Table S1. Yeast strains

Strain	Genotype
MJY3175	*MATa ura3-1, leu2-3,112, his3-11, trp1-1 can1-100 ade2-1 ndc10-1 CES4-MYC12::URA3* [cir$^+$, p5015]
MJY3176	*MATa ura3-1, leu2-3,112, his3-11, trp1-1 can1-100 ade2-1 ndc10-1 CES4-MYC12::URA 3* [cir$^+$, pSG5]
MJY3177	*MATa ura3-1, leu2-3,112, his3-11, trp1-1 can1-100 ade2-1 CES4-MYC12::URA3 bar1*Δ [cir$^+$, p5015]
MJY3178	*MATa ura3-1, leu2-3,112, his3-11, trp1-1 can1-100 ade2-1 CES4-MYC12::URA3 bar1*Δ[cir$^+$, pSG5]
MJY5693	*MATa ura3-1, leu2-3,112, his3-11, trp1-1 can1-100 ade2-1* [cir$^+$, p5015]
MJY5684	*MATa ura3-1, leu2-3,112, his3-11, trp1-1 can1-100 ade2-1* [cir$^+$, pSG5]
MJY3180	*MATa ura3-1, leu2-3,112, his3-11, trp1-1 can1-100 ade2-1 CES4-MYC12::URA3 bar1*Δ [cir^0 , pSG5]
MJY5687	*MATa ura3-1, leu2-3,112, his3-11, trp1-1 can1-100 ade2-1* [cir^0 , pSG5]
MJY3215	*MATa ura3-1, leu2-3,112, his3-11, trp1-1 can1-100 ade2-1 CES4-MYC12::URA3 rsc2*Δ*::KAN* [cir$^+$, p5015]
MJY3218	*MATa ura3-1, leu2-3,112, his3-11, trp1-1 can1-100 ade2-1 CES4-MYC12::URA3 rsc2*Δ*::KAN* [cir$^+$, pSG5]
MJY3210	*MATa ura3-1, leu2-3,112, his3-11, trp1-1 can1-100 ade2-1 CES4-MYC12::URA3 kip1*Δ*::HIS3* [cir$^+$, pSG5]
MJY3188	*MATa ura3-1, leu2-3,112, his3-11, trp1-1 can1-100 ade2-1 CES4-MYC12::URA3 his3::STB::HIS3 P_{GAL}-REP1,REP2::TRP1* [cir^0]
MJY3191	*MATa ura3-1, leu2-3,112, his3-11, trp1-1 can1-100 ade2-1 bar1*Δ *CES4-MYC12::URA3 his3::STB*-distal*::HIS3 P_{GAL}-REP1,REP2::TRP1* [cir^0]
MJY3193	*MATa ura3-1, leu2-3,112, his3-11, trp1-1 can1-100 ade2-1 bar1*Δ *CES4-Myc12::URA3 his3::STB*-proximal*::HIS3 P_{GAL}-REP1,REP2::TRP1* [cir^0]
MJY3181	*MATa ura3-1, leu2-3,112, his3-11, trp1-1 can1-100 ade2-1 bar1*Δ *CES4-MYC12::URA3* [cir$^+$, p*STB*-distal]
MJY3240	*MATa ura3-1, leu2-3,112, his3-11, trp1-1 can1-100 ade2-1 bar1*Δ *CES4-MYC12::URA3* [cir$^+$, p*STB*-proximal]
MJY3185	*MATa MCD1-MYC13::KAN his3::STB::HIS3 P_{GAL}-REP1,REP2::URA3* [cir^0]
MJY3220	*MATa ura3-1, leu2-3,112, his3-11, trp1-1 can1-100 ade2-1 bar1*Δ *CES4-MYC12::URA3 P_{GAL}-REP1,REP2::URA3* [cir^0, p$STB_{5.5}$]
MJY3222	*MATa ura3-1, leu2-3,112, his3-11, trp1-1 can1-100 ade2-1 bar1*Δ *CES4-MYC12::URA3 P_{GAL}-REP,REP2::URA3* [cir^0 pSTB_5]
MJY3224	*MATa ura3-1, leu2-3,112, his3-11, trp1-1 can1-100 ade2-1 bar1*Δ *CES4-MYC12::URA3 P_{GAL}-REP,REP2::URA3* [cir^0, pSTB_4]
MJY3226	*MATa ura3-1, leu2-3,112, his3-11, trp1-1 can1-100 ade2-1 bar1*Δ *CES4-MYC12::URA3 P_{GAL}-REP,REP2::URA3* [cir^0, pSTB_3]
MJY3197	*MATa ura3-1, leu2-3,112, his3-11, trp1-1 can1-100 ade2-1 bar1*Δ *CES4-MYC12::URA3 his3::*3 repeats proximal-*STB::HIS3 P_{GAL}-REP1,REP2::TRP1* [cir^0]
MJY3198	*MATa ura3-1, leu2-3,112, his3-11, trp1-1 can1-100 ade2-1 bar1*Δ *CES4-MYC12::URA3 his3::*4 repeats proximal-*STB::HIS3 P_{GAL}-REP1,REP2::TRP1* [cir^0]

MJY3199	*MATa ura3-1, leu2-3,112, his3-11, trp1-1 can1-100 ade2-1 bar1*Δ *CES4-MYC12::URA3 his3::*5.5 repeats proximal-*STB::HIS3* P_{GAL}*-REP1,REP2::TRP1* [cir^0]
MJY5729	*MATa ura3-1, leu2-3,112, his3-11, trp1-1 can1-100 ade2-1 SCM3-MYC13::HIS3* [cir^+]
MJY5732	*MATa ura3-1, leu2-3,112, his3-11, trp1-1 can1-100 ade2-1 SCM3-MYC13::HIS3* [cir^+, pSG5]
MJY5733	*MATa ura3-1, leu2-3,112, his3-11, trp1-1 can1-100 ade2-1 SCM3-MYC13::HIS3* [cir^+, p5015]
MJY5776	*MATa ura3-1, leu2-3,112, his3-11, trp1-1 can1-100 ade2-1 bar1*Δ *CES4-MYC12::URA3 bar1*Δ [cir^+ , pCH5]
RC82[1]	*MATa ura3-1, leu2-3,112, his3-11, trp1-1 can1-100 ade2-1 bar1*Δ *CES4-MYC12::URA3* P_{GAL}*-3HA-SCM3::KAN* [cir^+]

All strains listed, except for the last two, were engineered in our laboratory for the purpose of this study. The RC100 and RC82 strains were provided by J. L. Gerton[1]. The various plasmids harbored by several of these strains are described under 'Materials and Methods'.

1. **Camahort, R., B. Li, L. Florens, S. K. Swanson, M. P. Washburn, and J. L. Gerton.** 2007. Scm3 is essential to recruit the histone H3 variant Cse4 to centromeres and to maintain a functional kinetochore. Mol Cell **26:**853-65.

Table S2. Primers for analysis of micrococcal nuclease digested chromatin

	Sequence (5’ to 3’)
C1	GGTCACATGCTTATAATCAACTTTTTTAAA
C2	GGTTTTATGTTTCGGTAATCATAAACAATAAAT
C3	AATCTGGCTTAATAAAGTCTATAATATATATCTCAT
C4	GATATGGACTTAGTCAAAAGAAATTTTCTTAA
C5	AATAGGCATTATAGATCAGTTCGAGT
C6	ATAGGTTAACTCTAAGAGGTGATACTTA
C7	ACAATCAAATATCAAACTTAACTATTGACTTT
C8	AAAAGCTAGTACTGTTTTGCAGTAA
C9	TGACTTAGAACTATCTATCGGCAG
C10	GTTGAGATAATATATATATATATATATATATACTCCAGGTACAG
a	TGCTGCAAGGCGATTAAGTTGGGTAA
a’	AGCGTATTCGAATATCATTGAGAAGCTGCAGCG
b	GAGGAGATACAGCCTAATATCCGACAAAC
b’	TATGCTATTGAAGTGCAAGATGGAAACGCA
c	CGAAGCATCTGTGCTTCATTTTGTAGAACA
c'	GAACAAAAAAGAAGTATAGATTCTTTGTTG
d	AATGCATCCCGAGAGCGCTATTTTTCTAAC
c'	CAACGCAATTAATGTGAGTTACCTCACTCA

The primers listed here were used in PCR reactions for the analyses presented in Figure 6.

Table S3. Primers for chromatin immunoprecipitation (ChIP) walk across the chromosome locale of *STB* integration

	Sequence (5' to 3')
1	TTCTGAACGAGGCGCGCTTTCC
2	GGAGTCCACGTTCTTTAATAGTGG
3	CCAACGTCAAAGGGCGAAAAACCG
4	GCAGCCTGAATGGCGAATGGCGC
5	GCGCCATTCGCCATTCAGGCTGC
6	GCGTCACATCGGATAATAATGATGGC
7	GCCATCATTATTATCCGATGTGACGC
8	GAAAATGAACCGGGGATGCGACGTGC
9	TGCGTTTCCATCTTGCACTTCAATAGCATA
10	TGTAGAACAAAAAAGAAGTAT
11	ATACTTCTTTTTTGTTCTACA
12	GTAAAGCCTGGGGTGCCTAATGAG
13	CTCATTAGGCACCCCAGGCTTTAC
14	GTTCCTGGCCTTTTGCTGGCC
15	ACGAGCATCACAAAAATCGACGCTCA
16	GGGTTGGACTCAAGACGATAG

These primers were employed in ChIP experiments depicted in Figure 9.

S4. **Primers not listed in Tables S2 and S3**. Several primers, not listed in Tables S2 and S3, were utilized for various plasmid constructions and for ChIP analyses displayed in Figures 4, 5 and 8. Their sequences will be made available upon request.