Supplementary:

Additional Text:

Minichromosomes

Nomenclature
For clarity in presenting this study we renamed pSp-cc2+K"K" and CM3112 pHH-cc2 and pH-icc3i-H, respectively. pH-cc2 is a derivative of pSp-cc2+Kpn (see below). pH-icc3i-H contains a long inverted repeat of ~13 kb and therefore can not be propagated in E. coli. Total DNA was therefore prepared from fission yeast containing pH-icc3i-H and this was used to transform the minichromosome into wild-type and *clr4Δ* cells.

Minichromosome integrity following transformation into cells
Plasmid DNA can suffer rearrangement upon transformation into cells. Mitotically instability and selection might also result in rearranged minichromsomes. The intrgrity of the minichromosomes utilised for these studies in cells was assessed. Southern analyses confirmed that minichromosomes in wild-type and *clr4Δ* backgrounds used in our analyses were intact (fig. S2).

Minichromosome selection system and stability
pH-cc2 and pHH-cc2 (H denotes an *otr* heterochromatic element, cc denotes central domain DNA). pcc2 lacks *otr*/K repeats. These plasmids carry *ura4*$^+$ and *sup3-5* (suppressor of *ade6-704*) selection systems. pH-icc3i-H contains a complete central domain from *cen3* (cc denotes central domain, i denotes *imr* repeats), which is flanked by *otr* (H) on both sides. This plasmid carries only the *sup3-5* selection system. Cells without *ura4*$^+$ cannot grow on –uracil plates, whilst *ade6-704* cells do not grow without adenine and form red colonies on 1/10th adenine plates. The *sup3-5*-tRNA gene suppresses a premature stop in *ade6-704,* allowing growth on –adenine plates.
The acentric pcc2 plasmid is mitotically unstable and rapidly lost and therefore forms red colonies on 1/10th adenine indicator plates but is maintained in –ura –ade selective media. Cells containing minichromosomes pH-cc2, pHH-cc2 or pH-icc3i-H form a high percentage of white colonies, demonstrating their relative mitotic stability in wild-type cells. In cells lacking Clr4, however, their mitotic stability is lost due to a lack of heterochromatin dependent centromeric cohesion.

Minichromosome specific detection by PCR
Specific strains and primer pairs were used to distinguish otr and cc sequences on plasmids from those at endogenous centromeres. For pcc2, pH-cc2 and pHH-cc2 a strain was utilised in which a silent *his3*$^+$ gene was inserted within endogenous *cc2* of *cen2*. Primers across the insertion site therefore only detect the intact *cc2* of the minichromosomes. *cc1* and *cc3* share a DNA element, therefore for pH-icc3i-H experiments a strain was utilised in which silent *ura4*$^+$ and *arg3*$^+$ genes were inserted in *cc1* and *cc3*, respectively. Primers across the insertion site in strains containing pH-icc3i-H only detect the intact *cc3* of the minichromosome.

Reintroduction of Clr4 restores mitotic stability
To determine if the reintroduction of Clr4 concomitantly restored full centromere-kinetochore function, segregation fidelity as indicated by mitotic stability was assessed. Cells with a fully functional *ade6*$^+$ gene form white colonies on 1/10th adenine indicator plates, in contrast colonies formed by *ade6Δ* or *ade6-704* cells are completely red due to the accumulation and oxidation of CAIR. The *sup3-5* mutant

tRNA gene suppresses the premature stop in ade6-704. Thus a minichromosome with high mitotic stability and carrying *sup3-5* on an *ade6-704* background forms mainly white, ade$^+$ colonies. *clr4Δ*–X and *clr4Δ*–T cells the pH-icc3i-H circular minichromosome is mitotically unstable and rapidly lost, resulting in mainly red colonies on 1/10th adenine indicator plates (due to loss of *sup3-5*/ade$^+$). The unstable pH-icc3i-H plasmid can be maintained in the population by selecting cells on minimal medium that lacks supplementing adenine (– ade). Reintroduction of *clr4*$^+$ results in the formation of mainly white colonies grown on 1/10th adenine plates, indicating that the minichromosome gained centromere activity and thus segregation function resulting in mitotic stability.

Materials and Methods

Media, yeast growth and standard techniques

Chemicals were obtained from Sigma-Aldrich (St. Louis, MI) unless stated otherwise. Standard procedures were used for bacterial and fission yeast growth, genetics and manipulations *(S1).*

Serial Dilution Assay of Cell Growth

Strains containing minichromosomes were grown in PMG medium (Pombe Minimal Glutamate Medium) lacking adenine or non-selective minimal medium. For some purposes strains were grown in YES medium (Yeast extract with supplements). Serial (1:5) dilutions of cells grown at 32^{o}C up to 2-10x10^6/ml were spotted onto YES medium containing low adenine, full adenine or thiabenzadole (TBZ) 10 µg/ml with full adenine. Cells were grown at 25^{o}C for 5 days. The highest concentration plated contained ~4x10^3 cells.

Yeast strains

Strain	Genotype	Fig.
FY1645	*h+ ade6-210 arg3-D4 his3-D1 leu1-32 ura4-D18*	S1
FY7529	*h? clr4::LEU2 ade6-704 arg3-D4 his3-D1 leu1-32 ura4-D18/DSE cnt1:ura4 cnt:arg3arg3*	2, 3
FY7532	*h? ade6-704 arg3-D4 his3-D1 leu1-32 ura4-D18/DSE cnt1:ura4 cnt3:arg3arg3*	2, 3
FY7591	*h+ ade6-704 arg3-D4 his3-D1 leu1-32 ura4-DSE cc2:his3*	1, 4
FY7593	*h? clr4::LEU2 ade6-704 his3D1 leu1-32 ura4-D18 cc2:his3*	1
FY9473	*h? chp1::arg3 ade6-704 arg3-D4 his3-D1 leu1-32 ura4-DSE cc2:his3*	4
FY9477	*h? dcr1::nat ade6-704 arg3-D4 his-D1 leu1-32 ura4-DSE/D18 cc2:his3*	4
FY9481	*h? swi6::nat ade6-704 arg3-D4 his3-D1 leu1-32 ura4-DSE/D18 cc2:his3*	4
FY7524	*h? ade6-704 arg3-D4 his3-D1 leu1-32 ura4-D18/DSE cnt1:ura4 cnt3:arg3arg3* (+ pH-icc3i-H)	2, 3, S2

dcr1::nat (*dcr1Δ*) and *swi6::nat* (*swi6Δ*) parental strains were kindly provided by D. Moazed (Harvard University, MA).

The construction of strains with marker gene insertions in centromere central core regions (e.g. FY7532, 7591) was carried out as described *(S2*). The *cnt3:arg3*$^+$*arg3*$^+$ strain contains two partially functional *arg3*$^+$ genes inserted into the central core of *cen3*, and was obtained transformation of a cnt1(*Nco*I):arg3arg3 fragment *(S2)* into a strain containing *cnt3:ura4*$^+$ to direct replacement of *ura4*$^+$ by homologous

recombination (*cnt1* and *cnt3* sequences are highly homologous). Strains with an insertion of a non-functional *his3* gene inserted at *cc2* (*cc2:his3*) were obtained by transformation of a *cc2:ura4*$^+$ strain with the *cc2:his3* construct. To construct the *cc2:his3* fragment, primers K903 and K904 were used to amplify a *his3* fragment which included the TATA box but lacked other promoter elements. This was cloned into the *Sph*I site of cc2 in pBKS-cc2 which contains a 1.2 kb *Eco*RI-*Hin*dIII fragment from the central core of *cen2*. Strains were analysed by Southern blotting and used in crosses. Whilst *cnt3:arg3*$^+$*arg3*$^+$ strains are able to grow on media lacking arginine, the *cc2:his3* insertion does not allow growth on media lacking histidine.

Plasmids

For simplicity, in this study, pSp-cc2+K"K" *(S3)* and CM3112 *(S4)* are referred as pHH-cc2 and pH-icc3i-H, respectively. CM3112 cannot be propagated in *E. coli*. It was therefore was propagated in *S. pombe* strain FY7524. For the construction of pH-cc2, the *his3*$^+$ gene was excised from pAF1 *(S5)* as a *Bgl*II fragment and ligated to pSp-cc2+Kpn *(S3),* previously digested with *BamH*I. The resulting construct pH-cc2 contains two copies of the *his3*$^+$ gene. pcc2 was obtained by releasing the 2.0 kb *Kpn*I fragment from pSp-cc2+Kpn *(S3)*.

Primers and PCR conditions

Primer	Sequence	Expt	Fig
DF117	AAT CAT GAT CTG TGC CTT TC	ChIP (*J3*)	2-3, S4
DF122	TAA GGA GAA AAT ACC GCA TC	ChIP (*J3*)	2-3, S4
DF137	TAG GTA ATA CCG TCG TCG TC	*clr4 3'* probe	
DF138	ACT CAT TTT GCC TAT GAT GC	*clr4 3'* probe	
DF144	TGA GAT TAT GAA CGC TTG C	ChIP (*K'K'*)	1, 4, S4
DF146	CCC GAA TTG CTC TAG TAC AC	ChIP (*K'K'*)	1, 4, S4
DF151	GAC TGT TGT TGA GTG CTG TG	ChIP (*J2*)	1, S4
DF166	CTT TTG AAG TGC TTC ATT CC	*imr3* probe	S2
DF167	TGA AGT CAA CCT CAT TAT GG	*imr3* probe	S2
DF168	GAC GGT GAA AAC CTC TGA C	*pUC* probe	S2
DF169	CGC AAT TAA TGT GAG TTA GC	ChIP (*J2*) *pUC* probe	1, S2, S4
DF174	CCT GAG AAC CTT TCT GGA TG	*clr4 5'* probe	
DF175	GTA TCA TTC GTC GTC AGA GC	*clr4 5'* probe	
DF186	AAG GCG AGT TAC ATG ATC C	ChIP *(amp)*	S4
DF187	AAC GCT GGT GAA AGT AAA AG	ChIP *(amp)*	S4
DF191	GGT ATC AGC TCA CTC AAA GG	ChIP *(ori)*	S4
DF192	GGT TGG ACT CAA GAC GAT AG	ChIP *(ori)*	S4
DF201	GAT AGT AAG ATA AGA ACC AGT AAA C	qPCR (*cc2*)	1,4, S3, S5
DF202	GCC AGT GGG ATT TGT AGC	qPCR (*cc2*)	1,4, S3, S5
K903	CAT GAT GCA TGC CAT GGA TAT CAC CTG CAT CTT AGG	cc2:his3	
K904	GTC AGA GCA TGC CAT GGC ACG GGT TAT AAT CCT TT	cc2:his3	
qCnt1F	CAG ACA ATC GCA TGG TAC TAT C	qPCR-ChIP (*cc1/3*)	1,4, S3
qCnt1R	AGG TGA AGC GTA AGT GAG TG	qPCR-ChIP (*cc1/3*)	1,4, S3
R568	CTT CTT GAT GCC AAT GAA TG	*his3* probe	
R569	ACC GAA TTC CTG CTA GAC C	*his3* probe	
WA26	AAC AAT AAA CAC GAA TGC CTC	ChIP (*cc1/3*)	1-4
WA27	ATA GTA CCA TGC GAT TGT CTG	ChIP (*cc1/3*)	1-4

WA28	CAC ATC ATC GTC GTA CTA CAT	ChIP (*otr*)	1-4
WA29	GAT ATC ATC TAT ATT TAA TGA CTA CT	ChIP (*otr*)	1-4
WA33	AAT GAC AAT TCC CCA CTA GCC	ChIP & qPCR-ChIP (*fbp1*)	1-4, S3-S5
WA34	ACT TCA GCT AGG ATT CAC CTG G	ChIP & qPCR-ChIP (*fbp1*)	1-4, S3-S5
WA149	GGC ATC ACA CTT TCT ACA ACG	ChIP (*act1*)	2-3, S4
WA150	GAG TCC AAG ACG ATA CCA GTG	ChIP (*act1*)	2-3, S4
WA289	CAG AAG GTA TTA GTG GTC GG	ChIP (*cc3*)	2-3, S4
WA291	AAG TGT TTA ATT AGC TCA ACG	ChIP (*cc3*)	2-3, S4
WA293	CAA CTT TAC TAA TTT GAC TCC	ChIP (*cc2*)	1, 4, S4
WA295	CCA ACT GAT CCT TCA AAC TAC	ChIP (*cc2*)	1, 4, S4
Y393	TAC TAC GGA TCC CAC TCA GCT TTT CAA GCG G	GST-Cnp3	
Y394	TAC TAC CTC GAG TGT ATC TTC ACG TTC GAC CTG	GST-Cnp3	

$MgCl_2$ was added to 2.75 mM in all the PCR performed on ChIP samples.
A PCR program of 94°C 3'; 35 cycles of 94°C 30", 55°C 30" and 72°C 30"; 72°C 10' was used for the following duplexes:
- *cc2-fbp1*: 20 pmoles *cc2*, 5 pmoles f*bp1*
- *J2-fbp1*: 20 pmoles *J2*, 5 pmoles *fbp1*
- *J3-/act1*: 20 pmoles *J3*, 5 pmoles *act1*.

A PCR program of 94°C 4'; 30 cycles of 94°C 30", 55°C 30" and 72°C 1'; 72°C 5' was used for the following duplex:
- *cc1/3-fbp1*: 20 pmoles *cc1/3*, 5 pmoles *fbp1*.
- *K'K'-fbp1*: 20pmoles *K'K'*, 5 pmoles *fbp1*.
- *otr-fbp1*: 30 pmoles *otr*, 5 pmoles *fbp1*.
- *cc3-act1*: 20 (CENP-A^{Cnp1} ChIP) or 30 pmoles (CENP-C^{Cnp3} and Sim4 ChIP) *cc3*,
  5 pmoles *act1*.
- *amp-fbp1*: 10 pmoles *amp*, 5 pmoles *fbp1.*
- *ori-fbp1*: 10 pmoles *ori*, 5 pmoles *fbp1.*

Real-Time qPCR
Real-time PCR was performed in the presence of SYBR Green on a Bio-Rad iCycler with the following primer pairs: DF201/DF202 (*cc2*, minichromosome central core), qCnt1_fwd/qCnt1_rev (*cen,* endogenous centromere) and WA33/WA34 (*fbp1*, euchromatic locus). The standard curves were generated with pooled crude CENP-A^{Cnp1} ChIP samples. Data were analyzed with iCycler iQ Optical System Software. Enrichment of minichromosome central core (*cc2*) relative to endogenous centromere (*cc1/3*) in the CENP-A^{Cnp1} ChIPs was calculated. The ratio between the enrichment obtained at *cc2* (plasmid) and *cc1/3* (endogenous) is shown in Fig.1, 4 and S3.

***S. pombe* transformations**
Plasmids pcc2, pH-cc2, pHH-cc2 were introduced into *S. pombe* by electroporation and transformants were selected by growth on PMG –ura –ade at 32°C for 5-7 days. Since pH-icc3i-H cannot be propagated in *E. coli*, total genomic DNA from strains FY7524 were purified by QIAGEN Genomic-Tip 100/G (Qiagen, Germany). ~5 µg of this preparation was used for cotransformation with *his3*$^{+}$-containing pRO319 *(S6)* using lithium acetate transformation method *(S1).* Selection were performed on PMG –his–ade at 32°C. Subsequently, transformants were propagated in PMG –ade media.

Chromatin Immunoprecipitation

Cells were grown at 32°C either in PMG –ura–ade (pcc2, pH-cc2, pHH-cc2) or PMG –ade (pH-icc3i-H). To confirm that plasmids were behaving episomally and had not integrated, a plasmid stability test was performed at the time of fixation. Cells (100-1000) were plated onto YES 1/10 adenine and allowed to form colonies. Wild type strains containing plasmids typically exhibit 80-90% of white/sectored colonies, whereas the mutants used in this study show only red colonies due to the loss of the plasmids in non-selective conditions. Samples exhibiting less than 2% of integrations (i.e. white colonies in the mutants) were used for ChIP.

ChIP was performed as described *(S7)* except for the following modifications. For CENP-A^{Cnp1} ChIPs, cells were fixed 1% PFA, 10 min, at room temperature. For CENP-C^{Cnp3} and Sim4 ChIPs, cells were incubated 2 h at 18°C and then fixed for 30 min at 18°C with 3% PFA. Cells were spheroplasted at 1 x 10^8 cells/ml in PEMS + 0.4 mg/ml zymolyase-100T (MP Biomedicals, Inc.) for 30 min at 36°C. Cells were washed twice in PEMS and cell pellets frozen at -80°C. The chromatin was sheared using the Bioruptor (Diagenode) sonicator (20 min, 30 seconds ON and seconds OFF at "High" 200 W position). The extent of the shearing was checked by ethidium bromide on a 1.5% TBE-agarose gel. For H3K9me2 ChIP, cells were fixed with 1 % PFA for 15 min at room temperature. Cells were lysed using a bead beater (Biospec products) and sonicated using a Bioruptor (Diagenode) sonicator for a total of 15 min (30 seconds ON and OFF cycle). 10 μl of α-CENP-A^{Cnp1} antiserum *(S8)*, 1μl of H3K9me2 antibody (m5.1.1, *S9),* 10 μl of CENP-C^{Cnp3} and 10 μl of Sim4 antiserum *(S2)* were used in ChIPs. PCR products were run on 1.5% TBE-agarose gels and the quantitation of bands was performed using the Kodak EDAS 290 system and 1D Image Analysis Software (Eastman Kodak). Enrichment of *cc1/3*, *otr, K'K'*, *cc2* and *J2* bands in the ChIPs was calculated relative to the *fbp1* primers pair band, and then corrected for the ratio obtained in the input PCR. Enrichment of *cc3* and *J3* was calculated relative to *act1*. Some variation in fold-enrichments were observed, however, the pattern of enrichment versus no-enrichment was consistent between experiments. Representative examples are shown. For each experiment presented, 2-6 independent transformants or progeny (from crosses) were analysed and ChIP at least twice.

For production of antiserum against CENP-C^{Cnp3}, primers Y393 and Y394 were used to PCR-amplify a fragment of the *cnp3*$^+$ ORF from genomic DNA. This was cloned as a *Bam*HI-*Xho*I fragment into pGEX-4T1 and used to produce a GST-Cnp3 fusion protein which was injected into a sheep.

Southern Blots

Genomic DNA was purified as described (*S1*). 2-10 μg of DNA were digested overnight with 40-80U of the following restriction enzymes (NEB): *Xba*I for pcc2, pH-cc2 and pHH-cc2; double digestion with *Nco*I and *Not*I for pH-icc3i-H; *Hind*III for the *clr4*$^+$ reintroduction experiment. Samples were run on 0.4% SeaKem Gold agarose (Cambrex Biosciences, Rochester, USA) in 1X TAE buffer (for strains containing minichromosomes) or 0.8% TBE-agarose (for *clr4*$^+$ reintroduction experiments).

The following probes were used in the hybridisations:

For pcc2 and pHH-cc2: the ~1.1 kb *Sph*I-*Nru*I fragment from pcc2 (*cc2* probe) and the ~1.0 kb *Pvu*I fragment from pBKSII (*Bluescript* probe).

For pH-cc2: a 607 bp amplified from pH-cc2 with R568-R569 (*his3* probe) and the *Bluescript* probe.

For pH-icc3i-H: a 904 bp PCR-amplified fragment with DF166/DF167 from pNC *(S10)* (*imr3* probe) and a 568 bp PCR-amplified fragment from pUC19 (*pUC* probe).

For *clr4*$^+$ reintroduction: a 287 bp amplified with DF137-DF138 (*clr4 3'* probe) and a 281 bp amplified with DF174-DF175 (*clr4 5'* probe) both from pJP1084.

Reintroduction of *clr4+*

pJP1084 (Kindly provided by Janet Partridge, St Jude's Childrens Hospital, TN) containing the *clr4*$^{+}$ gene was linearised with *Hpa*I (NEB). 2 µg was introduced by chemical transformation with lithium acetate into *clr4*Δ cells harbouring pH-icc3i-H. Transformants were selected at 32^{o}C on PMG –his–ade or PMG –his low ade. White and pink colonies from both media were selected and streaked onto YES low ade. Strains exhibiting white colonies were assayed by Southern blotting (probes *clr4 3'* and *clr4 5'*) in order to determine the number of *clr4*$^{+}$ copies introduced and the genomic location of these. Only strains containing one copy correctly reintegrated at the *clr4* locus were used for the ChIP assays.

References

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S10. N. C. Steiner, L. Clarke, *Cell* **79**, 865 (1994)

Supplementary Figures:

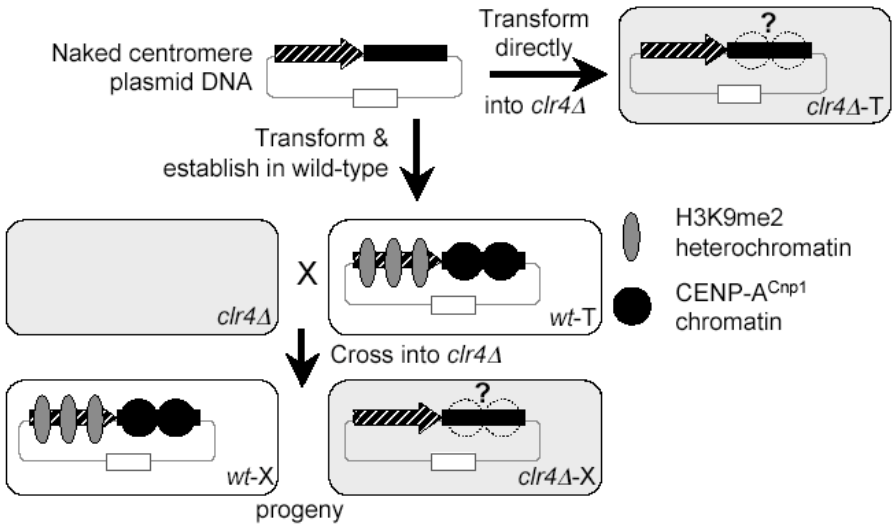

Figure S1. **Scheme to assess the role of outer repeat heterochromatin in CENP-A^{Cnp1} establishment and/or maintenance.**
Minichromosome centromeres established in wild-type cells are crossed into cells lacking Clr4-dependent heterochromatin (*clr4Δ*), or naked minichromosome DNA is transformed into *clr4Δ* cells. Clr4 dependent heterochromatin is indicated by the presence of H3K9me (ovals) over outer repeat (striped arrow) while the establishment of CENP-A (circle) on the central core (black rectangle) is assessed.

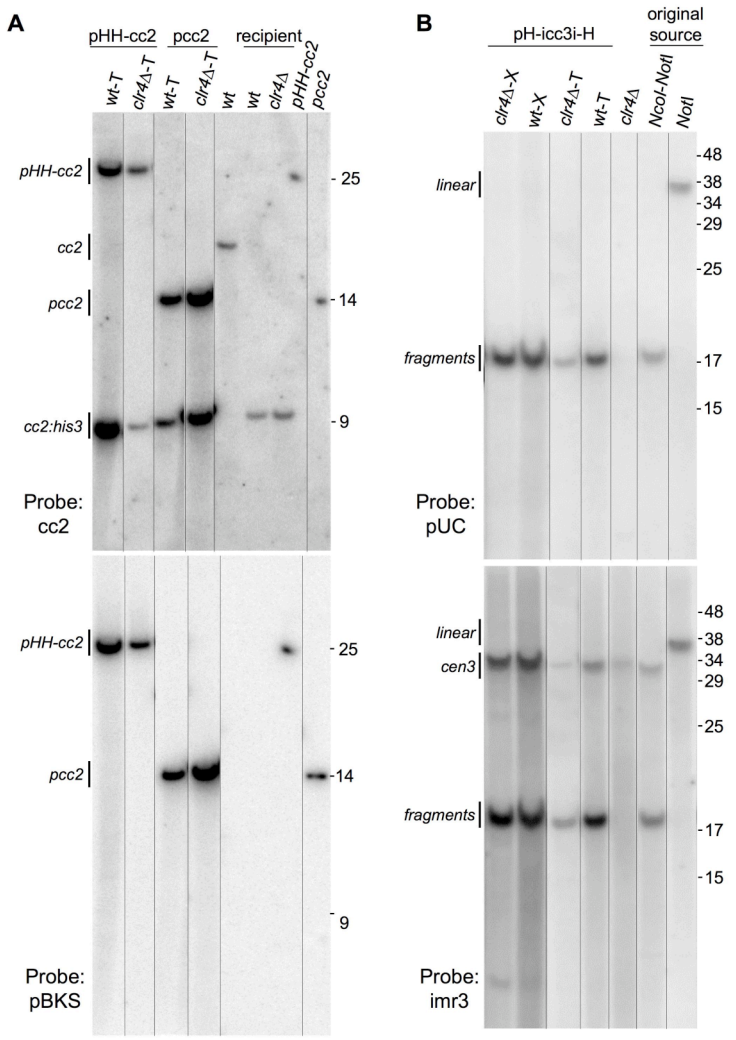

Figure S2. The DNA structure of minichromosomes remains intact in fission yeast.

Representative examples of Southern blots performed on strains containing minichromosomes used in the ChIP analyses. **(A)** Southern blot on strains harbouring either pHH-cc2 or pcc2 plasmids. Wild type (wt), recipient strains (wt, *clr4*Δ) as well as plasmids used as original source of DNA in transformations are shown. Samples were digested with *Xba*I, which linearises the plasmids. Probes detect either plasmid backbone (pBKS) or *cen2* central domain region (*cc2).* **(B)** Southern blot on various strains carrying pH-icc3i-H. Double digestion performed with *Nco*I and *Not*I produces two fragments of equivalent size. A recipient strain (*clr4*Δ) and the strain that provided the original source of pH-icc3i-H DNA used in transformation are also shown. For the latter sample, a single digestion with *Not*I which linearised the minichromosome and gave the expected size of 36 kb is shown. Probes detect either plasmid backbone (pUC) or *cen3* central domain region *(imr3).*

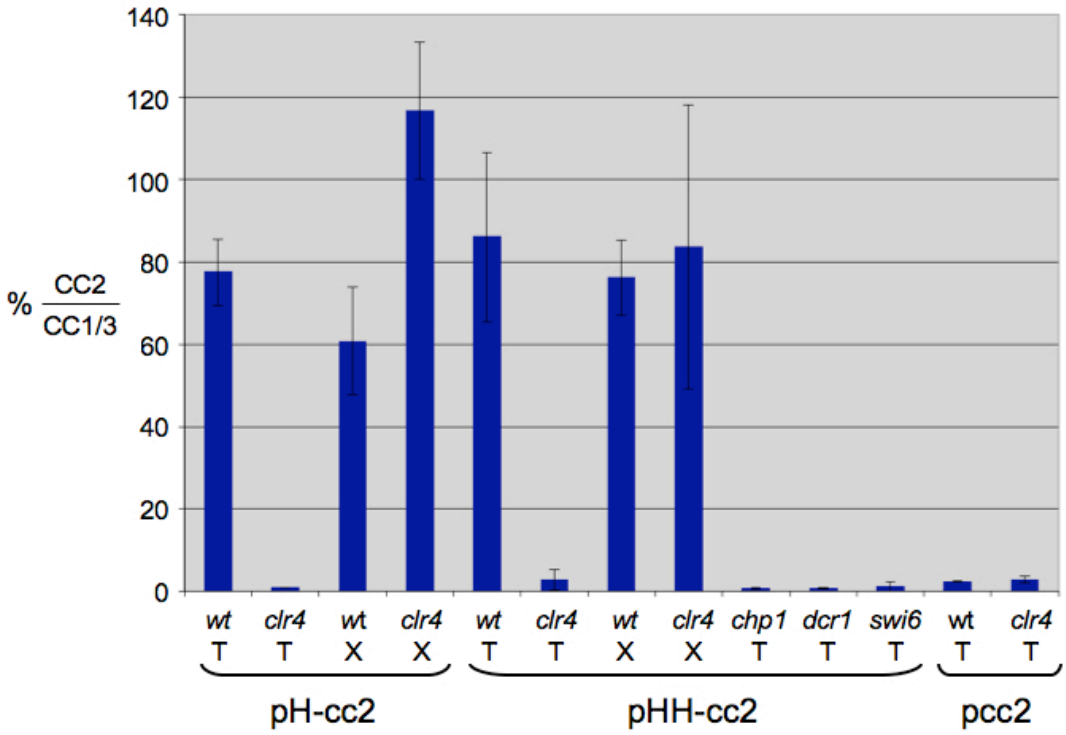

Figure S3: Quantitative PCR of relative enrichment of minichromosome versus endogenous centromere central domain DNA in CENP-A^{Cnp1} ChIP.
(Amalgamated data from Fig. 1B, C, D and Fig. 4.)
Minichromsomes were introduced in to *wild-type* (wt), *clr4Δ* (clr4), *chp1Δ* (chp1), *dcr1Δ* (dcr1) and *swi6Δ* (swi6) strains by crossing (X) or by direct transfromation with naked DNA (T) as indicated. The enrichment of minichromosome centromeric cc2 DNA was assessed relative to the enrichment of endogenous central cc1/3 DNA in the same samples. The % of cc2 immunoprecipitated relative to endogenous cc1/3 was plotted. The data presented are an average of at least three separate ChIPs. Note: more Cnp1 appears to associate with the minichromosome centromere in *clr4Δ* strains because in selective minimal medium less Cnp1 associates with the endogenous centromere relative to wild-type cells (See Figure S5).

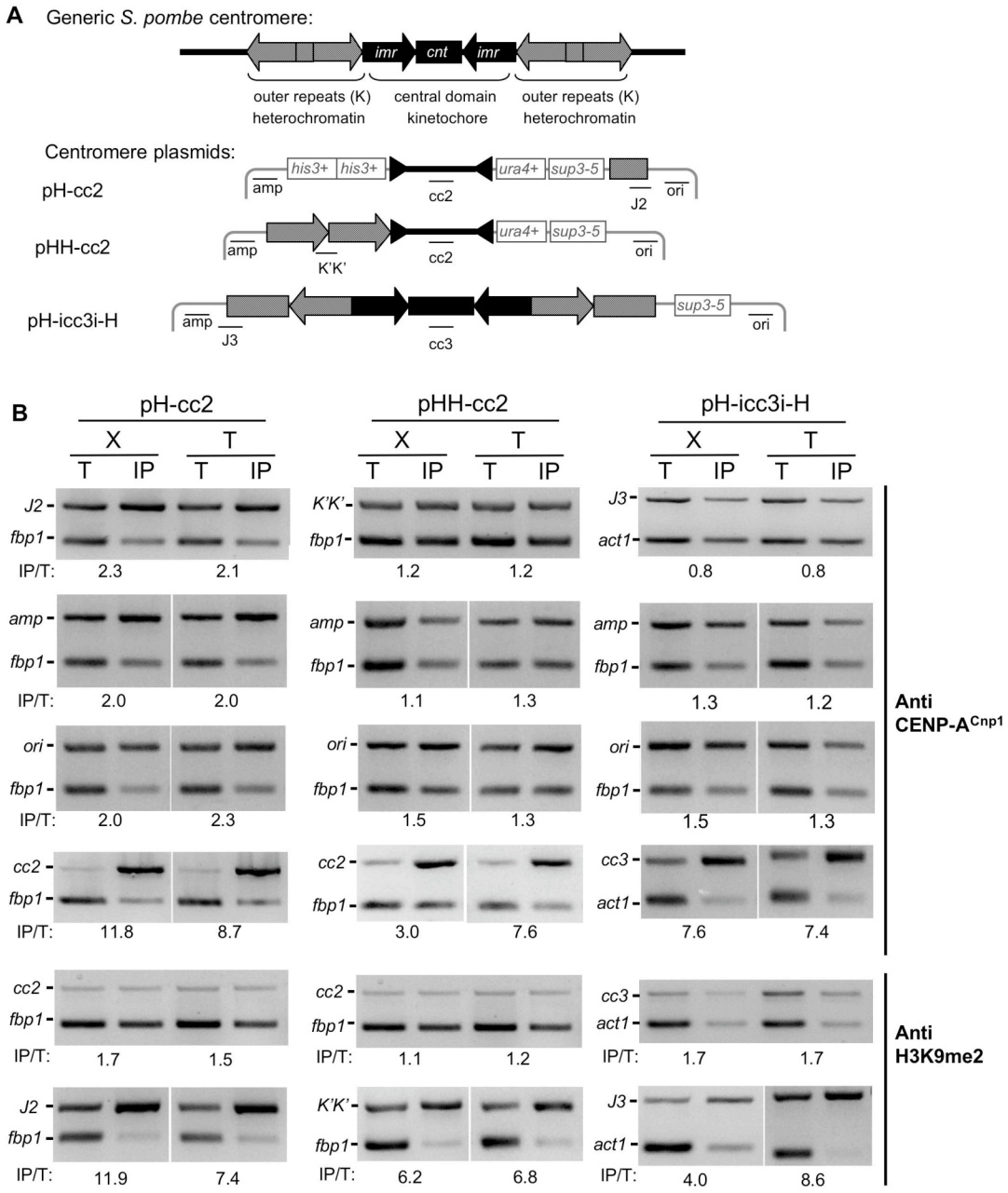

Fig S4:
CENP-A^{Cnp1} and H3K9me2 chromatin domains in minichromosomes.
(A) Fission yeast centromere DNA and minichromosomes used (*see SOM - Plasmids*).
(B) ChIP to determine association of CENP-A^{Cnp1} and H3K9me2 with minichromosomes pH-cc2, pHH-cc2 and pH-icc3i-H introduced into wild type cells by crossing (X) or transformation (T). PCR product positions as indicated (A). *fbp1* or *act1* gene primers were used as control non-centromeric loci. In CENP-A^{Cnp1} ChIP, enrichment at plasmid backbone *amp* and *ori* products were compared with input DNA **(T)** relative to *fbp1.* In both ChIPs (IP) the enrichment of *cc2*, *J2* and *K'K'* products was compared with input DNA **(T)** relative to the *fbp1* product. The enrichment at *cc3* and *J3* products was compared with input DNA **(T)** relative to the *act1* product.

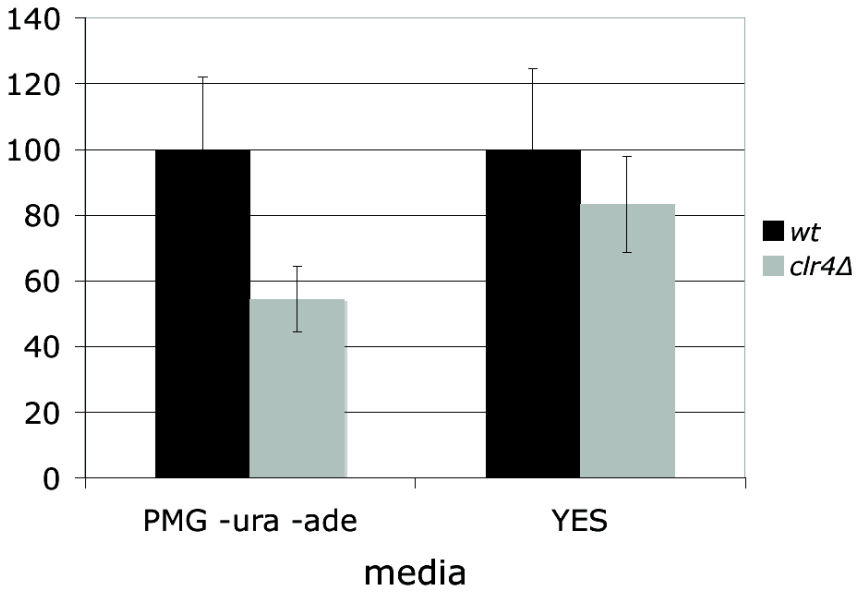

Figure S5: Association of CENP-A^{Cnp1} with endogenous centromeres in wild-type (*wt*) and *clr4*Δ cells determined by ChIP and qPCR.
Enrichment of endogenous central core domain sequences from *cen1* and *cen3* (*cc1/3*) was determined relative to the control non-centromeric locus *fbp1*. Cells were grown at 32^{o}C in minimal PMG –uracil –adenine (containing pH-cc2) or rich YES media. PMG –uracil –adenine (n=6 for *wild-type/wt* and *clr4Δ* strains); YES (n=3 for *wild-type/wt* and *clr4Δ* strains). These data indicate that in selective minimal media less CENP-A^{Cnp1} is detected at endogenous centromeres in *clr4*Δ cells relative to wild-type. This is not seen in rich medium. The reason for this difference is not known but these data may suggest that under these more demanding growth conditions less CENP-A^{Cnp1} is retained at endongenous centromeres in the absence of Clr4.

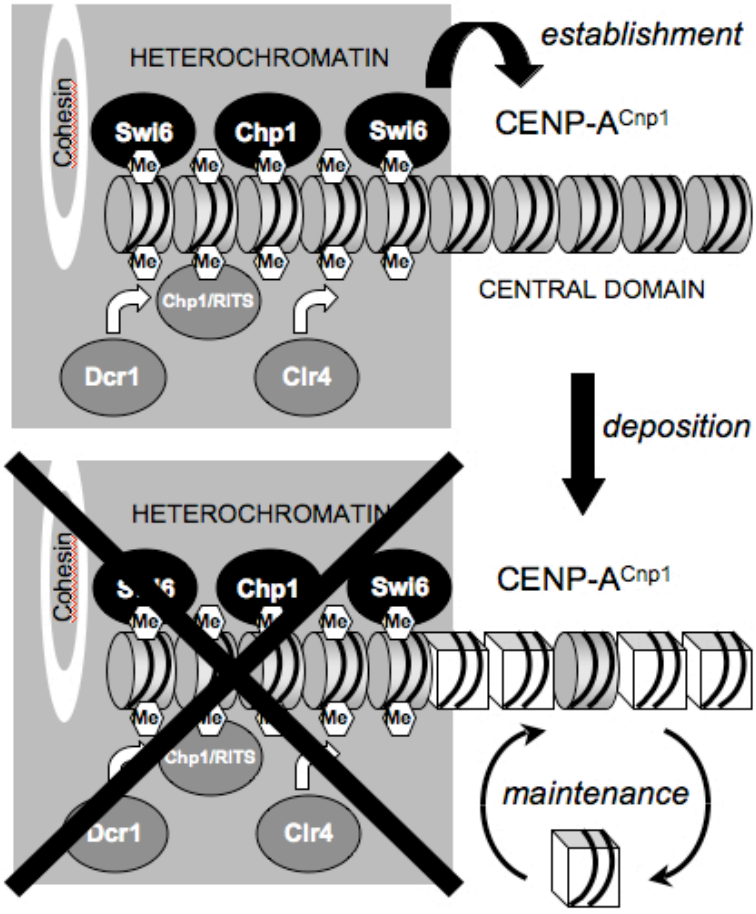

Figure S6. **Model.**
An intact domain of heterochromatin with all components (RNAi, Swi6, Clr4 H3K9me2) is required for the initial establishment of CENP-A^{Cnp1} chromatin and consequently kinetochore proteins on central domain DNA. However, once established, this specialised CENP-A^{Cnp1} structure can be propagated independently of heterochromatin. It is not known how heterochromatin effects CENP-A establishment on minichromsomes. It also remains to be shown that heterochromatin contributes to CENP-A establishment at endogenous centromeres. It is possible that the cohesin recruited by centromeric heteochromatin plays some role.