

# Supporting Information

Sperling and Grunstein 10.1073/pnas.0906866106



**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  $\text{Log}_2$  score of IP versus input. (d) Expression of euchromatic ORFs bound by Sir3 in wild-type and *SIR3* deletion (*sir3Δ*) 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.



Fig. S1 (continued).

oRap1



Fig. S1 (continued).

d.

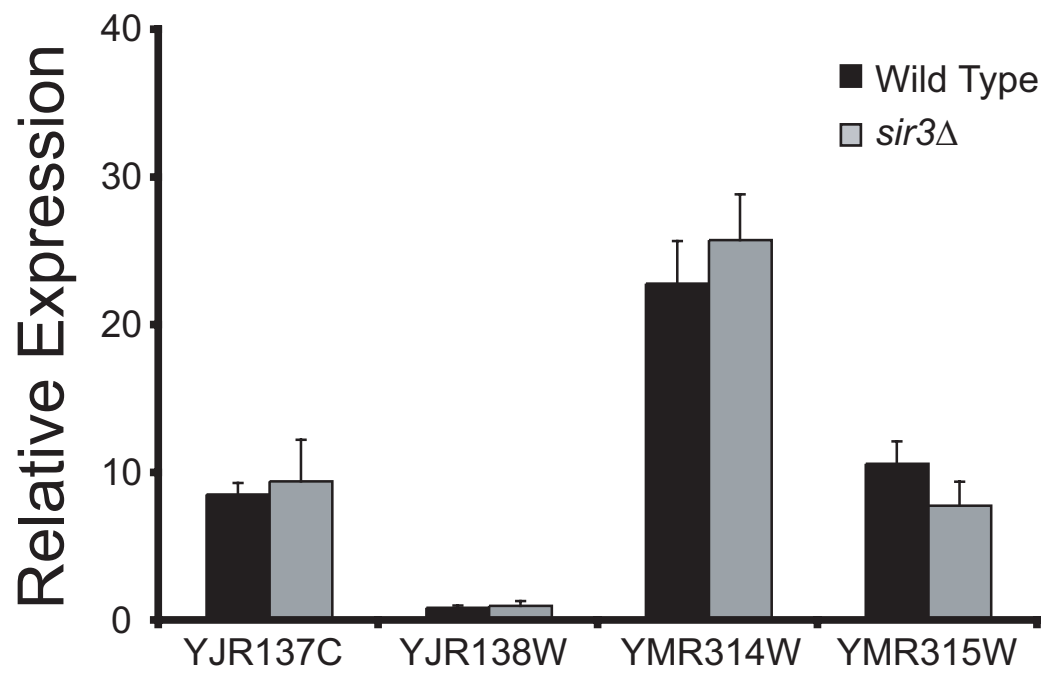
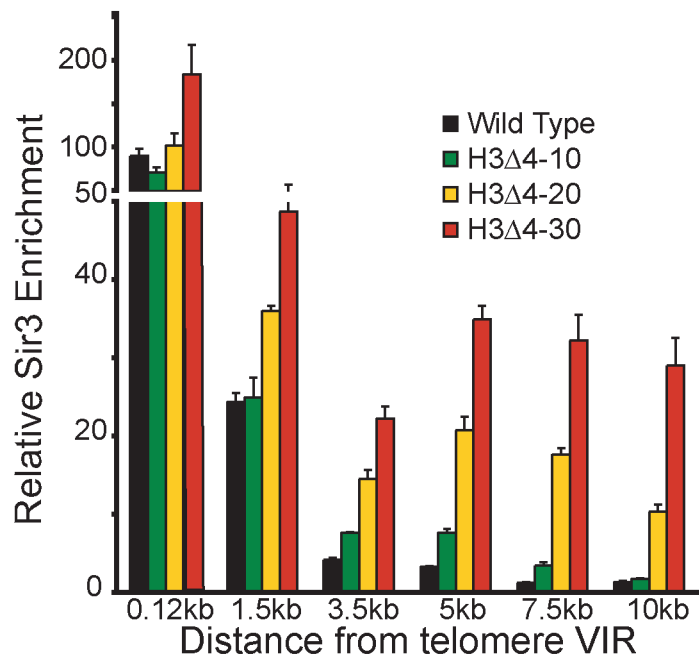


Fig. S1 (continued).





**Fig. S3.** H3 tail-mediated boundary formation requires the entire H3 tail. ChIP of Sir3 enrichment at telomere VIR in wild-type (black), H3Δ4-10 (green), H3Δ4-20 (yellow), and H3Δ4-30 (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.



Table S1. *S. cerevisiae* strains

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

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.

2. 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.

3. 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**

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

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.
2. Jin Y, Rodriguez AM, Wyrick JJ (2009) Genetic and Genomewide Analysis of Simultaneous Mutations in Acetylated and Methylated Lysine Residues in Histone H3 in *Saccharomyces cerevisiae*. *Genetics* 181:461–72.