Supporting Information

Huberts et al. 10.1073/pnas.1410024111

SI Text

Explanation of Statistical Analysis of the Data Using a Linear Mixed Model. In our statistical analysis, we explain changes in average replicative lifespan (RLS) by means of three variables: glucose concentration in the medium [2%, 0.5%, and 0.05% (wt/vol)], strain (BY4741, BY4742, and BY4741/2), and laboratory. In our model, we consider lifespan data obtained for the yeast strain BY4741 at 2% (wt/vol) glucose as the reference condition (model intercept, I). Using the data obtained for BY4741 at 2% (wt/vol) glucose as a reference implies that the effects of glucose levels and strain types on average RLS are always determined relative to this reference (Tables S1 and S2). The variable "lab" has a special role because it is not expected to have any effect on the average RLS. In particular, it reflects the random variation in the data between laboratories that cannot be explained by glucose concentration and strain in the model. Accordingly, we consider the laboratory as a random effect, whereas the differences between glucose levels and strains appear as fixed parameters in the model. The coefficients associated with the variable lab are Gaussian random variables, which means are assumed to be zero and variances are estimated from the data. In essence, the variable lab reflects the error term of linear models, which is typically Gaussian, and the variance (the so-called residual variance) is obtained from the data. We defined random effects per glucose level to account for the variability present in the data from individual laboratories at different glucose concentrations. Note, models that combine fixed (i.e., glucose concentration and strain) and random effects (i.e., laboratory) are called mixed models in the statistical literature.

The formulation of the model is

$$\begin{aligned} RLS = {} & I + B_{BY4742}X_{BY4742} + B_{BY4741/2}X_{BY4741/2} \\ & + (B_{0.05} + R_{0.05})X_{0.05} + (B_{0.5} + R_{0.5})X_{0.5} + R_1X_2 + E \end{aligned} \qquad \text{(model 1)}$$

where RLS is the average RLS of the cells; I is the model intercept [=average RLS of strain BY4741 at 2% (wt/vol) glucose]; B_{BY4742}, $B_{BY4741/2}$, $B_{0.05}$, and $B_{0.5}$ are the parameters associated with the levels of the fixed effects of the model (glucose concentration and strain); R_I, $R_{0.5}$, and $R_{0.05}$ are random effects of the model, which are added to account for the random variation produced by the laboratory within the three available glucose levels [R_I, $R_{0.5}$, and $R_{0.05}$ are Gaussian random variables with a mean of zero and variances s^2(Intercept|laboratory), s^2(0.5|laboratory) and s^2(0.05|laboratory)]; E is the residual of the model, which is assumed to follow a Gaussian distribution with a mean of zero and variance s^2 (residual); $X_{BY4742} = 1$ for strain BY4742; otherwise $X_{BY4742} = 0$; $X_{BY4741/2} = 1$ for strain BY4741/2; otherwise $X_{BY4741/2} = 0$; $X_2 = 1$ when the glucose concentration is 2% (wt/vol); otherwise $X_2 = 0$; $X_{0.5} = 1$ when the glucose concentration is 0.5%; otherwise $X_{0.05} = 0$; and $X_{0.05} = 1$ when the glucose concentration is 0.05%; otherwise $X_{0.05} = 0$.

Note that for strain BY4741 at 2% (wt/vol) glucose, model 1 reduces to $RLS = I + R_I + E$, where the average RLS is I, with a random variation on top that is modeled by R_I [the random variation produced by the laboratories in 2% (wt/vol) glucose experiments] and E (the model errors). In a similar fashion, the model reduces to $RLS = I + B_{BY4742}\ X_{BY4742} + (B_{0.5} + R_{0.5})\ X_{0.5} + E$ for strain BY4742 at 0.5% glucose. Initially, we used all of the available data to determine the parameters in model 1 (Table S1). Then, to validate that the obtained estimates are robust against outliers, we redetermined the parameters in model 1 using lifespan data that lies within the 95% CI for each glucose concentration (Table S2).

1. Kruegel U, et al. (2011) Elevated proteasome capacity extends replicative lifespan in Saccharomyces cerevisiae. *PLoS Genet* 7(9):e1002253.
2. Kaeberlein M, Kirkland KT, Fields S, Kennedy BK (2004) Sir2-independent life span extension by calorie restriction in yeast. *PLoS Biol* 2(9):E296.
3. Kaeberlein M, et al. (2005) Regulation of yeast replicative life span by TOR and Sch9 in response to nutrients. *Science* 310(5751):1193–1196.
4. Tsuchiya M, et al. (2006) Sirtuin-independent effects of nicotinamide on lifespan extension from calorie restriction in yeast. *Aging Cell* 5(6):505–514.
5. Chen XF, Meng FL, Zhou JQ (2009) Telomere recombination accelerates cellular aging in Saccharomyces cerevisiae. *PLoS Genet* 5(6):e1000535.

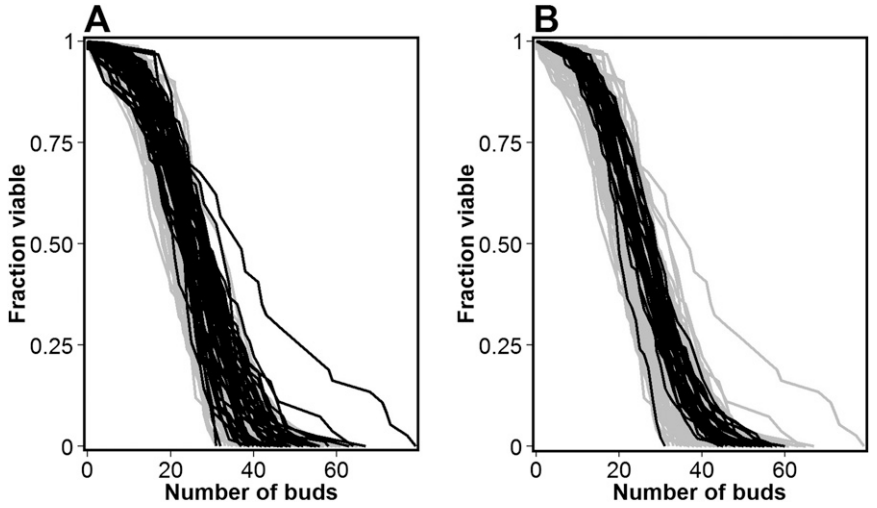

Fig. S1. Mating type does not affect RLS. Overview of RLS curves obtained at 2% (wt/vol) glucose presented by strain (black). For comparison all RLS curves obtained at 2% (wt/vol) glucose are plotted in the background in gray. (*A*) BY4741. (*B*) BY4742.

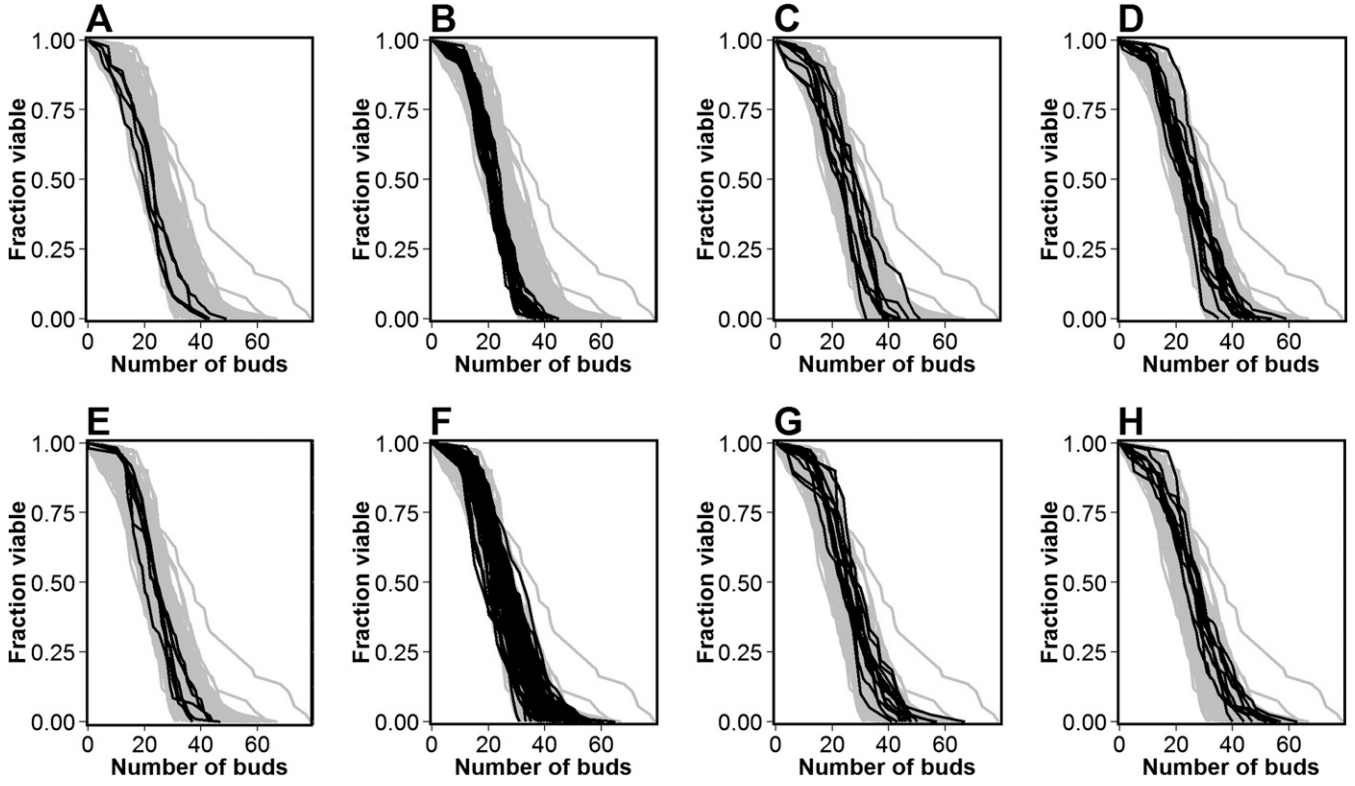

Fig. S2. There is a significant variation in RLS curves measured between laboratories. Overview of RLS curves at 2% (wt/vol) glucose per laboratory (black). For comparison all RLS curves obtained at 2% (wt/vol) glucose are plotted in the background in gray. (*A*) Lab 1. (*B*) Lab 2. (*C*) Lab 3. (*D*) Lab 4. (*E*) Lab 5. (*F*) Lab 6. (*G*) Lab 7. (*H*) Lab 8. For information on the laboratories, see Dataset S1.

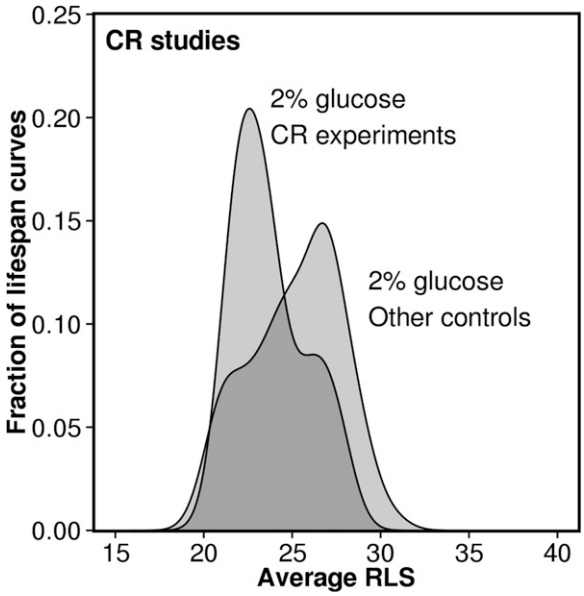

Fig. S3. The RLS of 2% (wt/vol) glucose calorie restriction (CR) control experiments is generally lower than that of other 2% (wt/vol) glucose controls. The average RLS of 2% (wt/vol) glucose CR controls reported by CR studies is 23.4 ± 0.3 buds, whereas the average RLS reported for other controls by CR studies is 25.9 ± 0.1 buds ($P = 0.001$).

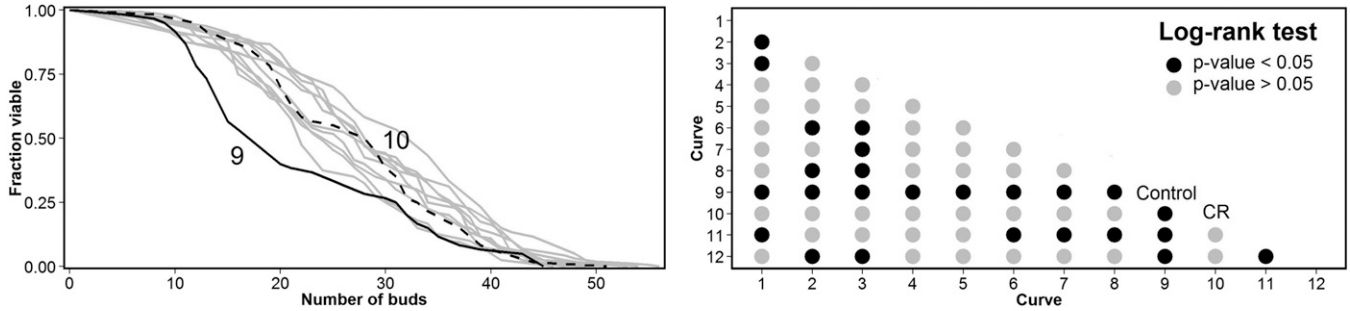

Fig. S4. (*Left*) The RLS curve used as a CR control is significantly shorter lived than the other 2% glucose controls. Overview of RLS data obtained from Kruegel et al. (1). The RLS curve measured under CR conditions is indicated with a dashed black line, whereas RLS curves measured at 2% (wt/vol) glucose are in black (used as CR control) and gray (used as other controls). One can observe that the RLS curve used as a CR control is shorter lived than the other 2% (wt/vol) glucose controls. (*Right*) Heatmap with pairwise comparisons showing the outcome of a pairwise comparison between all RLS curves within each paper using log-rank. $P < 0.05$ indictates that there a significant difference.

1. Kruegel U, et al. (2011) Elevated proteasome capacity extends replicative lifespan in Saccharomyces cerevisiae. *PLoS Genet* 7(9):e1002253.

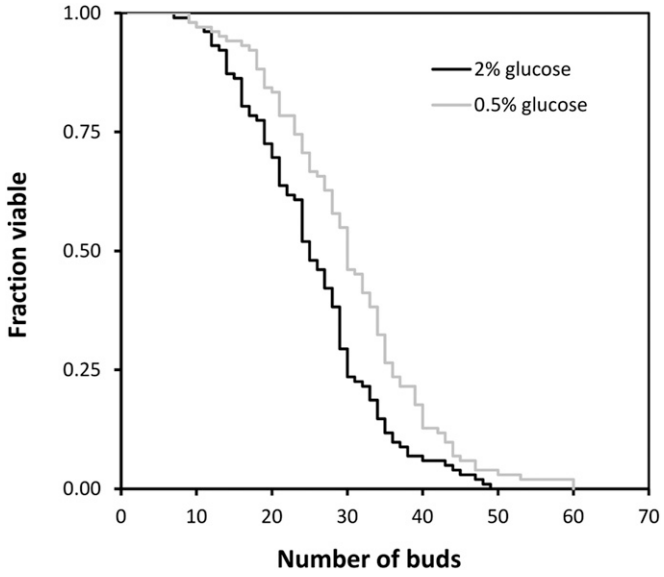

Fig. S5. CR extends the average RLS of BY4741 from 25.5 ± 0.9 buds at 2% (wt/vol) glucose (black; $n = 102$ cells) to 30.4 ± 1.0 buds at 0.5% glucose (gray; $n = 102$ cells; $P < 0.001$). RLS measurements were performed with the classical dissection method and yeast peptone dextrose (YPD) medium.

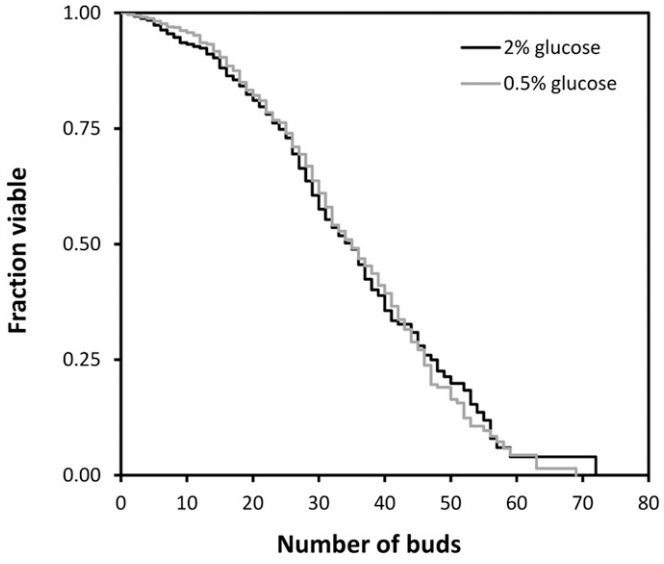

Fig. S6. CR does not extend the RLS of YSBN6 measured with the microfluidic dissection platform. We measured an identical average RLS for YSBN6 of 35.3 ± 0.9 buds at 2% (wt/vol) glucose (gray; five replicates; $n = 2,642$ of which 2,295 cells were washed out before death) and 35.3 ± 0.8 buds at 0.5% glucose (black; seven replicates; $n = 1,732$ cells of which 1,419 cells were washed out before death). Experiments were performed with yeast nitrogen base (YNB) medium.

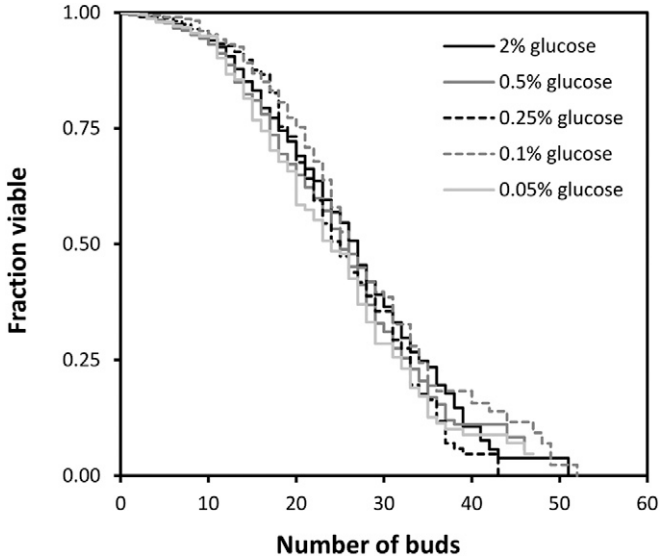

Fig. S7. Low concentrations of glucose do not extend RLS in the microfluidic dissection platform. We measured an RLS of 26.5 ± 0.5 buds at 2% (wt/vol) glucose (n = 2,806 cells of which 2,409 were washed out before death), 25.9 ± 0.7 buds at 0.5% glucose (n = 2,055 cells of which 1,716 were washed out before death), 25.5 ± 0.7 buds at 0.25% glucose (n = 601 cells of which 450 were washed out before death), 27.9 ± 1.0 buds at 0.1% glucose (n = 586 cells of which 472 were washed out before death), and 24.6 ± 0.8 buds at 0.05% glucose (n = 651 cells of which 476 were washed out before death). A reduction in the glucose concentration of the medium does not elicit an extension of RLS in the microfluidic dissection platform.

Table S1. Results of the mixed model analysis for the average RLS using glucose and strain as fixed factors and for laboratories, nested in glucose levels, as random factor

Parameter	Estimated value	SD	*P* value
I	25.650	0.659	<0.001
B_{BY4742}	−0.839	0.274	0.007
$B_{BY4741/2}$	−0.757	0.287	0.024
$B_{0.05}$	6.077	1.213	<0.001
$B_{0.5}$	7.385	1.081	<0.001
s^2 (intercept|laboratory)	7.505		
s^2 (0.05|laboratory)	9.237		
s^2 (0.5|laboratory)	6.164		
s^2 (residual)	88.569		

The reference level (intercept, I) is defined by cells in 2% (wt/vol) glucose medium belonging to strain BY4741.

Table S2. Results of the mixed model analysis for the average RLS using glucose and strain as fixed factors and for laboratories, nested in glucose levels, as random factor

Parameter	Estimated value	SD	*P* value
I	25.306	0.523	<0.001
B_{BY4742}	−0.565	0.275	0.095
$B_{BY4741/2}$	−0.527	0.287	0.146
$B_{0.05}$	5.872	0.959	<0.001
$B_{0.5}$	5.774	1.088	<0.001
s^2 (intercept|laboratory)	3.720		
s^2 (0.05|laboratory)	9.258		
s^2 (0.5|laboratory)	6.610		
s^2 (residual)	86.611		

In this case, we consider only data within the 95% CI at each glucose concentration. The reference level (intercept value, I) is defined by cells of strain type BY4741 cultured at 2% (wt/vol) glucose.

Dataset S1. Overview of median RLS determined for all lifespan data presented in this study; overview of the literature from which RLS curves were obtained; overview of RLS curves digitized from the literature; single cell lifespan data obtained from the literature; and single cell lifespan data generated in this study

Dataset S1