Methods

Strains: Unless otherwise stated, *C. elegans* strains were grown on NG2 medium inoculated with OP50 bacterial strain at 22.5°C. Following strains were used in the current study:

| Strain        | Genotype                                                                                                                                                                                                 | Reference         |
|---------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------|
| N2            | Wild-type Bristol strain                                                                                                                                                                               | CGC               |
| JK574         | fog-2(q71) V.                                                                                                                                                                                             | CGC               |
| TY2205        | xol-1(y9) X, sdc-3(y126) her-1(e1520) V.                                                                                                                                                               | Csankovski.2009a  |
| PMW162        | *ubsSi12*[baf-1::unc-543' UTR::rex-1•33; Cb unc-119] @ttTi5605 II. unc-119(ed3) III.                                                                                                                   | This study        |
| PMW288        | *ubsSi16*[baf-1::mCherry::unc-54 3' UTR::rex-1•33; Cb unc-119] @ttTi5605 II. unc-119(ed3) III.                                                                                                           | This study        |
| BN372/PMW337  | *ubsSi16*[baf-1::mCherry::unc-54 3' UTR::rex-1•33mA1; Cb unc-119] @ttTi5605 II. unc-119(ed3) III.                                                                                                          | This study        |
| PMW253        | *oxTi483* [256xLacO; Cbr-unc-119(+); PuroR] X. gwl539*[baf-1::gfp-lacI::let-858 3'UTR; vit-5::GFP] unc-119(ed3) III.                                                                                     | This study        |
| PMW278        | *oxTi481* [256xLacO; Cbr-unc-119(+); PuroR] I. gwl539*[baf-1::gfp-lacI::let-858 3'UTR; vit-5::GFP] unc-119(ed3) III.                                                                                  | This study        |
| BN195         | *bqSi195*[pBN65(unc-119(+); hsp16.41p::dam::myc::lmn-1)] II.                                                                                                                                           | Towbin 2012        |
| BN196         | *bqSi196*[pBN67(unc-119(+); hsp16.41p::gfp::myc::dam)] II.                                                                                                                                             | Towbin 2012        |
| BN208         | *bqSi208*[pBN69(unc-119(+); hsp16.41p::gfp::myc::mel-28)] II.                                                                                                                                       | This study        |

Identification of male embryos with autosomal lacO insertion was achieved by crossing males carrying a lacO-tagged array on the X (gwl54, Meister et al., 2010) to hermaphrodites with the autosomal lacO insertion. Embryos with a single lacO are cross progeny males. rex1•33 (5'-GGCTGCGGGTAATTGGGCAGGGAAAGAAGAAT) and rex-1•33mA1 (5'-GGCTGCGGGTAATTGGGTTTTTTAAAAGAAGAAT) sequences used in PMW162, PMW288 and PMW337 were taken from McDonel et al., 2006.

Probe Preparation for DNA FISH: Fosmid with 20-40kb region corresponding to the loci of interest was selected from a library containing the entire genome of *C. elegans* and amplified using Φ29 DNA polymerase overnight with amine-labeled dUTP. After sonication into 300-500 pb fragments, amplified DNA is crosslinked to NHS-ester of the selected dye (ATTO-488/565/647N). Dye coupled DNA fragments are then purified using a PCR clean up kit (Promega). Amount of probe and dye incorporation were estimated using a Nanodrop and then precipitated using 1 µl of tRNA (10 mg/ml) and 1 µl of Oyster glycogen (20 mg/ml). The precipitate is dissolved in deionized formamide to obtain a final concentration of 100 ng/ul probe. Probes were diluted to a final concentration of 2-3 ng/ul in 15 µl deionized formamide. FISH is carried out as in Meister et al., 2010. To ensure an equal number of XO and XX embryos on the slide, a fog-2(q71) male/female strain was used for FISH. The sex of the
embryos was determined by the number of X chromosomes using an X-specific probe (fos2 in Fig. 1C). For each individual probe/probe pair, the FISH experiment was repeated at least twice and for each experiment at least three different embryos for each sex/genotype were analyzed, except for rex-28 where the experiment was performed once.

Microscopy and Data analysis: For FISH, z-stacks of individual embryos were acquired using a Leica SP5/SP8 confocal microscope with a 60X 1.2 NA water/oil lens with 0.25 µm distance between individual slices. Individual stacks were then denoised using the PureDenoise plugin for ImageJ. The point picker plugin in ImageJ was used to measure inter-locus distances and analyze radial positions using DAPI staining as a marker for the nuclear periphery. Preparation of embryos for life imaging was carried out as described in (Askjaer et al. 2014). A spinning disc confocal microscope (FEI Andromeda) was used to image embryos with a 60X 1.4 NA oil objective. Z stacks with a slice separation of 200 nm were acquired using an EM-CCD camera (Andor iXon). Three zone scoring was used to analyze radial positions using unbound GFP-lacI signal to mark the nuclear shape. χ², Kolmogorov-Smirnov and Fishers tests were carried out in Excel or R. For live imaging, the experiment was performed at least twice and a minimum of 8 different embryos were imaged and analyzed.

RNAi: RNAi was conducted as in Towbin et al., 2012 using the Ahringer library clone for sdc-2 (C35C5.1, Source Bioscience) and dpy-27 (R13G10.1, Source Bioscience).

DamID: Worm strains expressing dam fusions under transcriptional control of the uninduced hsp-16.41 promoter, with either lamin (lmn-1, dam fused at the N terminus), nuceloporin (mel-28, dam fused at the N terminus) and GFP (free diffusible dam fused at the C terminus) were grown at 25°C on NG plates seeded with GM48 E. coli. Males and hermaphrodites L4 larvae were picked and transferred to plates without bacteria one hour before pooling 10 animals in a single tube containing 1µl of lysis buffer (10mM TrisAc, 10 mM MgAc, 50 mM KA, 0.67% Tween20, 0.67% Igepal), and snap frozen in liquid nitrogen. Additional 2 µl of lysis buffer with 1mg/ml Proteinase K was added and worms were lysed 4 hours at 42°C, followed by heat inactivation at 95°C for 15’. Methylated GATC were digested with 1U DpnI before ligation of DamID adapter (van Steensel et al. 2001) and amplification of ligated products by PCR using a single primer (5’-NNNNGTGGTCGCGGCCGAGGATC) complementary to the adapter with Advantage polymerase (Clontech) for 26 cycles. 2 tubes (20 worms) were pooled together and the resulting PCR product was purified, end repaired (End-It, Epicentre) and 3’-A-overhangs were added (Klenow fragment 3’->5’ exo-). Y-shaped Illumina adapters were ligated and the ligation product was PCR amplified using index-containing primers for multiplexing. Library was purified using AMPure XP 1.8 beads and subjected to HT sequencing in a HiSeq 2500 platform (Illumina, iGE3 Geneva). DamID adapters were removed from reads using cutadapt (Martin, 2011) using parameters -g CGCGGCGGAG -e 0.15. Resected reads were mapped to ce10 genome using bowtie, with parameters -m 1 –best. Bowtie output was then further analyzed using custom-made scripts in R (RCoreTeam, 2013), available upon request. In brief, unique mapped reads were assigned to individual GATC in the genome. Taking the results from 2 independent biological experiments with an average of 59’000 DamID reads per experiment (range 12’783-161’159), DamID reads per 100 kb regions were normalized to the total number of DamID reads. The signal was then further normalized to free dam (GFP-dam). Data presented is the average of the independent replicates. Libraries and processed data can be accessed online at GEO, accession GSE56270.
**Polymer modeling:** A chromosome (X or autosome) is modeled by self-avoiding bead-and-spring chain containing 100 beads. Each bead corresponds to about 180 kb and has an effective radius of 125 nm, consistent with a 30nm-chromatin fiber of density 0.1. The polymer is confined into a cubic box of typical size L where one of the cube faces represents the nuclear membrane. The first and last beads of the chain represent telomeres and are assumed to be always in contact with the envelope (Ferreira et al. 2013). In addition to steric interactions, we consider two types of interactions: 1) intra-chain non-specific interactions between beads accounting for cross-linking with condensin; 2) specific interactions between beads and discrete sites at the nuclear membrane accounting for putative anchoring of the rex sites to specific perinuclear sites. Dynamical properties of the chain were sampled using molecular dynamics simulations (for details about the force fields and the simulations, see the supporting materials). Each simulation started from a random compact conformation representing the post-mitotic chromosome state. From the simulations, we estimated the time evolution of the mean radius of gyration that represents the typical size of the chromosome, and the mean distance to the membrane.

**Model**
A chromosome is modeled by a self-avoiding bead-and-spring chain containing 100 beads. Each bead corresponds to about 180 kb (that implicitly defines our mass unit). Assuming a 30 nm-chromatin fiber and a spherical nucleus of diameter D=2.5 μm, conservation of the chromatin volume imposes a bead diameter of 125 nm. The polymer is confined into a periodic cubic box of typical size L (see Fig.4A of the main text). One face of the cube (z=0) represents the nuclear membrane and is impenetrable. We fix L=4.4 μm to simulate the nuclear membrane surface (L^2=πD^2). The chain is tethered at both ends to the surface. In absence of interaction with anchoring sites, the chain dynamics is driven by the Hamiltonian $H=H_{FENE}+H_{LJ}+H_{SURF}$ where:

- **$H_{LJ}$** models excluded volume interactions and attractive interactions between beads and is given by a truncated and shifted Lennard-Jones potential:
  $$H_{LJ} = \sum_{i\neq j} \left\{ 4\varepsilon \left[ \left( \frac{\sigma}{r_{ij}} \right)^{12} - \left( \frac{\sigma}{r_{ij}} \right)^{6} \right] + C, r_{ij} \leq 2^{7/6}\sigma \\
  0, r_{ij} > 2^{7/6}\sigma \right\}$$
  with $r_{ij}$ the distance between beads $i$ and $j$ and $C$ being chosen such that the pair-wise potential is continuous at the cut-off distance. We choose $\sigma=125$ nm and $\varepsilon=1$ as our unit of length and energy.

- **$H_{FENE}$** models the polymeric interactions between neighboring beads along the chain and is given by a FENE potential describing finitely extensible spring (Kremer and Grest, J. Chem. Phys. 92, 5057 (1990)):
  $$H_{FENE} = \sum_{i} \left\{ \frac{1}{2} k R_0^2 \ln \left( 1 - \frac{r_{ii+1}^2}{R_0^2} \right), r_{ii+1} < R_0 \right\}_{\infty, r_{ii+1} > R_0}$$
  with $k=30\varepsilon$ and $R_0=1.5\sigma$.

- **$H_{SURF}$** models the impenetrability of the membrane surface and is given by a Lennard-Jones like potential that we have truncated at its minimal value and shifted to insure continuity of the potential:
  $$H_{SURF} = \sum_{i} \left\{ 4\varepsilon_s \left[ \frac{1}{5} \left( \frac{\sigma_s}{z_i} \right)^{10} - \frac{1}{2} \left( \frac{\sigma_s}{z_i} \right)^{4} \right] + C', z_i \leq \sigma_s \right\}$$
  with $C'$ and $\sigma_s$ being chosen to give the correct surface tension and radius of the spherical nucleus.
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with \( z_i \) the distance between the membrane and bead \( i \), \( \epsilon_s = \epsilon \) and \( \sigma_s = 0.5\sigma \). The shape of this potential is justified as the result of integrating a standard 12-6 Lennard-Jones potential between beads and putative membrane atoms over a two-dimensional plane.

In presence of interactions with anchoring sites located onto the membrane surface, the chain dynamics follows the Hamiltonian \( H_{TOT} = H_P + H_{ANCHOR} \) in the nuclear pore hypothesis (sparse and discrete anchors) or \( H_{TOT} = H_{FENE} + H_{LJ} + H_{SURF} \) in the lamina hypothesis (dense and continuous anchors) where

- \( H_{ANCHOR} \) models the interaction between beads and discrete sites and is given by a truncated and shifted Lennard-Jones potential:
  \[
  H_{ANCHOR} = \sum_a \sum_i \left( 4\epsilon_a \left( \frac{\sigma_a}{r_{ia}} \right)^{12} - 4\epsilon_a \left( \frac{\sigma_a}{r_{ia}} \right)^6 \right) + C'', r_{ia} \leq 2^{7/6}\sigma_a
  \]
  with \( r_{ia} \) the distance between bead \( i \) and anchor \( a \) and \( \sigma_a = 0.65\sigma \) (≈80nm the typical radius of a pore). Anchors are assumed to be immobile but randomly distributed. Their number was fixed to 60, corresponding to a surface density of about 3 anchors per \( \mu \)m\(^2\).

- \( H_{SURF} \) models both the interactions with the continuous anchors as well as the impenetrability of the surface, and is given by a truncated and shifted Lennard-Jones-like potential:
  \[
  H_{SURF} = \sum_i \left( 4\epsilon_s \left[ \left( \frac{\sigma_s}{z_i} \right)^{10} - \frac{1}{2} \left( \frac{\sigma_s}{z_i} \right)^4 \right] + C''', z_i \leq 3\sigma_s \right)
  \]
  with \( \sigma_s = 0.5\sigma \).

**Simulations**

Dynamics of the polymer chain was simulated using a homemade Molecular Dynamics program. It implements a standard velocity Verlet algorithm coupled to an Andersen thermostat (Frenkel and Smit, *Understanding Molecular Simulations*, Academic Press). Tethered beads 1 and 100 were constrained to move only in the (z=0)-plane. Periodic boundary conditions were used in the x and y directions. The integration step was set to 0.002\( \tau \) as a compromise between accuracy and rapidity, with \( \tau \) the time unit.

Each simulation first starts by generating a compact "post-mitotic-like" conformation. This step is performed by integrating the polymer dynamics during 1,000\( \tau \) under \( H_P \) only and at a temperature \( T=0.5 \) (bad solvent regime). After this initialization, the polymer properties were monitored during 2,000\( \tau \):

- Autosomes were simulated using \( H_P \) at a temperature \( T_{auto}=6 \) (good solvent regime).
- X chromosomes in hermaphrodites were simulated using also \( H_P \) but for temperatures smaller than \( T_{auto} \) (\( \Theta \)-collapse and bad solvent regime) to account for DCC-mediated crosslinks.
- X chromosomes in males were simulated using \( H_{TOT} \) or \( H_{TOT} \) at a temperature \( T_{auto} \) for different values of \( \epsilon_a \) or \( \epsilon_s \) to account for interactions between chromosome and anchoring sites located at the nuclear periphery. Note that in the nuclear pore hypothesis the positions of the 60 pores onto the membrane were randomly generated before each simulation.
For each parameter set, we run 1,000 simulations. From them, we computed the time evolution of the mean values for the radius of gyration \( R_G \) and for the distance to the membrane \( Z \), with

\[
\begin{align*}
R_G^2 &= \frac{1}{2 \times 100^2} \sum_{i,j} r_{ij}^2 \\
Z &= \frac{1}{100} \sum_i z_i.
\end{align*}
\]

**Physical interpretation of the X chromosome compaction**

Our model suggests that the compaction of the X chromosome observed in hermaphrodites can be simply regarded as the \( \Theta \)-collapse of a polymer chain from a good solvent regime (coil phase) to a bad solvent regime (globular phase) (Doi and Edwards, *Theory of polymer dynamics*, Oxford University Press). This corresponds to a relative increase of pairwise interactions between individual beads due to DCC loading and spreading along the chromosome.

The compaction of the X chromosome in males is more subtle. We showed that this effect could be observed in the nuclear pore hypothesis but not in the lamina hypothesis. Indeed, in the lamina hypothesis, increasing the interaction with the surface leads to a wetting transition (Metzger, Muller, Binder and Baschnagel, J. Chem. Phys., **118**, 8489 (2003)). The polymer becomes 2D and spreads easily and quickly onto the surface (Fig. S4B), leading to less compact structures than autosomes. In the nuclear pore hypothesis, for weak interactions with the anchors, the polymer still spreads (Fig. S4C). However for strong interactions, there is a critical slowing-down of the polymer dynamics that becomes glassy-like (Jost and Vaillant, manuscript in preparation). Therefore, the chain stays trapped in compact conformations, signature of the initial compact (post-mitotic) state (Fig. S4C). Indeed, in this case, since beads that interact with the pores are strongly bound and since typical distances between pores (\( \approx 4.5\sigma \)) are greater than the bead diameter, it becomes unlikely for the polymer to explore extended conformations that would imply the cooperative dissociation of many beads.

**Supporting material references**

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Martin M. 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnetjournal* **17**: 10-12.
**Supplementary Figures legends**

**Figure S1.** Representative images for *rex-33/fos1* 2-color FISH (partial projections of z-stacks) in hermaphrodite (A) and male (B) embryos. DNA was stained with DAPI to define nuclear boundaries.

**Figure S2.** GRO-seq profiles in wild-type and *sdc-2(RNAi)* embryos in the vicinity of *lacO* insertions on an autosome (*A, oxTi481 I.*) or on chromosome X (*B, oxTi483 X.*). Upper strand transcripts coverage is shown in black, lower strand in red.

**Figure S3:** Boxplot representation of measured inter-probe distances. (A) Probes used to assess chromatin compaction and X chromosome localization. Two color FISH was performed with following combinations: *rex-31/rex-35* (4 Mb, X, black line), *rex-32/rex-23* and *rex-33* and *fos1* (1 Mb, black and grey lines, respectively), as well as two pairs of control loci on chromosome III and V (1 and 4 Mb, green lines). (B) Inter-locus distances were measured on z axis projections and normalized to the nuclear diameter. (C) (bottom panel) Box plots representing normalized inter-locus distances (Nd) for 1Mb probes combinations described in A. (Kolgomorov-Smirnov (ks) test for both 1Mb distances between males and hermaphrodites on *chr X*: p < 10^-3, n>190; on chromosome III and V: p > 0.4, n>110; same data as Fig. 3C,E. (top panel) Key denoting the representation of the data in the box plots. Boxes with solid outlines and dashed outlines show distances in hermaphrodites and males respectively. Different genomic regions are marked with a color code. (D) (bottom panel) Boxplots representing normalized inter-locus distances for 4 Mb probes combinations described in A. (ks for 4Mb on *chr X* and *chr V* between males and hermaphrodites: p=0.049, 0.25, respectively; same data as Fig. 3D). (top panel) same as C top panel. (E) Boxplots representing non-normalised distances (d) in nanometers for the same combinations as in C. (F) Boxplots representing non-normalised distances (d) in nanometers for the same combinations as in D. Refer to suppl. Table 1 for statistics and sample size.

**Figure S4.** Time evolution of the normalized mean radius of gyration <R_g> (left) and distance to the membrane <Z> (right). Normalization is performed by dividing by the corresponding autosome value in steady state (t=∞). (A) Autosome (dots) vs X chromosome in hermaphrodite (no anchoring interaction and temperature *T*< *T*_{auto}=6). (B) Autosome (dots) vs X chromosome in male in the lamina hypothesis (anchoring interaction *ε_s* and *T*=*T*_{auto}). (C) Autosome (dots) vs X chromosome in male in the nuclear pore hypothesis (anchoring interaction *ε_a* and *T*=*T*_{auto}).
**Supplementary table 1. Sample size and statistical tests**

For 3 zones scoring, $\chi^2$ is calculated for zone 1 and the sum of 2 and 3 versus random (33% zone 1, 66% zone 2 and 3, taking into account sample size).

| Strain | C site | sex | Total spots counted (n) | Total embryos counted | Age of the embryos (# Cells) (Range: 40<n<150 cells) | Expt | p values vs random | significance (ns: p>0.05, *: p<0.05, ** p<0.01) | p values for Fisher exact test (zone 1 males vs herm) |
|--------|-------|-----|------------------------|-----------------------|-----------------------------------------------|-----|-------------------|-----------------------------------------------|--------------------------------------------------|
| *fog-*2 | X | rex32 | Herm | 203 | 5 | 80, 84, 96, 73, 63 | 3 | 4.7E-09 | ** | \(3.061E-11\) |
| *fog-*2 | X | rex32 | Male | 110 | 5 | 45, 73, 68, 60, 90 | 2 | 8.5E-10 | ** | \(7.657E-10\) |
| *fog-*2 | X | rex32 | Herm | 209 | 5 | 80, 84, 96, 73, 63 | 3 | 1.0E-06 | ** | \(3.026E-09\) |
| *fog-*2 | X | rex33 | Male | 131 | 5 | 45, 73, 68, 60, 90 | 2 | 4.5E-12 | ** | \(0.0257\) |
| *fog-*2 | X | rex33 | Herm | 326 | 6 | 83, 48, 66, 47, 96, 104 | 3 | 1.7E-01 | ns | \(0.9084\) |
| *fog-*2 | X | rex33 | Male | 225 | 6 | 74, 45, 98, 69, 78, 75 | 3 | 2.4E-15 | ** | \(3.061E-11\) |
| *fog-*2 | X | fos1 | Herm | 258 | 5 | 42, 89, 112, 96, 104 | 3 | 3.5E-02 | * | \(7.657E-10\) |
| *fog-*2 | X | fos1 | Male | 218 | 6 | 91, 92, 96, 118, 78, 75 | 2 | 1.0E-11 | ** | \(7.657E-10\) |
| *fog-*2 | X | rex28 | Herm | 100 | 2 | 75, 50, 45, 68 | 1 | 1.3E-02 | * | \(0.01788\) |
| *fog-*2 | X | rex28 | Male | 68 | 4 | 75, 50, 45, 68 | 2 | 4.1E-08 | ** | \(0.01788\) |
| *fog-*2 | X | rex8 | Herm | 346 | 6 | 78, 91, 125, 108, 104, 117 | 2 | 2.0E-02 | * | \(3.026E-09\) |
| *fog-*2 | X | rex8 | Male | 276 | 6 | 120, 105, 111, 96, 108, 105 | 2 | 8.8E-10 | ** | \(0.7613\) |
| *fog-*2 | X | rex31 | Herm | 238 | 4 | 140, 150, 95, 70 | 3 | 4.4E-13 | ** | \(0.9084\) |
| *fog-*2 | X | rex31 | Male | 112 | 5 | 72, 44, 52, 100, 42 | 3 | 2.1E-06 | ** | \(0.04391\) |
| *fog-*2 | X | fos2 | Herm | 120 | 7 | 40, 97, 140, 47, 73, 63 | 2 | 2.0E-02 | * | \(0.163\) |
| *fog-*2 | X | fos2 | Male | 129 | 7 | 72, 75, 44, 50, 60, 90, 45 | 3 | 3.0E-06 | ** | \(0.04391\) |
| *fog-*2 | X | rex35 | Herm | 195 | 3 | 140, 150, 93 | 3 | 5.3E-39 | ** | \(0.04391\) |
| *fog-*2 | X | rex35 | Male | 94 | 4 | 72, 44, 100, 42 | 2 | 1.2E-21 | ** | \(0.7613\) |
| *fog-*2 | V | Herm | 287 | 5 | 50, 70, 116, 80, 110 | 2 | 3.7E-02 | * | \(0.04391\) |
| Ty22 V | rex33 | XO | Herm | 222 | 6 | 98, 98, 88, 105, 64, 60 | 2 | 1.3E-17 | ** | \(0.7613\) |
| Ty22 V | fos1 | XO | Herm | 196 | 6 | 98, 98, 88, 105, 64, 60 | 2 | 3.7E-11 | ** | \(0.7613\) |
| Ty22 V | XO | Herm | 107 | 4 | 45, 68, 45, 90 | 1 | 1.7E-01 | ns | \(0.7613\) |
| gene          | cross           | samples | mean  | std. mean | p-value | significance |
|---------------|-----------------|---------|-------|-----------|---------|--------------|
| **fog-2**     | X rex33 Herm    | 326     | 6     | 83, 48, 66, 47, 96, 104 | 3       | 1.7E-01 ns   |
| Ty22          | X rex33 XO     | 222     | 6     | 98, 98, 88, 105, 64, 60 | 2       | 1.3E-17 **   |
| **fog-2**     | X rex33 Male    | 225     | 6     | 74, 45, 98, 69, 78, 75 | 3       | 2.4E-15 **   |
| Ty22          | X rex33 XO     | 222     | 6     | 98, 98, 88, 105, 64, 60 | 2       | 1.3E-17 **   |
| **fog-2**     | X fos1 Herm     | 258     | 5     | 42, 89, 112, 96, 104 | 3       | 3.5E-02 *    |
| Ty22          | X fos1 XO      | 196     | 6     | 98, 98, 88, 105, 64, 60 | 2       | 3.7E-11 **   |
| **fog-2**     | X fos1 Male     | 218     | 6     | 91, 92, 96, 118, 78, 75 | 2       | 1.0E-11 **   |
| Ty22          | X fos1 XO      | 196     | 6     | 98, 98, 88, 105, 64, 60 | 2       | 3.7E-11 **   |
| **fog-2**     | V Herm          | 287     | 5     | 50, 70, 116, 80, 110 | 2       | 3.7E-02 *    |
| Ty22          | V XO            | 107     | 4     | 45, 68, 45, 90 | 1       | 1.7E-01 ns   |
| **fog-2**     | V Male          | 190     | 6     | 47, 70, 45, 78, 49, 82 | 3       | 3.8E-01 ns   |
| Ty22          | V XO            | 107     | 4     | 45, 68, 45, 90 | 1       | 1.7E-01 ns   |
| PMW           | II rex1-33 Herm | 287     | 8     | 82, 71, 58, 99, 45, 78, 96, 80 | 2       | 2.8E-01 ns   |
| PMW           | II rex1-33 Male | 414     | 10    | 70, 45, 42, 58, 63, 83, 48, 78, 47, 100 | 2       | 2.6E-04 **   |
| PMW           | II rex1-33 Herm | 471     | 7     | 100, 80, 134, 104, 100, 112, 81 | 2       | 4.3E-04 **   |
| PMW           | II rex1-33 Male | 528     | 8     | 68, 90, 123, 105, 72, 70, 96, 81, 110 | 2       | 3.0E-08 **   |
| PMW           | II rex1-33mut  | 339     | 6     | 76, 84, 114, 120, 105, 111 | 2       | 1.6E-02 *    |
| PMW           | II rex1-33mut  | 493     | 9     | 133, 90, 115, 81, 99, 100, 108, 104, 125 | 3       | 1.2E-04 **   |

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| Strain   | Chr | Site        | Sex   | RNAi | Total spots counted (n) | Total embryos counted | Age of the embryos (# Cells) | Expt | p values vs random | significance (ns: p>0.05, *: p<0.05, ** p<0.01) | p values for Fisher exact test (zone1 between lines) |
|----------|-----|-------------|-------|------|-------------------------|-----------------------|-----------------------------|------|-------------------|-----------------------------------------------|-------------------------------------------------|
| PMW 253  | X   | oxiTi 483   | Herm  | -    | 434                     | 20                    | 40-150                       | 2    | 8.7E-01           | ns                                             | 0.003                                           |
| PMW 253  | X   | oxiTi 483   | Male  | -    | 424                     | 22                    | 40-150                       | 4    | 2.8E-05           | **                                            |                                                 |
| PMW278   | I   | oxiTi 481   | Herm  | -    | 424                     | 14                    | 40-150                       | 2    | 4.0E-12           | **                                            | 0.06                                            |
| PMW278   | I   | oxiTi 481   | Male  | -    | 287                     | 13                    | 40-150                       | 2    | 3.0E-14           | **                                            |                                                 |
| PMW 253  | X   | oxiTi 483   | Herm  | L4440| 876                     | 32                    | 40-150                       | 4    | 7.2E-01           | ns                                             | 2.2E-10                                         |
| PMW 253  | X   | oxiTi 483   | Herm  | sdc-2| 587                     | 17                    | 40-150                       | 2    | 1.5E-16           | **                                            |                                                 |
| PMW 253  | X   | oxiTi 483   | Herm  | dpy-27| 632                    | 25                    | 40-150                       | 2    | 9.2E-05           | **                                            | 0.002                                           |
| PMW278   | I   | oxiTi 481   | Herm  | L4440| 916                     | 25                    | 40-150                       | 3    | 1.8E-51           | **                                            |                                                 |
| PMW278   | I   | oxiTi 481   | Herm  | sdc-2| 518                     | 16                    | 40-150                       | 2    | 1.3E-35           | **                                            | 0.51                                            |
| PMW278   | I   | oxiTi 481   | Herm  | dpy-27| 370                    | 8                     | 40-150                       | 1    | 2.0E-22           | **                                            | 0.91                                            |
| fog-2    | X   | 1           | rex 32-23 | Herm | 221                     | 6                     | 80, 84, 96, 73, 63, 47       | 3    | 1.6E-04           |                                               |                                                 |
| fog-2    | X   | 1           | rex 32-23 | Male | 191                     | 6                     | 45, 73, 68, 60, 90, 52       | 2    |                  |                                               |                                                 |
| fog-2    | X   | 1           | rex 33- fos1 | Herm | 316                     | 5                     | 50, 96, 110, 104, 112       | 2    | 1.4E-12           |                                               |                                                 |
| fog-2    | X   | 1           | rex 33- fos1 | Male | 281                     | 7                     | 78, 75, 50, 64, 60, 71, 108 | 2    |                  |                                               |                                                 |
| TY2205   | X   | 1           | rex 33- fos1 | XO Herm | 261                    | 6                     | 98, 98, 88, 105, 64, 60     | 2    |                  |                                               |                                                 |
| fog-2    | V   | 1           | 226-227   | Herm | 111                     | 5                     | 50, 70, 116, 80, 110        | 2    | 9.1E-01           |                                               |                                                 |
| fog-2    | V   | 1           | 226-227   | Male | 167                     | 9                     | 47, 70, 45, 78, 49, 42, 41, 82, 178 | 3    |                  |                                               |                                                 |
| fog-2    | III | 1           | 315-316   | Herm | 327                     | 5                     | 154, 94, 104, 90, 106       | 2    | 4.2E-01           |                                               |                                                 |
| fog-2    | III | 1           | 315-316   | Male | 354                     | 5                     | 125, 150, 156, 104, 58      | 2    |                  |                                               |                                                 |
| fog-2 | X  | 4  | rex 31-35 | Herm | 307 | 4  | 140, 200, 93, 200 | 3 | 4.9E-02 |
|-------|----|----|-----------|------|-----|----|-----------------|---|--------|
| fog-2 | X  | 4  | rex 31-35 | Male | 212 | 5  | 72, 44, 200, 100, 42 | 3 |         |
| fog-2 | V  | 4  | 237-248   | Herm | 273 | 5  | 82, 89, 116, 79, 82 | 2 | 2.5E-01 |
| fog-2 | V  | 4  | 237-248   | Male | 340 | 5  | 96, 116, 132, 83, 100 | 2 |         |
GENESDEV/2014/248864 Sharma et al., Suppl. Figure 2

A

GRO-seq reads plus strand minus strand

oxTi481

WT

sdc-2

chr1

7510 7520 7530 7540 7550

Position (kb)

chrI

oxTi483

WT

sdc-2

chrX

12390 12400 12410 12420

Position (kb)
