Supplemental figures

Figure S1

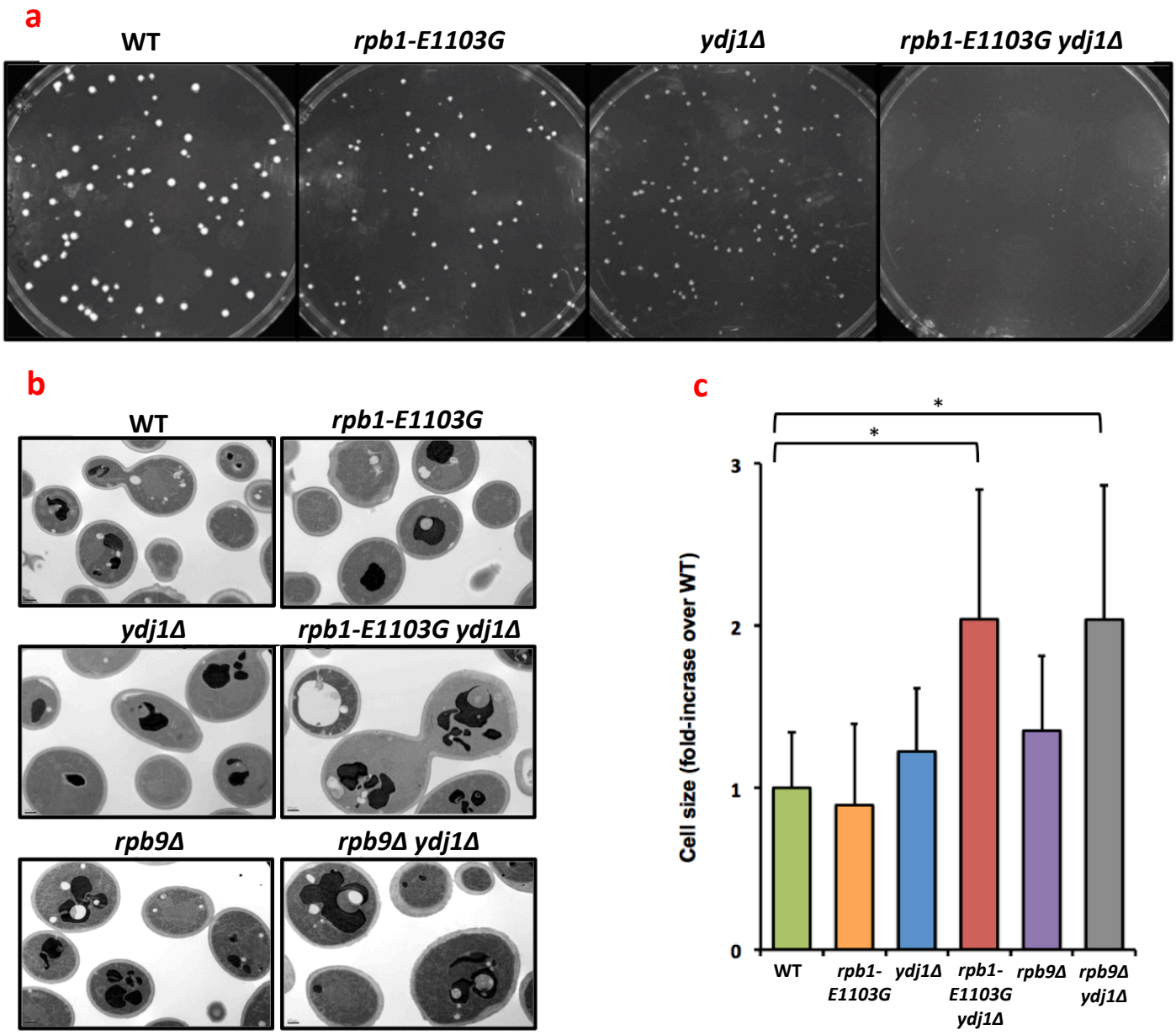

Fig. S1. Error prone cells grow slower than WT cells. (a) Cells were grown on a YAPD plate, and allowed to grow uninterrupted for 48 hours to form a colony. During that timespan, WT cells generate

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 fig. S1b. The volume of at least 50 cells was determined for each genotype (MVY0001-6).

Figure S2

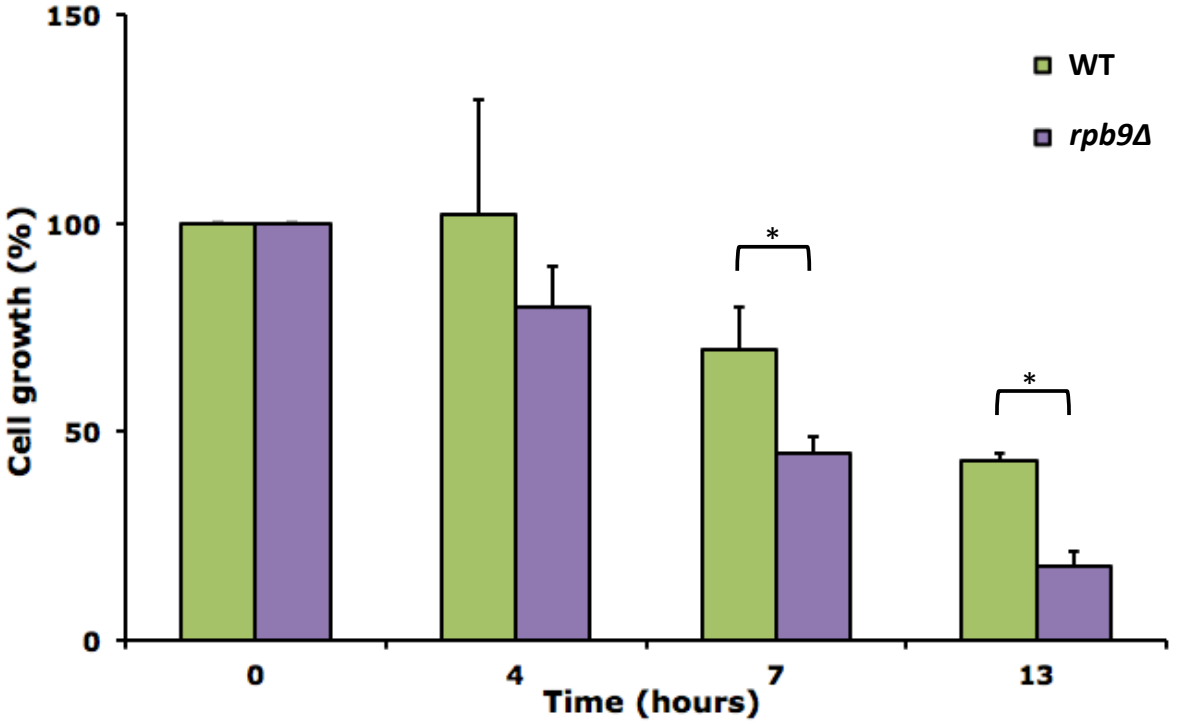

Fig. S2. *Rpb9Δ* cells are more sensitive to radicicol than WT cells. WT cells and *rpb9Δ* were diluted to OD 0.02, and allowed to grow for 13 hours in the presence of 200μM radicicol. The growth of the cells suspended in radicicol was then compared at three time intervals to cells that were grown in the absence of radicicol. *Rpb9Δ* cells exhibited a greater reduction in growth than WT cells (MVY0001, 3).

*= P<0.05, *= P<0.01

Figure S3

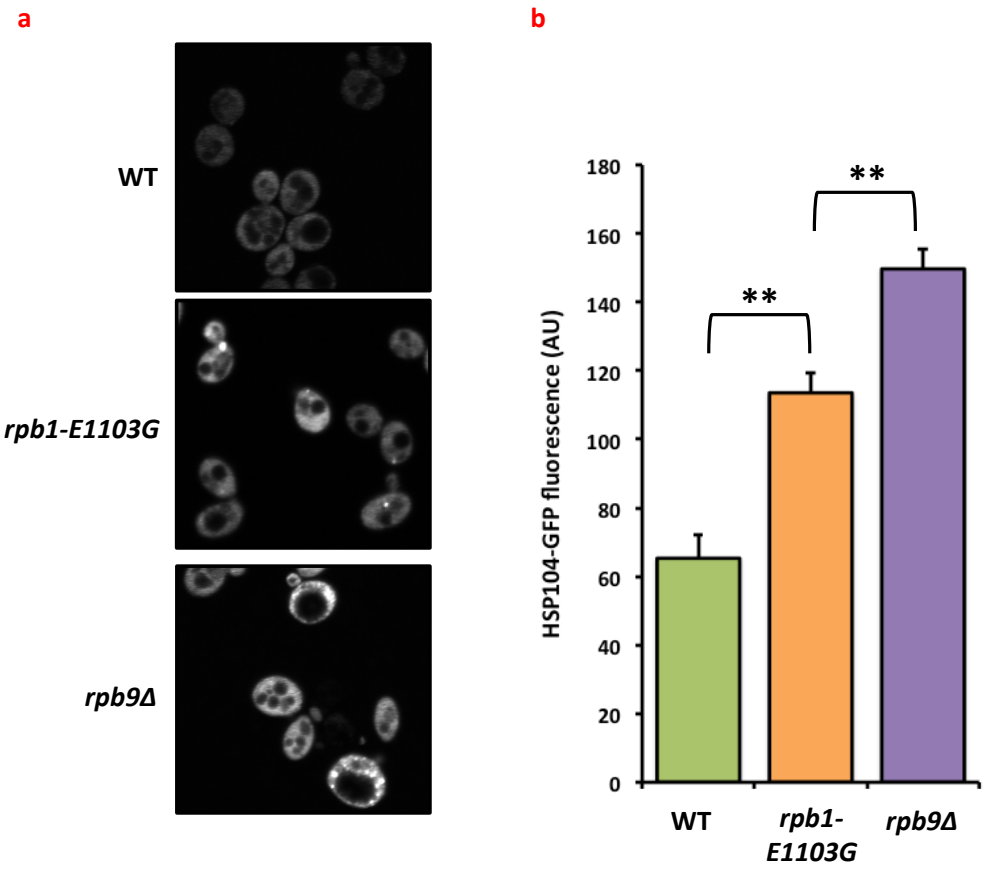

Fig. S3. Error prone cells exhibit greater Hsp104-GFP expression than WT cells (a) Cells received a chromosomally integrated copy of HSP104-GFP. The cell lines were grown into log phase and monitored under a con-focal microscope. The expression level of HSP104-GFP was then compared between the strains (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 genotype (MVY0001-3).

*= P<0.01

Figure S4

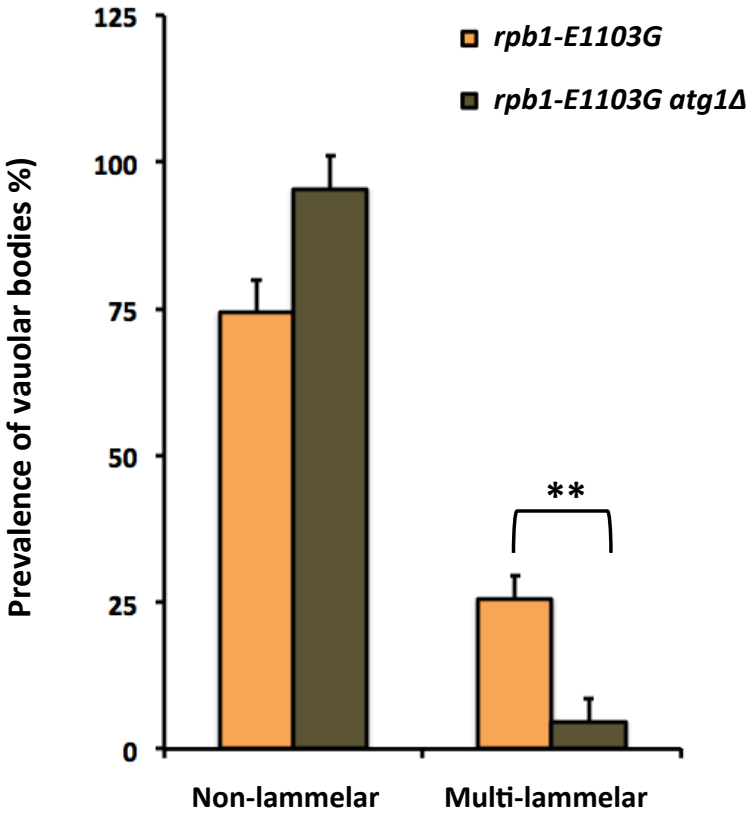

Fig. S4. *ATG1* deletion in error prone cells. Cells that have lost *ATG1* display a decreased amount of multi-lammelar structures inside vacuoles. Approximately 150 cells were monitored for each genotype (MVY0002, 12).

**= P<0.01

Figure S5

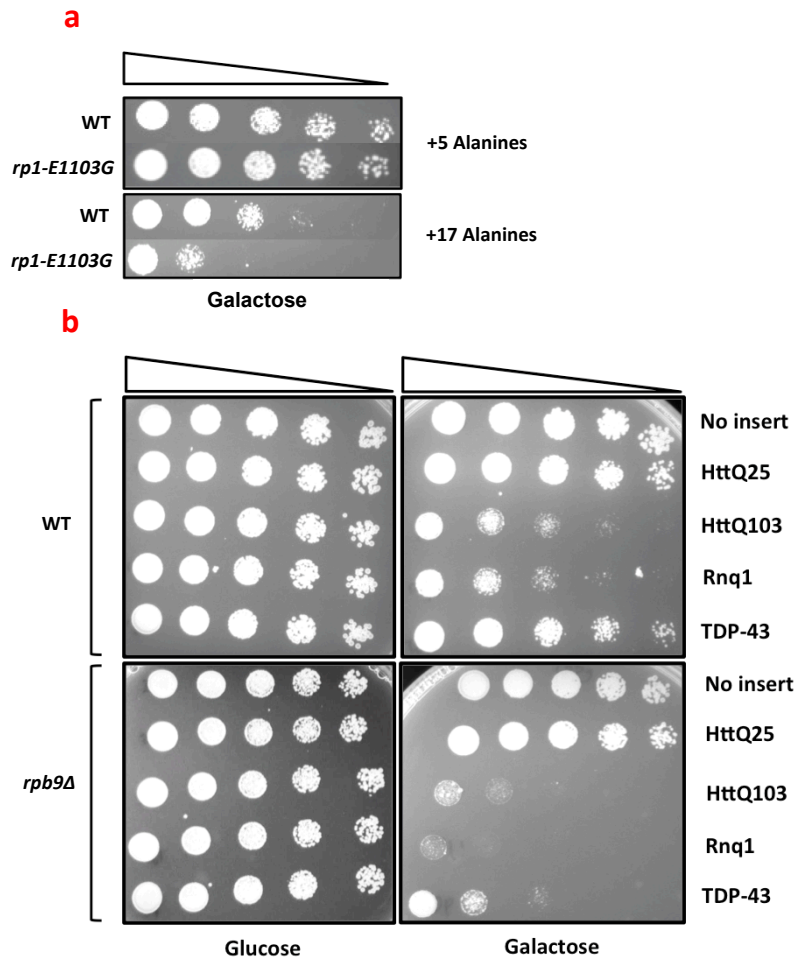

Fig. S5. Expression of proteotoxic genes in WT, *rpb1-E1103G* and *rpb9Δ* cells**.** (a) Cells were grown in raffinose and then plated on glucose and galactose. When the cells are grown on galactose (but not glucose) the proteotoxic genes are expressed. This experiment shows that a repeat sequence of 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).

Figure S6

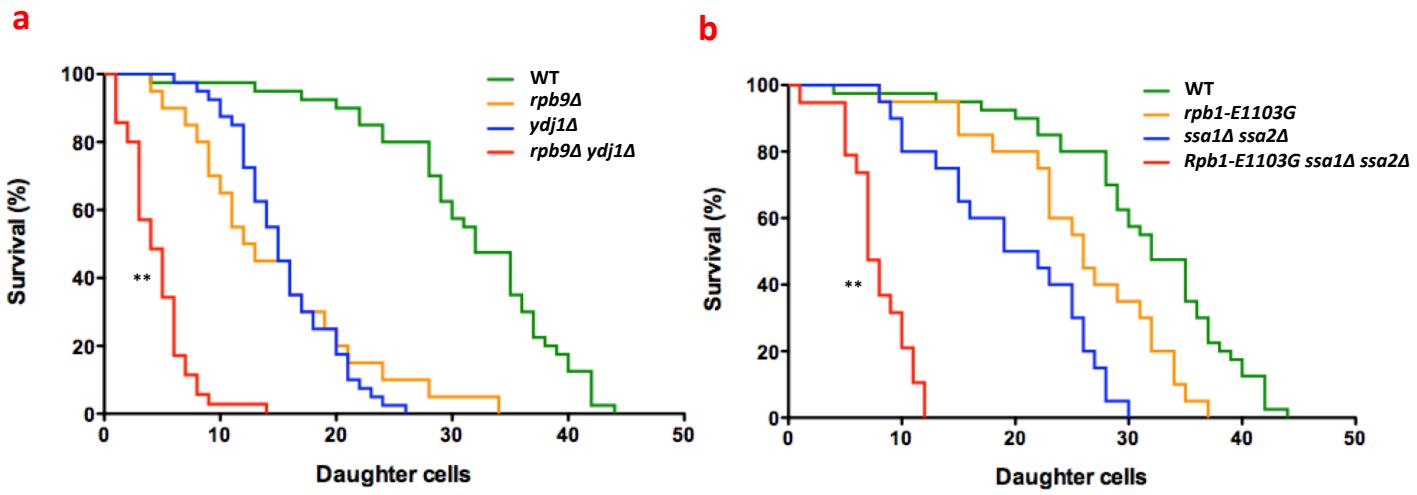

Fig. S6. Error prone cells have a shortened replicative lifespan in the absence of *YDJ1* or *SSA1* and *SSA2*. (a) When *YDJ1* is deleted in *rpb9Δ* cells, the lifespan of the *rpb9Δ* cells is reduced dramatically (MVY0001, 2, 4, 5). (b) When *SSA1* and *SSA2* are deleted in *rpb1-E1103G* cells, the lifespan of *rpb1-E1103G* cells is reduced dramatically as well (MVY0001, 2, 7, 8).

**= P<0.01

Figure S7

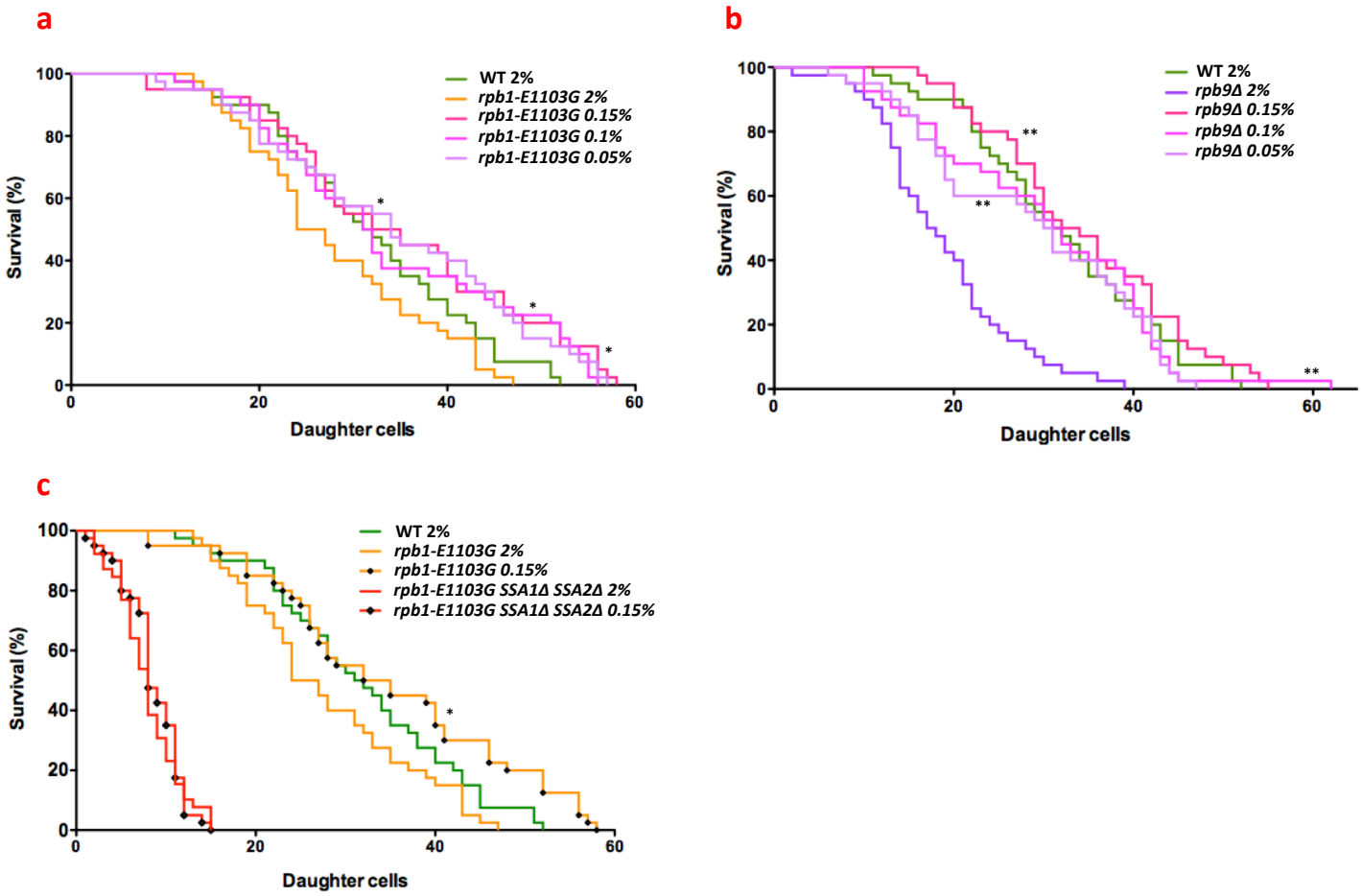

Fig. S7. Dietary restriction rescues the lifespan of error prone cells, but not error prone cells that have lost SSA1 and SSA2. (a) The lifespan of *rpb1-E1103G* cells is extended beyond the lifespan of WT cells when they are grown on 0.05, 0.1, or 0.15% glucose (MVY0001-2). (b) The lifespan of *rpb9Δ* 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 SSA2* cells (MVY0001, 2, 8).

*= P<0.05, **= P<0.01

Supplemental tables

Supplemental table 1

		rpb9		*rpb1-E1103G*	
Protein	**Function**	**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, macroautopphagy	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 gluthathione 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 suppreses 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
Idp1	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 Hso90 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

Table S1. 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 as well (blue, MVY0001-3)).