Figure S1. The morphology of the ER, vacuoles and the actin cytoskeleton is not affected by the absence of Mdm36. (A) Yeast cells expressing ER-targeted GFP were grown to logarithmic growth phase in glucose-containing medium (YPD) and analyzed by DIC and fluorescence microscopy. Bar, 5 µm. (B) Yeast cells were grown to logarithmic growth phase in glucose-containing medium (YPD), stained with 5-(and-6)-carboxy-2′,7′-dichlorofluorescein diacetate and analyzed by DIC and fluorescence microscopy. Bar, 5 µm. (C) Yeast cells expressing mitochondria-targeted GFP were grown to logarithmic growth phase in glucose-containing medium (YPD), fixed, stained with rhodamine-phalloidin and analyzed by DIC and fluorescence microscopy. Images from left to right: DIC, green fluorescence (mitochondria-targeted GFP), red fluorescence (rhodamine-stained actin shown as a reversed black and white image to better visualize faint actin cables and patches), merged image of GFP and rhodamine staining. Bar, 5 µm.

Figure S2. Mitochondrial ultrastructure is not affected by deletion of the *MDM36* gene. Yeast cells were grown to logarithmic growth phase in glucose (YPD) or glycerol (YPG) containing media and analyzed by transmission electron microscopy. All images are shown at the same magnification.

Figure S3. Mitochondrial motility is altered in the absence of Mdm36. Yeast cells expressing mitochondria-targeted GFP were grown to logarithmic growth phase in glucose-containing medium (YPD) and analyzed by confocal microscopy. For each cell, 50 confocal z-stacks consisting of 10 confocal planes were recorded over a time period of 355 sec (i.e. one z-stack was taken every ~7.2 sec) and displayed as maximum intensity projections. Bars, 5 µm. Top, bright field images with traces of mitochondrial tips (compare Figure 6A). Videos 1, 3, 5 and 7 have been generated with these data sets, Videos 2, 4, 6 and 8 show additional representative cells.

Figure S4. Yeast cells expressing Dnm1-GFP and Num1-RFP were grown to logarithmic growth phase in glucose (SD) or glycerol (YPG) containing media and analyzed by DIC and epifluorescence microscopy. Bars, 5 µm. Additional representative cells are shown in Figure 6C.
**Video 1.** Mitochondrial dynamics in wild type yeast. Yeast cells expressing mitochondria-targeted GFP were grown to logarithmic growth phase in glucose-containing medium (YPD) and analyzed by confocal microscopy. 50 confocal z-stacks consisting of 10 confocal planes were recorded over a time period of 355 sec (i.e. one z-stack was taken every ~7.2 sec) and displayed as maximum intensity projections. Traces of mitochondrial tips in this cell are shown in Figure 6A, and still images showing maximum intensity projections of this cell are shown in Figure S3.

**Video 2.** Mitochondrial dynamics in wild type yeast. Yeast cells expressing mitochondria-targeted GFP were grown to logarithmic growth phase in glucose-containing medium (YPD) and analyzed by confocal microscopy. 50 confocal z-stacks consisting of 10 confocal planes were recorded over a time period of 355 sec (i.e. one z-stack was taken every ~7.2 sec) and displayed as maximum intensity projections.

**Video 3.** Mitochondrial dynamics in \(\Delta mdm36\) yeast. Yeast cells expressing mitochondria-targeted GFP were grown to logarithmic growth phase in glucose-containing medium (YPD) and analyzed by confocal microscopy. 50 confocal z-stacks consisting of 10 confocal planes were recorded over a time period of 355 sec (i.e. one z-stack was taken every ~7.2 sec) and displayed as maximum intensity projections. Traces of mitochondrial tips in this cell are shown in Figure 6A, and still images showing maximum intensity projections of this cell are shown in Figure S3.

**Video 4.** Mitochondrial dynamics in \(\Delta mdm36\) yeast. Yeast cells expressing mitochondria-targeted GFP were grown to logarithmic growth phase in glucose-containing medium (YPD) and analyzed by confocal microscopy. 50 confocal z-stacks consisting of 10 confocal planes were recorded over a time period of 355 sec (i.e. one z-stack was taken every ~7.2 sec) and displayed as maximum intensity projections.

**Video 5.** Mitochondrial dynamics in \(\Delta num1\) yeast. Yeast cells expressing mitochondria-targeted GFP were grown to logarithmic growth phase in glucose-containing medium (YPD) and analyzed by confocal microscopy. 50 confocal z-stacks consisting of 10 confocal planes were recorded over a time period of 355 sec
(i.e. one z-stack was taken every ~7.2 sec) and displayed as maximum intensity projections. Traces of mitochondrial tips in this cell are shown in Figure 6A, and still images showing maximum intensity projections of this cell are shown in Figure S3.

**Video 6.** Mitochondrial dynamics in \(\Delta num1\) yeast. Yeast cells expressing mitochondria-targeted GFP were grown to logarithmic growth phase in glucose-containing medium (YPD) and analyzed by confocal microscopy. 50 confocal z-stacks consisting of 10 confocal planes were recorded over a time period of 355 sec (i.e. one z-stack was taken every ~7.2 sec) and displayed as maximum intensity projections.

**Video 7.** Mitochondrial dynamics in \(\Delta mmd36 \Delta num1\) yeast. Yeast cells expressing mitochondria-targeted GFP were grown to logarithmic growth phase in glucose-containing medium (YPD) and analyzed by confocal microscopy. 50 confocal z-stacks consisting of 10 confocal planes were recorded over a time period of 355 sec (i.e. one z-stack was taken every ~7.2 sec) and displayed as maximum intensity projections. Traces of mitochondrial tips in this cell are shown in Figure 6A, and still images showing maximum intensity projections of this cell are shown in Figure S3.

**Video 8.** Mitochondrial dynamics in \(\Delta mmd36 \Delta num1\) yeast. Yeast cells expressing mitochondria-targeted GFP were grown to logarithmic growth phase in glucose-containing medium (YPD) and analyzed by confocal microscopy. 50 confocal z-stacks consisting of 10 confocal planes were recorded over a time period of 355 sec (i.e. one z-stack was taken every ~7.2 sec) and displayed as maximum intensity projections.

**Video 9.** Mitochondrial dynamics in latrunculin A-treated wild type yeast. Yeast cells expressing mitochondria-targeted GFP were grown to logarithmic growth phase in glucose-containing medium (YPD), treated with 10 µM latrunculin A for 1 h at 30°C and analyzed by confocal microscopy. 50 confocal z-stacks consisting of 10 confocal planes were recorded over a time period of 355 sec (i.e. one z-stack was taken every ~7.2 sec) and displayed as maximum intensity projections.
**Video 10.** Mitochondrial dynamics in latrunculin A-treated Δmdm36 yeast. Yeast cells expressing mitochondria-targeted GFP were grown to logarithmic growth phase in glucose-containing medium (YPD), treated with 10 µM latrunculin A for 1 h at 30°C and analyzed by confocal microscopy. 50 confocal z-stacks consisting of 10 confocal planes were recorded over a time period of 355 sec (i.e. one z-stack was taken every ~7.2 sec) and displayed as maximum intensity projections.

**Video 11.** Mitochondrial dynamics in latrunculin A-treated Δnum1 yeast. Yeast cells expressing mitochondria-targeted GFP were grown to logarithmic growth phase in glucose-containing medium (YPD), treated with 10 µM latrunculin A for 1 h at 30°C and analyzed by confocal microscopy. 50 confocal z-stacks consisting of 10 confocal planes were recorded over a time period of 355 sec (i.e. one z-stack was taken every ~7.2 sec) and displayed as maximum intensity projections.

**Video 12.** Mitochondrial dynamics in latrunculin A-treated Δmdm36 Δnum1 yeast. Yeast cells expressing mitochondria-targeted GFP were grown to logarithmic growth phase in glucose-containing medium (YPD), treated with 10 µM latrunculin A for 1 h at 30°C and analyzed by confocal microscopy. 50 confocal z-stacks consisting of 10 confocal planes were recorded over a time period of 355 sec (i.e. one z-stack was taken every ~7.2 sec) and displayed as maximum intensity projections.
**Supplemental Table 1.** Cells lacking Mdm36 or Num1 have a reduced number of Dnm1-GFP clusters on mitochondrial tips.

| Medium | Strain     | Mitochondrial tips (total) | Dnm1-GFP on mitochondrial tips (total) | Mitochondrial tips per cell | Dnm1-GFP on mitochondrial tips per cell | Dnm1-GFP per mitochondrial tip |
|--------|------------|----------------------------|----------------------------------------|----------------------------|-----------------------------------------|---------------------------------|
| YPD    | WT         | 75                         | 57                                     | 1.50                       | 1.14                                    | 0.76                            |
|        | Δmdm36     | 40                         | 17                                     | 0.72                       | 0.31                                    | 0.42                            |
|        | Δnum1      | 57                         | 33                                     | 1.04                       | 0.60                                    | 0.58                            |
|        | Δmdm36 Δnum1 | 63                       | 20                                     | 1.23                       | 0.39                                    | 0.32                            |
| YPG    | WT         | 29                         | 18                                     | 0.56                       | 0.35                                    | 0.62                            |
|        | Δmdm36     | 19                         | 5                                      | 0.35                       | 0.09                                    | 0.26                            |
|        | Δnum1      | 39                         | 11                                     | 0.75                       | 0.21                                    | 0.28                            |
|        | Δmdm36 Δnum1 | 43                       | 11                                     | 0.80                       | 0.20                                    | 0.26                            |

Yeast strains coexpressing mitochondria-targeted RFP and Dnm1-GFP were grown to logarithmic growth phase in glucose (YPD) or glycerol (YPG) containing media and analyzed by confocal microscopy. At least 50 cells per strain were analyzed by inspection of single frames of confocal z-stacks for the presence of Dnm1-GFP spots located at free mitochondrial tips (compare Figure 5).
The images show the effects of different genetic conditions on cellular structures. Panel A compares wild-type (WT) and Δmdm36 cells in DIC and ER imaging, highlighting vacuole variations. Panel B focuses on vacuole comparison between WT and Δmdm36 cells. Panel C uses DIC, mtGFP, and actin imaging to show the integration of these markers and a merge image for Δmdm36 cells.
