RNA polymerase backtracking drives the accumulation of fission yeast condensin at active genes

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Running title: RNAP backtracking and condensin buildup

Abstract

The mechanisms leading to the accumulation of the SMC complex condensin around specific transcription units in mitosis remain unclear. Observations made in bacteria suggested that RNA polymerases (RNAP) constitute an obstacle to SMC translocation, particularly when RNAP and SMC travel in opposite directions. Whether this also applies to eukaryotic condensin remains unclear. Here we show in fission yeast that condensin remains focused at the 3' end of an RNAP2-transcribed gene after flipping its orientation, suggesting that gene termini harbor intrinsic condensin-positioning features whatever the orientation of transcription. Consistent with this, we provide evidence that transcription termination mechanisms position condensin whatever the RNAP involved. Moreover, to stabilize backtracked RNAP2 polymerases within gene bodies was sufficient to cancel the accumulation of condensin at gene termini and to redistribute it evenly within transcription units. Altogether, our results suggest that RNAP backtracking, which is frequent at gene termini, plays a key role in positioning condensin and strengthen the idea that dense arrays of proteins tightly-bound to DNA alter the distribution of condensin on mitotic chromosomes.

Keywords: backtracking, chromosomes, condensin, mitosis, Sen1

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INTRODUCTION

Structural Maintenance of Chromosomes (SMC) complexes are essential for the organization and stability of chromosomes from bacteria to humans (Hassler *et al*, 2018; van Ruiten & Rowland, 2018; Uhlmann, 2016). The SMC complex condensin is particularly important for the compaction and the structuration of chromosomes throughout mitosis and for their faithful segregation to daughter cells (Hirano, 2016). Condensin is a ring-shaped DNA translocase that uses the energy of ATP-hydrolysis to organize mitotic chromosomes into large consecutive loops of chromatin anchored around a central protein scaffold (Naumova *et al*, 2013; Gibcus *et al*, 2018). It has been shown *in vitro* that purified condensin hydrolyses ATP to extrude loops of naked DNA (Kong *et al*, 2020; Ganji *et al*, 2018), but the structural details of the formation and enlargement of such loops remain misunderstood (Cutts & Vannini, 2020). Furthermore, whether such loop extrusion activity is the only way condensin organizes mitotic chromosomes is still under debate as computer models have suggested that condensin could also organize chromosomes by sequentially capturing two DNA molecules brought into proximity by Brownian motion, thereby producing a chromatin loop (diffusion capture model, (Cheng *et al*, 2015; Sakai *et al*, 2018)). The relative contributions of loop extrusion and diffusion capture to chromosome organization remain to be understood.

Another fundamental question is to understand how chromatin and large DNA-bound protein assemblies impact the loop extrusion activity of condensin *in vivo*. Loop extrusion on chromatin *in vivo* is predicted to be roughly 10 times slower than on naked DNA *in vitro* (Banigan & Mirny, 2020) and it was recently suggested that arrays of proteins tightly bound to DNA could hinder the loop extrusion activity of condensin, possibly by constituting a steric obstacle to the reeling of chromatin (Guérin *et al*, 2019). An inability to bypass obstacles might result in the formation of unlooped chromatin gaps within mitotic chromosomes (Banigan *et al*, 2020). Whether and how condensin bypasses chromatin-associated obstacles is currently unclear.

Gene transcription has been shown to influence the distribution of SMC complexes in several organisms. In *Bacillus subtilis* and *Caulobacter crescentus*, a single SMC complex juxtaposes the arms of a circular chromosome by translocating in a unidirectional fashion from a single loading site (Tran *et al*, 2017; Wang *et al*, 2015; Le *et al*, 2013; Sullivan *et al*, 2009; Gruber & Errington, 2009). A highly active transcription unit in the opposite direction (head-on orientation) was shown to slow down the translocation of SMC, which transiently accumulates towards the 3' of the unit in a transcription-dependent manner (Brandão *et al*, 2019; Tran *et al*, 2017; Wang *et al*, 2017). It has been proposed that RNA polymerase (RNAP) molecules themselves constitute a directional albeit permeable barrier that impedes the translocation of SMC and each encounter with a RNAP molecule would force SMC to stall for a few seconds (Brandão *et al*, 2019). Great densities of RNAP (or other DNA-bound proteins) are therefore expected to impact the distribution of SMC along chromosome arms. Interestingly, specific mutations in *B. subtilis* SMC were shown to interfere with its ability to overcome transcription-dependent obstacles, suggesting that their bypass is an active process (Vazquez Nunez *et al*, 2019). Consistent with this, it was postulated that the bypass rate of different SMC complexes is a function of their intrinsic ATP hydrolysis rates (Brandão *et al*, 2019). In eukaryotes, transcription is also a positioning device for the SMC complex cohesin in interphase (Heinz *et al*, 2018; Busslinger *et al*, 2017; Bausch *et al*, 2007; Lengronne *et al*, 2004), suggesting that transcription is a conserved regulator of SMC occupancy. It is still unclear however whether the impact of transcription on the translocation of bacterial SMC complexes (Brandão *et al*, 2019) might also apply to SMC complexes in eukaryotes.

Transcription also impinges on the distribution of condensin complexes in eukaryotes, even in organisms where active transcription is strongly reduced in mitosis (Bernard & Vanoosthuyse, 2015). In mouse ES cells, the localisation of condensin II, which is nuclear throughout the cell cycle, correlates with RNAP2 occupancy (Dowen *et al*, 2013). In both chicken and human cells, condensin I, which only associates with predominantly transcriptionally-silent chromatin after nuclear envelope breakdown in mitosis, accumulates

towards the 5' of RNAP2-transcribed genes that were highly transcribed in the previous G2 phase (Kim *et al*, 2013; Sutani *et al*, 2015). By contrast, RNAP1 transcription was proposed to antagonize the accumulation of condensin within the 35S transcription unit in budding yeast (Clemente-Blanco *et al*, 2009; Johzuka & Horiuchi, 2007). Fission yeast is a very good model to understand how transcription affects the function of condensin because transcription remains active during mitosis, when the activity of condensin is maximal. A number of studies have established that fission yeast condensin accumulates in a transcription-dependent manner in the vicinity of genes that are highly expressed in mitosis, whatever the RNA polymerase involved (RNAP1, RNAP2 or RNAP3) (Kim *et al*, 2016; Sutani *et al*, 2015; Nakazawa *et al*, 2015; Kim *et al*, 2014; Nakazawa *et al*, 2008). Moreover, the drug-induced inhibition of transcription partially rescued the loss of viability of condensin-defective mutants (Sutani *et al*, 2015) and it was recently proposed that active transcription interferes locally with the condensin-dependent resolution of sister chromatids (Nakazawa *et al*, 2019b). Taken together, these observations suggest that transcriptionally active RNA polymerases, and/or features associated with ongoing transcription, might challenge condensin function and the assembly of mitotic chromosomes in fission yeast.

We have previously proposed that condensin might load onto DNA at gene promoters depleted of nucleosomes (Toselli-Mollereau *et al*, 2016). In fission yeast, condensin would subsequently accumulate particularly towards the 3' of genes actively transcribed by RNAP2 (Toselli-Mollereau *et al*, 2016; Sutani *et al*, 2015). Whether this is due to a head-on conflict between transcription and translocating condensin like in bacteria (see above) has not yet been investigated. On the other hand, there is evidence that the positioning of fission yeast condensin at the 3' of RNAP2-transcribed genes could be functionally linked to the process of transcription termination. First, a number of positive and negative genetic interactions have been reported between mutants of the transcription termination machinery and mutants of condensin (Nakazawa *et al*, 2019a; Vanoosthuyse *et al*, 2014). As lack of condensin does not directly impact transcription termination in fission yeast (Nakazawa *et al*, 2019a; Hocquet *et al*, 2018), these genetic interactions suggest that RNAP2 transcription termination mechanisms might impinge on the function of condensin. Consistent with this interpretation, it was shown recently that to inactivate Xrn2^{Dhp1}, an enzyme that is key for RNAP2 transcription termination, was sufficient to displace condensin further downstream of transcription units (Nakazawa *et al*, 2019a), strengthening the possibility of interplay between transcription termination mechanisms, the 3' edge of the RNAP2 domain and the positioning of condensin. To explain these observations, it was proposed that condensin is actively recruited at transcription termination regions because they accumulate single-stranded DNA (ssDNA) and/or chromatin-associated RNA molecules that interfere with the organization of mitotic chromosomes (Nakazawa *et al*, 2019a; Sutani *et al*, 2015). Condensin, thanks to its ability to re-anneal melted dsDNA molecules *in vitro* (Sutani *et al*, 2015; Akai *et al*, 2011; Sakai *et al*, 2003; Sutani & Yanagida, 1997), would suppress these structures, thereby allowing the formation of fully functional mitotic chromosomes. This hypothesis therefore posits that condensin plays a "clearing" role in the assembly of mitotic chromosomes (Yanagida, 2009) besides its role in the extrusion of chromatin loops. It remains unclear however how short chromosome regions that are rich in single-stranded DNA (ssDNA) and/or chromatin-associated RNA could interfere with the formation of segregation-competent mitotic chromosomes. Importantly, other models could also account for these observations: (i) the permeable moving barrier model could explain the accumulation of translocating condensin at the 3' border of the RNAP2 domain or (ii) the transcription termination machinery could play a more direct role in the positioning of moving condensin. These models haven't yet been tested experimentally.

Here we sought to better understand what features of transcription might influence the distribution of condensin in fission yeast mitosis. By switching the orientation of an RNAP2-transcribed gene expressed in mitosis, we tested whether or not gene transcription could be a directional barrier for condensin. Although these experiments neither confirmed nor infirmed

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that transcription might be a directional barrier in fission yeast, they strongly reinforced the idea that the 3' end of genes contain intrinsic condensin-positioning features. We then showed that to interfere with RNAP3 transcription termination also alters the distribution of condensin, suggesting that transcription termination defects impact the accumulation of condensin, whatever the RNA polymerase involved. This strengthened the idea that RNAP molecules rather than a specific transcription termination machinery could influence the positioning of condensin. Consistent with this, we provide evidence that to increase the stability of backtracked RNAP2 polymerases throughout the gene body was sufficient to shift condensin occupancy towards the 5' end of transcribed genes. This strongly suggests that backtracked RNA polymerase molecules are themselves positioning devices for condensin. Taken together, our data clarify the role of transcription in the accumulation of condensin and are consistent with the idea that proteins that are tightly-bound to DNA impact the distribution of condensin along mitotic chromosomes.

RESULTS AND DISCUSSION

One prediction of the permeable moving barrier model is that the orientation of transcription impacts the distribution pattern of condensin (Brandão *et al*, 2019). To test this prediction in fission yeast, we changed the orientation of *exg1*, a gene that is transcribed by RNAP2 in mitosis and where condensin was shown previously to accumulate strongly towards the 3' end (Kakui *et al*, 2017) (Fig 1A). Interestingly, it was shown previously that RNAP2 levels remain relatively constant throughout the gene (Sutani *et al*, 2015) (Fig 1A), arguing that the density of RNAP2 *per se* is unlikely to be sufficient to position condensin at the 3' end of this gene. Importantly, the reversal of orientation did not affect RNAP2 levels around *exg1* in mitotic cells (Fig 1BC). Strikingly, the peak of condensin accumulation was moved symmetrically with the flipping of *exg1* and coincided with the new genomic position of the 3' end of the gene (Fig 1BC). These observations could be interpreted in several ways: either (i) transcription is not a directional barrier for condensin in fission yeast, or (ii) transcription is a directional barrier for condensin but the chromatin around *exg1* can be reeled by condensin from both directions with equal probability; alternatively, (iii) the transcription termination process itself or its machinery forces the accumulation of condensin in the 3' end of transcribed genes.

To test the latter hypothesis, we wondered whether transcription termination at another class of genes could also modulate the distribution of condensin. Several ChIP-seq studies reported that fission yeast condensin accumulates at RNAP3-transcribed genes (Kakui *et al*, 2017; Kim *et al*, 2016; Sutani *et al*, 2015; Kim *et al*, 2014) and it was proposed that the B-box binding transcription factor TFIIIC and the TATA-binding protein Tbp1 were required for this accumulation by interacting directly with condensin (Iwasaki *et al*, 2015, 2010). Whether or not transcription termination at RNAP3-transcribed genes could impact the distribution of condensin was not investigated. We recently demonstrated that the conserved DNA&RNA helicase Sen1 is required for efficient transcription termination at RNAP3-transcribed genes in *-cis* (Rivosecchi *et al*, 2019). In the absence of Sen1, RNAP3 strongly accumulates downstream of most of its target genes and we showed that this accumulation of read-through RNAP3 molecules downstream of gene ends could be suppressed by strengthening the endogenous terminators by the use of long polyT sequences (Rivosecchi *et al*, 2019). We tested whether the RNAP3 termination defects associated with lack of Sen1 could impact the distribution of condensin around RNAP3-transcribed genes. Strikingly, condensin levels increased significantly at a subset of RNAP3-transcribed genes in synchronized mitotic cells lacking Sen1 (Fig 2A). This accumulation was specific because lack of Sen1 had no impact on the association of the heterologous *E. coli* protein LacI (Fig 2A). Importantly, the accumulation of condensin in *sen1Δ* cells could not be caused by an accumulation of either TFIIIC or Tbp1, because their levels on chromatin remained largely unaffected in the absence of Sen1 (Fig 2B and Fig EV2). In the absence of Sen1, condensin did not accumulate either at COC sites (Fig 2A), which recruit TFIIIC but not RNAP3 (Noma *et al*, 2006), consistent with a transcription-mediated effect. To further determine whether the accumulation of condensin was mechanistically linked to the transcription termination defects observed in the absence of

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Sen1, we corrected those defects by strengthening the terminator sequences at two tRNA genes by inserting long polyT sequences, as described previously (Rivosecchi *et al*, 2019). As expected, this strategy was sufficient to correct the accumulation of RNAP3 downstream of the terminator sequences in mitotic cells lacking Sen1 (Fig 2CD, lower panels). Strikingly, this was also sufficient to prevent the accumulation of condensin (Fig 2CD, top panels). These observations show that the increased accumulation of condensin at class III genes in the absence of Sen1 is a direct consequence of RNAP3 transcription termination defects. This is reminiscent of the data showing that to interfere with RNAP2 transcription termination mechanisms also altered the distribution of condensin (Nakazawa *et al*, 2019a) or cohesin (Heinz *et al*, 2018) in -*cis*. As the transcription termination machineries differ for RNAP2 and RNAP3, it seems unlikely that a component of the transcription termination machinery itself is involved in the positioning of condensin in the 3' of genes. Our data suggest instead that intrinsic properties of RNA polymerase molecules undergoing a termination process might explain their impact on the distribution of condensin.

What could be the intrinsic properties of RNAP molecules in the 3' end of genes that impact the position of condensin? We hypothesized that RNAP backtracking could be a contributing factor for two reasons: (i) RNAP molecules are often backtracked around termination sites (Sheridan *et al*, 2019; Lemay *et al*, 2014) and (ii) backtracking would conceivably strengthen the interaction of RNAP molecules with chromatin, making them less dynamic and possibly a harder obstacle to bypass by translocating condensin molecules (Guérin *et al*, 2019; Brandão *et al*, 2019). To test this hypothesis, we sought to prolong RNAP2 backtracking events by over-expressing a dominant-negative mutant of TFIIS (*tfs1D274AE275A* in fission yeast (Lemay *et al*, 2014), thereafter referred to as *tfs1DN*). This strategy was shown to interfere with transcription elongation throughout the gene in different organisms and to alter the distribution of RNAP2 (Zatreanu *et al*, 2019; Sheridan *et al*, 2019; Sigurdsson *et al*, 2010). Upon *tfs1DN* expression in mitotic fission yeast cells, the distribution of RNAP2 was reduced in the 3' and shifted towards the 5' of genes (Fig 3). Remarkably, the over-expression of *tfs1DN* had a similar impact on the distribution of condensin around RNAP2-transcribed genes in mitosis (Fig 3): the accumulation of condensin at the 3' of genes was significantly reduced but its accumulation towards the 5' increased significantly. Overall, condensin became evenly distributed throughout the gene body upon *tfs1DN* over-expression instead of being enriched at the transcription termination site. On the contrary, over-expression of *tfs1DN* had no impact on the association of condensin with the RNAP1-transcribed 18S (Fig EV3). Taken together, these observations are consistent with the idea that RNAP backtracking impacts the distribution of condensin within transcribed genes.

Our observations strongly suggest that the dynamics of elongating RNAP molecules and in particular RNAP backtracking impacts the positioning of condensin on chromosomes. Because backtracking is a prominent feature in the 3' end of genes (Sheridan *et al*, 2019; Lemay *et al*, 2014), this might explain why condensin accumulates particularly over the 3' of transcriptionally active genes in fission yeast (Toselli-Mollereau *et al*, 2016; Sutani *et al*, 2015). In the absence of Sen1, RNAP3 accumulates strongly over and downstream of class III genes (Rivosecchi *et al*, 2019). We speculate that those accumulated RNAP3 molecules are also often backtracked and that the increased levels of condensin downstream of class III genes in the absence of Sen1 depend on the size and the density of the domain occupied by these read-through polymerases. By introducing super-terminator sequences (Fig 2), we reduced the size of this domain and prevented the accumulation of condensin. Both the size and the density of this RNAP3-rich read-through domain would depend on the chromatin context and the transcription rate. This might explain why condensin did not accumulate at all RNAP3-transcribed genes in the absence of Sen1 (Fig 2). Our data are consistent with the observation that the number and density of DNA-bound Rap1 proteins influence condensin function in budding yeast (Guérin *et al*, 2019) and strengthen the idea that arrays of proteins that are tightly-bound to DNA could trigger the accumulation of condensin. Similarly, it is conceivable that a number of tightly-bound proteins (for example transcription factors or paused RNAP2

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molecules) contribute to position condensin complexes in the 5' of genes in mitosis in vertebrates (Sutani *et al*, 2015; Kim *et al*, 2013), even in the absence of significant transcriptional activity. It remains to be determined why tightly-bound proteins lead to the accumulation of condensin: is it that they oppose steric hindrance to the translocation of condensin or that they modify the local physical properties of the chromatin fibre or some properties of condensin itself in a way that would eventually challenge its translocation? Finally, our data confirm one prediction made by the permeable moving barrier model (Brandão *et al*, 2019), that to interfere with transcription elongation affects the distribution of condensin within genes. It would be interesting to determine experimentally by single molecule approaches whether SMC complexes pause for longer when facing backtracked RNAP molecules than when facing elongating, dynamic RNAP.

MATERIAL AND METHODS

Yeast strains. The strains used in this study are listed on Table EV1.

Cell synchronization. Two different methods were used to synchronize fission yeast cells in metaphase. The first method (Fig 1BC and Fig 2CD) used an analogue-sensitive version of the Cyclin-DK Cdc2 (*cdc2-asM17*, (Aoi *et al*, 2014)) which can be inhibited by 2 µM of 3-Br-PP1 (A602985, Toronto Research Chemicals). After 3 hours in the presence of the drug at 28°C in rich medium, 5.10^8 cells were filtered, washed 3 times with warm medium and released in fresh medium without BrPP1. After 10 minutes, ~80% of cells were in mitosis, as judged by the localization of GFP-tagged condensin (Cnd2) in the nucleus. The second synchronization method (Fig 2AB and Fig 3) relies on the inhibition of the expression of Slp1 (Petrova *et al*, 2013), a protein that is key to the metaphase to anaphase transition (Matsumoto, 1997). Cells expressing Slp1 under the control of the thiamine-repressible *nmt41* promoter were grown in minimal medium at 32°C until mid-log phase, when 60 µM of thiamine was added to the culture for 3 hours. Cell synchrony in mitosis was checked as above by the presence of GFP-tagged condensin (Cnd2) in the nucleus.

***Exg1* inversion.** *ura4* was first integrated at the *exg1* locus to generate the *exg1Δ*::ura4+ strain. PCR was then used to fuse the 3' of *exg1* to its 5' domain and its 5' to its 3' domain using the primers exg1 qL2/exg1 RV3 and exg1 qR2/exg1 FW3 (see Table EV2 for a list of the primers used in this study). An overlapping PCR was then used to amplify the whole inverted locus. The resulting 2.8 kb PCR product was then transformed into the *exg1Δ::ura4+* strain and stable integrants were selected by several rounds of FOA selection. The correct integration of the *exg1* gene in the reverse orientation was confirmed by PCR and sequencing.

***tfs1DN* over-expression.** The strains of interest were transformed with the pFB818 plasmid (a generous gift from François Bachand, University of Sherbrooke) that allows the inducible expression of *tfs1-DN* by addition of 7.5 µM of anhydrotetracycline hydrochloride (AhTET; Sigma-Aldrich, 94664), as described in (Lemay *et al*, 2014). AhTET was dissolved in DMSO. Cells were grown in PMG-Leu at 30°C until they reached a concentration of 5.10^6 cells/mL. AhTET or DMSO was added for 3 hours, at which point 60 µM of thiamine was added to repress the expression of Slp1 as above.

Chromatin Immunoprecipitation (ChIP). ChIP was carried out as described previously (Rivosecchi *et al*, 2019), using the primers listed in Table EV2. GFP-tagged proteins were immuno-precipitated with the A11122 antibody (Thermo Fisher Scientific); Myc-tagged proteins were immuno-precipitated with the 9E10 antibody (Merck); Rpb1 was immunoprecipitated using the 8WG16 antibody (Merck).

DATA AVAILABILITY
This study includes no data deposited in external repositories.

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CONFLICT OF INTEREST.

The authors declare that they have no conflict of interest

AUTHOR CONTRIBUTION

Conceptualization: VV; Formal analysis: JR and VV; Funding acquisition: PB and VV; Investigation: JR, LV, FG and VV; Supervision: VV; Writing -original draft: VV; Writing - review&editing: JR, PB and VV.

FIGURE LEGENDS

Figure 1: Distribution of condensin and RNAP2 upon flipping of *exg1*. A. ChIP-seq profiles of condensin (left) and the RNAP subunit Rpb5 (right) around *exg1* in mitotic cells. The ChIP-seq data are indicated by their SRA numbers and were generated in (Kakui *et al*, 2017) and (Sutani *et al*, 2015) respectively. **B.** Cells were synchronized in mitosis and ChIP-qPCR in two different biological replicates was used to determine the distribution of condensin (left) and Rpb1 (right) around *exg1*. **C.** Same as **(B)** when the orientation of *exg1* has been flipped over. The scheme above shows the organization of the chromosome around *exg1* in the wild-type (top) and in the reversed (bottom) orientations. Vertical dotted lines indicate the region of the chromosome that has been flipped over. Grey squares indicate the position of the *exg1* transcription unit. The % IP were normalized using the values given at the site within the gene body indicated by the red vertical dotted line (*exg1#1*). The raw data are shown on Fig EV1.

Figure 2: RNAP3 transcription defects induced by lack of Sen1 trigger the accumulation of condensin. A. Cells were synchronized in metaphase and the association of condensin (Cnd2-GFP) or the heterologous LacI (lacI-GFP) at the indicated loci was investigated by ChIP-qPCR in the presence and in the absence of Sen1 (mean ± std of 4 biological replicates; p-values determined by the test of Wilcoxon Mann-Whitney are indicated above the graph). **B.** The association of the TFIIIC component Sfc6 at the indicated loci was investigated by ChIP-qPCR in cells synchronized in metaphase (mean ± std of 5 biological replicates). **CD.** Distribution of condensin (cnd2-GFP, top) and RNAP3 (rpc37-flag, bottom) around *SPCTRNATHR.10* (**C**) and *SPCTRNAARG.10* (**D**) in mitotic cells, in the presence or not of super-terminator sequences (*thr10-20T* and *arg10-23T* respectively) which correct the transcription termination defects in the absence of Sen1 (Rivosecchi *et al*, 2019) (compare the yellow and red curves). Results are presented as (mean ± std) of 3 (**C**) or 4 (**D**) biological replicates.

Figure 3: The over-expression of *tfs1DN* alters significantly the distribution of condensin around RNAP2-transcribed genes. Cells were synchronized in metaphase and the association of condensin (cnd2-GFP) at the indicated loci was investigated by ChIP-qPCR (mean ± std of 3 biological replicates). Cells carried a plasmid allowing the AhTET-induced over-expression of *tfs1-DN*, as described previously (Lemay *et al*, 2014). DMSO was used as control. For each locus investigated, the normal distribution of condensin and RNAP2 as determined by ChIP-seq is shown above, as published in (Kakui *et al*, 2017) and (Sutani *et al*, 2015) respectively. The data were normalized to the %IP at a site within the gene body indicated by the dotted line. The raw data are shown on Fig EV4.

Figure EV1: **Raw data (not normalized) for the ChIP results presented on Fig 1.**

Figure EV2: Lack of Sen1 does not significantly alter the association of Tbp1 at RNAP3-transcribed genes. Cells were synchronized in metaphase by depleting Slp1 (see Methods). The association of Tbp1 at the indicated loci was investigated by ChIP-qPCR (mean ± std of 4 biological replicates).

Figure EV3: The over-expression of *tfs1DN* does not interfere with the occupancy of condensin at the rDNA. The samples described in Fig 3 were used to monitor the occupancy of condensin within the RNAP1-transcribed 18S transcription unit.

Figure EV4: Raw data (not normalized) for the ChIP results presented on Fig 3.

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REFERENCES

Akai Y, Kurokawa Y, Nakazawa N, Tonami-Murakami Y, Suzuki Y, Yoshimura SH, Iwasaki H, Shiroiwa Y, Nakamura T, Shibata E, *et al* (2011) Opposing role of condensin hinge against replication protein A in mitosis and interphase through promoting DNA annealing. *Open Biol* 1: 110023

Aoi Y, Kawashima SA, Simanis V, Yamamoto M & Sato M (2014) Optimization of the analogue-sensitive Cdc2/Cdk1 mutant by in vivo selection eliminates physiological limitations to its use in cell cycle analysis. *Open Biol* 4: 140063

Banigan EJ, van den Berg AA, Brandão HB, Marko JF & Mirny LA (2020) Chromosome organization by one-sided and two-sided loop extrusion. *eLife* 9: e53558

Banigan EJ & Mirny LA (2020) Loop extrusion: theory meets single-molecule experiments. *Curr Opin Cell Biol* 64: 124–138

Bausch C, Noone S, Henry JM, Gaudenz K, Sanderson B, Seidel C & Gerton JL (2007) Transcription alters chromosomal locations of cohesin in Saccharomyces cerevisiae. *Mol Cell Biol* 27: 8522–8532

Bernard P & Vanoosthuyse V (2015) Does transcription play a role in creating a condensin binding site? *Transcription* 6: 12–16

Brandão HB, Paul P, van den Berg AA, Rudner DZ, Wang X & Mirny LA (2019) RNA polymerases as moving barriers to condensin loop extrusion. *Proc Natl Acad Sci U S A* 116: 20489–20499

Busslinger GA, Stocsits RR, van der Lelij P, Axelsson E, Tedeschi A, Galjart N & Peters J-M (2017) Cohesin is positioned in mammalian genomes by transcription, CTCF and Wapl. *Nature* 544: 503–507

Cheng TMK, Heeger S, Chaleil RAG, Matthews N, Stewart A, Wright J, Lim C, Bates PA & Uhlmann F (2015) A simple biophysical model emulates budding yeast chromosome condensation. *eLife* 4: e05565

Clemente-Blanco A, Mayán-Santos M, Schneider DA, Machín F, Jarmuz A, Tschochner H & Aragón L (2009) Cdc14 inhibits transcription by RNA polymerase I during anaphase. *Nature* 458: 219–222

Cutts EE & Vannini A (2020) Condensin complexes: understanding loop extrusion one conformational change at a time. *Biochem Soc Trans* 48: 2089–2100

Dowen JM, Bilodeau S, Orlando DA, Hübner MR, Abraham BJ, Spector DL & Young RA (2013) Multiple structural maintenance of chromosome complexes at transcriptional regulatory elements. *Stem Cell Rep* 1: 371–378

Ganji M, Shaltiel IA, Bisht S, Kim E, Kalichava A, Haering CH & Dekker C (2018) Real-time imaging of DNA loop extrusion by condensin. *Science* 360: 102–105

Gibcus JH, Samejima K, Goloborodko A, Samejima I, Naumova N, Nuebler J, Kanemaki MT, Xie L, Paulson JR, Earnshaw WC, *et al* (2018) A pathway for mitotic chromosome formation. *Science* 359

Gruber S & Errington J (2009) Recruitment of condensin to replication origin regions by ParB/SpoOJ promotes chromosome segregation in B. subtilis. *Cell* 137: 685–696

Guérin TM, Béneut C, Barinova N, López V, Lazar-Stefanita L, Deshayes A, Thierry A, Koszul R, Dubrana K & Marcand S (2019) Condensin-Mediated Chromosome Folding and Internal Telomeres Drive Dicentric Severing by Cytokinesis. *Mol Cell* 75: 131-144.e3

Hassler M, Shaltiel IA & Haering CH (2018) Towards a Unified Model of SMC Complex Function. *Curr Biol CB* 28: R1266–R1281

Heinz S, Texari L, Hayes MGB, Urbanowski M, Chang MW, Givarkes N, Rialdi A, White KM, Albrecht RA, Pache L, *et al* (2018) Transcription Elongation Can Affect Genome 3D Structure. *Cell* 174: 1522-1536.e22

Hirano T (2016) Condensin-Based Chromosome Organization from Bacteria to Vertebrates. *Cell* 164: 847–857

Hocquet C, Robellet X, Modolo L, Sun X-M, Burny C, Cuylen-Haering S, Toselli E, Clauder-Münster S, Steinmetz L, Haering CH, *et al* (2018) Condensin controls cellular RNA levels through the accurate segregation of chromosomes instead of directly regulating transcription. *eLife* 7: e38517

Iwasaki O, Tanaka A, Tanizawa H, Grewal SIS & Noma K-I (2010) Centromeric localization of dispersed Pol III genes in fission yeast. *Mol Biol Cell* 21: 254–265

Iwasaki O, Tanizawa H, Kim K-D, Yokoyama Y, Corcoran CJ, Tanaka A, Skordalakes E, Showe LC & Noma K-I (2015) Interaction between TBP and Condensin Drives the Organization and Faithful Segregation of Mitotic Chromosomes. *Mol Cell* 59: 755–767

Johzuka K & Horiuchi T (2007) RNA polymerase I transcription obstructs condensin association with 35S rRNA coding regions and can cause contraction of long repeat in Saccharomyces cerevisiae. *Genes Cells Devoted Mol Cell Mech* 12: 759–771

Kakui Y, Rabinowitz A, Barry DJ & Uhlmann F (2017) Condensin-mediated remodeling of the mitotic chromatin landscape in fission yeast. *Nat Genet* 49: 1553–1557

Kim H-S, Mukhopadhyay R, Rothbart SB, Silva AC, Vanoosthuyse V, Radovani E, Kislinger T, Roguev A, Ryan CJ, Xu J, *et al* (2014) Identification of a BET family bromodomain/casein kinase II/TAF-containing complex as a regulator of mitotic condensin function. *Cell Rep* 6: 892–905

Kim JH, Zhang T, Wong NC, Davidson N, Maksimovic J, Oshlack A, Earnshaw WC, Kalitsis P & Hudson DF (2013) Condensin I associates with structural and gene regulatory regions in vertebrate chromosomes. *Nat Commun* 4: 2537

Kim K-D, Tanizawa H, Iwasaki O & Noma K-I (2016) Transcription factors mediate condensin recruitment and global chromosomal organization in fission yeast. *Nat Genet* 48: 1242–1252

Kong M, Cutts EE, Pan D, Beuron F, Kaliyappan T, Xue C, Morris EP, Musacchio A, Vannini A & Greene EC (2020) Human Condensin I and II Drive Extensive ATP-Dependent Compaction of Nucleosome-Bound DNA. *Mol Cell* 79: 99-114.e9

Le TBK, Imakaev MV, Mirny LA & Laub MT (2013) High-resolution mapping of the spatial organization of a bacterial chromosome. *Science* 342: 731–734

Lemay J-F, Larochelle M, Marguerat S, Atkinson S, Bähler J & Bachand F (2014) The RNA exosome promotes transcription termination of backtracked RNA polymerase II. *Nat Struct Mol Biol* 21: 919–926

Lengronne A, Katou Y, Mori S, Yokobayashi S, Kelly GP, Itoh T, Watanabe Y, Shirahige K & Uhlmann F (2004) Cohesin relocation from sites of chromosomal loading to places of convergent transcription. *Nature* 430: 573–578

Matsumoto T (1997) A fission yeast homolog of CDC20/p55CDC/Fizzy is required for recovery from DNA damage and genetically interacts with p34cdc2. *Mol Cell Biol* 17: 742–750

Nakazawa N, Arakawa O & Yanagida M (2019a) Condensin locates at transcriptional termination sites in mitosis, possibly releasing mitotic transcripts. *Open Biol* 9: 190125

Nakazawa N, Arakawa O & Yanagida M (2019b) Condensin facilitates sister chromatid separation in actively transcribed DNA regions by relieving the obstructive effect of transcription. *bioRxiv* doi: 10.1101/644799 [PREPRINT]

Nakazawa N, Nakamura T, Kokubu A, Ebe M, Nagao K & Yanagida M (2008) Dissection of the essential steps for condensin accumulation at kinetochores and rDNAs during fission yeast mitosis. *J Cell Biol* 180: 1115–1131

Nakazawa N, Sajiki K, Xu X, Villar-Briones A, Arakawa O & Yanagida M (2015) RNA pol II transcript abundance controls condensin accumulation at mitotically up-regulated and heat-shock-inducible genes in fission yeast. *Genes Cells Devoted Mol Cell Mech* 20: 481–499

Naumova N, Imakaev M, Fudenberg G, Zhan Y, Lajoie BR, Mirny LA & Dekker J (2013) Organization of the mitotic chromosome. *Science* 342: 948–953

Noma K, Cam HP, Maraia RJ & Grewal SIS (2006) A role for TFIIIC transcription factor complex in genome organization. *Cell* 125: 859–872

Petrova B, Dehler S, Kruitwagen T, Hériché J-K, Miura K & Haering CH (2013) Quantitative analysis of chromosome condensation in fission yeast. *Mol Cell Biol* 33: 984–998

Rivosecchi J, Larochelle M, Teste C, Grenier F, Malapert A, Ricci EP, Bernard P, Bachand F & Vanoosthuyse V (2019) Senataxin homologue Sen1 is required for efficient termination of RNA polymerase III transcription. *EMBO J* 38: e101955

van Ruiten MS & Rowland BD (2018) SMC Complexes: Universal DNA Looping Machines with Distinct Regulators. *Trends Genet TIG* 34: 477–487

Sakai A, Hizume K, Sutani T, Takeyasu K & Yanagida M (2003) Condensin but not cohesin SMC heterodimer induces DNA reannealing through protein-protein assembly. *EMBO J* 22: 2764–2775

Sakai Y, Mochizuki A, Kinoshita K, Hirano T & Tachikawa M (2018) Modeling the functions of condensin in chromosome shaping and segregation. *PLoS Comput Biol* 14: e1006152

Sheridan RM, Fong N, D'Alessandro A & Bentley DL (2019) Widespread Backtracking by RNA Pol II Is a Major Effector of Gene Activation, 5' Pause Release, Termination, and Transcription Elongation Rate. *Mol Cell* 73: 107-118.e4

Sigurdsson S, Dirac-Svejstrup AB & Svejstrup JQ (2010) Evidence that transcript cleavage is essential for RNA polymerase II transcription and cell viability. *Mol Cell* 38: 202–210

Sullivan NL, Marquis KA & Rudner DZ (2009) Recruitment of SMC by ParB-parS organizes the origin region and promotes efficient chromosome segregation. *Cell* 137: 697–707

Sutani T, Sakata T, Nakato R, Masuda K, Ishibashi M, Yamashita D, Suzuki Y, Hirano T, Bando M & Shirahige K (2015) Condensin targets and reduces unwound DNA structures associated with transcription in mitotic chromosome condensation. *Nat Commun* 6: 7815

Sutani T & Yanagida M (1997) DNA renaturation activity of the SMC complex implicated in chromosome condensation. *Nature* 388: 798–801

Toselli-Mollereau E, Robellet X, Fauque L, Lemaire S, Schiklenk C, Klein C, Hocquet C, Legros P, N'Guyen L, Mouillard L, *et al* (2016) Nucleosome eviction in mitosis assists condensin loading and chromosome condensation. *EMBO J* 35: 1565–1581

Tran NT, Laub MT & Le TBK (2017) SMC Progressively Aligns Chromosomal Arms in Caulobacter crescentus but Is Antagonized by Convergent Transcription. *Cell Rep* 20: 2057–2071

Uhlmann F (2016) SMC complexes: from DNA to chromosomes. *Nat Rev Mol Cell Biol* 17: 399–412

Vanoosthuyse V, Legros P, van der Sar SJA, Yvert G, Toda K, Le Bihan T, Watanabe Y, Hardwick K & Bernard P (2014) CPF-associated phosphatase activity opposes condensin-mediated chromosome condensation. *PLoS Genet* 10: e1004415
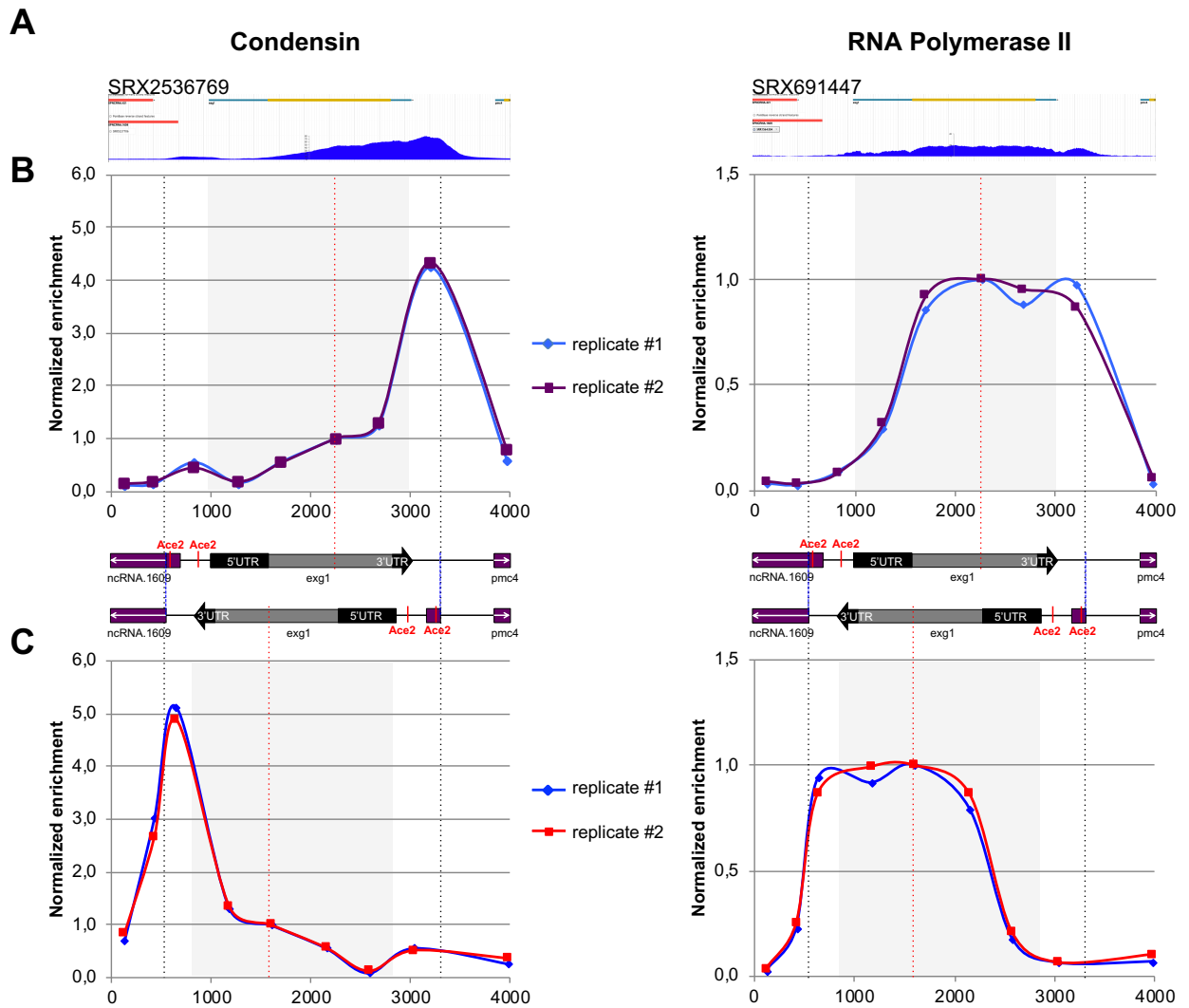
Vazquez Nunez R, Ruiz Avila LB & Gruber S (2019) Transient DNA Occupancy of the SMC Interarm Space in Prokaryotic Condensin. *Mol Cell* 75: 209-223.e6

Wang X, Brandão HB, Le TBK, Laub MT & Rudner DZ (2017) Bacillus subtilis SMC complexes juxtapose chromosome arms as they travel from origin to terminus. *Science* 355: 524–527
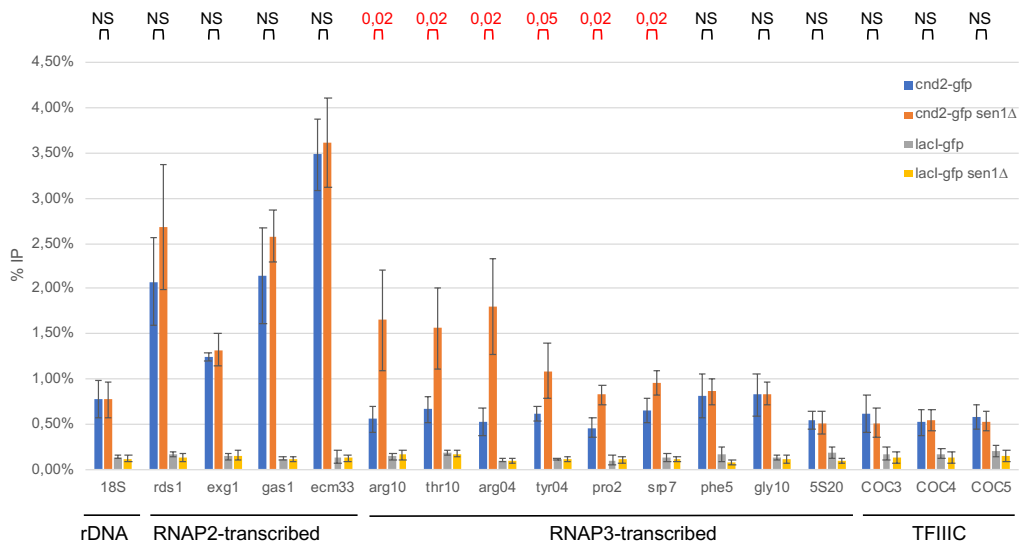
Wang X, Le TBK, Lajoie BR, Dekker J, Laub MT & Rudner DZ (2015) Condensin promotes the juxtaposition of DNA flanking its loading site in Bacillus subtilis. *Genes Dev* 29: 1661–1675

Yanagida M (2009) Clearing the way for mitosis: is cohesin a target? *Nat Rev Mol Cell Biol* 10: 489–496

Zatreanu D, Han Z, Mitter R, Tumini E, Williams H, Gregersen L, Dirac-Svejstrup AB, Roma S, Stewart A, Aguilera A, *et al* (2019) Elongation Factor TFIIS Prevents Transcription Stress and R-Loop Accumulation to Maintain Genome Stability. *Mol Cell* 76: 57-69.e9
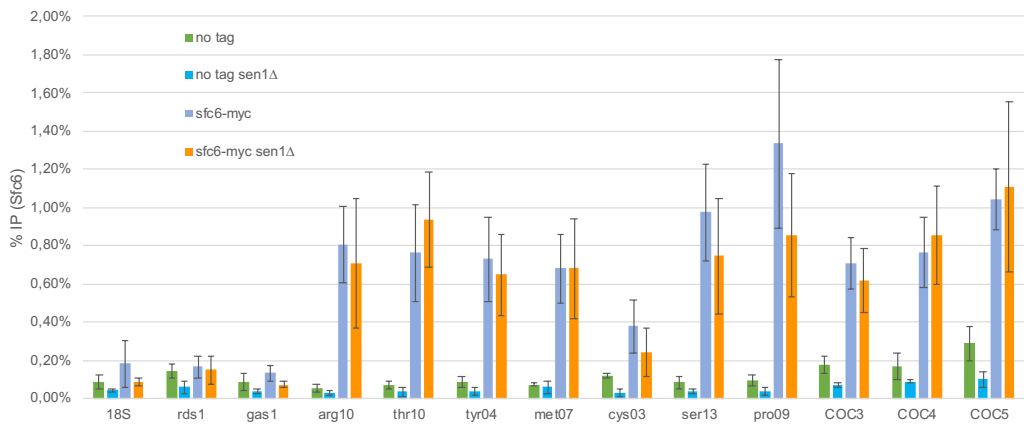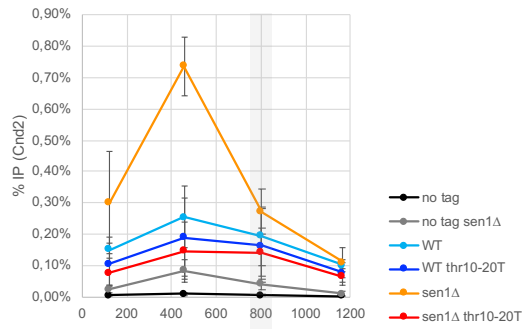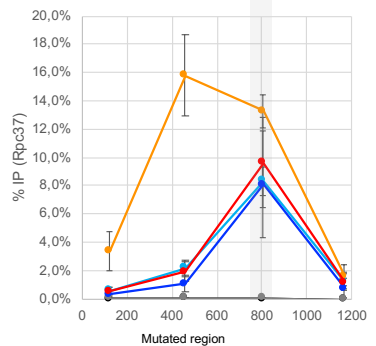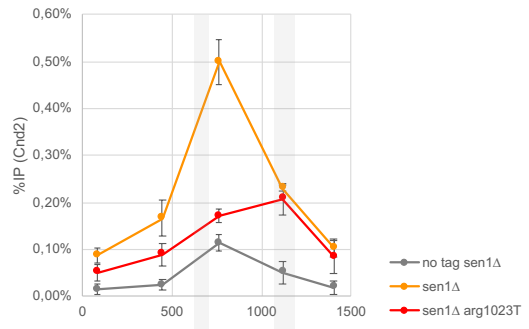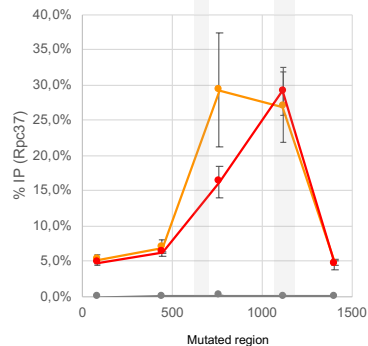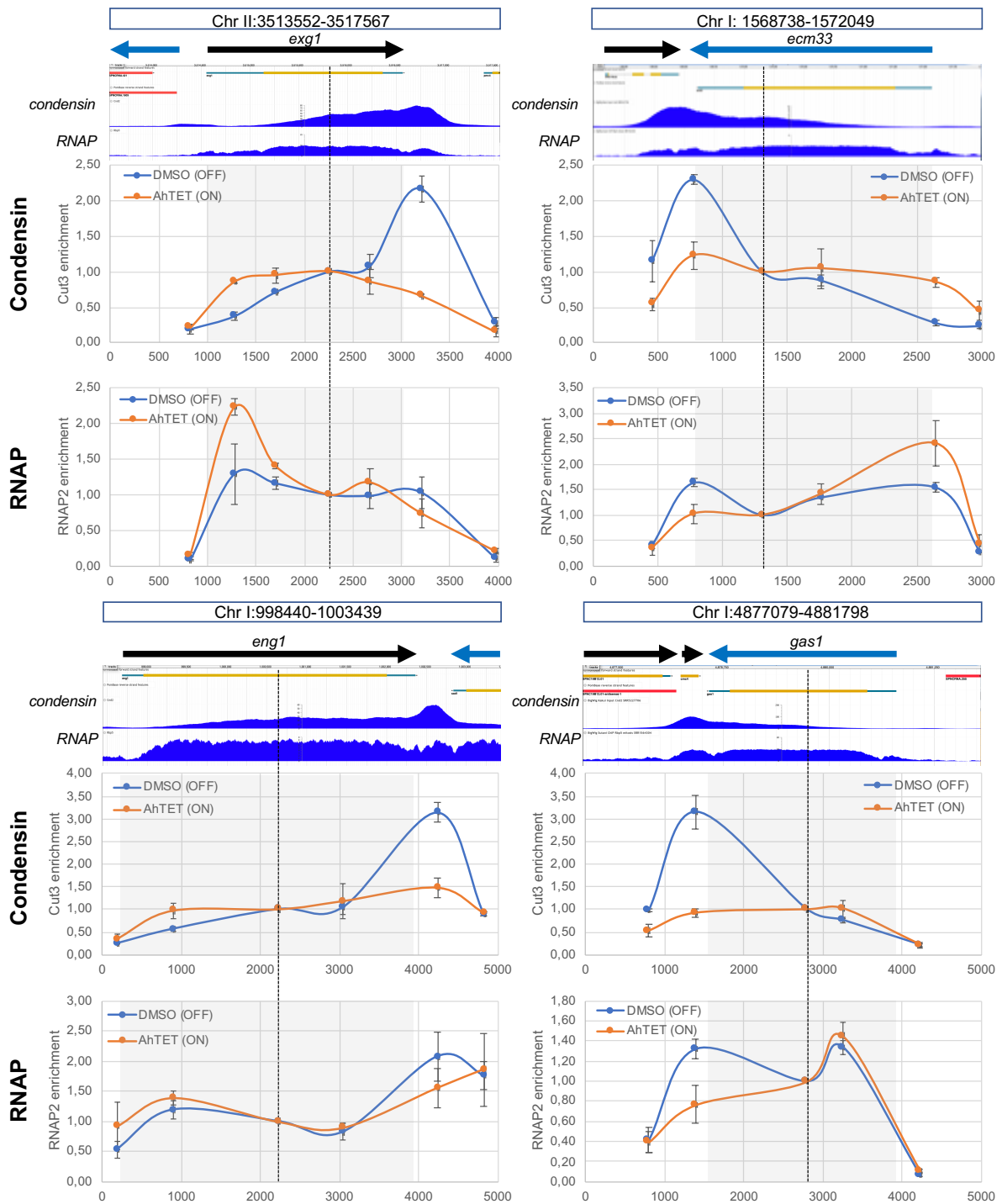
Raw data for Figure 1

ChIP condensin

Legend: no tag_#1, no tag_#2, cnd2-GFP_#1, cnd2-GFP_#2

ChIP RNAP2

Legend: no tag_#1, no tag_#2, cnd2-GFP_#1, cnd2-GFP_#2

ChIP condensin

Legend: cnd2-GFP_#1, cnd2-GFP_#2

ChIP RNAP2

Legend: cnd2-GFP_#1, cnd2-GFP_#2

ChIP Tbp1
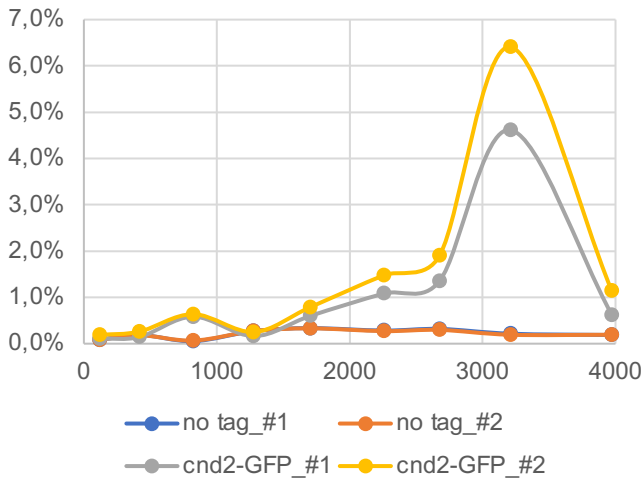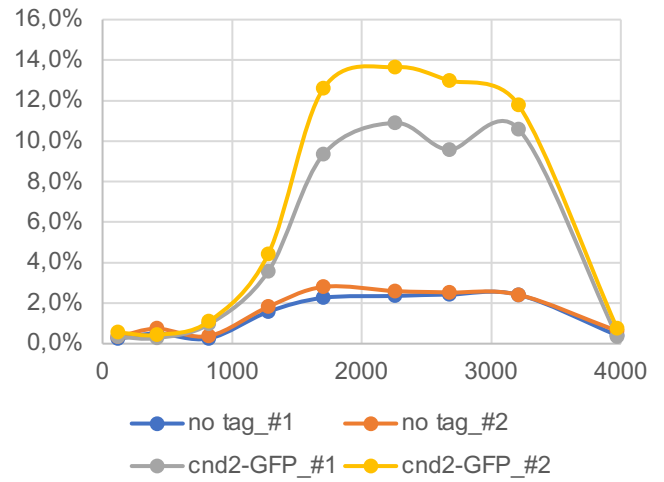
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Condensin
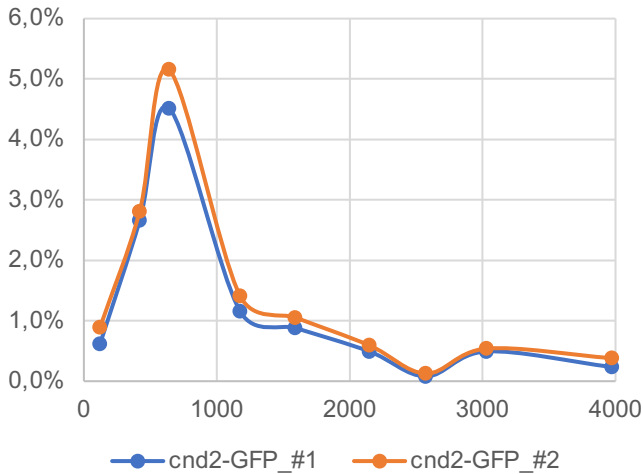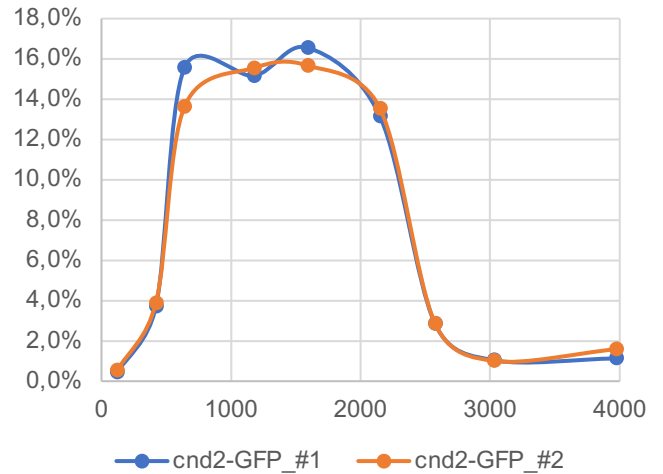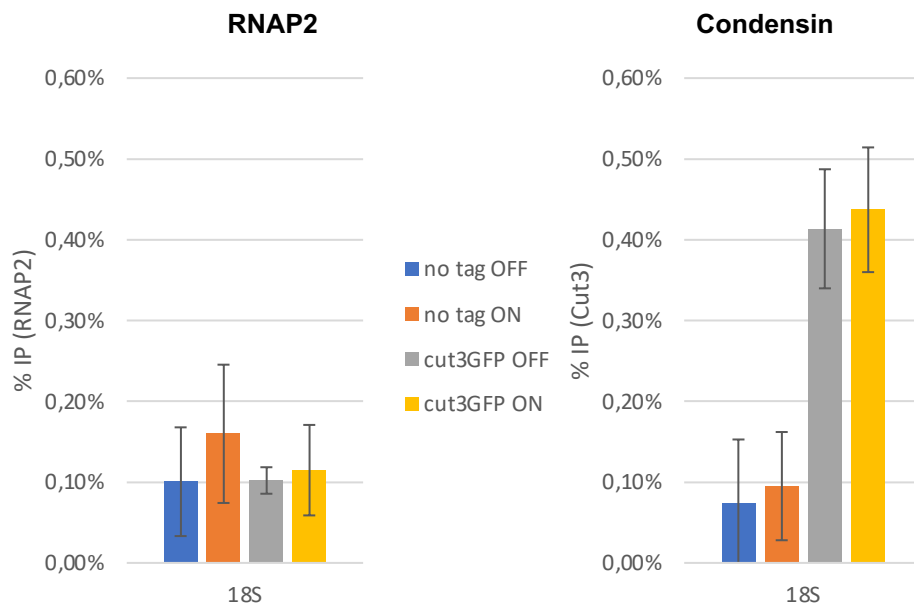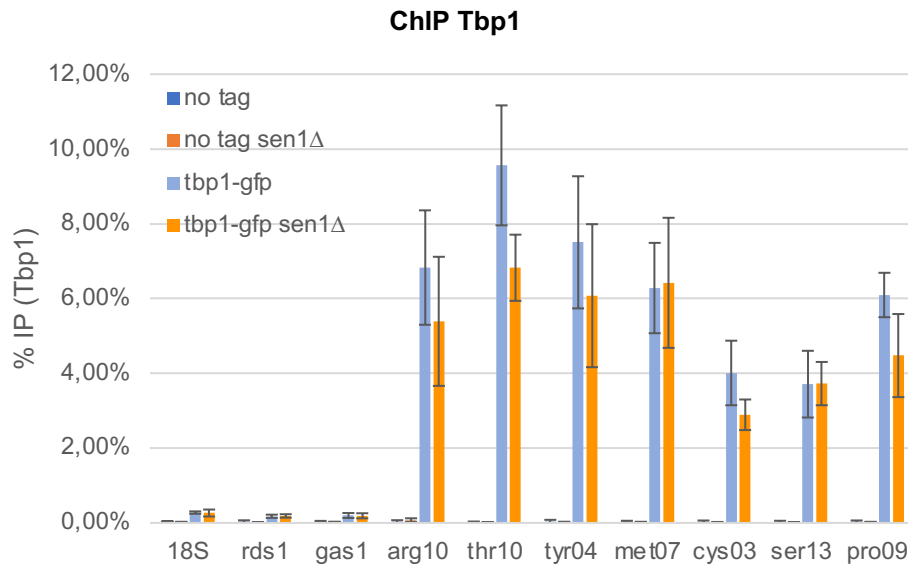
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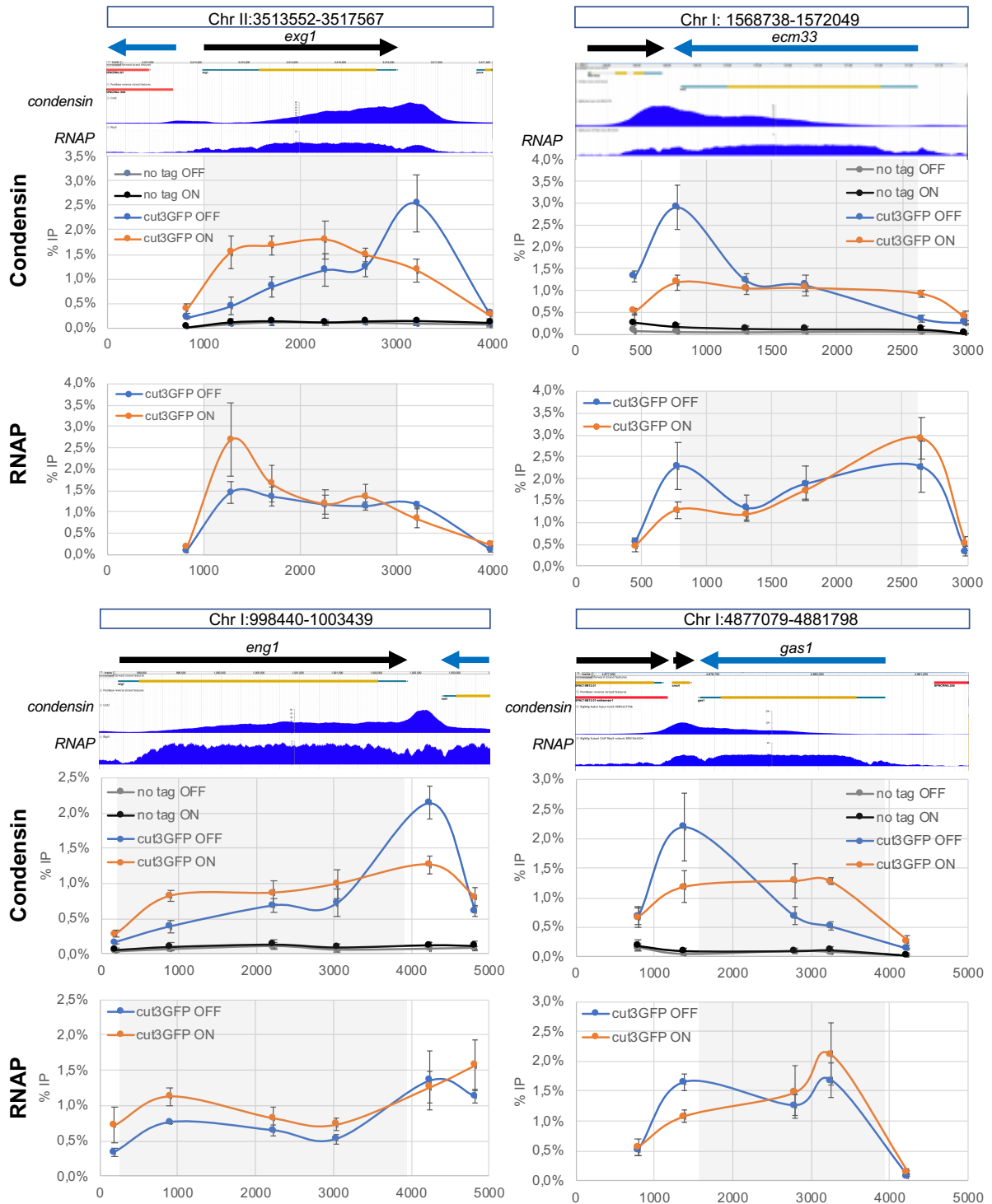
Table EV1: strains used in this study

LY112	h+	leu1-32 ura4D18 ade6-210	
LY4232	h-		KanR-Pnmt41-slp1+
LY4233	h-		KanR-Pnmt41-slp1+ sen1Δ::HygroR
LY4234	h-		KanR-Pnmt41-slp1+ cnd2-GFP-LEU2
LY4237	h+		KanR-Pnmt41-slp1+ sen1Δ::HygroR cnd2-GFP-LEU2
LY4339	h-	leu1-32	KanR-Pnmt41-slp1+ cut3-GFP-ura4+
LY4340	h+	leu1-32	KanR-Pnmt41-slp1+
LY4483	h-	leu1-32 ura4D	KanR-Pnmt41-slp1+ cnd2-GFP-LEU2
LY4488	h?		KanR-Pnmt41-slp1+ sen1Δ::HygroR cnd2-GFP-LEU2
LY4681	h+	leu1-32 ura4D18	cdc2asM17
LY4682	h-	leu1-32 ura4D18	cdc2asM17
LY4731	h-	leu1-32 ura4D	cdc2asM17 cnd2-GFP-LEU2
LY4732	h+	leu1-32 ura4D	cdc2asM17 cnd2-GFP-LEU2
LY4781	h-		cdc2asM17 sen1Δ::KanR
LY4979	h+	leu1-32 ade6-704	cdc2asM17 cnd2-GFP-LEU2 rpc37-3flag-NatR
LY4980	h-	leu1-32 ade6-704	cdc2asM17 cnd2-GFP-LEU2 rpc37-3flag-NatR
LY4981	h+	leu1-32 ade6-704	cdc2asM17 cnd2-GFP-LEU2 rpc37-3flag-NatR sen1Δ::KanR
LY4982	h-	leu1-32 ade6-704	cdc2asM17 cnd2-GFP-LEU2 rpc37-3flag-NatR sen1Δ::KanR
LY5034	h?	leu1-32 ura4- ade6-	his7::LacI-GFP ura4-Pnmt41-slp1+
LY5035	h-	leu1-32 ura4- ade6-	his7::LacI-GFP ura4-Pnmt41-slp1+ sen1Δ::KanR
LY5036	h?	leu1-32 ura4- ade6-	his7::LacI-GFP ura4-Pnmt41-slp1+ nda3-KM311
LY5038	h?	leu1-32 ura4- ade6-	his7::LacI-GFP ura4-Pnmt41-slp1+ nda3-KM311 sen1Δ::KanR
LY5609	h-	leu1-32	KanR-Pnmt41-slp1+
LY5610	h-	leu1-32	KanR-Pnmt41-slp1+ sen1D::HygroR
LY5615	h+	leu1-32	KanR-Pnmt41-slp1+ sfc6-13myc-KanR
LY5616	h+	leu1-32	KanR-Pnmt41-slp1+ sfc6-13myc-KanR
LY5617	h+	leu1-32	KanR-Pnmt41-slp1+ sfc6-13myc-KanR sen1Δ::HygroR
LY5618	h+	leu1-32	KanR-Pnmt41-slp1+ sfc6-13myc-KanR sen1Δ::HygroR
LY5764	h+	leu1-32 ura4D ade6-210/704?	cdc2-asM17 arg10-23T sen1Δ::KanR cnd2-GFP-LEU2 rpc37-3flag-NatR
LY5765	h-	leu1-32 ura4D ade6-210/704?	cdc2-asM17 arg10-23T sen1Δ::KanR cnd2-GFP-LEU2 rpc37-3flag-NatR
LY6201	h-	leu1-32 ura4D ade6-210/704	cdc2-asM17 thr10-20T sen1Δ::KanR cnd2-GFP-LEU2 rpc37-3flag-NatR
LY6203	h+	leu1-32 ura4D ade6-210/704	cdc2-asM17 thr10-20T sen1Δ::KanR cnd2-GFP-LEU2 rpc37-3flag-NatR
LY6215	h-	leu1-32 ura4D ade6-210/704	cdc2-asM17 thr10-20T cnd2-GFP-LEU2 rpc37-3flag-NatR
LY6585	h+	leu1-32 ura4D ade6-210	cdc2-asM17 inverted-exg1 cnd2-GFP-LEU2
LY6586	h-	leu1-32 ura4D ade6-210	cdc2-asM17 inverted-exg1 cnd2-GFP-LEU2

Table EV2: primers used in this study

Figure 1	
exg1qL1	GACGGTAAATGAGCCTTTGG
exg1qR1	AGCTGGAAAGAGGATTGACG
exg1qL2	CGCGGTAAGCCACAATAAAC
exg1qR2	CTGCTTGGATTTGCGTACTG
exg1 qL3	CACATAGACGGACCACTTTGAG
exg1 qR3	ATATGTCACCTGTGGCTGAGTG
Exg1qL4	CGAAATAACCCCGTTTTTGAC
Exg1qR4	AATTTTCCAGCACCAACCAG
Exg1qL5	GGGAGCTGTTCGTCTGTTTATC
Exg1qR5	GACTTGGTCCACCAGTTTTAGC
Exg1 qL6	GGAGAAAGTTGACCGAGAAATC
Exg1 qR6	CTCAAAGTGGTCCGTCTATGTG
Exg1 qL7	ATGCTCCCCAATGGAATATC
Exg1 qL8	ACGACTCGGTAAATGCTACCTC
Exg1 qR8	AAGGTTTGTCGCGCTAAGTC
Exg1_Del_FW3	GCATACTCTAGAGCGCTCCGTGAGTTGAGAAATTGGTATAAAGATACTCATGCCCAGTTCCTTTGTTCCATTGTTTAATTCAAAGACGctactcagcattctttctct
Exg1_Del_RV3	AATGTCTAAAGATAATGCCAAACAAACAAAGACTGAATGGATAGTAGATAGCAGCACGTTAAATGATAGTAGATGATAAATGCAAGGACtatcttgtttgtctacatgg
INV_EXG1_FW3	ATTACTTTACCAAGAAATCGCTCAATTTAAATGTAATTTTTATAAAGTATTTTAAACATATGTCACCTGTGGCTGAGTGGTAAACATTACCAAATTCATTACCG
INV_EXG1_RV3	ATGAAGTAAATAAAAGGGTTGATATCGTTAAATTGGTAAGTCACATGTTGGTTTCCGACATTGAGTTGAGAAGAATCATAAAACCTATTCTGTCGATTTGC

Figure 2	
18sqL1	TTTCTAGGACCGCCGTAATG
18sqR1	TGCTTTCGCAGTAGTTCGTC
rds1s-2qL1	TCTGGCTCCTCGTCATTTTC
rds1s-2qR1	TCATCGCTTCCAACCCTTAC
exg1qL1	GACGGTAAATGAGCCTTTGG
exg1qR1	AGCTGGAAAGAGGATTGACG
gas1qL3	AATAGCATGTCGAGGTTGTATGG
gas1qR3	TGTCATCGCGAAACCTTACC
ecm33qL4	TTGGCAAAGATGAGACATAAGG
ecm33qR4	AATAAACTCGGTAGTCTTGCAATC
Arg10 qL1	GGTGTGTAGCCTAATGGTTAAGG
Arg10 qR1	GAGTGTGACAGGACTCGAACC
Arg10 qL2	GGAAGGATACAATATCCACAACG
Arg10 qR2	GCTGTTATCCATCCACTTACGG

Arg10 qR3	GGCATTCGTCGATTTTGC
Arg10 qL4	AAGCCGCCTTTCGTTAACAC
Arg10 qR4	CTGCTTGACCAGCTTTTGTG
Arg10 qL5	ACCAAACTGCCCGATACAAC
Arg10 qR5	GCAAACAGAGTCCAATTGAGG
Thr10 qL1	CAATCAACGTTGCCCCTATG
Thr10 qR1	ATACAAATTGCCCCCACTCG
Thr10qR3	TCATGAGGGACGTGGTTTAG
Thr10 qL4	AGTCACGGACTTGGTCTTTACC
Thr10 qR4	TTCTTTGCACCCCATAGCAC
Thr10 qL5	AATGAGATTCATCCAGCGTTC
Thr10 qR5	GCAGGTACCGTAATTAGCCTTC
Thr10 qL6	GTGGGATGTACTAAACCACGTC
Thr10 qR6	ACGGATGACAGTAAAAGGAATG
Thr10 qL6	GTGGGATGTACTAAACCACGTC
Thr10 qR6	ACGGATGACAGTAAAAGGAATG
arg4qL1	CATTAATCCGCCGTGGATAG
arg4qR1	TTCACCTAATAGTTGCCAAACG
tyr4.2_FW	TGGTGTAGTTGGTTATCACATCC
tyr4.2_RV1	AATCTCCTGAGCCAGAATCG
tyr4qL2	TTGCCCTTGCATCCTATCTC
tyr4qR2	TGAATTTAGCACGTTTCTCTCAA
Pro.02 qL2	CTGGCAGCTAATGAGCAAATC
Pro.02 qR2	CGGTGTAATGCCTTTGCTAC
srp7qL1	TACCGATGGAGGTTGGAAAC
srp7qR1	ACATCCTGCGAAGGTGAATC
Phe05 qL1	TCGACTTCCCAAGATTCTGC
Phe05 qR1	CGTGCAATTGCTCAAGTAGG
gly10qL1	AGGGAAACCTGAAACTAACTGG
gly10qR1	GAGAAATACGGGCTAACAGTGG
5S 20 qL1	ATGGAATGCTTGGGTGATG
5S 20 qR1	AATGGTGTGACGCGAAATC
COC3qL	CGAATCGCCCTTATGGATTC
COC3qR	ATGCTACATCCCGATGATCC
COC4qL	TGTCGATATTAGACCGAGCAAC
COC4qR	TTCGAACCCACAGACTTTGC
COC5qL	CCGAATTGACGCTAGTCTCC
COC5qR	TTGTTGCTACCAGCGTATCC
c417qL1	AGGTTCAAATCCTGCTGGTG
c417qR1	TGGGACCTACGGGTTATGAG
cys03 qL1	TACTACTGATGCGGCTTTGC
cys03 qR1	TGTAGCCAAGCGAGAAATCG
Ser13 qL2	CGAGTGGTTTTAAGGCGTTC
Ser13 qR2	TCGACAACGGCAGGATTC
pro9 qL2	GCCGTTTGGTCTAGTGGTATG
pro9 qR2	TTGGGCTGTTGTGGGAATC

Figure 3	
exg1qL1	GACGGTAAATGAGCCTTTGG
exg1qR1	AGCTGGAAAGAGGATTGACG
exg1qL2	CGCGGTAAGCCACAATAAAC
exg1qR2	CTGCTTGGATTTGCGTACTG
exg1 qL3	CACATAGACGGACCACTTTGAG
exg1 qR3	ATATGTCACCTGTGGCTGAGTG
Exg1qL4	CGAAATAACCCCGTTTTTGAC
Exg1qR4	AATTTTCCAGCACCAACCAG

Exg1qL5	GGGAGCTGTTCGTCTGTTTATC
Exg1qR5	GACTTGGTCCACCAGTTTTAGC
Exg1 qL6	GGAGAAAGTTGACCGAGAAATC
Exg1 qR6	CTCAAAGTGGTCCGTCTATGTG
Exg1 qL7	ATGCTCCCCAATGGAATATC
Exg1 qL8	ACGACTCGGTAAATGCTACCTC
Exg1 qR8	AAGGTTTGTCGCGCTAAGTC
ecm33qL1	CAAAGTTTGGTCGCAAGCTC
ecm33qR1	AGGTGCGCAATTTCGTTG
ecm33qL2	TTCGGTTCCTTACCTTACCG
ecm33qR2	AACGAAACCACCACCAACAG
ecm33qL3	TCAGCTGCACGTTGTTTAGC
ecm33qR3	AGAAACAAGCCGGAGATCCTAC
Ecm33qL8	TGTGGTCAATCTCTGTCTTTTTG
Emc33qR8	TGCAAAAGGGAATATGAAGG
gas1qL1	ACGACAGAATTGCCACGTTC
gas1qR1	CGTTTTGGGGGAATACAGTG
gas1qL2	CCGTTCGTGACGTTAAATCC
gas1qR2	CATCATCACCGCAGTCAAAG
gas1qL3	AATAGCATGTCGAGGTTGTATGG
gas1qR3	TGTCATCGCGAAACCTTACC
gas1 qL4	TTCCAATCCTTAGCGACCAC
gas1 qR4	TGTTGAGGGAGCTTTGGAAC
gas1 qL5	AGCCTCAGTTTCTCCTGTTCAC
gas1 qR5	CCATCAGAAAGAGGATCAACG
eng1 qL4	GGGCATCTGGTTTGTTTGAG
eng1 qR4	GACCCCAAAGTTTCATACCG
eng1 qL5	ATATGGCTGCGACAATGGAG
eng1 qR5	TTGTGGGAGTAGGCGTAATG
eng1 qL6	CGAACTCAACGATGCAAAAC
eng1 qR6	CCTTAAGACTCTGCGTTCGAG
Eng1qL7	TGATGGCTGTCCAAGTTTCC
Eng1qR7	GGTTTCAAGATTTCCCGATTG
Eng1qL8	TGGTGGCAGTCTTCAGACAC
Eng1qR8	GAGCGAACAAATACCGATGG
sod1 qL3	GCGTGGTTTCCATATTCACC
sod1 qR3	GCATCGCTTTCCAAGTTACC