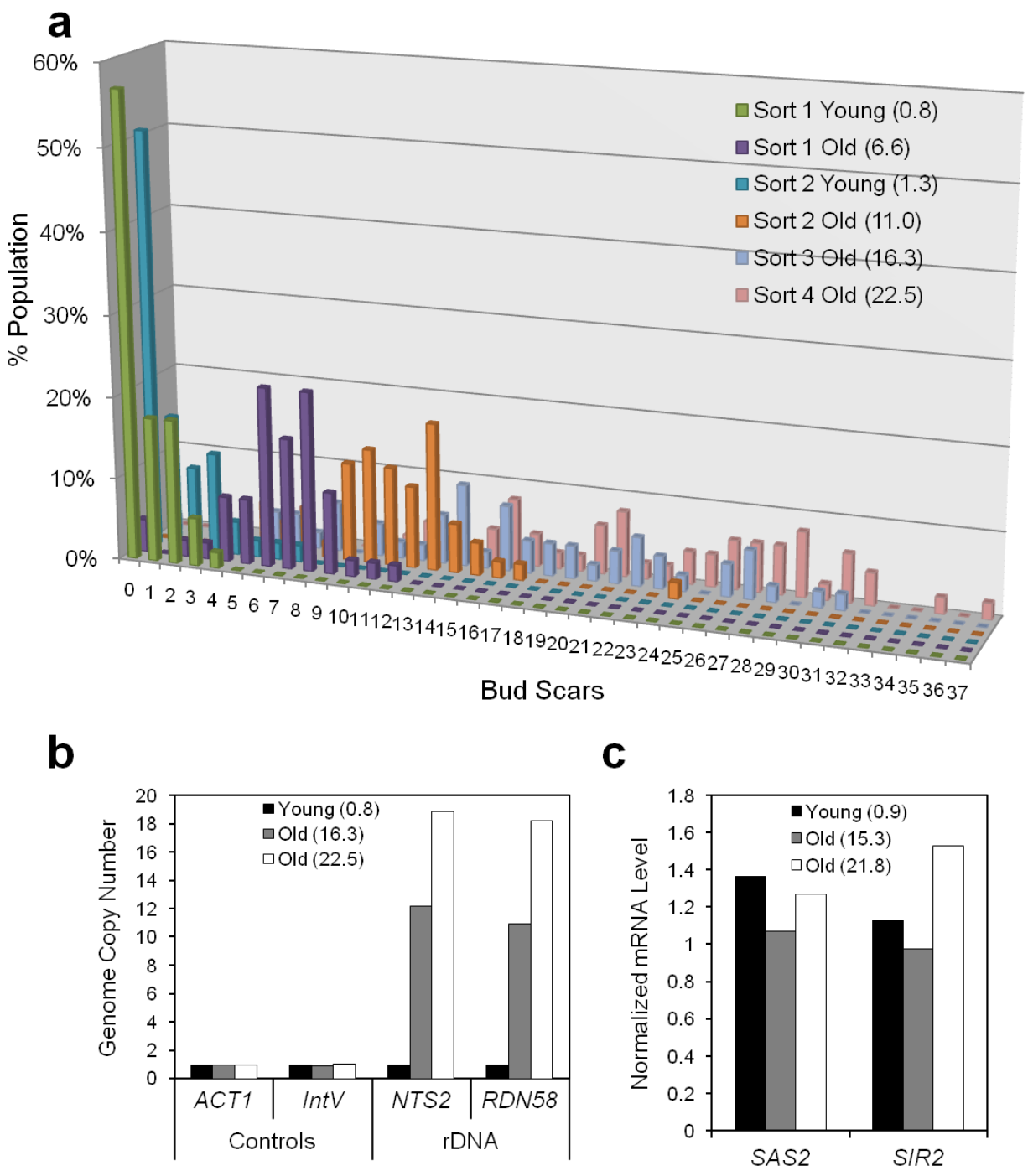
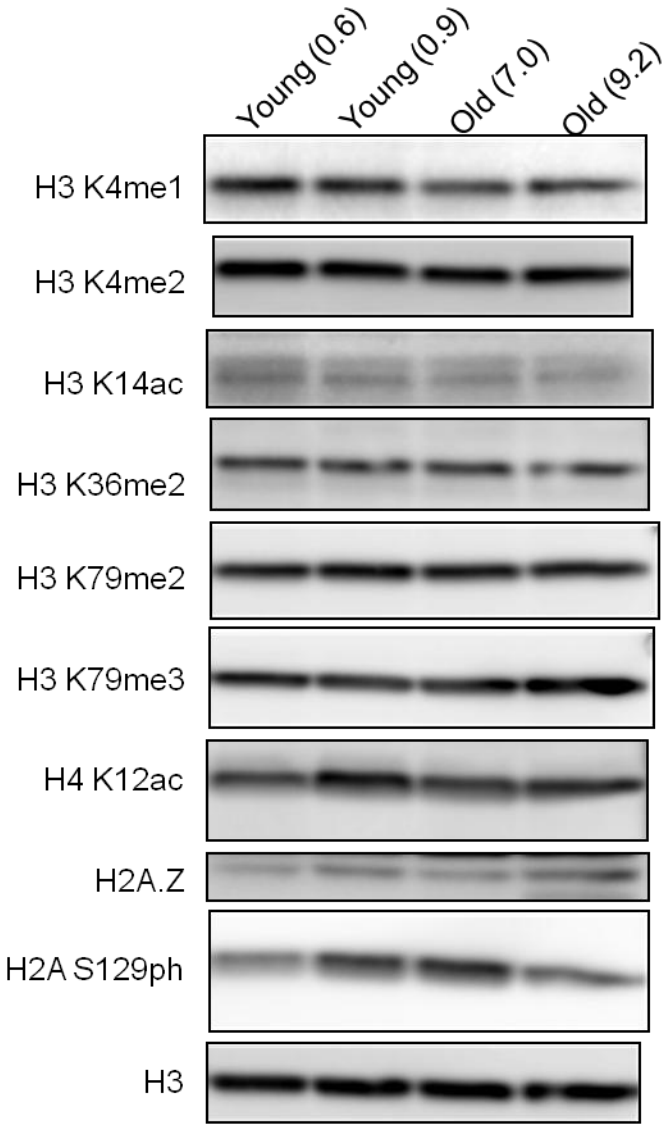
Supplementary Information

Supplementary Figures



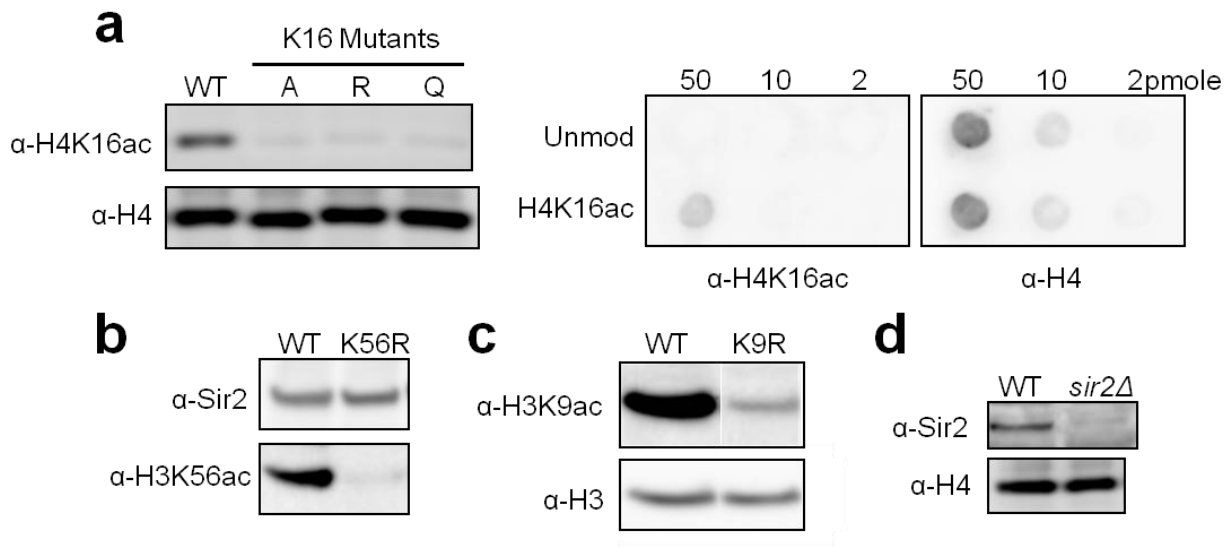
Supplementary Figure 1. Isolated old cells show increasing bud scars and rDNA copy numbers, but no change in *SIR2* expression levels.

(a) An example of age distribution of isolated young and old cells from four rounds of progressive sorting. Bud scars were stained with Calcofluor and visualized by fluorescence microscopy. At least 50 cells from each fraction were counted for total number of bud scars. (b) Total genomic DNA was purified from whole cell extracts and copy number was quantified by real-time PCR with primers specific to *ACT1* gene, an intergenic region on Chromosome V (*IntV*), non-transcribed region *NTS2* and *RDN58* gene on rDNA repeat. All signals were normalized to that of *ACT1* gene and then to young cell levels. (c) Quantitative real-time PCR analysis of mRNA expression levels for *SAS2* and *SIR2* in young and old cells of strain BY4741. Real-time PCR primers are listed in Supplementary Table 4. Signals were normalized to *ACT1* levels. Average bud scar counts are indicated in parenthesis.



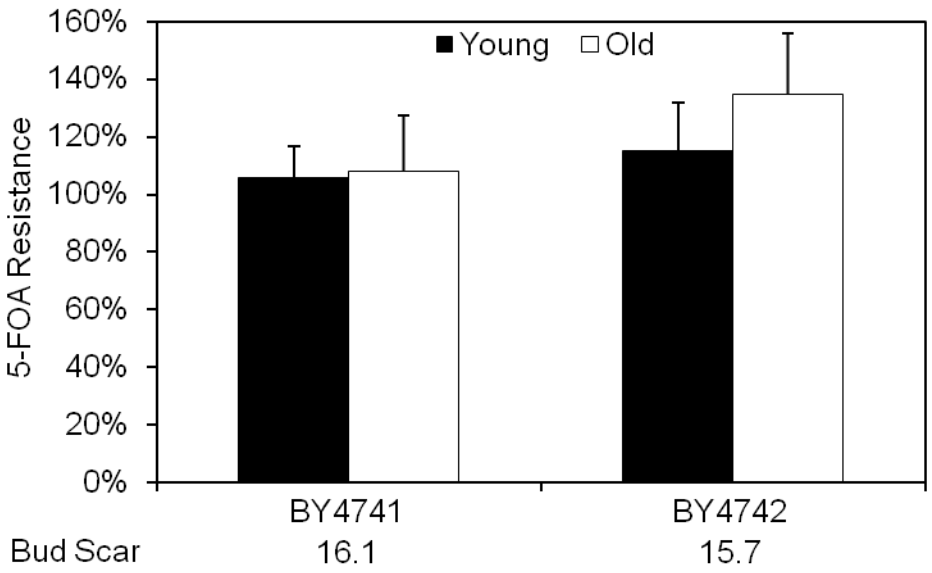
Supplementary Figure 2. Examples of other histone modifications remaining unchanged in old cells.

Samples as in Fig. 1a were analyzed with other antibodies with details listed in Supplementary Table 3. Average bud scar counts are indicated in parenthesis.



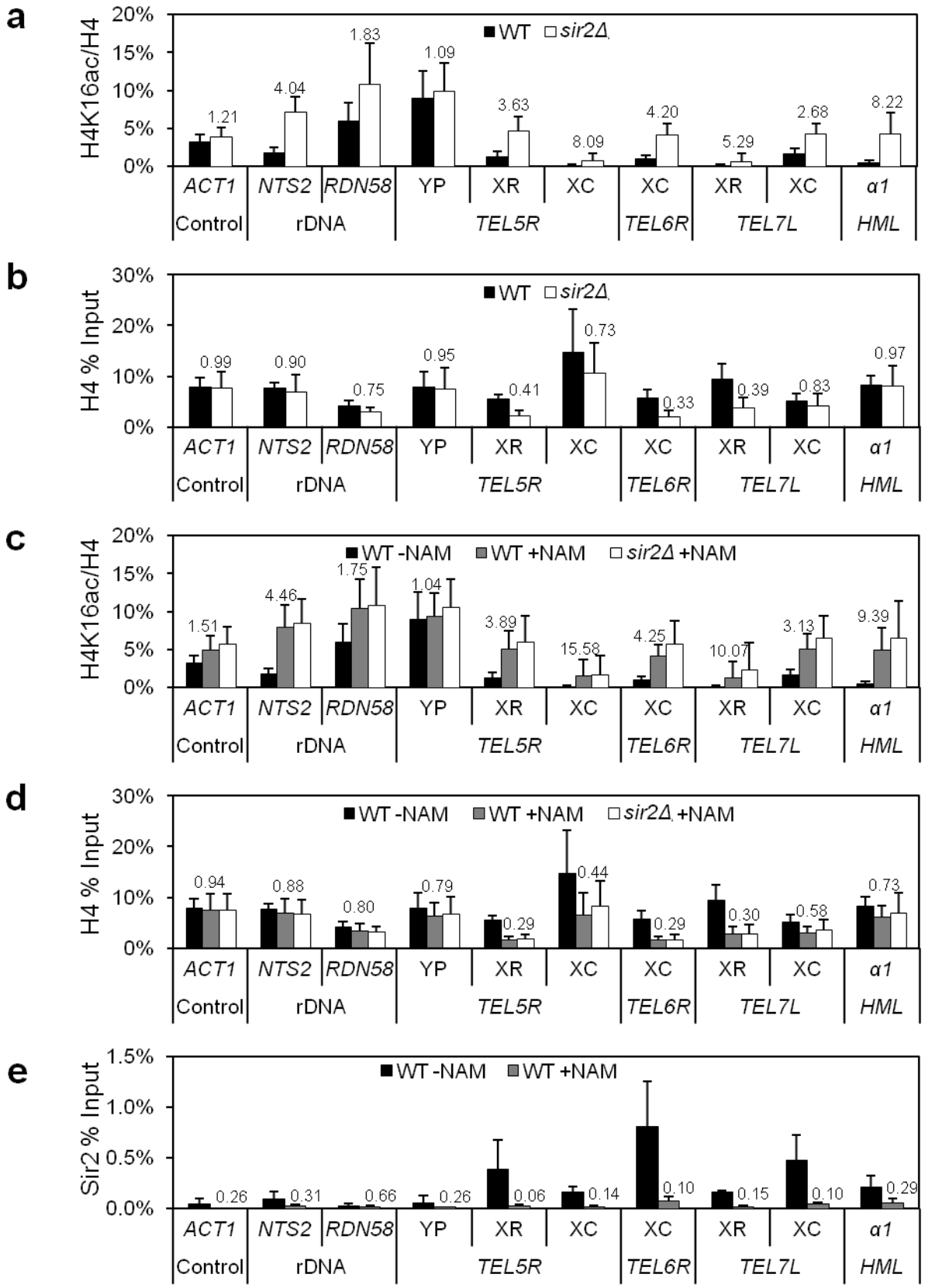
Supplementary Figure 3. Specificity of key antibodies used in this study.

(a) Left: Whole cell extracts from strains carrying WT histone H4, H4K16A, H4K16R, or H4K16Q plasmids were analyzed by western blot and probed with antibodies against H4K16ac and H4. Right: H4 N-terminal peptide containing the first 30 amino acids (unmod) and corresponding K16 acetylated (H4K16ac) peptides were dot-blotted onto a PVDF membrane with the indicated amount and probed with H4K16ac and H4 antibodies. (b) Whole cell extracts from strains carrying WT H3 or H3K56R plasmids were analyzed by western blot and probed with antibodies against H3K56ac and Sir2. (c) Whole cell extracts from strains carrying WT H3 or H3K9R plasmids were analyzed by western blot and probed with antibodies against H3K9ac and histone H3. (d) Whole cell extracts from WT (BY4741) or *sir2Δ* strains were analyzed by western blot and probed with antibodies against Sir2 and H4.



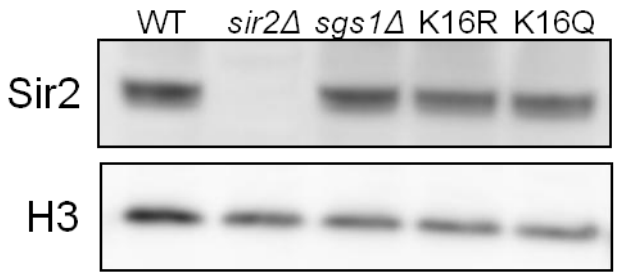
Supplementary Figure 4. *ura3*Δ old cells are not sensitive to 5-FOA.

Identical silencing assay as in Fig. 2b for young and old cells of strains containing *ura3*Δ. The extent of silencing is expressed as the fraction of cells resistant to 5-FOA (n=3). Average bud scar counts are listed for the old cell samples. Error bars show standard deviations.


a
H4K16ac/H4
20%
15%
10%
5%
0%
WT
sir2Δ
1.21
4.04
1.83
1.09
3.63
8.09
4.20
5.29
2.68
8.22
ACT1
NTS2
RDN58
YP
XR
XC
XC
XR
XC
α1
Control
rDNA
TEL5R
TEL6R
TEL7L
HML
b
H4 % Input
30%
20%
10%
0%
WT
sir2Δ
0.99
0.90
0.75
0.95
0.41
0.73
0.33
0.39
0.83
0.97
ACT1
NTS2
RDN58
YP
XR
XC
XC
XR
XC
α1
Control
rDNA
TEL5R
TEL6R
TEL7L
HML
c
H4K16ac/H4
20%
15%
10%
5%
0%
WT -NAM
WT +NAM
sir2Δ +NAM
1.51
4.46
1.75
1.04
3.89
15.58
4.25
10.07
3.13
9.39
ACT1
NTS2
RDN58
YP
XR
XC
XC
XR
XC
α1
Control
rDNA
TEL5R
TEL6R
TEL7L
HML
d
H4 % Input
30%
20%
10%
0%
WT -NAM
WT +NAM
sir2Δ +NAM
0.94
0.88
0.80
0.79
0.29
0.44
0.29
0.30
0.58
0.73
ACT1
NTS2
RDN58
YP
XR
XC
XC
XR
XC
α1
Control
rDNA
TEL5R
TEL6R
TEL7L
HML
e
Sir2 % Input
1.5%
1.0%
0.5%
0.0%
WT -NAM
WT +NAM
0.26
0.31
0.66
0.26
0.06
0.14
0.10
0.15
0.10
0.29
ACT1
NTS2
RDN58
YP
XR
XC
XC
XR
XC
α1
Control
rDNA
TEL5R
TEL6R
TEL7L
HML

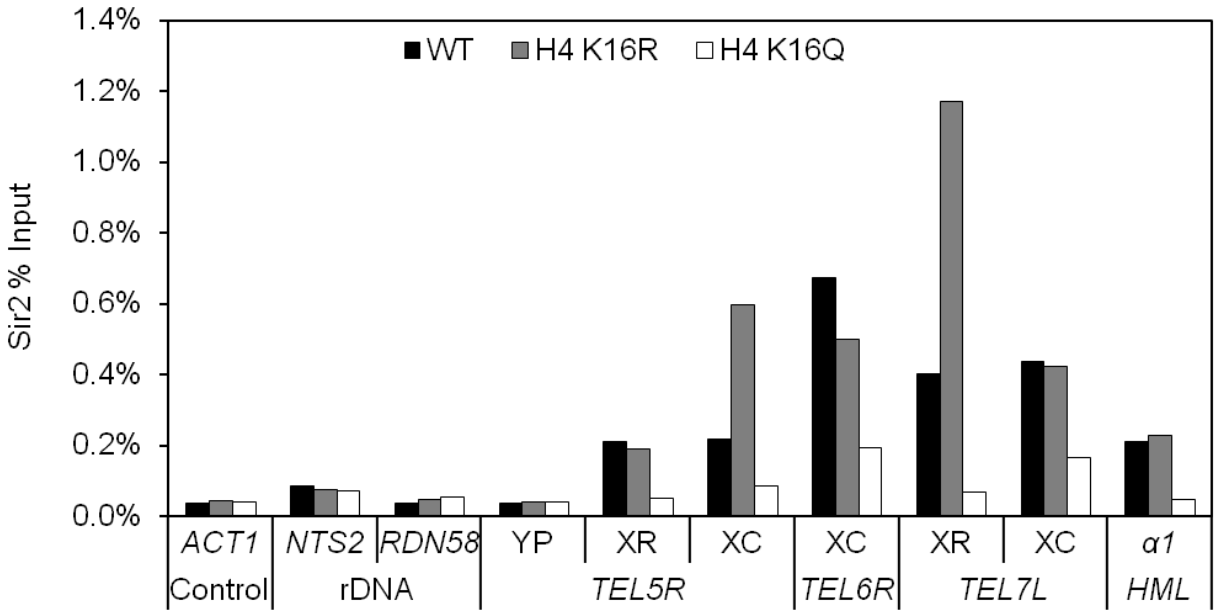
Supplementary Figure 5. Localized changes of H4K16 acetylation and histone levels when Sir2 is lost or inhibited.

Chromatin Immunoprecipitation was performed for wild-type (BY4741) and *sir2Δ* cells (n=3) with antibodies against (a) H4K16ac and (b) histone H4. Fold changes for *sir2Δ* compared to wild-type are indicated above the bars. The same analysis was carried out for wild-type (BY4741) cells with (WT+NAM) or without (WT-NAM) treatment of 5 mM nicotinamide (NAM) and *sir2Δ* cells with 5 mM nicotinamide (*sir2Δ*+NAM) (n=3) with antibodies against (c) H4K16ac and (d) histone H4. Fold changes for WT+NAM compared to WT-NAM are indicated above the bars. (e) Chromatin Immunoprecipitation was performed for wild-type (BY4741) cells with (WT+NAM) and without (WT-NAM) treatment of 5 mM nicotinamide (n=3) with antibodies against Sir2. The primer sets are shown in Fig. 1b. All error bars show standard deviations.



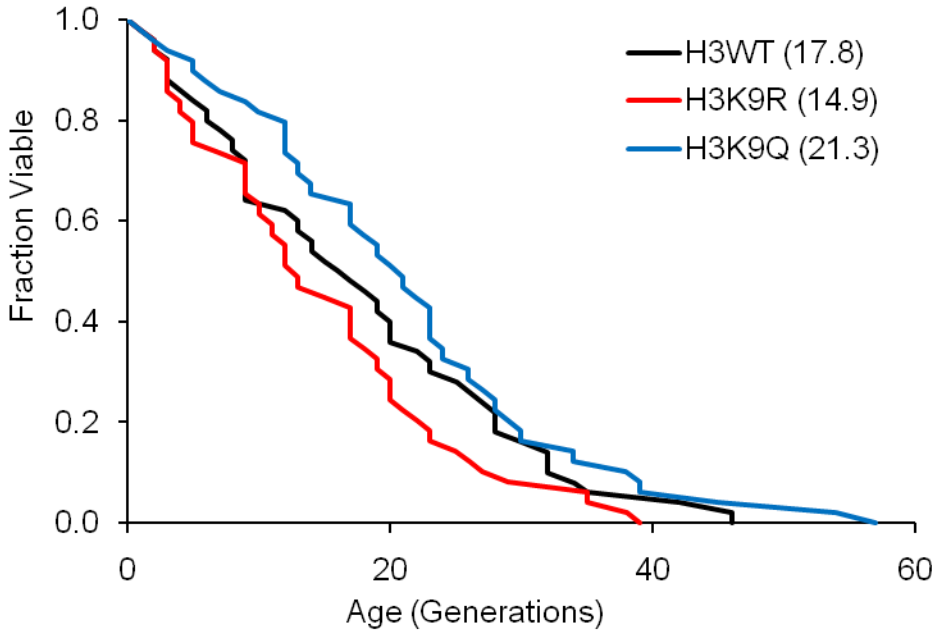
Supplementary Figure 6. Mutations of H4K16 does not affect availability of Sir2.

Western analysis with antibodies against Sir2 and H3 for cells bearing WT histone H4, H4K16R, or H4K16Q plasmids.

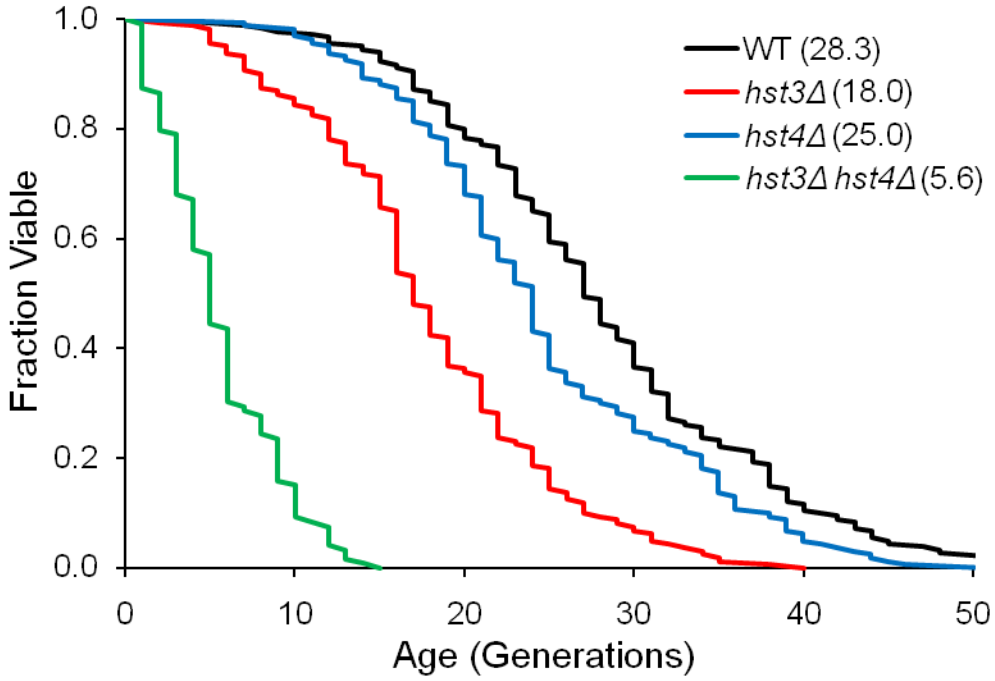


Supplementary Figure 7. H4K16Q mutation displaces Sir2 from its binding sites.

Chromatin Immunoprecipitation was performed for strains carrying WT histone H4, H4K16R, or H4K16Q plasmids with antibodies against Sir2 and analyzed by real-time PCR with primers shown in Fig. 1b.

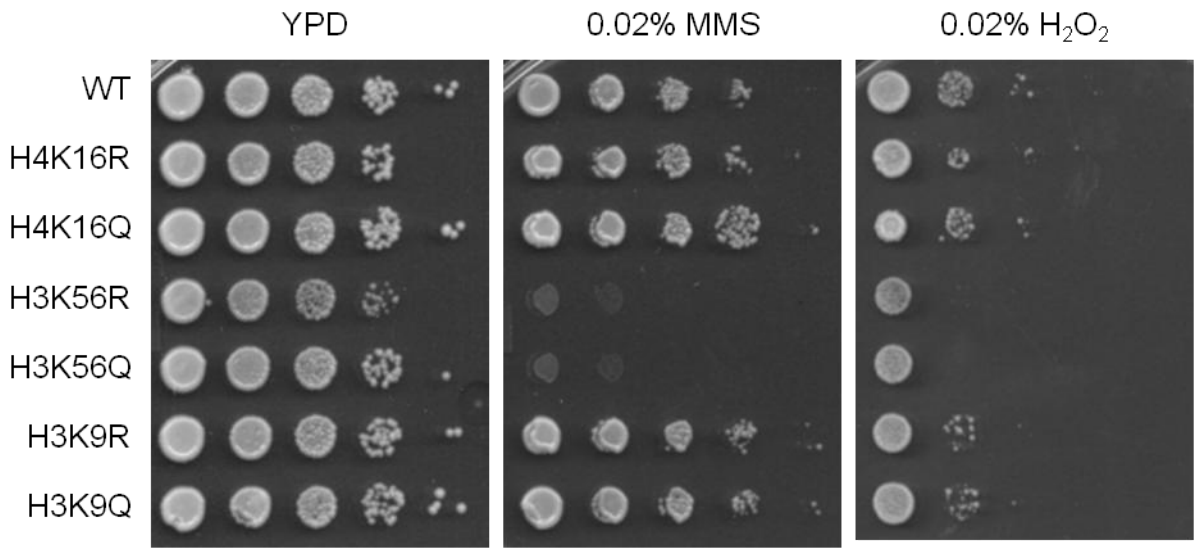


Supplementary Figure 8. H3K9 mutations do not significantly affect the replicative lifespan. Replicative lifespan analysis for strains carrying WT histone H3, H3K9R, or H3K9Q plasmids with MLS in parenthesis.



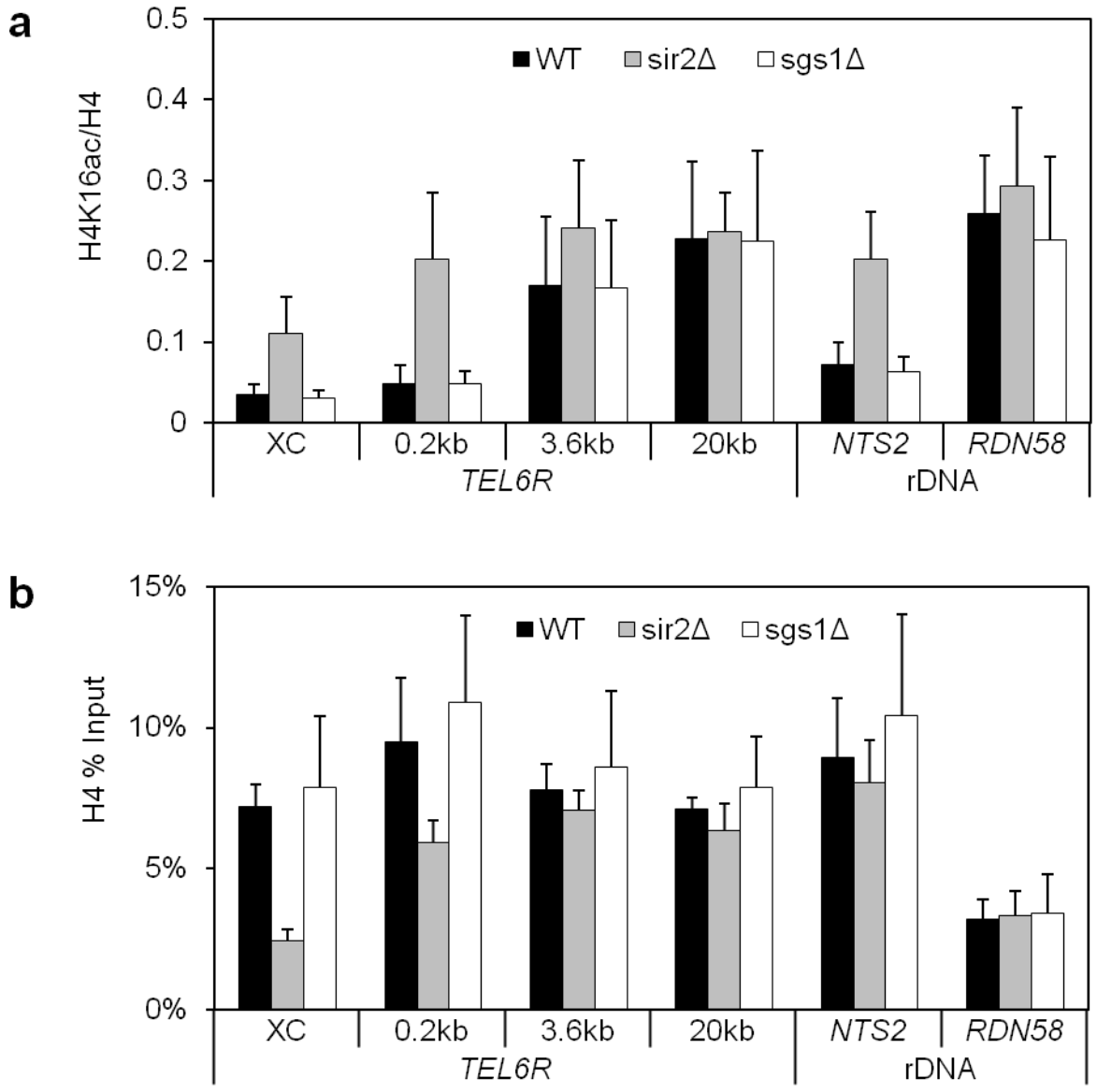
Supplementary Figure 9. Replicative lifespan for *HST3* or *HST4* deletion strains.

Replicative lifespan analysis for yeast strains WT (*MATα* 140 cells, *MATa* 40 cells), *hst3Δ* (*MATα* 120 cells, *MATa* 40 cells), *hst4Δ* (*MATα* 120 cells, *MATa* 40 cells), and *hst3Δ hst4Δ* (*MATα* 119 cells) with mean lifespan in parenthesis. The p-values for *hst3Δ*, *hst4Δ*, and *hst3Δ hst4Δ* compared to WT were 1.8×10^{-26}, 1.2×10^{-5}, 1.0×10^{-52}, respectively.



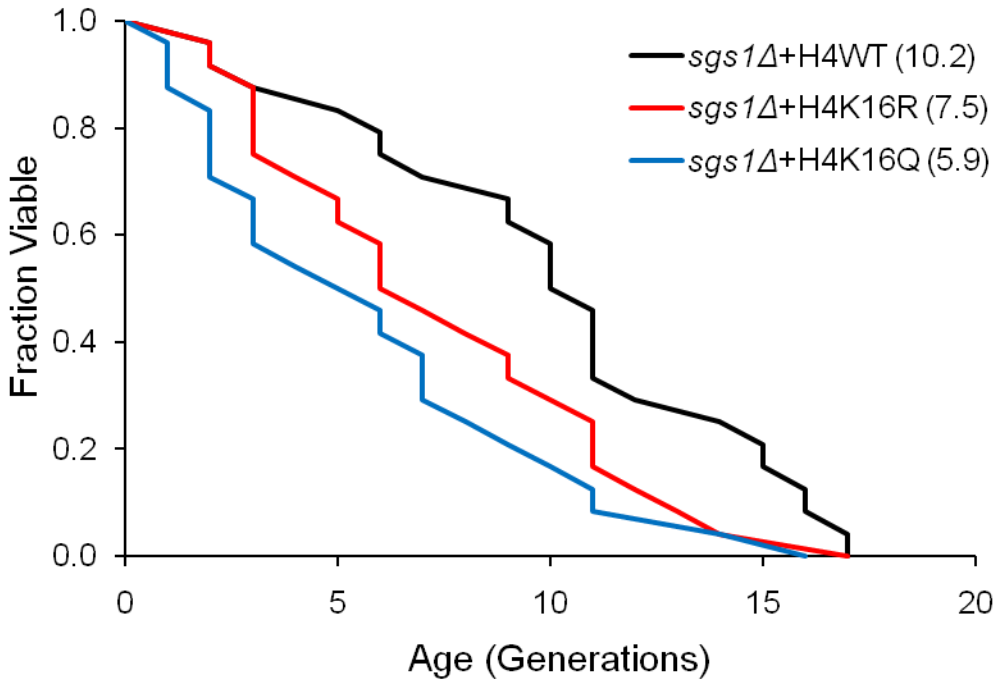
Supplementary Figure 10. Both R and Q mutations to H3K56 result in sensitivity to DNA damaging agents.

Strains bearing wild-type or mutant histone H3-H4 plasmids were 10-fold serial-diluted and plated on YPD, YPD containing 0.02% MMS, and YPD containing 0.02% H_2O_2.



Supplementary Figure 11. Deletion of SIR2, but not SGS1, causes changes to telomere chromatin.

ChIP analysis for wild-type, *sir2Δ*, and *sgs1Δ* cells with antibodies against H4K16ac (a) and H4 (b). Error bars show standard deviations (n=3).



Supplementary Figure 12. Replicative lifespan for H4K16 mutants in *sgs1Δ* background.

Replicative lifespan analysis for yeast strains containing integrated WT histone H4, H4K16R, or H4K16Q in *sgs1Δ* backgrounds with mean lifespan in parenthesis (n=24, each). The p-values for *sgs1Δ*+H4K16R and *sgs1Δ*+H4K16Q compared to *sgs1Δ*+H4WT were 7.6×10^{-3} and 1.7×10^{-3}, respectively.

Supplementary Tables

Supplementary Table 1. H4K16ac and histone occupancy change more dramatically at X elements of telomeres in old cells.

	TEL-X	Other Sir2	*ACT1*
H4K16ac, Old/Young	3.08±0.44	2.49±0.21	1.96
H3, Young/Old	6.52±1.07	3.53±0.69	3.30
H4, Young/Old	5.67±0.79	3.95±1.46	4.47
Sir2, Young/Old	13.71±2.28	12.27±3.44	2.09

ChIP analysis with primers for XC and XR elements of telomeres *TEL5R*, *TEL6L*, *TEL6R*, *TEL7L*, *TEL9L*, *TEL9R*, *TEL10R*, *TEL11L*, and *TEL15R*, as well as other Sir2 sites (*NTS2*, *RDN58*, and *HMLα1*) and *ACT1* control, using another set of age-sorted cells with average bud scar counts of 0.5 for young and 18.6 for old cells. Average fold changes between young and old cells are shown with margins estimated with 90% confidence intervals.

Supplementary Table 2. List of strains used in this study.

Strain Name	Genotype	Source	Note
Figure 1a			
W1588-4C	*MATa RAD5 leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3-11,15*	R. Rothstein	W303 with *RAD5*
Figure 1c			
BY4741	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*	Invitrogen	
Figure 2b			
FEP100-10	*MATa leu2Δ1 ura3-52 can1-1 ade2Δ, URA3_TELXIL position 1*	Ref. 26	
FEP180	*MATa ura3Δ851 leu2Δ1 his3Δ200 lys2Δ202 URA3_TELXIL position 2*	Ref. 26	
FEP184	*MATa ura3Δ851 leu2Δ1 his3Δ200 lys2Δ202 URA3_TELXIL position 3*	Ref. 26	
FEP193	*MATa ura3Δ851 leu2Δ1 his3Δ200 lys2Δ202 URA3_TELXIL position 4*	Ref. 26	
FEP210b	*MATa ura3Δ851 leu2Δ1 his3Δ200 lys2Δ202 URA3_TELXIL position 5*	Ref. 26	
Figure 3a			
BY4741	See Figure 1c		
BY4742	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0*	Invitrogen	
YKO6568	BY4741 *sas2Δ::kanMX4*	Invitrogen	
YKO16568	BY4742 *sas2Δ::kanMX4*	Invitrogen	
Figure 3b			
BY4741	See Figure 1c		
YWD250	BY4741 *sas2Δ::kanMX4*	This work	
YWD400	BY4741 *LEU2::SIR2*	This work	
YWD450	BY4741 *LEU2::SIR2 sas2Δ::kanMX4*	This work	
Figure 3cdef			
BY4741	See Figure 1c		
YWD250	See Figure 3b		
Figure 4ab			
YWD120	*MATa his3-200 leu2Δ1 ura3-52 trp1Δ63 lys2-128 (hht1-hhf1)Δ::LEU2 (hht2-hhf2)Δ::HIS3 pRM204[CEN TRP1 HHT2-HHF2]*	This work	From FY1716
YWD123	*MATa his3-200 leu2Δ1 ura3-52 trp1Δ63 lys2-128 (hht1-hhf1)Δ::LEU2 (hht2-hhf2)Δ::HIS3 pWD23[HHT2-hhf2(K16R) TRP1 CEN]*	This work	From FY1716
YWD125	*MATa his3-200 leu2Δ1 ura3-52 trp1Δ63 lys2-128 (hht1-hhf1)Δ::LEU2 (hht2-hhf2)Δ::HIS3 pWD25[HHT2-hhf2(K16Q) TRP1 CEN]*	This work	From FY1716

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Supplementary Table 2, continued

Strain Name	Genotype	Source	Note
Figure 4c			
YWD120	See Figure 4ab		
YWD156	*MATa his3-200 leu2Δ1 ura3-52 trp1Δ63 lys2-128 (hht1-hhf1)Δ::LEU2 (hht2-hhf2)Δ::HIS3 pWD43[hht2(K56R)-HHF2, TRP1, CEN]*	This work	From FY1716
YWD157	*MATa his3-200 leu2Δ1 ura3-52 trp1Δ63 lys2-128 (hht1-hhf1)Δ::LEU2 (hht2-hhf2)Δ::HIS3 pWD45[hht2(K56Q)-HHF2, TRP1, CEN]*	This work	From FY1716
Figure 4d			
BY4741	See Figure 1c		
BY4742	See Figure 3a		
YKO1490	BY4741 *rtt109Δ::kanMX4*	Invitrogen	
YKO11490	BY4742 *rtt109Δ::kanMX4*	Invitrogen	
Figure 5a			
YWD1000	*MATa his3-200 leu2Δ1 ura3-52 trp1Δ63 lys2-128 (hht1-hhf1)Δ::LEU2*	This work	From FY1716
YWD1116	YWD1000 *HHT2-hhf2(K16R)*	This work	
YWD1117	YWD1000 *HHT2-hhf2(K16Q)*	This work	
YWD1200	YWD1000 *sir2Δ::kanMX4*	This work	
YWD1276	YWD1000 *sir2Δ::kanMX4 HHT2-hhf2(K16R)*	This work	
YWD1277	YWD1000 *sir2Δ::kanMX4 HHT2-hhf2(K16Q)*	This work	
Figure 5b			
YWD1400	YWD1000 *leu2Δ::SIR2-natMX4*	This work	
YWD1476	YWD1000 *leu2Δ::SIR2-natMX4 HHT2-hhf2(K16R)*	This work	
YWD1477	YWD1000 *leu2Δ::SIR2-natMX4 HHT2-hhf2(K16Q)*	This work	
Figure 5c			
YWD1500	YWD1000 *fob1Δ::natMX4*	This work	
YWD1576	YWD1000 *fob1Δ::natMX4 HHT2-hhf2(K16R)*	This work	
YWD1577	YWD1000 *fob1Δ::natMX4 HHT2-hhf2(K16Q)*	This work	
Figure 5d			
BY4742	See Figure 3a	Invitrogen	
LF796	BY4742 *sir3::URA3*	This work	
LF797	BY4742 *LEU2::SIR2*	This work	
LF798	BY4742 *sir3::URA3 LEU2::SIR2*	This work	
Figure 5e			
BY4741	See Figure 1c		
YKO7110	BY4741 *sir3Δ::kanMX4*	Invitrogen	
YWD381	BY4741 *sir4Δ::hphMX4*	This work	

Supplementary Table 3. List of antibodies used in this study.

Specificity	Source	Catalog #	Applications in this work
H2AS129ph	Abcam	Ab15083	WB
H3	Abcam	Ab1791	WB, ChIP
H3K4me1	Millipore	07-436	WB
H3K4me2	Millipore	07-030	WB
H3K4me3	Abcam	Ab8580	WB
H3K9ac	Abcam	Ab4441	WB
H3S10ph	Millipore	05-817	WB
H3K14ac	Millipore	06-911	WB
H3K56ac	Shilatifard	N/A	WB
H3K79me2	Abcam	Ab3594	WB
H3K79me3	Abcam	Ab2621	WB
H4	Abcam	Ab31827	WB, ChIP
H4	Millipore	05-858	WB, ChIP
H4S1ph	Berger, G&D, 2006	N/A	WB
H4K12ac	Abcam	Ab1761	WB
H4K16ac	Millipore	07-329	WB, ChIP
H4K16ac	Active Motif	39167	WB, ChIP
Htz1	Abcam	Ab4626	WB
Sir2	Santa Cruz	sc-25753	WB, ChIP

N/A: Not applicable

Supplementary Table 4. List of real-time PCR oligos used in this study.

Location	Oligo Name	Oligo Sequence
ACT1	ACT1-RT-F	TCGTTCCAATTTACGCTGGTT
	ACT1-RT-R	CGGCCAAATCGATTCTCAA
HML	HML-Alpha1-RT-F	TCAATATTATTCGACCACTCAAGAAAG
	HML-Alpha1-RT-R	CGCTATCCTGTGAATTTGGATTT
RDN1	NTS2-1-RT-F	CGGATGCGGGCGATAAT
	NTS2-1-RT-R	GCCGACATTCTGTCCCACAT
	RDN58-1-RT-F	GCGAAATGCGATACGTAATGTG
	RDN58-1-RT-R	GGCGCAATGTGCGTTCA
SAS2	SAS2-qPCR-F	GGAGCGCCGTTTATTTCGA
	SAS2-qPCR-R	TGAAGCAGTATTCACAAACGAAAAG
SIR2	SIR2-qPCR-F	GTGCAGTGCCATGGCTCTT
	SIR2-qPCR-R	TCTCACCGGGTAGGTTCCAA
TEL5R	TEL5R-YP-RT-F	CGTTTGTTGAAGACGAACCAGAT
	TEL5R-YP-RT-R	TGTAGACCATCACGTGGTTTGTT
	TEL5R-XR-RT-F	TGGAGTTGGATATGGGTAATTGG
	TEL5R-XR-RT-R	CATCCATCCCTCTACTTCCTACCA
	TEL5R-XC-RT-F	CCATGGAGTGGAATGTGAGAGTAG
	TEL5R-XC-RT-R	TGCCATACTCACCCTCACTTGT
TEL6L	TEL6L-XC-RT-F	TGAGTACAAATGCACCCACATCA
	TEL6L-XC-RT-R	GCGTAAATGGCACAGGGTATAGA
	TEL6L-XR-RT-F	CCCTTCCATCCTGTCTCTCAACT
	TEL6L-XR-RT-R	GTGAGGTTGGATATGGGTAATTGG
TEL6R	TEL6R-RT-F	TGAGGCCATTTCCGTGTGTA
	TEL6R-RT-R	CCCAGTCCTCATTTCCATCAA
	TEL6R-0.2kb-RT-F	CCTTTTTTGATATAACTGTCGGAGAGT
	TEL6R-0.2kb-RT-R	TCCGAACGCTATTCCAGAAAGT
	TEL6R-3.6kb-RT-F	TCTCGGTGAACGGATGCA
	TEL6R-3.6kb-RT-R	CACGACAAAACCACCGATGA
	TEL6R-20kb-RT-F	CGCCTGCGTTCGCAAT
	TEL6R-20kb-RT-R	GCAGGCCCGCAGACTCTA
TEL7L	TEL7L-XR-RT-F	AACCACCATCCATCTCTCTACTTACTACTA
	TEL7L-XR-RT-R	AGAACAACAGTACAGTGAGTAGGACATG
	TEL7L-XC-RT-F	TCAGTACTAAATGCACCCACATCA
	TEL7L-XC-RT-R	TGGGTAAATGGCACAGGGTATAG
TEL9L	TEL9L-XC-RT-F	CACATCATTATGCACGGCACTT
	TEL9L-XC-RT-R	TGGATAATCGTGGGCTTTATGG
	TEL9L-XR-RT-F	CCTTGATTTGCAATGTCCCACTA
	TEL9L-XR-RT-R	TCCTGTTTGACGCAGATTTTAGC

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Supplementary Table 4, continued

Location	Oligo Name	Oligo Sequence
TEL9R	TEL9R-XC-RT-F	TGGGTAAATGGCACAGGGTATAG
	TEL9R-XC-RT-R	AAATCAGTACAAATGCACTCACATCA
	TEL9R-XR-RT-F	TGGTGGTAGTACGTAGAGAGATGGAT
	TEL9R-XR-RT-R	CCACTTGTTACCCTGTTCCATTC
TEL10R	TEL10R-XC-RT-F	TGGGTAAATGGCACAGGGTATAG
	TEL10R-XC-RT-R	TGCACTCGCATCATTATGCA
	TEL10R-XR-RT-F	TGGAGTTGGATATGGGTAATTGG
	TEL10R-XR-RT-R	CAACCACCATCCATCTCTCTACTTACT
TEL11L	TEL11L-XC-RT-F	CACTAAATCAGTACGATGCACTCACA
	TEL11L-XC-RT-R	TGGGTAAATTGCACAGGGTATAAA
	TEL11L-XR-RT-F	GTCTCCAAACCTACCCTCACATTAC
	TEL11L-XR-RT-R	TGGTGACAGTAGAGAGATGGATGGT
TEL15R	TEL15R-XC-RT-F	GCTGAGGCAAGTGCCGTTAA
	TEL15R-XC-RT-R	GGCCCATCTCTCACTTCATCA
	TEL15R-XR-RT-F	CAACATGGTGGGTAGAACAACAG
	TEL15R-XR-RT-R	CATATCCAACTCCACCACCACTT

Supplementary Table 5. List of Mean Lifespan (MLS) and p-values for replicative lifespan analysis in this study.

Figure	Strain A	Strain B	p-value
Fig. 3a	*sas2Δ* (32.4, n=105)	WT (26.7, n=125)	6.5×10^{-4}
Fig. 3b	*SIR2*-OE (31.4, n=39)	WT (24.2, n=20)	0.03
	sas2Δ SIR2-OE (29.0, n=40)	*sas2Δ* (28.4, n=20)	0.88
Fig. 4a	H4K16R (15.6, n=50)	H4WT (17.8, n=50)	0.234
	H4K16Q (11.7, n=50)	H4WT (17.8, n=50)	0.002
Fig. 4c	H3K56R (9.2, n=50)	H3WT (17.8, n=50)	9.0×10^{-6}
	H3K56Q (8.7, n=50)	H3WT (17.8, n=50)	2.4×10^{-6}
Fig. 4d	*rtt109Δ* (10.1, *MATα* n=45, *MATa* n=40)	WT (30.1, *MATα* n=25, *MATa* n=40)	1.33×10^{-21}
Fig. 5a	WT+H4K16R (30.4, n=120)	WT+H4WT (37.0, n=120)	2.0×10^{-6}
	WT+H4K16Q (24.6, n=120)	WT+H4WT (37.0, n=120)	1.7×10^{-19}
	sir2Δ+H4K16R (14.6, n=120)	*sir2Δ*+H4WT (15.0, n=120)	0.93
	sir2Δ+H4K16Q (13.6, n=110)	*sir2Δ*+H4WT (15.0, n=120)	0.053
	sir2Δ+H4WT (15.0, n=120)	WT+H4WT (37.0, n=120)	1.0×10^{-35}
Fig. 5b	*SIR2*-OE+H4K16R (32.7, n=120)	*SIR2*-OE+H4WT (38.5, n=130)	1.4×10^{-5}
	SIR2-OE+H4K16Q (25.0, n=120)	*SIR2*-OE+H4WT (38.5, n=130)	8.0×10^{-17}
Fig. 5c	*fob1Δ*+H4K16R (31.1, n=120)	*fob1Δ*+H4WT (37.0, n=120)	4.7×10^{-4}
	fob1Δ+H4K16Q (28.0, n=120)	*fob1Δ*+H4WT (37.0, n=120)	1.7×10^{-8}
Fig. 5d	*SIR2*-OE (31.2, n=160)	WT (24.1, n=200)	6.4×10^{-8}
	SIR2-OE *sir3Δ* (24.1, n=200)	WT (24.1, n=200)	1.0
	SIR2-OE (31.2, n=160)	*SIR2*-OE *sir3Δ* (24.1, n=200)	7.3×10^{-8}
	sir3Δ (21.8, n=160)	*SIR2*-OE *sir3Δ* (24.1, n=200)	8.5×10^{-3}
Fig. 5e	*sir3Δ* (19.2, n=40)	WT (29.1, n=40)	2.4×10^{-5}
	sir4Δ (21.0, n=40)	WT (29.1, n=40)	1.3×10^{-4}
Supp. Fig. 8	H3K9R (14.9, n=50)	H3WT (17.8, n=50)	0.11
	H3K9Q (21.3, n=50)	H3WT (17.8, n=50)	0.34
Supp. Fig. 9	*hst3Δ* (*MATα* n=120, *MATa* n=40)	WT (28.3, *MATα* n=140, *MATa* n=40)	1.8×10^{-26}
	hst4Δ (*MATα* n=120, *MATa* n=40)	WT (28.3, *MATα* n=140, *MATa* n=40)	1.2×10^{-5}
	hst3Δ hst4Δ (*MATα* n=119)	WT (28.3, *MATα* n=140, *MATa* n=40)	1.0×10^{-52}
Supp. Fig. 12	*sgs1Δ*+H4K16R (7.5, n=24)	*sgs1Δ*+H4WT (10.2, n=24)	7.6×10^{-3}
	sgs1Δ+H4K16Q (5.9, n=24)	*sgs1Δ*+H4WT (10.2, n=24)	1.7×10^{-3}

MLS and the number of cells analyzed (n) are listed in parenthesis.