SUPPLEMENTARY MATERIAL

Construction and characterization of an improved DeaD strain

K6001 is a W303-derived strain that was originally constructed for use in the study of mother cell-specific mating type switching [72, 73]. K6001 encodes two integrated copies of the essential gene *CDC6*, independently regulated by the repressible *GAL1* promoter and the mother cell-specific *HO* promoter, respectively (*GAL1:Ub:CDC6* and *HO:CDC6).* Previously, we exploited this strain as an alternative to the standard microdissection method of quantifying replicative lifespan (RLS), which is labor intensive and slow [74]. When K6001 cells are grown permissively in galactose-containing media (raffinose/galactose), *GAL1:CDC6* is expressed both in mother and daughter cells and the culture grows exponentially. When expression of the *GAL1:CDC6* gene is repressed by glucose, only the mother-cell-specific expression of *HO:CDC6* remains to support growth. Since *HO* expression is largely restricted to mother cells, daughters generally do not divide and growth of the culture is limited by the RLS of the initial cohort of mother cells. Since the success of this strategy depends on the conditional Death of Daughter cells, we call it the "DeaD" assay. Although promising, K6001 as a DeaD strain exhibited significant limitations. Its mother cells cease division on glucose after an average of only 3-4 divisions [74]. K6001 also has a short average lifespan under permissive conditions, presumably due to its W303 background (17 generations for K6001 [74], similar to 20.8 generations for W303R [18]). Early tests of the assay showed a reduction of DeaD lifespan by deletion of *SIR2* [74] but these tests ignored the fact that the *HO:CDC6* fusion is haploid-specific [75]. Deletion of *SIR2* prevents silencing of the mating type information at *HML* and *HMR* and thus renders cells pseudo-diploid [76, 77]. After a switch to glucose, *sir2Δ* mutants will thus be unable to express either copy of *CDC6* (see below for mechanism of HO gene expression) and will die rapidly, as was observed [74]. When the opposite mating type information was deleted from this strain, allowing *HO:CDC6* expression to sustain mother cells in glucose, no difference in DeaD lifespan was observed between *SIR2* and *sir2Δ* cells (data not shown). Combined with the other limitations, this finding indicated that the strategy exemplified by K6001 would have to be modified before it could be used as a platform for studying replicative lifespan.

We began by switching the parental background from W303 to the longer-lived S288C background [78]. For galactose-specific *CDC6* expression, we chose the strategy employed in K6001: a ubiquitin:*CDC6* fusion driven by the *GAL1* promoter. The N-terminal ubiquitin fusion allows modification of the amino terminus of Cdc6 to reduce protein stability and tighten control over Cdc6 activity. The ubiquitin moiety is co-translationally removed by ubiquitin processing proteases, and the amino acid serving as the new amino terminus of Cdc6 determines its half-life according to the N-end rule [79].

We cloned the *GAL:Ub:CDC6* fusion from K6001 to a plasmid vector. DNA sequencing revealed a tyrosine codon at the beginning of *CDC6*, rather than the expected arginine [73]. Multiple independent clones from two separately obtained K6001 isolates gave the same result. According to the N-end rule, tyrosine is less destabilizing than arginine [79]. Rapid turnover of *GAL1*-expressed Ub-Cdc6 is required for efficient death of daughters after a shift to glucose. Arginine, which is maximally destabilizing [79], is thus the desired N-terminal residue. All of the clones also carried a conservative mutation in the ubiquitin moiety: arginine 74 to lysine. We used PCR mutagenesis to correct this mutation and to change the N-terminal residue of Cdc6 to either arginine or methionine. We then integrated these alleles, and the original K6001 allele, in place of the endogenous *CDC6* in the S288C-derived strain Y7092 [80]. Since the *GAL:Ub:CDC6* allele is the only source of *CDC6* in these strains, all cells arrest when transferred to glucose, and the efficiency of this arrest is a function of the stability of Cdc6. Cells expressing arginine at the N-terminus of Cdc6 achieved the most efficient growth arrest. (Fig. S1A). This allele was incorporated into all subsequent DeaD strains.

The *HO:CDC6* allele in K6001 is an imprecise fusion of the open reading frame of *CDC6* to the *HO* promoter: the fusion leaves in place more than 90bp of the *CDC6* 5' untranslated region, and all of the 3' *CDC6* untranslated sequences. This construction excludes the 3' UTR of *HO*, which appears to play a role in mother-cell-specificity of *HO* expression [81]. For our new DeaD strain, we therefore created a precise replacement of the *HO* open reading frame with *CDC6*, leaving the large *HO* promoter and the *HO* 3' untranslated region in place. This *HO:CDC6:HO* fusion was integrated into a strain already carrying *GAL1:UbR:CDC6* to create DeaD strain BB573. BB573 mother cells have improved survival on glucose compared to K6001 mother cells: mean RLS increased from 3.4 to 8.3 generations (p=5.94 x 10^{-13}, Fig. S1B). However, 8.3 generations is still much shorter than the 26-28 generation mean lifespan of normal SC288c-derived cells [78].

To further improve mother cell survival in glucose, we sought to increase mother cell expression of *HO:CDC6*.

Insight into expression of this fusion can be gained from studies of normal *HO*. In cells that express the wild-type HO endonuclease, mother cells switch mating type at a rate of ~70% [82]. Inhibition of switching in the remaining ~30% of mothers is dependent on the transcriptional repressor Ash1, since deletion of *ASH1* increases mother cell switching to 95-100% [72, 82]. These results indicate that Ash1 is normally incompletely excluded from mother cells. Ideally, Ash1 would be completely partitioned into the incipient daughter cell (the bud), leaving none in the mother cell to repress *HO* expression.

ASH1 is expressed at the end of mitosis, when the transcription factors Swi5 and Ace2 enter the nucleus and promote ASH1 expression by binding to four putative Swi5/Ace2 binding sites (predicted by the nucleotide sequence kGCTGr, where "K" is G or T and "R" is A or G, [83] in the *ASH1* promoter. *ASH1* expression is dramatically decreased in *swi5 ace2* double mutants [72, 84]. We deleted the Swi5/Ace2 binding sites, either *in toto* or in pairwise combinations, in BB573 and assessed the effect on mother and daughter cell survival on glucose by pedigree analysis (Fig. S2). Deletion of the 1st two Swi5p binding sites (*ASH1-Δ12*) caused too drastic a drop in Ash1: daughter cell survival dramatically increased (represented by tall bars in Fig. S2 panel B), compare with parental strain BB573, Fig S2 panel A). This effect was caused to a lesser extent by deletion of the middle two sites (*ASH1-Δ23*, Fig. S2 panel C) or of all four binding sites (*ASH1-Δ14*, Fig S2 panel D). In contrast, deletion of the 3rd and 4th Swi5p binding sites produced a strain with enhanced mother cell survival without increased daughter cell "escape" (Fig S2 panel E). This strain, *ASH1-Δ34*, was renamed BB579 and it and its derivatives were used in all subsequent work. BB579 has a mean survival of 11.3 generations on glucose (longer than its parental strain, BB573, p=1.77 x 10^{-7}) corresponding roughly to a model combining Gompertzian senescence with a stochastic death rate of 7.5% (Fig S3 panel A).

Yeast cells become sterile near the end of their lifespan due to de-repression of the silent *HM* mating type loci and the resulting pseudo diploidy [85]. De-repression of the *HM* loci in a DeaD strain will result in failure to express haploid-specific *HO:CDC6*, as discussed above, and which might cause premature death in aging cells and an artificially short lifespan. To investigate the potential benefit of preventing pseudo diploidy by deleting one of the two mating type loci, *HMR*, we deleted HMR in *SIR2* and *sir2Δ* BB579 cells. We performed microdissection (Fig. S3 panels A and B) and DeaD lifespan assays (Fig. S3 panel C) of parental BB579 (*HMR*), *hmrΔ*, *sir2Δ* and *hmrΔ sirΔ* strains. Deletion of *SIR2* alone produced an extremely short

apparent RLS, presumably due to repression of HO expression resulting from pseudo diploidy, and deletion of *HMR* and *SIR2* together gave an intermediate phenotype (Fig. S3 panels A and C) in both the microdissection and DeaD lifespan assays. None of these deletions had significant effects on permissive growth in galactose, although the *hmrΔ sir2Δ* strain exhibited a minor growth defect (Fig. S3 panel D). By microdissection RLS assay, deletion of *HMR* slightly extended RLS: mean RLS increased from 11.3 to 12.1 generations (p=1.5 x 10^{-2}), and maximum increased from 32 to 45 (Fig. S3 panel B). Deletion of *SIR2* in *hmrΔ* BB579 cells shortened RLS to a mean of 8.6 generations (p=1.45 x 10^{-4}), with a maximum of 33 generations (Fig. S3 panel A).

The above results demonstrate that *SIR2* strains have longer DeaD assay lifespans than *sir2Δ* strains in the improved BB579 background. To test this relationship further, and to determine whether the DeaD assay is sensitive not only to lifespan shortening but also to lifespan extension, we asked whether we could detect a range of effects on RLS by altering the expression level of *SIR2* with a series of promoter fusions. Replicative lifespan varies with *SIR2* expression level in yeast, since deletion of *SIR2* shortens lifespan and an extra copy of *SIR2* extends it [18]. There is likely to be an upper limit to lifespan extension by up-regulation of *SIR2*, however, since *SIR2* overexpression from the *GAL1* promoter causes toxicity and elevated rates of chromosome loss [86]. For our test, we used PCR-based integration to replace the endogenous *SIR2* promoter with the *CYC*, *ADH*, *TEF*, or *GPD* promoter [87] in an *HMR*-deleted BB579 derivative. DeaD assay lifespan of the resulting strains paralleled the predicted promoter strength (*CYC<ADH<TEF<GPD*, [87]), with strains carrying the strongest two promoters showing extension of DeaD assay lifespan (Fig. S4 panel C).

As expected if strong overexpression of *SIR2* is toxic, the *GPDpr:SIR2* fusion, which we expect to be more strongly expressed than the *TEFpr:SIR2* fusion [87], confers no additional advantage for lifespan (Fig. S4 panel C). None of these deletions had significant affects on permissive growth in galactose, although the *hmrΔ sir2Δ* strain exhibited a minor growth defect (Fig. S3 panel B).

In a parallel set of experiments, we replaced the endogenous *SIR2* promoter with the same promoter series in a wild-type S288C strain and measured replicative lifespan using the standard microdissection assay. The results (Fig.4S Panel D) are a striking parallel to the DeaD assay findings. The *TEF* promoter extended mean lifespan by a robust 45%, while the stronger *GPD* promoter showed a lesser extension of

22%. We conclude that improvements to K6001 engineered into BB579 allow recapitulation of key features of yeast aging using high throughput capable liquid growth-based assays, including lifespan shortening and extension by under- and overexpression of *SIR2*.
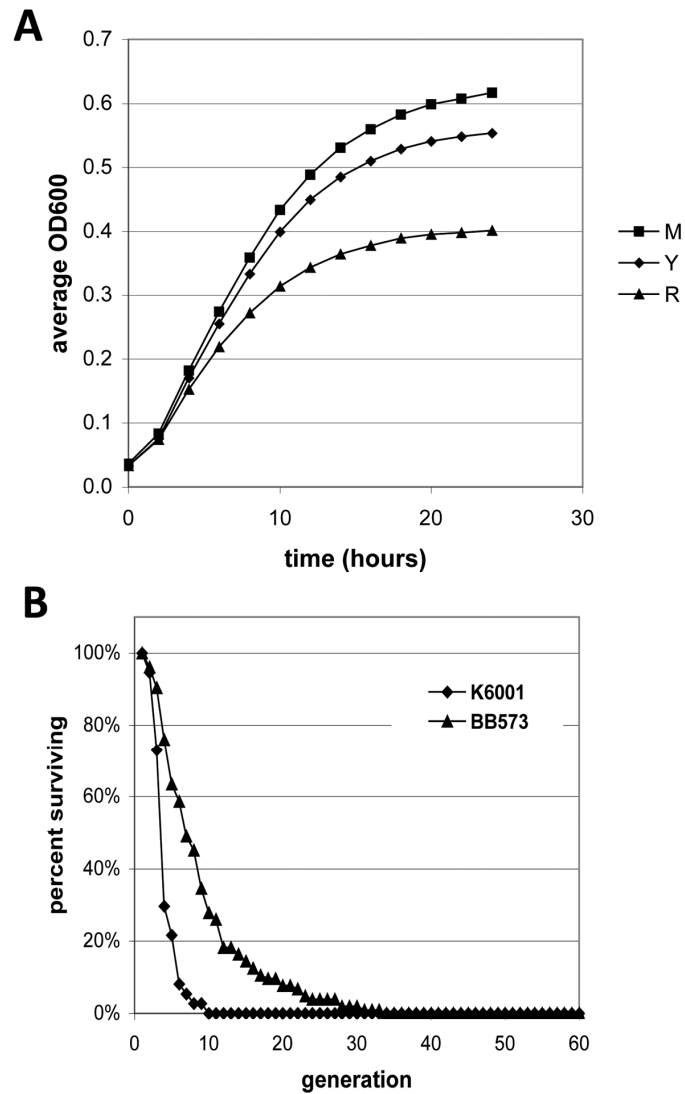

Figure S1. **Characterization of improved DeaD strain BB573.** (**A**) Arrest of *GAL:Ub:CDC6* strains in glucose is dependent on the N-terminal residue of Cdc6p. Cells were transferred to glucose at time 0 and growth arrest was monitored by measuring the culture OD600. The letter indicates the N-terminal Cdc6p residue: M: methionine, Y: tyrosine, or R: arginine. (**B**) BB573 mother cells have improved survival on glucose compared to K6001 mother cells: mean RLS increased from 3.4 to 8.3 generations (p=5.94 x 10^{-13}).
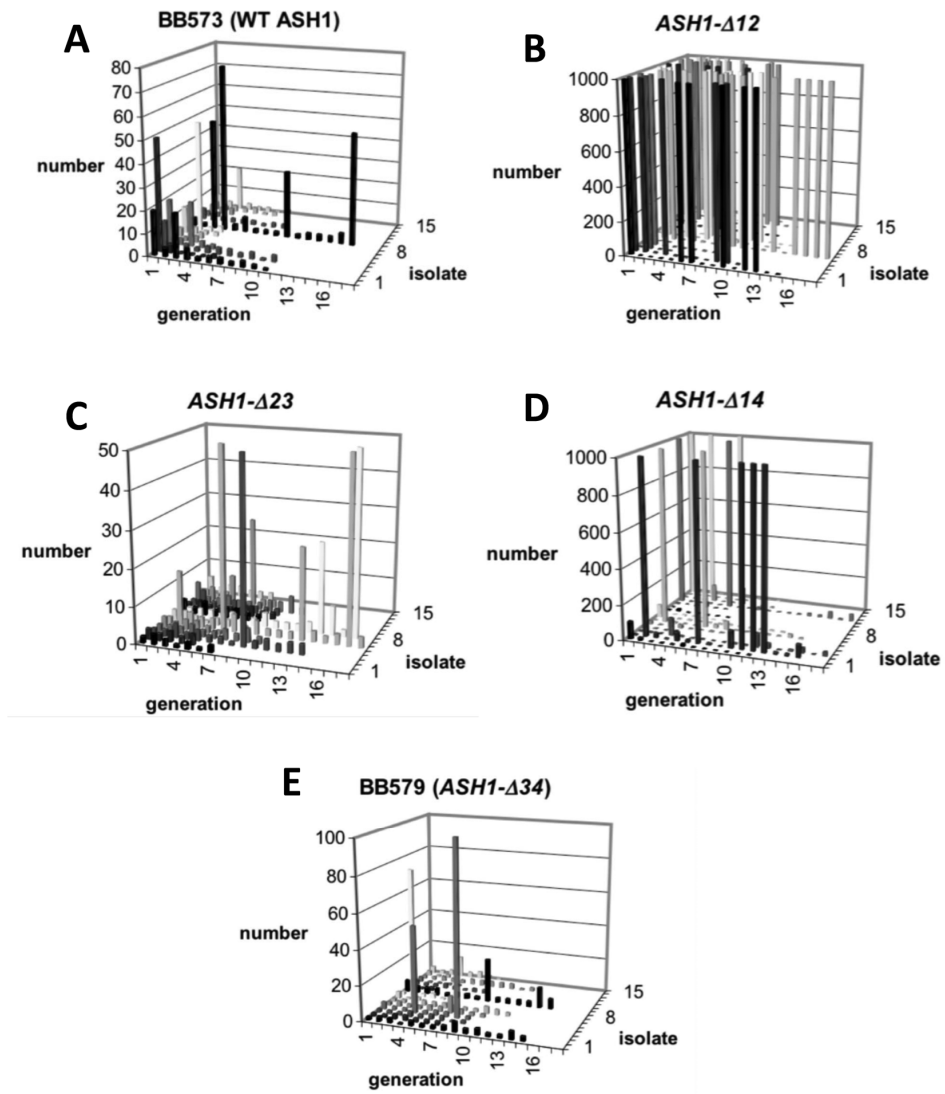
Figure S2. Pedigree analysis of BB573 and derivatives carrying *ASH1* promoter deletions. Strains were grown in a galactose-containing medium to mid-log phase and arrayed on glucose (SCD) plates for microdissection. Divisions of mother cells are represented on the x axis, and divisions of daughter cells by the height of the bars.
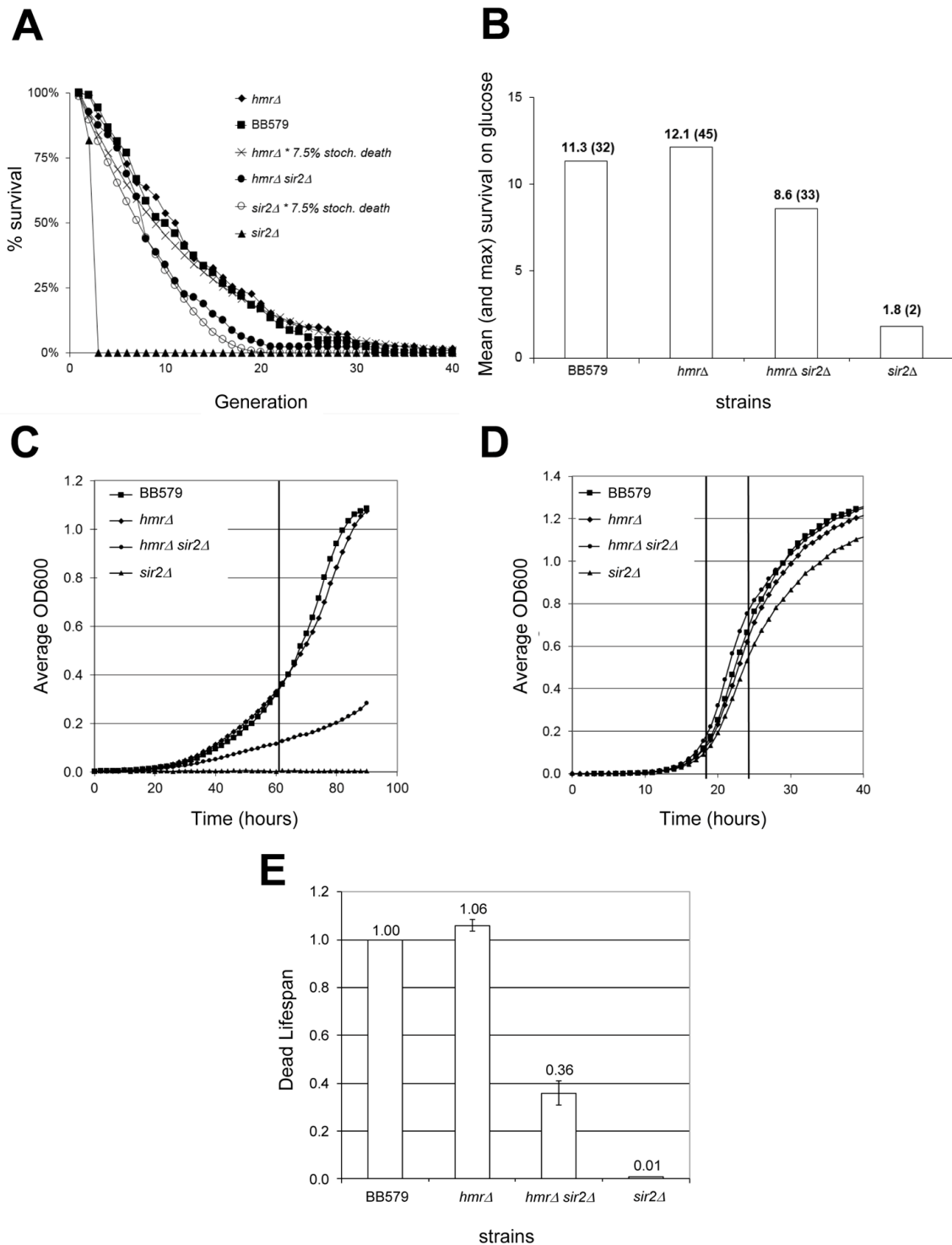
Figure S3. The effect of _hmr_ and _sir2_ deletions on mother cell survival and DeaD lifespan in new DeaD assay strain BB579. (**A**) Survival of BB579 and its _hmrΔ hmrΔsir2Δ_, and _sir2Δ_ derivatives on glucose. Strains were grown to mid-log phase in a galactose-containing medium and arrayed on glucose (SCD) plates. Mother cell survival was determined by microdissection. For comparison, models of BY4741 ("x"s) and its _sir2Δ_ derivative (open circles) with a 7.5% rate of stochastic death are included. (**B**) Mean and maximum replicative lifespans from (**A**). (**C**) Representative restrictive liquid cultures of the same four strains. (**D**) Parallel permissive liquid cultures. (**E**) Relative DeaD assay lifespans of the four strains, using data from (**C**) and (**D**) together with two more independent experiments. Error bars are +/- one standard error of the mean of the three experiments.
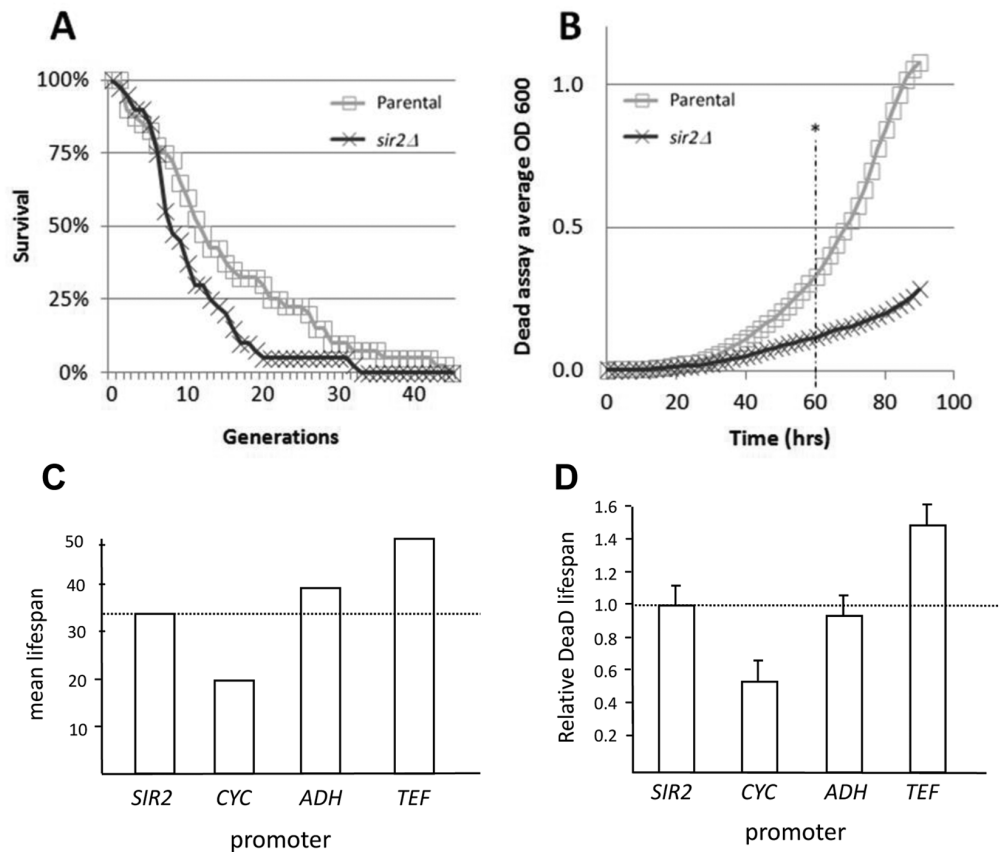
Figure S4. DeaD assay recapitulates microdissection replicative lifespan measurement. (**A**) In the traditional microdissection replicative lifespan method the DEAD strain deletion of *SIR2* results in a drop in average lifespan from 15.5 generations (□ Parental) to 10.5 generation (X *sir2Δ*), a 32.25% decrease. (**B**) In the DeaD assay the average lifespan decrease (* measured at 60hrs) from optical density of 0.331 (□ Parental) to 0.116 (X *sir2Δ*), a 65% drop in lifespan. (**C**) & (**D**) When *SIR2* expression levels are varied by promoters of varying strength from low (CYC), normal (ADH) and high (TEF), relative and mean lifespan as reported by the DeaD assay also show reduced, normal, and extended levels.
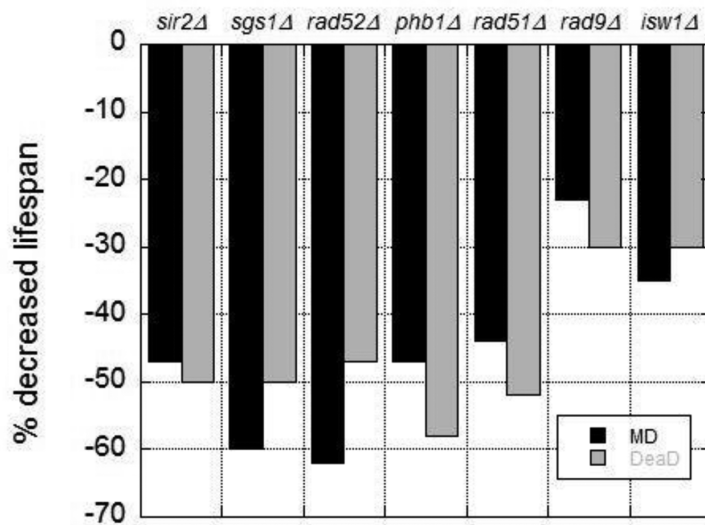

Figure S5. DeaD assay reports replicative lifespan for several strains that show reduced lifespan in the traditional microdissection assay. Yeast strains were obtained from the gene deletion collection. Replicative lifespan (%) for both microdissection and DeaD assay were determined as described in Methods.
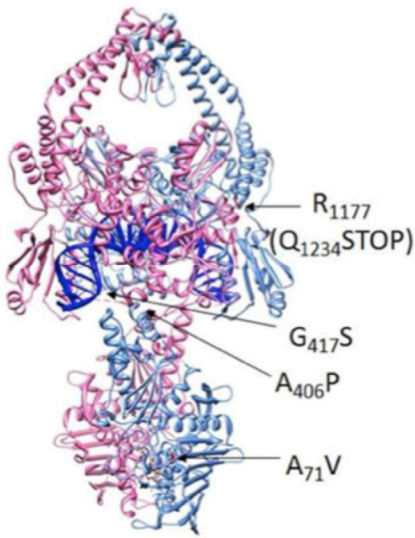
Figure S6. Map of LS1 resistant hypomorphic _top2_ alleles. UCSF Chimera was used to display the X-ray structure of yeast Top2 and map the location of each of the LS1 resistant hypomorphic alleles onto the protein structure. The dispersed locations of each mutation are consistent with other _top2_ alleles previously found to confer broad resistance to Top2 poisons such as DOX or ETOP.

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