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The SAGA Histone Deubiquitinase Module Controls Yeast Replicative Lifespan via Sir2 Interaction

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SUMMARY

We have analyzed the yeast replicative lifespan of a large number of open reading frame (ORF) deletions. Here, we report that strains lacking genes *SGF73*, *SGF11*, and *UBP8* encoding SAGA/SLIK complex histone deubiquitinase module (DUBm) components are exceptionally long lived. Strains lacking other SAGA/SALSA components, including the acetyltransferase encoded by *GCN5*, are not long lived; however, these genes are required for the lifespan extension observed in DUBm deletions. Moreover, the *SIR2*-encoded histone deacetylase is required, and we document both a genetic and physical interaction between DUBm and Sir2. A series of studies assessing Sir2-dependent functions lead us to propose that DUBm strains are exceptionally long lived because they promote multiple prolongevity events, including reduced rDNA recombination and altered silencing of telomere-proximal genes. Given that ataxin-7, the human Sgf73 ortholog, causes the neurodegenerative disease spinocerebellar ataxia type 7, our findings indicate that the genetic and epigenetic interactions between DUBm and SIR2 will be relevant to neurodegeneration and aging.

INTRODUCTION

Aging is a fundamental biological process of great interest. Although yeast cells may seem an unlikely model organism to study human aging, insights from the study of yeast replicative lifespan (RLS), in which the number of daughter cells that one mother produces through budding is determined (Mortimer and Johnston, 1959), have advanced our understanding of eukaryotic and even mammalian aging (Steinkraus et al., 2008). Early studies led to the identification of the protein deacetylase encoded by *SIR2* as a key modulator of aging, and subsequently Sirtuins have become a major focus of aging research (Guarente, 2011; Kaeberlein et al., 1999; Kennedy et al., 1995). Additionally, studies in yeast as well as other invertebrates have highlighted the importance of the TOR pathway in aging (Kaeberlein et al., 2005b; Stanfel et al., 2009). Furthermore, a quantitative comparison of aging genes in yeast and worms has demonstrated with high statistical significance that longevity pathways are conserved between yeast and *C. elegans* (Smith et al., 2008), two organisms more divergent than worms are from humans. Hence, there is strong evidence to conclude that the orthologs of other yeast aging genes may influence mammalian aging.

Spinocerebellar ataxia type 7 (SCA7) is an autosomal-dominant neurodegenerative disease resulting from the expansion of a polymorphic and unstable CAG tract in the ataxin-7 gene (David et al., 1997). In affected individuals, the CAG tract is translated into an abnormally long stretch of glutamine residues in the N terminus of the ataxin-7 protein. When containing a track of 37 or more glutamine residues, polyglutamine-expanded ataxin-7 is not readily degraded, accumulates in protein aggregates, and causes neuronal dysfunction and neuronal cell death in the retina, cerebellum, and associated brainstem structures. This results in blindness, a severe loss of coordination, and ultimately premature death (Lebre and Brice, 2003). Ataxin-7 is a highly conserved member of the SPT3-TAFII31-GCN5L acetylase (STAGA) complex, one of the major transcriptional

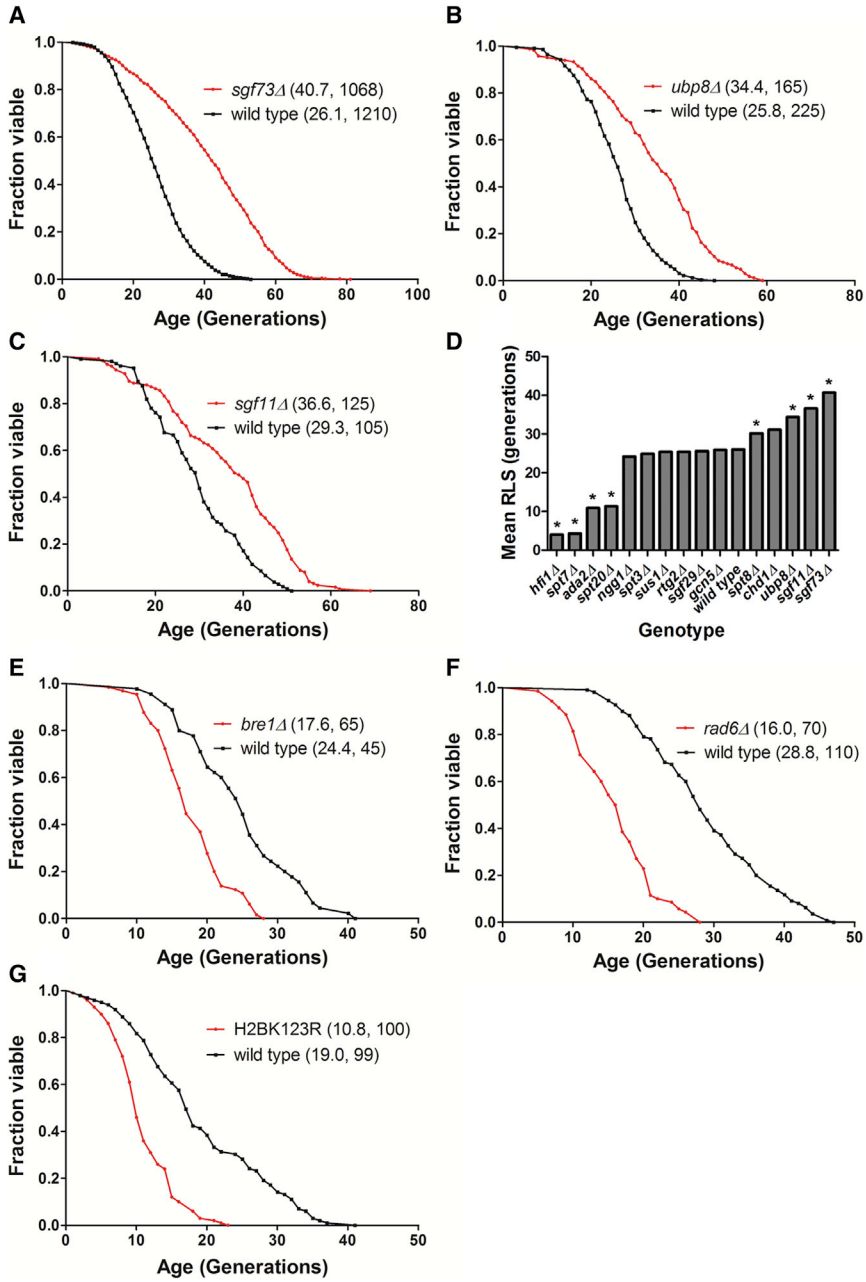

Figure 1. Deletion of SAGA DUBm Components *SGF73*, *UBP8*, and *SGF11* Significantly Increases Yeast RLS, and Mutation of H2B-K123 or Deletion of Its Monoubiquitinating Enzymes Shortens RLS

(A) *sgf73Δ*.
(B) *ubp8Δ*.
(C) *sgf11Δ*.
(D) Summary of remaining viable SAGA component deletions.
(E) *bre1Δ*.
(F) *rad6Δ*.
(G) H2B-K123R.
Legends show mean RLS and number of mother cells scored. See also Figures S1–S3.

coactivator complexes in mammalian cells (Helmlinger et al., 2004; Martinez et al., 2001). More specifically, ataxin-7 is a component of the USP22 histone deubiquitinase module (DUBm) of the complex.

Based on limited sequence homology, *SGF73* was proposed to be the yeast ortholog of ataxin-7 (Mushegian et al., 2000; Scheel et al., 2003), and later functional studies demonstrated this to be the case (Mal, 2006). In the DUBm of the yeast SAGA (Spt-Ada-Gcn5-acetyltransferase) complex, which is analogous to the mammalian STAGA (SPT3-TAFII31-GCN5L acetylase) complex, Sgf73 serves to link the histone deubiquitinase Ubp8 to the rest of the complex (Lee et al., 2009). This function has also been proposed for ataxin-7 in mammals, where USP22, the ortholog of Ubp8, possesses H2B deubiquitinase activity (Zhang et al., 2008; Zhao et al., 2008). Ubp8 deubiquitinates histone H2B-K123 to confer large-scale SAGA-mediated transcriptional changes (Daniel et al., 2004; Henry et al., 2003).

In an ongoing genome-wide screen for long-lived yeast open reading frame (ORF) deletions, we identified *sgf73Δ* as having a dramatically enhanced RLS, on par with the longest-lived single deletions that we found (Sutphin et al., 2012). Strains lacking other components of the DUBm also have exceptional RLS extension, but those lacking other SAGA components, including the histone acetyltransferase Gcn5, are not long lived. Unexpectedly, lifespan extension in *sgf73Δ* and *ubp8Δ* depends entirely on *SIR2*, and further analysis indicates that lifespan extension in strains lacking components of the SAGA deubiquitinase complex occurs through coordinate control of multiple longevity pathways. Sgf73 can also physically interact with Sir2 and Ubp8, a finding replicated in mammals by interaction of USP22 with human SIRT1 (Armour et al., 2013; Lin et al., 2012), suggesting that altered chromatin-modifying activities in the CNS may contribute to SCA7 neurodegeneration.

RESULTS

SAGA DUBm Components Limit Yeast Replicative Lifespan

As part of an ongoing assessment of the effects of nonessential yeast genes on yeast replicative lifespan, we discovered that the *sgf73Δ* strain was one of the longest-lived strains yet identified, extending median and maximum lifespan by 65% and 53%, respectively (Figure 1A). *SGF73* encodes a protein in the yeast

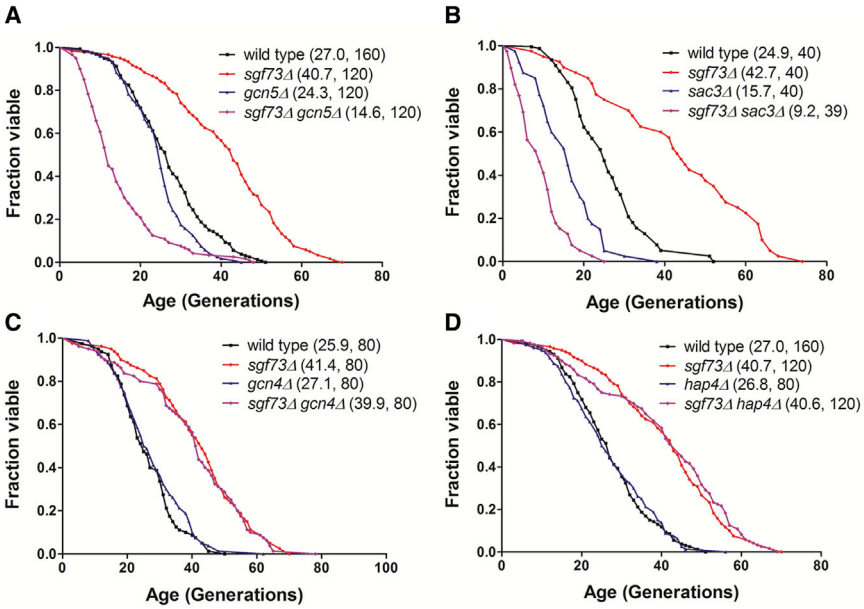

Figure 2. RLS Epistasis to Determine the Role of SAGA Components and Other Nuclear Transcription Factors in *sgf73Δ* Life Extension

Gene encoding the SAGA component *GCN5* (A) is necessary for the extended RLS of *sgf73Δ*, while those encoding transcription factors *GCN4* (B) and *HAP4* (C) are not. Legends show mean RLS and number of mother cells scored.

SAGA and SLIK (SAGA-like) complexes, chromatin-modifying machines that control transcription of a large set of genes. These complexes contain at least two enzymatic activities: Gcn5 has histone acetyltransferase activity, and Ubp8 is a histone deubiquitinase that targets the histone H2B-K123 residue. A component of a four-protein DUBm, Sgf73 serves as a linking factor keeping the DUBm connected with the rest of the SAGA or SLIK complex (Lee et al., 2009). Therefore, we determined the lifespans of the other three components of the DUBm, and found that both *sgf11Δ* and *ubp8Δ* strains had robust lifespan extension (Figures 1B and 1C). Strains lacking the fourth component, *SUS1*, are not long lived (Figure S1A). Sus1 has other functions in addition to its activity in SAGA, which may make its role in yeast RLS unpredictable. Strains lacking both *SGF73* and *UBP8* have lifespans identical to the *SGF73* single deletion, consistent with the prediction that both deletions enhance lifespan by a similar mechanism and cause increased levels of ubiquitinated H2B (Figure S1B) (Köhler et al., 2008).

We then determined the lifespan of yeast deletions lacking other nonessential components of SAGA (Figure 1D; Figure S2). For the most part, these strains were either not long lived or had shortened lifespans. Exceptions were *chd1Δ*, which encodes a chromodomain protein involved in maintaining chromatin structure during transcription by preventing histone exchange (Smolle et al., 2012), and *spt8Δ*, which encodes a protein unique to the SAGA complex, influencing the TBP-TATA interaction at promoters (Belotserkovskaya et al., 2000). Interestingly, the *gcn5Δ* strain had no detectable effect on RLS. We conclude from these studies that enhanced RLS derives not from reduced SAGA function, but instead from a more specific effect linked to reduced DUBm function or to uncoupling of the acetyltransferase and deubiquitinase sub-complexes.

A primary target for Ubp8-mediated deubiquitination is histone H2B-K123. Monoubiquitination of this residue is mediated by a heterodimeric complex composed of Bre1, an E3 ubiquitin ligase, and Rad6, an E2 ubiquitin-conjugating enzyme. We determined the lifespan of yeast strains lacking *BRE1* or *RAD6*, finding that they were both short-lived (Figures 1E and 1F). These data are consistent with the interpretation that replicative lifespan is mediated by H2B-K123 monoubiquitination and that higher levels of ubiquitination lead to enhanced lifespan. It should be noted that Rad6 serves as an E2 ubiquitin-conjugating enzyme for other E3s in addition to Bre1; hence, we cannot rule out the possibility that the short lifespan of *rad6Δ* could be attributable to other causes. To test the role of H2B-K123 monoubiquitination more directly, we determined the lifespan of a strain with one integrated copy of a *H2BK123R* mutation that is refractory to ubiquitination (and one wild-type *H2B* allele, since the gene is duplicated) (Figure 1G). Interestingly, this mutant was short-lived relative to a wild-type control, again consistent with an association of ubiquitination at H2B-K123 with enhanced longevity.

In addition to Ubp8, Ubp10 can also deubiquitinate histone H2B-K123 at alternative sites in the genome (Schulze et al., 2011). Therefore, we determined whether an *ubp10Δ* mutant would also exhibit enhanced lifespan. Instead, lifespan was reduced in this strain (Figure S3). This finding suggests that increased H2B-K123 ubiquitination at the sites normally regulated by Ubp8 and not Ubp10 mediates lifespan extension (Emre et al., 2005).

Lifespan Extension by *sgf73Δ* or *ubp8Δ* Is SAGA Dependent but Not SLIK Dependent

To further delineate the link between SAGA function and longevity, we performed epistasis studies to determine whether non-DUBm components of the SAGA complex are required for lifespan extension associated with *sgf73Δ* and *ubp8Δ*. For genes encoding most components of the complex, including strains such as *gcn5Δ* (Figure 2A), loss of *SGF73* not only fails to lengthen lifespan but also shortens it significantly. These findings are specific for SAGA components, as loss of other nuclear transcription factors, such as Gcn4 (Figure 2B), which blocks the lifespan extension of other long-lived mutants, and Hap4 (Figure 2C), has no impact on lifespan extension in *sgf73Δ* cells (Steffen et al., 2008).

Our data indicate that loss of SAGA components reverses the longevity benefits of *sgf73Δ*; in other words, loss of DUBm components shortens the lifespan of strains lacking SAGA

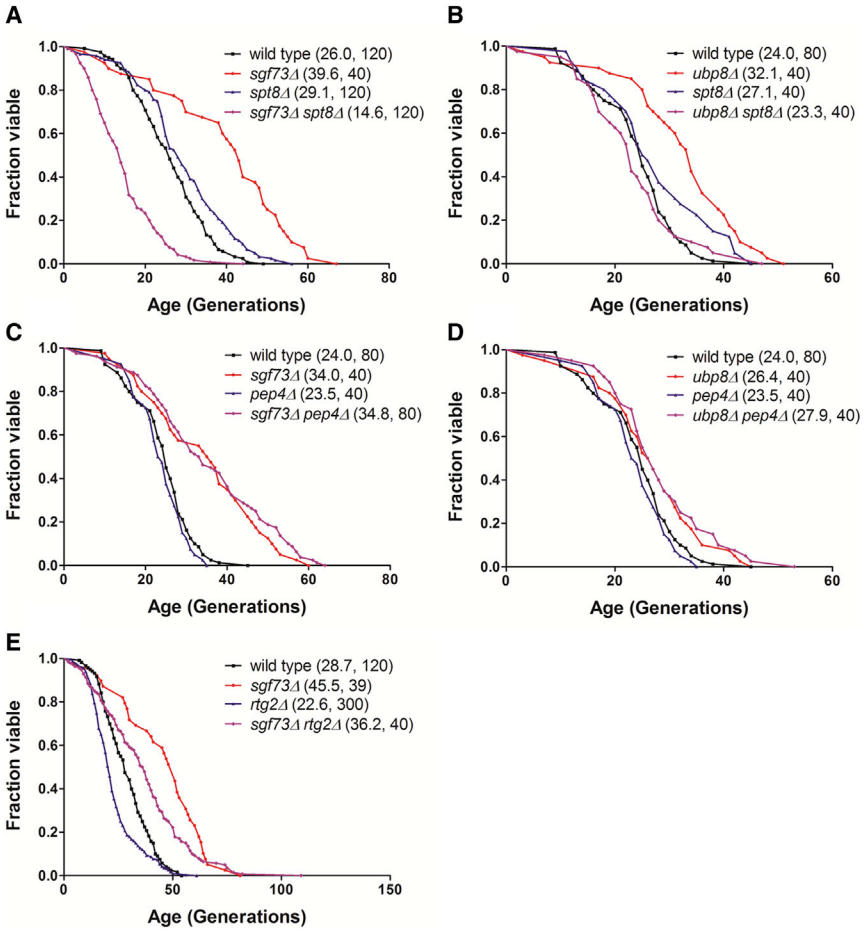

Figure 3. RLS Epistasis to Determine the Relative Roles of SAGA and SLIK in *sgf73Δ* and *ubp8Δ* Lifespan Extension

The SAGA-specific component *SPT8* is necessary for the extended RLS of (A) *sgf73Δ* and (B) *ubp8Δ*, while neither *PEP4*, the protease necessary for processing of the SLIK-specific form of Spt7 (C, D), nor SLIK-specific component *RTG2* (E) are necessary. Legends show mean RLS and number of mother cells scored. See also Figure S4.

components rather than lengthening them. We wanted to determine whether this effect was specific to SAGA or SLIK, both of which contain Gcn5. Therefore, we conducted a series of experiments to distinguish between the two complexes by using genetic approaches known to specifically disrupt each complex. First, we determined whether Spt8, a component of SAGA and not SLIK, was required for lifespan extension in *sgf73Δ* or *ubp8Δ*, finding that lifespan effects were reversed in this genetic background in a manner similar to that of *gcn5Δ* (Figures 3A and 3B). Second, we determined the consequences of deletion of *PEP4*, which encodes an endopeptidase that cleaves full-length Spt7 (associated with SAGA) into a smaller truncated form that associates specifically with SLIK (Spedale et al., 2010). In contrast to the findings with *SPT8*, both the *sgf73Δ pep4Δ* and *ubp8Δ pep4Δ* strains retained a long lifespan (Figures 3C and 3D). Together, these findings indicate that lifespan extension by loss of DUBm components requires an otherwise intact SAGA complex, but not SLIK complex. To confirm this, we also checked the effects of deleting *RTG2*, another SLIK-specific component that is of particular interest, because it is linked to the retrograde response that communicates mitochondrial stress signals to the nucleus to evoke transcriptional responses. This pathway has been linked to RLS regulation in other contexts (Jazwinski, 2005). However, we found similar results to those in the *pep4Δ* strain: loss of *SGF73* still resulted in lifespan extension to a comparable extent (Figure 3E). As expected, *rtg2Δ* alone was slightly short-lived in this strain background, as previously reported (Kaeberlein et al., 2005a). Two other components of retrograde signaling, *RTG1* and *RTG3*, are also not required for lifespan extension by *sgf73Δ* (Figure S4). These findings indicate that the integrity of the rest of the SAGA complex is required for lifespan extension by loss of deubiquitinase components.

The Increased RLS of *sgf73Δ* and *ubp8Δ* Requires *SIR2*

To explore the mechanism of DUBm-deficient lifespan extension, we performed epistasis analysis using several known lifespan-modulating genes. First, we determined whether *sgf73Δ* functions through the Sir2 longevity pathway by deleting *SGF73* or *UBP8* in a *sir2Δ* background. Strains lacking *SIR2* are very short-lived, due to ERC accumulation and possibly other *SIR2*-dependent mechanisms (Kaeberlein et al., 1999). Previous studies reported that other aging pathways do not make a significant contribution in the *sir2Δ* background; hence, genetic interventions that modulate these pathways no longer extend lifespan (Kaeberlein et al., 2004). We found a similar result for *sgf73Δ* and *ubp8Δ*, with these deletions failing to extend the *sir2Δ* lifespan (Figures 4A and 4B). However, this finding is difficult to interpret. Whereas loss of *SIR2* may indeed be epistatic to loss of *SGF73* and *UBP8*, more often we have found that longevity interventions that fail to extend RLS in the *sir2Δ* background retain their ability to do so in *sir2Δ fob1Δ* strains (Delaney et al., 2011; Kaeberlein et al., 2004), which have a RLS similar to wild-type strains, most likely due to the suppressive effect of *fob1Δ* on ERC formation (Defossez et al., 1999; Kaeberlein et al., 1999). Thus, it was possible that *sgf73Δ* and *ubp8Δ* strains would be long lived in a *sir2Δ fob1Δ* background. However, neither loss of *SGF73* nor loss of *UBP8* affected longevity in the *sir2Δ fob1Δ* background (Figures 4C and 4D). These findings are consistent with a model in which deletion of components of the DUBm influences aging by enhancing or altering Sir2 function. One surprising facet of this series of experiments is that the replicative lifespan of either *sgf73Δ* or *ubp8Δ* far surpasses the extension observed

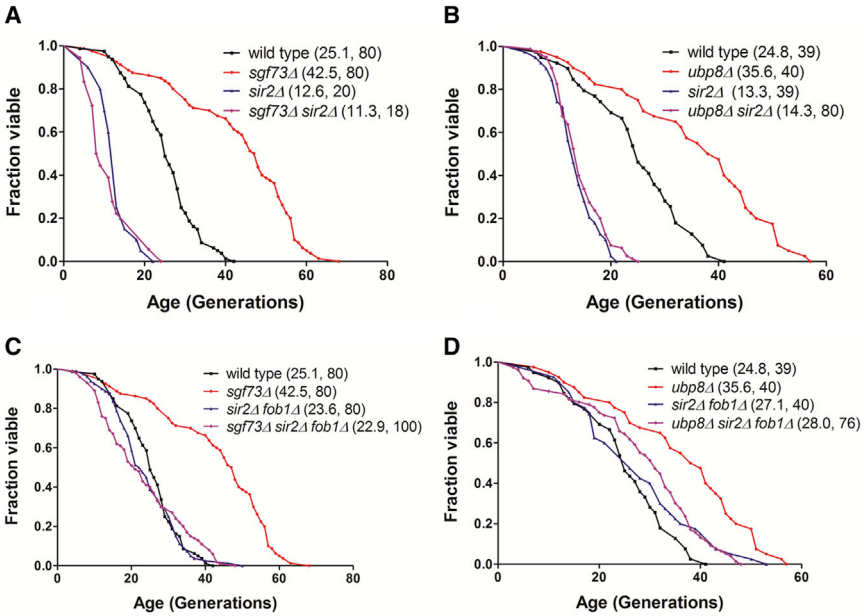

Figure 4. *SIR2* Is Necessary for the Extended RLS of *sgf73Δ* and *ubp8Δ* Even in Strains Lacking *FOB1*

(A) *sgf73Δ*.

(B) *ubp8Δ*.

(C and D) Strains Lacking *FOB1*.

(E) *SIR2OE ubp8Δ* double shows extended lifespan comparable to *sgf73Δ*. Legends show mean RLS and number of mother cells scored.

See also Figure S5.

by overexpression of *SIR2* or deletion of *FOB1* (Figure S5) (Defossez et al., 1999; Kaeberlein et al., 1999). A possibility is that overexpression of *SIR2* and deletion of *UBP8* might lead to lifespan extension by at least partially independent mechanisms, both of which are apparent in the *sgf73Δ* strain.

We also considered the alternate possibility that activity of the SAGA complex in the *sir2Δ fob1Δ* strain may counteract the longevity benefits associated with disrupting the deubiquitinase module. One way to think about this would be that unchecked Gcn5 acetyltransferase activity, when Sir2 is not there to compete, may have a negative impact on longevity that is independent of the benefits of the *sgf73Δ* strain. However, this appears not to be the case, as the *sgf73Δ sir2Δ fob1Δ spt8Δ* strain is not long lived, as is the case with *sgf73Δ sir2Δ fob1Δ* (Figure S6A). Therefore, we favor a model whereby loss of *SGF73* leads to enhanced Sir2 activity.

Sgf73 Regulates Telomere Silencing and rDNA Recombination

We reasoned that lifespan extension in strains lacking *SGF73* could be due, at least in part, to enhanced Sir2 function at regions linked to longevity. Since increased Sir2 expression is linked to lifespan extension, one simple model would be that Sir2 levels are elevated in the *sgf73Δ* strain. However, we did not detect any difference in Sir2 expression in this strain (Figure 5A). Therefore, we turned to regions of the genome where Sir2 function is associated with longevity: rDNA and telomeres (Dang et al., 2009; Kaeberlein et al., 1999). Interestingly, a subset of SAGA components, but not Gcn5, is required for Sir2-dependent silencing at both loci (Gottlieb and Esposito, 1989; Jacobson and Pillus, 2009).

The rDNA locus contains between 100 and 200 nine-kb repeats. Recombination within the repeats leads to production of extrachromosomal rDNA circles (ERCs) (Sinclair and Guarente, 1997), which can replicate due to the presence of an autonomously replicating sequence (ARS) element, but fail to segregate to daughter cells because they lack centromeric sequences. Accumulation of ERCs in mother cells is one factor that promotes aging (Sinclair and Guarente, 1997), possibly through competition for replication factors in other regions of the genome (Kwan et al., 2013). Sir2 suppresses recombination within repeats, leading to fewer ERCs and longer lifespan (Gottlieb and Esposito, 1989; Kaeberlein et al., 1999). Loss of the SAGA component Ada2 leads to reduced silencing at both telomeres and rDNA (Jacobson and Pillus, 2009), similar to loss of *SIR2* (Aparicio et al., 1991; Smith and Boeke, 1997). In these studies, rDNA recombination was not tested and other SAGA components have not been assessed for rDNA silencing. We tested whether the *sgf73Δ* strain had reduced rDNA recombination using a standard assay whereby the *ADE2* gene is inserted in one repeat and recombination is tracked by the frequency of red/white half-sectored colonies; daughter cells do not receive the *ADE2* locus when it is contained on an ERC. We found that rDNA-dependent recombination is dramatically reduced in the *sgf73Δ* strain, consistent with enhanced Sir2 function (Figure 5B). By examining double mutants lacking both *SGF73* and *SIR2*, we were able to ascertain that rDNA recombination remains high, although perhaps slightly lower than in the *sir2Δ* strain alone (Figure S6B). These findings indicate that whereas Ada2 is required for Sir2 function at the rDNA locus, Sgf73 counteracts Sir2 function and loss of Sgf73 enhances repression of rDNA recombination. We conclude that this phenotype contributes to the mechanism by which the *sgf73Δ* strain is long lived.

Sir2 silences reporter genes placed near telomeres (Aparicio et al., 1991). However, its effect on endogenous genes in these locations has to date been evaluated most thoroughly for *YFR057W*, where there is a clear requirement for *SIR2* (Vega-Palas et al., 2000). The deacetylase activity of Sir2 is required for silencing of this gene (Xu et al., 2007). Interestingly, disruption of SAGA components also affects expression of both reporter genes and *YFR057W* in a manner that indicates a complex interaction with Sir2. Loss of core SAGA components, *ADA2* and *ADA3*, leads to disruption of silencing of both *URA3* placed near the telomere of chromosome VIIL and *ADE2* near the telomere of chromosome VR (Jacobson and Pillus, 2009); however, components of the deubiquitinase module

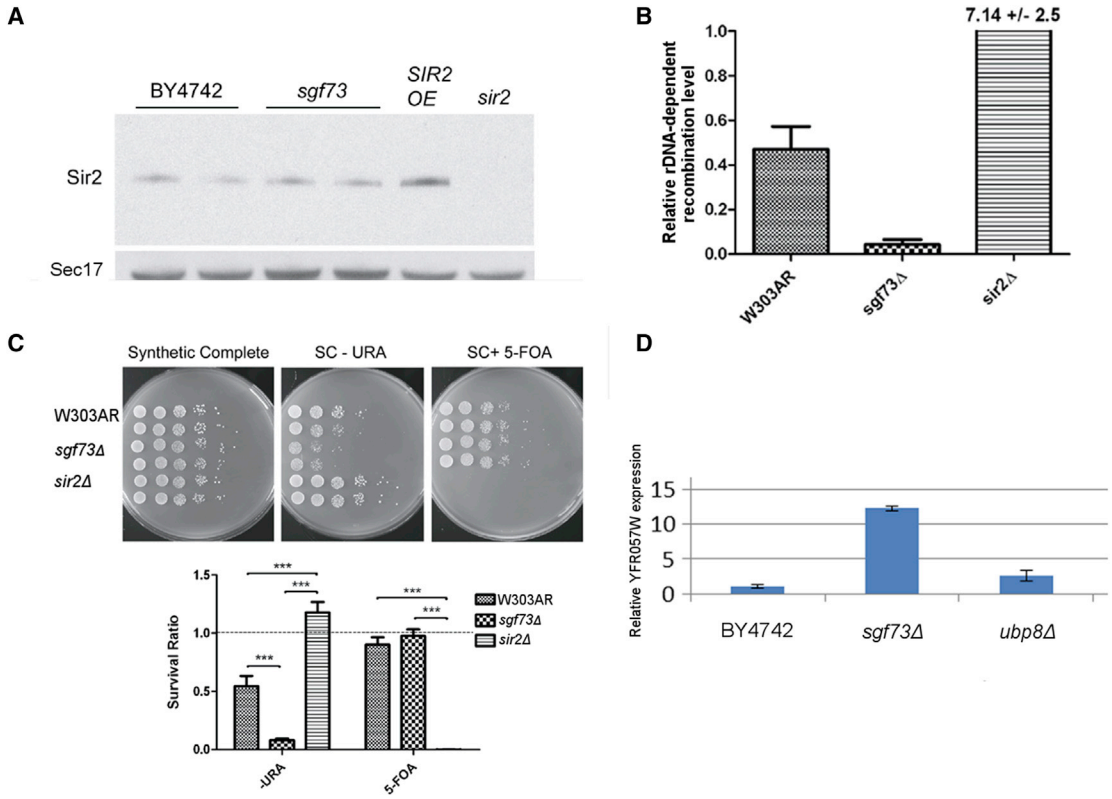

Figure 5. Sgf73 Influences Silencing at rDNA and Telomeres

(A) Sir2 protein levels are unchanged in *sgf73Δ*.

(B) rDNA-dependent recombination of an *ADE2* locus is dramatically reduced in *sgf73Δ*.

(C and D) At telomeres, *sgf73Δ* shows increased silencing of *URA3* marker on VIIL (C) but decreased silencing of endogenous ORF YFR057W on VIR (D). Error bars indicate SEM. See also Figure S6.

have not been assessed. We tested silencing of both *URA3* and *YFR057W* in a strain lacking *SGF73*, finding discordant results. Silencing of the *URA3* locus occurs in a discrete and semiheritable manner, with some of the cells and their progeny expressing the gene and other cells silencing it. We evaluated silencing of the *URA3* reporter locus in a strain lacking *SGF73* in two ways: (1) by spotting cell dilutions on plates either lacking uracil, where *URA3* expression is required for growth, and (2) on plates containing 5-FOA, where *URA3* expression is toxic. In wild-type cells, approximately half of the cells grow on each condition, and in the *sir2Δ* background, nearly all cells grow in the absence of uracil (Figure 5C). In contrast, *sgf73Δ* cells grow on 5-FOA but largely fail to grow on plates lacking uracil, indicating increased silencing. However, RT-PCR analysis of *sgf73Δ* cells indicates reduced silencing of *YFR057W* (Figure 5D). Thus, our findings demonstrate that the effects of the *sgf73Δ* strain on silencing of the endogenous locus are distinct from those of core SAGA components with respect to the reporter gene. This discrepancy is not unprecedented, as prior studies indicate that mutants can have opposing effects on genes located near different telomeres and there can be complex effects with different reporter genes (Fourel et al., 1999; Pryde and Louis, 1999; Rossmann et al., 2011; Vega-Palas et al., 1997; Wyrick et al., 1999).

Sir2 Interacts with Sgf73

Based upon our findings of altered *SIR2*-dependent properties in the *sgf73Δ* yeast and the dependence on *SIR2* for *sgf73Δ* RLS extension, we explored additional potential connections between Sgf73 and Sir2. Evidence exists in mammalian cells for an interaction between the ortholog of *SIR2*, SIRT1, and the ortholog of *UBP8*, USP22 (Armour et al., 2013; Lin et al., 2012). This suggests that (1) Sir2 may interact with DUBm components in yeast and that (2) the functional interactions between the deacetylase and the deubiquitinase components may be deeply conserved. We tested whether Sir2 interacted with Sgf73 in yeast, finding evidence of an interaction by two different assays. First, we examined the interaction in vitro by mixing yeast lysates expressing an endogenously tagged Sgf73-GFP with purified recombinant GST-tagged Sir2 protein. Immunoprecipitation of Sir2 by glutathione Sepharose was sufficient to recover Sgf73-GFP, upon immunoblot analysis of the precipitated material (Figure 6A). There is a nonspecific band that is reactive to anti-GFP, which is present in the WT input lanes but is not immunoprecipitated. An enzymatically dead Sir2-R139K mutant protein also retained the capacity to interact with Sgf73-GFP, indicating that the deacetylase activity of Sir2 is not required for interaction. In a second experiment, we detected an in vivo interaction between Sgf73-GFP and endogenous Sir2

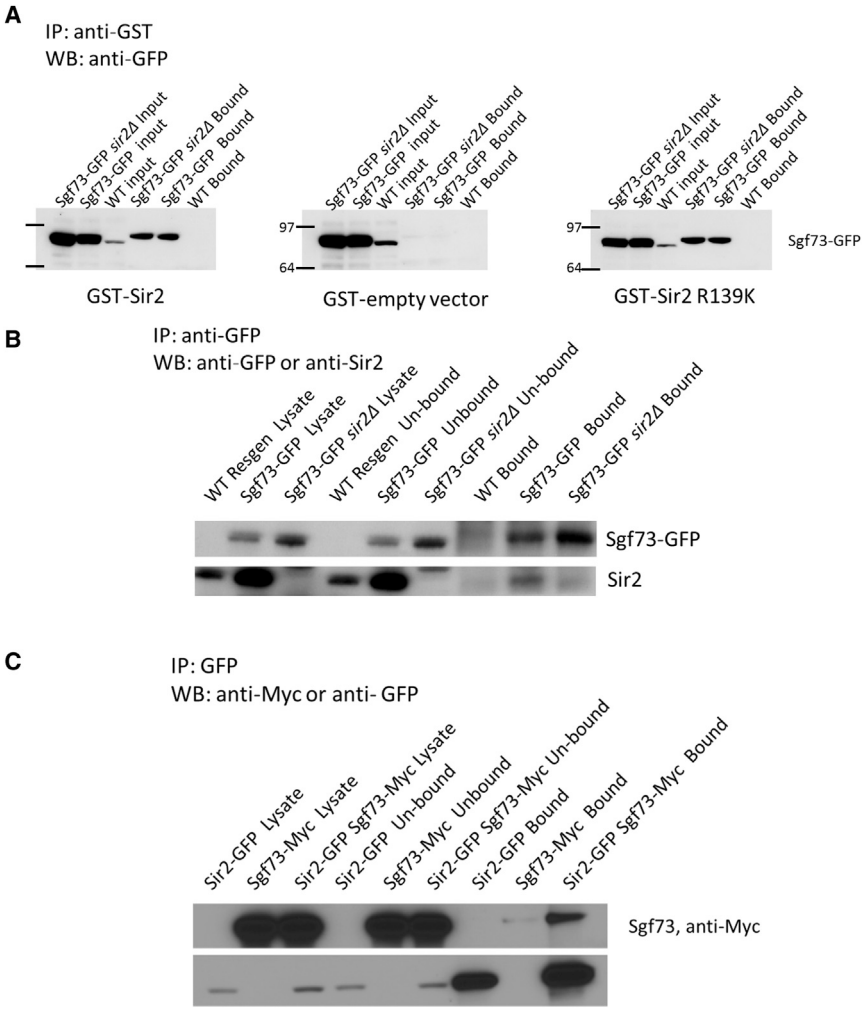

Figure 6. Sgf73 Interacts with Sir2

(A) Interaction of recombinant Sir2-GST and Sgf73-GFP in the presence and absence of Sir2. Immunoprecipitation was done with glutathione beads coupled to either GST tagged Sir2, GST tagged enzymatically dead Sir2R319K, or GST, and western blot was probed with anti-GFP.

(B) Interaction of endogenous Sir2 and Sgf73-GFP. Immunoprecipitation was performed with anti-GFP, and western blots were probed with anti-Sir2.

by coimmunoprecipitation of Sgf73-GFP, as well as between an endogenously tagged Sir2-GFP and endogenously tagged Sgf73-Myc by coimmunoprecipitation of Sir2-GFP (Figures 6B and 6C). Altogether, these experiments indicate an interaction between Sir2 and the DUBm of SAGA in yeast and provide evidence for the deeply conserved nature of the interaction, which has also been detected in mammalian cells.

DISCUSSION

In this study, we report that deletion of *SGF73*, the yeast ortholog of human ataxin-7, the causal gene in SCA7, results in one of the longest-lived mutant strains observed to date. Sgf73 is a transcriptional adaptor, serving as the linking factor that connects the core SAGA complex to the Ubp8 histone deubiquitinase module of the complex. Strains lacking the other DUBm components, encoded by *UBP8* and *SGF11*, are also dramatically long lived, whereas strains lacking *GCN5* or other core SAGA components have either normal or shortened replicative lifespans. Strikingly, lifespan extension by loss of DUBm components requires *SIR2*, suggesting that DUBm mutants enhance Sir2 function. This view is somewhat nuanced by the observation that the *sgf73Δ* mutant has a much longer replicative lifespan than either strains lacking *FOB1* or those overexpressing *SIR2*. From our studies and other reports describing their enzymatic activities, we propose that the extremely long lifespan of *sgf73Δ* is due to the contributions of multiple downstream pathways. The likelihood of involvement of each regulatory pathway is discussed below.

The foremost activity of Sir2 linked to extended RLS is repression of rDNA recombination. We find that a *sgf73Δ* strain has reduced rDNA recombination, consistent with enhanced longevity. A recent study indicates that polymorphisms in the ARS element, which is present in each rDNA repeat, can affect longevity (Kwan et al., 2013). A strong ARS element in the rDNA, possibly in combination with enhanced numbers of ERCs, competes with replication origins in other sites in the genome, leading to replication stress. In support of this idea, Ubp8 has been identified as the deubiquitinase responsible for removing ubiquitin linkages on histone H2B-K123 in newly assembled histone octamers that are generated on replicated DNA. Strains lacking Bre1, the E3 ligase for H2B-K123, have reduced H2B-K123 ubiquitination. This leads to reduced assembly or stability of nucleosomes around newly replicated DNA, which slows fork progression, thereby resulting in replication stress (Trujillo and Osley, 2012). In contrast, *ubp8Δ* strains have increased nucleosome deposition and improved replication fork progression. In the context of old cells, where replication stress may be a key component contributing to aging, the *ubp8Δ* and *sgf73Δ* strains may be long lived not only because they reduce ERC formation but also because they promote replication fork progression.

Understanding the role of telomere-proximal genes is more complicated. Although Sir2 activity in these regions has also been linked to longevity, we find that the *sgf73Δ* strain has enhanced silencing of a telomere-proximal reporter gene at chromosome VIIL but reduced repression of an endogenous gene clearly linked to Sir2-dependent silencing, *YFR075W*, located on chromosome VIR. This suggests that Sgf73 has a

context-dependent effect on silencing of genes near telomeres, as observed for other genes at other natural telomeres (Fourel et al., 1999; Pryde and Louis, 1999; Vega-Palas et al., 1997, 2000; Wyrick et al., 1999). Future studies of telomeres with different genomic composition and structural features will clarify whether these effects are linked to aging. In addition, given that SAGA and Sgf73 have roles in heterochromatin-euchromatin barrier formation (Oki et al., 2004), with the C terminus of the Sgf73 subunit of SAGA and SLIK important for retention of the larger complex and for heterochromatin boundary function (Kamata et al., 2013), it will be important to better understand the role of the DUBm in the context of the greater complex.

Finally, recent studies have linked SAGA activity to coordination of different metabolic phases of yeast growth in culture (Cai et al., 2011). This occurs through control of transcription, in part through H3K9 acetylation. Interestingly, SAGA has a direct role in regulating expression of ribosomal protein genes in a manner dependent on the transcription factor Ifh1 (Cai et al., 2013; Downey et al., 2013). Ribosomal protein gene deletions are highly enriched among long-lived mutants in yeast (Mehta et al., 2010). Thus, another potential link to enhanced longevity in DUBm mutants may be through reduced ribosome biogenesis. In summary, multiple mechanisms are likely to explain the extremely long lifespan of *sgf73Δ* and *ubp8Δ* strains, and further experimentation will be required to unravel the relative contribution of each component.

The discovery of the marked replicative lifespan extension in *sgf73Δ* mutants, together with the demonstration that the *sgf73Δ* strain lifespan extension phenotype stems from enhanced Sir2 function in combination with reduced Ubp8 function, may have important implications for the normal function of the mammalian Sgf73 ortholog, ataxin-7. ataxin-7 was originally discovered as the causal gene in a dominantly inherited neurodegenerative disorder, known as spinocerebellar ataxia type 7 (SCA7) (David et al., 1997). Patients with SCA7 exhibit cerebellar and retinal degeneration, display CAG repeat expansions within the coding regions of their ataxin-7 genes, and produce mutant ataxin-7 protein containing expanded polyglutamine tracts ranging in size from 37 to >400 glutamines. SCA7 is thus one member of a family of nine CAG-polyglutamine diseases and belongs to a large class of neurodegenerative disorders, including Alzheimer's and Parkinson's diseases. These ''neurodegenerative proteinopathies'' result from the production of misfolded proteins (La Spada and Taylor, 2010).

In the case of SCA7, polyglutamine-expanded ataxin-7 promotes transcriptional interference to produce neuropathology, as the mutant ataxin-7 protein localizes to the nucleus, where it disrupts transcription factor function (Holmberg et al., 1998; Kaytor et al., 1999; La Spada et al., 2001). Unbiased mass spectrometry analysis of the STAGA complex yielded ataxin-7 as a core STAGA complex component, and further studies determined that polyglutamine-expanded ataxin-7 interferes with the GCN5-dependent histone acetyltransferase activity of the STAGA complex to impair STAGA-dependent transactivation (Palhan et al., 2005). Although polyglutamine-expanded ataxin-7 exhibits gain-of-function toxicity due to a proteotoxicity effect, the discovery of altered STAGA function in SCA7 mutant mice indicated a role for altered ataxin-7 protein function in SCA7 disease pathogenesis. Hence, SCA7-dependent neurodegeneration may result from divergent effects of mutant ataxin-7 protein on the activities of interacting proteins in the context of the STAGA complex, akin to polyglutamine-expanded ataxin-1 effects on its protein complex partners in SCA1 (Zoghbi and Orr, 2009).

As the Ubp8 ortholog USP22 was recently shown to interact with, deubiquitinate, and stabilize the Sir2 ortholog Sirt1 (Lin et al., 2012), and conversely because Sirt1 has been shown to mediate deacetylation of USP22 and the SAGA coactivator complex (Armour et al., 2013), the discovery of genetic and functional relationships among Sgf73, Ubp8, and Sir2 in yeast strongly suggests that mutant ataxin-7 protein could be impairing the function of not only Gcn5 in the context of the STAGA complex but also USP22 in the deubiquitinase module. Given the recent proposed linkage between USP22 and Sirt1, our findings predict that polyglutamine-expanded ataxin-7 could interfere with the normal function of both USP22 and SIRT1 in neurons. Indeed, whether and how ataxin-7 affects pathways of mammalian aging relevant to neural function and neuroprotection will be key questions for future studies, with implications for both SCA7 disease pathogenesis and therapy development and for a deeper understanding of the biology of aging in mammals.

EXPERIMENTAL PROCEDURES

Strains and Media

All yeast strains were derived from the parent strains of the haploid yeast ORF deletion collections (Winzeler et al., 1999), BY4742 (*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0*) and BY4741 (*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*). *sgf73Δ* was reconstructed by deleting *SGF73* via homologous recombination of a selectable *URA3* marker in the deletion collection wild-type BY strain using standard PCR-mediated gene disruption, and this parent was used to generate all *sgf73Δ* containing double and triple mutants. *SIR2OE* (overexpression) strains contain a second integrated copy of *SIR2* under the control of its endogenous promoter. RGY43 was a gift of Richard Gardner. Cells were grown on standard YPD containing 1% yeast extract, 2% peptone, and 2% glucose.

Replicative Lifespan

RLS assays were performed as described previously (Steffen et al., 2009). Survival curves are pooled data from multiple experiments with accompanying experiment-matched controls, with mean RLS and number of mother cells scored shown for each curve. p values for RLS survival curve comparisons were calculated with a Wilcoxon rank-sum test (Wilcoxon, 1946). Kaplan-Meier survival curves (Kaplan and Meier, 1958) were plotted with Prism (GraphPad).

Yeast Interaction Studies

Yeast lysis

Standard yeast glass bead lysis protocol was followed. Briefly, yeast were grown to an absorbance at 600 nm (A_{600}) of 0.8 and lysed in 50 mM HEPES (pH 7.5), 0.1 M NaCl, 0.5% Np40, 10% glycerol, and 1 mM EDTA, with protease inhibitors including phenylmethylsulfonylfluoride (PMSF). Lysates were precleared with 10 μl of protein A/G (Dynabeads, Life Technologies) magnetic beads at 4°C for one hour.

Strains

All tagged protein strains used were endogenously tagged with either GFP or 13Myc. Tagged strains were demonstrated to be functional by mating for tagged Sir2 and by checking cycloheximide resistance for tagged Sgf73.

Recombinant Protein Purification

BL21 cells were transformed with one of three plasmids (Sir2-GST, Sir2-R139K-GST, or GST empty vector). A 100 ml culture was grown to A_{600} 0.6 and isopropyl β-D-1-thiogalactopyranoside was added to a final concentration of 0.5 mM. The culture was then incubated with shaking at room temperature

for 4 hr. Cells were collected and lysed (20 mM Tris [pH 8], 1 mM EDTA, 1 mM EGTA, 1% NP-40, 350 mM NaCl, 10 mM dithiothreitol, protease inhibitors [Roche 04693124001], PMSF, and 200 mg/ml lysozyme) for 30 min on ice, sonicated three times for 1 min with 5 s on/0.5 s off cycles, and then spun at 4°C for 20 min. Glutathione agarose was prepared and added to the bacterial lysates and incubated at 4°C for 1 hr.

Immunoprecipitation

In vitro, conjugated beads were added to prepared yeast lysates and rotated at 4°C overnight. Beads were washed twice with yeast lysis buffer and twice with immunoprecipitation (IP) wash buffer (50 mM HEPES [pH 7.5], 150 mM NaCl, 1 mM EDTA). Proteins were analyzed by western blot; 2.5% of input was loaded, 12.5% of bound material was loaded. In vivo, anti-GFP (living colors JL8 632381) was added to yeast lysates and rotated at 4°C overnight, and 50 μl of protein A/G beads was then added and the sample rotated for 1 hr at 4°C. Beads were washed twice with lysis buffer and twice with wash buffer. For Sgf73-GFP IP 2% of input and unbound, and 30% of bound material was loaded, for Sir2-GFP IP 1.25% of input and unbound, and 100% of bound material was loaded. Protein was analyzed via immunoblotting.

Immunoblot

Standard SDS-PAGE was used to separate proteins on 4%–12% gels. After transfer to nitrocellulose, membrane was blocked with 5% milk and incubated with either anti-GFP (living colors JL8 632381) or anti-Sir2 (Garcia and Pillus, 2002).

SUPPLEMENTAL INFORMATION

Supplemental Information includes six figures and can be found with this article online at http://dx.doi.org/10.1016/j.celrep.2014.06.037.

AUTHOR CONTRIBUTIONS

M.A.M., A.G.M., S.J.G., S.L.B., M.K., L.P., A.R.L.S., and B.K.K. wrote and revised the manuscript, figures, captions, and Supplemental Information along with all authors. Yeast strain generation and RLS experiments were conducted by M.A.M., S.J.G., W.D., M.K.T., and R.M.M. Coimmunoprecipitation experiments were conducted by A.G.M.

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REFERENCES

Aparicio, O.M., Billington, B.L., and Gottschling, D.E. (1991). Modifiers of position effect are shared between telomeric and silent mating-type loci in S. cerevisiae. Cell *66*, 1279–1287.

Armour, S.M., Bennett, E.J., Braun, C.R., Zhang, X.Y., McMahon, S.B., Gygi, S.P., Harper, J.W., and Sinclair, D.A. (2013). A high-confidence interaction map identifies SIRT1 as a mediator of acetylation of USP22 and the SAGA coactivator complex. Mol. Cell. Biol. *33*, 1487–1502.

Belotserkovskaya, R., Sterner, D.E., Deng, M., Sayre, M.H., Lieberman, P.M., and Berger, S.L. (2000). Inhibition of TATA-binding protein function by SAGA subunits Spt3 and Spt8 at Gcn4-activated promoters. Mol. Cell. Biol. *20*, 634–647.

Cai, L., Sutter, B.M., Li, B., and Tu, B.P. (2011). Acetyl-CoA induces cell growth and proliferation by promoting the acetylation of histones at growth genes. Mol. Cell *42*, 426–437.

Cai, L., McCormick, M.A., Kennedy, B.K., and Tu, B.P. (2013). Integration of multiple nutrient cues and regulation of lifespan by ribosomal transcription factor Ifh1. Cell Rep *4*, 1063–1071.

Dang, W., Steffen, K.K., Perry, R., Dorsey, J.A., Johnson, F.B., Shilatifard, A., Kaeberlein, M., Kennedy, B.K., and Berger, S.L. (2009). Histone H4 lysine 16 acetylation regulates cellular lifespan. Nature *459*, 802–807.

Daniel, J.A., Torok, M.S., Sun, Z.W., Schieltz, D., Allis, C.D., Yates, J.R., 3rd, and Grant, P.A. (2004). Deubiquitination of histone H2B by a yeast acetyltransferase complex regulates transcription. J. Biol. Chem. *279*, 1867–1871.

David, G., Abbas, N., Stevanin, G., Dürr, A., Yvert, G., Cancel, G., Weber, C., Imbert, G., Saudou, F., Antoniou, E., et al. (1997). Cloning of the SCA7 gene reveals a highly unstable CAG repeat expansion. Nat. Genet. *17*, 65–70.

Defossez, P.A., Prusty, R., Kaeberlein, M., Lin, S.J., Ferrigno, P., Silver, P.A., Keil, R.L., and Guarente, L. (1999). Elimination of replication block protein Fob1 extends the life span of yeast mother cells. Mol. Cell *3*, 447–455.

Delaney, J.R., Sutphin, G.L., Dulken, B., Sim, S., Kim, J.R., Robison, B., Schleit, J., Murakami, C.J., Carr, D., An, E.H., et al. (2011). Sir2 deletion prevents lifespan extension in 32 long-lived mutants. Aging Cell *10*, 1089–1091.

Downey, M., Knight, B., Vashisht, A.A., Seller, C.A., Wohlschlegel, J.A., Shore, D., and Toczyski, D.P. (2013). Gcn5 and sirtuins regulate acetylation of the ribosomal protein transcription factor Ifh1. Curr. Biol. *23*, 1638–1648.

Emre, N.C., Ingvarsdottir, K., Wyce, A., Wood, A., Krogan, N.J., Henry, K.W., Li, K., Marmorstein, R., Greenblatt, J.F., Shilatifard, A., and Berger, S.L. (2005). Maintenance of low histone ubiquitylation by Ubp10 correlates with telomere-proximal Sir2 association and gene silencing. Mol. Cell *17*, 585–594.

Fourel, G., Revardel, E., Koering, C.E., and Gilson, E. (1999). Cohabitation of insulators and silencing elements in yeast subtelomeric regions. EMBO J. *18*, 2522–2537.

Garcia, S.N., and Pillus, L. (2002). A unique class of conditional sir2 mutants displays distinct silencing defects in Saccharomyces cerevisiae. Genetics *162*, 721–736.

Gottlieb, S., and Esposito, R.E. (1989). A new role for a yeast transcriptional silencer gene, SIR2, in regulation of recombination in ribosomal DNA. Cell *56*, 771–776.

Guarente, L. (2011). Sirtuins, aging, and metabolism. Cold Spring Harb. Symp. Quant. Biol. *76*, 81–90.

Helmlinger, D., Hardy, S., Sasorith, S., Klein, F., Robert, F., Weber, C., Miguet, L., Potier, N., Van-Dorsselaer, A., Wurtz, J.M., et al. (2004). Ataxin-7 is a subunit of GCN5 histone acetyltransferase-containing complexes. Hum. Mol. Genet. *13*, 1257–1265.

Henry, K.W., Wyce, A., Lo, W.S., Duggan, L.J., Emre, N.C., Kao, C.F., Pillus, L., Shilatifard, A., Osley, M.A., and Berger, S.L. (2003). Transcriptional activation via sequential histone H2B ubiquitylation and deubiquitylation, mediated by SAGA-associated Ubp8. Genes Dev. *17*, 2648–2663.

Holmberg, M., Duyckaerts, C., Dürr, A., Cancel, G., Gourfinkel-An, I., Damier, P., Faucheux, B., Trottier, Y., Hirsch, E.C., Agid, Y., and Brice, A. (1998). Spinocerebellar ataxia type 7 (SCA7): a neurodegenerative disorder with neuronal intranuclear inclusions. Hum. Mol. Genet. *7*, 913–918.

Jacobson, S., and Pillus, L. (2009). The SAGA subunit Ada2 functions in transcriptional silencing. Mol. Cell. Biol. *29*, 6033–6045.

Jazwinski, S.M. (2005). Rtg2 protein: at the nexus of yeast longevity and aging. FEMS Yeast Res. *5*, 1253–1259.

Kaeberlein, M., McVey, M., and Guarente, L. (1999). The *SIR2/3/4* complex and *SIR2* alone promote longevity in *Saccharomyces cerevisiae* by two different mechanisms. Genes Dev. *13*, 2570–2580.

Kaeberlein, M., Kirkland, K.T., Fields, S., and Kennedy, B.K. (2004). Sir2-independent life span extension by calorie restriction in yeast. PLoS Biol. *2*, E296.

Kaeberlein, M., Kirkland, K.T., Fields, S., and Kennedy, B.K. (2005a). Genes determining yeast replicative life span in a long-lived genetic background. Mech. Ageing Dev. *126*, 491–504.

Kaeberlein, M., Powers, R.W., 3rd, Steffen, K.K., Westman, E.A., Hu, D., Dang, N., Kerr, E.O., Kirkland, K.T., Fields, S., and Kennedy, B.K. (2005b). Regulation

of yeast replicative life span by TOR and Sch9 in response to nutrients. Science *310*, 1193–1196.

Kamata, K., Hatanaka, A., Goswami, G., Shinmyozu, K., Nakayama, J., Urano, T., Hatashita, M., Uchida, H., and Oki, M. (2013). C-terminus of the Sgf73 subunit of SAGA and SLIK is important for retention in the larger complex and for heterochromatin boundary function. Genes Cells *18*, 823–837.

Kaplan, E.L., and Meier, P. (1958). Nonparametric estimation from incomplete observations. J. Am. Stat. Assoc. *53*, 457–481.

Kaytor, M.D., Duvick, L.A., Skinner, P.J., Koob, M.D., Ranum, L.P., and Orr, H.T. (1999). Nuclear localization of the spinocerebellar ataxia type 7 protein, ataxin-7. Hum. Mol. Genet. *8*, 1657–1664.

Kennedy, B.K., Austriaco, N.R., Jr., Zhang, J., and Guarente, L. (1995). Mutation in the silencing gene SIR4 can delay aging in S. cerevisiae. Cell *80*, 485–496.

Köhler, A., Schneider, M., Cabal, G.G., Nehrbass, U., and Hurt, E. (2008). Yeast Ataxin-7 links histone deubiquitination with gene gating and mRNA export. Nat. Cell Biol. *10*, 707–715.

Kwan, E.X., Foss, E.J., Tsuchiyama, S., Alvino, G.M., Kruglyak, L., Kaeberlein, M., Raghuraman, M.K., Brewer, B.J., Kennedy, B.K., and Bedalov, A. (2013). A natural polymorphism in rDNA replication origins links origin activation with calorie restriction and lifespan. PLoS Genet. *9*, e1003329.

La Spada, A.R., and Taylor, J.P. (2010). Repeat expansion disease: progress and puzzles in disease pathogenesis. Nat. Rev. Genet. *11*, 247–258.

La Spada, A.R., Fu, Y.H., Sopher, B.L., Libby, R.T., Wang, X., Li, L.Y., Einum, D.D., Huang, J., Possin, D.E., Smith, A.C., et al. (2001). Polyglutamine-expanded ataxin-7 antagonizes CRX function and induces cone-rod dystrophy in a mouse model of SCA7. Neuron *31*, 913–927.

Lebre, A.S., and Brice, A. (2003). Spinocerebellar ataxia 7 (SCA7). Cytogenet. Genome Res. *100*, 154–163.

Lee, K.K., Swanson, S.K., Florens, L., Washburn, M.P., and Workman, J.L. (2009). Yeast Sgf73/Ataxin-7 serves to anchor the deubiquitination module into both SAGA and Slik(SALSA) HAT complexes. Epigenetics Chromatin *2*, 2.

Lin, Z., Yang, H., Kong, Q., Li, J., Lee, S.M., Gao, B., Dong, H., Wei, J., Song, J., Zhang, D.D., and Fang, D. (2012). USP22 antagonizes p53 transcriptional activation by deubiquitinating Sirt1 to suppress cell apoptosis and is required for mouse embryonic development. Mol. Cell *46*, 484–494.

Mal, A.K. (2006). Histone methyltransferase Suv39h1 represses MyoD-stimulated myogenic differentiation. EMBO J. *25*, 3323–3334.

Martinez, E., Palhan, V.B., Tjernberg, A., Lymar, E.S., Gamper, A.M., Kundu, T.K., Chait, B.T., and Roeder, R.G. (2001). Human STAGA complex is a chromatin-acetylating transcription coactivator that interacts with pre-mRNA splicing and DNA damage-binding factors in vivo. Mol. Cell. Biol. *21*, 6782–6795.

Mehta, R., Chandler-Brown, D., Ramos, F.J., Shamieh, L.S., and Kaeberlein, M. (2010). Regulation of mRNA translation as a conserved mechanism of longevity control. Adv. Exp. Med. Biol. *694*, 14–29.

Mortimer, R.K., and Johnston, J.R. (1959). Life span of individual yeast cells. Nature *183*, 1751–1752.

Mushegian, A.R., Vishnivetskiy, S.A., and Gurevich, V.V. (2000). Conserved phosphoprotein interaction motif is functionally interchangeable between ataxin-7 and arrestins. Biochemistry *39*, 6809–6813.

Oki, M., Valenzuela, L., Chiba, T., Ito, T., and Kamakaka, R.T. (2004). Barrier proteins remodel and modify chromatin to restrict silenced domains. Mol. Cell. Biol. *24*, 1956–1967.

Palhan, V.B., Chen, S., Peng, G.H., Tjernberg, A., Gamper, A.M., Fan, Y., Chait, B.T., La Spada, A.R., and Roeder, R.G. (2005). Polyglutamine-expanded ataxin-7 inhibits STAGA histone acetyltransferase activity to produce retinal degeneration. Proc. Natl. Acad. Sci. USA *102*, 8472–8477.

Pryde, F.E., and Louis, E.J. (1999). Limitations of silencing at native yeast telomeres. EMBO J. *18*, 2538–2550.

Rossmann, M.P., Luo, W., Tsaponina, O., Chabes, A., and Stillman, B. (2011). A common telomeric gene silencing assay is affected by nucleotide metabolism. Mol. Cell *42*, 127–136.

Scheel, H., Tomiuk, S., and Hofmann, K. (2003). Elucidation of ataxin-3 and ataxin-7 function by integrative bioinformatics. Hum. Mol. Genet. *12*, 2845–2852.

Schulze, J.M., Hentrich, T., Nakanishi, S., Gupta, A., Emberly, E., Shilatifard, A., and Kobor, M.S. (2011). Splitting the task: Ubp8 and Ubp10 deubiquitinate different cellular pools of H2BK123. Genes Dev. *25*, 2242–2247.

Sinclair, D.A., and Guarente, L. (1997). Extrachromosomal rDNA circles—a cause of aging in yeast. Cell *91*, 1033–1042.

Smith, J.S., and Boeke, J.D. (1997). An unusual form of transcriptional silencing in yeast ribosomal DNA. Genes Dev. *11*, 241–254.

Smith, E.D., Tsuchiya, M., Fox, L.A., Dang, N., Hu, D., Kerr, E.O., Johnston, E.D., Tchao, B.N., Pak, D.N., Welton, K.L., et al. (2008). Quantitative evidence for conserved longevity pathways between divergent eukaryotic species. Genome Res. *18*, 564–570.

Smolle, M., Venkatesh, S., Gogol, M.M., Li, H., Zhang, Y., Florens, L., Washburn, M.P., and Workman, J.L. (2012). Chromatin remodelers Isw1 and Chd1 maintain chromatin structure during transcription by preventing histone exchange. Nat. Struct. Mol. Biol. *19*, 884–892.

Spedale, G., Mischerikow, N., Heck, A.J., Timmers, H.T., and Pijnappel, W.W. (2010). Identification of Pep4p as the protease responsible for formation of the SAGA-related SLIK protein complex. J. Biol. Chem. *285*, 22793–22799.

Stanfel, M.N., Shamieh, L.S., Kaeberlein, M., and Kennedy, B.K. (2009). The TOR pathway comes of age. Biochim. Biophys. Acta *1790*, 1067–1074.

Steffen, K.K., MacKay, V.L., Kerr, E.O., Tsuchiya, M., Hu, D., Fox, L.A., Dang, N., Johnston, E.D., Oakes, J.A., Tchao, B.N., et al. (2008). Yeast life span extension by depletion of 60s ribosomal subunits is mediated by Gcn4. Cell *133*, 292–302.

Steffen, K.K., Kennedy, B.K., and Kaeberlein, M. (2009). Measuring replicative life span in the budding yeast. J. Vis. Exp., ().

Steinkraus, K.A., Kaeberlein, M., and Kennedy, B.K. (2008). Replicative aging in yeast: the means to the end. Annu. Rev. Cell Dev. Biol. *24*, 29–54.

Sutphin, G.L., Olsen, B.A., Kennedy, B.K., and Kaeberlein, M. (2012). Genome-wide analysis of yeast aging. Subcell. Biochem. *57*, 251–289.

Trujillo, K.M., and Osley, M.A. (2012). A role for H2B ubiquitylation in DNA replication. Mol. Cell *48*, 734–746.

Vega-Palas, M.A., Venditti, S., and Di Mauro, E. (1997). Telomeric transcriptional silencing in a natural context. Nat. Genet. *15*, 232–233.

Vega-Palas, M.A., Martín-Figueroa, E., and Florencio, F.J. (2000). Telomeric silencing of a natural subtelomeric gene. Mol. Gen. Genet. *263*, 287–291.

Wilcoxon, F. (1946). Individual comparisons of grouped data by ranking methods. J. Econ. Entomol. *39*, 269.

Winzeler, E.A., Shoemaker, D.D., Astromoff, A., Liang, H., Anderson, K., Andre, B., Bangham, R., Benito, R., Boeke, J.D., Bussey, H., et al. (1999). Functional characterization of the S. cerevisiae genome by gene deletion and parallel analysis. Science *285*, 901–906.

Wyrick, J.J., Holstege, F.C., Jennings, E.G., Causton, H.C., Shore, D., Grunstein, M., Lander, E.S., and Young, R.A. (1999). Chromosomal landscape of nucleosome-dependent gene expression and silencing in yeast. Nature *402*, 418–421.

Xu, F., Zhang, Q., Zhang, K., Xie, W., and Grunstein, M. (2007). Sir2 deacetylates histone H3 lysine 56 to regulate telomeric heterochromatin structure in yeast. Mol. Cell *27*, 890–900.

Zhang, X.Y., Varthi, M., Sykes, S.M., Phillips, C., Warzecha, C., Zhu, W., Wyce, A., Thorne, A.W., Berger, S.L., and McMahon, S.B. (2008). The putative cancer stem cell marker USP22 is a subunit of the human SAGA complex required for activated transcription and cell-cycle progression. Mol. Cell *29*, 102–111.

Zhao, Y., Lang, G., Ito, S., Bonnet, J., Metzger, E., Sawatsubashi, S., Suzuki, E., Le Guezennec, X., Stunnenberg, H.G., Krasnov, A., et al. (2008). A TFTC/STAGA module mediates histone H2A and H2B deubiquitination, coactivates nuclear receptors, and counteracts heterochromatin silencing. Mol. Cell *29*, 92–101.

Zoghbi, H.Y., and Orr, H.T. (2009). Pathogenic mechanisms of a polyglutamine-mediated neurodegenerative disease, spinocerebellar ataxia type 1. J. Biol. Chem. *284*, 7425–7429.

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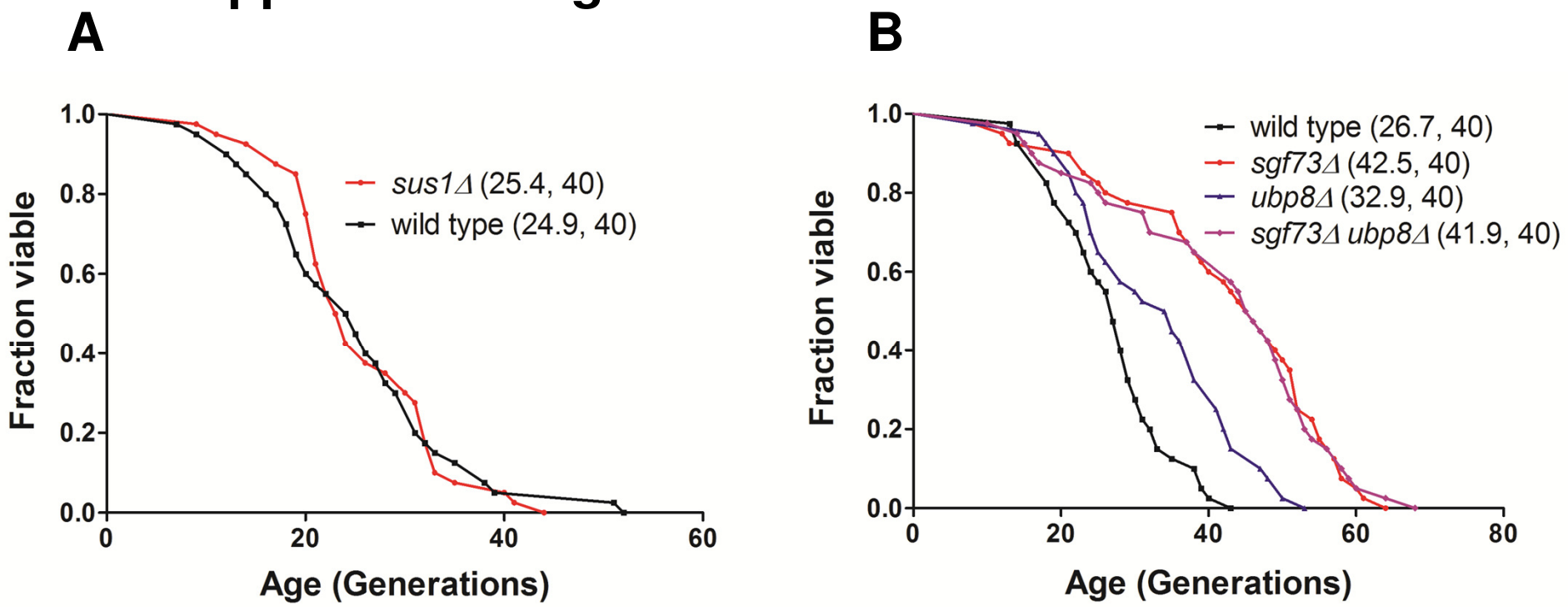
Supplemental Information

The SAGA Histone Deubiquitinase Module Controls Yeast Replicative Lifespan via Sir2 Interaction

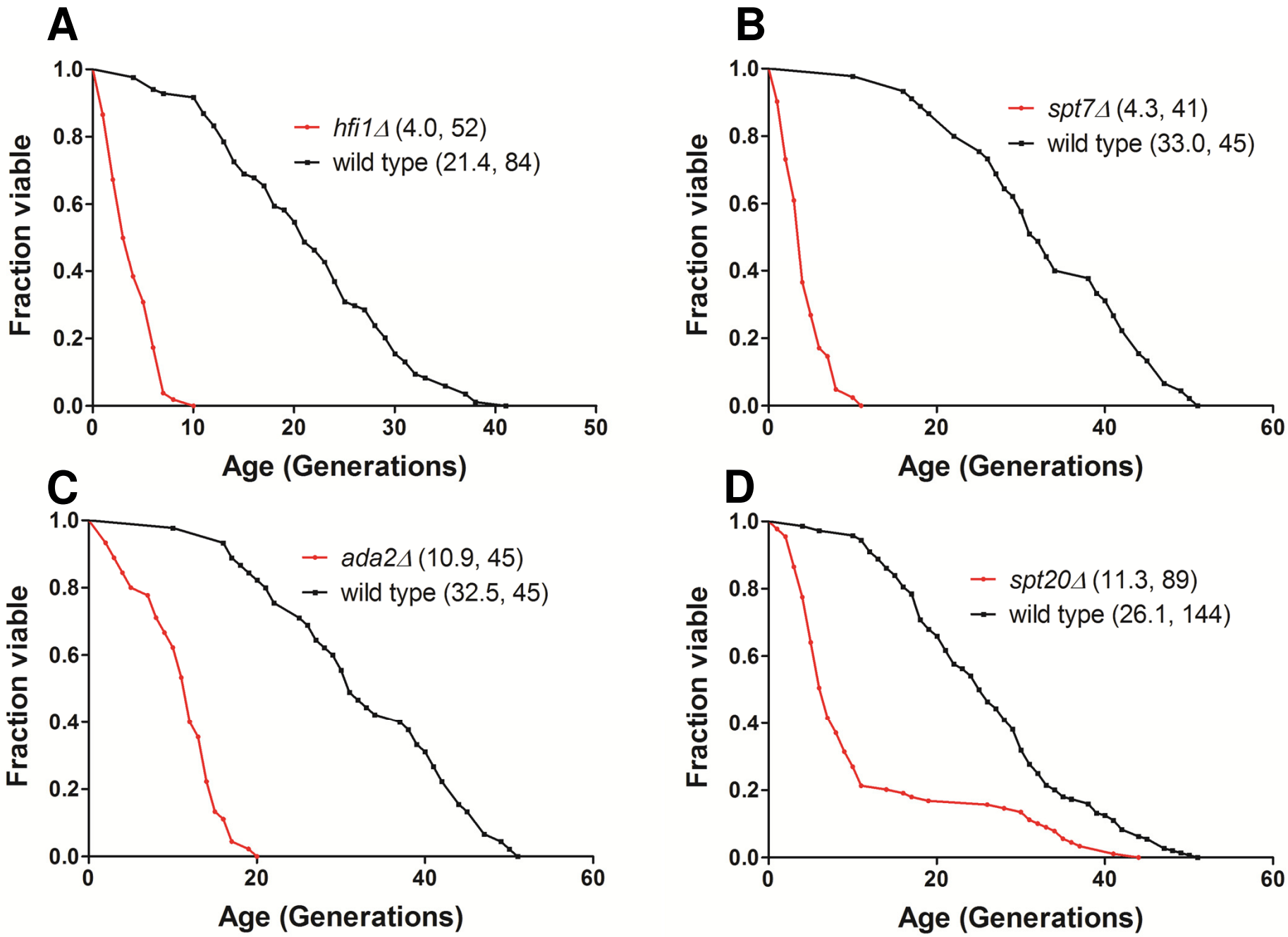
Mark A. McCormick, Amanda G. Mason, Stephan J. Guyenet, Weiwei Dang, Renee M. Garza, Marc K. Ting, Rick M. Moller, Shelley L. Berger, Matt Kaeberlein, Lorraine Pillus, Albert R. La Spada, and Brian K. Kennedy

Supplemental Figure S1

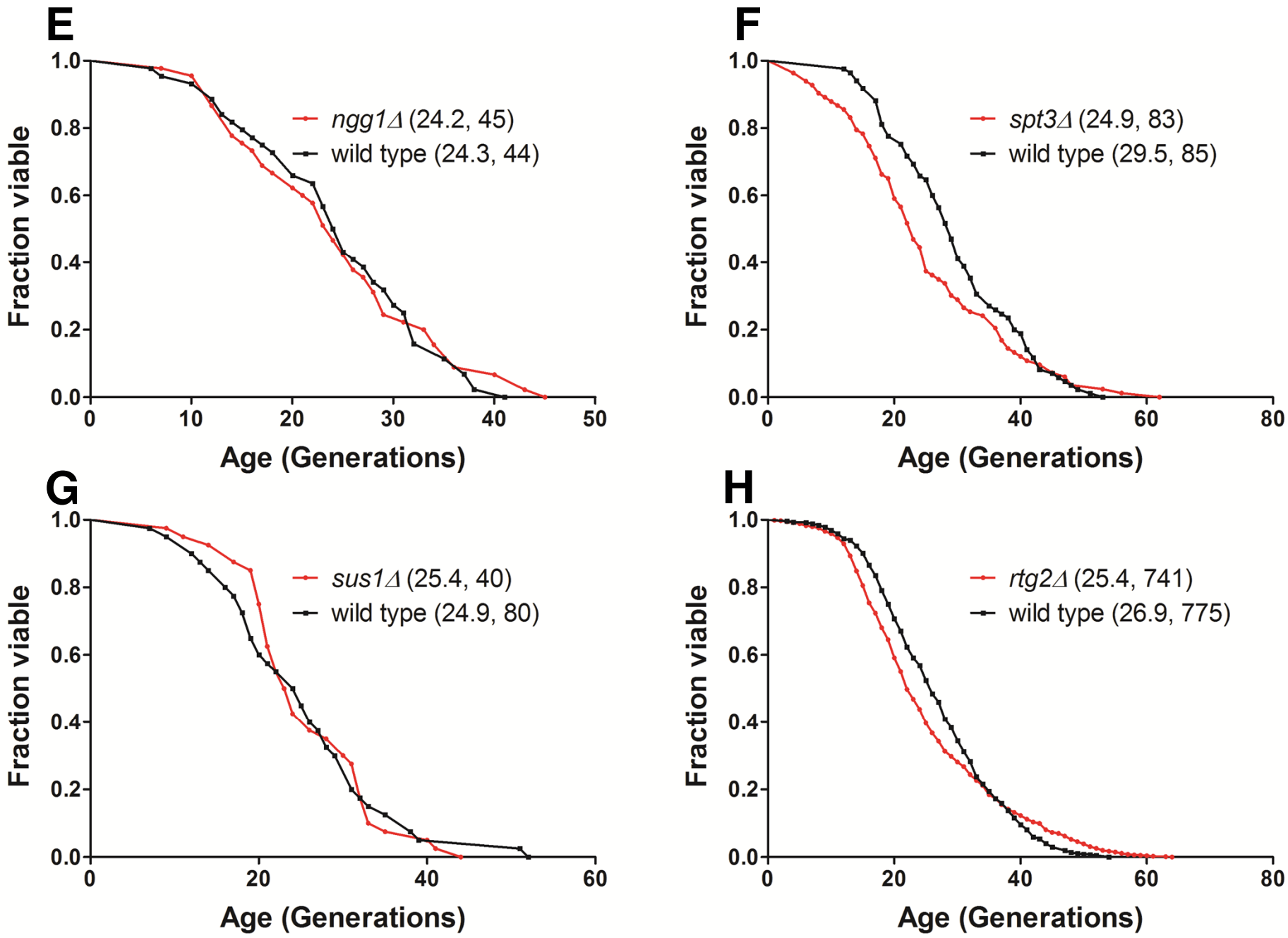


Supplemental Figure S1. Effect of *sus1Δ* and of combined *sgf73Δ* and *ubp8Δ* deletions on yeast RLS. (A), deletion of DUBm component *SUS1* does not extend RLS. (B), deletion of *UBP8* does not further extend RLS of *sgf73Δ*. Legends show (mean RLS, number of mother cells scored), related to Figure 1.

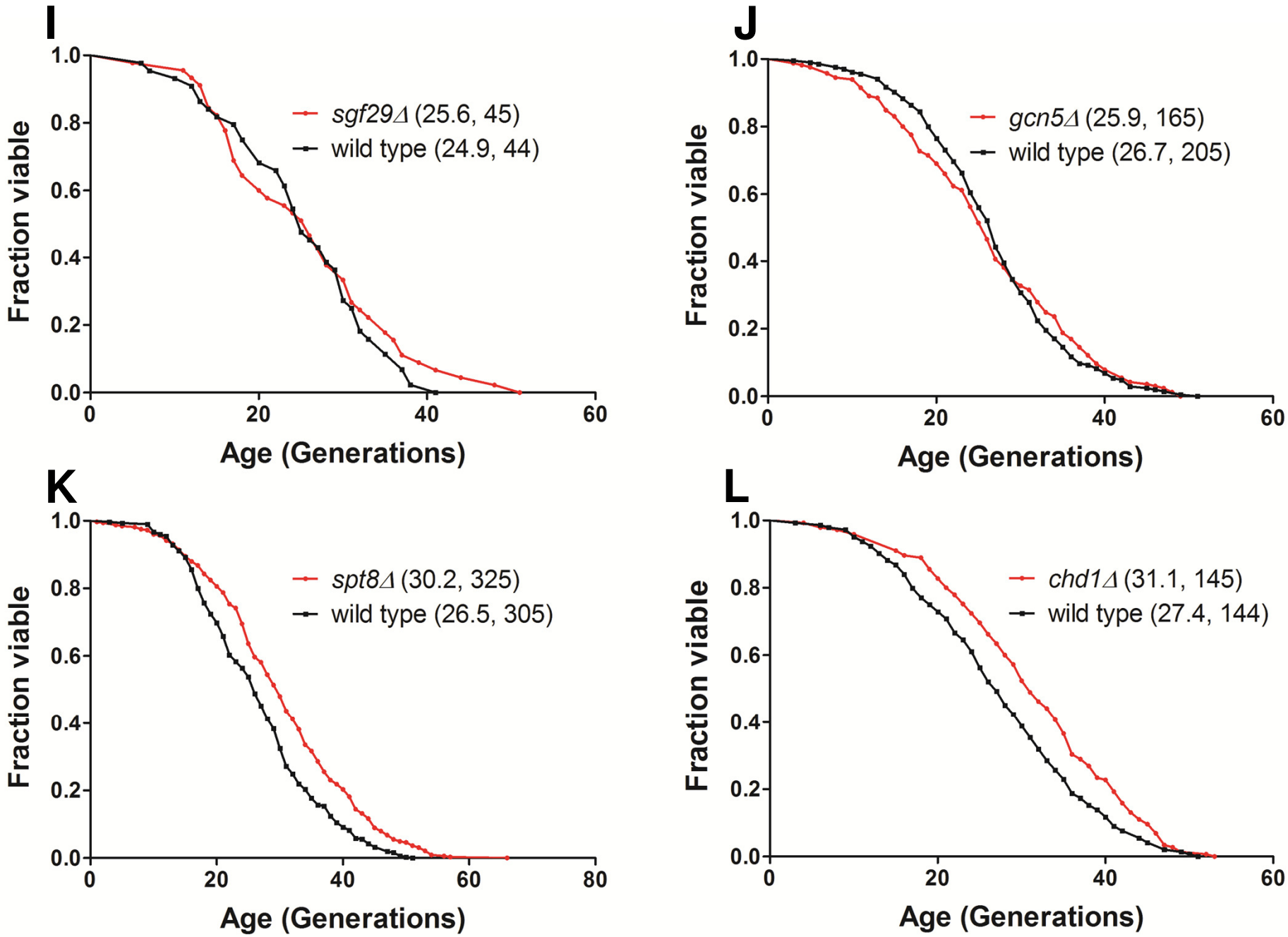
Supplemental Figure S2



Supplemental Figure S2 continued

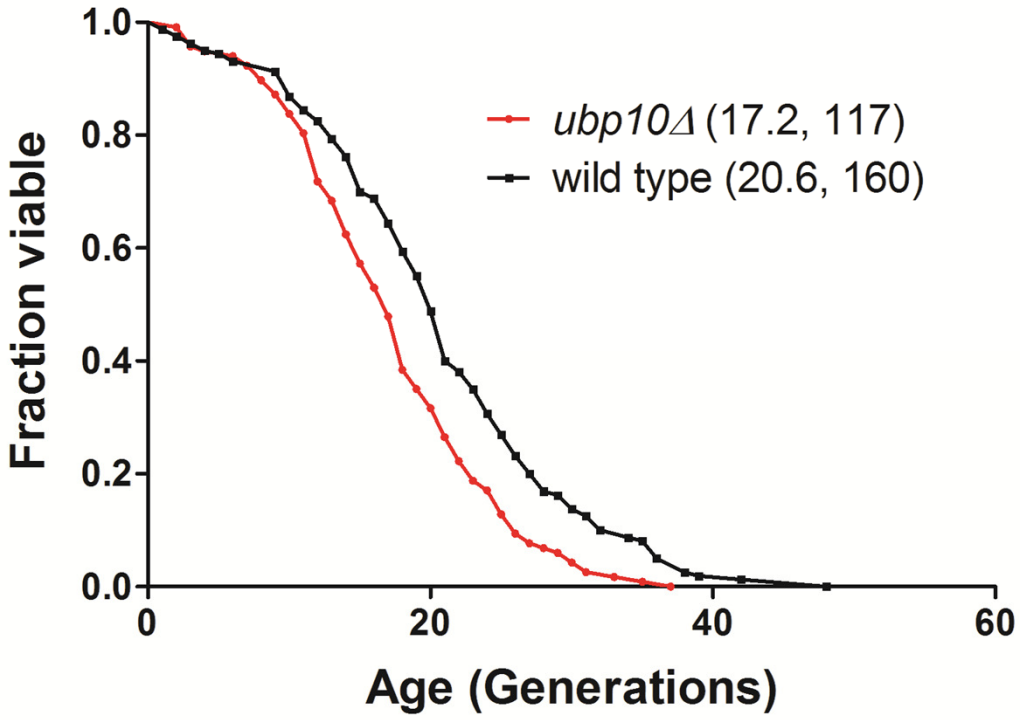


Supplemental Figure S2 continued



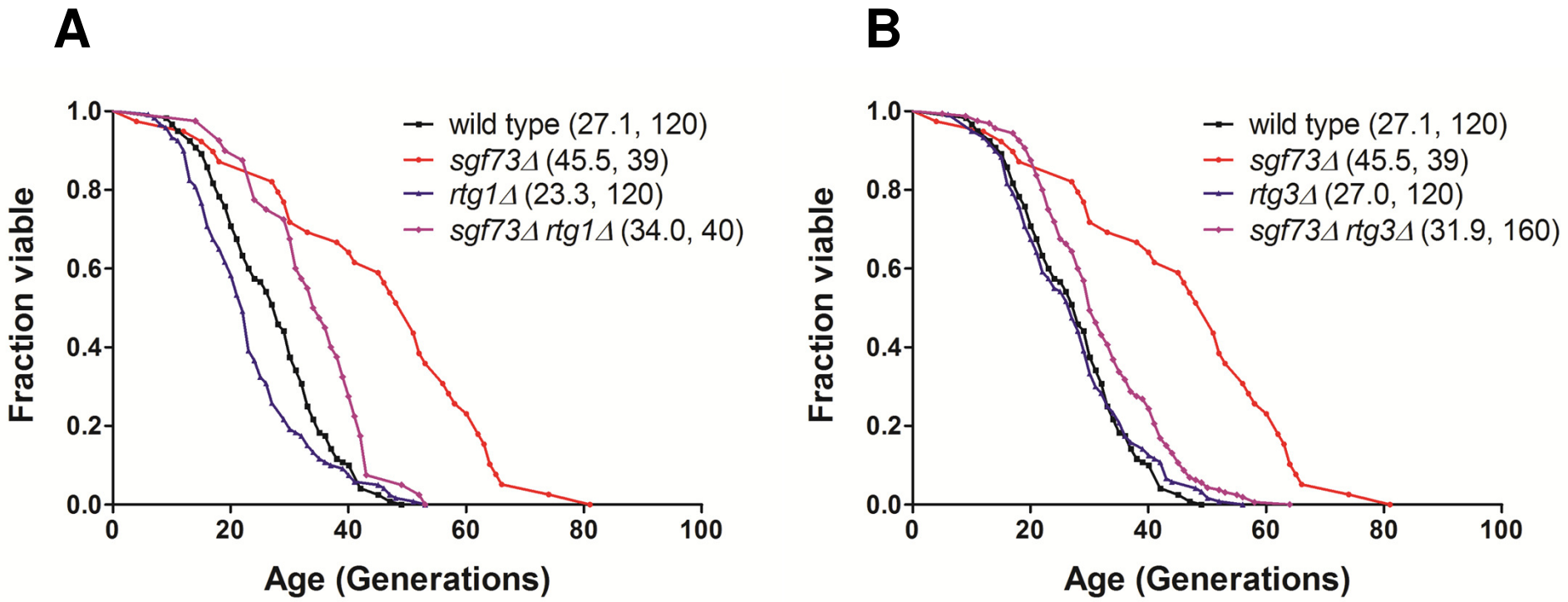
Supplemental Figure S2. Effect on yeast RLS of deletion of other non-essential components of SAGA. Legends show (mean RLS, number of mother cells scored), related to Figure 1.

Supplemental Figure S3



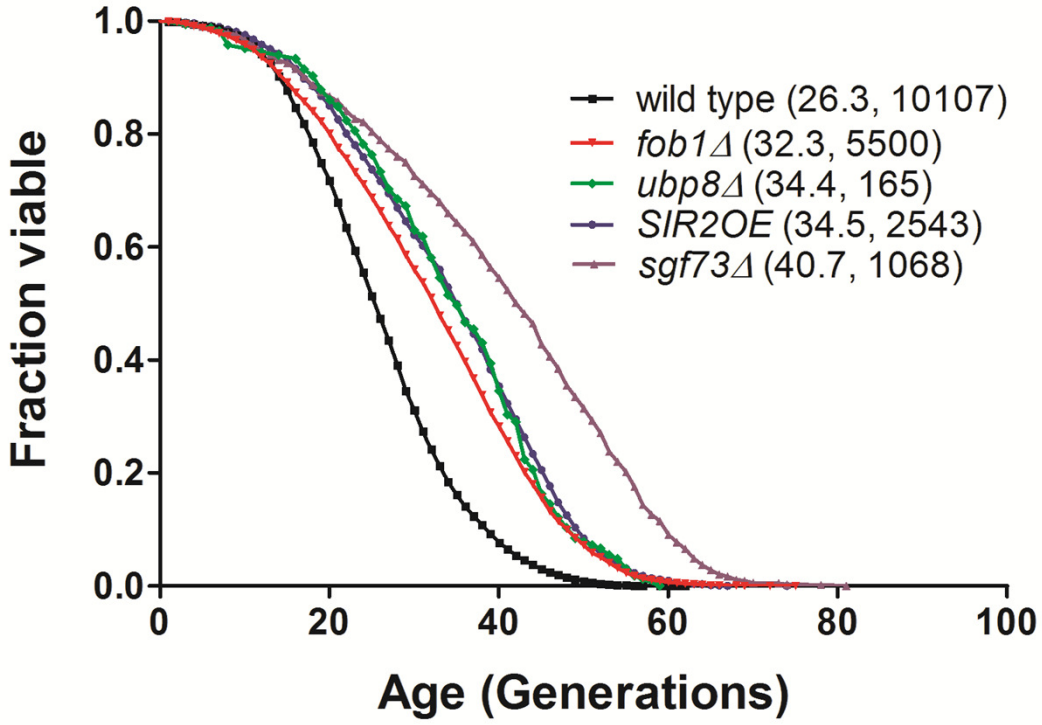
Supplemental Figure S3. Deletion of *UBP10* does not increase RLS, and in fact decreases it. Legend shows (mean RLS, number of mother cells scored), related to Figure 1.

Supplemental Figure S4



Supplemental Figure S4. *RTG1* (A) and *RTG3* (B) are not required for the extended RLS of *sgf73Δ*. Legends show (mean RLS, number of mother cells scored), related to Figure 3.

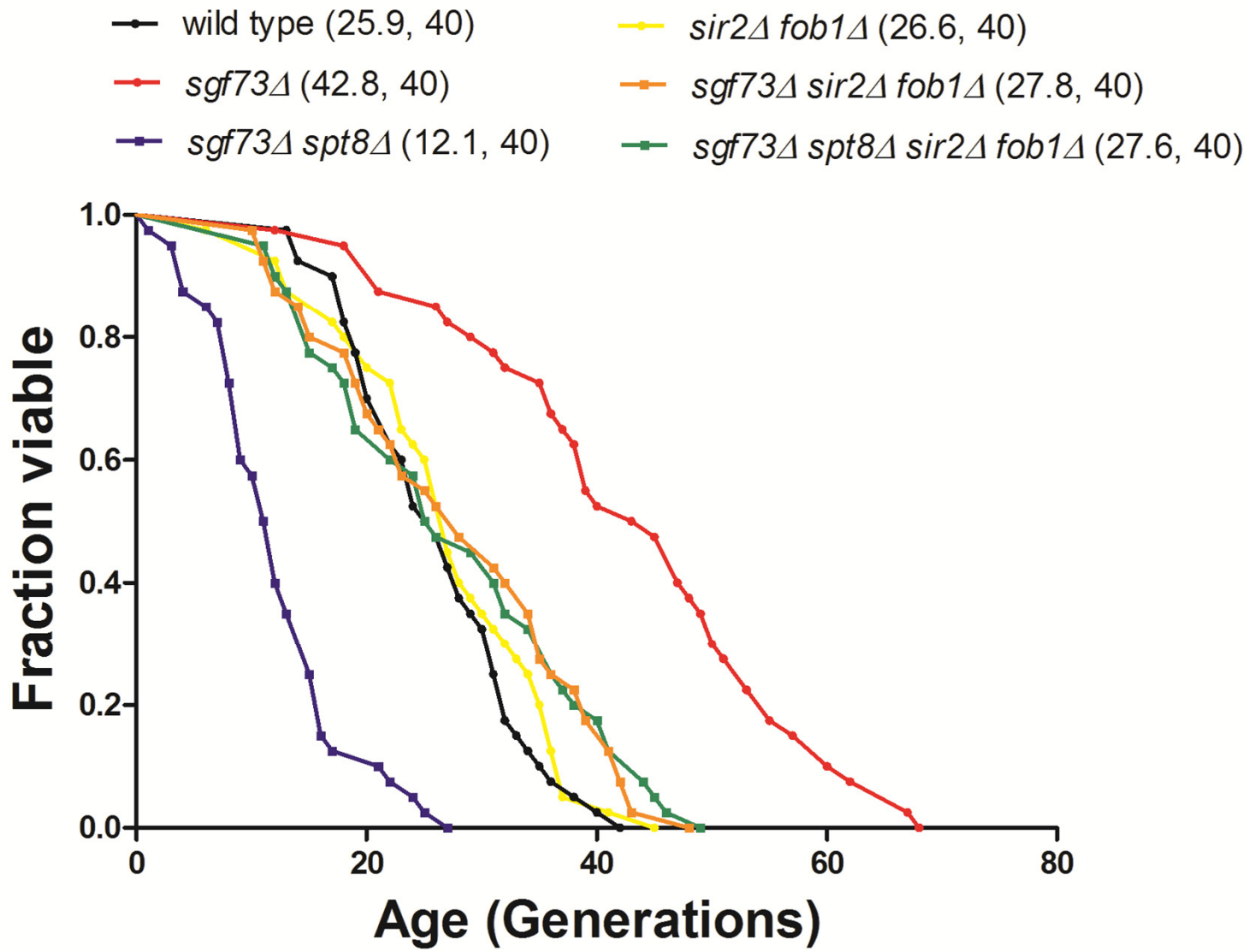
Supplemental Figure S5



Supplemental Figure S5. *sgf73Δ* and *ubp8Δ* are much longer-lived than *fob1Δ* or *SIR2*-OE. Legend shows (mean RLS, number of mother cells scored), related to Figure 4.

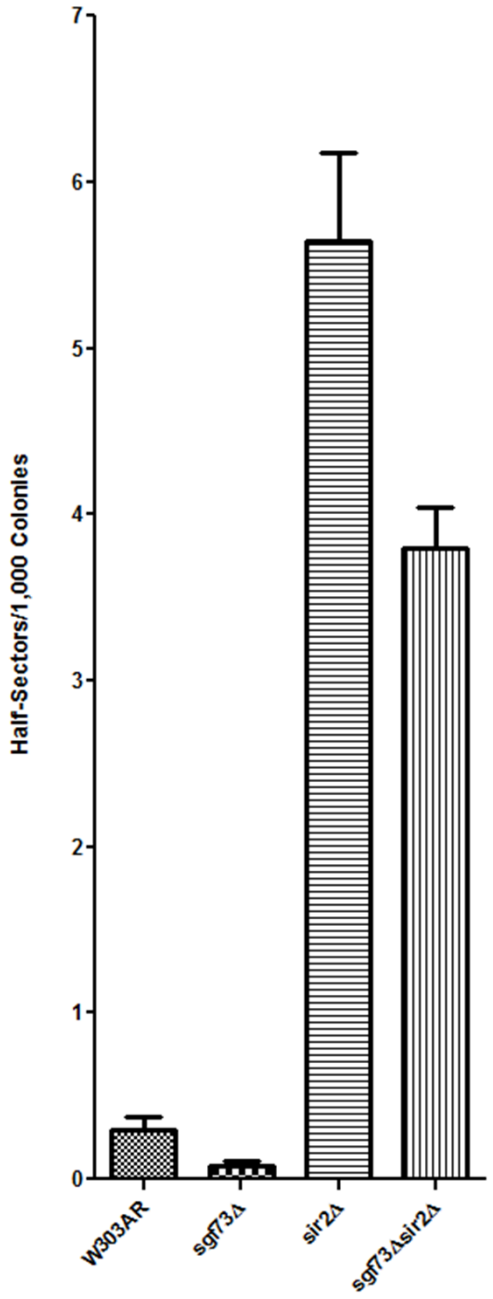
Supplemental Figure S6

A



Supplemental Figure S6, continued

B



Supplemental Figure S6. (A), The *sgf73Δ sir2Δ fob1Δ spt8Δ* strain is not long-lived, as is the case with *sgf73Δ sir2Δ fob1Δ*. (B), rDNA recombination remains high in double mutants lacking both *SGF73* and *SIR2*, related to Figure 5.