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CENP-B preserves genome integrity at replication forks paused by retrotransposon LTR

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Centromere-binding protein B (CENP-B) is a widely conserved DNA binding factor associated with heterochromatin and centromeric satellite repeats¹. In fission yeast, CENP-B homologues have been shown to silence long terminal repeat (LTR) retrotransposons by recruiting histone deacetylases². However, CENP-B factors also have unexplained roles in DNA replication^{3,4}. Here we show that a molecular function of CENP-B is to promote replication-fork progression through the LTR. Mutants have increased genomic instability caused by replication-fork blockage that depends on the DNA binding factor switch-activating protein 1 (Sap1), which is directly recruited by the LTR. The loss of Sap1-dependent barrier activity allows the unhindered progression of the replication fork, but results in rearrangements deleterious to the retrotransposon. We conclude that retrotransposons influence replication polarity through recruitment of Sap1 and transposition near replication-fork blocks, whereas CENP-B counteracts this activity and promotes fork stability. Our results may account for the role of LTR in fragile sites, and for the association of CENP-B with pericentromeric heterochromatin and tandem satellite repeats.

In fission yeast, CENP-B proteins are encoded by three homologues, autonomously replicating sequence binding protein 1 (*abp1*), cenp-B homologue 1

(*cbh1*) and *cbh2*, and were previously characterized as DNA binding factors at origins of replication and centromeric repeats, respectively^{3,5}. Mutants of *abp1* grow slowly, whereas double mutants with *cbh1* or *cbh2* have severely stunted growth, abnormal mitosis and morphological defects, and triple deletion mutants are inviable^{6,7}. As a result, double $\Delta abp1\Delta cbh1$ mutants form microcolonies on solid media (Fig. 1a and Supplementary Table 1) and exhibit high levels of cell death (Supplementary Fig. 1). We observed the spontaneous appearance of faster growing cells in a culture of $\Delta abp1\Delta cbh1$ that grew at rates similar to the $\Delta abp1$ single mutant, lacked morphological defects (Fig. 1a and Supplementary Table 1) and showed lower levels of cell death (Supplementary Fig. 1). Genetic analysis revealed the presence of a single essential locus that also suppressed the lethality of the triple mutant $\Delta abp1\Delta cbh1\Delta cbh2$ (not shown). We performed whole-genome resequencing in the mutant strain⁸ and isolated a missense mutation in the coding sequence of the DNA binding factor Sap1 (*sap1E101D*, henceforth called *sap1-c*; Supplementary Fig. 2) that co-segregated with suppression of slow growth in $\Delta abp1\Delta cbh1$ and resulted in lethality in a wild-type background. Sap1 is a protein with essential roles in chromosome stability⁹. Sap1 has been implicated in a programmed replication-fork block in the ribosomal DNA (rDNA) monomer that ensures directional replication to prevent mitotic recombination between rDNA repeats¹⁰⁻¹².

To test the effects of CENP-B and *sap1-c* mutations on genome integrity, we examined chromosomes by pulsed-field gel electrophoresis. Although single $\Delta abp1$ and $\Delta cbh1$ mutants had wild-type chromosome lengths, the double $\Delta abp1\Delta cbh1$ mutant had a smear of DNA fragments indicating double-strand breaks in all three chromosomes (Fig. 1b). Treatment of the $\Delta abp1\Delta cbh1$ sample plugs with the restriction enzyme NotI allowed migration of the chromosomes into the gel, and detection of telomeric and centromeric sequences (Supplementary Fig. 3), suggesting the presence of scattered unresolved replication or recombination intermediates that interfere with the migration of full-length chromosomes, but not with NotI-digested DNA, into the pulsed field gel. This indicates that Abp1 and Cbh1 have roles in the maintenance of genome integrity.

Surprisingly, the *sap1-c* mutation restored genome integrity to all chromosomes, with chromosome 3 exhibiting size variability in several isolates of $\Delta abp1\Delta cbh1\Delta sap1-c$

mutant (Fig. 1b). In fission yeast, chromosome 3 harbours the rDNA repeats. Temperature-sensitive alleles of *sap1* exhibit changes in the size of chromosome 3 attributable to loss of fork barrier activity and an increase in mitotic recombination at rDNA¹². The changes in the size of chromosome 3 size in the *sap1-c* mutants are associated with altered rDNA copy number (Supplementary Fig. 4). The temperature-sensitive alleles *sap1-1* and *sap1-48* (ref. 12) suppressed slow growth in the $\Delta abp1\Delta cbh1$ double mutant, mimicking *sap1-c* (Supplementary Fig. 5). Consistent with a reduction in fork barrier activity, a probe containing a canonical Sap1-binding sequence had reduced electrophoretic mobility shift in crude extracts from *sap1-c* mutants (Supplementary Fig. 6). We conclude that the suppression of the $\Delta cbh1\Delta abp1$ phenotype is not specific to the *sap1-c* mutation but a result of defective function of Sap1, and therefore that the loss of genome integrity in $\Delta abp1\Delta cbh1$ mutants is a consequence of Sap1 activity.

Blocked replication forks are potential sources of genome instability because they can lead to collapse of the replisome and double-strand break formation¹³. The fact that Sap1 activity leads to DNA damage in the absence of Abp1/Cbh1 suggests that the function of CENP-B is to manage Sap1-arrested replication forks. In the absence of Sap1, loss of replication-fork blockage would render Abp1/Cbh1 activity unnecessary and lead to increased genome stability in $\Delta abp1\Delta cbh1$ mutants. This model predicts that CENP-B and Sap1 would co-localize to the regions where they acted on the replication fork, and that these regions would engage in homologous recombination and degrade to double-strand breaks in the absence of CENP-B. To test this hypothesis, we performed chromatin immunoprecipitation of Sap1, Abp1 and Cbh1 followed by high-throughput sequencing (ChIP-seq). Abp1 has previously been shown to localize and recruit Cbh1 to the LTRs of Tf1 and Tf2 retrotransposons, where the LTRs play a role in their transcriptional silencing². We demonstrated a strong co-localization of Sap1 with Abp1 and Cbh1 at these LTRs as well as at solo LTRs scattered throughout the genome (Fig. 2a, c and Supplementary Fig. 7a, b) and at the mating type locus (Supplementary Fig. 8), where Sap1 and Abp1 have been described to regulate mating-type switching^{14,15}. Both Sap1 and Abp1/Cbh1 also localized to genomic regions independently of each other, suggesting that they do not form a stable complex or mediate their mutual recruitment. In particular, Abp1 exhibited binding to transfer RNA (tRNA) genes (Fig.

2b and Supplementary Fig. 7b), known to be potent replication pause sites^{13,16}. Abp1 and Cbh1 co-localize to a highly A/T-rich region located in positions 100–150 of the LTR (Fig. 2c and Supplementary Fig. 7a, b). The localization of Sap1 within the LTR was concentrated in the first 50 base pairs) of sequence (Fig. 2c), coinciding with a predicted Sap1-binding site¹⁷ (Supplementary Fig. 7a, c). We tested this sequence by electrophoretic mobility shift assay and detected specific binding in wild-type extracts (Fig. 2d) as well as decreased binding and altered mobility in extracts from *Δabp1Δcbh1sap1-c* mutants (Supplementary Fig. 7d). Interestingly, solo LTR and full-length Tf2 insertions were associated with a prominent peak of Sap1 binding located outside the 3' end of the transposon sequence (Fig. 2c). These observations indicate that Sap1 binding precedes and possibly guides Tf element integration. To test this prediction, we plotted the average enrichment of Sap1, Abp1 and Cbh1 around more than 70,000 *de novo* Tf1 integration sites recently reported¹⁸. We observed a dramatic association of these integration sites, with a peak of Sap1 binding immediately downstream of the insertion site (Fig. 2e and Supplementary Fig. 8) and no appreciable CENP-B enrichment. These results strongly suggest that Sap1-binding sequences determine the targeting and orientation of Tf retroelement transposition.

To evaluate the mutual influence of Sap1 and Abp1/Cbh1 on LTR binding, we performed ChIP analysis of Sap1 in *Δabp1Δcbh1* mutants and of Abp1 in temperature-sensitive *sap1* mutants that affect DNA-binding activity¹². Sap1 binding to the LTR was unaffected in *Δabp1* and *Δcbh1* mutants, and was slightly increased in *Δabp1Δcbh1* double mutants (Fig. 2f), but consistently reduced (twofold) in *Δabp1Δcbh1sap1-c*. Conversely, Abp1 binding to the LTR was increased between two and three times at the permissive temperature in *sap1-1* and *sap1-48* mutants (Fig. 2g). These results indicate that Sap1 and Abp1/Cbh1 bind to the LTR independently of each other and mutually counteract their recruitment, and that the *sap1-c* mutation impairs its binding to the LTR *in vivo* as well as *in vitro* (Supplementary Fig. 4).

A failure of replication-fork stability at LTRs, which are distributed throughout the genome, would explain the widespread DNA damage in *Δabp1Δcbh1* mutants. We assessed the behaviour of the replication fork as it traversed the LTR using two-dimensional agarose gel electrophoresis. Sap1-dependent programmed fork blocks are

directional and only hinder fork progression in one orientation^{10,11}. We cloned a full-length LTR and its first 50 base pairs (containing the Sap1-binding site) in a plasmid in both orientations with respect to the replication origin *arsI*. Two-dimensional gel electrophoresis in a wild-type strain transformed with this episomal system showed a modest accumulation of fork signal at the location of the cloned LTR (Fig. 3a), but only when the Sap1-binding site was proximal to the origin, and not in the opposite orientation (Supplementary Fig. 9). The Sap1-binding site was sufficient for this blocking activity, with the same orientation requirement (Supplementary Fig. 9). We next assayed the LTR for pausing activity in $\Delta abp1$, $\Delta cbh1$ and *sap1-c* mutants (Fig. 3a). Strikingly, the paused fork signal was consistently enhanced and always at the same location in $\Delta abp1$ and $\Delta cbh1$ mutants, whereas the $\Delta abp1\Delta cbh1$ double mutant exhibited additional signals outside the replication arc, suggestive of recombination intermediates¹⁹. The fork-blocking activity of the LTR disappeared in $\Delta abp1\Delta cbh1\text{sap1-c}$ mutants. Unresolved fork blocks can collapse and undergo homologous recombination for fork recovery. We confirmed the presence of homologous recombination in the $\Delta abp1\Delta cbh1$ double mutants by measuring the increase in the formation of Rad22 (homologous to Rad52 in *Saccharomyces cerevisiae*) foci in a Rad22–yellow fluorescent protein (YFP) strain²⁰ (Fig. 3b). We observed that $\Delta abp1\Delta cbh1$ double-mutant cells accumulated the homologous recombination protein Rad22 at the LTR (Fig. 3c). Consistently, the recombination factor Rhp51 (Rad51 homologue) was essential for viability of $\Delta abp1\Delta cbh1$ double mutants (Supplementary Fig. 10), indicating that homologous recombination is necessary for recovery from fork stalling at LTRs. These results indicate that Abp1/Cbh1 counteract Sap1 barrier activity and stabilize the replication fork at LTRs. This results in loss of genome integrity and homologous recombination at the LTR in $\Delta abp1\Delta cbh1$ mutants.

The Sap1-binding sequence is conserved in Tf1 and Tf2 retrotransposon LTRs (Supplementary Fig. 6c), suggesting that it plays a role in the retrotransposon life cycle. We assayed the effect of *sap1* and *abp/cbh1* on Tf2 stability by measuring the frequencies of loss of a *ura4* reporter transgene inserted in the Tf2-6 transposon²¹. Mutation of *abp1* resulted in a dramatic decrease of Tf2 ectopic recombination, which returned to normal levels when *sap1* was also mutated (Fig. 4). In the presence of *sap1+*

there is a preference for gene conversion, which normally constitutes most ectopic recombination events²²; however, in $\Delta abp1sap1-c$ and $\Delta cbh1sap1-c$ mutants the proportion of eviction and conversion events is similar (Fig. 4). Therefore we propose that the LTR recruits Sap1 to control the direction of transposon replication and increase transposon persistence in the genome, perhaps by coordinating lagging strand synthesis, which prevents single-strand annealing from complementary direct repeats (Supplementary Fig. 11a, b). CENP-B counteracts this activity, possibly by promoting replication-fork progression through the Sap1-dependent barrier. Thus CENP-B and Sap1 promote genome and transposon integrity, respectively, in a ‘tug-of-war’ between transposon and host. Abp1 stimulates fork progression by recruiting the fork-restart protein MCM10 (ref. 4), which has primase activity, and the histone deacetylase Mst1, which has roles in replication-fork stability and interacts directly with Cbh1 (ref. 23) (Supplementary Fig. 11a). In *S. cerevisiae* the histone deacetylase Sir2 silences and inhibits recombination in repetitive DNA²⁴. CENP-B factors recruit the histone deacetylases Clr3 and Clr6, which perform LTR silencing². The result of these functions would be to preserve genome integrity at LTRs by preventing DNA damage and recombination. This novel role of CENP-B may not be limited to LTR and tDNA, as mutation of the replication-fork blocking factor *reb1*, which is specific to rDNA repeats, also suppresses the slow growth of the *abp1* mutant²⁵. Similarly, our ChIP-seq data indicate that Sap1 may also be implicated in the functionality of the replication terminator RTS1 (Supplementary Fig. 8) in collaboration with Rtf1. In this manner, the function and regulation of the Sap1-bound regions is determined by the binding in their vicinity of different factors affecting replication-fork progression.

Because of their repetitive nature, transposons have a close relationship with replication and recombination. For example, the IS608 transposon of *Escherichia coli* is targeted to the lagging strand and always replicated in the same direction²⁶. This might prevent recombination between tandemly arranged copies. We have shown that retrotransposons influence DNA replication through recruitment of directional fork blocking factor Sap1 and that activity of CENP-B is required for replication-fork management. Additionally, retrotransposition is targeted to the genomic localization of Sap1. These mechanisms influence the replicative dynamics of the host genome. The genomes of eukaryotes show widespread colonization by retrotransposons, and

pericentromeric satellite repeats are often of transposon origin²⁷. When such sequences are arranged as tandem repeats, control of replication direction by CENP-B would prevent chromosome breaks and preserve genome integrity. This mechanism accounts for the role of other regulators of fork progression in inter-LTR recombination^{28,29}. In contrast, when flanked by LTR in opposite orientations, fragile sites fail to replicate and result in chromosome breaks^{13,30}.

METHODS SUMMARY

ChIP was performed using tagged TAP-Abp1 and TAP-Cbh1 strains with an **Anti-Calmodulin Binding Protein** antibody (**Millipore**) and a polyclonal serum against the native Sap1 protein⁹. High-throughput sequencing was performed on an **Illumina G2** genome analyser, and analysed for polymorphism detection or statistical analysis of enrichment. Two-dimensional gel electrophoresis was performed as described¹¹; see Supplementary Information for construction of the episomal system. Electrophoretic mobility shift assay was performed as described previously¹⁷.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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Author Contributions M.Z., B.A. and R.A.M. designed the experiments presented and wrote the paper. M.Z. performed and analysed the experiments. M.W.V. provided bioinformatic analysis. D.G. and D.V.I. provided strains. S.W. and J.B. performed additional experiments.

Author Information The sequences from the ChIP-seq experiments are deposited in Sequence Read Archive (www.ncbi.nlm.nih.gov/sra) under accession number SRA024710.2. Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of this article at www.nature.com/nature. Correspondence and requests for materials should be addressed to R.A.M. (martiens@cshl.edu).

Figure 1 DNA damage in CENP-B mutants is suppressed by *sap1* mutation. a, Images of 10^3 plated cells of wild type (WT), $\Delta abp1$ and $\Delta abp1\Delta cbh1$ with $\Delta abp1\Delta cbh1sap1-c$ colonies. Microscopy image inserts: branched phenotype in $\Delta abp1\Delta cbh1$ background (right) and $\Delta abp1\Delta cbh1sap1-c$ mutant (left). Scale bar, 10 μm . **b,** Pulsed-field gel blot analysis of WT, CENP-B mutants ($\Delta abp1$, $\Delta cbh1$, $\Delta abp1\Delta cbh1$) and five CENP-B/*sap1-c* mutant isolates ($\Delta abp1\Delta cbh1sap1-c$). The position of the three chromosomes is indicated on the right. The image is a false-coloured composite of hybridizations for all three chromosomes.

Figure 2 Sap1 and CENP-B co-localize at the LTR of retrotransposons *in vivo*. Average genome-wide enrichment by ChIP-seq of Sap1, Abp1 and Cbh1 on (a) all *Tf2* elements, (b) euchromatic tRNA and (c) solo LTR. Error bars, s.e.m. **d,** Left panel, competition electrophoretic mobility shift assay; right panel, inactivation by incubation with anti-Sap1 serum⁹. **e,** Average Sap1, Abp1 and Cbh1 enrichment around *Tf1 de novo* insertion points¹⁸. **f,** ChIP of Sap1 with LTR of *Tf2* in CENP-B and *sap1-c* mutants and (g) of Abp1 with LTR of *Tf2* in *sap1* temperature-sensitive mutants. Error bars, s.d. for triplicates.

Figure 3 CENP-B promotes replication-fork progression through the Sap1-dependent barrier present at the LTR and prevents homologous recombination. a, Two-dimensional gel electrophoresis of a plasmid fragment containing the *Tf2* LTR oriented towards (left) and away (right) from the *ars1* origin. Arrows, paused replication intermediates; open arrows, recombination intermediates. The percentage of signal over the LTR is indicated below each panel. **b,** Quantification of Rad22–green fluorescent protein foci ($n > 400$ nuclei for all mutants). Error bars, s.e.m. **c,** Rad22–YFP ChIP with LTR in wild type, $\Delta abp1$ and $\Delta abp1\Delta cbh1$ mutants. Error bars, s.d. for triplicates.

Figure 4 CENP-B and Sap1 have opposite effects on *Tf2* stability. a, Ectopic recombination fluctuation assay. Two potential mechanisms of *ura4* loss from the marked *Tf2-6::ura4* are indicated: gene conversion and eviction by LTR recombination. Columns represent total median *ura4* loss frequency in wild type, $\Delta abp1$, $\Delta cbh1$, $\Delta abp1sap1-c$ and $\Delta cbh1sap1-c$ mutants; error bars, 95% confidence intervals. Tints indicate distribution of mode of ectopic recombination events in the *ura4-* colonies

obtained from wild type ($n = 93$), $\Delta abp1$ ($n = 88$), $\Delta cbh1$ ($n = 94$), $\Delta abp1sap1-c$ ($n = 91$) and $\Delta cbh1sap1-c$ ($n = 89$) mutants. **b**, Model for the interactions between Abp1, Cbh1, Sap1 and the replication fork at the LTR.

Figure 1

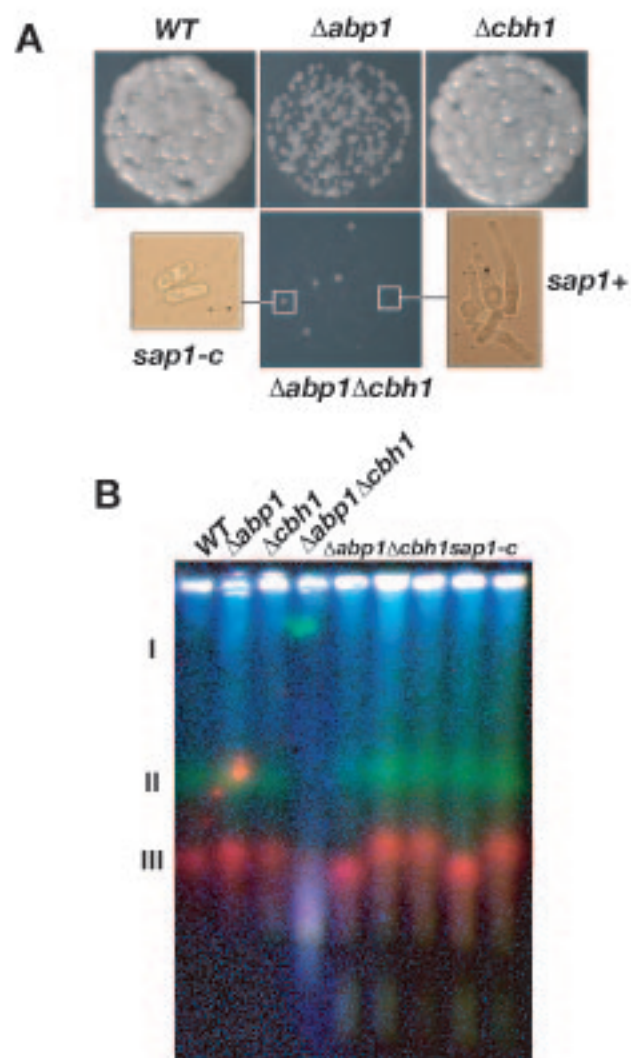


Figure 2

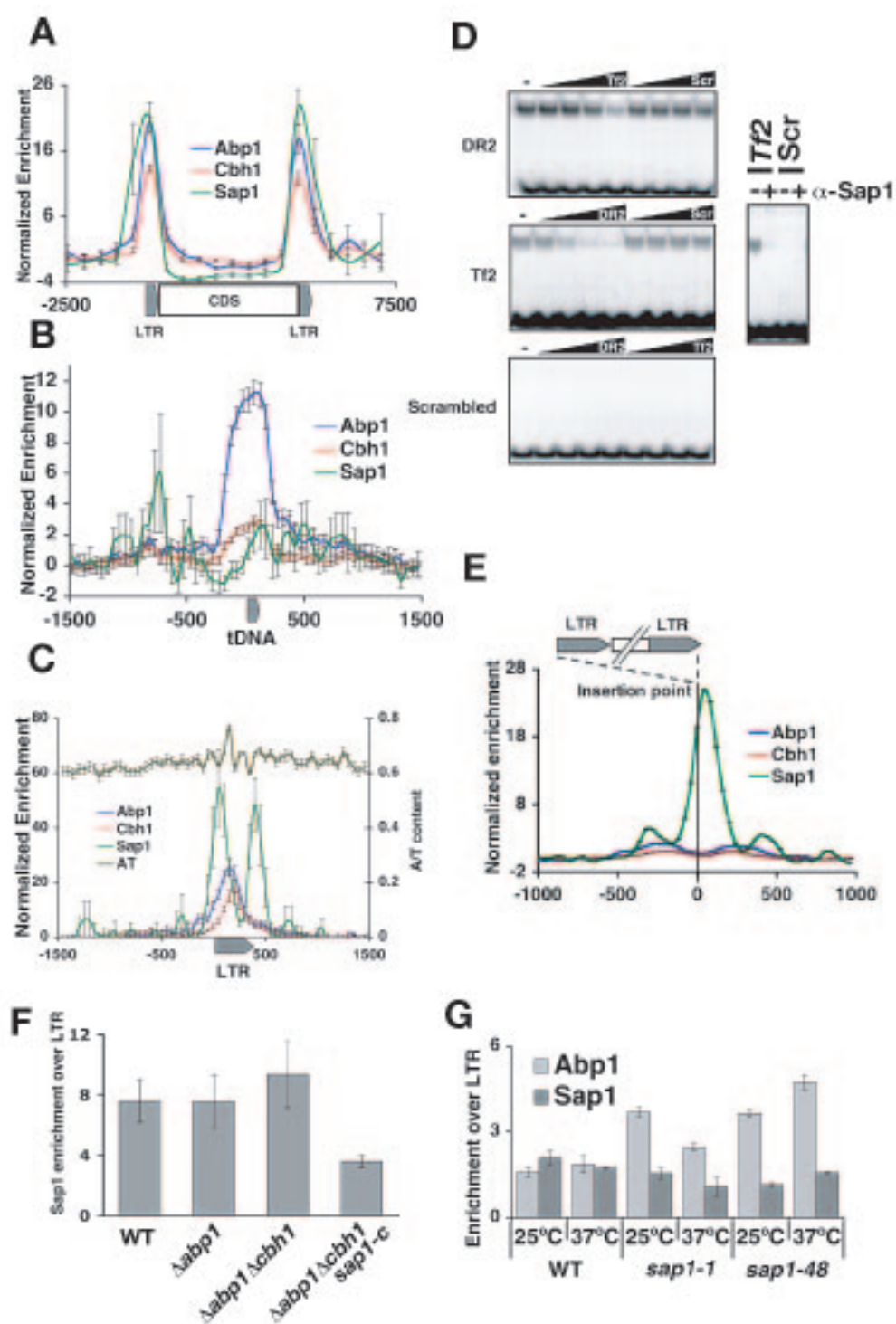


Figure 3

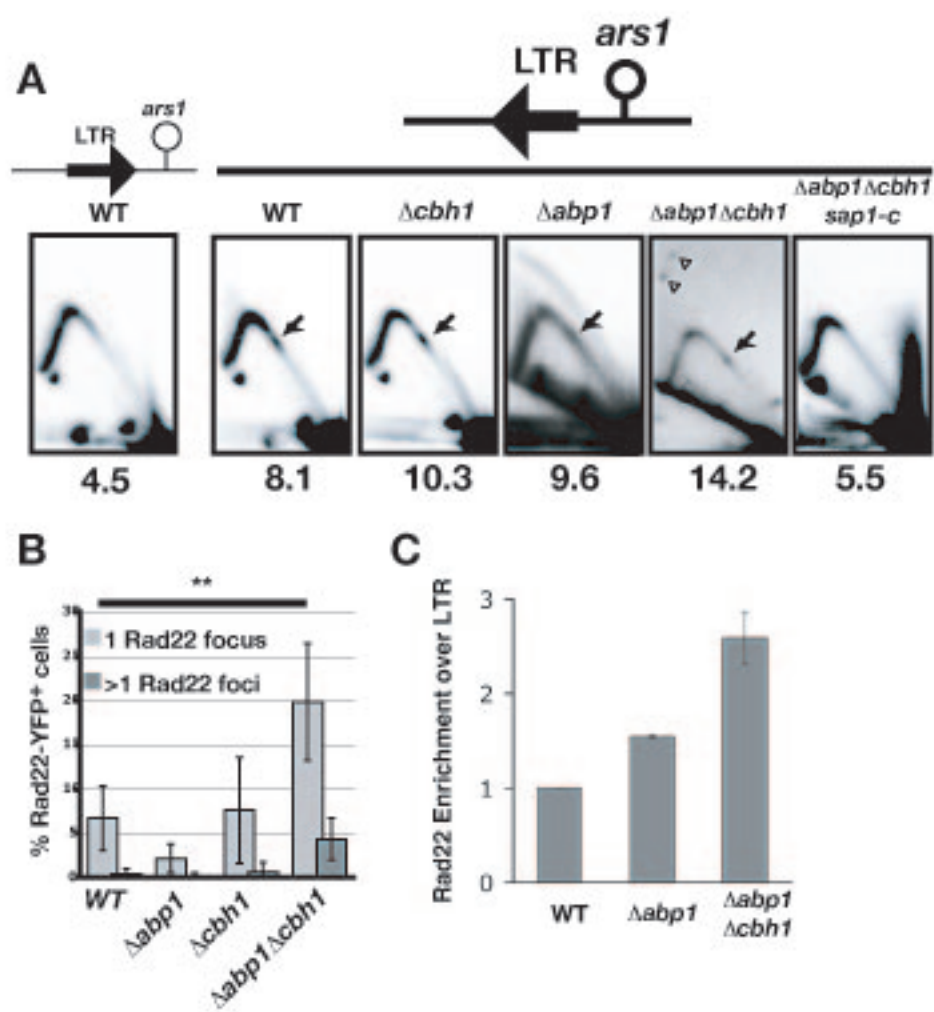
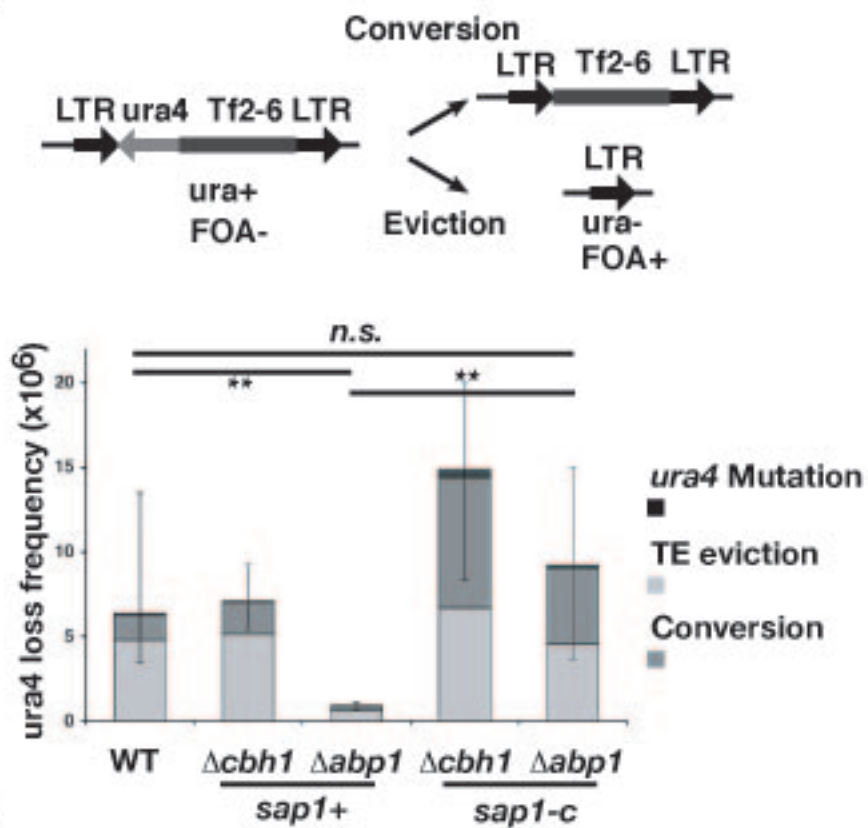
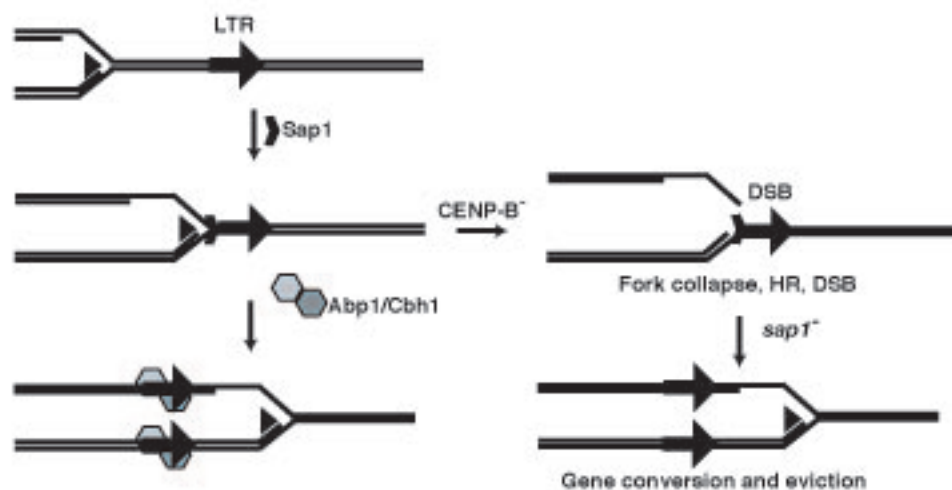


Figure 4

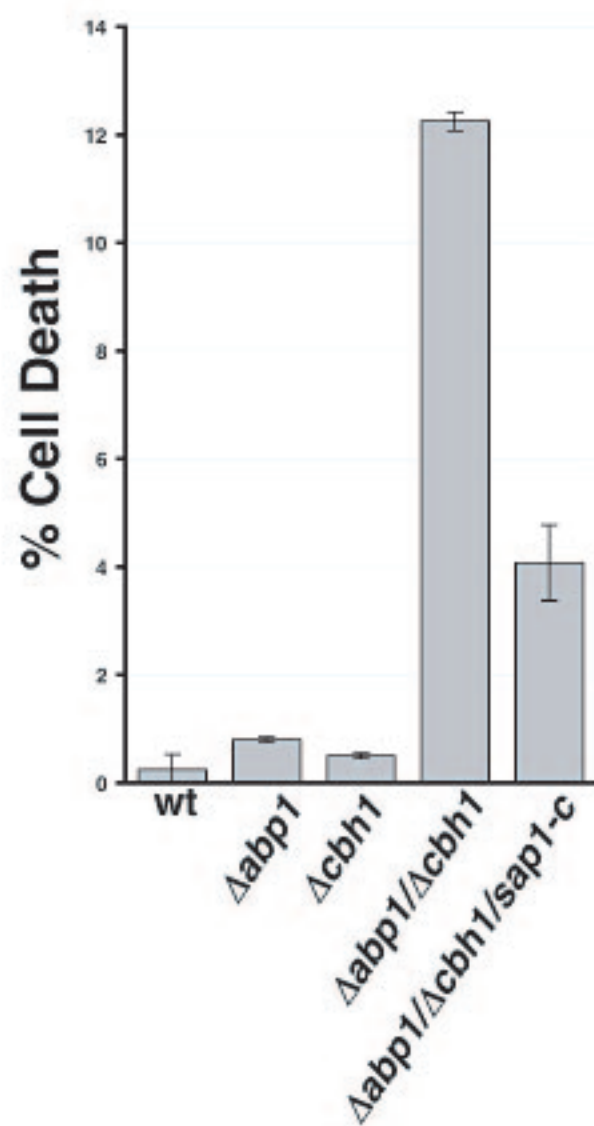
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B

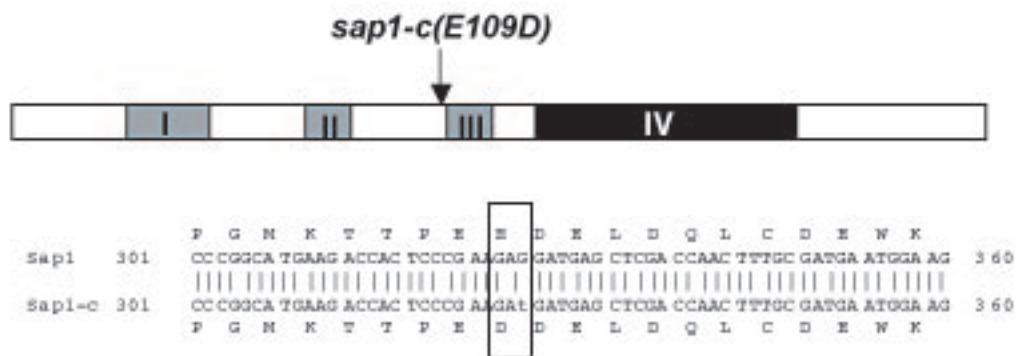


Supplementary Fig. 1

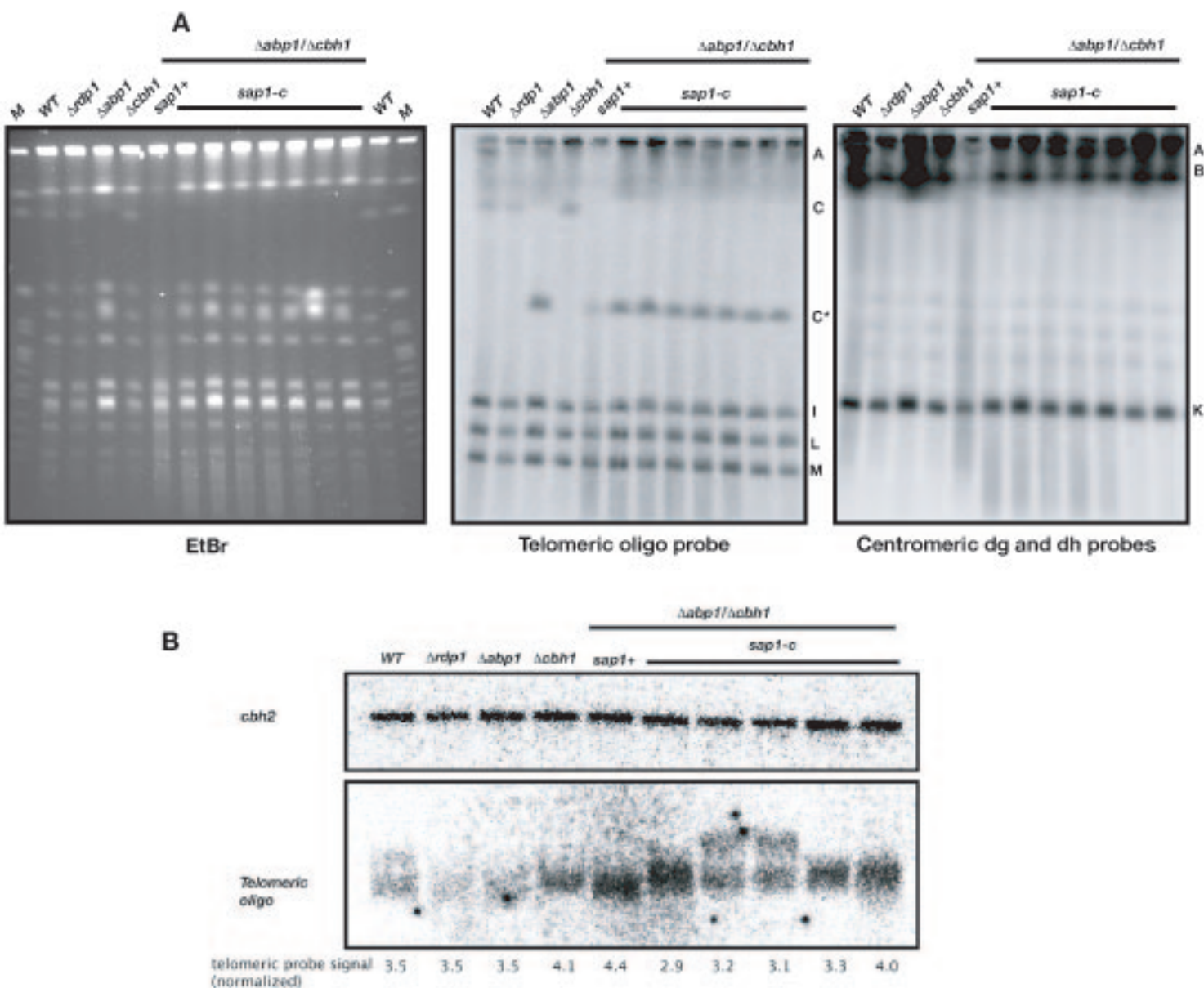


Cell death assays on the indicated mutants. Strains were harvested and analyzed at OD=0.3-0.5. The increased cell death phenotype in the $\Delta abp1\Delta cbh1$ mutant is partially suppressed by the *sap1-c* mutation.

Supplementary Fig. 2

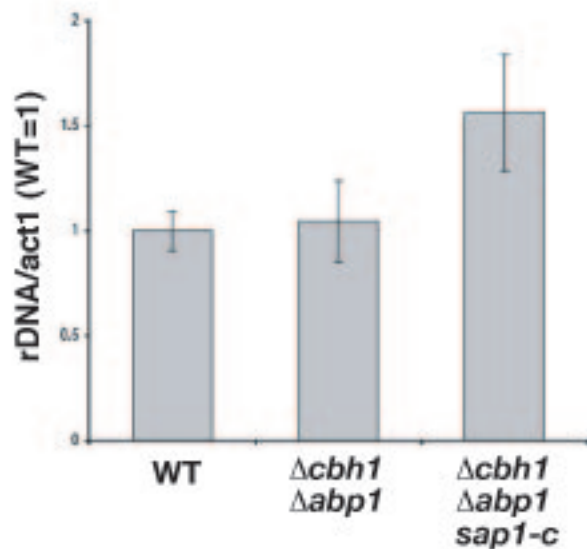


Characterization of the *sap1-c* mutation. a, Location of the *sap1-c* mutation on the coding sequence. Domains I, II and III are DNA binding and Domain IV is the dimerization domain. The *sap1-c* mutation is marked by a rectangle.

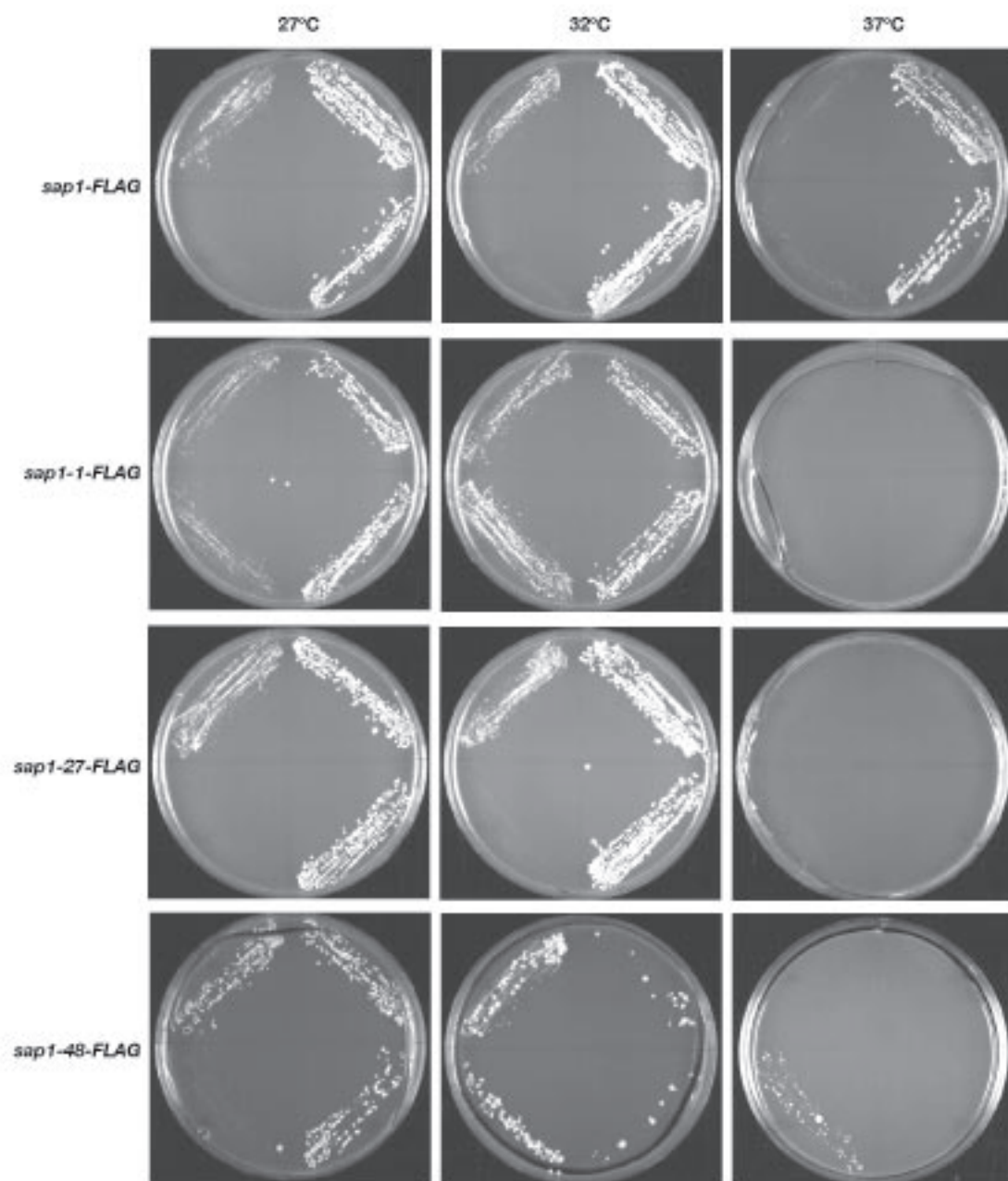
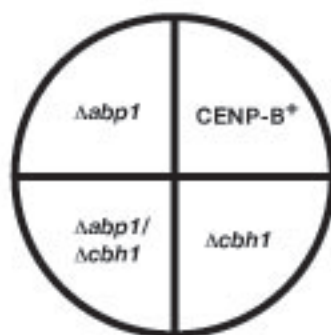


Structural characterization of the genomes on CENP-B and *sap1* mutants. a, Digestion by the rare cutter NotI resolves the fission yeast genome to 17 bands labeled A (complete chromosome III) to Q. Telomere signals are expected from bands A (tel 3 L and R), C (tel2 R), I, (tel1 R) L (tel1 L) and M (tel2 L). Band C* results from the digestion of band C into two fragments due to the introduction of a new NotI target by the *abp1* deletion construct. Centromeric signals are expected in bands A (cenIII), B (cenII) and K (cenI). Digestion by NotI releases intact bands from $\Delta abp1 \Delta cbh1$ agarose plugs indicating recombination or replication intermediates.

B. Quantification of telomeric repeat number and size. Total genomic DNA was subjected to EcoRI digestion, followed by electrophoresis in 1% agarose, southern blotting and hybridisation with a probe against the gene *cbh2* or an oligonucleotide complementary to the fission yeast telomeric repeat. Signal from telomeric repeats is in arbitrary units normalized to signal from *cbh2*. In two isolates of $\Delta abp1 \Delta cbh1 sap1-c$ there is a shift in size of telomeric signal that is attributable to a subtelomeric translocation detected by high throughput sequencing.

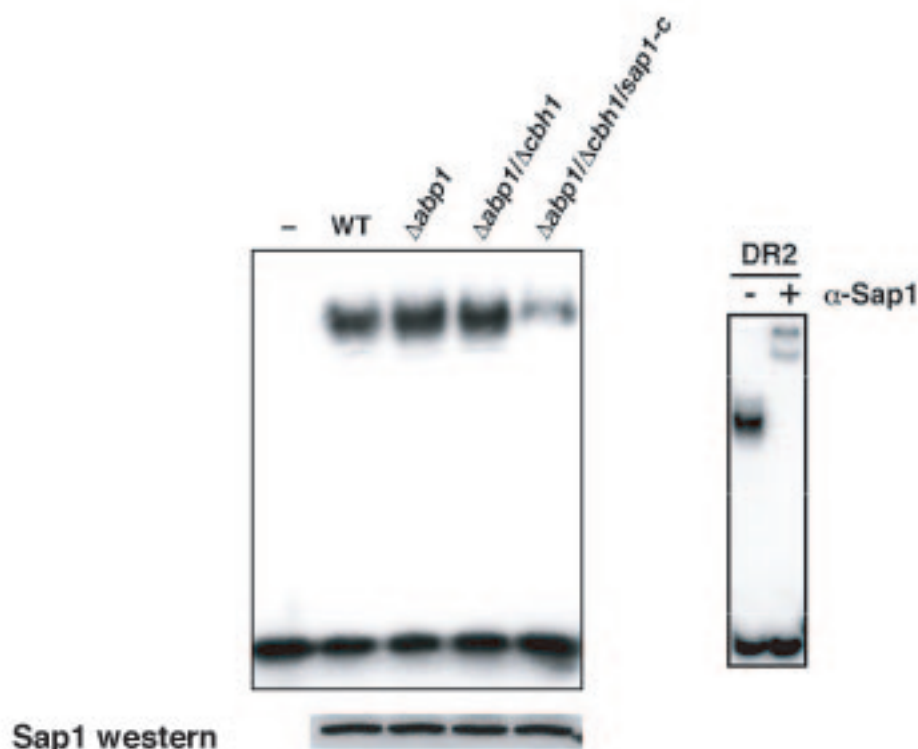


Quantification by Real timeqPCR of rDNA copy number. 4 independent colonies of each genotype from WT, $\Delta abp1 \Delta cbh1$ double mutant and a $\Delta abp1 \Delta cbh1 sap1-c$ triple mutant isolate that showed increased Chromosome 3 size by PFGE (Figure 1) were grown in rich media and the DNA was isolated. Real time qPCR rDNA copy number levels were normalized to act1 copy number levels, and each data-point was normalized to the average of WT copy numbers.

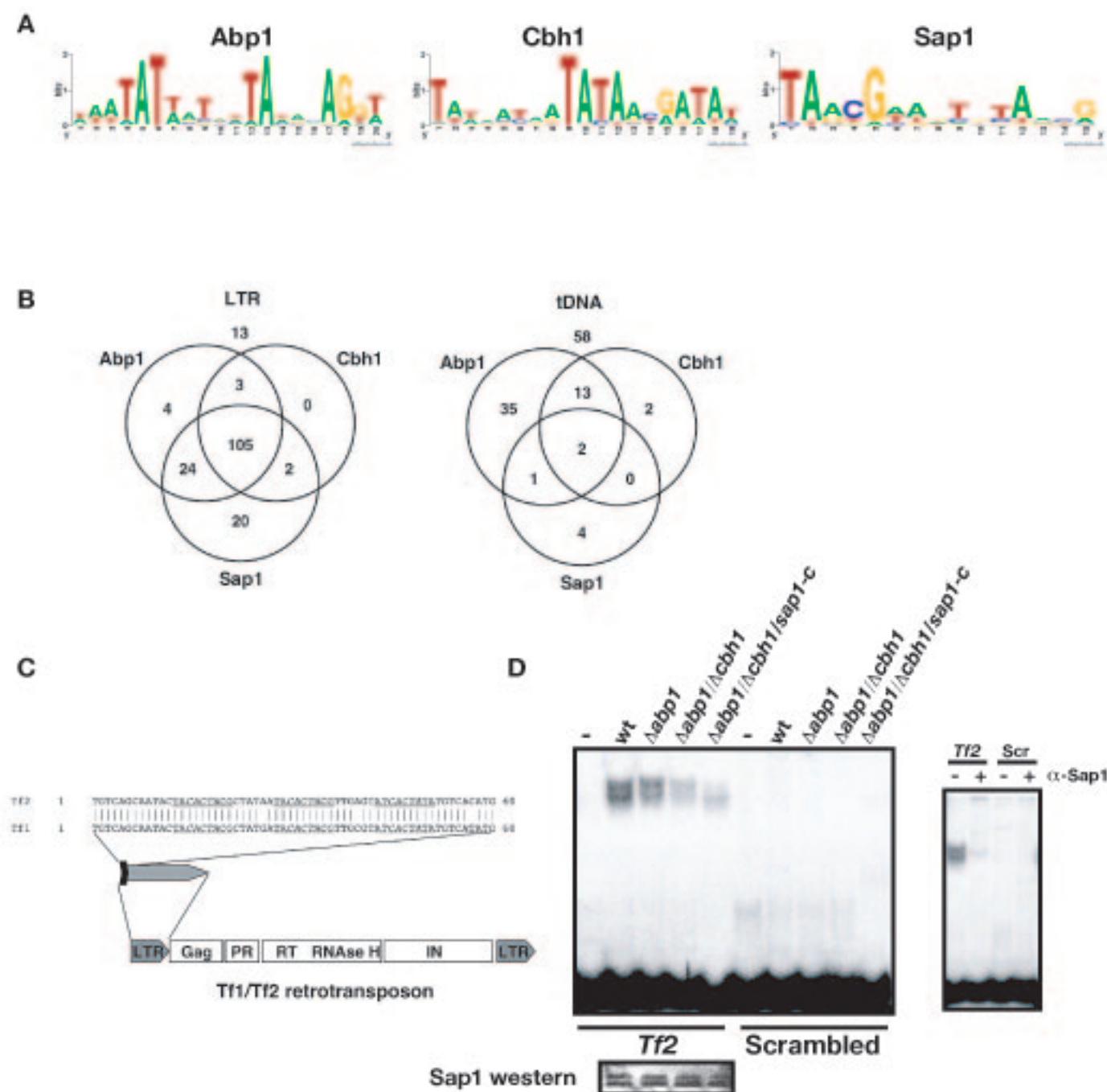


Growth phenotype of CENP-B mutants in combination with *sap1* temperature sensitive alleles. Approximately 10^4 cells were streaked onto Rich media and grown for 3 days at the indicated temperatures. While FLAG-tagged *sap1* and the weak allele *sap1-27* do not interact with *abp1* or *cbh1*, *sap1-1* and *sap1-48* suppress the slow growth of $\Delta abp1\Delta cbh1$ at the semipermissive temperature. Furthermore, the lethality of *sap1-48* at the restrictive temperature is suppressed by $\Delta abp1\Delta cbh1$, phenocopying *sap1-c* which is only viable in a $\Delta abp1\Delta cbh1$ background.

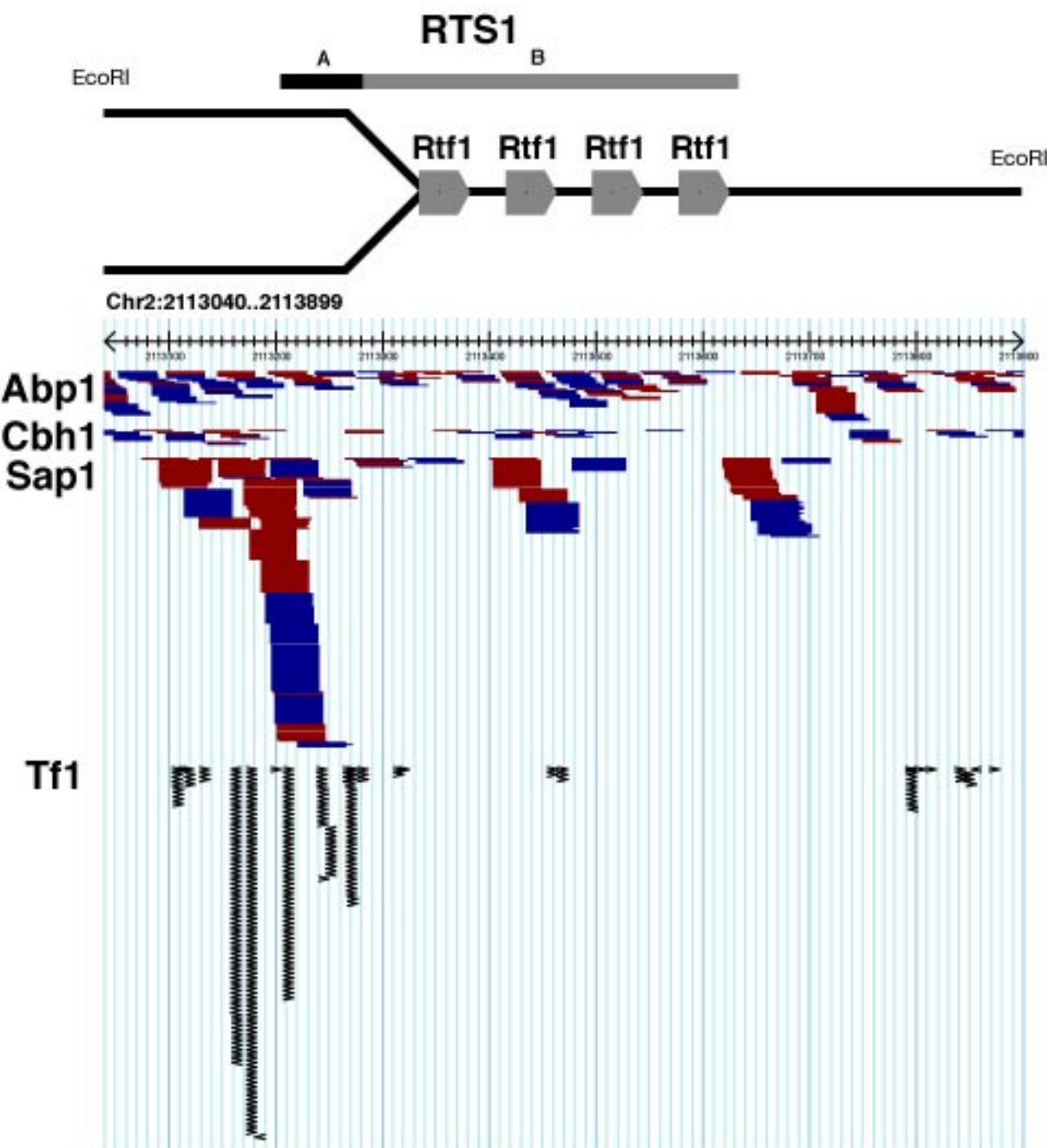
Supplementary Fig. 6



Assay of Sap1 DNA binding activity. a, EMSA (electrophoretic mobility shift assay) of a probe containing the Sap1-binding motif (DR2, see Supplementary Materials and Methods) in extracts from WT, CENP-B mutants ($\Delta abp1$, $\Delta abp1/\Delta cbh1$) and CENP-B/sap1 mutants ($\Delta abp1/\Delta cbh1/sap1-c$). b, EMSA of the DR2 probe in the absence (-) or presence (+) of anti-Sap1 serum. The anti-Sap1 serum supershifts the mobility of the bound probe and reduces its abundance, demonstrating that the binding activity in the extract is due to Sap1.

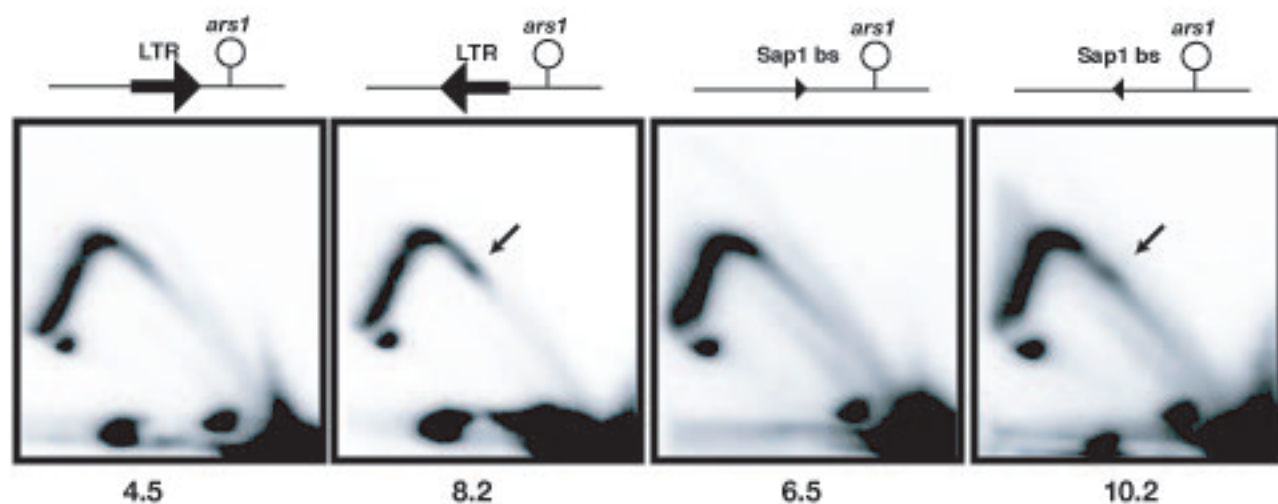


Abp1, Cbh1 and Sap1 binding sites. a, Motifs found by analysis of the sequence of significant peaks in the ChIP-seq. The motifs found in Abp1 and Cbh1 are found in an A/T rich region in the LTR. b, Distribution of Abp1, Cbh1 and Sap1 significant peaks in LTR and tDNA genes. c, Alignment of the 5' end of Tf2 and Tf1 retrotransposon LTR. The putative Sap1 binding sites are underlined. c, EMSA of Tf2 probe and scrambled sequence control probe in extracts from WT, CENP-B mutants (Δ abp1, Δ abp1 Δ cbh1) and CENP-B/sap1 mutants (Δ abp1 Δ cbh1sap1-c). Incubation with an anti-Sap1 serum abrogates binding.

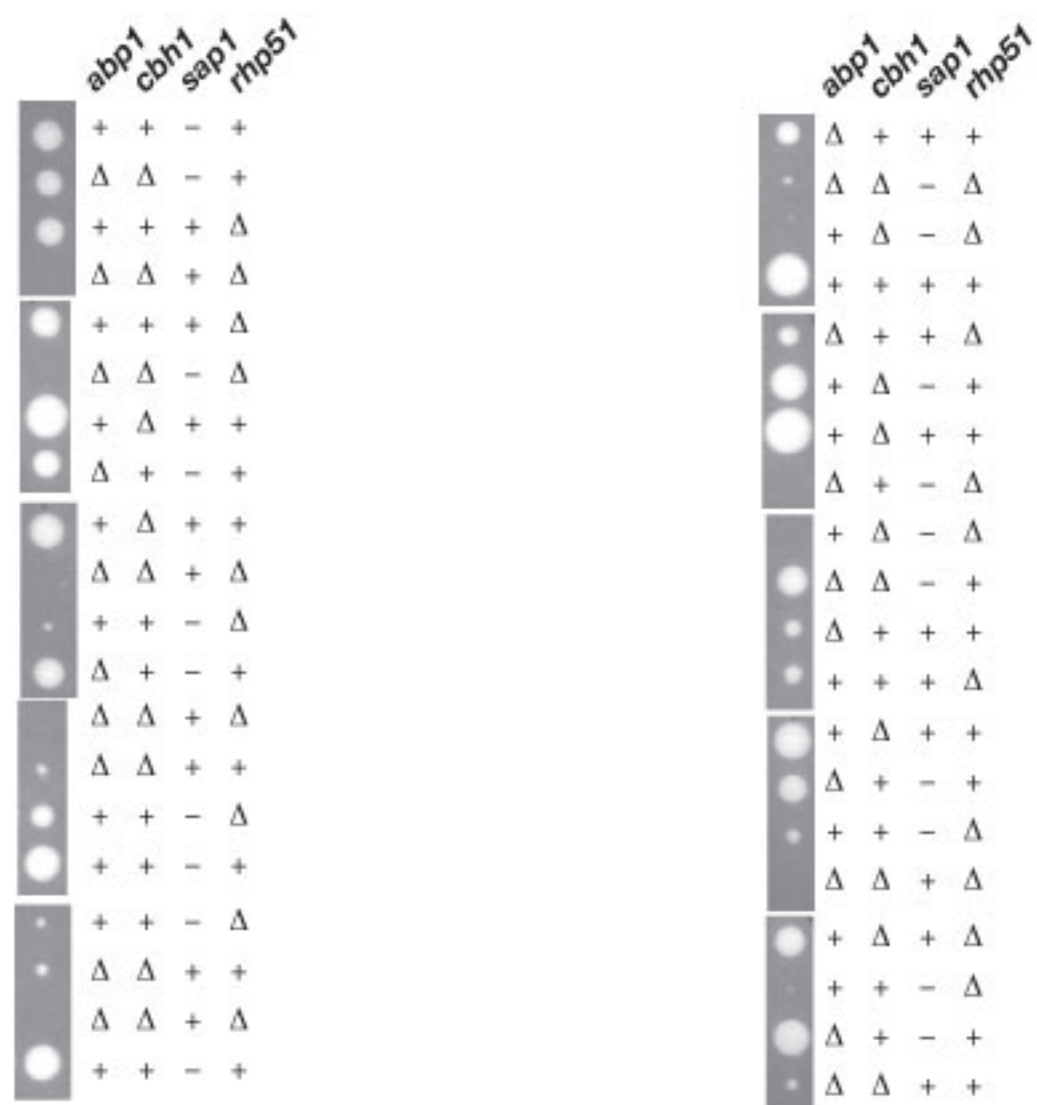


Close-up of the replication terminator RTS1, near the *mat1* locus at the mating type region. Abp1, Cbh1 and Sap1: ChIP-seq reads with position corrected for peak location (red forward, blue reverse reads). Tf1: position and orientation of Tf1 insertions sequenced in (Guo 2010). Above, a schematic representation of the region, showing the orientation of the directionality of the replication fork block, as well as the functional components identified in (Codlin 2003). Rtf1 binding sites indicated with block arrows. A Sap1 binding site is located in the A region, which enhances the blocking activity of RTS1, and determines the presence of a Tf1 transposition hotspot upstream of the barrier.

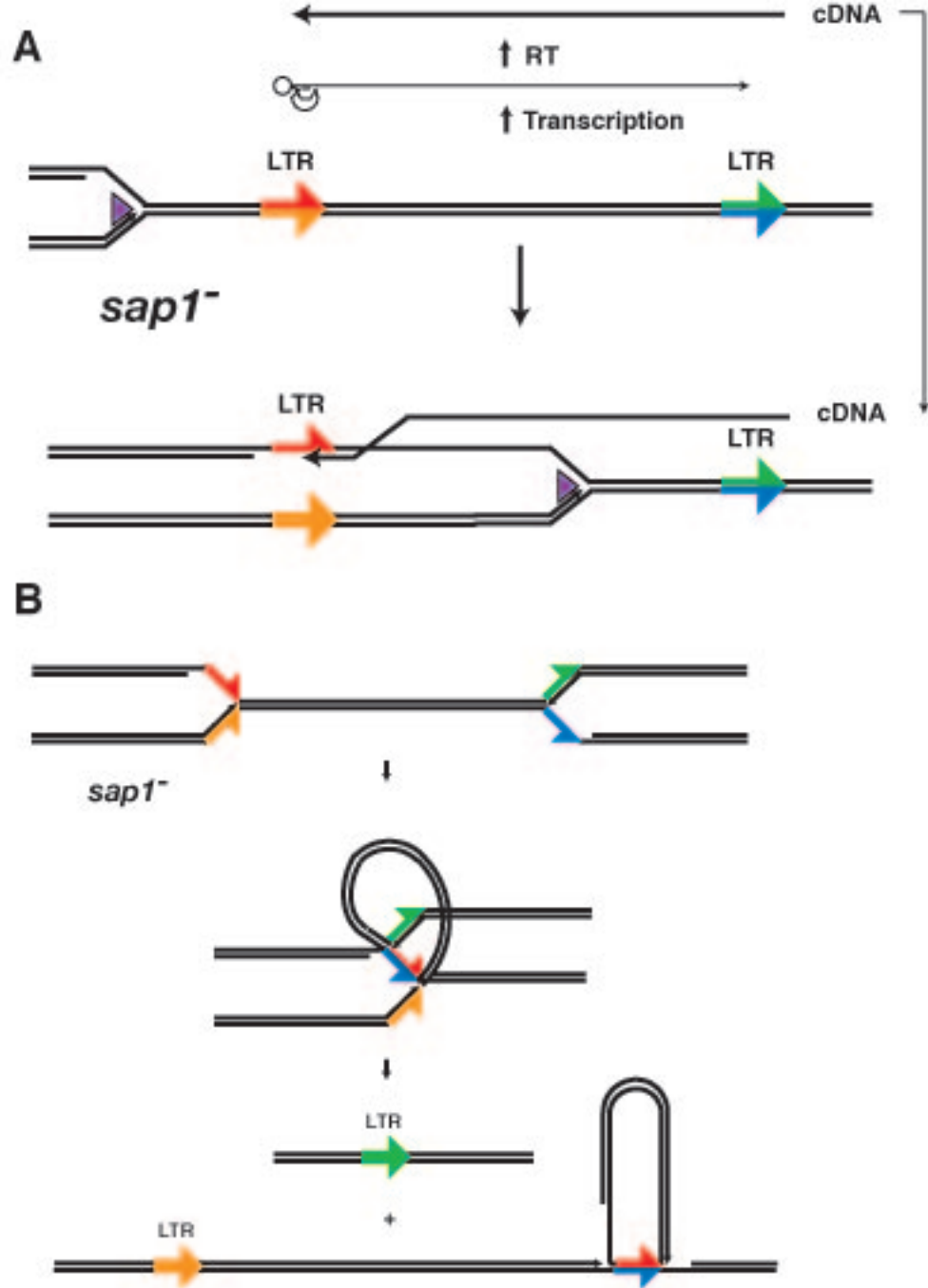
Supplementary Fig. 9



2D gel electrophoresis of plasmid fragments containing the Tf2 LTR or the first 50 base pairs of the Tf2 LTR in both orientations with respect to the *ars1* origin, in a WT strain. Accumulation of paused replication intermediates is indicated (arrows). The percentage of hybridization signal within the replication arch over the LTR is indicated below each panel. The presence of an LTR or the Sap1 binding site in the reverse orientation pauses the replication fork, but not in the forward orientation. This is in agreement with the orientation requirement of the Sap1 binding site present in the Ter1 replication terminator in the rDNA.



Segregation of a cross between a $\Delta abp1\Delta cbh1sap1-c$ triple mutant and a $\Delta rhp51$ Knockout mutant. Triple $\Delta abp1\Delta cbh1\Delta rhp51$ mutants are inviable, while $\Delta abp1\Delta cbp1\Delta rhp51sap1-c$ quadruple mutants form very slow growing microcolonies. $sap1-c\Delta rhp51$ double mutants are also slow growing, suggesting that defective *sap1* function also elicits homologous recombination.



Supplemental Fig. 11

A model for the control of ectopic recombination at LTR by directional replication fork barriers.

A. cDNA mediated recombination is promoted by lagging strand replication complementary to the cDNA.

B. Inter-LTR recombination occurs by simultaneous replication of both LTR in opposite directions.