Supporting Information

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Sperling and Grunstein 10.1073/pnas.0906866106

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Fig. S1. A high resolution map of silencing proteins. ChIP DNA of Sir3 (a), Sir4 (b) and Rap1 (c) and input from wild-type cells were amplified, fragmented, labeled and hybridized to Affymetrix Tiling 1.0R arrays. ORFs (blue), ARSs (orange), and the centromere (green) are shown. Binding data has been divided into 500-bp bins and values >1 SD above the average (yellow line) have been colored in red. Enrichment is measured as the Log_2 score of IP versus input. (d) Expression of euchromatic ORFs bound by Sir3 in wild-type and *SIR3* deletion (*sir3* Δ) strains. Relative level of *YJR137C*, *YJR138W*, *YMR314W*, and *YMR315W* mRNA were calculated by normalizing to the *SCR1* gene. Loss of the *SIR3* gene does not effect the expression of these euchromatic ORFs. Error bars represent the SEM.

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Fig. S2. Silent chromatin remains hypoacetylated when the H3 tail is deleted. ChIP of H4 K16 (*a*), H4 K8 (*b*), H2A K7 (*c*), and H2B K11 (*d*) acetylation at chromosome VIR in wild-type (black line) and H3 Δ 4–30 (gray line) strains. Telomeric sites remains hypoacetylated and subtelomeric chromatin becomes hypoacetylated when the Sir complex spreads ectopically in the H3 Δ 4–30 mutant. All data are normalized to the level of histone acetylation at a euchromatic internal control (*ACT1*) and to input.



Fig. S3. H3 tail-mediated boundary formation requires the entire H3 tail. ChIP of Sir3 enrichment at telomere VIR in wild-type (black), H3 Δ 4–10 (green), H3 Δ 4–20 (yellow), and H3 Δ 4–30 (red) strains. All data are normalized to the level of Sir3 binding at a euchromatic internal control (*ACT1*) and to input. Error bars represent the SEM.

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Fig. S4. Rap1 cross-links to distal subtelomeric sites even in the absence of the H3 tail. ChIP DNA of Rap1 binding and input were amplified, fragmented, labeled and hybridized to Affymetrix Tiling 1.0R arrays. The moving average (block = 500 bp, window = 20) of Rap1 binding in wild-type (black line), H3 Δ 4–30 (light gray line), and H4 K16Q (dark gray line) at all 32 yeast telomeres was plotted as a function of distance from the chromosome end. Rap1 enrichment is measured as the Log₂ score of IP versus input.

Table S1. S. cerevisiae strains

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Name	Genotype	Ref.
RMY200	MATa ade2–101 ^(och) his3 ²⁰⁰ lys2–801 ^(amp) trp1 ⁹⁰¹ ura3–52	1
	hht1,hhf1::LEU2 hht2,hhf2::HIS3 plus pRM200	
ASY151	MAT α ade2–101 ^(och) his3 ²⁰⁰ lys2–801 ^(amp) trp1 ⁹⁰¹ ura3–52	This study
	hht1,hhf1::LEU2 hht2,hhf2::HIS3 plus pRM200	
RMY430	MATa ade2–101 ^(och) his3 ²⁰⁰ lys2–801 ^(amp) trp1 ⁹⁰¹ ura3–52	1
	hht1,hhf1::LEU2 hht2,hhf2::HIS3 plus pRM430	
ASY159	MAT α ade2–101 ^(och) his3 ²⁰⁰ lys2–801 ^(amp) trp1 ⁹⁰¹ ura3–52	This study
	hht1,hhf1::LEU2 hht2,hhf2::HIS3 plus pRM430	
ASY240	MATa ade2–101 ^(och) his3 ²⁰⁰ lys2–801 ^(amp) trp1 ⁹⁰¹ ura3–52	This study
	hht1,hhf1::LEU2 hht2,hhf2::HIS3 plus pYJ031	
RMY410	MATa ade2–101 ^(och) his3 ²⁰⁰ lys2–801 ^(amp) trp1 ⁹⁰¹ ura3–52	1
	hht1,hhf1::LEU2 hht2,hhf2::HIS3 plus pRM410	
RMY420	MATa ade2–101 ^(och) his3 ²⁰⁰ lys2–801 ^(amp) trp1 ⁹⁰¹ ura3–52	1
	hht1,hhf1::LEU2 hht2,hhf2::HIS3 plus pRM420	
ASY163	MATa ade2–101 ^(och) his3 ²⁰⁰ lys2–801 ^(amp) trp1 ⁹⁰¹ ura3–52	This study
	hht1,hhf1::LEU2 hht2,hhf2::HIS3 plus pCM307	-
ASY153	MAT α ade2–101 ^(och) his3 ²⁰⁰ lys2–801 ^(amp) trp1 ⁹⁰¹ ura3–52	This study
	hht1,hhf1::LEU2 hht2,hhf2::HIS3 plus pCM307	
ASY155	MAT α ade2–101 ^(och) his3 ²⁰⁰ lys2–801 ^(amp) trp1 ⁹⁰¹ ura3–52	This study
	hht1,hhf1::LEU2 hht2,hhf2::HIS3 plus pAS006	
GFY3000	MAT α ade2–101 his3 Δ 201 leu2–3 trp1 Δ 901 ura3–52	2
	lys2–801:dam ⁺ :LYS2 hht1,hhf1::LEU2 hht2,hhf2::HIS3 plus pRM200	
ASY220	MAT α ade2–101 his3 Δ 201 leu2–3 trp1 Δ 901 ura3–52	This study
	lys2–801:dam ⁺ :LYS2 hht1,hhf1::LEU2 hht2,hhf2::HIS3 plus pRM430	
FXY59	MAT α ade2–101 his3 Δ 201 leu2–3 trp1 Δ 901 ura3–52	3
	lys2–801:dam ⁺ :LYS2 hht1,hhf1::LEU2 hht2,hhf2::HIS3 sir2::KanMX6	
	plus pRM200	
ASY197	MATa ade2–101 ^(och) his3 ²⁰⁰ lys2–801 ^(amp) trp1 ⁹⁰¹ ura3–52	This study
	hht1,hhf1::LEU2 hht2,hhf2::HIS3 gcn5::KanMX6 plus pRM200	
	hht1,hhf1::LEU2 hht2,hhf2::HIS3 gcn5::KanMX6 plus pRM200	

1. Mann RK, Grunstein M (1992) Histone H3 N-terminal mutations allow hyperactivation of the yeast GAL1 gene in vivo. EMBO J 11:3297-306.

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Fisher-Adams G, Grunstein M (1995) Yeast histone H4 and H3 N-termini have different effects on the chromatin structure of the GAL1 promoter. *EMBO J* 14:1468–77.
Xu F, Zhang Q, Zhang K, Xie W, Grunstein M (2007) Sir2 deacetylates histone H3 lysine 56 to regulate telomeric heterochromatin structure in yeast. *Mol Cell* 27:890–900.

Table S2. Plasmids

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Name	Description	Ref.
pRM200	CEN4 ARS1 TRP1 HHT2 HHF2	1
pRM430	CEN4 ARS1 TRP1 hht2∆4–30 HHF2	1
pRM420	CEN4 ARS1 TRP1 hht2∆4–20 HHF2	1
pRM410	CEN4 ARS1 TRP1 hht2∆4–10 HHF2	1
pCM307	CEN4 ARS1 TRP1 HHT2 hhf2-K16Q	This study
pAS006	CEN4 ARS1 TRP1 hht2∆4–30 <i>hhf2</i> -K16Q	This study
pYJ031	CEN TRP1 HHF2 hht2-K9,14,18,23,27G	2

Mann RK, Grunstein M (1992) Histone H3 N-terminal mutations allow hyperactivation of the yeast GAL1 gene in vivo. *EMBO J* 11:3297–306.
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