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Detecting chromatin interactions between and along sister chromatids with SisterC

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Abstract

Chromosome segregation requires both compaction and disentanglement of sister chromatids. We describe SisterC, a chromosome conformation capture assay that distinguishes interactions between and along identical sister chromatids. SisterC employs BrdU incorporation during S-phase to label newly replicated strands, followed by Hi-C and then the destruction of BrdU-containing strands by UV/Hoechst treatment. After sequencing of the remaining intact strands, this allows for assignment of Hi-C products as inter- and intra-sister interactions based on the strands that reads are mapped to. We performed SisterC on mitotic *S. cerevisiae* cells. We find precise alignment of sister chromatids at centromeres. Along arms, sister chromatids are less precisely aligned with inter-sister connections every ~35kb. Inter-sister interactions occur between cohesin binding sites that often are offset by 5 to 25kb. Along sister chromatids, cohesin forms loops of up to 50kb. SisterC allows study of the complex interplay between sister chromatid compaction and their segregation during mitosis.

Introduction

During S-phase, when sister chromatids are formed, they are held together by cohesin. During the subsequent mitosis, sister chromatids become compacted and, in the process, become disentangled from each other, although they remain aligned side by side[1]. This process has been studied using microscopy by labeling sister chromatids differently using thymidine analogues[2,3]. It has been difficult to study relationships between sister chromatids using genomic techniques such as Hi-C, as sequencing-based methods cannot distinguish the

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Author contributions

M.E.O. and J.D. conceived and designed the project. M.E.O. cultured yeast, generated and analyzed SisterC and Hi-C datasets and performed flow cytometry experiments. A.K.H. and J.K.W. designed HPLC and mass spectrometry experiments, A.K.H. performed and analyzed HPLC and MS experiments. M.E.O and J.D. wrote the manuscript.

Ethics Declaration

All authors declare no conflict of interest.

identical sister chromatids, and therefore cannot differentiate interactions between and along sister chromatids. Recently an assay detecting sister chromatid exchange events allowed mapping of sister chromatid interactions genome wide in bacteria[4]. However, this approach requires extensive genome editing to introduce sister chromatid exchange markers throughout the genome.

Here we present a Hi-C-based assay, SisterC that can distinguish inter- and intra-sister interactions in mitotic budding yeast cells. In *S. cerevisiae* the cohesin complex mediates inter-sister interactions at the centromere and along the chromosome arms ("cohesive cohesin")[5,6]. In addition, the cohesin complex compacts the sister chromatid arms by forming intra-sister loops by dynamic loop extrusion ("extruding cohesin")[7–11]. SisterC reveals the alignment of sister chromatids, the positioning of inter-sister interactions and intra-sister loops and how the interplay between cohesive and extruding cohesin shapes the mitotic chromosome in yeast.

Results

SisterC library production after induction of single strand breaks at BrdU incorporation sites

Sister chromatids are identical in sequence but differ in which strand was newly replicated in S-phase. This difference can be leveraged to differentiate interactions between and within sister chromatids. BrdU containing DNA strands can be selectively degraded after Hoechst treatment and radiation with UV[3,12]. This property has been used before in Strand-seq[13], which allows the detection of sister chromatid exchange events[14,15], and the mapping of structural genomic variants such as polymorphic inversions[16] onto completely phased human genomes[17]. Here we describe SisterC, a method that combines Hi-C[18,19] on BrdU incorporated DNA and UV/Hoechst treatment to distinguish interactions between sister chromatids (inter-sister interactions) and along sister chromatids (intra-sister interactions). SisterC works as follows (figure 1a–c). When cells go through S-phase in the presence of BrdU, this results in cohesed pairs of sister DNA molecules where one molecule contains BrdU in the − strand (assigned as sister A), and the other molecule contains BrdU in the + strand (sister B) (figure 1a). Hi-C ligation of crosslinked and digested fragments of these DNA molecules results in 16 possible ligation products between and within sister A and B that differ in the orientation of ligated fragments and the strand or strands that contain BrdU (figure 1b–c). Treatment of ligation products with Hoechst and UV light creates nicks in strands containing BrdU. Upon PCR amplification, this results in specific depletion of Hi-C products for which BrdU was present in both the + and − strands as for these products no intact template strand remains. Only 8 possible ligation products will still have one completely intact strand after UV irradiation, and these can therefore be PCR amplified (figure 1c). Four of these amplifiable ligation products will be interactions between sister chromatids and four products will be interactions along a sister chromatid, but they differ in fragment orientation. Fragment orientations can be assigned after paired-end sequencing and mapping of genomic locations to the + or – strands. The orientation of SisterC fragments can then be used to differentiate interactions between and along sister chromatids: + + or − −

read orientations represent inter-sister interactions, while + − or − + orientations represent intra-sister interactions.

Here we studied mitotic sister chromatid interactions in *S. cerevisiae* using SisterC. Budding yeast has a relatively small and haploid genome. Furthermore, it can be synchronized in both late G1 and mitosis, which allows incorporation of BrdU for one S-phase. Lastly, its mappable centromeres and known cohesin binding sites along arms offer sites of particular interest, as these are sites at which sister chromatids are connected and intra-sister chromatid loops may form. As wild-type *S. cerevisiae* cells do not take up nucleosides from the environment, we use a strain that expresses human equilibrative nucleoside transporter (hENT) and *Drosophila* deoxyribonucleoside kinase DmdNK, which allows cells to take up BrdU from the environment and incorporate it into their DNA[14,20]. Cells were synchronized in late G1 using alpha factor, the culture was split and released in media containing either BrdU or thymidine. This was followed by nocodazole treatment to obtain cells arrested in mitosis or was followed by a second alpha factor incubation to obtain cells arrested in the subsequent G1 (extended data figure 1).

To investigate the efficiency of SisterC, we performed several control experiments. First, to determine the amount of BrdU incorporation required for efficient Hoechst/UV-induced strand destruction, we PCR amplified DNA fragments using a range of dTTP to BrdUTP ratios, resulting in products with BrdU incorporated in both strands. After treatment with Hoechst and UV radiation, we amplified these products again (extended data figure 2). This showed that amplification efficiency of DNA fragments containing more than 10 to 50% BrdU after treatment with UV and Hoechst is greatly reduced, indicating that template strands were successfully broken. Second, we performed flow cytometry to detect BrdU incorporation and cell cycle profile of the synchronized yeast cultures (extended data figure 1b and supplemental figure 1). We observe proper cell synchronization in mitosis and late G1 and uniform BrdU incorporation across the cell population. Third, we directly measured the BrdU incorporation efficiency by determining the base composition of genomic DNA using HPLC (figure 1d and supplemental figure 2). We identified peaks in the HPLC spectra by mass spectrometry (supplemental figure 3). After adjustment of the peak area in the spectra by each extinction coefficient (supplemental figure 4), we find that 82.7 up to 98.1% of all Ts in the newly replicated strand are replaced by BrdU (table 1). Lastly, we estimated the efficiency of selective depletion of Hi-C ligation products as result of UV/Hoechst treatment by producing SisterC libraries for cells synchronized in late G1 after BrdU incorporation during the previous S-phase. Using these cells to perform SisterC, we were able to estimate the percentage of false inter-sister interactions as these should no longer be present in a G1 SisterC library. Although we do not detect full depletion of all inter-sister interactions in G1 libraries, we do see a depletion down to approximately 20% (table 1 and supplemental table 1). In contrast, in mitotic SisterC libraries we typically see around 35–40% captured inter-sister interactions (table 1 and supplemental table 1), supporting that in SisterC inter-sister interactions are enriched in pairs with − − and + + read orientation and intra-sister interactions are enriched in pairs with − + and + − read orientation.

SisterC allows observation of interactions between and along sister chromatids

We carried out three biological replicates of SisterC experiments using DpnII with highly concordant results and one SisterC experiment using HindIII (extended data figure 3 and supplemental table 1). After mapping and standard Hi-C data processing (see methods), we examined the SisterC interaction frequency (P) as a function of genomic distance (s) separated by read orientation (figure 2a–b and extended data figure 4a–b). Here the definition of genomic distance for interactions between sister chromatids is the difference between their respective genomic coordinates, even though this involves two different DNA molecules. We compared the results to Hi-C controls (extended data figure 4c–d) and SisterC negative controls of cells cultured in thymidine instead of BrdU (extended data figure 4e–h). As expected, read orientation of interactions between loci separated by less than 1500bp are influenced by technical artifacts, such as unligated dangling ends (+ − orientation) and self-ligated fragments (− + orientation)[21]. Therefore, we exclude any interactions between loci separated by less than 1500bp in all our analyses (extended data figure 4a–b). In the G1 SisterC libraries (figure 2a), we see a clear depletion in $P_{inter}(s)$ of inter-sister interactions as expected, as there are no longer sister chromatids in G1.

We find a very different $P_{inter}(s)$ for inter-sister interactions in mitosis (figure 2b). Inter-sister interactions are less frequent than intra-sister interactions for loci separated by short genomic distances (below 30kb). $P(s)$ for inter-sister and intra-sister interactions converge for distances larger than 35kb. This describes two phenomena. First, sister chromatids are not perfectly aligned, as that would result in minimal differences in $P(s)$ between inter-sister and intra-sister interactions (figure 2c, top). Instead interactions with genomic distance below 35kb occur more frequently within the same sister than between sisters, suggesting sister chromatids are loosely aligned (figure 2c, bottom left). Second, interaction frequencies of inter-sister and intra-sister interactions between loci separated by more than 35kb distance converge (figure 2c, bottom model, right panel). Together, this suggests that although the sister chromatids are not perfectly aligned, sisters are held together mediated by inter-sister interactions that are spaced every 35kb on average (figure 2c, bottom panel).

When mitotic SisterC data are visualized as an interaction heatmap, we observe similar characteristics as seen in $P_{inter}(s)$ (figure 2d). Inter-sister interactions show a weaker interaction signal around the diagonal compared to intra-sister interactions, while at larger distances this difference is no longer detectable. However, zooming in, we observe features that appear stronger in either the inter-sister heatmap or the intra-sister heatmap (figure 2e). In the combined SisterC interaction map (the sum of inter- and intra-sister interactions, figure 2e left panel), we observe dots that represent interactions between cohesin binding sites as detected by ChIP-seq[22]. When inter-sister or intra-sister interactions are plotted separately, we observe that some of these interactions are more prominent in the inter-sister dataset (black arrow, figure 2e), while others are more prominent in the intra-sister dataset (green arrow, figure 2e). This difference is further highlighted after correction for the distance dependent expected interaction frequency (extended data figure 5). The finding that some of these interactions between cohesin binding sites are detected in both inter- and intra-sister datasets, albeit with different frequencies, could be related to the fact that Hoechst/UV depletion is not complete. Perhaps more interestingly, this could also be due to

variability in the population in positioning of inter- and intra-sister interactions. Where in one cell a given cohesin site interacts with a second cohesin site along the same chromatid to mediate an intra-sister interaction, in another cell this cohesin site interacts with the same second cohesin site but located on the other sister chromatid to form an inter-sister interaction.

Sister chromatid interactions at centromeres

We set out to explore SisterC data around centromeres as we expect enrichment of inter-sister interactions at these sites. Centromeres display very prominent binding of both cohesin and condensin, where they mediate inter-sister interactions and possibly intra-sister interactions[7]. When we aggregate all inter- and intra-sister interactions combined around all 16 centromeres (figure 3a), we observe a striking pattern. Regions directly adjacent on either side of the centromere interact frequently with sequences up to 10 to 15kb away from the centromere. When interactions between sisters and interactions along the sisters are plotted separately (figure 3b–c), we find several differences. First, intra-sister interactions are depleted in a 4kb window centered precisely at the centromere. Second, we observe that, compared to the genome-wide average expected level, inter-sister interactions at centromeric regions are enriched up to 10kb away from the diagonal (figure 3b and 3d). Third, both intra- and inter-sister interactions contribute to the line-like features emanating from the centromere in the Hi-C maps. Line-like features have been interpreted as dynamic or variable loops with one fixed anchor and with the second anchor at various distances away[23] (figure 3e). In this instance the fixed inter-sister connection and intra-sister loop anchor are located directly adjacent to the centromere. This fixed site then is engaged in an inter-sister interaction with a site located on the other sister chromatid some variable distance away from the centromere but on the same chromosome arm. Similarly, within a sister chromatid the fixed loop anchor would engage with a second anchor at some variable distance on the same arm. Averaged over 16 centromeres, lines appear, but at individual centromeres such intra-sister looping and inter-sister interactions at the same site can be observed as an enriched dot, an example is shown in extended data figure 6.

We then examined $P_{\text{inter}}(s)$ and $P_{\text{intra}}(s)$ for all interactions anchored at centromeres (figure 3d, solid lines) and compare this to $P_{\text{inter}}(s)$ and $P_{\text{intra}}(s)$ along chromosome arms (figure 3d, dashed lines). Where the frequency of inter-sister and intra-sister interactions along the arms only converge at distances larger than 35kb, we observe near identical interaction frequencies at all distances for interactions anchored at centromeres. This indicates that at centromeres sisters chromatids are precisely aligned. This can be explained at least in part because cohesin binding sites are spaced more closely at centromeres than along arms[22,24]: spacing between ChIP-seq cohesin peaks is about 5kb around centromeres but along arms the spacing is ~ 15kb. Second, cohesin ChIP peaks at centromeres are much higher than along arms, indicating that many or most cells in the population will have cohesin bound to those sites, while along arms there may be more cell-to-cell variation in cohesin site occupancy.

Combined these observations lead us to propose that around centromeres interactions between and along sister chromatids are organized differently from those along chromosome

arms. We note that we cannot differentiate between a situation where extruding loops are actively formed by loop extruding factors such as extruding cohesin and condensin or if the observation of loops in peri-centromeric regions is a result of offset cohesive cohesin binding, which would passively expulse a loop on one sister chromatid (figure 3e, marked loops by asterisks).

Inter-sister and intra-sister interactions along arms are mediated by independent cohesin complexes that act at different genomic distances

Cohesin-mediated interactions along chromosome arms have been identified by Hi-C before[7,25,26], however prior to SisterC it has not been possible to differentiate between interactions between and along sister chromatids. When we aggregate all inter-sister interactions (figure 4a) and intra-sister interactions (figure 4b) at and around individual cohesin sites (far left panels), we see that cohesin sites preferentially interact with sites located at least 5kb away on either side. Interestingly, visual inspection of these aggregate interaction maps reveals that inter-sister interactions occur at shorter distances than intra-sister interactions. This becomes more evident when we calculate the difference between the two aggregate interaction maps (figure 4c). We observe an enrichment for inter-sister interactions over intra-sister interaction for cohesin sites separated by less than 20kb. Figure 4d illustrates this difference in another way by plotting the enrichment of inter and intra-sister signal over expected as a function of genomic distance from cohesin binding sites (figure 4d). Inter-sister interactions at cohesin sites preferentially occur at a distance up to 20kb, whereas, intra-sister interactions become more abundant at distances larger than 20kb. This can also be seen at an individual cohesin site (extended data figure 7).

To explore and quantify these differences between inter-sister and intra-sister interactions in more detail, we analyzed pairwise cohesin-cohesin site interactions at different genomic distances. Interactions of cohesin sites within 10kb from each other are preferentially inter-sister interactions (figure 4a–c, second panel from left). This difference is also observed when cohesin sites are separated by 10–20kb (figure 4a–c, middle panel). Interestingly, this preference switches for pairwise cohesin site interactions for sites separated by more than 20kb: for cohesin sites separated by 20 to 35kb we observe a slight preference for intra-sister interactions (figure 4a–c, second panel from right). This difference for intra-sister interactions becomes much more prominent for pairwise cohesin interactions for sites separated by 35 to 50kb (figure 4a–c, far right panel). Thus, cohesive cohesin enables interactions between sites at sister chromatids that are separated by 5 to 25kb, whereas extruding cohesin generates loops along sister chromatids that can be as large as 50kb.

Above we described that findings in the SisterC data suggest that a given cohesin binding site can be engaged in an inter-sister interaction in one cell and an intra-sister interaction in another cell. To investigate this further, we leveraged the fact that these two types of interactions occur at different length scales. Specifically, we ranked SisterC signal of pairs of cohesin binding sites at different distances by their intensity (extended data figure 8). We identified 284 cohesin binding sites that are engaged in the strongest inter-sister interactions (top 10 percent) with other cohesin binding sites that are located 10 to 20kb away. When we explore intra-sister interactions for this set of cohesin binding sites, we observed that these

cohesin binding site pairs also display enriched intra-sister interactions 10 to 20kb away, albeit at lower frequency compared to their inter-sister cohesin-cohesin binding site interactions (extended data figure 8a–c). Similarly, we identified 284 cohesin binding sites that mediate the strongest intra-sister interactions between cohesin sites separated by 35 to 50kb. These cohesin-cohesin binding site pairs also show enriched inter-sister interactions, although at a lower frequency compared to their intra-sister interaction signal (extended data figure 8d–f). This suggests that there are cohesin sites that mediate both strong intra-sister interaction and strong inter-sister interactions. Further, we find an overlap of 65 cohesin binding sites) between cohesin binding sites that mediate strong inter-sister interactions with distal cohesin binding sites located at 10 to 20kb distance as well as strong intra-sister interactions with cohesin binding sites located at 35 to 50kb distance (extended data figure 8g). This provides further support that a given cohesin binding site can engage in either inter-sister interactions or intra-sister interactions in different cells in the population.

Discussion

Here we describe SisterC, a chromosome conformation capture technique that allows detection of interactions between and along sister chromatids separately. SisterC reveals the extent to which sister chromatids are aligned. At centromeres the alignment is rather precise, possibly as a result of high density of cohesin binding sites that are engaged in inter-sister interactions at centromeric regions in every cell. The alignment is more loose along chromosome arms with inter-sister interactions spaced every 35kb on average. Analysis of cohesin binding sites in cell populations shows that cohesin binding sites occur every 10 to 15kb. Combined with our data, this observation suggests that not every cohesin site will be bound in every cell or not every cohesin site will be engaged in inter-sister interactions. The alignment of sisters observed here resembles the locus-dependent loose or precise pairing of homologues observed in *Drosophila*[27]. We see a clear difference in alignment between centromeric regions and chromosome arms in mitotic yeast sister chromatids. However, we do not observe different degrees of alignment along chromosome arms, while such differences were observed in alignment of homologs in *Drosophila*.

Inter-sister interactions occur mostly between cohesin binding sites separated by less than 25kb. This distance is smaller than the distance between inter-sister interaction sites, which we estimated to occur every 35kb on average. One explanation for this can be that inter-sister interactions get established during S-phase every 35 kb or so and relatively close to the replication fork that generates the sister chromatid pair[28–30]. Possibly, inter-sister interactions are initially very precise, but can possibly move to the closest cohesin binding site at convergent gene pairs on both sister chromatids[24,31,32], producing a spacing that can be up to 25kb. Along these lines, we explored whether this offset of inter-sister chromatid interactions would differ at and around origins of replications. However, we found no such differences (extended data figure 9). Interestingly, we do see a clear boundary at origins of replication in G1 Hi-C libraries (extended data figure 9c).

Intra-sister interactions, possibly mediated by extruding cohesin, form larger loops ranging from 25 to 50kb. These cohesin-mediated extruded loops are established during G2/M-phase[7]. In agreement with these observations, previous polymer simulation showed that

yeast Hi-C data from mitotic cells is consistent with the formation of dynamic loops of around 35kb in size[7].

Cohesive cohesin and extruding cohesin are preferentially interacting at different genomic distances, mediate interactions independent from each other and are loaded and established in different phases in the cell cycle[7,33]. This leads us to propose that these cohesin complexes are distinct, possibly having different subunit compositions or modifications. For instance, cohesin can be bound by either Scc2/4 or Pds5[34–36]. Further, the acetylation status of a cohesin complex is important for establishment of cohesion[37,38]. Distinct behavior of cohesive and extruding cohesin complexes was also observed in G2-arrested mammalian cells using another Hi-C based technique that can distinguish interactions between and along sister chromatids, scsHi-C[39]. Where in yeast both inter-sister interactions and intra-sister interactions in mitosis are mediated by cohesin complexes, in vertebrates these are formed by two different protein complexes: cohesin establishes sister chromatid cohesion and condensin I and II mediate intra-sister looping formation to compact chromosomes in mitosis[7,40].

In the current SisterC procedure, selective depletion of DNA strands containing BrdU is not complete. Therefore, there is some level of cross contamination of inter- and intra-sister interactions. We did not identify particular types of molecules that are resistant to selective depletion. For instance, A-T content of the genomic site (extended data figure 10a–b), distance from a digestion site (extended data figure 10c–d) or regions near replication origins (extended data figure 10e–f) do not affect assignment of interactions as being inter-sister or intra-sister. More efficient strand depletion using alternative approaches, such as enzymatic or chemical depletion of T-analogues, could result in more sensitive differentiation of inter-sister and intra-sister interactions.

BrdU incorporation as measured by HPLC is near to complete in the newly replicated strand. In order to achieve this in budding yeast, an engineered strain was used. However, we note that in order to apply SisterC in mammalian cells, no special cell lines are required as mammalian cell lines are able to take up and incorporate BrdU without experimental manipulations[14,41], which makes application of SisterC to any mammalian cell type straightforward.

SisterC allows the study of the significant topological challenge each cell faces during the cell cycle: the concordant chromosome compaction and sister chromatid separation during mitosis, particularly prophase. This process has been difficult to study by conventional Hi-C, because of its inability to distinguish between inter- and intra-sister interactions. We believe SisterC will have a broad applicability in different model organisms and during different phases of the cell cycle from late S-phase to mitosis.

Online Methods

We have made our SisterC protocol available online at protocol exchange (10.21203/rs.3.pex-1021/v1).

Yeast synchronization and culture conditions

The YLV11 strain[20] was used for all experiments in this study. For normal growth, cells were cultured in YP media with 2% galactose and 100μM thymidine at 30°C. Prior to synchronization, cultures were diluted to OD ~0.15 and allowed to double. To synchronize cells in late G1, 5 μM alpha factor mating pheromone (zymoresearch #Y1001) was added for 2.5–3 hours until cells started schmoo formation. SisterC DpnII R2 and R3 received a boost of 500uM thymidine or BrdU after 2 hours of alpha factor synchronization. G1 arrested cells were washed 3 times and released in prewarmed media containing 1mM BrdU or Thymidine. For mitotic arrested cells, 1% DMSO was added 15 minutes after release and 10μg/mLnocodazole was added 30 minutes later. Mitotic cells were harvested approximately 4.5 hours after alpha factor release. For G1 arrested cells, the culture was released for 2 hours, followed by a second alpha factor arrest for 3 hours. Cells were washed and pelleted for genomic DNA extraction for HPLC detection and stored at −80°C until further processing. For Hi-C and SisterC, cells were fixed with 3% formaldehyde for 20 minutes at 30°C while in shaker incubator. Fixing was quenched by adding 2.5M glycine for an additional 5 minutes at 30°C. Cells were washed twice in MilliQ and pelleted cells were stored at −80°C till further processing. Throughout synchronization protocol, cells were washed and fixed in 95% ethanol for flow cytometry analysis.

Flow cytometry

Ethanol fixed cells were resuspended and washed with 50mM NaCitrate. After mild sonication, cell walls were degraded with 10 units of zymolyase in PBS for 30 min at 30°C, followed by a lysis using 2M HCl and 0.5% Triton Tx-100 for 30 min at room temperature, followed by 30 min incubation at room temperature in 0.1M NaB_4O_7. Cells were then washed and resuspended in PBS, 1% milk, 0.2% Tween and 1:20 anti-BrdU-FITC (ThermoFisher #11-5071-42) and incubated for 30 min at RT. Cells were again washed and resuspended in PBS, 1% milk, 0.2% Tween, 0.25mg/mL RNase A and 10mg/mL propidium iodide and incubated at 37°C for 30 min. Cells were washed and resonicated before flow cytometry detection using a MACSquant flow cytometer. Data was analyzed using FlowJo software.

Amplification of DNA fragments containing after UV/Hoechst treatment

DNA fragments (686 bp length) were amplified for 15 cycles using DreamTaq (ThermoFisher # EP1701) in presence of 100%, 90%, 50%, 10% or 0% BrdUTP, supplemented with dTTP. Amplified DNA fragments were incubated in TLE with 100ng/uL Hoechst 33342 (ThermoFisher #H3570) for 15 min at room temperature while protecting from light, followed by UV radiation at 2.7kJ/m^2. Samples were washed 3 times in 30KDa amicon columns (MilliPore # UFC5030BK) and amplified with 10 PCR cycles.

HPLC separation and LCMS analysis

Cells were harvested, pelleted and frozen at −80°C for HPLC analysis from 300mL yeast cultures at OD ~0.3 (approximately 600 million cells). Frozen cells were washed in 1mL spheroplasting buffer and lysed for 10 minutes at 35°C using 0.5% beta-mercaptoethanol and 10ug/mL zymolyase (Zymoresearch # E1005). Cells were washed in 1x NEBuffer 3.1

(NEB #B7203S) and incubated with proteinase K twice for 12 hours and an additional 2 hours at 65°C. DNA was extracted with phenol:chloroform, followed by ethanol precipitation and RNA digestion using RNase A. DNA samples were first washed with milliQ water using a 3 kDa cut-off Amicon filter. Digestion into nucleosides was carried out on 3 μg aliquots using 'nucleoside digestion enzyme mix' from New England Biolabs (NEB#M0649) and incubated overnight at 37°C. Once digested, aliquots from the same sample were pooled, filtered, and rinsed through a 3 kDa Amicon filter with milliQ water. The flow through was concentrated and quantified by A260 for subsequent HPLC injection. Digested deoxynucleosides were resolved using an Agilent 1260 Infinity HPLC with a Synergi C18 4-μm Fusion-RP 80Å 250 × 4.6 mm LC column. Nucleosides were resolved over 35 minutes using an isocratic gradient of 2–22% [95% acetonitrile, 5% 20 mM ammonium acetate pH 4.5] in [20 mM ammonium acetate] at 25°C, using a flow rate of 0.5 mL/min. This was followed by wash steps at higher eluent strengths between runs. Absorbance was recorded at 260 nm and 279 nm. Normal deoxynucleosides were quantitated using HPLC peak areas over three repeats, and published values for molar extinction coefficients at 260 nm [ε260 (M-1 cm-1) adenosine = 15400, cytidine = 7300, guanosine = 11700, thymidine = 8800[42]. The molar extinction coefficients for bromodeoxyuridine at 260 and 279 nm were empirically measured and found to be 9229 and 5003, respectively. For mass spectrometry analysis, deoxynucleosides were first resolved using the HPLC method above, and UV peaks were manually collected in separate vials. These were evaporated to dryness, resuspended in 50 μL milliQ water, and desalted on the same HPLC column using an isocratic gradient of 0–40% [98% acetonitrile, 2% water] in [90% water, 10% acetonitrile] over 15 min at 25°C, using a flow rate of 1 mL/min. The desalted peak was collected, evaporated to dryness, then resuspended in 20 μL milliQ water and analysed by high resolution liquid chromatography mass spectrometry. Mass spectrometry was carried out in positive ESI mode on an Agilent 6530 Accurate-Mass Q-TOF LC/MS linked to a pre-injection Agilent 1260 infinity HPLC. Samples were run in milliQ water containing 0.1 % formic acid. Mass spectrometry settings to detect nucleosides were as follows; gas temperature 350°C, nebulizer gas rate 45 psig, drying gas 10 L/min, VCap 4000 V, fragmentor voltage 120 V, skimmer voltage 65 V. 5 mM stock solutions of RNA nucleosides A, U, C, and G (ChemGenes) were made up in milliQ water. HPLC injections of digested deoxynucleosides were doped with the addition of 2 μL of each of the RNA nucleosides. Nucleosides were resolved using the standard separation method above.

SisterC and Hi-C library preparation

For each mitotic SisterC or Hi-C library approximately 300 million cells or 100mL culture at OD ~0.3 was used. For each G1 SisterC or Hi-C library this was double, approximately 600 million cells or 100mL culture at OD ~0.6. There were 3 biological replicates produced using DpnII as restriction enzyme and one replicate using HindIII. SisterC and Hi-C were preformed according to previously published Hi-C protocol for yeast[25], with several major modifications. Samples were split for Hi-C and SisterC library production before treatment with Hoechst/UV. Briefly, cells were fixed and stored as described above. Crosslinked cells were thawed, washed and resuspended in spheroplasting buffer (1M Sorbitol, 50mM Tris pH 7.5). Cells were lysed by addition of 0.5% beta-mercaptoethanol and 10ug/mL zymolyase (Zymoresearch # E1005) and incubated for 10 min at 35°C. Cells were washed twice with

1x NEBuffer 3.1 (for DpnII libraries) or NEBuffer 2.1 (for HindIII libraries). Chromatin was solubilized with 0.1% SDS for 10 minutes at 65°C, followed by quenching with 1% Triton X-100. Chromatin was digested with 400U HindIII or DpnII overnight at 37°C. After inactivation of restriction enzyme for 20 min at 65°C, DNA ends were filled in with nucleotides and supplemented with biotin-14-dCTP (LifeTech #19518018) for HindIII libraries and biotin-14-dATP (LifeTech #19524016) for DpnII libraries for 4 hours at 23°C. DNA fragments were ligated with T4 DNA ligase (LifeTech #15224090) for 4 hours at 16°C in reactions of 75 μL each. All ligation reactions were combined and samples were treated with proteinase K overnight at 65°C. DNA was purified using 1:1 phenol:chloroform and ethanol precipitation. Samples treated with RNAse A and biotin from unligated ends were removed using T4 DNA polymerase. DNA was sonicated and size-selected using AMpure XP beads (Bedman coulter #A63881) to obtain fragments sized 600–800bp. We performed end repair and a-tailing prior to illumina TruSeq adapter ligation. Each sample was split in two to obtain one SisterC library treated with UV and Hoechst and one Hi-C library without treatment from the same biological sample. SisterC libraries were treated in two reaction volumes of 50uL each with 100ng/uL Hoechst 33342 (ThermoFisher #H3570) for 15 min at room temperature while protecting from light, followed by UV radiation at 2.7kJ/m^2. Samples were washed with TLE 3 times in 30KDa amicon columns (MilliPore # UFC5030BK). Both SisterC and Hi-C libraries were then enriched for biotin-containing fragments by pull down with MyOne Streptavidin C1 beads (Life Tech #65–001). Libraries were amplified, cleaned from pcr primers and sequenced using paired end 50bp reads on an Illumina HiSeq4000 platform. All libraries within a set of replicates were amplified with the same number of PCR cycles.

SisterC and Hi-C analysis

Hi-C and SisterC FASTQ sequencing files were mapped to saccer3 yeast reference genome using publicly available distiller-nf mapping pipeline (https://github.com/mirnylab/distiller-nf) and downstream analysis tools pairtools (https://github.com/mirnylab/pairtools) and cooltools (https://github.com/mirnylab/cooltools). Briefly, reads were mapped with bwa-mem, deduplicated and filtered for mapping quality, resulting in only “valid reads”. Reads were classified as inter-sister reads when one read end was mapped as + orientation and the other end as – read orientation. Reads were classified as intra-sister reads when both read ends mapped as + or – read orientation. For downstream analysis, interactions at a shorter distance than 1500bp were removed. Interactions were binned at 1kb, 2kb and 10kb resolution using cooler[43]. Iterative balancing was applied to all matrices, individually for inter-sister and intra-sister interactions, while ignoring two bins from the diagonal[44]. Hi-C and SisterC statistics for all samples are provided in supplemental table 1.

Distance decays were plotted from valid pairs separated by read orientation were used to calculate contact frequency (P) as a function of genomic distance (s) using cooltools code. Pile up plots on genomic loci were produced using valid interactions binned at 1kb resolution separated for inter- or intra-sister interactions and contained only interactions at distances larger than 1500bp. Observed over expected values were calculated using expected files of matching conditions (e.g. expected file of inter-sister interactions of SisterC library for matching observed interactions of inter-sister interactions of SisterC library). Heatmaps

were plotted using modified cooltools code. For centromere pile up plot, the directionality of the centromere DNA elements was taken into account. Anchor plots were plotted from valid interactions binned at 2kb resolution, which were separated for inter or intra-sister interactions and contained only interactions at distances larger than 1500bp.

Data availability

All genomic data generated for this study are publicly available on the NCBI Gene Expression Omnibus (GEO; https://www.ncbi.nlm.nih.gov/geo/) under accession number GSE145695.

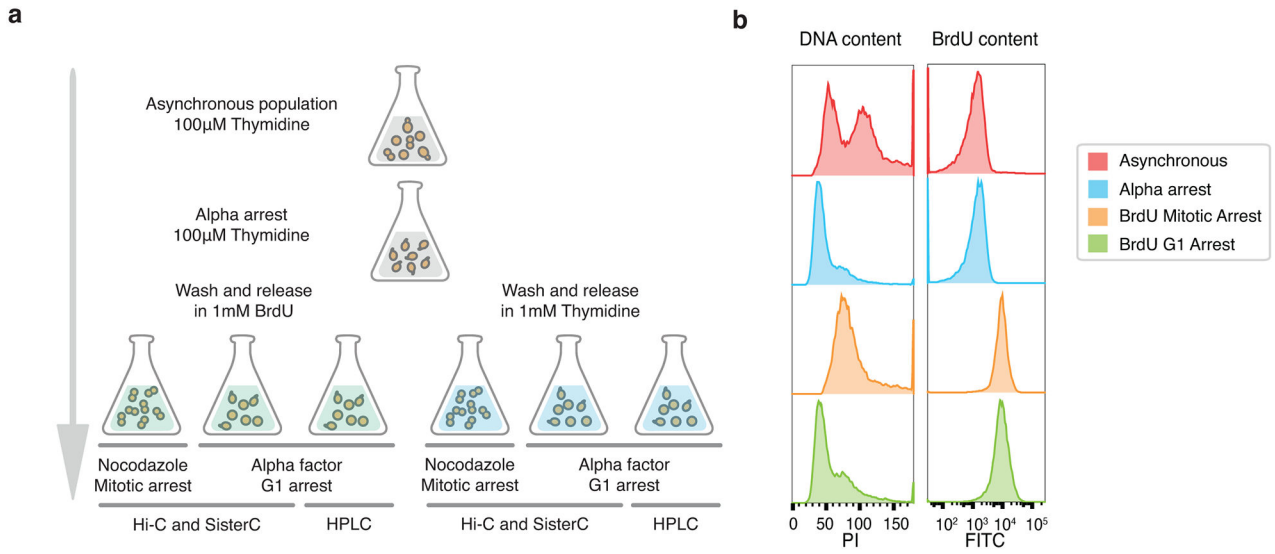
Publicly available datasets used in this manuscript

Scc1 calibrated ChIP-seq tracks from Hu et al[22] were used for cohesin pile up SisterC heatmaps and ChIP-seq tracks in figure 1. This dataset is available on GEO under accession number GSM1712309. Peaks were called on this dataset using MACS2. Pairwise cohesin interactions were compiled by listing all possible pairwise combinations of cohesin peak sites in cis, followed by separation on distance between cohesin pairs (smaller than 10kb, 10 to 20kb, 20 to 35kb and 35 to 50kb). Cohesin sites in a 50kb window around centromeres and on all of chrXII and chrIV were removed from the dataset. Additionally Hi-C samples from cdc45 mutant cells were used from Schalbetter et al[7] to investigate distance decay. This dataset is available on GEO under accession number GSM2327664. This data was processed identical to Hi-C libraries produced for this study. Sites of origin of replication were downloaded from OriDB (http://www.oridb.org/)[45].

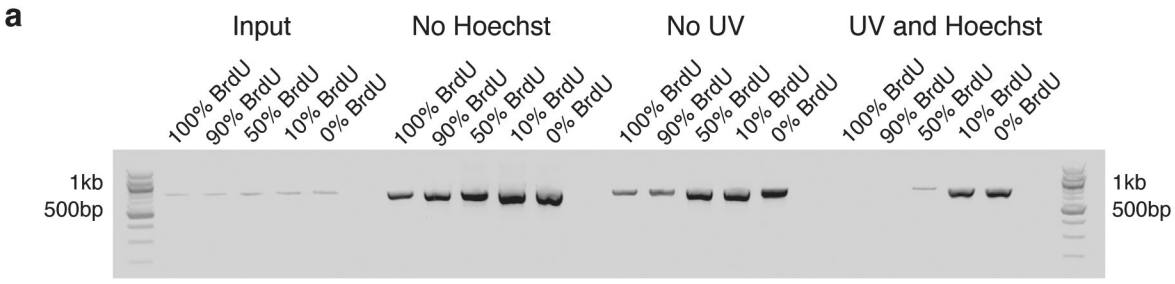
Code availability

Hi-C mapping pipeline distiller-nf is available on https://github.com/mirnylab/distiller-nf. Downstream analysis tools pairtools and cooltools are available through https://github.com/mirnylab/pairtools and https://github.com/mirnylab/cooltools.

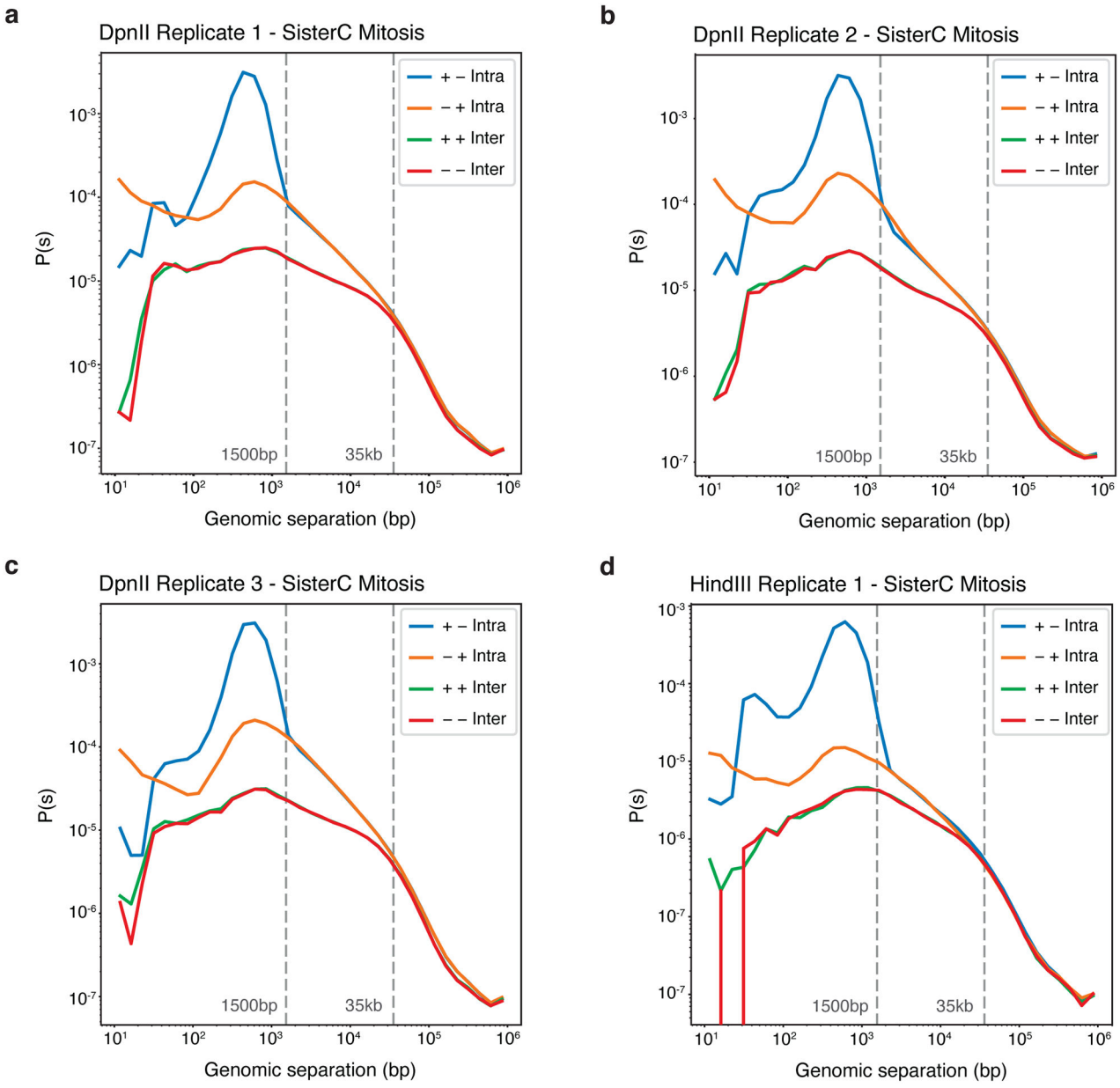
Extended Data



Extended Data Fig. 1. Outline of SisterC yeast culture conditions and flow cytometry analysis.
(a) Asynchronous yeast cultures are cultured and synchronized in late G1 using alpha factor. Cells are released in media containing BrdU or Thymidine, followed by an arrest in mitosis using nocodazole or G1 using a second alpha factor arrest. Cells are harvested and prepared for Hi-C or SisterC library production or processed for BrdU detection using HPLC. (b) Flow cytometry analysis of cell cycle profile and BrdU incorporation of harvested yeast cultures for preparation of SisterC libraries.

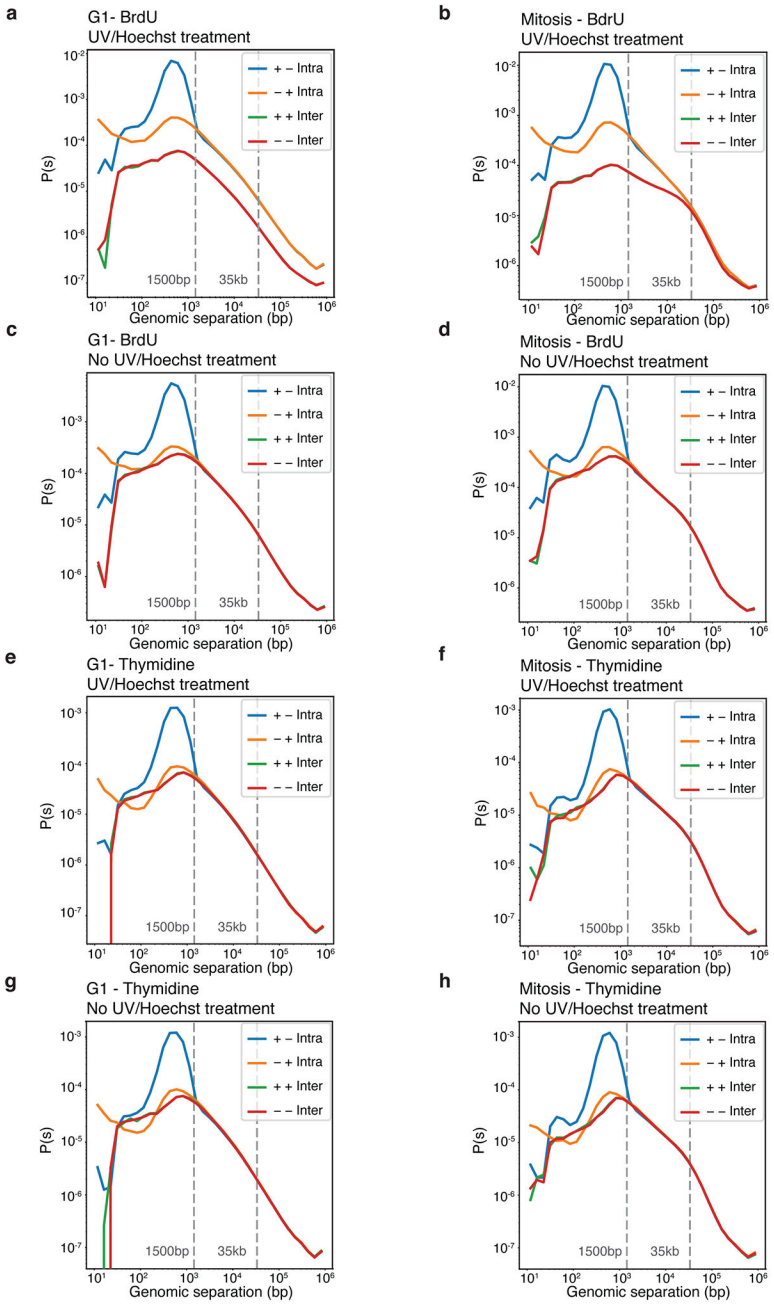


Extended Data Fig. 2. Depletion of BrdU containing DNA molecules by PCR
DNA fragments were amplified in presence of 0%, 10%, 50%, 90% and 100% BrdU to allow for incorporation in both strands (first 5 lanes). This was followed by treatment of UV only (second 5 lanes), Hoechst only (third 5 lanes) or treatment with both UV and Hoechst (last 5 lanes). Fragments containing more than 10% BrdU did not get amplified after UV/ Hoechst treatment. This experiment was repeated twice with highly similar results.



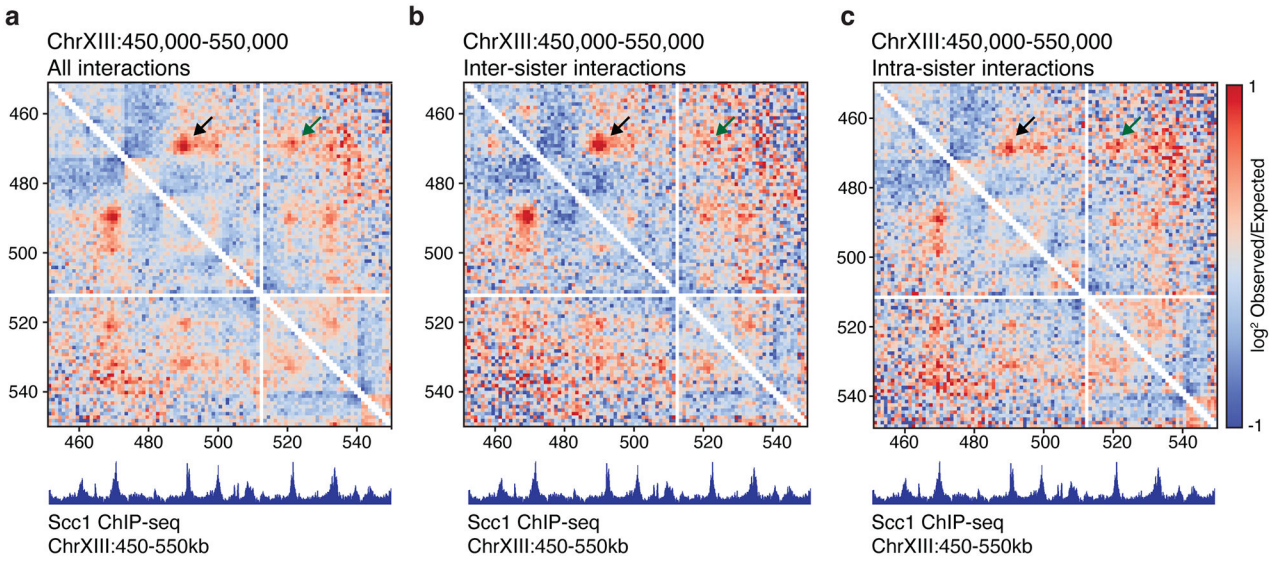
Extended Data Fig. 3. Distance decay plots of all SisterC replicates

Distance decay plots of all SisterC mitotic libraries: DpnII replicate 1 (**a**), DpnII replicate 2 (**b**), DpnII replicate 3 (**c**) and HindIII replicate 1 (**d**)



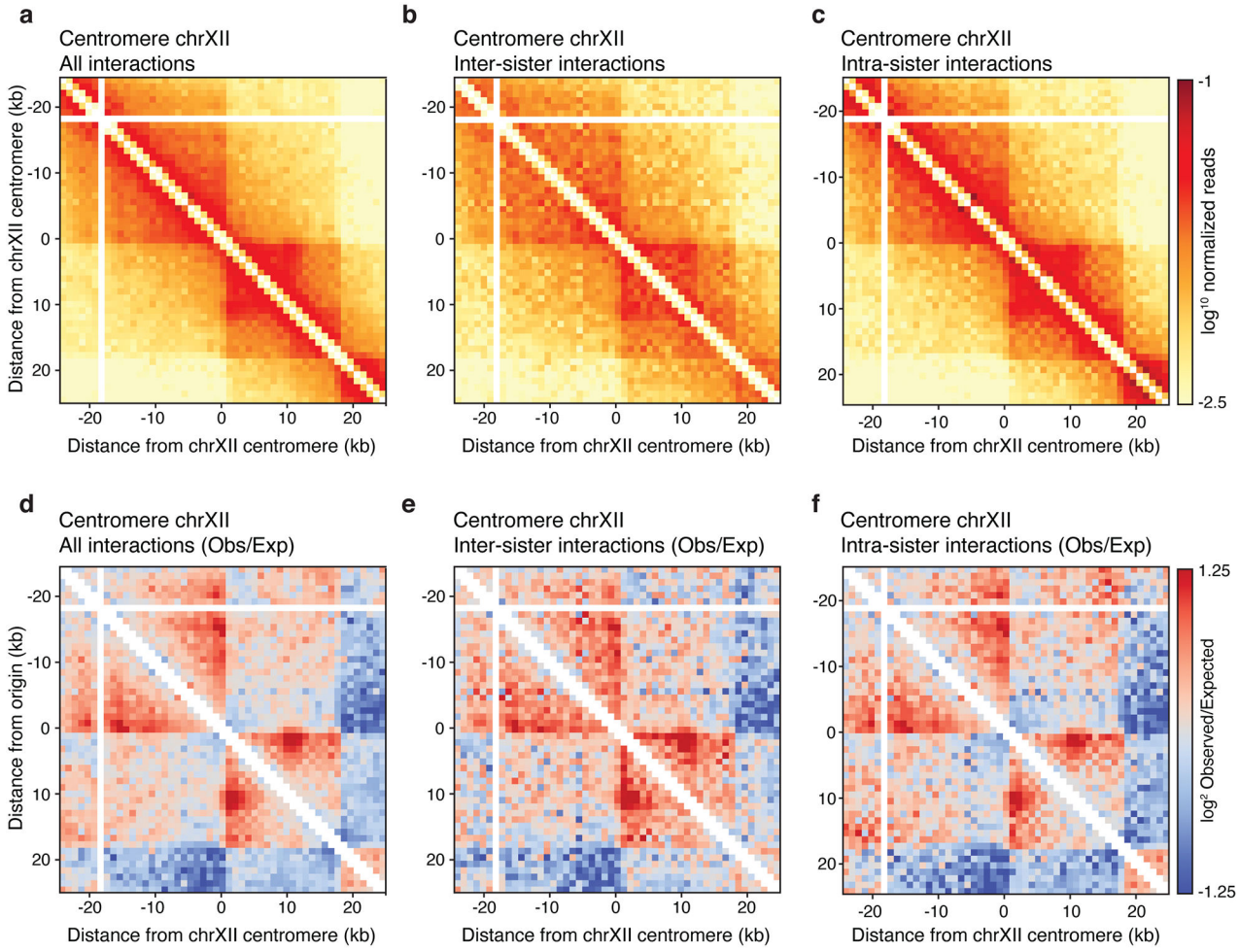
Extended Data Fig. 4. Distance decay plots of all SisterC control experiments

Distance decay plots of all SisterC control libraries: (**a-b**) G1 (a) and mitotic (b) arrested cells grown in BrdU and treated with UV/Hoechst. (**c-d**) G1 (c) and mitotic (d) arrested cells grown in BrdU, not treated with UV/Hoechst. (**e-f**) G1 (e) and mitotic (f) arrested cells grown in Thymidine, treated with UV/Hoechst. (**g-h**) G1 (g) and mitotic (h) arrested cells grown in Thymidine, not treated with UV/Hoechst.



Extended Data Fig. 5. Observed/expected interaction heatmap of chrXIII:450,000–550,000

Log2 observed over expected interaction frequency on region ChrXIII:450,000–550,000 (as shown in main figure 2) for all interactions (**a**), inter-sister interactions (**b**) and intra-sister interactions (**c**).

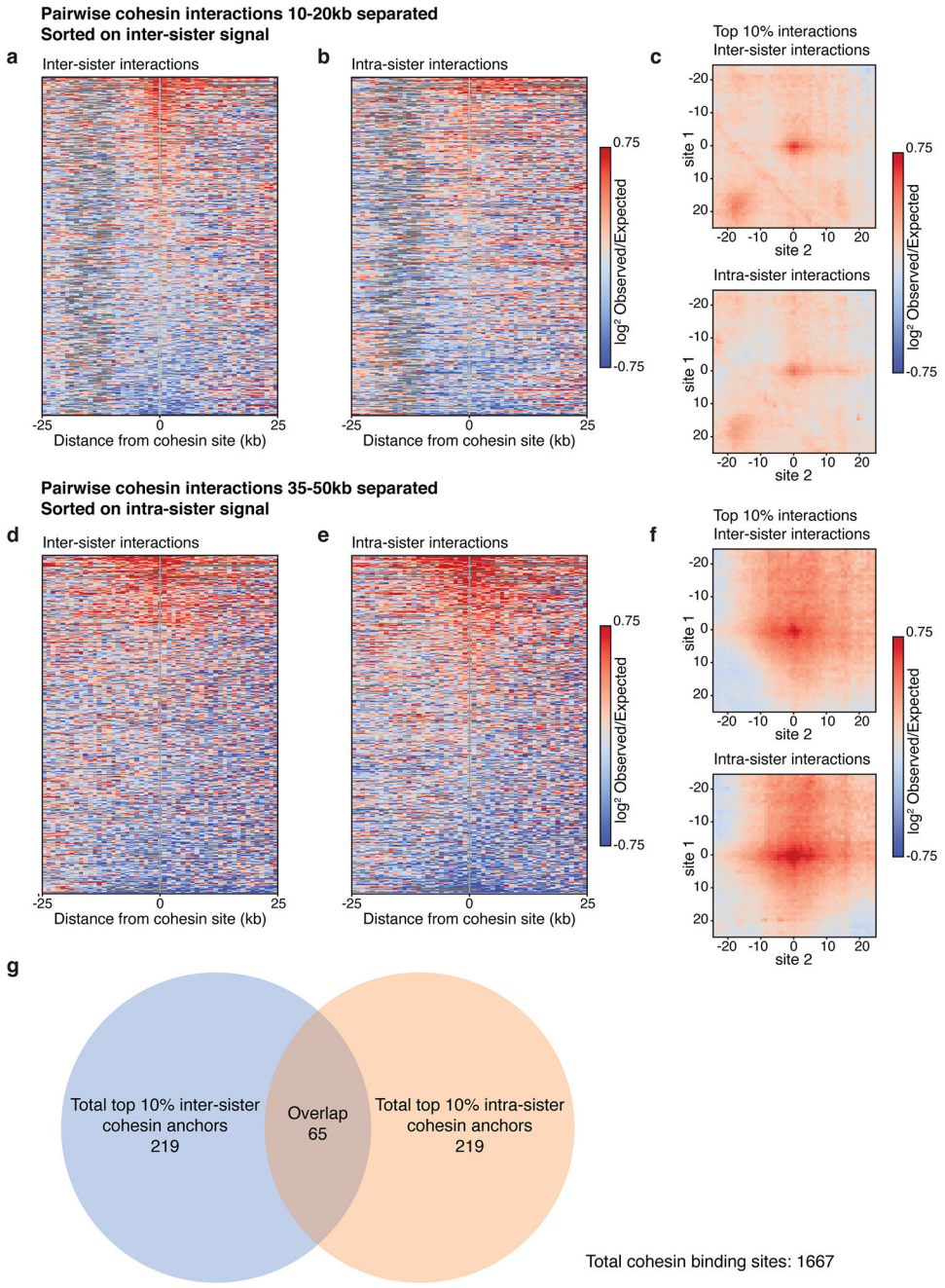


Extended Data Fig. 6. Centromeric region of chrXII

(a-c) Interaction heatmaps of 50kb window around the centromeric region of chrXII, for all interactions (a), inter-sister interactions (b) and intra-sister interactions (c). (**d-f**) Log2 observed over expected interaction heatmaps of all interactions (d), inter-sister interactions (e) and intra-sister interactions (f) on the centromeric region on chrXII.

Extended Data Fig. 7. Anchor plot on ChrXIII:469000

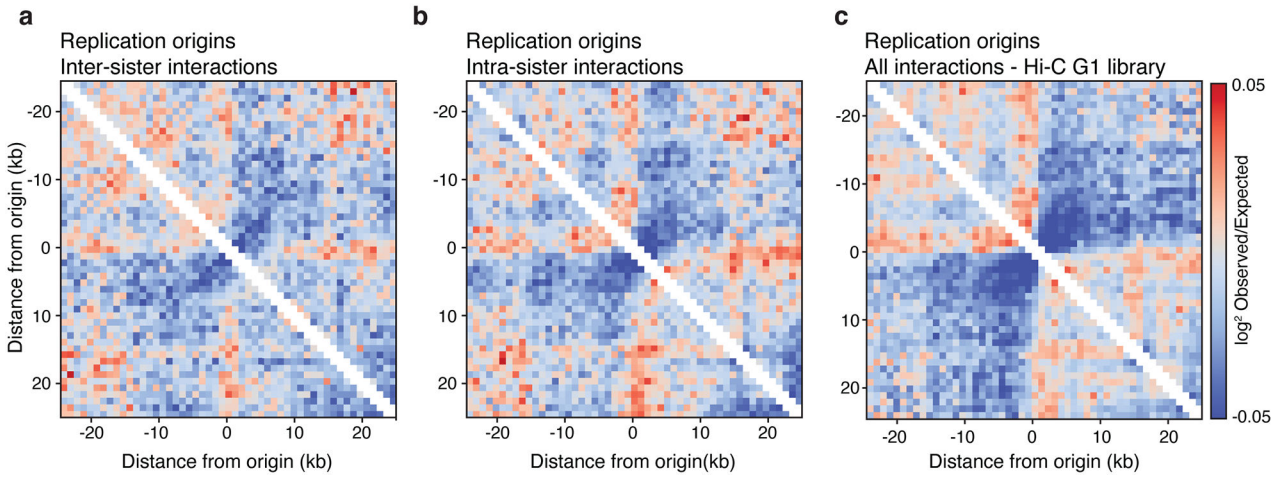
Interaction frequency of inter-sister and intra-sister interactions anchored on chrXIII:469,000 shows higher frequency of inter-sister interactions at 25kb distance from the anchor (highlighted in green). Annotated below the plot are the positions of Scc1 peaks.



Extended Data Fig. 8. Ranking of SisterC signal on pairs of cohesin binding sites at different genomic distances

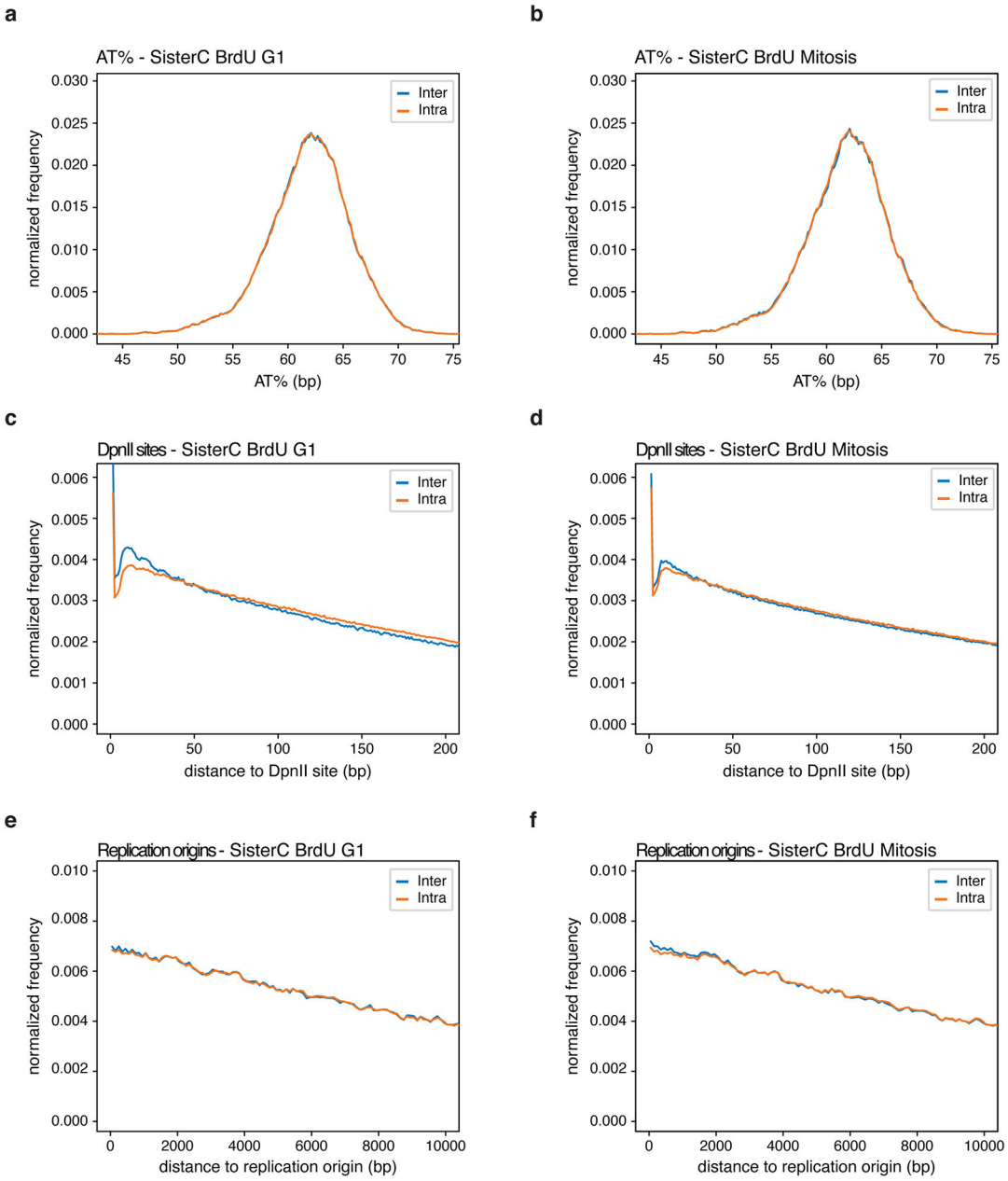
(a-b) Inter-sister interaction (a) and intra-sister interaction (b) frequency of pairs of cohesin binding sites separated by 10 to 20kb were ranked by inter-sister interaction intensity for 10kb window around the cohesin binding site. (**c**) Interaction pile up plots of inter-sister and intra-sister interactions of the top 10 percent sites that were ranked in (a). (**d-e)** Inter-sister interaction (d) and intra-sister interaction (e) frequency of pairs of cohesin binding sites separated by 35 to 50kb were ranked by intra-sister interaction intensity for 10kb window around the cohesin binding site. (**f**) Interaction pile up plots of inter-sister and intra-sister

interactions of the top 10 percent sites that were ranked in (e). (**g**) 284 cohesin sites that anchor the top 10% inter-sister interacting cohesin pairs at 10 to 20kb distance were identified as well as again 284 cohesin sites that anchor the top 10% intra-sister interacting cohesin pairs at 35 to 50kb distance. 65 cohesin sites are found to mediate both top 10% inter-sister cohesin-cohesin interactions and top 10% intra-sister cohesin-cohesin interactions.



Extended Data Fig. 9. SisterC pile up plots of origins of replication

(a-b) Mitotic SisterC inter-sister interactions (a) and intra-sister interactions (b) plotted on replication origins. (**c**) Pile up plot of Hi-C data of G1 alpha arrested cells on replication origins.



Extended Data Fig. 10. Efficiency of SisterC inter- and intra-sister interaction assignment

(**a-b**) Normalized frequency SisterC G1 (a) and mitotic (b) inter-sister and intra-sister interactions as a function of AT percentage. (**c-d**) Normalized frequency SisterC G1 (c) and mitotic (d) inter-sister and intra-sister interactions as a function of distance to DpnII digestion site. (**e-f**) Normalized frequency SisterC G1 (e) and mitotic (f) inter-sister and intra-sister interactions as a function of distance to origins of replication.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Yatskevich S, Rhodes J & Nasmyth K Organization of Chromosomal DNA by SMC Complexes. 1–38 (2019).
2. Nagasaka K, Hossain MJ, Roberti MJ, Ellenberg J & Hirota T Sister chromatid resolution is an intrinsic part of chromosome organization in prophase. Nat. Cell Biol (2016). doi:10.1038/ncb3353
3. Falconer E et al. Identification of sister chromatids by DNA template strand sequences. Nature 463, 93–97 (2010). [PubMed: 20016487]
4. Espinosa E, Paly E & Barre F-X High-Resolution Whole-Genome Analysis of Sister-Chromatid Contacts. Mol. Cell 1–13 (2020). doi:10.1016/j.molcel.2020.06.033
5. Haering CH, Farcas AM, Arumugam P, Metson J & Nasmyth K The cohesin ring concatenates sister DNA molecules. Nature 454, 297–301 (2008). [PubMed: 18596691]
6. Tanaka T, Cosma MP, Wirth K & Nasmyth K Identification of Cohesin Association Sites at Centromeres and along Chromosome Arms. Cell 98, 847–858 (1999). [PubMed: 10499801]
7. Schalbetter SA et al. SMC complexes differentially compact mitotic chromosomes according to genomic context. Nat. Cell Biol 19, 1071–1080 (2017). [PubMed: 28825700]
8. Lazar-Stefanita L et al. Cohesins and condensins orchestrate the 4D dynamics of yeast chromosomes during the cell cycle. EMBO J. 36, 2684–2697 (2017). [PubMed: 28729434]
9. Kim Y, Shi Z, Zhang H, Finkelstein IJ & Yu H Human cohesin compacts DNA by loop extrusion. Science (80-.). 366, 1345–1349 (2019).
10. Ganji AM et al. Title : Real-time imaging of DNA loop extrusion by condensin. 7831, 1–9 (2018).
11. Golfier S, Quail T, Kimura H & Brugues J Cohesin and condensin extrude loops in a cell-cycle dependent manner. bioRxiv 821306 (2019). doi:10.1101/821306
12. Alves P & Jonasson J New staining method for the detection of sister-chromatid exchanges in BrdU-labelled chromosomes. J. Cell Sci Vol. 32, 185–195 (1978). [PubMed: 81211]
13. Falconer E et al. DNA template strand sequencing of single-cells maps genomic rearrangements at high resolution. Nat. Methods 9, (2012).
14. Claussin C et al. Genome-wide mapping of sister chromatid exchange events in single yeast cells using strand-seq. Elife 6, 1–17 (2017).
15. Van Wietmarschen N & Lansdorp PM Bromodeoxyuridine does not contribute to sister chromatid exchange events in normal or Bloom syndrome cells. Nucleic Acids Res. 44, 6787–6793 (2016). [PubMed: 27185886]
16. Chaisson MJP et al. Multi-platform discovery of haplotype-resolved structural variation in human genomes. Nat. Commun 10, 1–16 (2019). [PubMed: 30602773]
17. Porubsky D et al. Dense and accurate whole-chromosome haplotyping of individual genomes. Nat. Commun 8, (2017).
18. Lieberman-Aiden E et al. Comprehensive mapping of long-range interactions reveals folding principles of the human genome. Science 326, 289–93 (2009). [PubMed: 19815776]
19. Belaghzal H, Dekker J & Gibcus JH Hi-C 2.0: An optimized Hi-C procedure for high-resolution genome-wide mapping of chromosome conformation. Methods 123, 56–65 (2017). [PubMed: 28435001]
20. Vernis L, Piskur J & Diffley JFX Reconstitution of an efficient thymidine salvage pathway in Saccharomyces cerevisiae. Nucleic Acids Res. 31, 120e–120 (2003).
21. Lajoie BR, Dekker J & Kaplan N The Hitchhiker ' s guide to Hi-C analysis : Practical guidelines. Methods 72, 65–75 (2015). [PubMed: 25448293]

22. Hu B et al. Biological chromodynamics: A general method for measuring protein occupancy across the genome by calibrating ChIP-seq. Nucleic Acids Res. 43, 1–20 (2015). [PubMed: 25505162]
23. Fudenberg G, Abdennur N, Imakaev M, Goloborodko A & Mirny LA Emerging Evidence of Chromosome Folding by Loop Extrusion. Cold Spring Harb. Symp. Quant. Biol LXXXII, 034710 (2018).
24. Lengronne A et al. Cohesin relocation from sites of chromosomal loading to places of convergent transcription. Nature 430, 573–578 (2004). [PubMed: 15229615]
25. Schalbetter SA, Neale MJ, Fudenberg G, Baxter J & Pollard KS Principles of meiotic chromosome assembly revealed in S. cerevisiae. Nat. Commun 1–12 (2019). doi:10.1038/s41467-019-12629-0 [PubMed: 30602773]
26. Muller H et al. Characterizing meiotic chromosomes' structure and pairing using a designer sequence optimized for Hi-C. Mol. Syst. Biol 14, 1–19 (2018).
27. AlHaj Abed J et al. Highly structured homolog pairing reflects functional organization of the Drosophila genome. Nat. Commun 10, 1–14 (2019). [PubMed: 30602773]
28. Zheng G, Kanchwala M, Xing C & Yu H MCM2–7-dependent cohesin loading during s phase promotes sister-chromatid cohesion. Elife 7, 1–25 (2018).
29. Lengronne A et al. Establishment of Sister Chromatid Cohesion at the S. cerevisiae Replication Fork. Mol. Cell (2006). doi:10.1016/j.molcel.2006.08.018
30. Moldovan GL, Pfander B & Jentsch S PCNA Controls Establishment of Sister Chromatid Cohesion during S Phase. Mol. Cell (2006). doi:10.1016/j.molcel.2006.07.007
31. Borrie MS, Campor JS, Joshi H & Gartenberg MR Binding, sliding, and function of cohesin during transcriptional activation. Proc. Natl. Acad. Sci. U. S. A 114, E1062–E1071 (2017). [PubMed: 28137853]
32. Bausch C et al. Transcription Alters Chromosomal Locations of Cohesin in Saccharomyces cerevisiae. Mol. Cell. Biol 27, 8522–8532 (2007). [PubMed: 17923700]
33. Lazar-Stefanita L et al. Cohesins and condensins orchestrate the 4D dynamics of yeast chromosomes during the cell cycle. EMBO J. 1–14 (2017). doi:10.15252/embj.201797342
34. Makrantoni V & Marston AL Cohesin and chromosome segregation. Curr. Biol 28, R688–R693 (2018). [PubMed: 29920258]
35. Kikuchi S, Borek DM, Otwinowski Z, Tomchick DR & Yu H Crystal structure of the cohesin loader Scc2 and insight into cohesinopathy. Proc. Natl. Acad. Sci. U. S. A 113, 12444–12449 (2016). [PubMed: 27791135]
36. Lee BG et al. Crystal Structure of the Cohesin Gatekeeper Pds5 and in Complex with Kleisin Scc1. Cell Rep. 14, 2108–2115 (2016). [PubMed: 26923598]
37. Tóth A et al. Yeast cohesin complex requires a conserved protein, Eco1p(Ctf7), to establish cohesion between sister chromatids during DNA replication. Genes Dev. 13, 320–333 (1999). [PubMed: 9990856]
38. Skibbens RV, Corson LB, Koshland D & Hieter P Ctf7p is essential for sister chromatid cohesion and links mitotic chromosome structure to the DNA replication machinery. Genes Dev. 13, 307–319 (1999). [PubMed: 9990855]
39. Mitter M et al. Sister-chromatid-sensitive Hi-C reveals the conformation of replicated human chromosomes. bioRxiv 2020.03.10.978148 (2020). doi:10.1101/2020.03.10.978148
40. Gibcus JH et al. A pathway for mitotic chromosome formation. Science (80-.). 359, eaao6135 (2018).
41. Falconer E & Lansdorp PM Strand-seq: A unifying tool for studies of chromosome segregation. Semin. Cell Dev. Biol 24, 643–652 (2013). [PubMed: 23665005]
42. Andrus A & Kuimelis RG Base Composition Analysis of Nucleosides Using HPLC. Curr. Protoc. Nucleic Acid Chem 1, (2000).
43. Abdennur N & Mirny LA Cooler: scalable storage for Hi-C data and other genomically labeled arrays. Bioinformatics 36, 311–316 (2019).
44. Imakaev M et al. Iterative correction of Hi-C data reveals hallmarks of chromosome organization. Nat. Methods 9, 999–1003 (2012). [PubMed: 22941365]

45. Siow CC, Nieduszynska SR, Müller CA & Nieduszynski CA OriDB, the DNA replication origin database updated and extended. Nucleic Acids Res. 40, 682–686 (2012). [PubMed: 21926160]

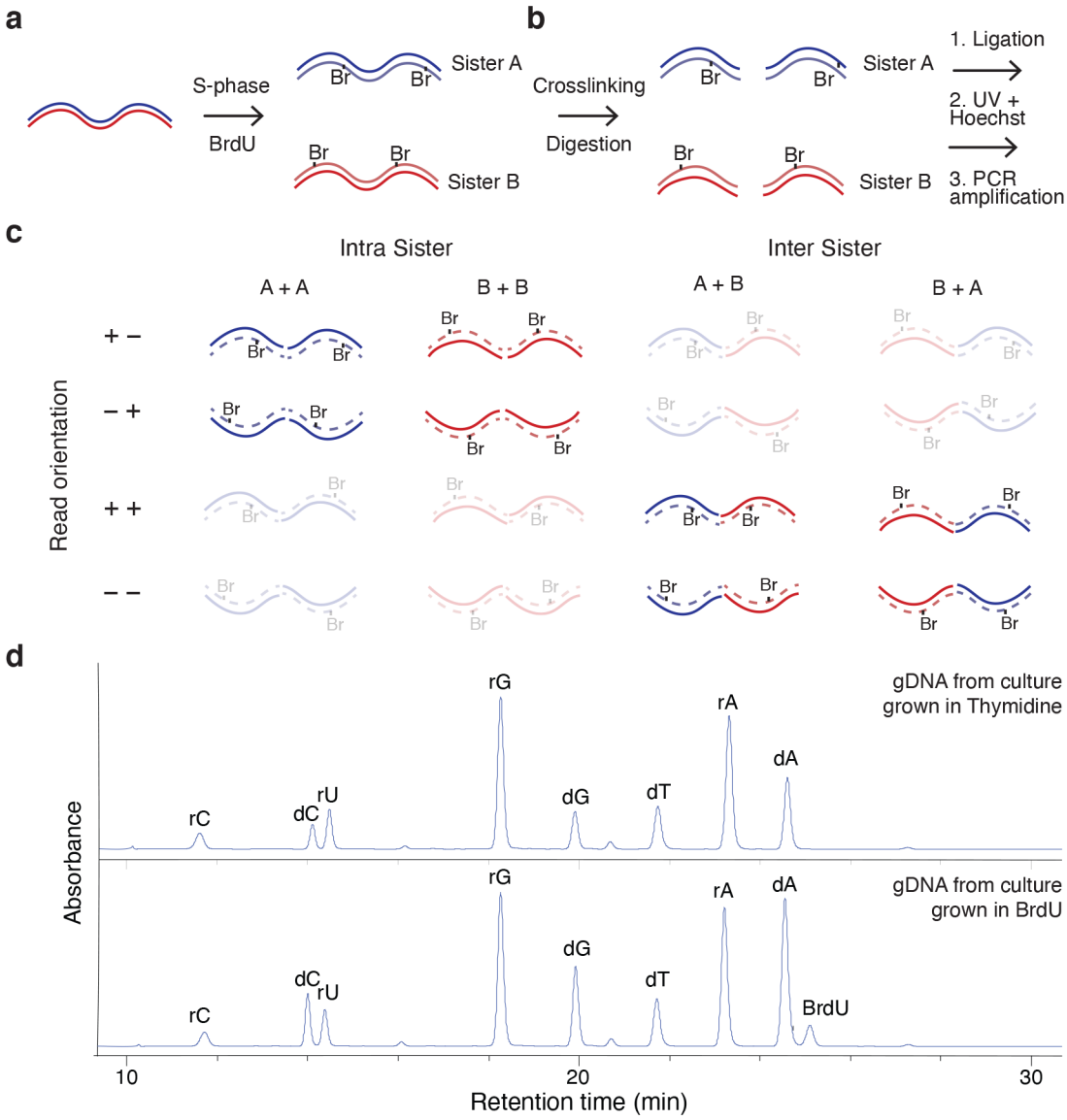

Figure 1 –. Outline of SisterC chromosome conformation capture technique.

(a) As cells go through replication BrdU is incorporated in the − strand for sister A and in the + strand for sister B. (**b**) During Hi-C/SisterC DNA is digested followed by proximity ligation, UV-Hoechst treatment and PCR-amplification. (**c**) Proximity ligation leads to 16 possible Hi-C products of inter- and intra-sister interactions. 8 different ligation products are amplifiable. After paired-end sequencing, we can identify inter-sister (+ + and − −) and intra-sister interactions (− + and + −) by read orientation. (**d**) HPLC spectra of digested DNA isolated from yeast cultures that progressed through 1 round of DNA replication in BrdU- or thymidine-containing media; this enables quantification of BrdU incorporation. Percentages of each nucleoside are calculated using the extinction coefficient of each nucleoside (compare to supplemental figure 4).

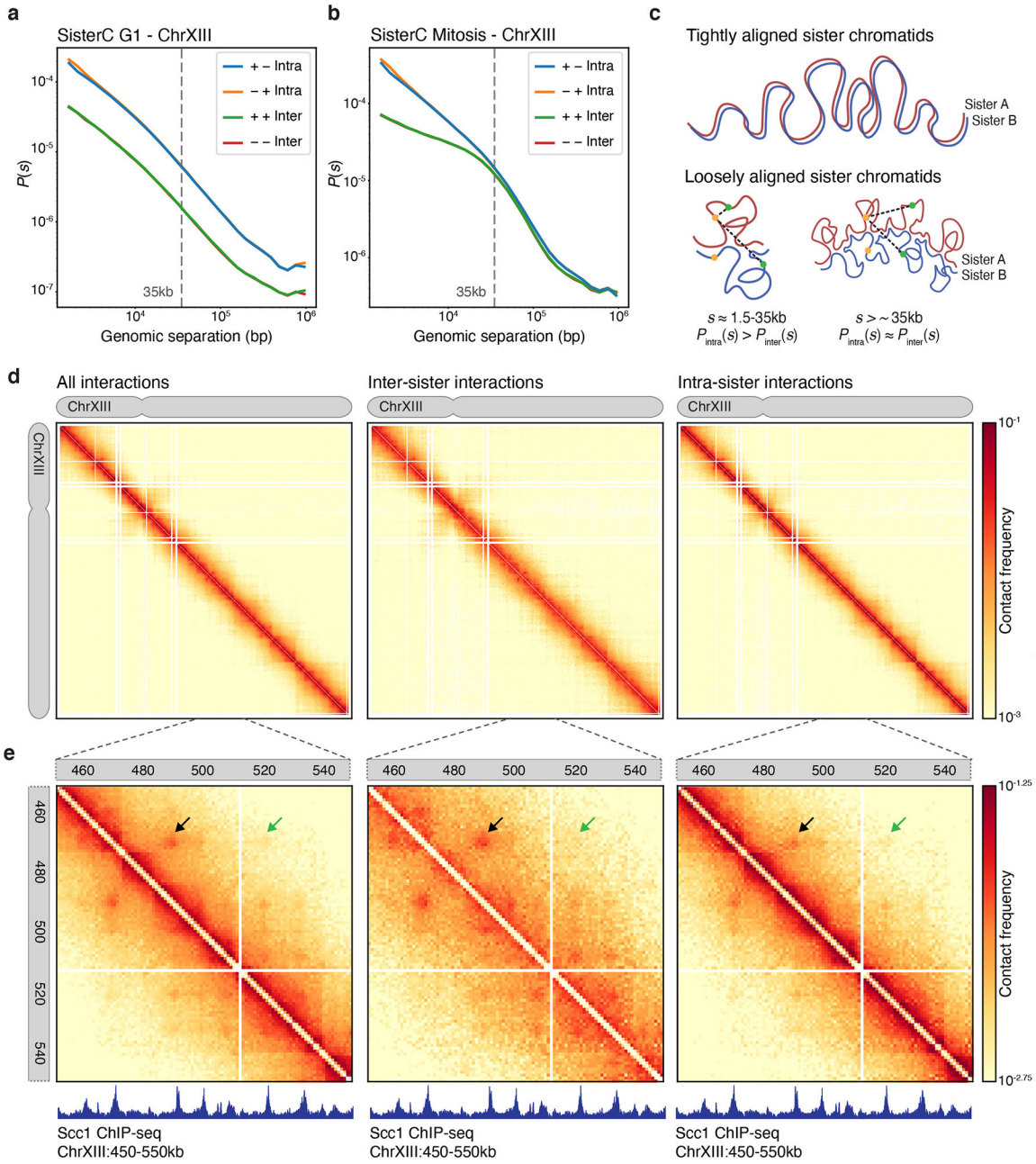

Figure 2 –. SisterC allows differentiation of inter-sister and intra-sister interactions in mitotic chromosomes.

(a) Chromatin interaction distance-dependent decay shows uniform depletion of read pairs with + + and − − orientation in G1 synchronized yeast (loci separated by more than1500bp). (**b**) Mitotic SisterC libraries show an offset of inter-sister interactions up to 35kb genomic separation, with interactions between loci separated by less than 35 kb depleted compared to intra-sister interactions. (**c**) Model of tightly aligned sister chromatids (top panel) and loosely aligned sister chromatids (bottom panel). (**d**) Chromosome-wide SisterC interactions on chrXIII of all read pairs (left panel), all read pairs assigned as inter-sister interactions (middle panel) and all read pairs assigned as intra-sister interactions (right panel), binned in

2kb bins. (**e**) SisterC interactions at 1kb resolution of all reads on zoomed in region of chrXIII:450,000–550,000 of all reads (left panel), inter-sister reads (middle panel) and intra-sister reads (right panel). Arrows highlight interaction of cohesin sites that are more prevalent as inter-sister interaction (black) or intra-sister interaction (green). Lower panels show Scc1 ChIP-seq track, a subunit of the cohesin complex.

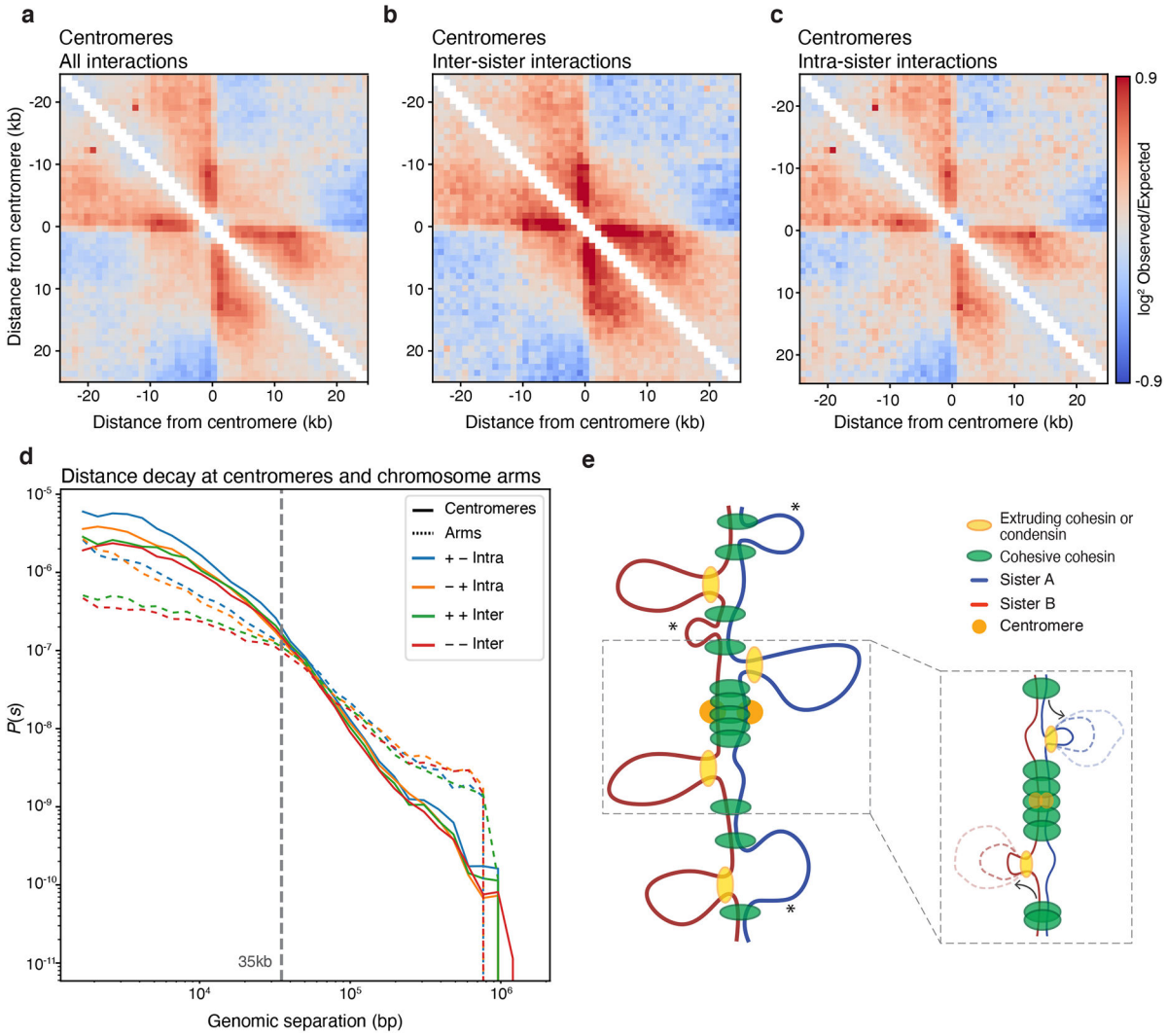

Figure 3 –. SisterC data show that centromeres are more precisely and closely aligned than loci along chromosome arms.

(**a-c**) Pile up plot on all yeast centromeres at 1kb resolution of all interactions (**a**), inter-sister interactions (**b**) and intra-sister interactions (**c**). (**d**) Distance decay of interactions anchored on centromeres shows inter- and intra-sister interactions are very similar for all genomic distance, while interactions along the chromosome arms show reduced inter-sister interaction for loci separated by up to 35kb (dashed lines; dashed vertical line indicates the 35 kb offset). (**e**) Proposed model of centromeric sister chromatid conformation where closely spaced binding by cohesin and condensin molecules mediate tight and aligned interactions of the sister chromatids at the centromere.

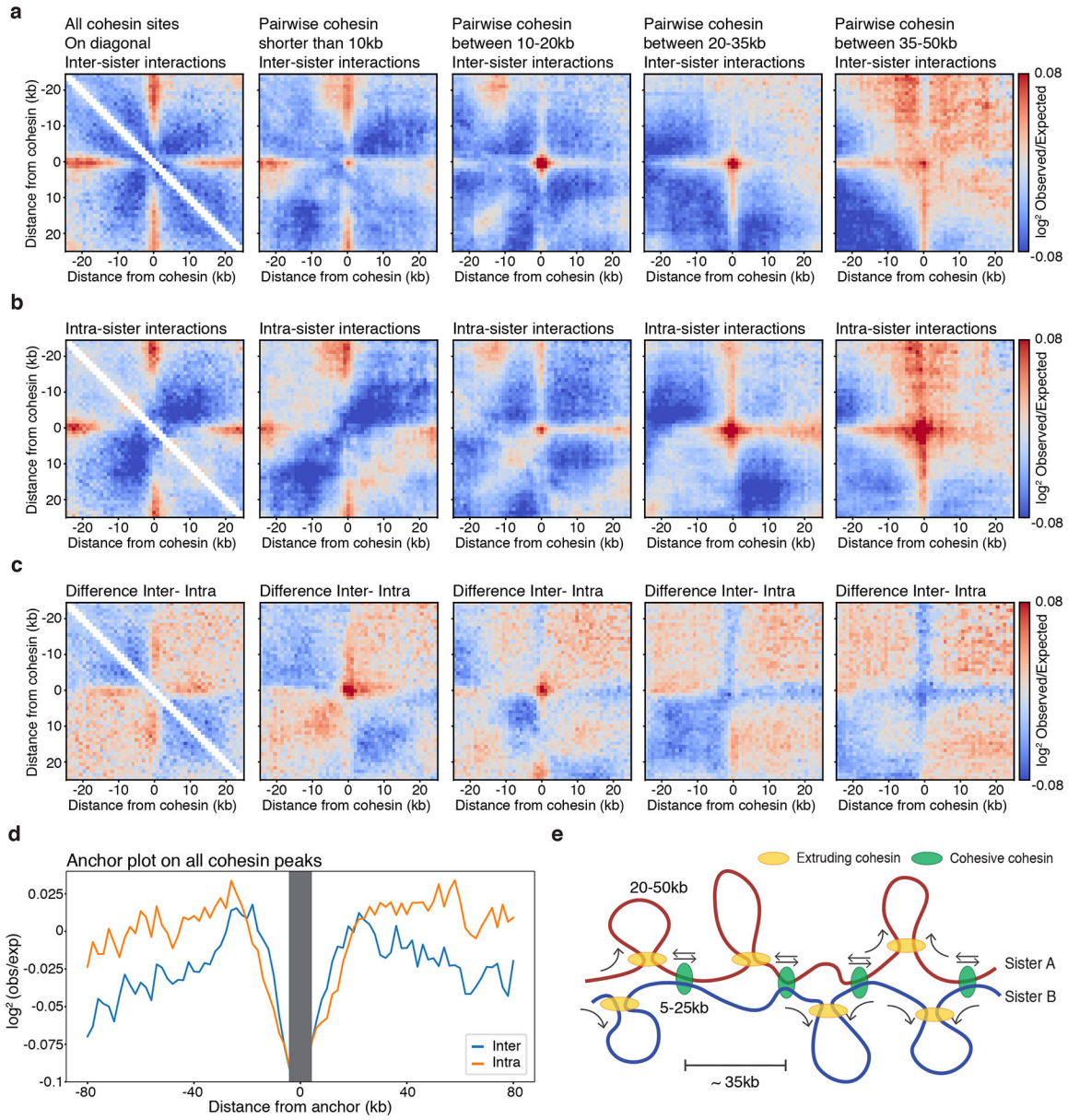

Figure 4 –. SisterC shows that cohesive cohesin mediates interactions between sister chromatids at shorter genomic distances than loops formed within sister chromatids by extruding cohesin.

(**a-b**) Aggregate pile up plot of inter-sister interactions (**a**), intra-sister interactions (**b**) and the difference between inter-sister and intra-sister interactions (**c**) of all cohesin sites (left panel), of all pairwise cohesin interactions for pairs separated by less than 10kb (second to left panel), all pairwise interactions for sites separated by 10–20kb (middle panel), all pairwise interactions between cohesin sites separated by 20–35kb (second to right panel), and for cohesin sites separated by 35–50kb (far right panel). (**d**) Anchor plot of all cohesin binding sites show that inter-sister interactions are preferentially formed at distances shorter than 30kb, whereas intra-sister interactions at cohesin sites predominantly interact with sites further than 25kb away. (**e**) Proposed model of intra-sister interactions formed by extruding cohesin and inter-sister interactions formed by cohesive cohesin. Loops of different sizes are

made within sisters by extruding cohesin with loop sizes ranging from 10–50 kb. Inter-sister interactions are mediated by cohesive cohesin, are spaced by ~35 kb apart, and occur between sites that can be off-set by 5–25 kb.

Table 1 -

Table of nucleotide content and percentage inter-sister interactions in each SisterC replicate performed in this study and in the budding yeast genome SacCer3 as comparison.

	Release in:	G/C%	A/T/BrdU%	BrdU% in new strand	% inter sister in G1	% inter sister in mitosis
SacCer3		37.7%	62.3%			
HindIII R1	Thymidine	36.0%	64.0%	0%	50.2%	50.2%
	BrdU	36.7%	63.3%	82.7%	24.9%	41.9%
DpnII R1	Thymidine	n/a	n/a	n/a	49.8%	49.9%
	BrdU	n/a	n/a	n/a	21.1%	37.6%
DpnII R2	Thymidine	35.3%	64.7%	0%	49.6%	49.7%
	BrdU	37.0%	63.0%	98.1%	20.1%	38.1%
DpnII R3	Thymidine	36.3%	63.7%	0%	49.8%	49.8%
	BrdU	36.2%	63.8%	93.6%	21.0%	34.1%