Supplemental Data for *Feng Xu et al.*

Figure S1

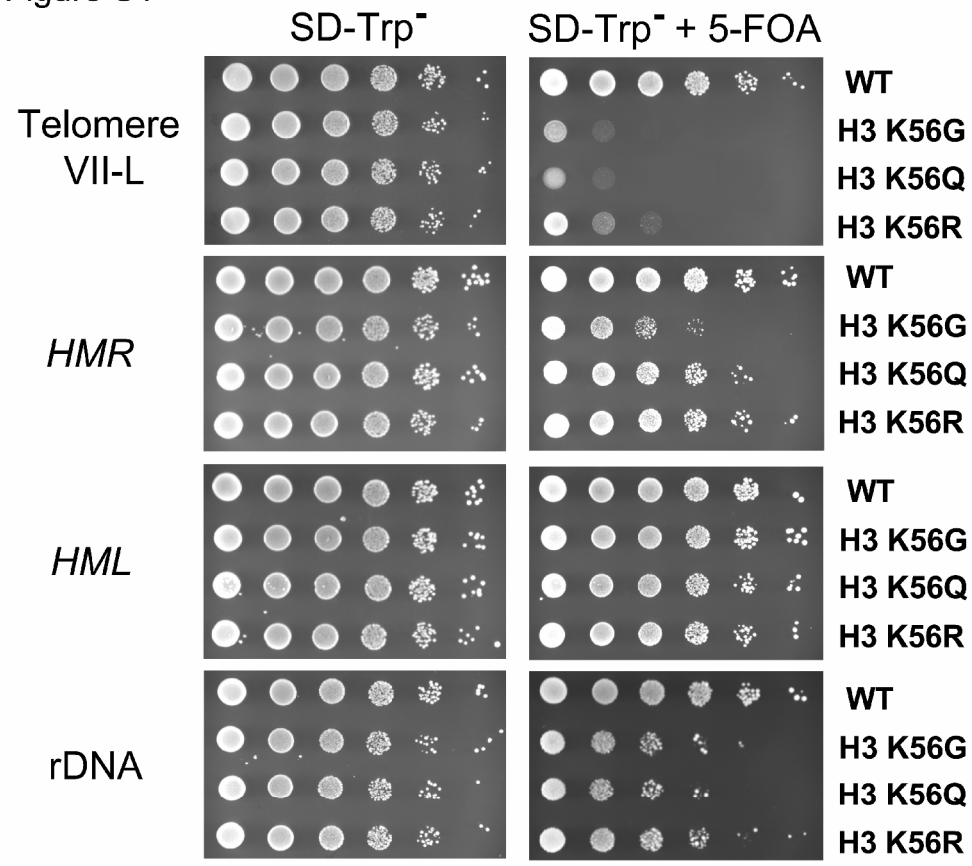

Figure S1. Effects of histone H3 lysine 56 substitutions on gene silencing in yeast

Plasmids expressing either wild type or K56 substituted histone H3 were transformed into yeast strains containing an *URA3* reporter inserted at telomere VII-L, *HMR*, *HML* and the ribosomal DNA (rDNA) repeats. Tenfold serial dilutions of wild type and K56 substitution strains were spotted onto SD-Trp$^-$ plate lacking or containing 0.1% 5-FOA. Silencing of the *URA3* reporter was evaluated by cell growth on the 5-FOA plates. The absence of growth indicates expression of *URA3* that enables conversion of 5-FOA into toxic 5-fluorouracil.

Figure S2

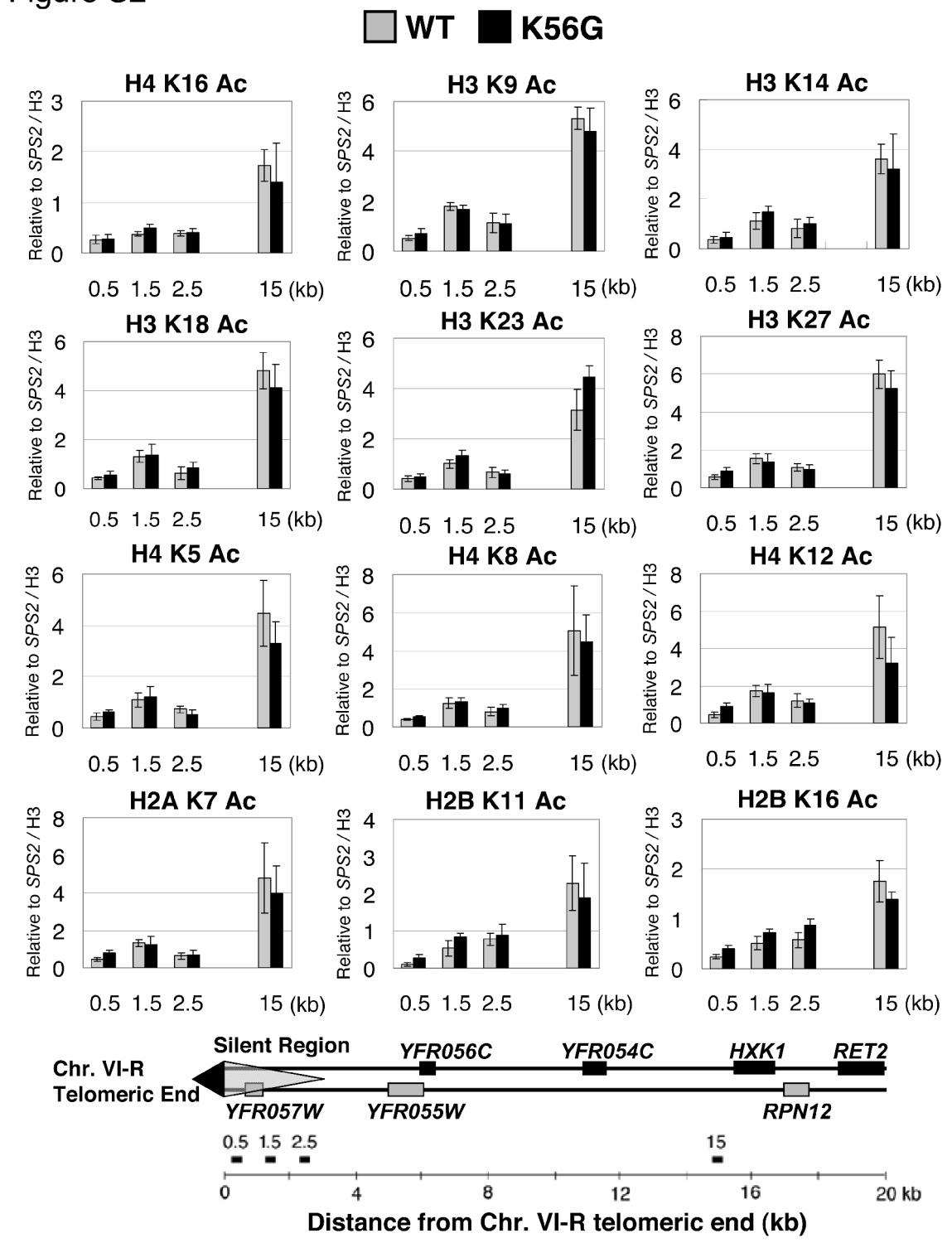
WT
K56G
H4 K16 Ac
H3 K9 Ac
H3 K14 Ac
H3 K18 Ac
H3 K23 Ac
H3 K27 Ac
H4 K5 Ac
H4 K8 Ac
H4 K12 Ac
H2A K7 Ac
H2B K11 Ac
H2B K16 Ac
Relative to SPS2 / H3
0.5 1.5 2.5 15 (kb)
Silent Region
Chr. VI-R
Telomeric End
YFR057W
YFR056C
YFR055W
YFR054C
HXK1
RPN12
RET2
0.5 1.5 2.5
15
0 4 8 12 16 20 kb
Distance from Chr. VI-R telomeric end (kb)

Figure S2. H3 K56G substitution doesn't affect the acetylation of histone N-terminal sites at telomere

Levels of histone acetylation were evaluated by ChIP assay using antibodies specific for individual sites on histone tails in both WT and K56G cells. Acetylation sites examined are indicated above the graph. Acetylation state at 15 kb from telomere VI-R were shown as a control of euchromatic region. All ChIP data are first normalized to an internal control (*SPS2*) and the input DNA, then to the level of histone H3. Gene map under the graphs shows the positions of fragments amplified in PCR. These results are averages of three independent ChIPs with error bars shown for standard deviations.

Figure S3

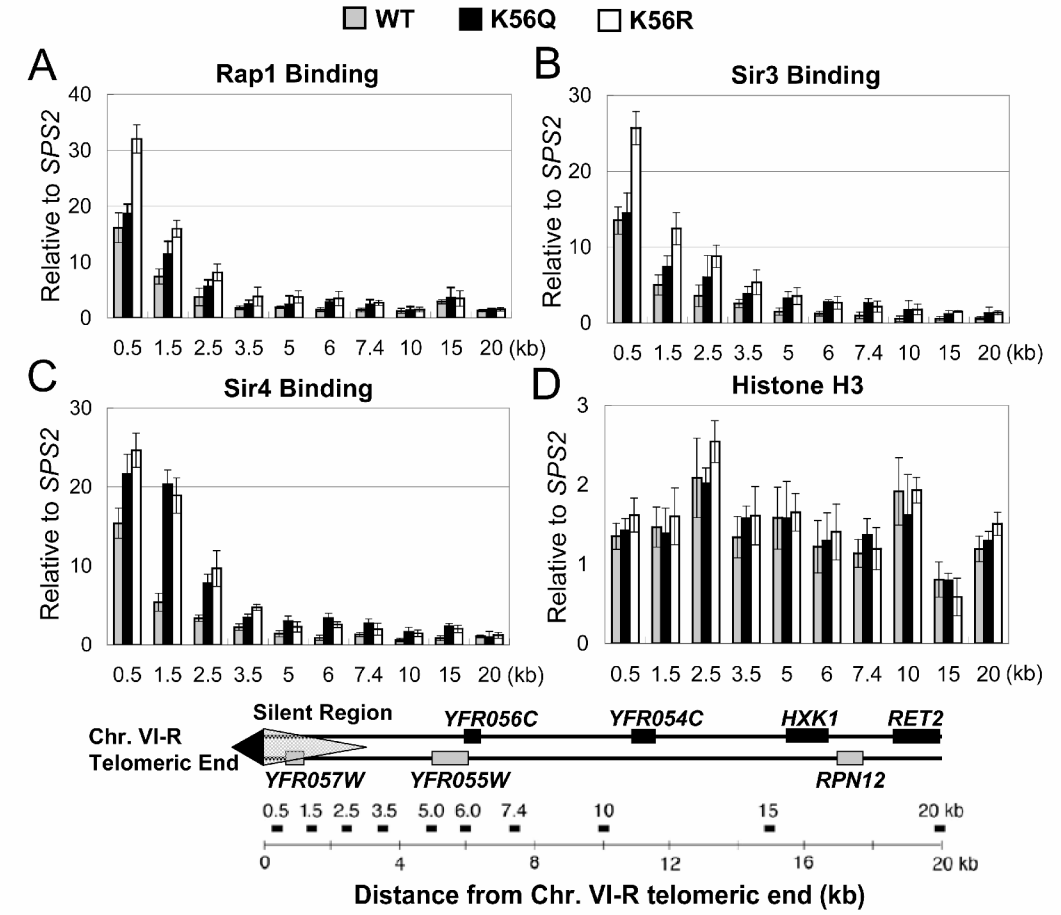

Figure S3. Substitutions of H3 K56 don't lead to decreased Rap1, Sir3, Sir4 binding and histone H3 level at telomere

Rap1 (A), Sir3 (B), Sir4 (C) binding and H3 level (D) were examined by ChIP assay using antibodies against individual proteins in WT and K56 substitution strains. The ChIP data were normalized to an internal control (*SPS2)* and the input DNA. Generally, Rap1, Sir3 and Sir4 binding at the telomeric silent region and the adjacent euchromatic region don't decrease in K56 substitution mutants (K56Q and K56R) as compared to WT cells. Histone H3 level at this 20-kb region in the mutants is also comparable to the WT strain. Gene map under the graphs

shows the positions of fragments amplified in PCR. The results are averages of three independent ChIPs with error bars shown for standard deviations.

Figure S4

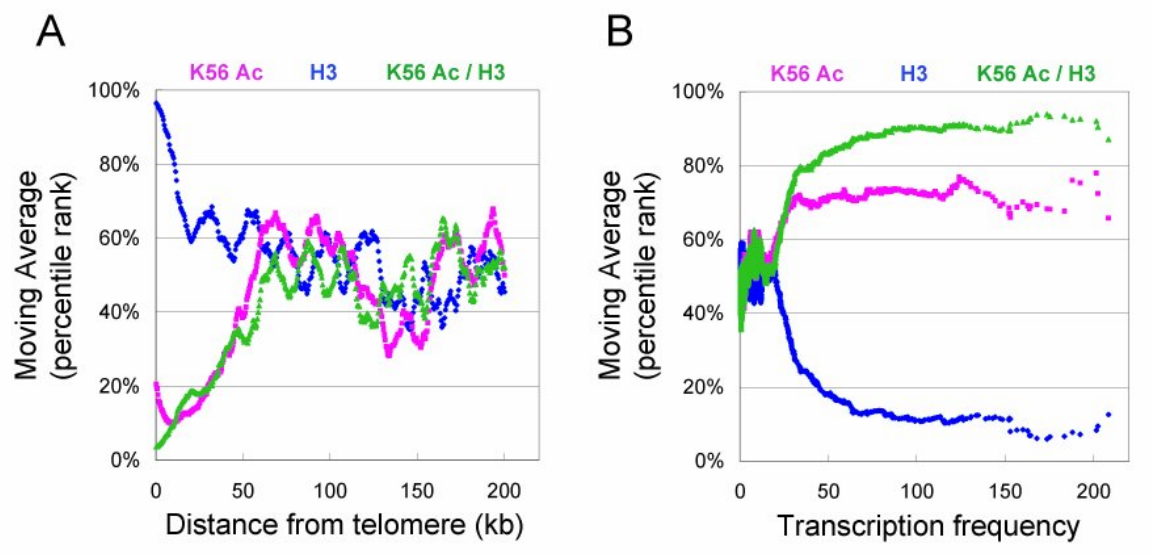

Figure S4. H3 K56 is hypo-acetylated at telomeres and hyper-acetylated at active genes

ChIP DNA of K56 acetylation and H3 C-terminus antibodies and input were amplified, fragmented, labeled and hybridized to GeneChip *S.cerevisiae* Tiling 1.0R Array. A percentile rank was assigned for each 500 bp chromosomal region or each gene exclusively according to its actual K56 Ac or H3 intensity value and the moving averages of these percentile ranks were plotted against (A) the distance from telomere end (window size, 20; step size, 500 bp) or (B) gene transcription rate (window size, 100; step size, 1).

Figure S5

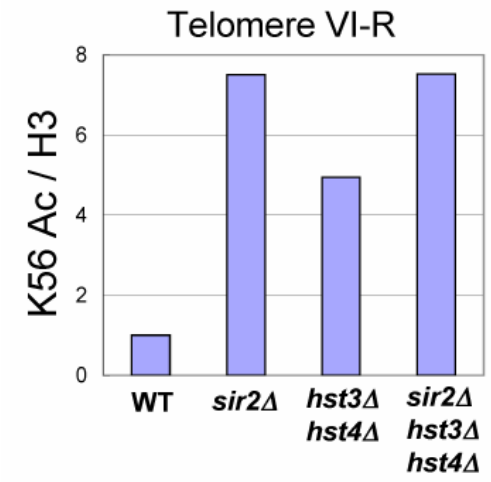

Figure S5. Sir2 is required for deacetylating K56 Ac maximally at the telomere

ChIP assay was performed using H3 K56 acetylation and H3 antibodies in the WT, *sir2Δ*, *hst3Δhst4Δ* and *sir2Δhst3Δhst4Δ* strains. Levels of K56 acetylation and H3 at the telomere (Chr. VI-R) were determined by analyzing the ChIP DNA using real-time PCR and the data were normalized to the corresponding input. After normalizing to the histone H3 level, K56 acetylation increases significantly in the *sir2Δ* strain and less so in the *hst3Δhst4Δ* double mutant as compared to the WT. In the triple mutant, K56 acetylation doesn't increase further than in the *sir2Δ* strain, which argues that Sir2 is required for deacetylating K56 acetylation maximally at the telomere.

Figure S6

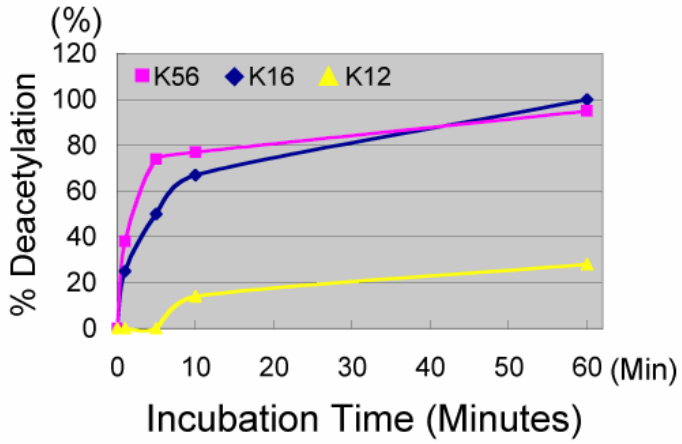

Figure S6. Sir2 deacetylates H3 K56 to a similar extent as H4 K16 *in vitro*

In vitro deacetylation assays were performed by incubating 10 μg recombinant yeast Sir2 and various acetylated peptides in the presence of 1 mM NAD^+. Samples were taken from reaction mixture at different time points and subjected to MALDI-TOF mass spectrometry analysis to determine the percentage of peptide deacetylation.

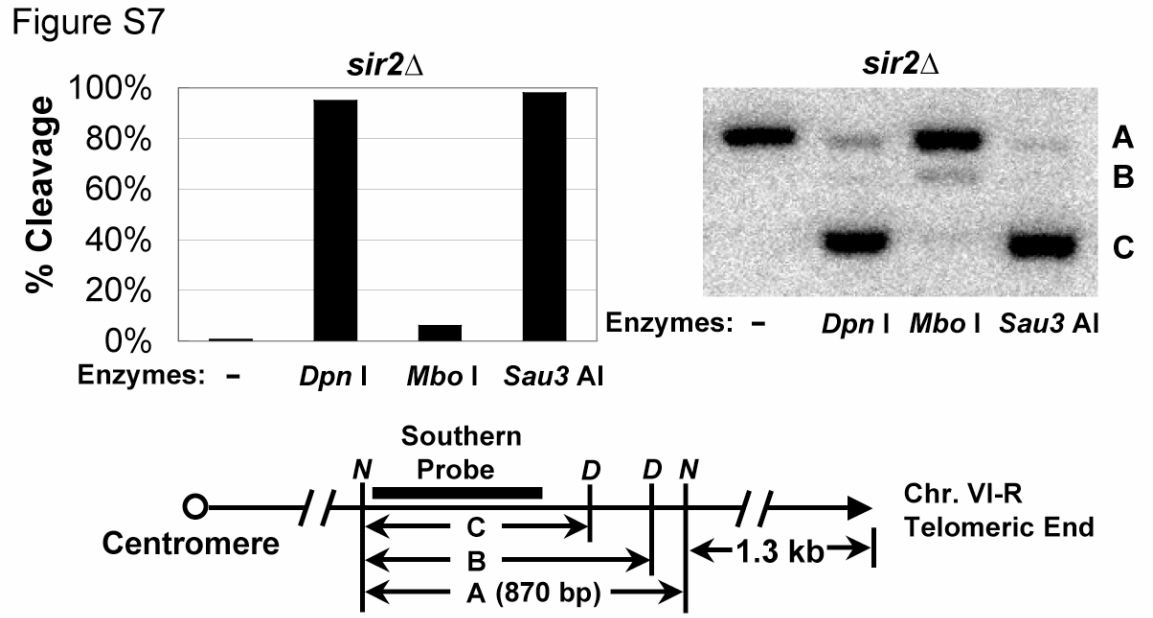

Figure S7. Telomeric chromatin is accessible to *dam* methylase in *sir2Δ* mutant

Yeast genomic DNA was isolated from *sir2Δ* cells expressing the *E.coli dam* methyltransferase. DNA samples were first digested with *Nde* I (lanes -) to yield a 870 bp telomeric fragment (fragment A), then a fraction of *Nde* I digested DNA was further cleaved with *Dpn* I or *Mbo* I or *Sau3* AI. Enzyme digested DNA samples were subjected to southern blot analysis using a 544 bp telomere VI-R DNA probe (indicated by a solid box in the gene map under the graphs). Percentile of enzyme cleavage in *sir2Δ* strain was calculated by dividing the sum of the intensities of B and C bands by the sum of the intensities of all the bands in the corresponding lane ((B+C)/(A+B+C)). Right panel shows a representative

southern blot. Position of the telomeric southern blot region was shown under the graph. D, *Dpn* I, *Mbo* I and *Sau3* AI site; N, *Nde* I site.

Supplemental Experimental Procedures

Yeast strains and plasmids

All plasmids used in this study are listed in supplemental table 1. Histone H3 amino acids substitution plasmids pFX04 (H3 K56G), pFX05 (H3 K56Q) and pFX06 (H3 K56R) were constructed using the QuikChange Site-Directed Mutagenesis Kit (Stratagene) and confirmed by sequencing. Plasmid pMW102L is a kind gift from Jeff Thompson. The 1.7 kb yeast *SIR2* gene was cloned into *Nhe* I-*Xho* I sites of pET-24a(+) vector (Novagen) to yield Sir2 expression plasmid pFX21.

All yeast strains used in this study are listed in supplemental table 2. Silencing testing strains UCC7262 (*hmr::URA3*), UCC7266 (*hml::URA3*), UCC1188 (*RDN1::URA3*) and MWY102LU (*adh4::URA3-TEL*) were kindly provided by Daniel Gottschling and Jeff Thompson. To test the silencing phenotypes of K56 mutants at individual silent loci, plasmid pRM200 (WT), pFX04 (K56G), pFX05 (K56Q) and pFX06 (K56R) were shuffled (Mann and Grunstein, 1992) into UCC7262, UCC7266, UCC1188 and MWY102LU to generate FXY7262WT (H3, *hmr::URA3*), FXY7262G (K56G, *hmr::URA3*), FXY7262Q (K56Q, *hmr::URA3*),

FXY7262R (K56R, *hmr::URA3*); FXY7266WT (H3, *hml::URA3*), FXY7266G (K56G, *hml::URA3*), FXY7266Q (K56Q, *hml::URA3*), FXY7266R (K56R, *hml::URA3*); FXY1188WT (H3, *RDN1::URA3*), FXY1188G (K56G, *RDN1::URA3*), FXY1188Q (K56Q, *RDN1::URA3*), FXY1188R (K56R, *RDN1::URA3*); JTY200U (H3, *adh4::URA3-TEL*) (Thompson et al., 1994), QZY013 (K56G, *adh4::URA3-TEL*), QZY014 (K56Q, *adh4::URA3-TEL*), QZY015 (K56R, *adh4::URA3-TEL*), respectively. FXY18 (K56Q) was made by transforming plasmid pFX05 into RMY102 to replace plasmid pRM102. To test the effects of K56 substitutions on telomeric heterochromatin structure, plasmids pFX04 (K56G), pFX05 (K56Q) and pFX06 (K56R) were shuffled into GFY3001 to generate FXY55 (K56G, *dam*$^+$), FXY56 (K56Q, *dam*$^+$) and FXY57 (K56R, *dam*$^+$), respectively. As a control, *SIR2* was disrupted with a *KanMX6* fragment from GFY3000 to yield FXY59. MW83-6A (WT) and NLM7 (*hst3Δ, hst4Δ*) strains are kind gifts from David Toczyski. *SIR2* was disrupted with a *HIS3* fragment from MW83-6A and NLM7 background to yield FXY58 and FXY60, respectively. All yeast cultures were grown in YEPD medium unless otherwise noted.

Silencing assay

Silencing of an *URA3* reporter gene integrated at telomere VII-L, *HML*, *HMR* and rDNA was examined by growth of cells on media containing 5-fluoroorotic acid (5-FOA). Cells with silenced *URA3* are resistant to 5-FOA, but cells with expressed *URA3* are sensitive to 5-FOA due to conversion of 5-FOA into toxic 5-

fluorouracil. Early log phase cells were collected and spotted in a 10-fold dilution series onto SD-Trp$^-$ plate lacking or containing 0.1% 5-FOA. Then the plates were incubated at 30 °C for 2-3 days before photographing.

RT-PCR for mRNA quantitation

Total RNA was prepared from logarithmically growing cells using hot-phenol extraction (Rundlett et al., 1998) and 0.5-1 μg total RNA was treated with 1 unit DNase I (Invitrogen) to remove genomic DNA contaminations. We carried out a 25 μl RT reaction using 1x First-Strand Buffer (Invitrogen), 10 mM DTT, 0.75 mM dNTPs, 1 U μl^{-1} RNasin (Promega), 0.165 μg random 9-mer, 0.1 μg total RNA and 8 U μl^{-1} M-MLV reverse transcriptase (Invitrogen) at 23 °C for 10 min, 37 °C for 60 min and 70 °C for 15 min. One microliter of this RT reaction was used in the subsequent quantitative PCR reaction. Sequences of the primers used in RT-PCR were listed in supplemental table 3.

Supplemental Table 1. Plasmids

Plasmid	Description	Source
pRM200	Wildtype *HHT2* and *HHF2* under native promoter control, *CEN4/ARS1/TRP1*	(Mann and Grunstein, 1992)

pRM102	Wildtype *HHT2* and *HHF2* under *GAL1-10* promoter control, *CEN4/ARS1/URA3*	(Mann and Grunstein, 1992)
pMW102L	Wildtype *HHT2* and *HHF2* under *GAL1-10* promoter control, *CEN4/ARS1/LYS2*	Jeff Thompson Unpublished
pFX04	same as pRM200 except H3 K56G	(Xu et al., 2005)
pFX05	same as pRM200 except H3 K56Q	This study
pFX06	same as pRM200 except H3 K56R	(Xu et al., 2005)
pFX21	*SIR2* 1.7kb fragment cloned into *Nhe* I-*Xho* I sites of pET-24a(+)	This study

Supplemental Table 2. Yeast Strains

Strain	Genotype	Source
UCC7262	*MATa ade2 his3 leu2 lys2 ura3 hhf1-hht1::LEU2 hhf2-hht2::MET15 ADE2-TEL-VR hmr::URA3*, pMP9	(van Leeuwen et al., 2002)
FXY7262WT	*MATa ade2 his3 leu2 lys2 ura3 hhf1-hht1::LEU2 hhf2-hht2::MET15 ADE2-TEL-VR hmr::URA3*, pRM200	This study
FXY7262G	*MATa ade2 his3 leu2 lys2 ura3 hhf1-hht1::LEU2 hhf2-hht2::MET15 ADE2-TEL-VR hmr::URA3*, pFX04	This study
FXY7262Q	*MATa ade2 his3 leu2 lys2 ura3 hhf1-hht1::LEU2 hhf2-hht2::MET15 ADE2-TEL-VR hmr::URA3*, pFX05	This study
FXY7262R	*MATa ade2 his3 leu2 lys2 ura3 hhf1-hht1::LEU2 hhf2-hht2::MET15 ADE2-TEL-VR hmr::URA3*, pFX06	This study
UCC7266	*MATa ade2 his3 leu2 lys2 ura3 hhf1-hht1::LEU2 hhf2-hht2::MET15 ADE2-TEL-VR hml::URA3*, pMP9	(van Leeuwen et al., 2002)
FXY7266WT	*MATa ade2 his3 leu2 lys2 ura3 hhf1-hht1::LEU2 hhf2-hht2::MET15 ADE2-TEL-VR hml::URA3*, pRM200	This study
FXY7266G	*MATa ade2 his3 leu2 lys2 ura3 hhf1-hht1::LEU2 hhf2-hht2::MET15 ADE2-TEL-VR hml::URA3*, pFX04	This study
FXY7266Q	*MATa ade2 his3 leu2 lys2 ura3 hhf1-hht1::LEU2 hhf2-hht2::MET15 ADE2-TEL-VR hml::URA3*, pFX05	This study
FXY7266R	*MATa ade2 his3 leu2 lys2 ura3 hhf1-hht1::LEU2 hhf2-hht2::MET15 ADE2-TEL-VR hml::URA3*, pFX06	This study
MWY102LU	*MATa ade2-101(och) his3Δ300 lys2-801(amb) trp1Δ901 ura3-52 hht1, hhf1::LEU2 hht2, hhf2::HIS3 adh4::URA3-TEL*, pMW102L	Jeff Thompson Unpublished
JTY200U	*MATa ade2-101(och) his3Δ300 lys2-801(amb) trp1Δ901*	(Thompson et al.,

	ura3-52 hht1, hhf1::LEU2 hht2, hhf2::HIS3 adh4::URA3-TEL, pRM200	1994)
QZY013	*MATa ade2-101(och) his3Δ300 lys2-801(amb) trp1Δ901 ura3-52 hht1, hhf1::LEU2 hht2, hhf2::HIS3 adh4::URA3-TEL*, pFX04	This study
QZY014	*MATa ade2-101(och) his3Δ300 lys2-801(amb) trp1Δ901 ura3-52 hht1, hhf1::LEU2 hht2, hhf2::HIS3 adh4::URA3-TEL*, pFX05	This study
QZY011	*MATa ade2-101(och) his3Δ300 lys2-801(amb) trp1Δ901 ura3-52 hht1, hhf1::LEU2 hht2, hhf2::HIS3 adh4::URA3-TEL*, pFX06	This study
UCC1188	*MATα leu2Δ1 lys2-801 trp1 ura3 hhf1-hht1::LEU2 hhf2-hht2::HIS3 RDN1::URA3*, pMP9	(van Leeuwen et al., 2002)
FXY1188WT	*MATα leu2Δ1 lys2-801 trp1 ura3 hhf1-hht1::LEU2 hhf2-hht2::HIS3 RDN1::URA3*, pRM200	This study
FXY1188G	*MATα leu2Δ1 lys2-801 trp1 ura3 hhf1-hht1::LEU2 hhf2-hht2::HIS3 RDN1::URA3*, pFX04	This study
FXY1188Q	*MATα leu2Δ1 lys2-801 trp1 ura3 hhf1-hht1::LEU2 hhf2-hht2::HIS3 RDN1::URA3*, pFX05	This study
FXY1188R	*MATα leu2Δ1 lys2-801 trp1 ura3 hhf1-hht1::LEU2 hhf2-hht2::HIS3 RDN1::URA3*, pFX06	This study
RMY102	*MATa ade2-101(och) his3Δ300 lys2-801(amb) trp1Δ901 ura3-52 hht1, hhf1::LEU2 hht2, hhf2::HIS3*, pRM102	(Thompson et al., 1994)
RMY200	*MATa ade2-101(och) his3Δ300 lys2-801(amb) trp1Δ901 ura3-52 hht1, hhf1::LEU2 hht2, hhf2::HIS3*, pRM200	(Thompson et al., 1994)
FXY17	*MATa ade2-101(och) his3Δ300 lys2-801(amb) trp1Δ901 ura3-52 hht1, hhf1::LEU2 hht2, hhf2::HIS3*, pFX04	(Xu et al., 2005)

FXY18	*MATa ade2-101(och) his3Δ300 lys2-801(amb) trp1Δ901 ura3-52 hht1, hhf1::LEU2 hht2, hhf2::HIS3*, pFX05	This study
FXY19	*MATa ade2-101(och) his3Δ300 lys2-801(amb) trp1Δ901 ura3-52 hht1, hhf1::LEU2 hht2, hhf2::HIS3*, pFX06	(Xu et al., 2005)
GFY3001	*MATα, ade2-101, his3Δ201, leu2-3, 112, trp1Δ901, ura3-52, lys2-801:dam$^+$:LYS2, hht1, hhf1::LEU2, hht2, hhf2::HIS3*, pRM102	(Fisher-Adams and Grunstein, 1995)
GFY3000	*MATα, ade2-101, his3Δ201, leu2-3, 112, trp1Δ901, ura3-52, lys2-801:dam$^+$:LYS2, hht1, hhf1::LEU2, hht2, hhf2::HIS3*, pRM200	(Fisher-Adams and Grunstein, 1995)
FXY55	*MATα, ade2-101, his3Δ201, leu2-3, 112, trp1Δ901, ura3-52, lys2-801:dam$^+$:LYS2, hht1, hhf1::LEU2, hht2, hhf2::HIS3*, pFX04	This study
FXY56	*MATα, ade2-101, his3Δ201, leu2-3, 112, trp1Δ901, ura3-52, lys2-801:dam$^+$:LYS2, hht1, hhf1::LEU2, hht2, hhf2::HIS3*, pFX05	This study
FXY57	*MATα, ade2-101, his3Δ201, leu2-3, 112, trp1Δ901, ura3-52, lys2-801:dam$^+$:LYS2, hht1, hhf1::LEU2, hht2, hhf2::HIS3*, pFX06	This study
FXY59	*MATα, ade2-101, his3Δ201, leu2-3, 112, trp1Δ901, ura3-52, lys2-801:dam$^+$:LYS2, hht1, hhf1::LEU2, hht2, hhf2::HIS3*, *sir2::KanMX6,* pRM200	This study
MW83-6A	*MATa, his3Δ0, leu2Δ0, met15Δ0, ura3Δ0*	(Maas et al., 2006)
FXY58	*MATa, his3Δ0, leu2Δ0, met15Δ0, ura3Δ0, sir2::HIS3*	This study
FXY60	*MATa, his3Δ0, leu2Δ0, met15Δ0, ura3Δ0, sir2::HIS3, hst3::URA3, hst4::KanMX*	This study
NLM7	*MATa, his3Δ0, leu2Δ0, met15Δ0, ura3Δ0, hst3::URA3,*	(Maas et al., 2006)

	hst4::KanMX	
YDS2	*MATa, ade2-1, can 1-100, leu2-3,-112, his 3-11,-15, trp1-1, ura3-52*	(Laman et al., 1995)

Supplemental Table 3. Primers

Primer Pair	Sequences	Application
Chromosome VI-R, 0.5 kb	0.5 kb-F 5'-GCGTAACAAAGCCATAATGCCTCC 0.5 kb-R 5'-CTCGTTAGGATCACGTTCGAATCC	ChIP
Chromosome VI-R, 1.5 kb	1.5 kb-F 5'-TGGTTAGTTATTGGGGATCATCATGC 1.5 kb-R 5'-GTACAGTCCAGAAATCGCTCCTTTA	ChIP
Chromosome VI-R, 2.5 kb	2.5 kb-F 5'-CGCAGTACCTTGGAAAAATCTAGGC 2.5 kb-R 5'-GAGTCTCTTCGCGGTATCTTCCTT	ChIP
Chromosome VI-R, 3.5 kb	3.5 kb-F 5'-GAATTTACTCCTAGCGAGCCTAGTG 3.5 kb-R 5'-GGCTTCATCTGATCTAGGAACCTC	ChIP
Chromosome VI-R, 5 kb	5 kb-F 5'-CGGACATGAATACTGGGTTCGTGA 5 kb-R 5'-CGAGACCCACTTGTATTCTTAGTGC	ChIP
Chromosome VI-R, 6 kb	6 kb-F 5'-TGCTCTAGTGTTGGGACACCACAA 6 kb-R 5'-GGTTGACCCCTTATACAAAGGTGG	ChIP
Chromosome VI-R, 7.4 kb	7.4 kb-F 5'-GCTATTAGTGTAGACTTCCCACTG 7.4 kb-R 5'-CCTTTCAGAAGATAGCGATGCCGT	ChIP
Chromosome VI-R, 10 kb	10 kb-F 5'-TCATCCGTACACACACAGAGACCA 10 kb-R 5'-TCCAATTGTCAATGAGCAGGTTGA	ChIP
Chromosome VI-R, 15 kb	15 kb-F 5'-TCCCAAGGAAGTGAAACCGATTGC 15 kb-R 5'-GCGAAATACCTTACTGGAGCAACC	ChIP
Chromosome VI-R, 20 kb	20 kb-F 5'-CAAAGAGGTGATGACGTCGTAAGG 20 kb-R 5'-GCAAAATTGCTGCCAACGATGCAG	ChIP

SPS2	SPS2A 5'-ACTGTCCCGTCATTGATGCGTCTC	ChIP
	SPS2PB 5'-GGGATCGTTGCATTAGTGTTAACC	
HML-E	HML-E-F 5'-GAATCAAATAGGTGTATCGCAATGG	ChIP
	HML-E-R 5'-GTTAGATTTGGCCCCCGAAATCG	
HML-I	HML-I-F 5'-AACTTACTTCAACATGAAAGCCCG	ChIP
	HML-I-R 5'-AAGAACGTACATAGTGTGCCCAGC	
HMR-E	HMR-1 5'-AATACGAACGATCCCCGTCCAAGT	ChIP
	HMR-2 5'-CAAAAACCAGGAGTACCTGCGCTT	
HMR-I	HMR-G 5'-TTGAAACTCCCCATCGTCTTGCTC	ChIP
	HMR-H 5'-CGCCATATACGAAAATGTTGGTGAC	
SCR1	SCR1-F 5'-GCTGGTAAAGACTGAAACTGGGCC	RT-PCR
	SCR1-R 5'-GAAACTTGTAAGGGACTTTCGTCG	
YFR057W	YFR057W-F 5'-CTAGTGTCTATAGTAAGTGCTCGG	RT-PCR
	YFR057W-R 5'-GGTATATTGCCACGCAAAGAAAGG	
Chr. VI-R,	Chr. VI-R-damF 5'-ATGGCTTTCTGCCCTCTTTCCG	Southern Blot
Southern probe	Chr. VI-R-damR 5'-CTTTTGGTGTGTTTATTTGCCTGC	

Supplemental References

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