



Supplemental Figure S2. Inactive Ypt11 mutant proteins have a different phosphorylation pattern than the active forms. Whole cell extracts from *ypt11Δ* cells expressing WT from the *MET25* promoter or the indicated mutant FLAG-Ypt11 proteins were incubated with or without calf alkaline phosphatase (CIP). Samples were separated in acrylamide + Phos-tag™ mini gels, and analyzed by anti-FLAG immunoblotting. An arrowhead marks the lowest molecular weight (presumed dephosphorylated) protein band observed for inactive proteins. A double arrowhead marks the predominant band observed for active proteins. An asterisk marks modified (phosphorylated) species, which disappear upon CIP treatment.



Supplemental Figure S3. Abundance of Ypt11 variants correlates inversely with their activity in mitochondrial inheritance. To compare activity and abundance of the variants see Figures 3, E and F and 5, A and B.



Supplemental Tables

Table S1. Yeast strains used in this study

ID	Mating type	Genotype	Reference
JSY7000	MATa	*ade2-1 leu2-3 his3-11,15 trp1-1 ura3-1 can1-100*	(Frederick *et al.*, 2008)
JSY8563	MATa	*ade2-1 leu2-3 his3-11,15 trp1-1 ura3-1 can1-100 ypt11D::NatMX4*	(Frederick *et al.*, 2008)
JSY8571	MATa	*ade2-1 leu2-3 his3-11,15 trp1-1 ura3-1 can1-100 mmr1D::HIS3 ypt11D::NatMX4*	(Frederick *et al.*, 2008)
JSY5148 (PJ69-4A)	MATa	*trp1-901 leu2-3,112 ura3-52 his3-200 gal4Δ gal80Δ LYS2::GAL1-HIS3 GAL2-ADE2 met2::GAL7-lacZ*	(James *et al.*, 1996)

Table S2. Plasmids used in this study

ID	Plasmid*	Promoter	Protein	Reference
B61	pRS416	-	-	(Sikorski and Hieter, 1989)
B2160	p416*(MET25)-YPT11*	*MET25*	Ypt11	(Frederick *et al.*, 2008)
B2801	pRS416-*YPT11*	*YPT11*	Ypt11	this study
B2802	pRS416-*GFP-YPT11*	*YPT11*	GFP-Ypt11	this study
B2195	p416*(MET25)-GFP-YPT11*	*YPT11*	GFP-Ypt11	this study
B3128	pRS416-*FLAG-OneSTrEP-YPT11*	*YPT11*	FLAG-Ypt11	this study
B3127	p416*(MET25)-FLAG-OneSTrEP-YPT11*	*MET25*	FLAG-Ypt11	this study
B3021	pRS416-*ypt11-M1(ATC)*	*YPT11*	ypt11-M1(ATC)	this study
B3037	pRS416-*GFP-ypt11(63-417)*	*YPT11*	ypt11Δ62N	this study
B3048	p416*(MET25)-GFP-ypt11Δ62N*	*MET25*	ypt11Δ62N	this study
B2819	pRS416-*ypt11(1-414)*	*YPT11*	ypt11ΔCCV**	this study
B3022	p416*(MET25)-ypt11ΔCCV*	*MET25*	ypt11ΔCCV	this study
B3023	p416*(MET25)-GFP-ypt11ΔCCV*	*MET25*	GFP-ypt11ΔCCV	this study
B3122	p416*(MET25)-FLAG-OneSTrEP-ypt11ΔCCV*	*MET25*	FLAG-ypt11-ΔCCV	this study
B2981	pRS416-*ypt11(1-414)-fis1(128-155)*	*YPT11*	ypt11-Mt**	this study
B3024	p416*(MET25)-ypt11-Mt*	*MET25*	ypt11-Mt	this study
B3025	p416*(MET25)-GFP-ypt11-Mt*	*MET25*	GFP-ypt11-Mt	this study
B3123	p416*(MET25)-FLAG-OneSTrEP-ypt11-Mt*	*MET25*	FLAG-ypt11-Mt	this study
B3084	p416*(MET25)-GFP-ypt11-Mt(T104N)*	*MET25*	GFP-ypt11-Mt(T104N)	this study

B3362	p416*(MET25)-FLAG-OneSTrEP-ypt11-Mt(T104N)*	*MET25*	FLAG-ypt11-Mt(T104N)	this study
B2982	pRS416-*ypt11(1-414)-frt1(568-602)*	*YPT11*	ypt11-ER**	this study
B3026	p416*(MET25)-ypt11-ER*	*MET25*	ypt11-ER	this study
B3027	p416*(MET25)-GFP-ypt11-ER*	*MET25*	GFP-ypt11-ER	this study
B3124	p416*(MET25)-FLAG-OneSTrEP-ypt11-ER*	*MET25*	FLAG-ypt11-ER	this study
B2963	pRS416-*ypt11(Q232L)*	*YPT11*	ypt11(Q232L)	this study
B2964	p416*(MET25)-ypt11(Q232L)*	*MET25*	ypt11(Q232L)	this study
B3253	p416*(MET25)-FLAG-OneSTrEP-ypt11(Q232L)*	*MET25*	FLAG-ypt11(Q232L)	this study
B2966	pRS416-*ypt11(T104N)*	*YPT11*	ypt11(T104N)	this study
B2967	p416*(MET25)-ypt11(T104N)*	*MET25*	ypt11(T104N)	this study
B3254	p416*(MET25)-FLAG-OneSTrEP-ypt11(T104N)*	*MET25*	FLAG-ypt11(T104N)	this study
B3371	p416*(MET25)-GFP-ypt11(T104N)*	*MET25*	GFP-ypt11(T104N)	this study
B2455	pGAD-c1	*ADH1*	Gal4AD	(James *et al.*, 1996)
B2456	pGBD-c1	*ADH1*	Gal4BD	(James *et al.*, 1996)
B3151	pGBD-c1-*ypt11(1-414)*	*ADH1*	BD-Ypt11	this study
B3153	pGBD-c1-*ypt11(1-414)(Q232L)*	*ADH1*	BD-ypt11(Q232L)	this study
B3155	pGBD-c1-*ypt11(1-414)(T104N)*	*ADH1*	BD-ypt11(T104N)	this study
B3232	pGBD-c1-*ypt11(1-414) (S8A)*	*ADH1*	BD-ypt11(S8A)	this study
B3233	pGBD-c1-*ypt11(1-414)(S158A,S159A)*	*ADH1*	BD-ypt11-2xS/A	this study
B3234	pGBD-c1-*ypt11(1-414)(S8A,S158A,S159A)*	*ADH1*	BD-ypt11-3xS/A	this study
B3349	pGBD-c1-*ypt11(1-414)(S8A,S77A,S79A,S80A,S158A,S159A)*	*ADH1*	BD-ypt11-6xS/A	this study
B3074	p416*(MET25)-ypt11(S8A)*	*MET25*	ypt11(S8A)	this study
B3075	p416*(MET25)-ypt11(S158A)*	*MET25*	ypt11(S158A)	this study
B3343	p416*(MET25)-FLAG-OneSTrEP-ypt11(S158A,S159A)*	*MET25*	FLAG-ypt11 (S158A,S159A)	this study
B3350	p416*(MET25)-FLAG-OneSTrEP-ypt11(S77A,S79A,S80A,S158A,S159A)*	*MET25*	FLAG-ypt11-5xS/A	this study
B2371	pGAD-c1-*MMR1*	*ADH1*	AD-Mmr1	this study

B2375	pGAD-c1-*myo2(1031-1574)*	*ADH1*	AD-Myo2t	this study
B1220	pYX142-*Su9(1-69)-GFP*	*TPI1*	mtGFP	(Frederick *et al.*, 2004)
B1590	YIpLAC204/TKC-*DsRed-HDEL*		ER-DsRed	(Bevis *et al.*, 2002)
B1504	pYX142-*Su9(1-69)-RFPff*	*TPI1*	mtRFP	(Frederick *et al.*, 2004)

*Numbers in parentheses in plasmid names refer to codon numbers.
**For these Ypt11 variants, the full description of the cloned region is included in the Plasmid column the first time it appears. The corresponding clone name (i.e. Ypt11-mt) occurs in the Protein column and is subsequently used to describe the plasmid and protein for all derived variants (i.e. GFP-ypt11-Mt(T104N)).

SUPPLEMENTAL METHODS

Plasmid construction

To create the *YPT11* native promoter vector (pRS416-*YPT11*, B2801), the *YPT11* gene was PCR amplified from W303 genomic DNA together with 500 bp upstream and 60 bp downstream sequence, and cloned into pRS416 using SpeI and XhoI restriction sites. Only a short downstream sequence fragment was cloned due to a close proximity of a neighboring gene in the *S. cerevisiae* genome.

For generation of GFP-tagged *YPT11* with the native promoter (pRS416-*GFP-YPT11*, B2802), the *GFP* sequence followed by eight glycine codons was introduced at the 5'-end of the *YPT11* ORF by homologous recombination in a *ypt11::URA3* yeast strain. The tagged *YPT11* sequence was cloned from the genomic DNA of the resulting strain as described above. For generation of GFP-tagged *YPT11* under the control of the *MET25* promoter (p416(*MET25*)-*GFP-YPT11*, B2195) a PCR amplified *GFP* sequence followed by eight glycine codons was introduced at the 5'-end of the *YPT11* gene in the B2160 plasmid (p416(*MET25*)-*YPT11*) using SpeI and XhoI restriction sites.

For generation of FLAG-tagged *YPT11* under control of the *MET25* promoter (p416(*MET25*)-*FLAG-OneSTrEP-YPT11*, B3127), the N-terminal XbaI/BamHI fragment of the tagged gene sequence was excised from pYSG167-*YPT11* (created by recombination in a StarGate® cloning system (IBA BioTAGnology) according to the manufacturer's protocol) and ligated in place of the XbaI/BamHI fragment of the GFP-tagged gene in B2195.

To create the *ypt11Δ62N* variant under the control of the native promoter (pRS416-*GFP-ypt11Δ62N*; B3037), a two step cloning approach was used. First, a SfoI/KasI restriction site was created directly upstream of the first *YPT11* ATG codon in B2802 (within the 8xGly linker) (yielding B2802-SfoI/KasI). Next, the short *YPT11* fragment (codons 63-417) was PCR amplified with a primer containing Sfo/KasI sites at the 5'-end. The PCR product was digested with KasI and XhoI restriction ezymes and cloned into the B2802-SfoI/KasI plasmid in place of the WT *YPT11* sequence. To create the construct for expression of *ypt11Δ62N* from the *MET25* promoter, the *GFP-ypt11Δ62N* sequence was PCR amplified from B3037 using a primer containing an XmaI restriction site at the 5'-end. The PCR fragment was digested with XmaI and XhoI and cloned into the p416(*MET25*) vector.

For generation of the mitochondrial variant (*ypt11-Mt*) under the control of the native promoter (pRS416-*ypt11(1-414)-fis1(128-155)*, B2981) a three-step cloning approach was used. First, in a vector encoding *GFP* fused to the mitochondrial targeting Fis1 transmembrane domain (*GFP-Fis1(128-155)*, B1457) the SpeI/HindIII *GFP* fragment was replaced with the PCR amplified *ypt11(1-414)* sequence (the full length gene missing the last three codons). Second, a BglII restriction site was introduced by site-directed mutagenesis in place of the stop codon in the B2801 plasmid (pRS416-*YPT11*) to create B2801-BglII. Third, the BamHI/BglII fragment of the resulting plasmid was replaced with the BamHI/BglII fragment of the *ypt11(1-414)-fis1(128-155)-BglII* construct (product of the first step). To create a GFP-tagged

mitochondrially-targeted variant, the same steps were followed using a B2802-BglII plasmid as the target vector (instead of B2801-BglII).

For generation of the ER variant (*ypt11-ER*) under the control of the native promoter (pRS416-*ypt11(1-414)-frt1(568-602)*, B2982), the *FRT1* gene was PCR amplified from W303 genomic DNA and cloned into the p416(*MET25*) vector. The last 35 codons plus a stop codon from the *FRT1* ORF were PCR amplified using primers that introduced BglII sites on both ends of the fragment. The PCR product was digested with BglII and cloned into the BglII site in the B2801-BglII plasmid (see above) or B2802-BglII (to create a GFP-tagged version).

The cytoplasmic variant *ypt11ΔCCV* (pRS416-*ypt11(1-414)*) was created by a single site-directed mutagenesis reaction designed to introduce two stop codons in place of the *CCV* sequence.

To create the localization variants of Ypt11 expressed from the *MET25* promoter, the respective constructs were PCR amplified and cloned into BamHI/XhoI sites of B2160 or B2195.

To create the FLAG-tagged localization variants of Ypt11, the XbaI/BamHI 5'-fragment of the FLAG-tagged *YPT11* sequence was excised from pYSG167-*YPT11* and ligated in place of the XbaI/BamHI fragment in vectors containing the respective localization variants under *MET25* promoter.

For generation of plasmids for the two-hybrid assay, *YPT11* (codons 1-414), *MMR1* (full length) and *MYO2* (codons 1031-1574) were PCR amplified and cloned into pGBD and pGAD using XmaI and ClaI restriction sites.

The following *YPT11* mutants were created by site directed mutagenesis using the respective source plasmids: M1(ATC); Q232L; T104N; S8A; S158A; S158A,S159A; S77A,S79A,S80A. Combinations of mutations were achieved by sequential mutagenesis.

REFERENCES

Bevis BJ, Hammond AT, Reinke CA, Glick BS (2002). De novo formation of transitional ER sites and Golgi structures in *Pichia pastoris*. Nat Cell Biol 4, 750-756.

Frederick RL, McCaffery JM, Cunningham KW, Okamoto K, Shaw JM (2004). Yeast Miro GTPase, Gem1p, regulates mitochondrial morphology via a novel pathway. J Cell Biol 167, 87-98.

Frederick RL, Okamoto K, Shaw JM (2008). Multiple pathways influence mitochondrial inheritance in budding yeast. Genetics 178, 825-837.

James P, Halladay J, Craig EA (1996). Genomic libraries and a host strain designed for highly efficient two-hybrid selection in yeast. Genetics 144, 1425-1436.

Sikorski RS, Hieter P (1989). A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. Genetics 122, 19-27.