Supplementary information

Supplementary Material and Methods

Genotype analysis

Genomic DNA was digested with PstI and PacI enzymes, separated on a 0.7% agarose gel, blotted and hybridized with radiolabelled 5´ internal and 3´ external probes. The internal probe (462 bp) was generated by PCR amplification of genomic DNA using GGTTCCTGTGAGCAAGGTCGTAAGC and CAGAGATTGCCTCACACCCTTCCTGG primers. The external probe (503 bp) was amplified with GCAATTCTCTCTATGTGTCTCTCTAATACTCC and GAATTTTCTTACCTGGAAACCAAGCC primers. For PCR genotyping, a combination of three primers was used (P1: GACTGGTTTAATCCTAGGATAACTTCG, P2: CTACCAGTCTTGGTGTTGATGCAT, P3: TGTGCACATCTTATGGACAGGTGG). Amplified fragments were 279 bp (endogenous Eco2 allele, P1/P3), 347 bp (floxed allele, P2/P3) and 204 bp (Cre-excised allele, P2/P3) long.

Analysis of 2-cell stage embryos

2-cell stage embryos were obtained from timed mating of heterozygous Eco2+/- parents by flushing oviducts of pregnant females 30 hrs post coitum. Flushed embryos were maintained in M-16 medium (Gibco) supplemented with BSA (5 mg mL⁻¹) for additional 4 h and synchronized in the prometaphase stage of the second cell division by supplementing the culture medium with nocodazole (75 ng mL⁻¹). 3 h later, nocodazole-arrested embryos were transferred into the fresh M-16 medium and allowed to reach anaphase (1 h after nocodazole block release). Embryos were fixed in 2% PFA for 30 min and mounted with DAPI containing Vectashield medium (Vector). After image
acquisition using the Leica DMI4000B microscope, the genotype of embryos was confirmed by PCR.

For chromosomes spreads preparation, embryos were treated as described above. Nocodazole arrested embryos were incubated in the 1% sodium citrate for 10 min and spotted in a drop (1-2 µL) of sodium citrate onto the marked area of microscope glass. A drop (3-5 µL) of methanol:acetic acid (3:1) solution was used to spread the prometaphase chromosomes.

**Immunofluorescence and STED microscopy**

6 µm thick frozen or paraffin sections fixed in 4% PFA were repeatedly boiled in citrate buffer for 5 min and incubated in 0.5% Triton X-100 in PBS at room temperature (RT) for 30 min. Incubation in blocking reagent (5% BSA, 5% donkey serum, 0.2% fish skin gelatin in PBS) was carried out at RT for 1 h. The primary antibody was applied overnight at 4°C and the sections were labeled with appropriate secondary donkey antibody conjugated with Alexa Fluor 488 or Alexa Fluor 555 (Invitrogen, 1:500). For immunocytochemistry on cells, mouse embryonic fibroblasts grown on Lab-Tek II chambered cover glasses (Nunc) were fixed with 90% methanol in MeS buffer (100 mM 4-Morpholineethanesulfonic acid, pH 6.9; 1 mM EGTA, 1 mM MgCl₂) for 5 min at 4°C and incubated with blocking reagent (5% BSA, 5% donkey serum in PBS) for 1 h at RT. The remaining steps were conducted as described above. The samples were viewed in inverted Leica DMI4000B or confocal Leica TCS SP5 microscope. Data was acquired and analyzed with Leica Advance Fluorescence software. For STED microscopy PFA-fixed frozen sections were processed as described above, the secondary goat antibody had been conjugated with Atto 647N (Sigma-Aldrich, 1:50). Analysis was done on Leica TCS STED microscope. Data was normalized relative to the background fluorescence.
and quantified using the Leica Advance Fluorescence software and ImageJ.

**Immunofluorescence combined with FISH**

Cortical sections were labeled with the primary and Alexa Fluor 488 conjugated secondary antibodies as described above and post-fixed in 2% PFA for 25 min at RT. After sequential dehydration in 70%, 80%, 95% and 2 x 100% ethanol, slides were air dried and 60µL of hybridization solution (70% formamide, 0.5% blocking reagent [Roche], 10 mM Tris-HCl, pH 7.2, 0.5µM PNA probe TelG-Cy3/TelC-Cy3 [Eurogentec]) were applied on each slide. Following cover slipping, slides were denatured at 70°C for 5 min and incubated at 4°C overnight. Washing was performed in 10 mM Tris-HCl, pH 7.2 containing 70% formamide. Slides were mounted in DAPI containing Vectashield (Vector) and viewed with confocal Leica TCS SP5 microscope. Overlap between the channels was measured in single focal plane using the Leica AF and Image J software.

**Chip analysis**

Cells synchronized for S and G2 phases as described in experimental procedures were sequentially cross-linked with 2 mM Di(N-succinimidyl)glutarate (Sigma-Aldrich) for 30 min and with 1% formaldehyde for 15 min in PBS (pH 7.4) containing 1 mM MgCl2. To remove free cytoplasmic protein, cell were lysed with L1 buffer (50 mM Tris-HCl, pH 8.0, 2 mM EDTA, 0.1% IGEPAL® 630 (Sigma-Aldrich), 10% glycerol, 1 mM dithiothreitol (DTT), supplemented with EDTA-free protease inhibitor cocktail (PIC, Roche), and 1mM PMSF) for 15 min on ice. Nuclei were collected by centrifugation (1500× g for 5 min) and resuspended in sodium dodecyl sulfate (SDS) lysis buffer (50 mM Tris-HCl, pH 8.0, 10 mM EDTA, 1% SDS) at room temperature. Chromatin was sonicated with Bioruptor water bath sonicator (Diagenode) for 25 min with a 30 sec on-
off cycle. The samples were centrifuged (14000×g for 5 min) and soluble chromatin was diluted in 1:9 ratio with low ionic strength ChIP dilution buffer (50 mM NaCl, 10 mM HEPES, pH 7.4, 1% IGEPAL 630, 10% glycerol, 1 mM DTT, 1× PIC and 1mM PMSF) and incubated with antibody overnight at 4°C with constant rotation. Immunocomplexes were captured with Magnetic protein G beads (Dynal), pre-blocked in low ionic strength ChIP dilution buffer in the presence of 5 mg/ml of BSA, using the magnetic stand (Dynal). Beads were sequentially washed twice with low ionic strength ChIP dilution buffer, once with high-salt ChIP wash buffer (500 mM NaCl, 0.1% SDS, 1% IGEPAL 630, 2 mM EDTA, 20 mM Tris-HCl, pH 8.0), once with LiCl ChIP wash buffer (0.25 M LiCl, 1% IGEPAL 630, 1% deoxycholate, 1 mM EDTA, 10 mM Tris-HCl, pH 8.0), twice with TE (10 mM Tris-HCl, pH 8.0, 1 mM EDTA) and transferred to a fresh tube. Decross-linking was performed using 10% (wt/vol) Chelex 100 slurry (Bio-Rad) and Proteinase K digestion as described before (Nelson et al, 2006). DNA concentration of the samples was measured using the Quibit 2.0 Fluorometer (Invitrogen) and was adjusted according to input. qPCR analysis was performed using the Sybr Green and iCycler iQ Real-Time PCR Detection System (Bio-Rad). Data was analyzed with Bio-Rad CFX manager software and presented as fold enrichment relative to the input.

**Primers used from qPCR**

Primers for cohesin binding sites were designed on the basis CTCF/Smc1a Chip on chip data for MEF published previously (Kagey et al, 2010) using Primer 3 software and were tested in silico using the UCSC bioinformatic tools. Sequence of amplicon was further validated by standard sequencing of qPCR product. Primer sequences are as follows:
Chr:11 Forw: 5’-TTAGGACAAAGCTCTGGAAGC-3’
Chr:11 Rev: 5’-AGAATCAGCAAGTACAGTCGGC-3’
Chr: 10 Forw: 5’-ACCTCTCCACACACACATATGG-3’
Chr: 10 Rev: 5’-TTGTCACATCGACACCTCTGC-3’
Chr. 13 Forw: 5’-CCTTTTGAGTTGCATTGCAGG-3’
Chr. 13 Rev: 5’-CTTACTATATGTCAGAATGTCCCATGTC-3’
Chr. 15a Forw: 5’-TTGCAGCCAGAGGACACATTGC-3’
Chr. 15a Rev: 5’-CCCAACATTCACTAGGATGCTTAGG-3’
Chr. 15b Forw: 5’-TCCTTCCTTCTTCTCCAGACC-3’
Chr. 15b Rev: 5’-TTAGGAGAGGAAGGCCTCAGG-3’

For major and minor satellite, rDNA, MmICR (cohesin-bound locus) and telomeres primers described previously were used (Cawthon, 2009; Koch et al, 2008; Martens et al, 2005).

**Determination of chromatin bound Sororin-LAP**

Following the deletion of the floxed Esco2 using AdCre, cells were synchronized in G2 phase with aphidicolin. Efficiency of gene deletion was assessed using PCR. To remove soluble proteins, cells growing on fibronectin coated coverslips (BD Biocoat) were incubated with PBS 0.1% Triton X-100 and fixed using 4% PFA. Image acquisition was performed using semiautomated spinning disc confocal microscope Zeiss / PelkinElmer UltraVIEW VoX with Volocity software. Automated image quantification was done using Definiens image analysis software. Statistical evaluation of obtained data was performed with SPSS software using Mann-Whitney Rank Sum test.
Live cell microscopy

Immortalized MEFs expressing LAP-tagged Smc3 were imaged in CO2 independent medium (Gibo) containing 125 nM Syto59 for visualization of nuclei. Imaging was performed using a Zeiss / PelkinElmer UltraVIEW VoX spinning disc confocal with Volocity software.

Tunnel assay

Tunnel assay on the paraffin sections was performed using APO-BrdU Tunnel Assay Kit (Invitrogen) according to the manufacturer’s instruction.

Synchronization primary mouse embryonic fibroblasts by double thymidine block

For the double thymidine block, primary fibroblasts passage two were maintained in the presence of 2 mM thymidine for 14 h. Cells were trypsinized, split in ratio 1:3 and cultured in DMEM supplemented with 10% FBS for at least 9 h. A second thymidine block was applied for an additional 14 h.

Preparation of mitotic cells for immunofluorescence analysis

Mitotic cells were shaken off 13 h after thymidine block release and spun onto positively charged microscope slides using a Shandon cytopsin 4 centrifuge at 900g for 5 min. After fixation in 4% PFA for 20 min at RT, cell spreads were incubated in 0.5% Triton X-100 in PBS for 15 min and in blocking reagent (5% BSA, 5% donkey serum in PBS) 1 h at RT. Tubulin, Pericentrin, Cyclin B1 and CenpA were detected as described above. For immunofluorescence on prometaphase chromosomes, 48 h after AdCre infection, cells were incubated in presence of nocodazole (100 ng mL⁻¹) for additional 4 h. Mitotic cells were shaken off, spun on positively charged microscope slides using Shandon
cytospin 4 at 1000xg for 5 min and pre-extracted with 0.1% Triton X-100 in PBS for 2 min at RT. The detection of Scc1-myc, Sgo1, Incenp, CREST and AuroraB was conducted as described above. Measuring the fluorescence intensities was carried out as described previously (Kitajima et al, 2006). Briefly, stack of in-focus images of prometaphase cells were taken with confocal microscope, Zeiss 710 using MetaMorph imaging software (Universal Imaging) and superimposed using Image J. The maximum intensity among the centromeric CREST signals within the cell was measured and subtracted with the background intensity of the region directly adjacent to the centromere.

**Metaphase chromosome spreads**

For metaphase chromosome spreads, cells were synchronized as described above. 11.5 h after thymidine block release, mitotic cells were shaken off and the remaining MEFs were maintained in the presence of nocodazole (100 ng mL⁻¹). 1, 3 or 5 h after the nocodazole arrest, mitotic cells were exposed to 60 mM KCl for 20 min at 37°C. Metaphase chromosomes fixed in methanol:acetic acid (3:1) were spread on humidified positively charged microscope slides (Thermo Scientific) and visualized using propidium iodide containing mounting medium Vectashield (Vector Laboratories) or Giemsa stain.

**Determination of Smc3 acetylation by quantitative Western blotting**

MEFs from *Esco2^{fl/Δ}* embryos were arrested in G₀ by serum starvation/contact inhibition and conversion of the floxed into a recombined allele was induced using either tamoxifen or adenovirus Cre as described above. For control either wild type cells expressing CreERT2, treated with tamoxifen, or *Esco2^{fl/Δ}* cells, treated with adenovirus EGFP were used. MEFs were then released from G₀ by splitting and arrested at the
G_{1}/S transition using either thymidine as describe above or aphidicolin (1 \, \mu\text{g mL}^{-1}). Cells were released and harvested at different time points by trypsinization. Correct synchronization was confirmed using propidium iodide staining and FACS analysis or immunofluorescence (see description of synchronization of primary MEFs). The efficiency of \textit{Esco2} deletion was determined by Southern blot and chromosome spreads. Harvested cells were lysed in extraction buffer (25 mM Tris-HCl pH 7.5, 100 mM NaCl, 5 mM MgCl2, 0.2% NP-40, 10% glycerol, 10 mM sodium butyrate, Complete protease inhibitor mix without EDTA [Roche]). The insoluble fraction was pelleted for 5 min at 5000xg, washed once with extraction buffer and resuspended in one pellet volume of extraction buffer supplemented with 20 mM CaCl2, and 20 units/mL DNase I. DNA was digested for 15 min in an ice-cooled sonication water bath. The volume of different samples was adjusted to the same protein concentration and NuPage LDS Sample Buffer (Invitrogen) was added. Remaining protein was solubilized by heating and tip sonication. Samples were passed through Ultrafree centrifugal filters (Millipore) and separated by SDS Page (Invitrogen). Following immunoblotting, acetylated Smc3 was detected using an antibody specific for the modified form of the protein as described (Nishiyama et al, 2010). The membrane was then stripped and total Smc3 levels were determined. Using ImageJ, band intensities for acetylated and total Smc3 were measured and the ratio for each lane calculated. Average and standard deviation were determined. The ratio of AcSmc3 to total Smc3 in the cells deleted for \textit{Esco2} was then calculated as a fraction of AcSmc3 to total Smc3 in control cells.

\textbf{Antibodies used in this study}

The following primary antibodies and dilutions were used in this study: rabbit anti-Esco2 (1:400 for immunofluorescence [IF]; 1:5000 for Western blot [WB]), guinea pig anti-Esco2
(1:10 IF; 1:1000 WB), rabbit anti-GFP (Abcam ab290, 5 µg per Chip), chicken anti-GFP (Abcam ab13970, 1:1000), rabbit anti-H3K9me3 and normal rabbit IgG (Millipore 17625, 4 µg per Chip), rabbit anti-Rad21 (Abcam ab992, 6 µg per Chip), anti-Sgo1 (1:250), mouse anti-Pcna (USBiologicals P3115-11A.500, 1:50), mouse anti-Brdu (Becton Dickinson 347580, 1:75), mouse anti-HP1α (LifeSpan BioSciences LS-C24208200, 1:10), mouse anti-H3S10ph (USBiological H5110-13K.100, 1:10), rabbit anti-Aurora B (Sigma-Aldrich A5102, 1:100), rabbit anti-Pericentrin (Abcam ab4448, 1:75), mouse anti-Tubulin (Abcam ab56676, 1:50), mouse anti-Cyclin B1 (Cell Signaling 4138S, 1:50 IF, 1:1000 WB), rabbit anti-CepA (Cell Signaling C51A7, 1:100), mouse anti-myc (Abcam 9E10, 1:1000), rabbit anti-myc (peptide: CEQKLISEEDLN, 1:1000), mouse anti-Aim-1 (BD Transduction Laboratories 611082, 1:250), rabbit anti-Incenp (provided by S. Hauf, 1:200), rabbit anti-Smc3 (Bethyl Laboratories A300-060A, 1:1000), mouse anti-acetyl-Smc3 (provided by K. Shirahige, 1:1000), rabbit anti-H2AT120ph (provided by Y. Watanabe, 1:1000), mouse anti-BUB1 (MBL K0168-3, 1:500), human anti-CREST (provided by A. Kromminga, 1:500).

In situ hybridization

Robotic in situ hybridization of brain sections was performed as described previously (Yaylaoglu et al, 2005) using the probes whose sequence can be retrieved from www.genepaint.org under the following Set IDs: Baiap3: MH3224; Col6a3: MH3225, Dlk1: ES1853; Drd1a: BA41, Tcf7l2: HD19.

Animal experiments

Experiments on living animals were approved by LAVES (Lower Saxony State Office for Consumer Protection and Food Safety, Document Number: 33.11.42502-04-095/07)
Supplemental references


Supplemental Figure Legends

Figure S1. Comparing the brain structures of Emx1-CRE;Esco2\textsuperscript{fl/fl} and Emx1-CRE;Esco2\textsuperscript{+/+} (wild type) brains using molecular markers. Amygdala and hypothalamus: Baiap3; septum: Col6a3, Dlk1; striatum: Drd1a; thalamus: Tcf7l2. Abbreviations: A–amygdala, H–hypothalamus, S–septum, St–striatum, T–thalamus. Besides the cortical and hippocampal agenesis we observed that the corpus callosum was absent and the medial septal nuclei and thalamus were impaired in mutants. Scale bar: 150 µm.

Figure S2. Expression of Esco2 peaks in mid S phase, when the bulk of Esco2 localizes to PCH

(A) Spatial separation of cells in S and M phases makes the E10.5 ventricular zone of the neocortex an exceptional system to investigate cell-cycle regulated processes. We found that at this embryonic stage, punctuate Esco2-IF is restricted to basally positioned nuclei of the VZ (for specificity of Esco2 antiserum see Fig. 3C, S3A) suggesting an S phase specific expression of Esco2 protein. Abbreviations: MZ–marginal zone, VZ–ventricular zone, Scale bars D,E: 10 µm.

(B) Esco2 protein is highly expressed in ~70% of BrdU-positive cells suggesting that Esco2 can be predominantly detected during a specific period of S phase. BrdU marks S phase cells several of which show a characteristic punctuate Esco2-IF (filled arrowheads), while other S phase nuclei are devoid of Esco2-IF (empty arrowheads).

(C) Western blot showing that Esco2 protein levels in double thymidine block synchronized MEFs sharply peak in mid to late S phase and then drop prior to cyclin A destruction. The center row shows cyclin A expression, which culminates in G\textsubscript{2} and delineates the S/G\textsubscript{2} phase boundary.
(D, F) Centromere is devoid of Esco2-IF. E14.5 cortisxrdcal progenitors were double labeled with Esco2 and CenpA antibody. Overlap between Esco2 and CenpA IF was measured using single focal plane images and Leica AF software. Graph in (F) shows relative intensity of Esco2 (red) and CenpA (green) fluorescence measured in single line (ROI1) driven through the nucleus (bottom right, D).

(E, G) FISH combined with Esco2-IF revealed absence of Esco2 at the telomeric repeats. Graph in (G) shows relative intensity of Esco2 (green) and telomeric FISH (red) fluorescence measured in single line (ROI2) driven through the nucleus (right, E).

**Figure S3. MEFs lacking Esco2 delay in prometaphase/metaphase.**

(A) Esco2-deficiency in primary CAGGCRE;Esco2ffff MEFs was induced by tamoxifen treatment of serum starved/contact inhibited cells. A Western blot shows a nearly complete absence of Esco2 signal during S phase in MEFsEsco2Δ/Δ but not in controls.

(B) Growth curves of synchronized Esco2-deficient and control cells. While the growth of control MEFs (dashes) was exponential, mutant cells (solid) failed to proliferate in culture (n=6).

(C) Elevated Cyclin B1 levels in MEFsEsco2Δ/Δ suggest a prometaphase delay.

(D) Relative to controls, MEFsEsco2Δ/Δ show ~2-fold increase in the number of cells in prometaphase/metaphase. 13 h after thymidine block release, cells grown on cover slips were classified according to DAPI stain and mitotic spindle morphology into different stages: P-prophase; PM/M–prometaphase/metaphase; A-anaphase; T-telophase.

**Figure S4. Chromosomes isolated from MEFsEsco2Δ/Δ are characterized by railroad track appearance.**
(A) Examples of prometaphase chromosomes. The two top panels display control cells which show either type 1 or 2. In MEFs\textsuperscript{Esco2\,\Delta} predominantly chromosomes of type 3 and 4 (bottom panels) were observed. Inserts show a high power view of a typical chromosome. Scale bar: 5 µm.

(B) The frequency of prometaphase chromosome types observed in MEFs\textsuperscript{Esco2\,\Delta} and control MEFs exposed to nocodazole. Prolonged nocodazole arrest in MEFs\textsuperscript{Esco2\,\Delta} resulted in increased frequency of spreads with single sister chromatids (n=600 for each type).

**Figure S5. Characterization of the Sgo1 antibody by immunofluorescence and western blotting.**

(A) HeLa cells transfected with control siRNA against GL2 firefly luciferase or siRNAs against Sgo1 were treated with nocodazole for 4 h. Mitotic cells were collected by shake off. Sgo1 depleted HeLa cells show loss of Sgo1-immunoreactivity at the centromeres marked by Aurora B. Scale bar: 10 µm.

(B) Specificity of the Sgo1 antibody was further validated by Western blotting of HeLa cell protein extracts prepared 24 h after the transfection with control or Sgo1 siRNAs.

(C) Sgo1-immunoreactivity is centromere specific in wild type mouse prometaphase chromosomes. Triton X-100 pre-extracted MEFs, stably expressing Scc1-myc, were labeled with anti-Sgo1 and anti-myc antibodies.

**Figure S6. Deficiency in Esco2 results in partial re-localisation of Aurora B and Sgo1 to the chromosome arms.**

Serum starved/contact inhibited MEFs were treated with tamoxifen. Prometaphase cells arrested in nocodazole for 4 h were analyzed.
(A, C) Triton X-100 pre-extracted prometaphase cells, labeled with Aurora B (A, red) or Sgo1 (C, green) antibody. Scale bar: 10 µm.

(B, D) The frequency of prometaphase cells with Aurora B (B) or Sgo1 (D) enriched at the centromeres and cells with Aurora B or Sgo1 present at centromeres and chromosome arms. More than 90% of Esco2-deficient prometaphase cells show partial relocalization of Aurora B/Sgo1 to the arms (n=200).

Figure S7. Intensity of Bub1 and H2ApT120 IF at the prometaphase kinetochores is reduced in ESCO2 depleted HeLa cells.

(A) Western blot demonstrating significant depletion (absence of slower running band) of ESCO2 in HeLa cells 48 h after the transfection with siRNA. Note that antibody raised against mouse Esco2 protein recognizes also faster running unspecific band in human cell lysates.

(B, D) Depletion of ESCO2 is HeLa cells results in reduction of BUB1 (B) and H2ApT120 (D) fluorescence intensity at the kinetochores. This reduction was not seen in HeLa cells transfected with siRNA against GL2 (firefly luciferase). Cells were analyzed 48 h after transfection with siRNA. Kinetochores were visualized by CREST IF. Scale bars: 10 µm.

(C, E) Frequency of prometaphases cells with strong or weak BUB1 (C) and H2ApT120 (E) fluorescence intensity at the kinetochores. >75% of ESCO2 depleted cells show reduced BUB1/ H2ApT120 IF (n = 200).
Emx1-CRE;Esco2+/+  Emx1-CRE;Esco2fl/fl

Baiap3

Col6a3

Dlk1

Drd1a

Tcf7l2
A. Western blot analysis showing Esco2 and β-actin expression in Esco2Δ/Δ and Esco2+/+ cells at 3, 5, 7, and 9 hours after release from thymidine block.

B. Graph depicting the cell numbers (E+06) over 96 hours after release from thymidine block, showing a comparison between Esco2Δ/Δ and Esco2+/+ cells.

C. Western blot analysis showing Cyclin B1 and β-actin expression in Esco2Δ/Δ and Esco2+/+ cells at 3, 7, 9, 12, 15, and 18 hours after release from thymidine block.

D. Bar graph showing the percentage of different mitotic stages (P, PM/M, A, T) in Esco2Δ/Δ and Esco2+/+ cells.
A

![Image of chromosome types] 

- Type 1
- Type 2
- Type 3
- Type 4

B

![Graph showing chromosome types over hours of nocodazole arrest] 

- Esco2Δ/Δ
- Esco2+/+

% of chromosome types

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<thead>
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A

DAPI  Sgo1  Aurora B  merge

control  

Sgo1 RNAi

B

RNAi

control  Sgo1

Sgo1  
tubulin

C

DAPI  Sgo1  Scc1  merge

merge
A

[Images of DAPI, Aurora B, and merge for Esco2Δ/Δ and Esco2+/+ conditions.]

B

[Bar graph showing % of prometaphases for Esco2Δ/Δ and Esco2+/+ conditions.]

C

[Images of DAPI, Sgo1, and merge for Esco2Δ/Δ and Esco2+/+ conditions.]

D

[Bar graph showing % of prometaphases for Esco2Δ/Δ and Esco2+/+ conditions.]
A

GL2 siRNA ESCO2 siRNA

Esco2

α-tubulin

B

ESCO2 siRNA GL2 siRNA

DAPI

CREST

Bub1

C

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D

ESCO2 siRNA GL2 siRNA

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CREST

H2ApT120

E

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