Defects Associated with Mitochondrial DNA Damage Can Be Mitigated by Increased Vacuolar pH in *Saccharomyces cerevisiae*

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ABSTRACT While searching for mutations that alleviate detrimental effects of mitochondrial DNA (mtDNA) damage, we found that disrupting vacuolar biogenesis permitted survival of a sensitized yeast background after mitochondrial genome loss. Furthermore, elevating vacuolar pH increases proliferation after mtDNA deletion and reverses the protein import defect of mitochondria lacking DNA.

LOSS of the mitochondrial genome prevents oxidative phosphorylation, yet there are additional biochemical consequences of mtDNA mutation. Damage to mtDNA perturbs the tricarboxylic acid cycle, a major hub for biosynthetic reactions (Liu and Butow 1999). Moreover, mitochondrial dysfunction can result in genomic instability in the nucleus (Veatch *et al.* 2009) or in increased apoptosis (Kujoth *et al.* 2005). Importantly, mtDNA mutation or deletion impairs mitochondrial protein import, an essential process, by lowering the electrochemical potential ($\Delta\Psi^{\text{mito}}$) across the mitochondrial inner membrane (Reid and Schatz 1982; Veatch *et al.* 2009).

Experiments using *Saccharomyces cerevisiae* offer an opportunity to study aspects of mitochondrial dysfunction in a genetically and biochemically tractable organism. Although mitochondria are essential, mtDNA is not required for viability of *S. cerevisiae* on fermentable medium, since sufficient ATP can be made by glycolysis (Chen and Clark-Walker 2000). However, mutation of certain nuclear genes is not compatible with mtDNA loss. Death or extremely slow proliferation after mtDNA loss is called the “petite-negative” phenotype (Bulder 1964).

In the hope of finding ways to relieve detrimental effects of mtDNA damage, we sought spontaneous mutations reverting

the petite-negative phenotype of cells lacking *Mgr1p*, a substrate-binding adaptor for the i-AAA protease (Dunn *et al.* 2006; Dunn *et al.* 2008) (methods for suppressor isolation and identification are found in [Supporting Information, File S1](#)). Mutation of *VPS16* allowed *mgr1Δ* cells to survive EtBr-precipitated mtDNA loss (Figure 1A), with absence of functional mtDNA confirmed by failed proliferation on medium requiring respiration after mating with a ρ^- strain (Figure 1B). *VPS16* deletion also permitted more rapid proliferation of *MGR1* cells after mtDNA loss (Figure 1A). *VPS16* encodes a member of the conserved, vesicle-tethering class C vacuolar protein sorting (C-Vps) complex, which plays a key role in endosomal trafficking (Nickerson *et al.* 2009). Similarly to *vps16Δ*, deletion of other genes encoding C-Vps complex members permitted *mgr1Δ ρ^-* viability (Figure 1C).

The petite-negative phenotypes caused by deletion of mitochondrial import receptor *Tom70p* or of the prohibitin complex, mutations not known to affect the i-AAA protease, could also be suppressed by *vps16Δ* (Figure 1D). Like *mgr1Δ*, the petite-negative phenotypes of *phb1Δ* and *tom70Δ* are dependent upon strain background or medium conditions (Berger and Yaffe 1998; Dunn and Jensen 2003; Dunn *et al.* 2008). However, “stronger” petite-negative mutations whose need for mtDNA appears less dependent on strain background or medium, such as *yme1Δ*, *atp2Δ*, or *aac2Δ*, could not be suppressed by *VPS16* deletion (Figure S1).

The C-Vps complex functions at multiple SNARE-dependent steps during endosomal trafficking, and combined deletion of the SNARE proteins *Pep12* and *Vam3* partially recapitulates the loss of C-Vps (Robinson *et al.* 1988). Therefore, we asked whether *pep12Δ* or *vam3Δ* would permit survival of *mgr1Δ ρ^-*.

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Figure 1 Mutation of the C-Vps complex can suppress the petite-negative phenotype. (A) Deletion of *VPS16* suppresses the petite-negative phenotype of *mgr1Δ*. Strains BY4742 (WT), CDD364 (*mgr1Δ*), CDD244 (*vps16Δ*), and CDD361 (*mgr1Δ vps16Δ*) were assayed for petite-negativity as described in File S1. (B) Viable, EtBr-treated *mgr1Δ vps16Δ* cells lack mtDNA. CDD361 (*mgr1Δ vps16Δ*) cells either untreated or treated for 1 day with EtBr were crossed to a ρ^- isolate of BY4742 transformed with a plasmid conferring leucine prototrophy. After mating on YEPD, cells were struck to SD media lacking uracil. This plate was then replica plated to SMM media lacking leucine to confirm diploidy and to YEPGE media to assay the ability to respire. (C) Deletion of any core C-Vps complex member permits survival of *mgr1Δ* ρ^- cells. Strains CDD364 (*mgr1Δ*), CDD390 (*mgr1Δ pep5Δ*), CDD391 (*mgr1Δ vps33Δ*), and CDD408 (*mgr1Δ pep3Δ*) were tested for a petite-negative phenotype. (D) Mutation of *VPS16* suppresses petite-negative mutations affecting mitochondrial protein import or assembly. Strains CDD348 (*phb1Δ*), CDD347 (*phb1Δ vps16Δ*), CDD338 (*tom70Δ*), and CDD331 (*tom70Δ vps16Δ*) were subjected to a petite-negativity assay as described in File S1; however, CDD338 and CDD331 were incubated for 4 days at 30° after loss of mtDNA. Strains and the oligonucleotides used for their construction are found in Table S1 and Table S2, respectively.

Only deletion of *PEP12* effectively suppressed *mgr1Δ* ρ^- lethality (Figure 2A), concentrating our attention on the consequences of deleting this SNARE protein.

Previously, microscopy-based assays suggested that *pep12Δ* mutants might be defective for vacuolar acidification (Preston *et al.* 1989; Palanisamy *et al.* 2010). In agreement with these reports, we found that *pep12Δ* was sensitive to alkaline YEPD medium (Figure 2B) (Nelson and Nelson 1990; Yamashiro *et al.* 1990) and synthetically lethal with *end3Δ* (no *pep12Δ end3Δ* colonies arising from 22 tetrads of strain CDD477), both

Figure 2 Removal of the t-SNARE protein Pep12p recapitulates the petite-negative suppressive phenotype of *vps16Δ* and causes defective proliferation on alkaline medium. (A) Deletion of *PEP12* allows survival of *mgr1Δ* ρ^- cells. Strains CDD364 (*mgr1Δ*), CDD361 (*mgr1Δ vps16Δ*), CDD362 (*mgr1Δ vam3Δ*), CDD363 (*mgr1Δ pep12Δ*), and CDD365 (*mgr1Δ pep12Δ vam3Δ*) were tested for the petite-negative phenotype as described in File S1. (B) *vps16Δ* and *pep12Δ* cells are sensitive to increased extracellular pH. Strains BY4742 (WT), CDD496 (*vma2Δ*), CDD244 (*vps16Δ*), CDD493 (*pep12Δ*), and CDD494 (*vam3Δ*) were cultured to early log phase in YEPD and then serially diluted and either plated to YEPD at pH 5.9 or YEPD buffered to pH 7.6. Plates were incubated for 2 days at 30°.

known phenotypes of mutations inhibiting vacuolar acidification (Munn and Riezman 1994). Moreover, *VPS16* deletion also caused sensitivity to elevated medium pH (Figure 2B), and the control mutant *vma2Δ*, as expected, did not proliferate in rich medium buffered to pH 7.6 (Nelson and Nelson 1990; Yamashiro *et al.* 1990).

Consequently, we focused upon vacuolar pH and its impact on ρ^- cells. *Vma2p* plays a structural role (Liu *et al.* 1996) and *Vma13p* plays a regulatory role in V_1V_O -ATPase assembly and function (Ho *et al.* 1993). Deletion of either protein successfully suppressed the inviability of *mgr1Δ* ρ^- (Figure 3A). Previous studies suggested that alkaline medium conditions can raise vacuolar pH (Nelson and Nelson 1990; Klionsky *et al.* 1992; Plant *et al.* 1999; Padilla-Lopez and Pearce 2006; Brett *et al.* 2011). Indeed, culture in medium buffered to pH 7.6 also suppressed the petite-negative phenotype of *mgr1Δ* ρ^- (Figure 3B). The division time of ρ^- cells not containing a petite-negative mutation was also reduced by mutation of the V_1V_O -ATPase (Figure 3C and Table S3A) or by culture in alkaline medium (Figure 3D and Table S3B). Finally, treatment with the V_1V_O -ATPase inhibitor concanamycin A, while initially inhibiting cell division in the first 10 hr of treatment (Table S3D), reduced the doubling time of ρ^- cells after adaptation to the drug (Figure 3E and Table S3C). The beneficial effects on ρ^- cells provided by raising vacuolar pH or V_1V_O -ATPase mutation may be specific to the genetic background tested; ρ^- cells from the W303 background do not appear to benefit from raised vacuolar pH (Table S3E and Table S3F).

Mitochondria lacking mtDNA exhibit a very low $\Delta\Psi_{\text{mito}}$, which is dependent upon exchange of cytosolic ATP^{4-} for

Figure 3 Increasing vacuolar pH by mutation or by medium conditions suppresses the petite-negative phenotype of *mgr1Δ* and raises the proliferation rate of otherwise WT ρ^- cells. (A) Abrogation of proton pumping by the V_1V_0 -ATPase permits *mgr1Δ* ρ^- viability. Strains CDD364 (*mgr1Δ*), CDD427 (*mgr1Δ vma2Δ*), and CDD440 (*mgr1Δ vma13Δ*) were tested for the petite-negative phenotype as described in File S1. (B) Alkaline extracellular pH rescues the *mgr1Δ* petite-negative phenotype. Strain CDD364 (*mgr1Δ*) was adapted overnight in either YEpd at pH 5.9 or YEpd buffered to pH 7.6. Cells were then, in the same buffer, cultured overnight in EtBr to logarithmic phase, serially diluted as for the standard petite-negativity test, plated on the indicated medium, and incubated at 30° for 3 days. (C) Disruption of the V_1V_0 -ATPase raises the proliferation rate of ρ^- cells. Strains BY4742 (WT) and CDD496 (*vma2Δ*) were either patched to YEpd + EtBr for 2 days to force mtDNA loss or left untreated. Densities of log-phase cultures were then followed at 600 nm and doubling times were determined (***, $P < 0.0005$ by two-tailed Student's *t*-test, unequal variance; $n = 3$; error bars relate standard deviation). Detailed information about doubling time calculations can be found in File S1. (D) Higher extracellular pH raises the division rate of ρ^- cells relative to ρ^+ cells. ρ^+ and ρ^- isolates of BY4742 (WT) were cultured overnight in YEpd at pH 5.9 or in YEpd buffered to pH 7.6. The next day, proliferation in the same medium was monitored as in C (*, $P < 0.05$ by two-tailed Student's *t*-test, unequal variance; $n = 3$; error bars relate standard deviation). (E) The V_1V_0 -ATPase antagonist concanamycin A provides a late-acting, beneficial effect upon ρ^- cells. BY4742 (WT) cells lacking mtDNA were cultured in YEpd containing 2 μ M concanamycin A or DMSO vehicle. After 10 hr, culture density was monitored as in C (*, $P < 0.05$ by two-tailed Student's *t*-test, unequal variance; $n = 4$; error bars relate standard deviation).

F_1 -ATPase-produced ADP^{3-} across the inner membrane (Dupont *et al.* 1985; Giraud and Velours 1997; Jazayeri

et al. 2003). Because $\Delta\Psi^{mito}$ is low, ρ^- mitochondria cannot efficiently import proteins from the cytosol (Schleyer *et al.* 1982; Veatch *et al.* 2009; Dasari and Kolling 2011). Fluorescent proteins linked to the mitochondria-targeting presequence of Cox4p (Sesaki and Jensen 1999) can serve as *in vivo* reporters of protein import (Veatch *et al.* 2009), and as expected, Cox4(1–21)–GFP accumulates in the cytosol in ρ^- cells (Figure 4, A and B). However, the *ATP3-5* allele, thought to raise $\Delta\Psi^{mito}$ in ρ^- cells (Kominsky *et al.* 2002; Dasari and Kolling 2011), caused relocation of Cox4(1–21)–GFP to ρ^- mitochondria (Figure 4, A and B). Deletion of V_1V_0 -ATPase components *Vma2p* or *Vma13p* also relocated Cox4(1–21)–GFP to ρ^- mitochondria (Figure 4, C and D), suggesting increased $\Delta\Psi^{mito}$ in ρ^- cells when vacuolar pH is raised. We note that the use of cationic dyes to assess $\Delta\Psi^{mito}$ when vacuolar pH is altered is contra-indicated by resultant effects on cellular dye uptake and distribution (Koning *et al.* 1993; Rottenberg and Wu 1998; Feldkamp *et al.* 2005; Martinez-Munoz and Kane 2008).

If $\Delta\Psi^{mito}$ is increased when vacuolar pH changes, then by what mechanism? We envision two nonexclusive scenarios by which vacuolar pH might impinge upon $\Delta\Psi^{mito}$. First, ATP hydrolysis in the mitochondrial matrix drives $\Delta\Psi^{mito}$ generation in ρ^- mitochondria (Chen and Clark-Walker 2000; Kominsky *et al.* 2002; Clark-Walker 2003), so transcriptional or post-transcriptional changes triggered by raised vacuolar pH may increase ATP hydrolysis in the matrix. We are currently investigating this possibility.

Second, changes in ion distribution or quantity resulting from higher vacuolar pH (Eide *et al.* 2005) could impinge upon the electrogenic circuit that is required for $\Delta\Psi^{mito}$ generation by ρ^- cells, resulting in a more negatively charged mitochondrial matrix in comparison to the cytosol. A recent proteomic survey of purified vacuolar membranes identified a comprehensive list of nearly 150 proteins, including numerous ion transporters (Wiederhold *et al.* 2009). We compared this list of vacuolar proteins with the responses of more than 3700 deletion mutants to loss of the mitochondrial genome (Dunn *et al.* 2006). Yet, for mutants for which data are available, deletion of no individual vacuolar transporter corresponded with a significant increase in the relative division rate of ρ^- cells compared to ρ^+ cells.

We reasoned that inorganic polyphosphate, a highly charged species localized to the vacuole (Urech *et al.* 1978), might play a role in determining $\Delta\Psi^{mito}$ in ρ^- cells. Indeed, inorganic polyphosphate depends upon a functional V_1V_0 -ATPase for synthesis (Freimoser *et al.* 2006). However, deletion of *PHM4*, required for vacuolar polyphosphate synthesis (Ogawa *et al.* 2000), did not rescue *mgr1Δ* ρ^- viability (Figure S2A). We anticipate that unbiased genetic screens might uncover specific vacuolar transporters or other proteins relevant to vacuolar pH effects on ρ^- mitochondria.

In the course of our work, we tested other potential consequences of *vps16Δ* deletion, in addition to increased vacuolar pH, that might influence the proliferation of ρ^- cells. These experiments provided no evidence that blocked

Figure 4 Disruption of the V_1V_O -ATPase increases mitochondrial protein import in cells lacking mtDNA. (A) A mutation raising the electrochemical potential of ρ^- mitochondria increases protein import. Strain CDD472 (WT) containing pHS1 [Cox4 (1–21)-GFP] (Sesaki and Jensen 1999) and lacking functional mtDNA was transformed with pM487 (ATP3) or pM488 (ATP3-5) (Weber *et al.* 1995; Dunn *et al.* 2006). Mitochondrial precursor localization was scored for >200 cells per strain as outlined in File S1. (B) Representative images from A. (C) Mutations blocking vacuolar acidification increase the import of substrate into ρ^- mitochondria. ρ^- isolates of BY4742 (WT) or CDD496 ($vma2\Delta$) carrying pHS1 were scored as in A. (D) Representative images from C. Bar, 5 μ m.

vacuolar luminal proteolysis (Figure S2B), reduced autophagy (Figure S2C), perturbed vacuolar iron transport (Figure S2D), or reduced TOR pathway activity (Figure S2E) are key mechanisms by which the fitness of ρ^- cells is improved by impeding vacuolar biogenesis. Moreover, although deletion of C-Vps complex member *Vps33p* results in fragmented mitochondria (Wang and Deschenes 2006), blocking mitochondrial fusion by *FZO1* deletion (Hermann *et al.* 1998; Rapaport *et al.* 1998) did not permit survival of *mgr1Δ* ρ^- cells generated by dissection of 21 tetrads from *mgr1Δ/MGR1 fzo1Δ/FZO1* strain CDD379. Finally, *end3Δ* ρ^- cells inefficiently imported substrate into mitochondria, suggesting that reduced endocytosis after raising vacuolar pH (Perzov *et al.* 2002) is not the primary mechanism by which vacuolar pH influences ρ^- mitochondria (Figure S3).

Numerous mutations have been shown to both extend replicative lifespan (RLS) and suppress the petite-negative phenotype, prompting us to speculate that mtDNA damage might limit RLS in yeast and that most or all mutations suppressing the petite-negative phenotype might forestall replicative senescence (Dunn 2011). However, a recent report demonstrates that V_1V_O -ATPase mutation leads to lower RLS, and, conversely, higher vacuolar acidity leads to extended RLS and a higher $\Delta\Psi^{\text{mito}}$ in aged, presumably ρ^+ cells (Hughes and Gottschling 2012). Therefore, it seems unlikely that mtDNA loss is the proximal event causing replicative

senescence in yeast. Moreover, the effects of increased vacuolar pH on ρ^+ and ρ^- cells clearly differ drastically, perhaps as a result of the divergent mechanisms by which $\Delta\Psi^{\text{mito}}$ is generated in these two cell types.

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Defects Associated with Mitochondrial DNA Damage Can Be Mitigated by Increased Vacuolar pH in *Saccharomyces cerevisiae*

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Figure S1 Deletion of *VPS16* does not suppress the lethality of all petite-negative mutants after mtDNA loss. (A) *vps16Δ* does not suppress the petite-negative phenotype of *yme1Δ*. Strains CDD329 (*mgr1Δ*), CDD328 (*mgr1Δ vps16Δ*), CDD344 (*yme1Δ*), and CDD343 (*yme1Δ vps16Δ*) were subjected to a petite-negativity test, except that strains were cultured at 34°C, the plate containing ρ^+ isolates was incubated for 3 d, and the plate containing ρ^- isolates was incubated for 4 d. (B) Cells lacking the F_1 -ATPase remain petite-negative in the absence of *VPS16*. Strains CDD329 (*mgr1Δ*), CDD328 (*mgr1Δ vps16Δ*), CDD218 (*atp2Δ*), and CDD219 (*atp2Δ vps16Δ*) were tested for a petite-negative phenotype as in (A), except cells were cultured at 30°C. (C) Mutation of *VSP16* does not suppress the petite-negative phenotype of *aac2Δ* cells. Strains CDD329 (*mgr1Δ*), CDD328 (*mgr1Δ vps16Δ*), CDD407 (*aac2Δ*), and CDD406 (*aac2Δ vps16Δ*) were tested for the petite-negative phenotype. The plate containing ρ^+ cells was incubated at 30°C for 2 d, and the plate containing ρ^- cells was incubated for 4 d.

Figure S2 The effects of vacuolar pH on mitochondrial biogenesis is not through impaired inorganic polyphosphate accumulation, vacuolar proteolysis, autophagy, iron transport, or TORC1 signaling. (A) Removal of vacuolar inorganic polyphosphate does not permit *mgr1Δ* p^- viability. Strains CDD364 (*mgr1Δ*), CDD361 (*mgr1Δ vps16Δ*), and CDD505 (*mgr1Δ phm4Δ*) were tested for a petite-negative phenotype. (B) Severely reducing vacuolar proteolysis does not rescue *mgr1Δ* p^- inviability. Strains CDD329 (*mgr1Δ*), CDD328 (*mgr1Δ vps16Δ*), and CDD327 (*mgr1Δ pep4Δ*) were assayed for petite-negativity. (C) Blocking autophagy does not rescue the petite-negative phenotype of *mgr1Δ* p^- . Strains CDD329 (*mgr1Δ*), CDD328 (*mgr1Δ vps16Δ*), and CDD326 (*mgr1Δ atg1Δ*) were tested for the petite-negative phenotype. (D) Disruption of a putative iron transporter has no effect on the petite-negative phenotype. Strains CDD329 (*mgr1Δ*), CDD388 (*mgr1Δ vps16Δ ccc1Δ*), and CDD389 (*mgr1Δ ccc1Δ*) were tested for viability following mtDNA loss. (E) Rescue of the petite-negative phenotype by *vps16Δ* is not correlated with reduced TORC1 signaling, as defined by rapamycin sensitivity. Strains CDD364 (*mgr1Δ*), CDD361 (*mgr1Δ vps16Δ*), CDD362 (*mgr1Δ vam3Δ*), CDD363 (*mgr1Δ pep12Δ*), and CDD427 (*mgr1Δ vma2Δ*) were cultured in YEPD to log phase at 30°C, and serial dilutions were spotted to either YEPD or YEPD containing 20nM rapamycin. Plates were incubated for 2 d (no rapamycin) or 5 d (20nM rapamycin) at 30°C.

Figure S3 Defective endocytosis does not increase mitochondrial protein import of ρ^- cells. ρ^- isolates of BY4742 (WT), CDD495 ($end3\Delta$), and CDD496 ($vma2\Delta$) were assayed as in Figure 4.

Table S1 - Strain genotypes and construction.

Strain	Genotype	Source	Parental strain(s)	Method Used
BMA64-1A	<i>MATa ura3-1 trp1Δ2 ade2-1 leu2-3,112 his3-11,15 can1-100</i>	Baudin-Baillieu et al. (1997)		
BMA64-1B	<i>MATa ura3-1 trp1Δ2 ade2-1 leu2-3,112 his3-11,15 can1-100</i>	Baudin-Baillieu et al. (1997)		
BY4741	<i>MATa leu2Δ0 met15Δ0 ura3Δ0 his3Δ1</i>	Brachman et al. (1998)		
BY4742	<i>MATa leu2Δ0 lys2Δ0 ura3Δ0 his3Δ1</i>	Brachman et al. (1998)		
RJ1854	<i>MATa/MATa met15Δ0/met15Δ0 his3Δ200/his3Δ200 leu2Δ0/leu2Δ0 trp1Δ63/trp1Δ63 ura3Δ0/ura3Δ0 atp2Δ::LEU2/ATP2 ydl047wΔ::HIS3/YDL047W</i>	Dunn et al. (2008)		
RJ2058	<i>MATa leu2Δ0 lys2Δ0 ura3Δ0 his3Δ1 mgr1Δ::kanMX4</i>	Dunn et al. (2006)		
RJ2059	<i>MATa leu2Δ0 lys2Δ0 ura3Δ0 his3Δ1 mgr1Δ::kanMX4</i>	Dunn et al. (2006)		
RJ2093	<i>MATa met15Δ0 his3Δ200 leu2Δ0 trp1Δ63 ura3Δ0 atp2Δ::LEU2</i>	This study	RJ1854	sporulation
RJ2204	<i>MATa/MATa his3Δ1/his3Δ1 leu2Δ0/leu2Δ0 lys2Δ0/LYS2 met15Δ0/MET15 ura3Δ0/ura3Δ0 mgr1Δ::kanMX4/mgr1Δ::kanMX4 sup1/SUP1</i>	This study	RJ2058, RJ2059	treated RJ2058 with EtBr, and crossed survivor colony containing the <i>smp1</i> allele to RJ2059
RJ2221	<i>MATa leu2Δ0 ura3Δ0 his3Δ1 mgr1Δ::kanMX4 sup1</i>	This study	RJ2204	sporulation
RJ2272	<i>MATa leu2Δ0 lys2Δ0 ura3Δ0 his3Δ1 vps16Δ::kanMX4</i>	Giaever et al. (2002)		
RJ2282	<i>MATa/MATa his3Δ1/his3Δ1 leu2Δ0/leu2Δ0 lys2Δ0/LYS2 met15Δ0/MET15 ura3Δ0/ura3Δ0 mgr1Δ::kanMX4/MGR1 vps16Δ::kanMX4/VPS16</i>	This study	RJ2058, RJ2272	cross RJ2058 to RJ2272
RJ2283	<i>MATa leu2Δ0 met15Δ0 ura3Δ0 his3Δ1 vps16Δ::kanMX4</i>	This study	RJ2282	sporulation
RJ2288	<i>MATa/MATa leu2Δ0/leu2Δ0 his3Δ200/his3Δ1 met15Δ0/met15Δ0 trp1Δ63/TRP1 ura3Δ0/ura3Δ0 atp2Δ::LEU2/ATP2 vps16Δ::kanMX4/VPS16</i>	This study	RJ2093, RJ2283	cross RJ2093 to RJ2283
CDD8	<i>MATa/MATa leu2Δ0/leu2Δ0 lys2Δ0/LYS2 met15Δ0/MET15 ura3Δ0/ura3Δ0 his3Δ1/his3Δ1 yme1Δ::kanMX4/YME1</i>	EUROSCARF Y27144		
CDD14	<i>MATa/MATa leu2Δ0/leu2Δ0 lys2Δ0/LYS2 met15Δ0/MET15 ura3Δ0/ura3Δ0 his3Δ1/his3Δ1 mgr1Δ::kanMX4/MGR1</i>	EUROSCARF Y23451		
CDD18	<i>MATa/MATa leu2Δ0/leu2Δ0 lys2Δ0/LYS2 met15Δ0/MET15 ura3Δ0/ura3Δ0 his3Δ1/his3Δ1 phb1Δ::kanMX4/PHB1</i>	EUROSCARF Y24762		
CDD24	<i>MATa/MATa leu2Δ0/leu2Δ0 lys2Δ0/LYS2 met15Δ0/MET15 ura3Δ0/ura3Δ0 his3Δ1/his3Δ1 tom70Δ::kanMX4/TOM70</i>	EUROSCARF Y27244		
CDD51	<i>MATa ura3-1 trp1Δ2 ade2-1 leu2-3,112 his3-11,15 can1-100 cyh2</i>	This study	BMA64-1B	selection on 10 mg/L cycloheximide
CDD53	<i>MATa/a ura3-1/ura3-1 trp1Δ2/trp1Δ2 ade2-1/ade2-1 leu2-3,112/leu2-3,112 his3-11,15/his3-11,15 can1-100/can1-100 cyh2/CYH2</i>	This study	CDD51, BMA64-1A	cross CDD51 to BMA64-1A
CDD58	<i>MATa/a ura3-1/ura3-1 trp1Δ2/trp1Δ2 ade2-1/ade2-1 leu2-3,112/leu2-3,112 his3-11,15/his3-11,15 can1-100/can1-100 cyh2/CYH2 aac2Δ::URA3/AAC2</i>	This study	CDD53	replace <i>AAC2</i> with <i>URA3</i> using primers 32/33 and pRS306 template
CDD59	<i>MATa/a ura3-1/ura3-1 trp1Δ2/trp1Δ2 ade2-1/ade2-1 leu2-3,112/leu2-3,112 his3-11,15/his3-11,15 can1-100/can1-100 cyh2/CYH2 aac2Δ::URA3/AAC2 ybr179cΔ::LEU2/YBR179C</i>	This study	CDD58	replace <i>YBR179C</i> with <i>LEU2</i> using primers 30/31 and pRS305 template
CDD63	<i>MATa/a ura3-1/ura3-1 trp1Δ2/trp1Δ2 ade2-1/ade2-1 leu2-3,112/leu2-3,112 his3-11,15/his3-11,15 can1-100/can1-100 cyh2/CYH2 aac2Δ::URA3/AAC2 ybr179cΔ::LEU2/YBR179C pTRP1-CYH2-YBR179C</i>	This study	CDD59	transformation of CDD59 with pTRP1-CYH2-YBR179C
CDD68	<i>MATa ura3-1 trp1Δ2 ade2-1 leu2-3,112 his3-11,15 can1-100 aac2Δ::URA3</i>	This study	CDD63	sporulation
CDD214	<i>MATa leu2Δ0 his3Δ met15Δ0 ura3Δ0 vps16Δ::kanMX4</i>	This study	RJ2288	sporulation
CDD218	<i>MATa leu2Δ0 his3Δ met15Δ0 trp1Δ63 ura3Δ0 atp2Δ::LEU2</i>	This study	RJ2288	sporulation
CDD219	<i>MATa leu2Δ0 his3Δ met15Δ0 ura3Δ0 atp2Δ::LEU2 vps16Δ::kanMX4</i>	This study	RJ2288	sporulation

CDD233	<i>MATa/MATa leu2Δ0/leu2Δ0 lys2Δ0/LYS2 met15Δ0/MET15 ura3Δ0/ura3Δ0 his3Δ1/his3Δ1 ydl134cΔ::kanMX4/YDL134C</i>	EUROSCARF Y23831		
CDD244	<i>MATa leu2Δ0 lys2Δ0 ura3Δ0 his3Δ1 vps16Δ::kanMX4</i>	EUROSCARF Y12783		
CDD246	<i>MATa/MATa leu2Δ0/leu2Δ0 lys2Δ0/LYS2 met15Δ0/MET15 ura3Δ0/ura3Δ0 his3Δ1/his3Δ1 ydl134cΔ::URA3/YDL134C</i>	This study	CDD233	replacement of <i>kanMX4</i> marker with <i>URA3</i> using primers 115/116 and pRS306 template
CDD250	<i>MATa met15Δ0 his3Δ200 leu2Δ0 trp1Δ63 ura3Δ0 ydl047wΔ::HIS3</i>	This study	RJ1854	sporulation
CDD258	<i>MATa leu2Δ0 lys2Δ0 ura3Δ0 his3Δ1 vps33Δ::kanMX4</i>	EUROSCARF Y15305		
CDD260	<i>MATa leu2Δ0 lys2Δ0 ura3Δ0 his3Δ1 pep3Δ::kanMX4</i>	EUROSCARF Y14105		
CDD264	<i>MATa leu2Δ0 lys2Δ0 ura3Δ0 his3Δ1 ydl188cΔ::kanMX4</i>	EUROSCARF Y13886		
CDD307	<i>MATa/MATa leu2Δ0/leu2Δ0 lys2Δ0/LYS2 met15Δ0/MET15 ura3Δ0/ura3Δ0 his3Δ1/his3Δ1 ydl134cΔ::URA3::YDL134C ydl188cΔ::kanMX4/YDL188C</i>	This study	CDD246, CDD264	cross CDD246 to CDD264
CDD309	<i>MATa/MATa leu2Δ0/leu2Δ0 lys2Δ0/LYS2 met15Δ0/MET15 ura3Δ0/ura3Δ0 his3Δ1/his3Δ1 mgr1Δ::kanMX4/MGR1 atg1Δ::URA3/ATG1</i>	This study	CDD14	replace <i>ATG1</i> with <i>URA3</i> using primers 164/165 and pRS306 template
CDD310	<i>MATa/MATa leu2Δ0/leu2Δ0 lys2Δ0/LYS2 met15Δ0/MET15 ura3Δ0/ura3Δ0 his3Δ1/his3Δ1 mgr1Δ::kanMX4/MGR1 pep4Δ::URA3/PEP4</i>	This study	CDD14	replace <i>PEP4</i> with <i>URA3</i> using primers 158/159 and pRS306 template
CDD311	<i>MATa/MATa leu2Δ0/leu2Δ0 lys2Δ0/LYS2 met15Δ0/MET15 ura3Δ0/ura3Δ0 his3Δ1/his3Δ1 yme1Δ::kanMX4/YME1 vps16Δ::URA3/VPS16</i>	This study	CDD8	replace <i>VPS16</i> with <i>URA3</i> using primers 179/180 and pRS306 template
CDD313	<i>MATa/MATa leu2Δ0/leu2Δ0 lys2Δ0/LYS2 met15Δ0/MET15 ura3Δ0/ura3Δ0 his3Δ1/his3Δ1 mgr1Δ::kanMX4/MGR1 vps16Δ::URA3/VPS16</i>	This study	CDD14	replace <i>VPS16</i> with <i>URA3</i> using primers 179/180 and pRS306 template
CDD314	<i>MATa/MATa leu2Δ0/leu2Δ0 lys2Δ0/LYS2 met15Δ0/MET15 ura3Δ0/ura3Δ0 his3Δ1/his3Δ1 phb1Δ::kanMX4/PHB1 vps16Δ::URA3/VPS16</i>	This study	CDD18	replace <i>VPS16</i> with <i>URA3</i> using primers 179/180 and pRS306 template
CDD315	<i>MATa/MATa leu2Δ0/leu2Δ0 lys2Δ0/LYS2 met15Δ0/MET15 ura3Δ0/ura3Δ0 his3Δ1/his3Δ1 tom70Δ::kanMX4/TOM70 vps16Δ::URA3/VPS16</i>	This study	CDD24	replace <i>VPS16</i> with <i>URA3</i> using primers 179/180 and pRS306 template
CDD324	<i>MATa/MATa met15Δ0/MET15 his3Δ200/his3-11,15 leu2Δ0/leu2-3,112 trp1Δ63/trp1Δ2 ura3Δ0/ura3-1 ade2-1/ADE2 can1-100/CAN1 ydl047wΔ::HIS3/YDL047W aac2Δ::URA3/AAC2</i>	This study	CDD250, CDD68	cross CDD250 to CDD68
CDD326	<i>MATa leu2Δ0 ura3Δ0 his3Δ1 mgr1Δ::kanMX4 atg1Δ::URA3</i>	This study	CDD309	sporulation
CDD327	<i>MATa leu2Δ0 ura3Δ0 his3Δ1 mgr1Δ::kanMX4 pep4Δ::URA3</i>	This study	CDD310	sporulation
CDD328	<i>MATa leu2Δ0 ura3Δ0 his3Δ1 mgr1Δ::kanMX4 vps16Δ::URA3</i>	This study	CDD313	sporulation
CDD329	<i>MATa leu2Δ0 ura3Δ0 his3Δ1 mgr1Δ::kanMX4</i>	This study	CDD313	sporulation
CDD331	<i>MATa leu2Δ0 ura3Δ0 his3Δ1 tom70Δ::kanMX4 vps16Δ::URA3</i>	This study	CDD315	sporulation
CDD338	<i>MATa leu2Δ0 ura3Δ0 his3Δ1 tom70Δ::kanMX4</i>	This study	CDD315	sporulation
CDD339	<i>MATa/MATa leu2Δ0/leu2Δ0 lys2Δ0/LYS2 met15Δ0/MET15 ura3Δ0/ura3Δ0 his3Δ1/his3Δ1 mgr1Δ::kanMX4/MGR1 vps16Δ::URA3/VPS16 end3Δ::LEU2/END3</i>	This study	CDD313	replace <i>END3</i> with <i>LEU2</i> using primers 161/162 and pRS305 template
CDD343	<i>MATa leu2Δ0 ura3Δ0 his3Δ1 yme1Δ::kanMX4 vps16Δ::URA3</i>	This study	CDD311	sporulation
CDD344	<i>MATa leu2Δ0 ura3Δ0 his3Δ1 yme1Δ::kanMX4</i>	This study	CDD311	sporulation
CDD347	<i>MATa leu2Δ0 ura3Δ0 his3Δ1 phb1Δ::kanMX4 vps16Δ::URA3</i>	This study	CDD314	sporulation
CDD348	<i>MATa leu2Δ0 ura3Δ0 his3Δ1 phb1Δ::kanMX4</i>	This study	CDD314	sporulation
CDD350	<i>MATa/MATa leu2Δ0/leu2Δ0 lys2Δ0/LYS2 met15Δ0/MET15 ura3Δ0/ura3Δ0 his3Δ1/his3Δ1 mgr1Δ::kanMX4/MGR1 vam3Δ::LEU2/VAM3 pep12Δ::URA3/PEP12</i>	This study	CDD14	replace <i>VAM3</i> with <i>LEU2</i> using primers 152/153 and pRS305 template, then replace <i>PEP12</i> with <i>URA3</i> using primers 155/156 and pRS306 template
CDD358	<i>MATa leu2Δ0 met15Δ0 ura3Δ0 his3Δ1 pep5Δ::kanMX4</i>	EUROSCARF Y00817		
CDD361	<i>MATa leu2Δ0 lys2Δ0 ura3Δ0 his3Δ1 mgr1Δ::kanMX4 vps16Δ::URA3</i>	This study	CDD339	sporulation

CDD362	<i>MATa leu2Δ0 lys2Δ0 ura3Δ0 his3Δ1 mgr1Δ::kanMX4 vam3Δ::LEU2</i>	This study	CDD350	sporulation
CDD363	<i>MATa leu2Δ0 lys2Δ0 ura3Δ0 his3Δ1 mgr1Δ::kanMX4 pep12Δ::URA3</i>	This study	CDD350	sporulation
CDD364	<i>MATa leu2Δ0 lys2Δ0 ura3Δ0 his3Δ1 mgr1Δ::kanMX4</i>	This study	CDD339	sporulation
CDD365	<i>MATa leu2Δ0 lys2Δ0 ura3Δ0 his3Δ1 mgr1Δ::kanMX4 vam3Δ::LEU2 pep12Δ::URA3</i>	This study	CDD350	sporulation
CDD379	<i>MATa/MATa leu2Δ0/leu2Δ0 lys2Δ0/LYS2 met15Δ0/MET15 ura3Δ0/ura3Δ0 his3Δ1/his3Δ1 mgr1Δ::kanMX4/MGR1 tzo1Δ::URA3/FZO1</i>	This study	CDD14	replace <i>FZO1</i> with <i>URA3</i> using primers 30/31 and pRS306 template
CDD388	<i>MATa leu2Δ0 lys2Δ0 ura3Δ0 his3Δ1 mgr1Δ::kanMX4 vps16Δ::URA3 ccc1Δ::LEU2</i>	This study	CDD313	replace <i>CCC1</i> with <i>LEU2</i> using primers 235/236 and pRS305 template, then sporulation
CDD389	<i>MATa leu2Δ0 lys2Δ0 ura3Δ0 his3Δ1 mgr1Δ::kanMX4 ccc1Δ::LEU2</i>	This study	CDD313	replace <i>CCC1</i> with <i>LEU2</i> using primers 235/236 and pRS305 template, then sporulation
CDD390	<i>MATa leu2Δ0 ura3Δ0 his3Δ1 mgr1Δ::URA3 pep5Δ::kanMX4</i>	This study	CDD358 and BY4742	cross CDD358 to BY4742, replace <i>MGR1</i> with <i>URA3</i> using primers 184/185 and pRS306, then sporulation
CDD391	<i>MATa leu2Δ0 ura3Δ0 his3Δ1 mgr1Δ::URA3 vps33Δ::kanMX4</i>	This study	CDD258 and BY4741	cross CDD258 to BY4741, replace <i>MGR1</i> with <i>URA3</i> using primers 184/185 and pRS306, then sporulation
CDD406	<i>MATa his3 leu2 ura3 aac2Δ::URA3 vps16Δ::kanMX4</i>	This study	CDD324, CDD214, BY4742	sporulate CDD324, cross Ura+ His- spore to CDD214, sporulate, cross Ura+ G418+ spore to BY4742, sporulate
CDD407	<i>MATa his3 leu2 ura3 aac2Δ::URA3</i>	This study	CDD324, CDD214, BY4742	sporulate CDD324, cross Ura+ His- spore to CDD214, sporulate, cross Ura+ G418+ spore to BY4742, sporulate
CDD408	<i>MATa leu2Δ0 lys2Δ0 ura3Δ0 his3Δ1 mgr1Δ::URA3 pep3Δ::kanMX4</i>	This study	CDD260	replace <i>MGR1</i> with <i>URA3</i> using primers 184/185 and pRS306
CDD427	<i>MATa leu2Δ0 ura3Δ0 his3Δ1 mgr1Δ::kanMX4 vma2Δ::URA3</i>	This study	CDD14	replace <i>VMA2</i> with <i>URA3</i> using primers 268/269 and pRS306, then sporulation
CDD440	<i>MATa leu2Δ0 lys2Δ0 ura3Δ0 his3Δ1 mgr1Δ::kanMX4 vma13Δ::URA3</i>	This study	CDD14	replace <i>VMA13</i> with <i>URA3</i> using primers 271/272 and pRS306, then sporulation
CDD472	<i>MATa leu2Δ0 met15Δ0 lys2Δ0 ura3Δ0 his3Δ1</i>	This study	CDD307	replace <i>YHR205W</i> with <i>LEU2</i> using primers 263/264 and pRS305, then sporulation
CDD477	<i>MATa/MATa leu2Δ0/leu2Δ0 lys2Δ0/LYS2 met15Δ0/MET15 ura3Δ0/ura3Δ0 his3Δ1/his3Δ1 mgr1Δ::kanMX4/MGR1 vam3Δ::LEU2/VAM3 pep12Δ::URA3/PEP12 end3Δ::HIS3/END3</i>	This study	CDD350	replace <i>END3</i> with <i>HIS3</i> using primers 161/162 and pRS303 template
CDD493	<i>MATa leu2Δ0 lys2Δ0 ura3Δ0 his3Δ1 pep12Δ::kanMX4</i>	EUROSCARF Y11812		
CDD494	<i>MATa leu2Δ0 lys2Δ0 ura3Δ0 his3Δ1 vam3Δ::kanMX4</i>	EUROSCARF Y12362		
CDD495	<i>MATa leu2Δ0 lys2Δ0 ura3Δ0 his3Δ1 end3Δ::kanMX4</i>	EUROSCARF Y12992		
CDD496	<i>MATa leu2Δ0 lys2Δ0 ura3Δ0 his3Δ1 vma2Δ::kanMX4</i>	EUROSCARF Y13266		
CDD505	<i>MATa leu2Δ0 lys2Δ0 ura3Δ0 his3Δ1 mgr1Δ::kanMX4 phm4Δ::URA3</i>	This study	CDD364	replace <i>PHM4</i> with <i>URA3</i> using primers 353/354 and pRS306 template
CDD552	<i>MATa ura3-1 trp1Δ2 ade2-1 leu2-3,112 his3-11,15 can1-100 vma2Δ::URA3</i>	This study	BMA64-1B	replace <i>VMA2</i> with <i>URA3</i> using primers 268/269 and pRS306
CDD553	<i>MATa ura3-1 trp1Δ2 ade2-1 leu2-3,112 his3-11,15 can1-100 vma2Δ::URA3</i>	This study	BMA64-1B	replace <i>VMA2</i> with <i>URA3</i> using primers 268/269 and pRS306

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Table S2 - Oligonucleotides used in this study.

Number	Sequence
30	ATGTCTGAAGGAAAACAACAATTCAAAGACAGCAATAAACAGATTGTACTGAGAGTGCAC
31	CTAATCGATGTCTAAATTTATTTCTTCCACCATCAATTTTCTGTGCGGTATTTACACCG
32	ATGTCTTCCAACGCCCAAGTCAAACCCCATTACCTCCAGAGATTGTACTGAGAGTGCAC
33	TTATTTGAACTTCTTACCAAACAAGATCATTGTCAGTTGGCTGTGCGGTATTTACACCG
115	GGATATAAATTATCGCATAAAACAATAAACAAAAAGAAAAGATTGTACTGAGAGTGCAC
116	GTGAATATATATCTATATAGATGCATATATGTATACATACCTGTGCGGTATTTACACCG
152	CAAATTGGCCAACTAATATCCACTGCAGAAAGTTGAGATTAGATTGTACTGAGAGTGCAC
153	TACCAGAAAGTCTGTGCTCAATGCGCGTTTAAGGAGATTACTGTGCGGTATTTACACCG
155	AGAAGAATATAACGTAAATTACTACAATAATTGTGTTGAGAGATTGTACTGAGAGTGCAC
156	TATTATGTATATGATATTTGACGACGTGTGTTGGTTGGCTGTGCGGTATTTACACCG
158	ATTTAATCCAAATAAAATTCAAACAAAAACAAAACCTAACAGATTGTACTGAGAGTGCAC
159	GGCAGAAAAGGATAGGGCGGAGAAGTAAGAAAAGTTAGCCTGTGCGGTATTTACACCG
161	TATTGGAAAGGCCGGTAAAGATAACAGGGATCTCTGAAAAAGATTGTACTGAGAGTGCAC
162	AAATATTACACATTCATGTACATAAAATTAATTATCGGTGCTGTGCGGTATTTACACCG
164	TTCAAATCTCTTTTACAACACCAGACGAGAAATTAAGAAAAGATTGTACTGAGAGTGCAC
165	GGTCATTTGTACTTAATAAGAAAACCATATTATGCATCACCTGTGCGGTATTTACACCG
179	ACAGACGGATAGGCCTCCCTTTTGTTCGCACTAATAAAAAGATTGTACTGAGAGTGCAC
180	TATGCGGCTAGATATCCACAACGTCTATAAATTATGAGTTCTGTGCGGTATTTACACCG
184	TCCTCCATTCCCTCTCCTTTTCCAATTACCGTAATAAAAAGAGATTGTACTGAGAGTGCAC
185	TTTAATATACGCACGGTACAACCTAAGCAATCCGCAAAGACCTGTGCGGTATTTACACCG
235	CTTTTTTTTTGTCTCCCATATCTCGTGACACAAATATTAGATTGTACTGAGAGTGCAC
236	TTTCTTTACAATAAATATGTATAGTGTGTTACTTTACACCTGTGCGGTATTTACACCG
263	ATACTCGTATAAGCAAGAAATAAAGATACGAATATACAATAGATTGTACTGAGAGTGCAC
264	GCGATTGAGAAATCATATTTTGAATCTTCCACTGACAAATCTGTGCGGTATTTACACCG
268	AGAGTAGACAGTACATCAAGCGAAAATAAATATTGCAGGAAGATTGTACTGAGAGTGCAC
269	GCCTTTTTCTTCAGCAACCGTCCTCTTAGATTAGAGATTCCTGTGCGGTATTTACACCG
271	AGACAAGAGATATAAAAGTGCAGTGGCGATTTTCAAGGAACAAGATTGTACTGAGAGTGCAC
272	AGGCTTCAAACCCACTACTACTTTACGGTCTTCTATATCTCTGTGCGGTATTTACACCG
353	TTATCGAATACGATTAAACACTACGCCAGATTTCCACAATAGATTGTACTGAGAGTGCAC
354	TTTGTGCGTAACCCACGCTTACGATATTGGAATTACAATTCTGTGCGGTATTTACACCG

Table S3 - Raw culture proliferation data

A. Individual doubling times for Figure 3C	Exp I	Exp II	Exp III	Average doubling time (min) [n=3]	
BY4742 [WT] ρ+	91	92	94	92	
CDD496 [<i>vma2Δ</i>] ρ+	143	150	147	146	
BY4742 [WT] ρ-	198	198	192	196	
CDD496 [<i>vma2Δ</i>] ρ-	138	136	143	139	
B. Individual doubling times for Figure 3D	Exp I	Exp II	Exp III	Average doubling time (min) [n=3]	
BY4742 [WT] ρ+ YEPD pH 5.9	91	89	97	92	
BY4742 [WT] ρ+ YEPD pH 7.6	109	107	123	113	
BY4742 [WT] ρ- YEPD pH 5.9	189	195	223	202	
BY4742 [WT] ρ- YEPD pH 7.6	153	160	165	160	
C. Individual doubling times for Figure 3E (after 620 minutes of culture in drug)	Exp I	Exp II	Exp III	Exp IV	Average doubling time (min) [n=4]
BY4742 [WT] ρ- + DMSO vehicle	239	224	193	202	215
BY4742 [WT] ρ- + 2μM concanamycin A	162	160	148	147	155
D. Early proliferation of ρ- cells treated with concanamycin A (through 600 minutes)	Exp I	Exp II	Exp III	Average doubling time (min) [n=3]	
BY4742 [WT] ρ- + DMSO vehicle	176	172	220	189	
BY4742 [WT] ρ- + 2μM concanamycin A	240	259	490	330	
E. Proliferation of WT cells from the W303 background in YEPD at pH 5.9 or pH 7.6	Exp I	Exp II	Exp III	Average doubling time (min) [n=3]	
BMA64-1A [WT] ρ+ YEPD pH 5.9	104	105	103	104	
BMA64-1A [WT] ρ- YEPD pH 5.9	120	117	119	118	
BMA64-1A [WT] ρ+ YEPD pH 7.6	135	136	149	140	
BMA64-1A [WT] ρ- YEPD pH 7.6	195	200	185	193	
F. Proliferation of WT and V _i V _o -ATPase knockouts from the W303 background	Exp I	Exp II	Exp III	Average doubling time (min) [n=3]	
BMA64-1B [WT] ρ+	103	103	107	104	
BMA64-1B [WT] ρ-	129	127	125	127	
CDD552 [<i>vma2Δ</i>] ρ+	169	167	165	167	
CDD552 [<i>vma2Δ</i>] ρ-	154	160	162	159	
CDD553 [<i>vma2Δ</i>] ρ+	147	160	152	153	
CDD553 [<i>vma2Δ</i>] ρ-	168	167	169	168	

File S1 - Supplemental methods

Yeast strains and culture conditions: Yeast media was as described previously (ADAMS *et al.* 1997). Gene disruptions were performed as in (SIKORSKI and HIETER 1989). Strain genotypes, along with construction details, are listed in Table S1. Oligonucleotides used for strain construction are listed in Table S2. EtBr (Thermo Fisher Scientific, Waltham, MA) was used at a concentration of 25µg/ml, and concanamycin A (Santa Cruz Biotechnology, Santa Cruz, CA) was used at a concentration of 2µM. Buffering of YEPD to pH 7.6 was performed using 50 mM NaH₂PO₄. Serial dilution assays were performed by diluting cultures to an OD₆₀₀ of 0.02, then performing three additional five-fold dilutions. 4 µl of each dilution was spotted to YEPD medium or an alternative medium, if specifically indicated.

Isolation and identification of *spm1*: We cultured *mgr1Δ* strain RJ2058 for one day in YEPD medium containing EtBr (GOLDRING *et al.* 1970; SLONIMSKI *et al.* 1968), then spread overnight cultures onto YEPD + EtBr and incubated for 2 d at 30°C. Six surviving colonies containing spontaneous *spm* mutations (suppressor of petite-negativity of *mgr1Δ*) were crossed to ρ^+ *mgr1Δ* strain RJ2059 to test for dominance. Four mutations (*SPM2*, *SPM3*, *SPM5*, and *SPM6*) were dominant, while mutations *spm1* and *spm4* were recessive. *spm1* and *spm4* exhibited a collateral slow-division phenotype on YEPGE medium. Since *spm1* and *spm4* were generated from the same initial culture, we reasoned that these suppressors might not be independent mutations, and we set

aside *spm4* for future analysis. We transformed *mgr1Δ spm1* strain RJ2221 with a plasmid library containing random yeast genomic fragments of approximately six to eight kilobases (a gift of Professor Phillip Hieter, University of British Columbia) within a *URA3*-bearing vector and tested ~50,000 transformants for the ability to survive on YEPGE. A single colony grew at a WT rate, suggesting complementation of *spm1*. We isolated the pM538 plasmid, which we subjected to partial sequencing, thereby revealing the presence of *VPS16*, *ELC1*, *SGF11*, and *CAM1*. Only deletion of *VPS16* was previously reported to cause a proliferation defect on YEPGE medium, immediately focusing our attention upon this gene.

Assays of the petite-negative phenotype: Strains were cultured overnight in YEPD medium either lacking or containing EtBr to maintain or destroy mtDNA, respectively. Logarithmic phase cultures were subjected to serial dilution, as described above, spotted to solid YEPD medium, and incubated for 2 d (ρ^+) or 3 d (ρ^-) at 30°C, except where temperature or incubation time is otherwise noted. All plate imaging was accomplished using a Colony Doc-It system (UVP, Upland, CA).

Microscopy: Overnight cultures were diluted from selective medium into rich medium and incubated until reaching early log phase. Cells were visualized using an Eclipse 80i fluorescent microscope fixed with a 100X Plan Fluor objective (Nikon, Tokyo, Japan). Cells were scored for any perceptible fluorescent mitochondria within each cell (>200 cells for each strain), and scoring was performed blind to genotype. Digital photography

was performed with a DS-Qi1Mc camera (Nikon, Tokyo, Japan). Exposure times were automatically determined and images were captured using NIS-Elements software version AR 3.1.

Cell proliferation rate measurement: For determining strain doubling times in Figure 3C and 3D, overnight cultures were diluted to an OD₆₀₀ of 0.05 in the indicated medium. For following late proliferation of ρ^- cells in concanamycin A (Figure 3E), overnight cultures were first diluted to an OD₆₀₀ of 0.015 in the indicated medium. For experiments following the early proliferation of ρ^- cells in concanamycin A (Table S3D), overnight cultures were first diluted to an OD₆₀₀ of 0.1. Turbidity measurements were collected by a Mecasys Optizen POP UV-VIS spectrophotometer at 600nm (Mecasys Co. Ltd., Daejeon, South Korea). For each experiment, at least four measurements were taken from the same culture over the course of at least eight hours of proliferation after dilution of overnight cultures in fresh medium. Measurements obtained after the culture OD₆₀₀ reached 1.5 were discarded in order to ensure that proliferation was measured within the logarithmic phase of culture growth. To calculate doubling times from OD₆₀₀ measurements, OD₆₀₀ readings were converted to log₂, and a slope was determined using Microsoft Excel 2011 (Microsoft Corp., Redmond, WA). The reciprocal of the slope provided the doubling time in minutes.