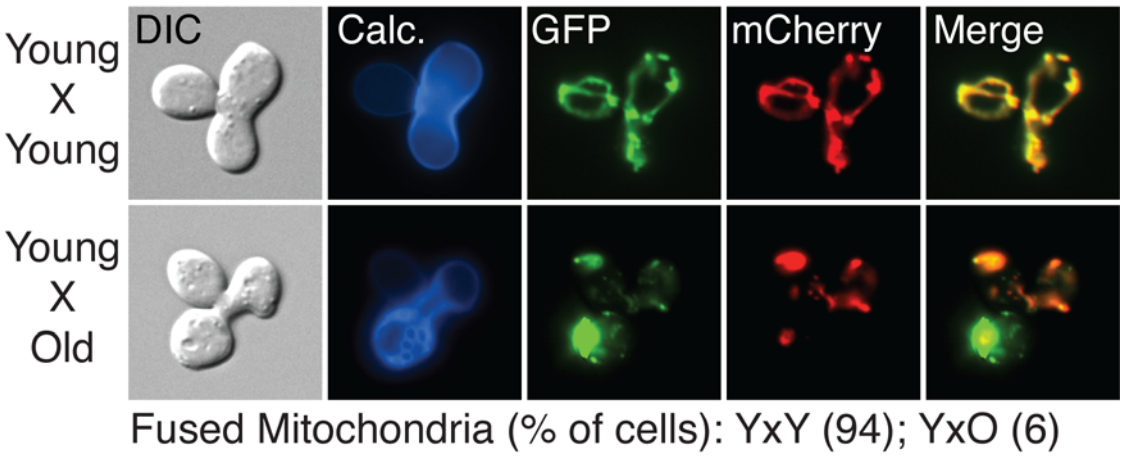
Supplemental Materials

Molecular Biology of the Cell

Goodrum et al.

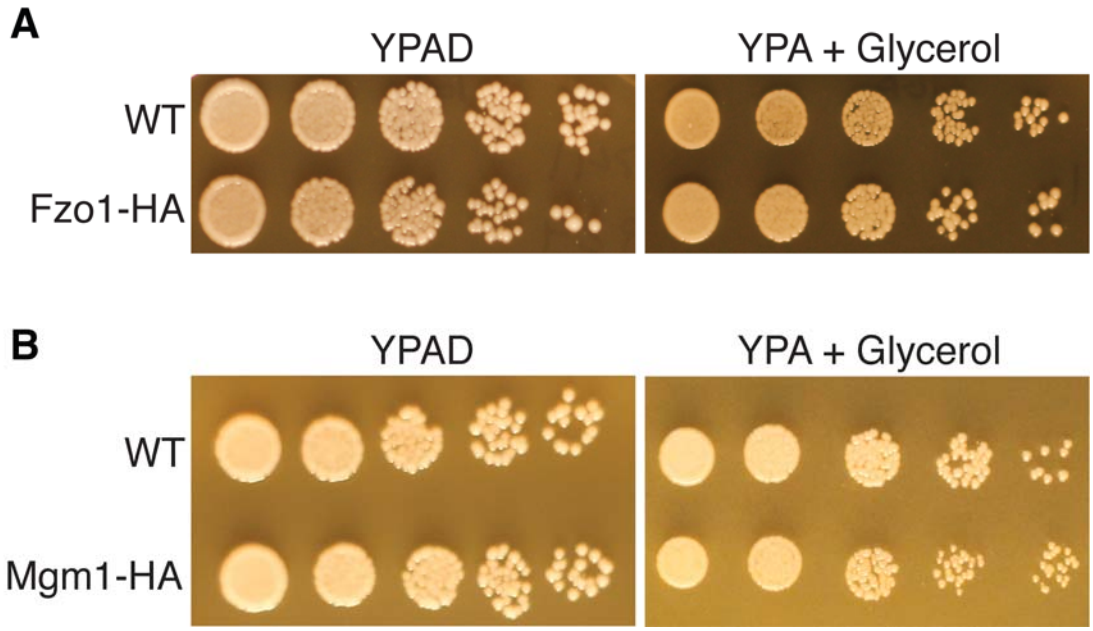
Supplemental Figure 1



Supplemental Figure 1. Mitochondrial fusion is impaired during mating of young and old zygote pairs.

Representative single Z-plane fluorescent images of wild-type zygotes derived from mating of young Tom70-mCherry and old Tom70-GFP expressing haploid cells. *n* = 50 cells. Young and old cells identified by bud scar levels after calcofluor staining.

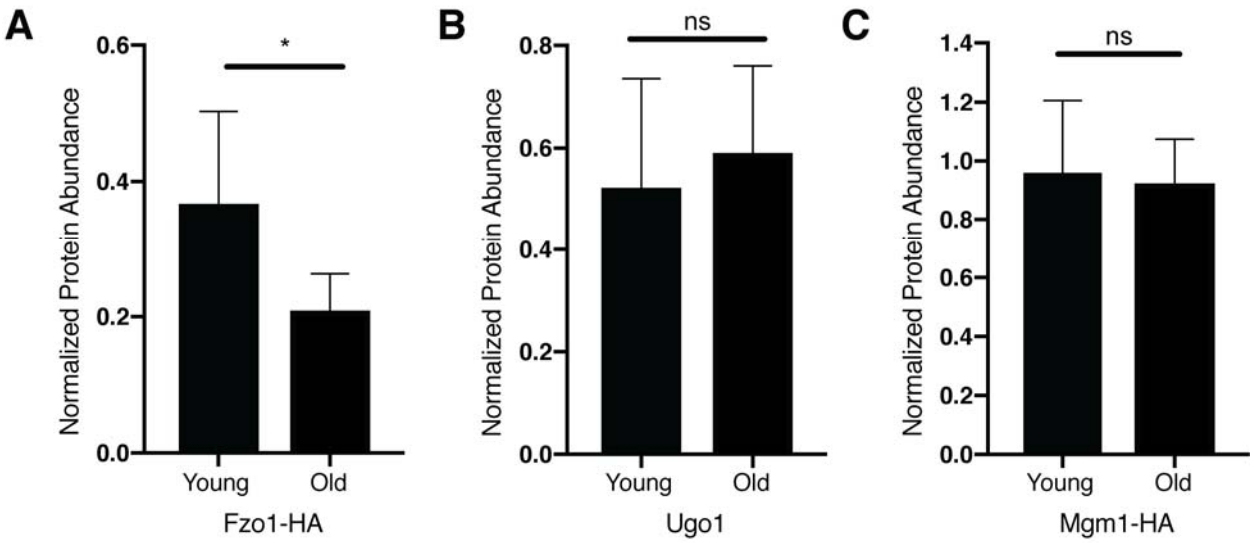
Supplemental Figure 2



Supplemental Figure 2. HA-epitope tagged Fzo1 and Mgm1 are functional.

A-B) Serial dilutions of wild-type (WT) and Fzo1-HA(A) or Mgm1-HA(B) expressing strains were plated on rich medium containing glucose (YPAD) or glycerol (YPA + Glycerol).

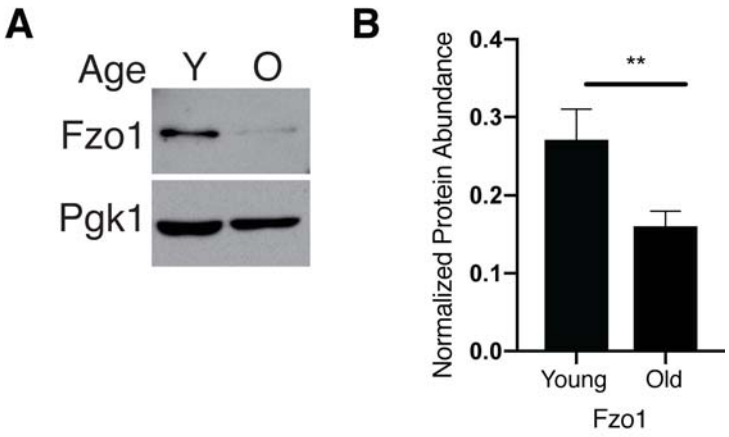
Supplemental Figure 3



Supplemental Figure3. Western blot quantification of mitochondrial fusion protein levels.

Fzo1-HA(A), Ugo1(B), and Mgm1-HA(C) protein levels from four technical replicate western blots run in parallel to Figure 1D were quantified by measuring the integrated density of each band in Image J/Fiji, and normalized to levels of Pgk1.Error bars represent standard errorof the mean of four technical replicates. pvalues were determined via paired t-test. ns = not significant, * = $p < 0.05$).

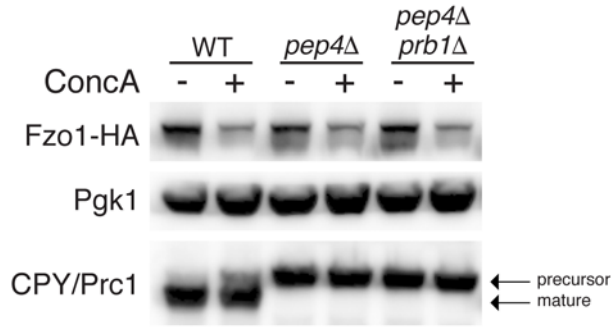
Supplemental Figure 4



Supplemental Figure4. Endogenous Fzo1 is reduced in aged cells.

A) Whole cell extracts from young (Y) and old (O) cells were analyzed by Western blot with anti-Fzo1and anti-PGK antibodies. Age ranges (*n* = 30 cells): Y = 0-3, O = 12-15. B)Endogenous Fzo1 protein levels from five technical replicate western blots run in parallel toSupplemental Figure 4A were quantified by measuring the integrated density of each band in Image J/Fiji, and normalized to levels of Pgk1. Error bars represent standard error of the mean of five technical replicates. p values were determined via paired t-test. ** = $p < 0.005$).

Supplemental Figure 5

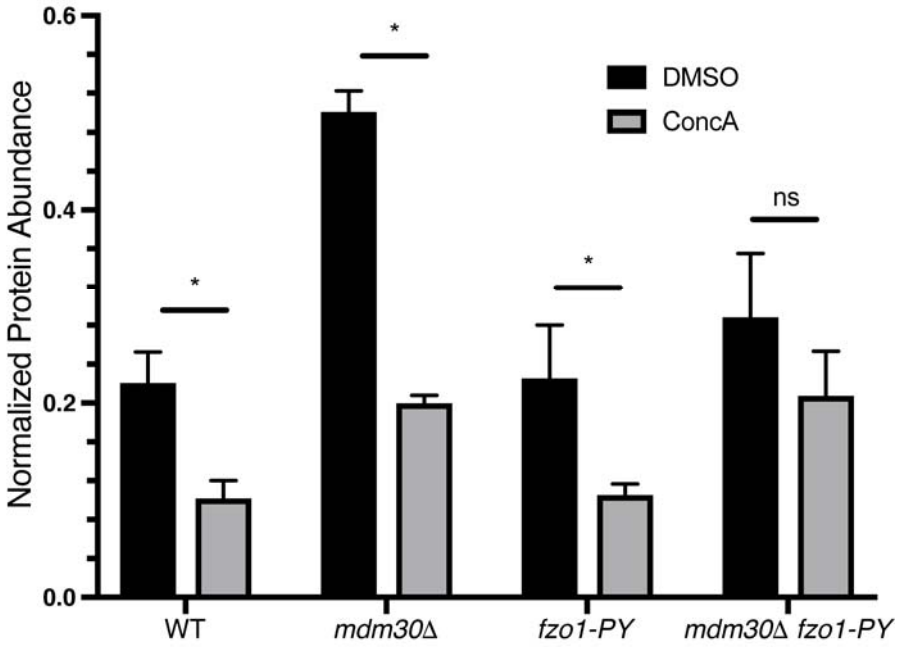


Supplemental Figure 5. *pep4Δ*and *pep4Δprb1Δ*yeast are deficient in vacuole function and still exhibitConcA-mediated Fzo1 decline.

Whole cell extracts from wild-type (WT), *pep4Δ,* and *pep4Δprb1Δ*yeast expressing Fzo1-HAgrown in the absence or presence of concanamycin A for four hourswere analyzed by Western blot with anti-HA, anti-Pgk1, and anti-Prc1 (CPY) antibodies.

Accumulation of the precursor form of Prc1 is an indicator of impaired vacuolar proteolysis.

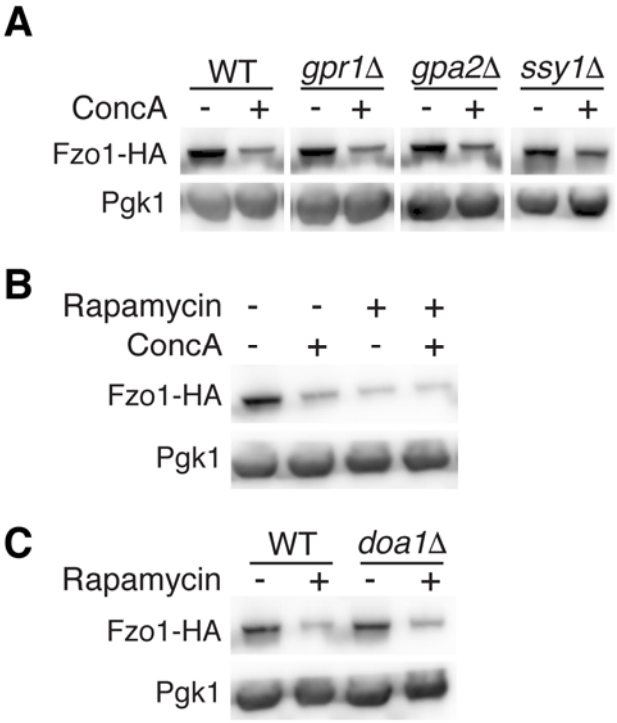
Supplemental Figure 6



Supplemental Figure 6. Western blot quantification of Fzo1-HA levels in *mdm30Δfzo1-PY* mutant yeast.

Fzo1-HA protein levels from three technical replicate western blots run in parallel to Figure 4D were quantified by measuring the integrated density of each band in Image J/Fiji, and normalized to levels of Pgk1. Error bars represent standard error of the mean of three technical replicates. p values were determined via paired t-test. ns = not significant, * = $p < 0.05$).

Supplemental Figure 7



Supplemental Figure 7. Common nutrient sensing pathways are not involved in vacuole-induced Fzo1 decline.

A)Whole cell extracts from wild-type (WT), *gpr1Δ, gpa2Δ,* and *ssy1Δ*yeast expressing Fzo1-HAgrown in the absence or presence of concanamycin A for four hourswere analyzed by Western blot with anti-HA and anti-Pgk1 antibodies.

B) Whole cell extracts from wild-type (WT)yeast expressing Fzo1-HAgrown in the absence or presence of concanamycin A and/or rapamycin for four hourswere analyzed by Western blot with anti-HA and anti-Pgk1 antibodies.

C) Whole cell extracts from wild-type (WT)and *doa1Δ*yeast expressing Fzo1-HAgrown in the absence or presence of rapamycin for four hourswere analyzed by Western blot with anti-HA and anti-Pgk1 antibodies.