Supplementary Figures legend

Supplementary Figure 1. BACs replication in egg extract (**a**) L10 DNA BAC or Sperm chromatin incubated in *Xenopus* egg extract with ^{32}PdCTP. DNA synthesis was monitored by measuring the percentage of radiolabelled nucleotide incorporation relative to the input DNA. Error bars represent ± sd of the mean. *n*=3 independent experiments; $p<0.05$ when comparing L10 and B18 mean values; unpaired two-tailed t-test. (**b**) L10 BAC was replicated in egg extract in the presence of geminin or roscovitine and ^{32}PdCTP. Incorporation of radiolabelled nucleotides was monitored by autoradiography and relative intensities were plotted on the graph, considering maximum values as 1. Error bars represent ± sd of the mean. *n*=3 independent experiments; $p<0.001$ when comparing mean values for all the indicated treatments; One-way Anova. (**c**) Replicating L10 BAC was pulse labelled with ^{32}PdCTP for 20 minutes and samples were fixed and analyzed at the indicated time by autoradiography. Quantification of one representative experiment is shown. (**d**) Chromatin was isolated at different times using L10 BAC DNA incubated in egg extracts treated with geminin or roscovitine and then analyzed by WB as indicated. (**e**) Graph showing incorporation of ^{32}PdCTP in fractions derived from CsCl gradient centrifugation of sperm and L10 BAC DNA replicated in egg extract in the presence of ^{32}PdCTP and BrdUTP. Heavy-Light (HL) and Heavy-Heavy (HH) DNA fractions are indicated. Quantification performed as in (b) from one representative experiment is shown. (**f**) Molecular combing of RP11-1051L10 (L10) and RP11-5B18 (B18) BACs fully replicated in the presence of digoxygenin-dUTP (green). (**g**) Table representing the nomenclature, the size and the chromosome regions of the BACs used in the present study. (**h**) Chromosome mapping of the centromere BACs. (**i**) Graph showing the percentage of alpha-satellite sequences in each centromeric BAC used. (**j**) Graph showing the GC base content in the BAC DNA sequences. (**k**) Replication of B18 and L10 DNA BACs incubated for five hours in *Xenopus* egg extracts supplemented with ^{32}PdCTP. DNA synthesis was monitored by measuring the percentage of radiolabelled nucleotides incorporation relative to the input DNA. Error bars represent ± sd of the mean. *n*=3 experiments; $p<0.001$ when

comparing L10 and B18 mean values; unpaired two-tailed t-test. (**l**) DNA replication of the different BACs DNA described in (g). Average percentage of ^{32}PdCTP incorporation. L10 values were considered as 100%. Error bars represent ± sd of the mean. *n*=3 experiments; p<0.001 when comparing DNA replication mean values for all the indicated BACs; Two-way Anova. (**m**) and (**n**) Control and centromeric chromatin was isolated at the indicated times and then analyzed by WB using the indicated antibodies. (**o**) Analysis of the average inter-origin distance (IODs) relative to at least hundred fibres of DNA in replicated L10 and B18 BACs. Error bars represent ± sd of the mean. *n*=3 experiments; p<0.001 when comparing L10 and B10 mean values; unpaired two tailed t-test.

Supplementary Figure 2. **Proteomic analysis and validation of control and centromeric chromatin.** (**a**) Scheme of MS experiments (see text). (**b**) STRING analysis of DNA repair factors enriched on centromeric chromatin. (**c**) WB analysis of L10 and B18 chromatin isolated at 150 minutes from DNA addition to egg extract and probed with the indicated antibodies. *MSH2* antibodies used here were different from the ones used in Fig 3. Graphs showing *TopBP1* (**d**) and *MSH2* (**e**) relative abudance on control L10 and centromeric B18 chromatin compared to *ATR*. Error bars represent ± sd of the mean. *n*=3 experiments; p<0.05 when comparing L10 and B10 mean values for TopBp1-ATR and MSH2-ATR ratios; One-way Anova.

Supplementary Figure 3. Visualization by EM of L10 and B18 DNA intermediates. (**a**) EM of a segment of L10 DNA isolated after 150 minutes incubation in interphase egg extract. (**b**) EM of intact circular centromeric B18 DNA molecule not incubated in egg extract. (**c**) A typical replication bubble made of doubled stranded DNA (dsDNA). (**d**) Typical ssDNA bubble observed on centromeric b18 DNA. Differences in ssDNA and dsDNA fiber thickness can be appreciated. (**e**) Graph showing the frequency of ssDNA bubbles for each mega base of non-centromeric DNA (L10) or centromeric B18 DNA incubated in egg extracts that were untreated (B18) or treated with geminin (B18 + Geminin). Experiments were repeated three times and one mega base of total DNA was

scored. Error bars represent ± sd of the mean. *n*=3 experiments; p<0.001 when comparing L10, B10 and B10 + Geminin mean values; One-way Anova. (**f**) Typical replication fork intermediate present on B18 DNA.

Supplementary Figure 4. DNA isolation procedure for EM. (**a**) Nuclei were isolated at different times from addition of L10 or B18 BAC DNA to egg extract and subjected to psoralen photo-crosslinking with 365 nm UV light. Chromatin was completely digested with 1 mg ml^{-1} Proteinase K for 2 hours at 37°C. DNA was isolated by phenol:chloroform extraction (25:25 v v^{-1}) and spread in the presence of 1.5 % formamide on carbon coated EM grids, which were subjected to rotary shadowing. (**b**) Scheme showing DNA positive supercoil (+) mediated inhibition of psoralen crosslinking, resulting in ssDNA bubbles after melting in mild denaturing conditions used for the EM preparation.

Supplementary Figure 5. Condensins accumulation and structural arrangement of centromeric chromatin. (**a**) WB analysis showing *SMC2* loading on L10 and B18 chromatin isolated at 150 and 300 minutes from BAC DNA addition to egg extract. *MCM7* was used as loading control (**b**) Graphs showing *SMC2* relative abundance on control L10 and centromeric B18 chromatin compared to *MCM7*. Error bars represent ± sd of the mean. *n*=3 experiments; p<0.01 when comparing L10 and B18 mean values for the indicated times; One-way Anova. (**c**) Tunel assay showing ^{32}PdCTP incorporation catalysed by Terminal Transferase (TdT) on DNA derived from L10 and B18 nuclei isolated from egg extracts that were untreated, treated with topotecan (TPT) or HAEIII restriction enzyme. The latter treatment was used as positive control of double strand breaks formation. Error bars represent ± sd of the mean. *n*=3 experiments; p<0.05 when comparing L10 and B18 mean values for all the indicated treatments; One-way Anova. (**d**) WB analysis showing *SMC2* loading on B18 chromatin isolated at 0 and 300 minutes from addition of DNA to egg extract supplemented with topotecan (TPT) (+) or left untreated (-). *MCM7* was used as loading control. A representative result is shown. (**e**) Modified EM protocol used to analyse BAC chromatin**.** Nuclei were isolated at different times from addition of L10 or B18 DNA to egg extracts and subjected to psoralen

photo-crosslinking with 365 nm UV light. Chromatin was treated with 1 mg ml^{-1} Proteinase K for 5 minutes at 30°C. DNA was isolated by phenol:chloroform extraction (25:5 v v^{-1}) and spread in the presence of 1.5 % formamide on carbon coated EM grids, which were subjected to rotary shadowing. (**f**) WB showing levels of *SMC2* protein present on B18 derived centromeric chromatin following procedures for EM staining performed at low (5 minutes) and high (2 hours) proteinase K treatment (See Methods). A typical result of is shown.

Supplementary figure 6. Whole scans relative to cropped immunoblot images. Red boxes indicate the cropped regions used to assemble images in the present study. Black bars indicate position of size markers (kDa). Some panels include samples not used.

Table titles and legend

Table S1. Total proteins identified by MS. The table contains raw data for proteins and peptides identified by MS.

Table S2. Proteins differentially represented on centromeric and non-centromeric chromatin in biological replicates.
The table contains proteins differentially represented on centromeric and non centromeric DNA (B18_L10) and on centromeric DNA in the absence or in the presence of geminin (B18_B18+) in three biological replicates (Bio1, Bio2, and Bio3).

Table S3. Proteins differentially represented on centromeric and non-centromeric chromatin considered for heatmap. The table contains protein differentially represented on centromeric (B18) and non centromeric (L10) DNA and on centromeric DNA in the presence of geminin (B18+) considered for the heatmap analysis shown in Fig 2c. Color code is described in Fig 2.