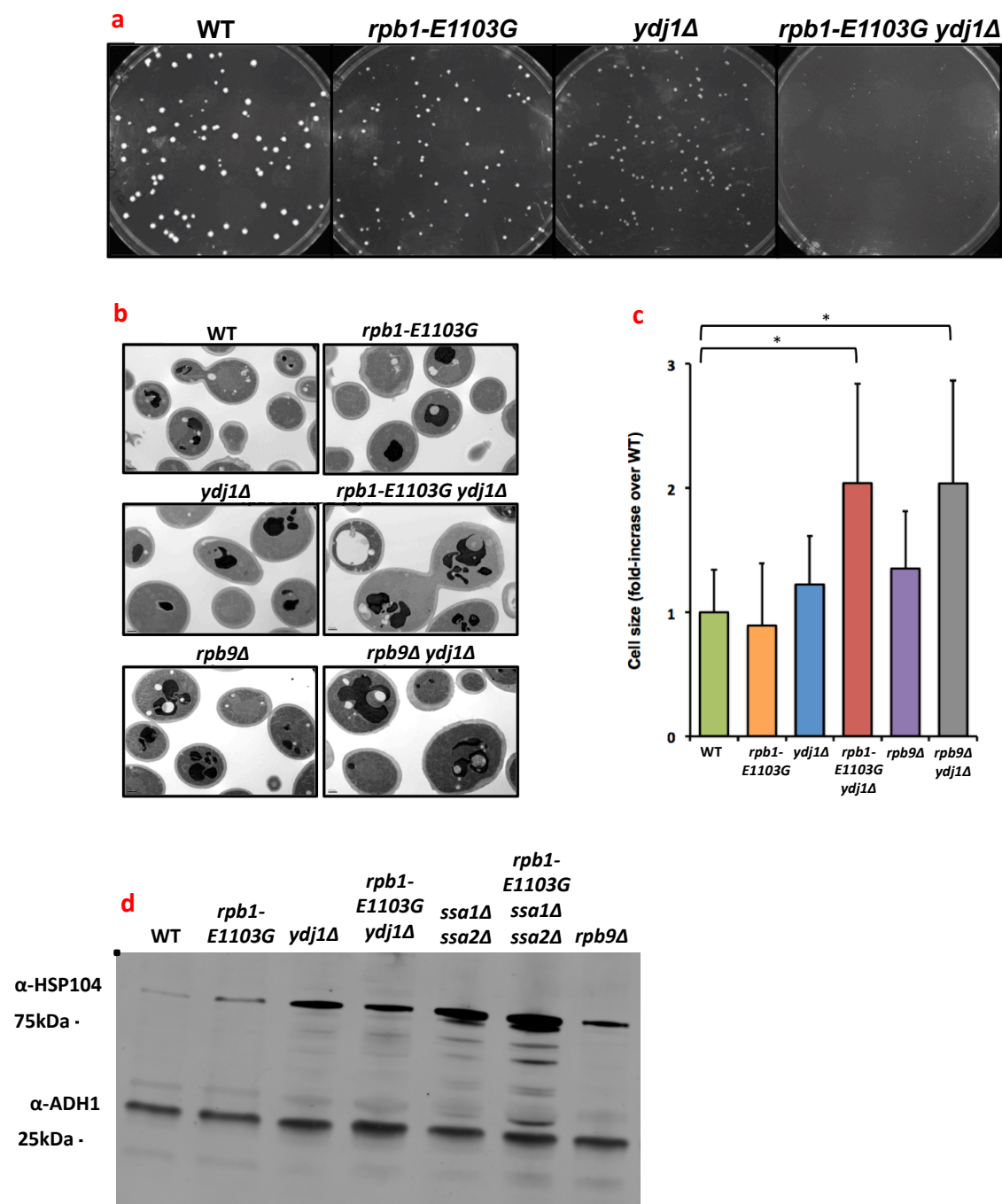


Supplementary Figures

Supplementary Figure 1

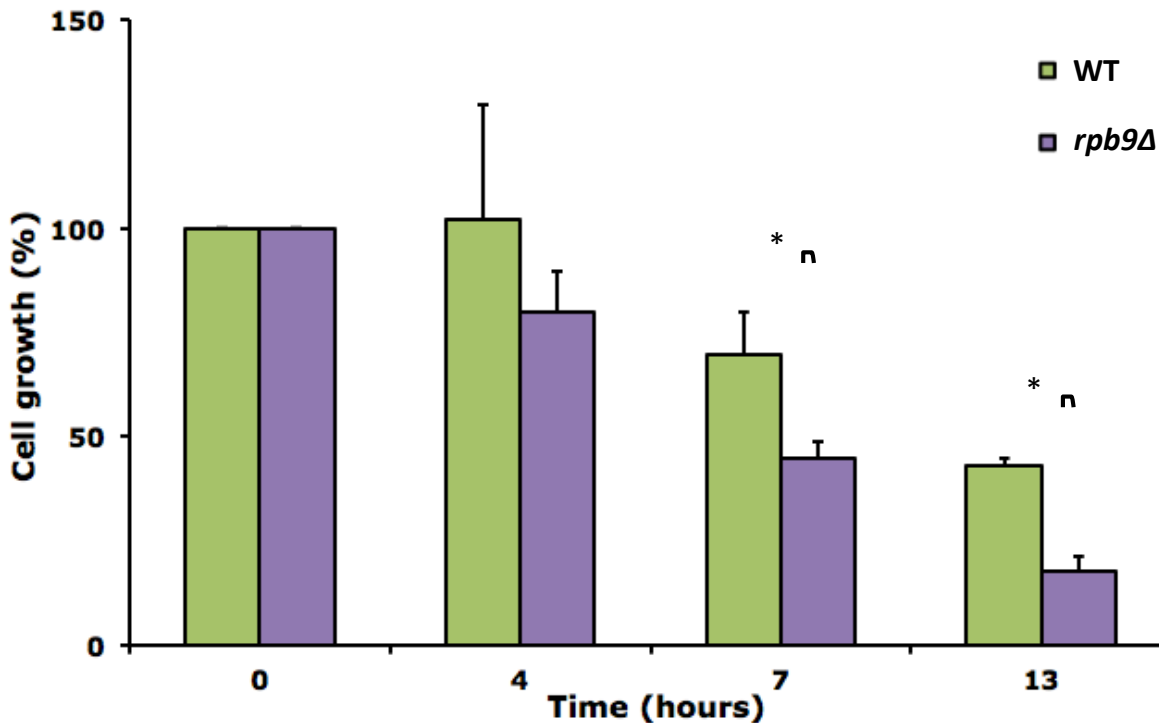


Supplementary figure 1. Error prone cells grow slower than WT cells

(a) Cells were grown on a YAPD plate, and allowed to grow uninterrupted for 48 hours to form a colony. During that timespan, WT cells generate larger colonies than *rpb1-E1103G* cells, *hsp40Δ*

cells and *rpb1-E1103G ydj1Δ* cells. The growth defect of *rpb1-E1103G* cells is sufficient to genotype cells after sporulation (MVY0001, 2, 4, 5). (b) *rpb1-E1103G ydj1Δ* and *rpb9Δ ydj1Δ* cells are significantly larger than WT cells (MVY0001-6). (c) Quantification of cell size, as shown in Supplementary Figure 1b. The volume of at least 50 cells was determined for each genotype (MVY0001-6). (d) Full gel picture of figure 1d. At least 3 biological replicates were used for each genotype. All statistical analyses were performed with Prism software, using an unpaired, two tailed t-test. Error bars indicate one standard deviation from the mean. *= P<0.05

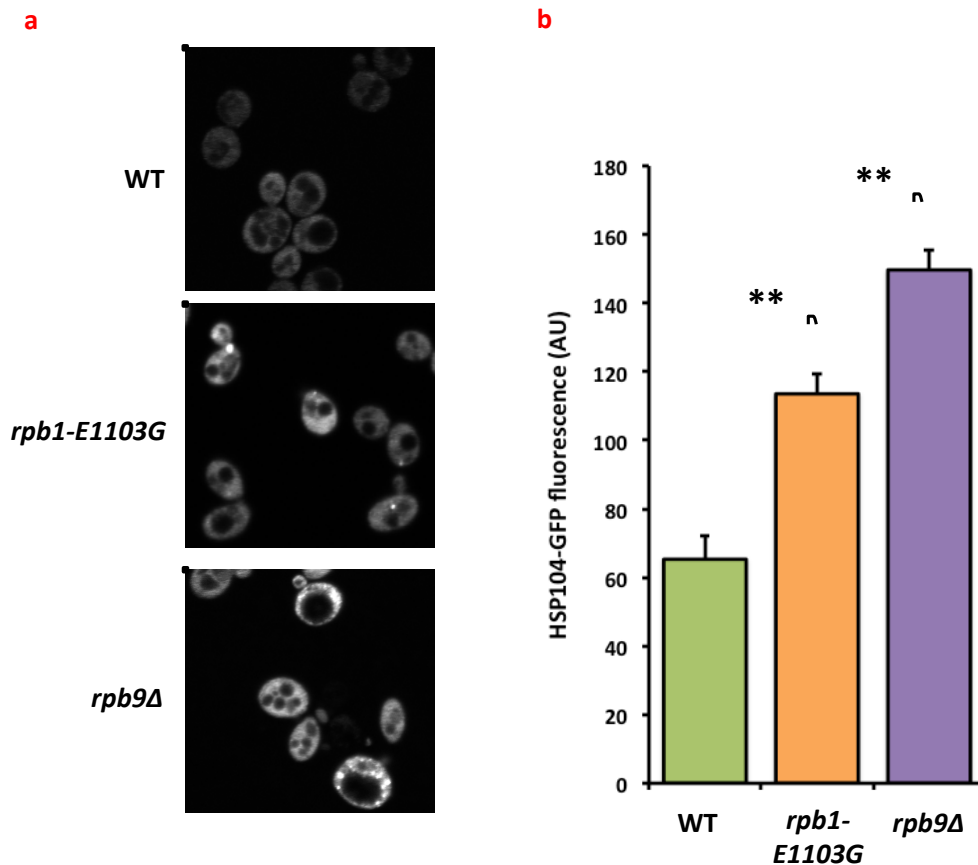
Supplementary Figure 2



Supplementary figure 2. *Rpb9 Δ* cells are more sensitive to radicicol than WT cells

WT cells and *rpb9Δ* were diluted to OD 0.02, and allowed to grow for 13 hours in the presence of 200μM radicicol. The growth of the cells suspended in radicicol was then compared at three time intervals to cells that were grown in the absence of radicicol. *Rpb9Δ* cells exhibited a greater reduction in growth than WT cells (MVY0001, 3). At least 3 biological replicates were used for each genotype. All statistical analyses were performed with Prism software, using an unpaired, two tailed t-test. Error bars indicate one standard deviation from the mean. *= P<0.05

Supplementary Figure 3

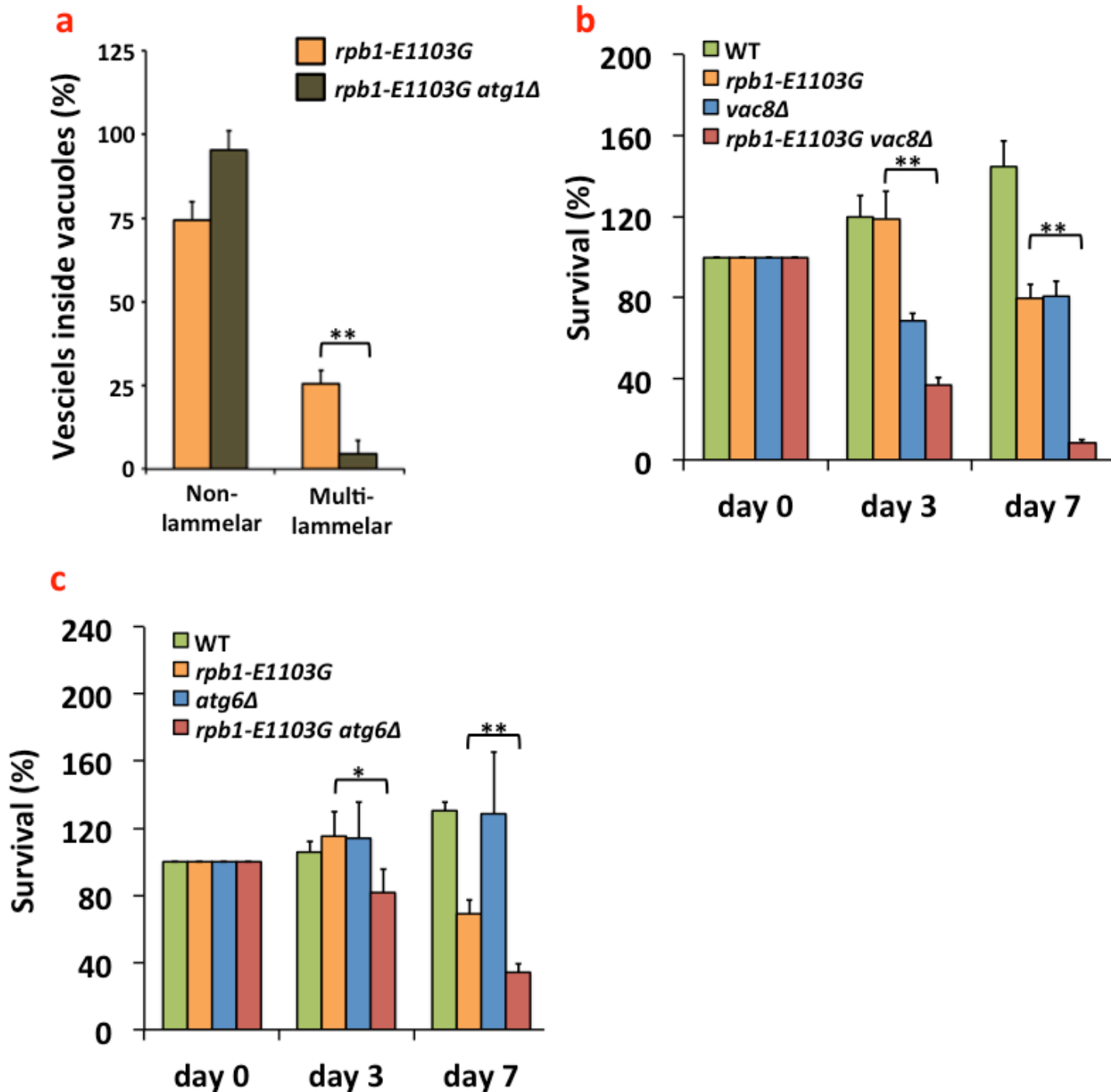


Supplementary figure 3. Error prone cells exhibit greater Hsp104-GFP expression than WT cells.

(a) Cells received a chromosomally integrated copy of HSP104-GFP. The cell lines were grown into log phase and monitored under a con-focal microscope. The expression level of HSP104-GFP was then compared between the strains (MVY0001-3). (b) Quantification of images presented in figure 1a. We found that *rpb1-E1103G* and *rpb9Δ* cells exhibited greater expression than WT cells (AU= arbitrary units). Over a 100 cells were analyzed for each sample, and 3 biological replicates were analyzed per genotype. All statistical analyses were performed with Prism software, using an unpaired, two tailed t-test. Error bars indicate one standard deviation from the mean. **= P<0.01

Supplementary Figure 4

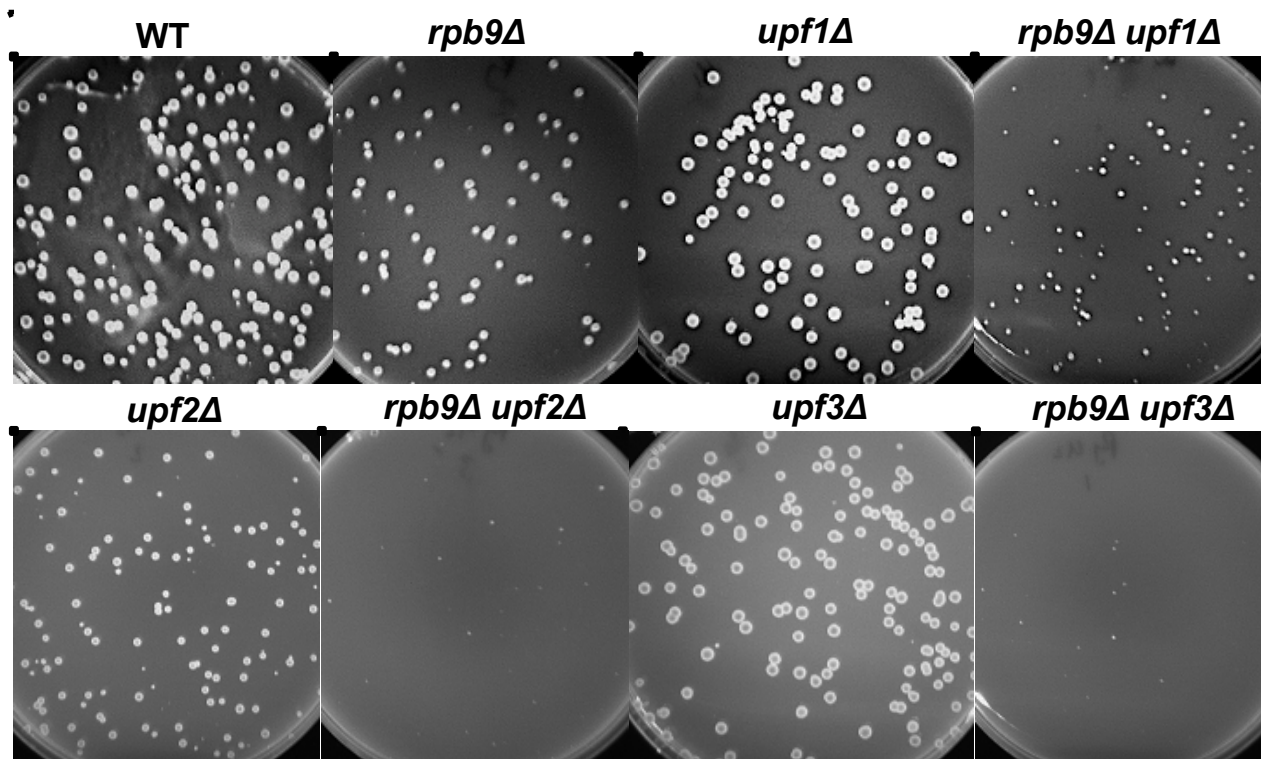
Figure S4



Supplementary figure 4. Autophagy safeguards the health of error prone cells

(a) *ATG1* deletion in error prone cells. Cells that have lost *ATG1* display a decreased amount of multi-lammellar structures inside vacuoles. Approximately 150 cells were monitored for each genotype (MVY0002, 12). (b-c) Cells were allowed to grow into stationary phase for 48 hours (day 0) and the number of colony forming units (CFUs) determined. After 3 and 7 days of incubation and constant shaking at 30°C, the number of CFUs were determined again and compared to day 0. At least 3 biological replicates were used for each genotype for each experiment presented above. All statistical analyses were performed with Prism software, using an unpaired, two tailed t-test. Error bars indicate one standard deviation from the mean. *= $P < 0.05$, **= $P < 0.01$

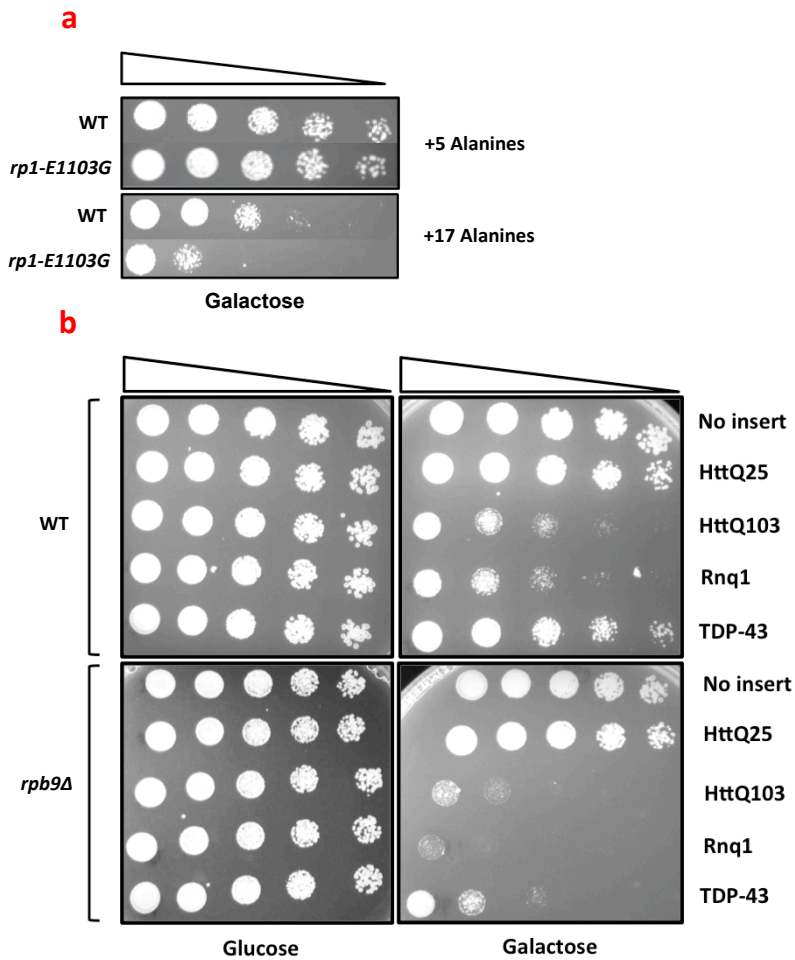
Supplementary Figure 5



Supplementary figure 5. Deletion of upf1-3 slows the growth of *rpb9Δ* cells

(a-c) Cells were grown on a YAPD plate, and allowed to grow uninterrupted for 48 hours to form a colony. During that timespan, *rpb9Δ* cells generate smaller colonies in the absence of *UPF1*, 2 or 3.

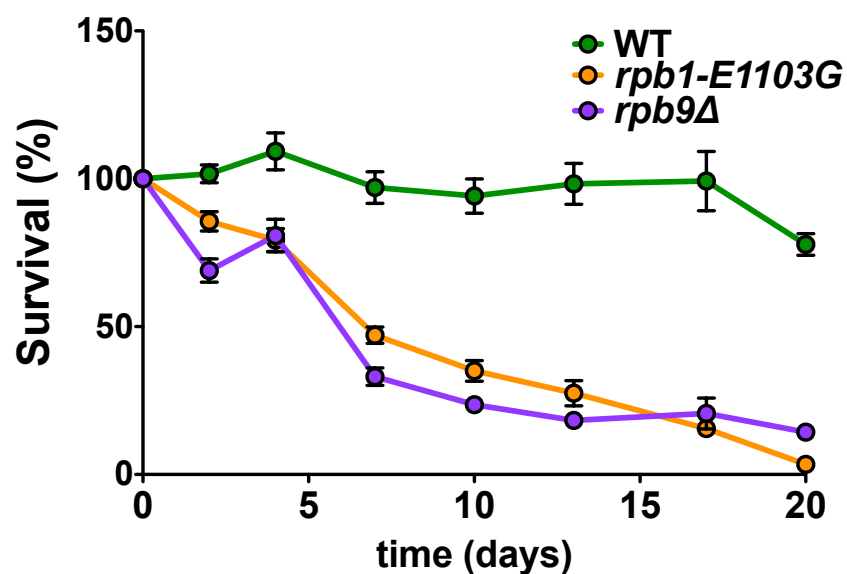
Supplementary Figure 6



Supplementary figure 6. Expression of proteotoxic genes in WT, *rpb1-E1103G* and *rpb9Δ* cells

(a) Cells were grown in raffinose and then plated on glucose and galactose. When the cells are grown on galactose (but not glucose) the proteotoxic genes are expressed. This experiment shows that a repeat sequence of 17 alanines is more toxic to *rpb1-E1103G* cells than WT cells, while a non-toxic repeat of 8 alanines had no effect (MVY0001-2). (b) A similar experiment shows that Htt103Q, Rnq1 and TDP-43 cause a greater drop in the viability of *rpb9Δ* cells than WT cells (MVY0001, 3).

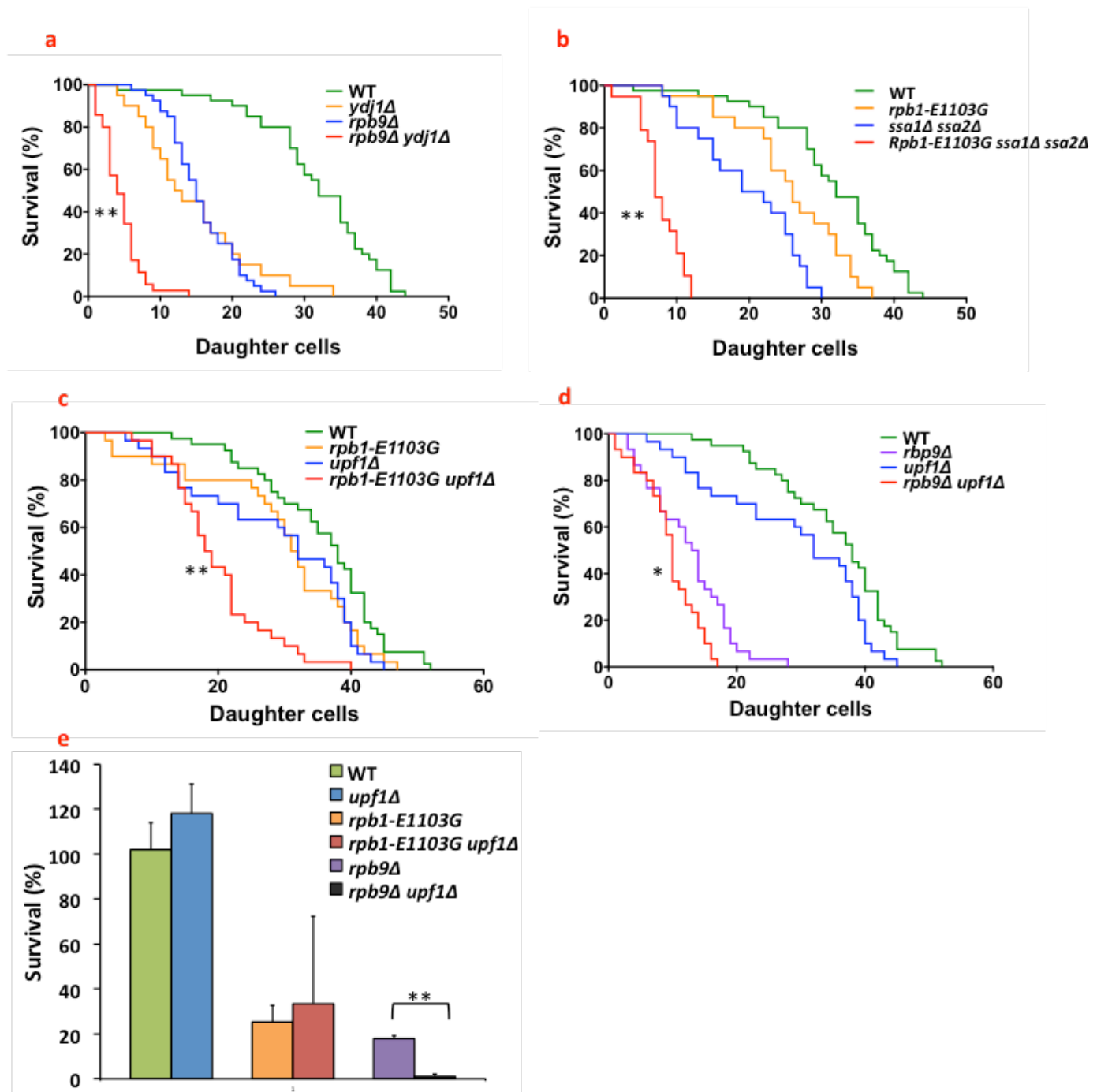
Supplementary Figure 7



Supplementary figure 7. Chronological lifespan of WT cells during fidelity measurements

At day 20, when the fidelity measurement was taken, the WT cells still showed >75% survival. The chronological lifespans of *rpb1-E1103G* and *rpb9Δ* cells are also listed for comparison. At least 3 biological replicates were used for each genotype.

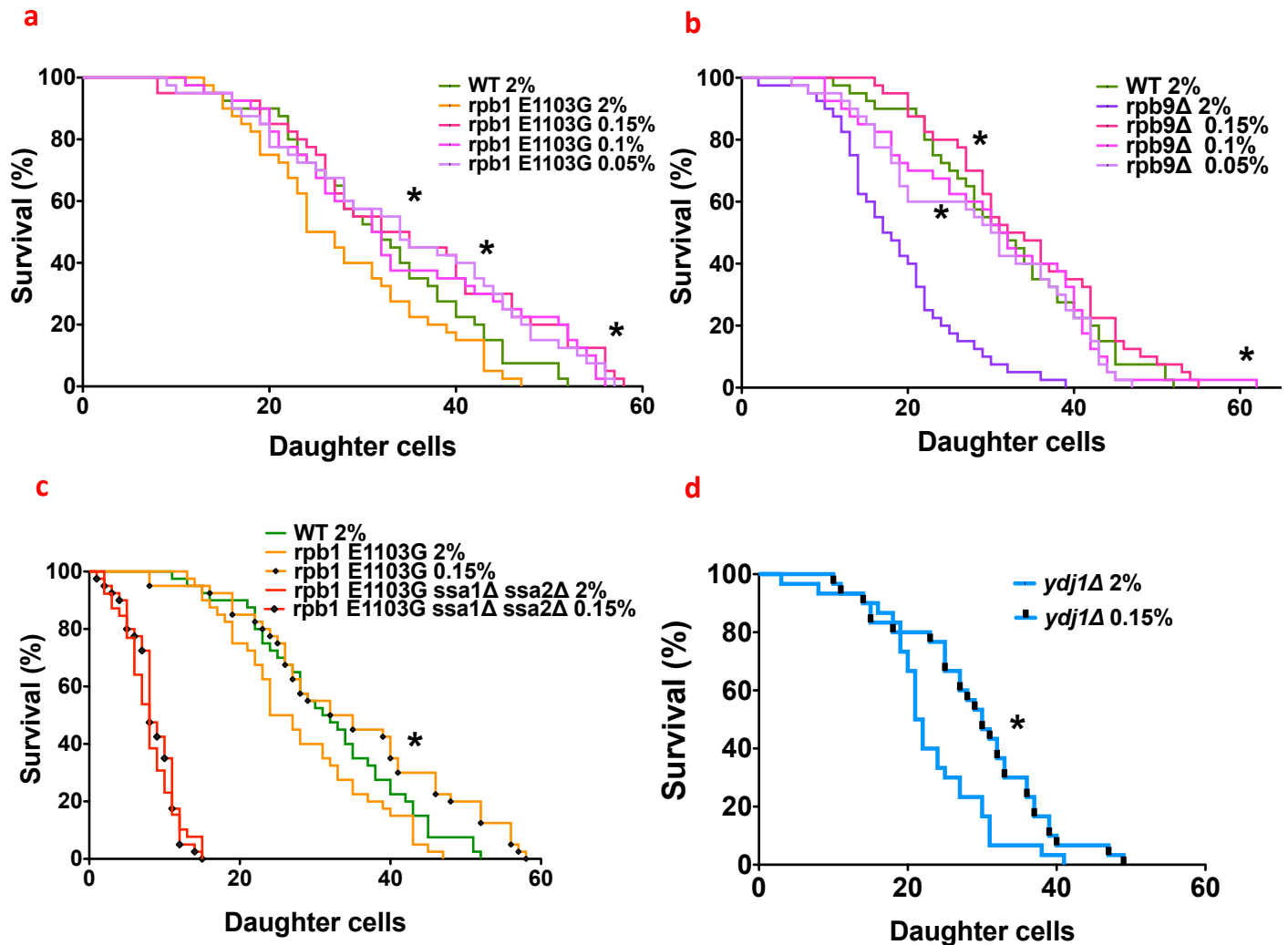
Supplementary Figure 8



Supplementary figure 8. Shortened replicative lifespan of the error prone cells in the absence of molecular chaperones. (a) When *YDJ1* is deleted in *rpb9Δ* cells, the lifespan of the *rpb9Δ* cells is reduced dramatically (MVY0001, 2, 4, 5). (b) When *SSA1* and *SSA2* are deleted in *rpb1-E1103G*

cells, the lifespan of *rpb1-E1103G* cells is reduced dramatically as well (MVY0001, 2, 7, 8). (c)
Deletion of *UPF1* further reduces the replicative lifespan of *rpb1-E1103G* and *rpb9Δ* cells. (e)
Deletion of *UPF1* further reduces the chronological lifespan of *rpb9Δ* cells after 13 days, but not *rpb1-E1103G* cells. At least 3 biological replicates were used for each genotype. All statistical analyses on the chronological lifespan of cells were performed with Prism software, using an unpaired, two tailed t-test. Error bars indicate one standard deviation from the mean. All statistical analyses on the replicative lifespan of cells were performed with Prism software using a log rank (Mantel-Cox) test. *= $P < 0.05$, **= $P < 0.01$.

Supplementary Figure 9



Supplementary figure 9. Dietary restriction rescues the lifespan of error prone cells, but not error prone cells that have lost SSA1 and SSA2 (a) The lifespan of *rpb1-E1103G* cells is extended beyond the lifespan of WT cells when they are grown on 0.05, 0.1, or 0.15% glucose (MVY0001-2). (b) The lifespan of *rpb9Δ* cells is rescued by dietary restriction on 0.05, 0.1 and 0.15% glucose (MVY0001, 3). (c) Dietary restriction rescues the shortened lifespan of *rpb1-E1103G* cells, but not *rpb1-E1103G SSA1Δ SSA2Δ* cells (MVY0001, 2, 8). (d) Dietary restriction extends the lifespan of *ydj1Δ* cells. At least 3 biological replicates were used for each genotype. All statistical analyses on the replicative lifespan of cells were performed with Prism software using a log rank (Mantel-Cox) test. * = $P < 0.05$, ** = $P < 0.01$.

Supplementary Table 1

| Strain | Genotype | Origin |
|----------------|--|--|
| MVY0001 | ura3 Δ 0, leu2 Δ 0, lys2 Δ 0, his3- Δ 1, MAT α | Yeast Knock Out MAT α collection from Thermo Scientific (YSC1053) |
| MVY0002 | rpb1-E1103G, ura3 Δ 0, leu2 Δ 0, lys2 Δ 0, his3- Δ 1, MAT α | Generated in the laboratory of Jeffrey Strathern, backcrossed 15x into BY4741 background |
| MVY0003 | rpb9::kanMX, ura3 Δ 0, leu2 Δ 0, lys2 Δ 0, his3- Δ 1, MAT α | Sporulated from the Yeast Knock Out heterozygous Collection (YSC1055) |
| MVY0004 | ydj1::kanMX, ura3 Δ 0, leu2 Δ 0, lys2 Δ 0, his3- Δ 1, MAT α | Yeast Knock Out MAT α collection from Thermo Scientific (YSC1053) |
| MVY0005 | rpb1-E1103G, ydj1::kanMX, ura3 Δ 0, leu2 Δ 0, lys2 Δ 0, his3- Δ 1, MAT α | Sporulated from a cross between MVY0002 (MAT α) and MVY0004 |
| MVY0006 | rpb9::kanMX ydj1::kanMX, ura3 Δ 0, leu2 Δ 0, lys2 Δ 0, his3- Δ 1, MAT α | Sporulated from a cross between MVY0003(MAT α) and MVY0004 |
| MVY0007 | ssa1::kanMX, ssa2::kanMX, ura3 Δ 0, leu2 Δ 0, lys2 Δ 0, his3- Δ 1, MAT α | Sporulated from a cross between ssa1::kanMX and ssa1::kanMX clones (YSC1053, YSC1054) |
| MVY0008 | rpb1-E1103G ssa1::kanMX, ssa2::kanMX, ura3 Δ 0, leu2 Δ 0, lys2 Δ 0, his3- Δ 1, MAT α | Sporulated from a cross between MVY0002 (MAT α) and MVY0007 |
| MVY0009 | HSP104-GFP-His3MX6, ura3 Δ 0, leu2 Δ 0, lys2 Δ 0, his3- Δ 1, MAT α | Yeast GFP Clone Collection (invitrogen, 95700, 95701, 95702) |
| MVY0010 | rpb1-E1103G, HSP104-GFP-His3MX6, ura3 Δ , leu2 Δ 0, lys2 Δ 0, his3- Δ 1, MAT α | Sporulated from a cross between MVY0002(MAT α) and MVY0009 |
| MVY0011 | rpb9::kanMX, HSP104 -GFP-His3MX6, ura3 Δ 0, | Sporulated from a cross between MVY0003(MAT α) and MVY0009 |
| MVY0012 | rpb1-E1103G, atg1::kanMX, ura3 Δ 0, leu2 Δ 0, lys2 Δ 0, his3- Δ 1, MAT α | Sporulated from a cross between MVY0003(MAT α) and atg1::KanMX clone (YSC1053) |
| MVY0013 | pep4::kanMX, ura3 Δ 0, leu2 Δ 0, lys2 Δ 0, his3- Δ 1, MAT α | Yeast Knock Out MAT α collection from Thermo Scientific (YSC1053) |
| MVY0014 | rpb1-E1103G, pep4::kanMX, ura3 Δ 0, leu2 Δ 0, lys2 Δ 0, his3- Δ 1, MAT α | Sporulated from a cross between MVY0002(MAT α) and MVY00013 |
| MVY0015 | rpb9::kanMX upf1::kanMX, ura3 Δ 0, leu2 Δ 0, lys2 Δ 0, his3- Δ 1, MAT α | Sporulated from a cross between MVY0003(MAT α) and upf1::KanMX clone (YSC1053) |
| MVY0016 | rpb9::kanMX upf2::kanMX, ura3 Δ 0, leu2 Δ 0, lys2 Δ 0, his3- Δ 1, MAT α | Sporulated from a cross between MVY0003(MAT α) and upf2::KanMX clone (YSC1053) |
| MVY0017 | rpb9::kanMX upf3::kanMX, ura3 Δ 0, leu2 Δ 0, lys2 Δ 0, his3- Δ 1, MAT α | Sporulated from a cross between MVY0003(MAT α) and upf3::KanMX clone (YSC1053) |
| GRY3337 | ade2 Δ , ade6-AI2floxkanMX, his3- Δ 1, leu2 Δ 0, met15 Δ 0, trp1- Δ 1, ura3 Δ 0, rpb1::kanMX, Mata | Generated in the laboratory of Jeffrey Strathern |
| GRY3724 | ade2 Δ , ade6 Δ 0::zeoMX, his3- Δ 1, leu2::PHIS3malE-C10EB-cre:hygMX, ura3 Δ 0, MAT α | Generated in the laboratory of Jeffrey Strathern |

Supplementary table 1. List of strains used in this manuscript. Please note that many additional strains were generated for this work, containing unique combinations of mating types and markers.

These strains were used for mating purposes, future strain constructions and control experiments.

Although not listed here, these strains can be requested by contacting M.V.

Supplementary Table 2

| Protein | Function | <i>rpb9</i> | | <i>rpb1-E1103G</i> | |
|---------|---|---------------|---------|--------------------|---------|
| | | Fold increase | P-value | Fold increase | P-value |
| Abp1 | Actin-binding protein | 3.5 | 0.001 | N/A | N/A |
| Aco1 | Required for the tricarboxylic acid cycle | 2.9 | 0.001 | 2.4 | 0.0027 |
| Act1 | Actin, structural protein | 1.9 | 0.001 | N/A | N/A |
| Ahp1 | Peroxiredoxin, protects cells from oxidative damage | 3.4 | 0.001 | N/A | N/A |
| Asn2 | Asparagine biosynthesis | N/A | N/A | 2.9 | 0.041 |
| Bmh1 | 14-3-3 protein, controls proteome at post-transcriptional level | 2.3 | 0.001 | N/A | N/A |
| Cdc48 | Subunit of complex involved in ER-associated protein degradation, macroautophagy | 2.2 | 0.006 | 2.4 | 0.0063 |
| Cdc60 | Cytosolic leucyl tRNA synthetase | 3.3 | 0.001 | 2.4 | 0.015 |
| Dug1 | Metallo-peptidase involved with glutathione degradation | 2.3 | 0.023 | 2.3 | 0.03 |
| Gpm1 | Phosphoglycerate mutase involved in glycolysis and gluconeogenesis | 2 | 0.003 | N/A | N/A |
| Hsc82 | Cytoplasmic chaperone | 1.5 | 0.015 | 2 | 0.00031 |
| Hsp104 | Disaggregase, helps refold and reactive previously denatured, aggregated proteins | 2.8 | 0.003 | N/A | N/A |
| Hsp26 | Small heat shock protein that suppresses unfolded protein aggregation | 2.4 | 0.019 | N/A | N/A |
| Hsp60 | Mitochondrial chaperonin | 2.4 | 0.001 | N/A | N/A |
| Hxk2 | Catalyzes phosphorylation of glucose in the cytosol | 4.3 | 0.001 | 3.5 | 0.0049 |
| Ildp1 | Mitochondrial isocitrate dehydrogenase | N/A | N/A | 3 | 0.0041 |
| Ipp1 | Cytoplasmic pyrophosphatase | 1.7 | 0.018 | N/A | N/A |
| Leu4 | Enzyme responsible for leucine biosynthesis | 3.0 | 0.001 | N/A | N/A |
| Met6 | Methionine synthase | N/A | N/A | 2.6 | 0.00059 |
| Pfk1 | Involved in glycolysis | 2.3 | 0.007 | 2.5 | 0.0069 |
| Pfk2 | Involved in glycolysis | 4.2 | 0.001 | 2.6 | 0.011 |
| Pgk1 | Key enzyme in glycolysis and gluconeogenesis | 1.6 | 0.001 | N/A | N/A |
| Pma1 | Regulator of cytoplasmic pH and membrane potential | 1.7 | 0.009 | 1.9 | 0.0087 |
| Por1 | Mitochondrial porin | 1.7 | 0.015 | N/A | N/A |
| Rcy1 | Involved in recycling of endocytosed proteins | N/A | N/A | 2.2 | 0.014 |
| Rnr4 | Ribonucleotide reductase subunit involved in dNTP synthesis | 2.3 | 0.007 | N/A | N/A |
| Rpl7b | 60S Ribosomal subunit | 2 | 0.014 | N/A | N/A |
| Sac6 | Actin-bundling protein | 4.4 | 0.002 | 4.2 | 0.0074 |
| Ssa1 | Chaperone of the Hsp70 family | 4.5 | 0.001 | 2.2 | 0.0001 |
| Ssa2 | Chaperone of the Hsp70 family | N/A | N/A | 1.7 | 0.0011 |
| Ssb2 | Ribosome associated chaperone | 2.4 | 0.001 | 2.2 | 0.00073 |
| Ssc1 | Hsp70 family ATPase | 1.7 | 0.016 | 1.8 | 0.021 |
| Sse1 | ATPase component of the Hsp90 chaperone complex | 2.1 | 0.001 | 2.7 | 0.0001 |
| Sti1 | Hsp90 co-chaperone | 2.9 | 0.006 | 4 | 0.00033 |
| Sub2 | Involved in spliceosome assembly, RNA helicase | N/A | N/A | 2.1 | 0.046 |
| Tdh2 | Dehydrogenase involved in glycolysis and gluconeogenesis | 3.4 | 0.01 | 4.1 | 0.0032 |
| Tpi1 | Glycolytic enzyme involved in redox metabolism | 1.6 | 0.001 | N/A | N/A |
| Vas1 | Valyl tRNA synthetase | 5.0 | 0.001 | N/A | N/A |
| Wtm1 | Transcriptional modulator involved in RNR expression | 3.4 | 0.001 | N/A | N/A |
| Yef3 | Translational elongation factor | N/A | N/A | 1.6 | 0.0023 |

Supplementary table 2. Proteins upregulated in *rpb1-E1103G* and *rpb9Δ* cells compared to

WT cells

Cells were grown into log-phase, lysed in liquid nitrogen, and analysed using mass spectrometry. All proteins that were significantly upregulated more than 1.5 fold in *Rpb9* and *rpb1-E1103G* cells are displayed. The error prone cells exhibit increased levels of heat shock proteins (red). In addition, a substantial number of genes involved in energy metabolism were upregulated (blue, MVY0001-3).

Supplementary Table 3

| Plasmid name | Genetic contents | Origin |
|---------------------------------|--|------------------------------|
| pRNQ1 | pRS416, Gal1-Rnq1-YFP (<i>URA3</i> , CEN) | Summers, Cyr, JBC, 2009 |
| pHTT25 | pYES, Gal1-HTT25Q-GFP (<i>URA3</i> , 2 μ) | Wolfe, Cyr, PLOSone, 2014 |
| pHtt103Q | pYES, Gal1-HTT103Q-GFP (<i>URA3</i> , 2 μ) | Wolfe, Cyr, PLOSone, 2014 |
| pTDP43 | pRS416, Gal1-TDP-43-YFP (<i>URA3</i> , CEN) | Johnson, Gitler, PNAS 2008 |
| pYFP | pRS416, Gal1-YFP (<i>URA3</i> , CEN) | Cyr, JBC, 2009 |
| pslGFP | pRS426, ADH1-slGFP (<i>URA3</i> , 2 μ) | Cyr, JBC, 2009 |
| pGFP | pRS416, PGK1- GFP (<i>URA3</i> , CEN) | Caine, Macreadie, FEMS 2007 |
| pAβ1-42 | pRS416, PGK1-A β 1-42-GFP (<i>URA3</i> , CEN) | Caine, Macreadie, FEMS 2007 |
| pATG8 | pRS426, ATG8-GFP (<i>URA3</i> , 2 μ) | Lang, Duncan, JBC 2014 |
| pJS725 | <i>RPB1</i> (<i>URA3</i> , 2 μ) | Strathern, Kashlev, JBC 2013 |
| pRG1361 | pRS416, Gal1-Pab1 ^{8A} -GFP (<i>URA3</i> , CEN) | Konopka, Gardner, MBC, 2011 |
| pRG1365 | pRS416, Gal1-Pab1 ^{17A} -GFP (<i>URA3</i> , CEN) | Konopka, Gardner, MBC, 2011 |

Supplementary table 3. Table of all plasmids used in this study.