

Natural genetic variation in yeast longevity

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Supplemental Material

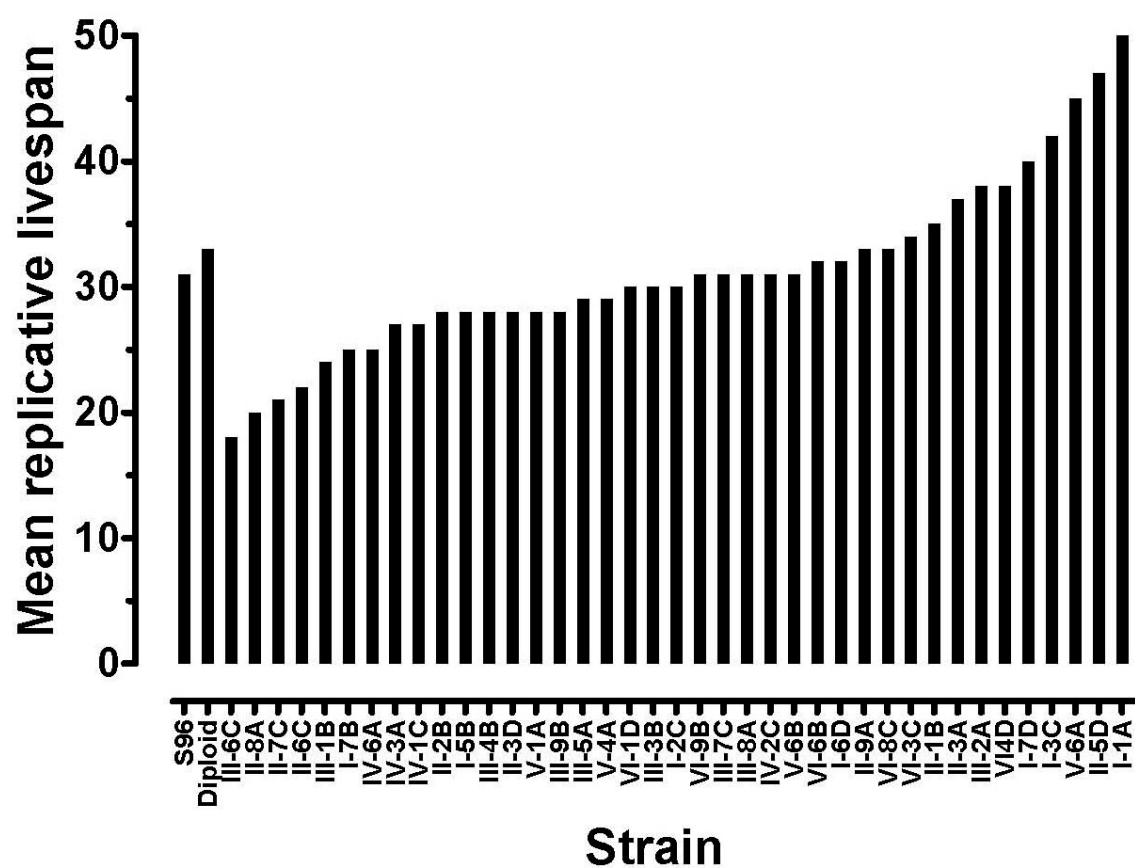


Figure S1: Mean replicative life spans of recombinant lines. The mRLS of all 39 recombinant lines of the haploid segregants of the S96 x YJM789 cross used for the QTL analysis are shown. For comparison, the parental S96 strain and S96 x YJM789 diploid mRLS are shown.

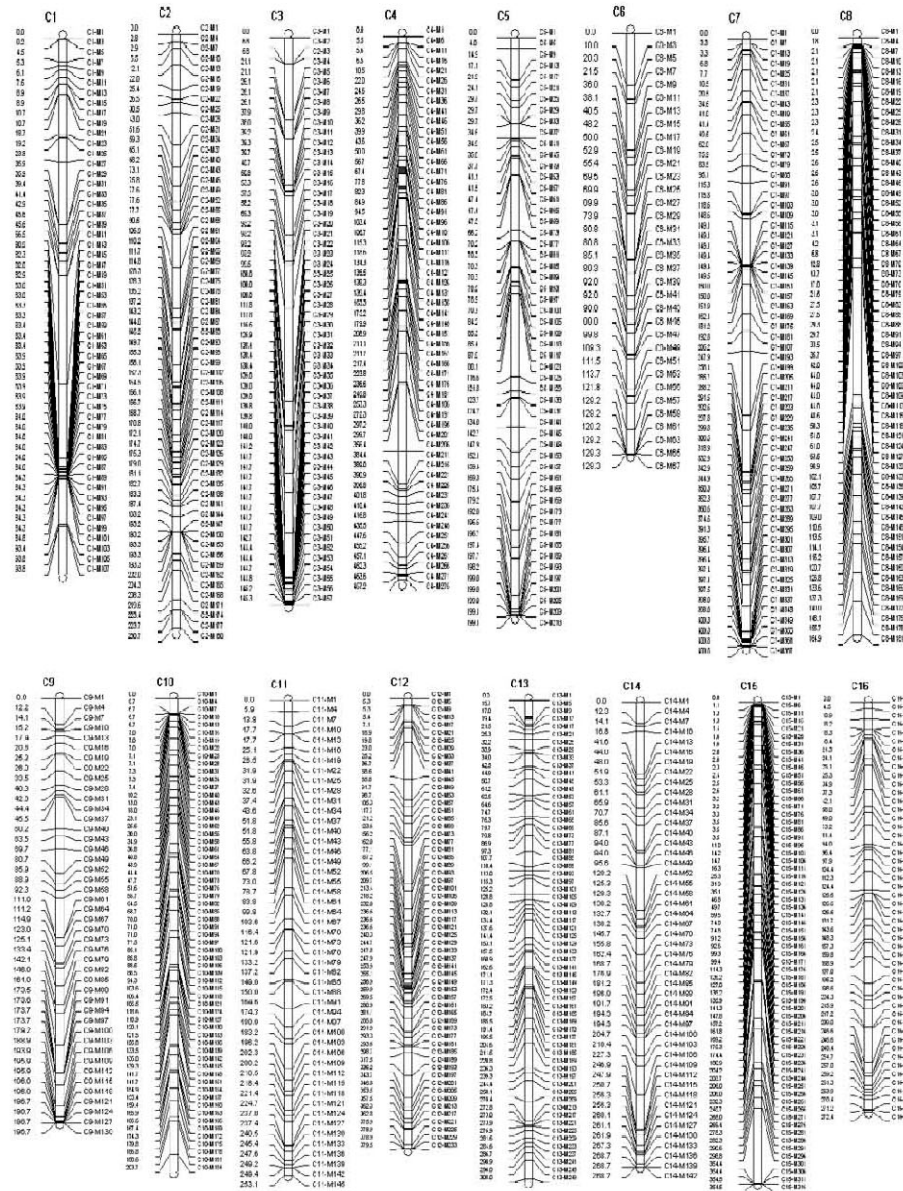


Figure S2: Chromosomal map of the 3,048 molecular markers in the *S. cerevisiae* genome assigned by Allelescan. Genetic distance is shown in cM on the left of each chromosome and marker label is shown on the right. Only 873 of the 3,048 markers used are shown for clarity.

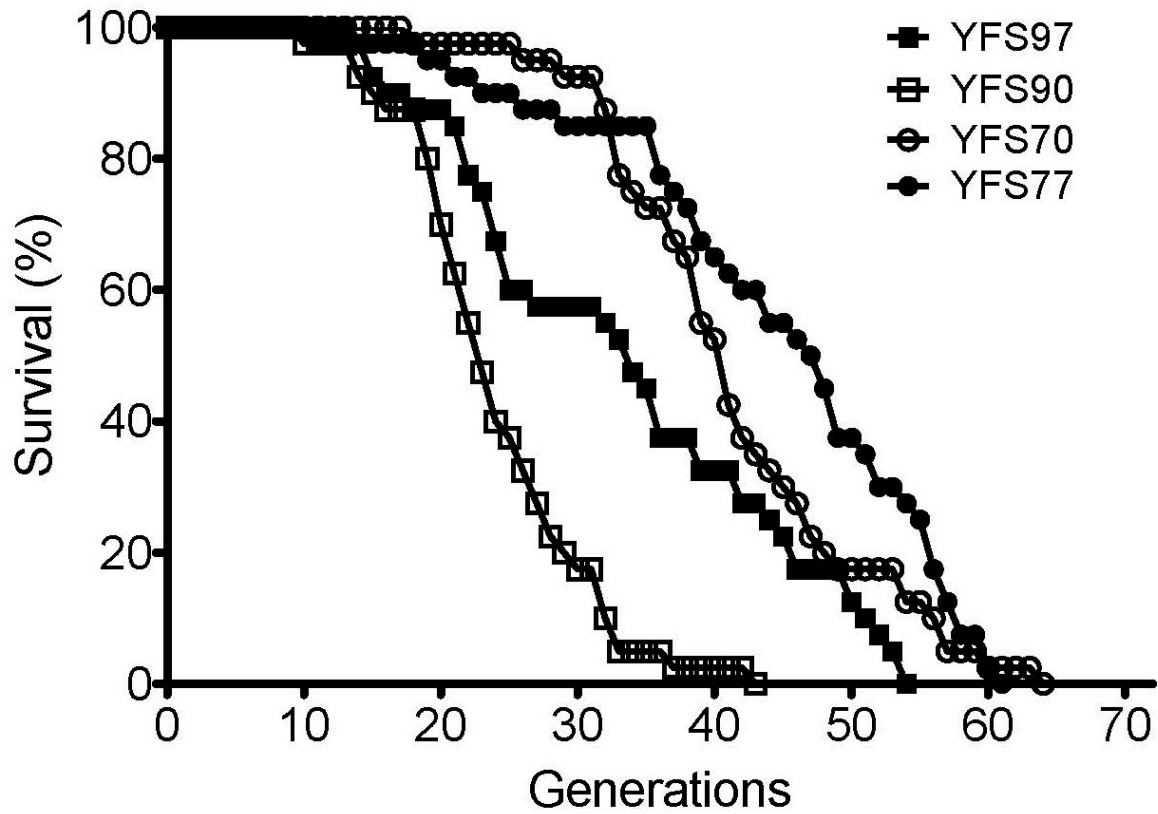


Figure S3: Reciprocal hemizygosity analysis of *SIR2* alleles. Cumulative survival curves were determined for 40 cells of each strain. Strains YFS97, YFS90, YFS70, and YFS77 have mRLS of 33.5, 24.1, 41.6, and 44.6 generations, respectively. The RLS of strains YFS90, YFS70, and YFS77 were each significantly different from that of YFS97, with p-values of 4.47×10^{-4} , 5.39×10^{-3} , and 2.1×10^{-4} respectively. The difference between the RLS of YFS90 and YFS70 was significant ($p = 1.58 \times 10^{-11}$) as well. There was no significant difference ($p = 9.48 \times 10^{-2}$) between the RLS of strains YFS70 and YFS77. Strain YFS97 is the diploid (S96xYJM789) heterozygous for *SIR2*. Strain YFS90 is this diploid hemizygous for the *SIR2* allele from strain S96. Strain YFS70 is this diploid hemizygous for the *SIR2* allele from strain YJM789. Strain YFS77 is the S96xYJM789 diploid homozygous for the *SIR2* allele from strain YJM789.

rDNA (S96) 1 ctcatgtttgccgctctgatggtgcggaaaaaactgctccatgaagcaaactgtccgggcaaatcctttc
 rDNA (YJM789) 1 ctcatgtttgccgctctgatggtgcggaaaaaactgctccatgaagcaaactgtccgggcaaatcctttc
 5' end RFB

rDNA (S96) 71 acgctcgggaagcctttgtgaaagcccttctctttcaaccatctttgcaacgaaaaa
rDNA (YJM789) 71 acgctcgggaagcctttgtgaaagcccttctctttcaaccatctttgcaacg---aaaaa
TERFs

ITS2:

rDNA (S96)	3781	g c c t a g a c g c t c t c t t c t t a t c g a t a a c g t t c c a a t a c g c t c a g t a t a a a a a a g a t t a g c c g c a g t t g g
rDNA (YJM789)	3778	g c c t a g a c g c t c t c t t c t t a t c g a t a a c g t t c c a a t a c g c t c a g t a t - a a a a a a g a t t a g c c g c a g t t g g

ITS1:

rDNA (S96)	4131	caagagatccggtgtgtgaaagtttttaatatattt	taaaatttccagttacgaaaattctgtttttgacaa
rDNA (YJM789)	4127	caagagatccggtgtgtgaaagtttttaatatattt	taaaatttccagttacgaaaattctgtttttgacaa

rDNA (S96)	4201	aaattttaa	gaataga	ataaaaattgtttgtgtttgttacctctgggccccgattgctcgaatgcccaaaga
rDNA (YJM789)	4197	aaattttaa	gaatara	ataaaaattgtttgtgtttgttacctctgggccccgattgctcgaatgcccaaaga

rDNA (S96)	4341	gcacagaaatctctcaccgtttggaatagcaagaagaaacttacaagcctagcaggaccgcgcacttaa
rDNA (YJM789)	4337	gcacagaaatctctcaccgtttggaatagcaagaagaaacttacaagcctagcaggaccgcgcacttaa

ETS1:

rDNA (S96) 6301 aggatcaaccagataaactatctttaaagaagaagcaacaagcagtaaaaaaagaaagaaaccgaaatctct
 rDNA (YJM789) 6297 aggatcaaccagataaactatctttaaagaagaagcaacaagcagtaaaaa---aaagaaaccgaaatctc-

rDNA (S96)	6371	ttt	ttttttttccacacctattccctcttgctagaagatacttattgagtttggaacagctgaaattccag
rDNA (YJM789)	6363	---	ttttttttccacacctattccctcttgctagaagatacttattgagtttggaacagctgaaattccag

NTS2:

rDNA (S96)	7561	cctaccaataacggttaactattctatgttttcttactcctatgtctattcatctttcatctgactacctta
rDNA (YJM789)	7550	cctaccaataatcggttaactattctatgttttcttactcctatgtctattcatctttcatctgactacctta

rDNA (S96)	7631	a	t	a	c	t	a	t	g	c	a	a	a	a	t	g	t	a	a	a	t	c	a	t	c	a	c	a	c	a	a	a	a	c	a	a	a	c	a	a	a	t	c	a	g	c	c	a	t	t	t	c	g	c	a	c	t	t	t	t
rDNA (YJM789)	7620	a	t	a	c	t	a	t	g	c	a	a	a	a	t	g	t	a	a	a	t	t	a	t	c	a	c	a	c	a	a	a	a	c	a	a	a	c	a	a	a	t	c	a	g	c	c	a	t	t	t	c	g	c	a	c	t	t	t	t

ACS

rDNA (S96)	7771	tactataaaacaacctttagacttacggttgctactctcatgggtctcaatactgccgccgaattctgtc
rDNA (YJM789)	7760	tactataaaacaacctttagacttacggttgctactctcatgggtctcaatactgccgccgaattctgtc

rDNA (S96)	8121	ctttttcacctgtcatatcctattgctattagatgaaatataataaaaaattgtcctccaccataacacc
rDNA (YJM789)	8110	ctttttcacctgtcatatcctattgcvttagatgaaatataataaaaaattgtcctccaccataacacc

NTS1-2:

rDNA (S96)	8331	ccgagtagtgtagtgggtgaccatacgcgaaactcaggtgctgcaatctttatttct	tttttttttttttt
rDNA (YJM789)	8320	ccgagtagtgtagtgggtgaccatacgcgaaactcaggtgctgcaatctttatttct	-----t

rDNA (S96)	8401	tttttttttttttctagtttcttggcttcctatgctaaatcccataactaacctaccattcgattcag
rDNA (YJM789)	8377	tttttttttttttctagtttcttggcttcctatgctaaatcccataactaacctaccattcgattcag

rDNA (S96)	8471	aaaaattcgca	tatccagctgc	actcttctct	tctgaagagtt	aagcactccat	tatgctcattg	gggttgc
rDNA (YJM789)	8447	aaaaattcgca	tatccagctgc	actcttctct	tctgaagagtt	aagcactccat	tatgctcattg	gggttgc

rDNA (S96)	8541	tactacttgatattgtacaacaatattctcctccgatattcctac	aaaaaaaaaaaaaaaaaacactccgg
rDNA (YJM789)	8517	tactacttgatattgtacaacaatattctcctccgatattcctac	-----aaaaaaaaaacactccgg

rDNA (S96)	8611	ttttgttctcttccctccatttccctctcttctacggttaatactttcctcttcgtctttttctacacc
rDNA (YJM789)	8579	ttttgttctcttccctccatttccctctcttctacggttaatactttcctcttcgtctttttctacacc

rDNA (S96)	8681	tcgcttagttgcttcttattccttcccgctttcctgcactaacattttgccgcattacactatatgatcg
rDNA (YJM789)	8649	tcctvtagtgtccttcttattccttcccgctttcctgcactaacattttgccgcattacactatatgatcg

rDNA (S96)	8751	tagtacatcttacaactccgcata	ccgcgctgcgcgctcg	ccgcgctgcgcaaaaatttacttcgccaacc
rDNA (YJM789)	8719	tagtacatcttacaactccgcata	-----ccgcgctgcgcaaaaatttacttcgccaacc	

rDNA (S96)	8821	attccatatctgttaagtatacatgtatatattgcactggctattcatcttgcacttttcctctttcttc
rDNA (YJM789)	8773	attccatatctgttaagtatacatgtatatattgcactgactattcatcttgcacttttcctctttcttc

rDNA (S96)	8891	ttccagtagcctcatccttttacgctgcctctctggaacttgccatcatcattccctagaaactgccat
rDNA (YJM789)	8843	ttccagtagcctcatccttttacgctgcctctctggaacttgccatcatcattccctagaaactgccat

rDNA (S96) 8961 ttacttt[aaaaaaaaaaaa](#)aaaaaaaaaaaatgtccccactggttcactgttcactgttcacttgtctcttaca
rDNA (YJM789) 8913 ttacttt-----aaaaaaaaaaaatgtccccacta-ttcactgttcactgttcacttgtctcttaca

rDNA (S96) 9031 tctttcttggtaaaatcgtagttcgtagta**t**ttttttcatatcaaaggcattgctct**g**tttaactatagga
rDNA (YJM789) 8971 tctttcttggtaaaatcgtagttcgtagta- tttttttcatatcaaaggcattgctctatttaactatagga

LaacLaayga
3' end RFB

rDNA (S96) 9101 aatgagcttttctcaattctctaaacttatacaagca 9137
rDNA (YJM789) 9040 aatgagcttttctcaattctctaaacttatacaagca 9076

Figure S4: Sequence alignment of one rDNA repeat. Only regions of the rDNA containing sequence differences between strains S96 and YJM789 are shown. These polymorphisms are highlighted in yellow. The six loci highlighted in green are polymorphic in YJM789, as indicated by the single-letter IUPAC code (m=a or c; r=a or g; y=c or t). The replication fork block (RFB) is indicated and underlined. The TERfs designates the *RDN5-1* Fail safe terminator, which is also underlined. The position of the 11-bp ACS (double-underlined) is shown as part of the 107-bp ARS1200 (underlined).

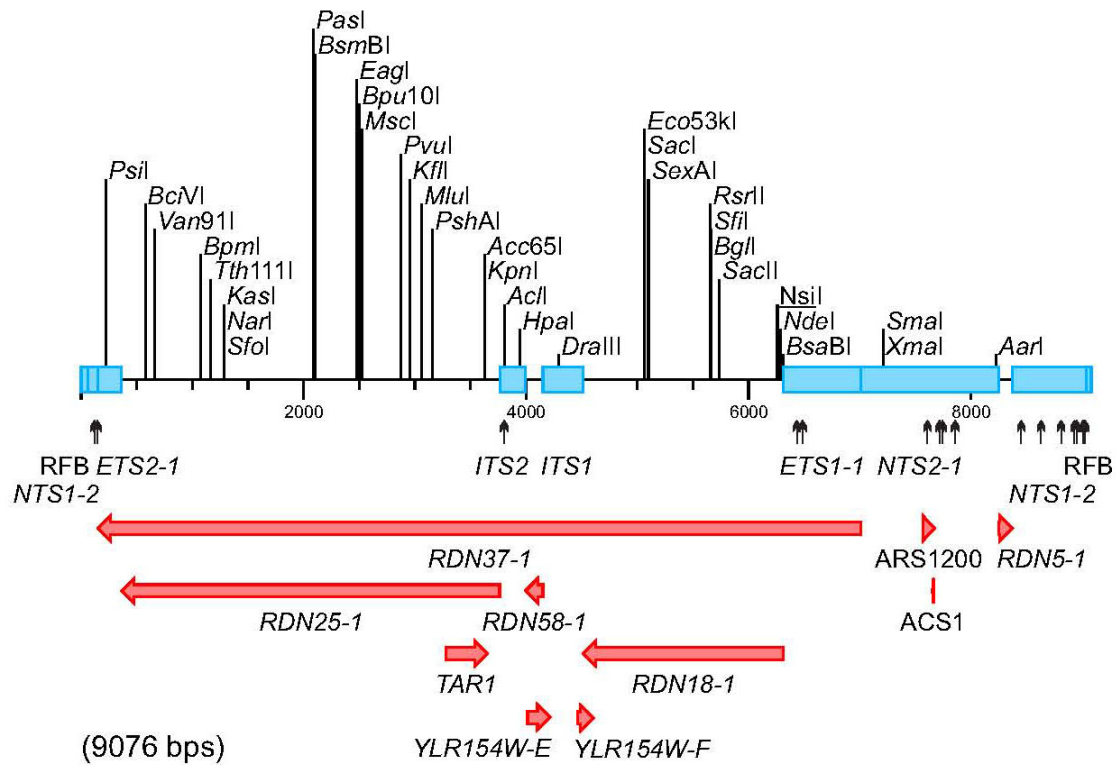


Figure S5: Schematic of a single repeat of the yeast rDNA cluster. The *RDN1* sequence of strain YJM789 was used to generate this figure. The ARS1200 origin of replication contains the ARS consensus sequence (ACS). *ETS* = external transcribed spacer, *ITS* = internal transcribed spacer, *NTS* = non-transcribed spacer, RFB = replication fork barrier. The arrows point to polymorphisms.

ACS in ARS1200 of S96...: GTTTATGT<u>T</u>TT ACS in ARS1200 of YJM789: GTTTATGT<u>C</u>TT ARS consensus sequence...: WTTTAYRT<u>T</u>TW
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Figure S6: ACS sequence comparison. The ACS present in ARS1200 of the rDNA cluster of strains S96 and YJM789 are compared. The ACS in all S288c-derived *S. cerevisiae* strain ARS is shown as reference. The ninth bp is C in YJM789, while it is T in S96 and the consensus. The polymorphism is underlined. W=A or T, Y=C or T and R=A or G.

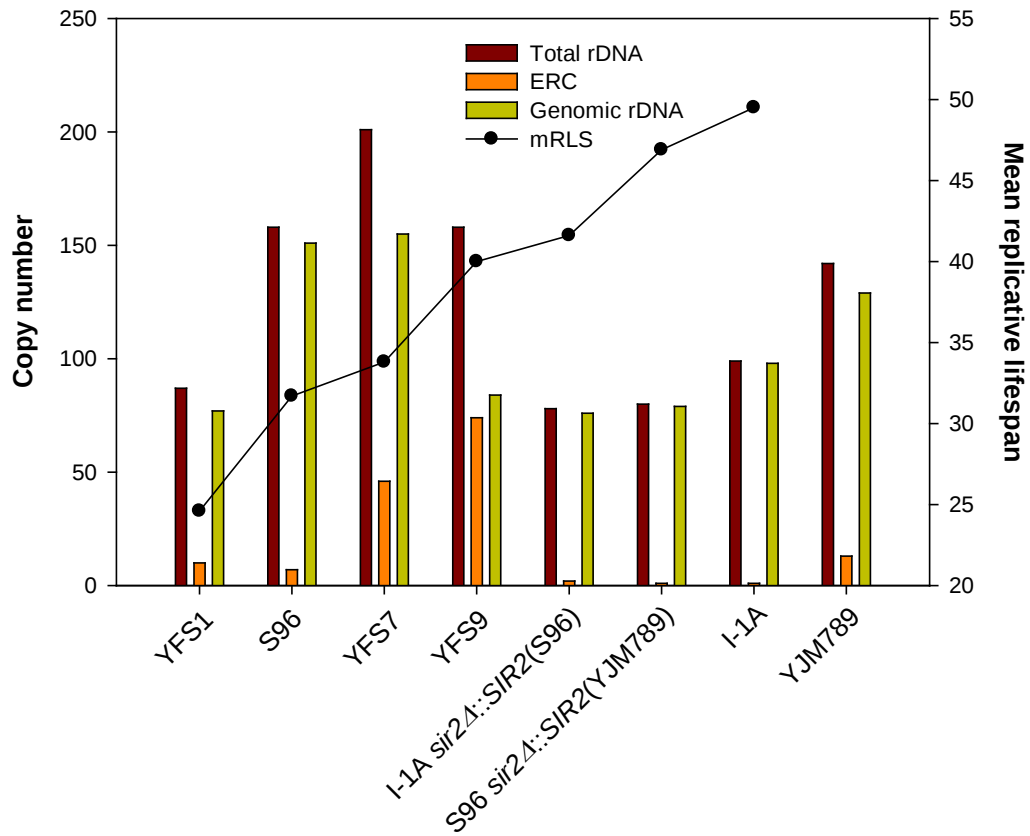


Figure S7: Comparison of rDNA copy number and mean replicative life span in selected strains and lines. Total rDNA and ERC copy number were determined as described in Methods, and the genomic rDNA is expressed as the difference. mRLS is plotted alongside copy number as one of the quantities for each strain/line. YFS1 (S96 background with *RDN1* from YJM789), S96, YFS7 (YJM789 background with *RDN1* from YJM789), YFS9 (YJM789 background with *RDN1* from S96), I-1A *sir2Δ::SIR2*(S96), S96 *sir2Δ::SIR2*(YJM789), I-1A, and YJM789 (for which RLS cannot be determined) were compared. I-1A is the recombinant line with the longest RLS.