Supplementary Information

Supplementary Figures

a

% Population

Bud Scars

b

Genome Copy Number

ACT1 | IntV | NTS2 | RDN58

Controls | rDNA

Young (0.8) | Old (16.3) | Old (22.5)

20 | 18 | 16 | 14 | 12 | 10 | 8 | 6 | 4 | 2 | 0

C

Normalized mRNA Level

SAS2 | SIR2

Young (0.9) | Old (15.3) | Old (21.8)

1.8 | 1.6 | 1.4 | 1.2 | 1.0 | 0.8 | 0.6 | 0.4 | 0.2 | 0 | 1
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.** *ura3Δ* old cells are not sensitive to 5-FOA.

Identical silencing assay as in Fig. 2b for young and old cells of strains containing *ura3Δ*. 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.
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, MATα 40 cells), hst3Δ (MATα 120 cells, MATα 40 cells), hst4Δ (MATα 120 cells, MATα 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% H₂O₂.
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 Information
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.

| Figure Name | Strain Name | Genotype | Source | Note |
|-------------|-------------|----------|--------|------|
| Figure 1a   | W1588-4C    | MATα RAD5 leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3-11,15 | R. Rothstein | W303 with RAD5 |
| Figure 1c   | BY4741      | MATα his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 | Invitrogen | |
| Figure 2b   | FEP100-10   | MATα leu2Δ1 ura3-52 can1-1 ade2Δ, URA3_TELXIL position 1 | Ref. 26 | |
|             | FEP180      | MATα ura3Δ851 leu2Δ1 his3Δ200 lys2Δ202 URA3_TELXIL position 2 | Ref. 26 | |
|             | FEP184      | MATα ura3Δ851 leu2Δ1 his3Δ200 lys2Δ202 URA3_TELXIL position 3 | Ref. 26 | |
|             | FEP193      | MATα ura3Δ851 leu2Δ1 his3Δ200 lys2Δ202 URA3_TELXIL position 4 | Ref. 26 | |
|             | FEP210b     | MATα ura3Δ851 leu2Δ1 his3Δ200 lys2Δ202 URA3_TELXIL position 5 | Ref. 26 | |
| Figure 3a   | BY4741      | See Figure 1c | Invitrogen | |
|             | BY4742      | MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 | Invitrogen | |
|             | YKO6568     | BY4741 sas2Δ::kanMX4 | Invitrogen | |
|             | YKO16568    | BY4742 sas2Δ::kanMX4 | Invitrogen | |
| Figure 3b   | BY4741      | See Figure 1c | This work | |
|             | 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 | This work | |
|             | YWD250      | See Figure 3b | This work | |
| Figure 4ab  | YWD120      | MATα 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      | MATα 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      | MATα 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 |

To be continued on next page
| 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 | Invitrogen | |
| YKO1490 | BY4741 rtt109Δ::kanMX4 | | |
| 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 | Invitrogen | |
| 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-RF    | TCGTTCACATTTACGCTGTT |
|          | ACT1-R    | CGGCCAAATCGATATTCTCAA |
| HML      | HML-Alpha1RF | TCAATATTATTCGACCACCTCAAGAAAG |
|          | HML-Alpha1R | CGCTTATCTGTGAATTTGGATTT |
| RDN1     | NTS2-1RF   | CGGATCGGGGCGATAAT |
|          | NTS2-1R    | GCCAAATGCATACGTAATGTG |
|          | RDN58-1RF  | GCCAAATGCATACGTAATGTG |
|          | RDN58-1R   | GCCAAATGCATACGTAATGTG |
| SAS2     | SAS2-qPCRF | GGAGCCGCGTTATTTTCA |
|          | SAS2-qPCR  | TGAAGCAGTAGTACAAAGAA |
| SIR2     | SIR2-qPCRF | GTGCAGTGCCATGGCTCTT |
|          | SIR2-qPCR  | TCTCACCAGGTAGTTTCAA |
| TEL5R    | TEL5R-YPF  | CGTTTGTTGAGACGAGACGAT |
|          | TEL5R-YP   | TGTAGACATACGTTGATTTT |
|          | TEL5R-XRF  | TGGAGTTGAGATGGTAAATGG |
|          | TEL5R-XR   | CATCCATCTTCTACTTCTCA |
|          | TEL5R-XCF  | CCGTGAGTGGAGATGGTAG |
|          | TEL5R-XCR  | TCCGATACTCCCTACTCTT |
| TEL6L    | TEL6L-XRF  | TGAGTACAAATGCACCCAT |
|          | TEL6L-XR   | CCCCTCATCCTGTCTCTCA |
|          | TEL6L-XR   | GTGAGGTTGAGATGGTAATGG |
| TEL6R    | TEL6R-F    | TGAGGCCATTTCCGTGTA |
|          | TEL6R-R    | CCCAGTCTCATTCCCATCAA |
|          | TEL6R-0.2kbF | CCTTTTTGATATAACTTACGAGGAT |
|          | TEL6R-0.2kbR | TCCGAACGCATTCTCCAGAGGAT |
|          | TEL6R-3.6kbF | TCTCGGTGAACGGATGCA |
|          | TEL6R-3.6kbR | CAGCAAAAACCCCGATGA |
|          | TEL6R-20kbF | GCTTGCGTTCGCAAT |
|          | TEL6R-20kbR | GCAGGCCGCCAGACTCTA |
| TEL7L    | TEL7L-XRF  | AACCACCATCCATCTCTTACTTACTACTA |
|          | TEL7L-XR   | AGAACAACAGTACAGTGAGTAGGACATG |
|          | TEL7L-XCF  | TTAGACTAATATGCACCCACATCA |
|          | TEL7L-XCR  | TGGGTAAATGGCAACAGGGTAG |
| TEL9L    | TEL9L-XRF  | CGAGGCCGCCAGACCTT |
|          | TEL9L-XR   | TCCTGTGTTGACGAGATTTAG |
|          | TEL9L-XRF  | TCCTGTTGACGAGATTTAG |

To be continued on next page
**Supplementary Table 4, continued**

| Location | Oligo Name     | Oligo Sequence               |
|----------|----------------|-----------------------------|
| TEL9R    | TEL9R-XC-RT-F  | TGGGTAAATGGCACAGGGTATAG     |
|          | TEL9R-XC-RT-R  | AAATCAGTACAAATGCACCTCACTCA |
|          | TEL9R-XR-RT-F  | TGGTTGATAGTACGAGAGATGGAT   |
|          | TEL9R-XR-RT-R  | CCACTTTGTTACCCCTGTTCCATTC  |
| TEL10R   | TEL10R-XC-RT-F | TGGGTAAATGGCACAGGGTATAG     |
|          | TEL10R-XC-RT-R | TGGAGTTGATATGGTAATGGG       |
|          | TEL10R-XR-RT-F | CACCACCATCCACCTCTCTACTTACT  |
|          | TEL10R-XR-RT-R | TGGAGTTGATATGGTAATGGG       |
| TEL11L   | TEL11L-XC-RT-F | CACTAAAACTACGATGCACTACACA  |
|          | TEL11L-XC-RT-R | TGGGTAAATGGCACAGGGTATAG     |
|          | TEL11L-XR-RT-F | GTCTCAAAACCTACCTACATTAC     |
|          | TEL11L-XR-RT-R | TGGGACTGAGTAGAGATGGAATG     |
| TEL15R   | TEL15R-XC-RT-F | GCTGAGGCAAGTGCCTTAAG        |
|          | TEL15R-XC-RT-R | GGGCATCTCCACTTCATCA         |
|          | TEL15R-XR-RT-F | CAACTGGTGAGTAGAAACACAG      |
|          | TEL15R-XR-RT-R | CATATCCACACTCCACACACTT      |
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 |
| 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, MATα n=40) | WT (30.1, MATα n=25, MATα 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 |
| 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 sir3Δ (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, MATα n=40) | WT (28.3, MATα n=140, MATα n=40) | 1.8×10^{-26} |
| | hst4Δ (MATα n=120, MATα n=40) | WT (28.3, MATα n=140, MATα n=40) | 1.2×10^{-5} |
| | hst3Δ hst4Δ (MATα n=119) | WT (28.3, MATα n=140, MATα 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^{-4} |

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