Supplementary figure legends

Figure S1. Vms1 mitochondrial targeting domain conservation.

Sequence alignment of the mitochondrial targeting domain (MTD) of Vms1 from diverse species.

Figure S2. The vms1Δ growth defects cannot be rescued by Vms1 deletion mutants.

(A) WT and the vms1Δ strains were transformed with empty vector (ev), a plasmid containing the VMS1 gene (pVMS1), or plasmids containing various deletion mutants of Vms1 as described in Figure 2 (pvms1-182-end to 182-417). Each strain was grown to saturation in liquid medium and serial dilutions were spotted on both SD-Ura and SD-Ura + rapamycin (20ng/ml) and grown at 30° for 3 and 7 days, respectively. (B) The vms1Δ strain was transformed with plasmids encoding Cdc48-myc and either Vms1^{1-182}-HA, Vms1-HA, or empty vector as indicated. Each strain was grown to log phase in SD-Leu-Trp and harvested. The crude lysates from each strain were immunoprecipitated with anti-HA antibody and Western blots were performed with anti-HA or anti-myc antibodies as indicated.
Figure S3. Schematic representation of Vms1 deletion mutants generated and tested for interaction in the yeast two-hybrid assay

A series of Vms1 mutants was generated as above. Mutants exhibiting interaction are shown in black while those that failed to interact are shown in red.

Figure S4. Vms1\textsuperscript{11-end} exhibits similar localization pattern to the Vms1 wild type and Vms1\textsuperscript{11-end, MutA} is constitutively localized to mitochondria.

The \textit{vms1}\textsuperscript{Δ} strain was transformed with C-terminus GFP tagged full-length wild type Vms1, Vms1\textsuperscript{11-end}, or Vms1\textsuperscript{11-end, MutA}. Each transformant was grown to log phase in SD-Ura media and subjected to fluorescence microscopy. Representative images are shown.

Figure S5. Vms1\textsuperscript{11-end, MutA} is dysfunctional due to its instability.

(A) WT and the \textit{vms1}\textsuperscript{Δ} strains were transformed with either empty vector (ev), a plasmid containing C-terminus HA tagged full-length wild type Vms1, Vms1\textsuperscript{11-end}, or Vms1\textsuperscript{11-end, MutA}. Each strain was then grown to saturation in liquid medium and serial 5-fold dilutions were spotted on both SD-Ura and SD-Ura + rapamycin (20ng/ml) and grown at 30° for 3 and 7 days, respectively. (B) The same transformed cells grown to mid log phase in SD-Ura media were either left as
they were or treated with rapamycin (200ng/ml) for 3 hours. The same amount of cells was then harvested to prepare a whole cell extract (WCE). Identical amounts of WCE were subjected to SDS-PAGE followed by Western blot. The levels of each Vms1 variant were determined by using anti-HA antibody. An antibody against actin was utilized as a loading control.

**Figure S6.** Vms1$^{\text{MutC}}$ and Vms1$^{\text{MutD}}$ can rescue the *vms1* mutant phenotype.

WT and the *vms1*Δ strains were transformed with empty vector (ev), a plasmid containing C-terminus HA-tagged wild type VMS1 (Vms1$^{\text{WT-HA}}$), VMS1$^{\text{MutC}}$ and VMS1$^{\text{MutD}}$. Each strain was grown to saturation in SD-Ura media and a serial 5-fold dilution of equal numbers of cells was spotted on both SD-Ura (control) and SD-Ura+rapamycin (20ng/ml) and grown at 30° for 3 and 7 days, respectively.

**Figure S7.** Vms1$^{1-182, T48A, S127A, S132A, T137A, S138A, S139A}$ mutant exhibits almost identical affinity to Vms1$^{\text{MTD}}$ compared with that of Vms1$^{1-182, \text{WT}}$.

(A) A schematic representation of phosphorylation sites identified in purified Vms1. (B) The *vms1*Δ strain was transformed with either empty vector (ev), Vms1$^{\text{MTD-myc}}$, Vms1$^{1-182, \text{WT-HA}}$ or Vms1$^{1-182, T48A, S127A, S132A, T137A, S138A, S139A}$-HA as indicated. Each strain was then grown to log phase and harvested. The crude lysates from each strain were immunoprecipitated with anti-HA antibody and
Western blots were performed with anti-HA or anti-myc antibodies as indicated.

(C) The $vms1\Delta$ strain was transformed with both mtRFP and C-terminus GFP-
 fused full-length wild type Vms1 or Vms1$^{S/T->A}$ mutant. Each cell was then grown
to log phase in SD-Ura-Leu medium and subjected to imaging. Representative
images are shown.

**Figure S8. Irradiation of mitochondrial RFP does not promote Vms1**
localization to mitochondria.

Yeast cells expressing Vms1-GFP were transformed with a mitochondrially-
targeted RFP. Cells were irradiated for 15 minutes with 572nm light. Following
irradiation, Vms1-GFP localization was monitored by wide field fluorescence
microscopy every 10 minutes for 60 minutes.

**Figure S9. Killer Red is not detected in the green channel.**

WT yeast strain was transformed with mitochondrially-targeted Killer Red. Cells
were imaged by wide field fluorescence microscopy with the identical conditions
used when imaging Vms1-GFP.
Figure S10. Vms1 exhibits preferential localization to ROS-exposed mitochondria

(A, B) Yeast cells expressing Vms1-GFP were transformed with mitochondrially-targeted Killer Red. These cells were irradiated with a 543nm HeNe laser at 100% intensity for one minute and only in a small region of the cell (indicated by the yellow dot), which contained mitochondria. Vms1-GFP localization was monitored by confocal fluorescence microscopy for 60 minutes after irradiation.
Heo, Figure S4
A

SD-Ura

SD-Ura + rap (10ng/ml)

WT + e.v.

vms1Δ + e.v.

vms1Δ + Vms1WT-HA

vms1Δ + Vms111-end-HA

vms1Δ + Vms111-end,MutA-HA

B

|                | empty vector | Vms1WT-HA | Vms1MutD-HA | Vms111-end-HA | Vms111-end,MutA-HA |
|----------------|--------------|-----------|-------------|---------------|--------------------|
| no rapamycin   |              |           |             |               |                    |
| α-HA           |              |           |             |               |                    |
| α-actin        |              |           |             |               |                    |
| with rapamycin |              |           |             |               |                    |
| α-HA           |              |           |             |               |                    |
| α-actin        |              |           |             |               |                    |

Heo, Figure S5
WT + e.v.

vms1Δ + e.v.

vms1Δ + Vms1\textsuperscript{WT-HA}

vms1Δ + Vms1\textsuperscript{MutC-HA}

vms1Δ + Vms1\textsuperscript{MutD-HA}

SD-Ura

SD-Ura + Rap 20ng/ml

Heo, Figure S6
Heo, Figure S7
Heo, Figure S8

- DIC
- mtRFP
- Vms1-GFP

Pre irradiation 0' 20' 40' 60'
Figure S9

(A) pre irradiation 0' 60'

DIC

Vms1-GFP

Mito-KillerRed

(B) pre irradiation 0' 60'

DIC

Vms1-GFP

Mito-KillerRed