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 ypt11Δ 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 ypt11D::NatMX4             | (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 gal4A gal80A 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 |
| Entry  | Vector/Plasmid/Tag                      | Gene   | Mutation/Tag          | Notes |
|--------|----------------------------------------|--------|-----------------------|-------|
| B3362  | p416(MET25)-FLAG-OneSTrEP-ypt11-Mt(T104N) | MET25  | FLAG-ypt11-Mt(T104N)  | this study |
| B2982  | pRS416-ypt11(1-414)-frr1(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-MMRI                           | ADH1   | AD-Mmr1               | this study |
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 ypt11Δ::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 Xbal/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 Xbal/BamHI fragment of the GFP-tagged gene in B2195.

To create the ypt11ΔA62N variant under the control of the native promoter (pRS416-GFP-ypt11ΔA62N; 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 enzymes and cloned into the B2802-SfoI/KasI plasmid in place of the WT YPT11 sequence. To create the construct for expression of ypt11ΔA62N from the MET25 promoter, the GFP-ypt11ΔA62N 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-Mt(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-BglIII. 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 Xbal/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.

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