Supplementary information

Mammalian SGO2 appears at the inner centromere domain and redistributes depending on tension across centromeres during meiosis II and mitosis

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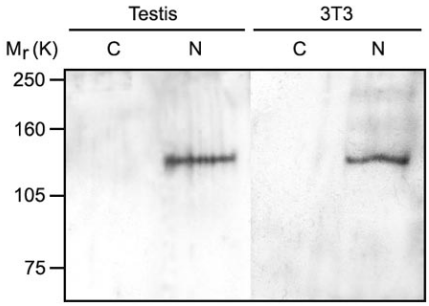
Supplementary Methods

Materials. Testes from adult male C57BL/6 mice were used for this study. In some experiments, mice weighing approximately 30 g, were intra-peritoneally injected with 40 mg/kg of colchicine (Sigma) in PBS and sacrificed at 3 hours. Mouse 3T3 cells were grown in Dulbecco's Modified Eagle's Medium with 4.5 g/l glucose (DMEM, Cambrex) supplemented with 10% foetal bovine serum (Gibco) and 2 mM L-glutamine (Cambrex), at 37°C in a humidified atmosphere containing 5% CO_2.

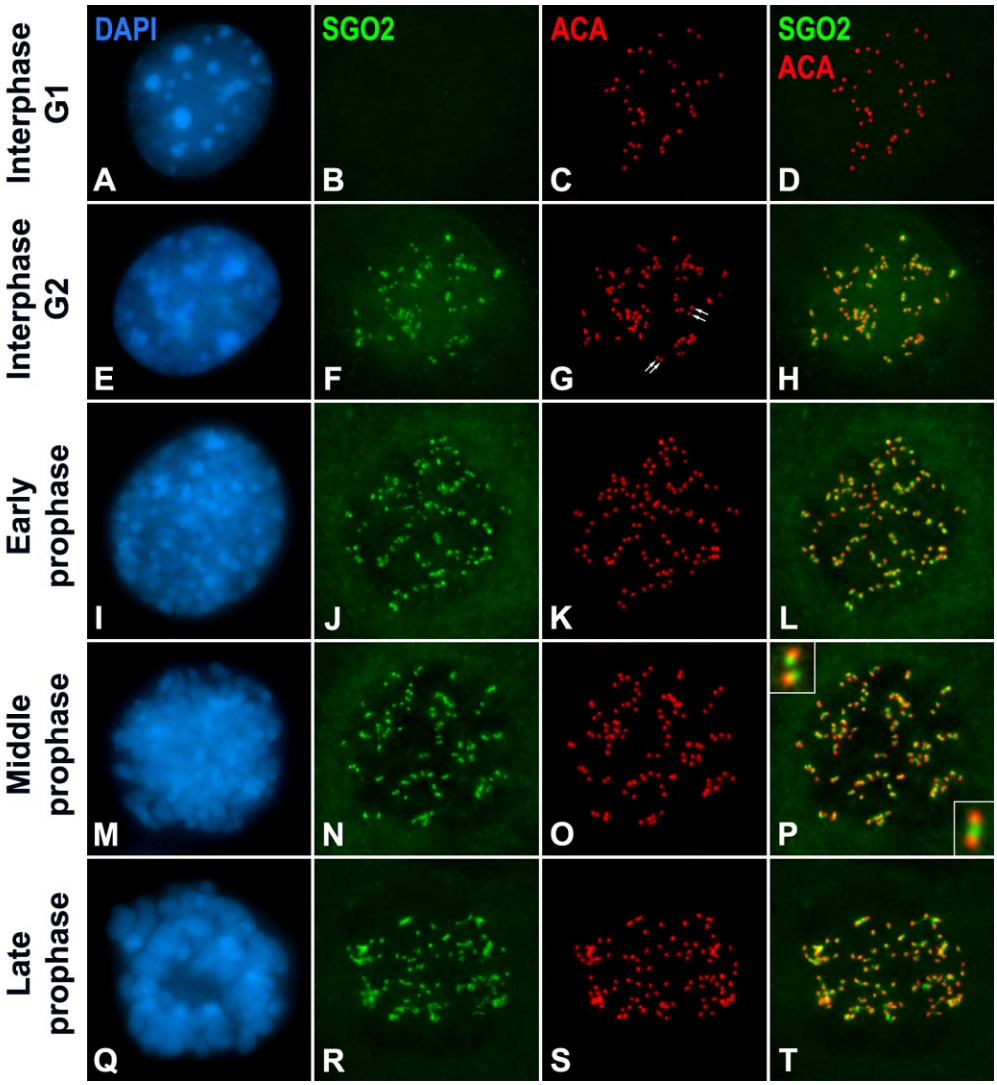
Western blotting. To determine the immunoreactivity of anti-SGO2 polyclonal antibodies we performed Western blot analysis of mouse testis and 3T3 culture cells using nuclear and cytoplasmic fractions. Mice testes were removed and washed with PBS. 3T3 cells ($5x10^6$ cells) were harvested and washed with PBS. Nuclear and cytoplasmic extracts were obtained using the NE-PER Nuclear and Cytoplasmic Extraction Reagent Kit (Pierce) according to the manufacturer's instructions. For Western blotting, proteins were resolved by 8% SDS-PAGE. The blot was incubated with anti-SGO2 (K1058 or K1059; dilution 1:500) antibodies, followed by incubation with HRP-conjugated donkey anti-rabbit IgG at a dilution of 1:2000 (Amersham). Visualization was performed using ECL detection system (Amersham).

Immunofluorescence on somatic cells. Mouse 3T3 cells were grown on coverslips for 48 h. Cells were washed with PBS and fixed in freshly prepared 2% formaldehyde in PBS containing 0.1% Triton X-100 (Sigma). After washing with PBS, cells were blocked for 15 min in PBS with 10% goat serum (Gibco). The coverslips were incubated with primary antibodies diluted in 5% goat serum-PBS for 45 min at room temperature. After rinsing in PBS, the coverslips were incubated with the corresponding secondary antibodies for 30 min. The DNA was counterstained with DAPI and then coverslips were washed in PBS and mounted with Vectashield (Vector Laboratories). Image stacks and their processing were as previously described for mouse spermatocytes.

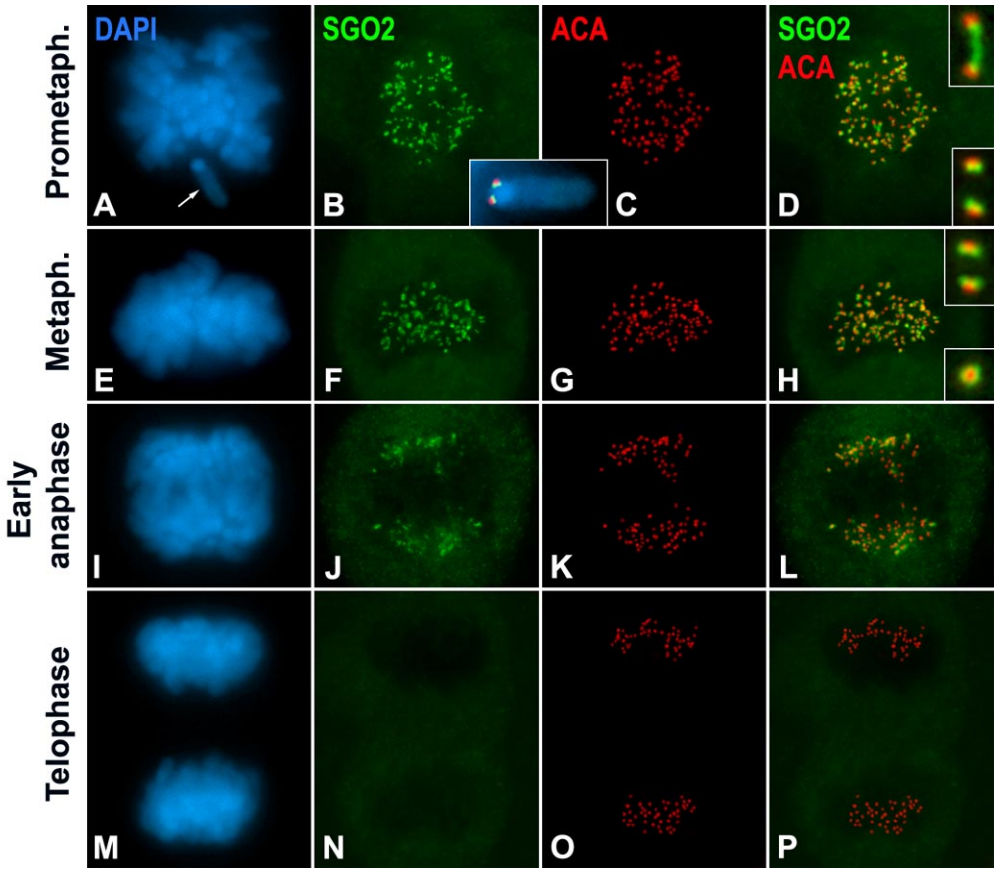
Supplementary Figures



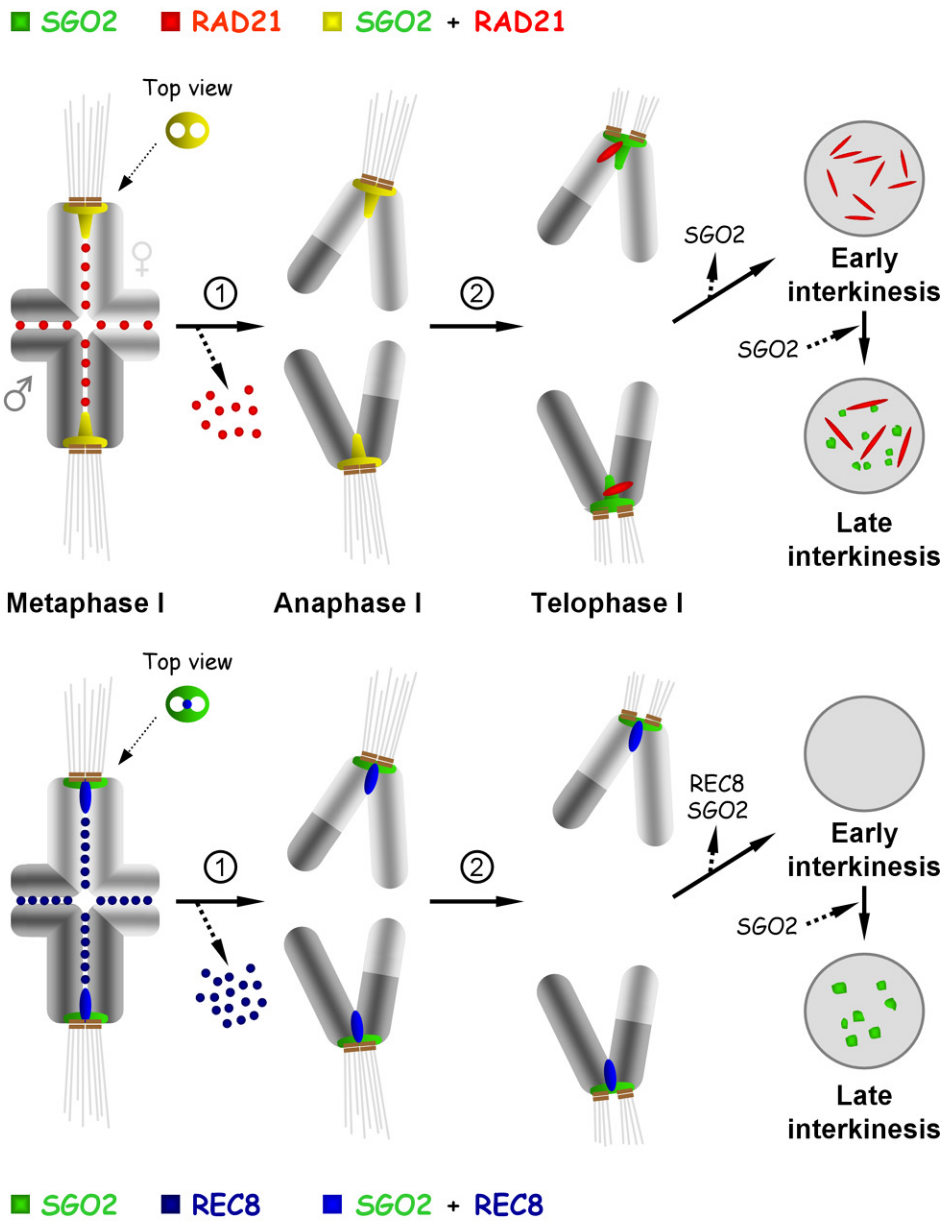
Supplementary Fig 1 Immunoblot analysis of mouse testis and 3T3 cells cytoplasmic (C) and nuclear (N) protein extracts. Both anti-mSGO2 K1058 and K1059 antibodies showed identical results. The figure only shows the western blot with the K1059 serum. The positions of molecular mass markers (M_r K) are indicated.



Supplementary Fig 2 SGO2 accumulates at centromeres during late interphase in somatic 3T3 cells. Double immunolabelling of SGO2 (green) with kinetochores (ACA) (red), and counterstaining of chromatin with DAPI (blue). (**A-D**) Interphase nucleus in G1. SGO2 is not detected. (**E-H**) Interphase nucleus in G2. Kinetochores (arrows in **G**) are observed as pairs of dots. SGO2 is detected at centromeres but do not completely overlap with kinetochores. (**I-T**) Early (**I-L**), middle (**M-P**), and late (**Q-T**) prophase nuclei. SGO2 appears either as a single signal below each sister kinetochore (upper inset in **P**) or as a patch between them (lower inset in **P**).

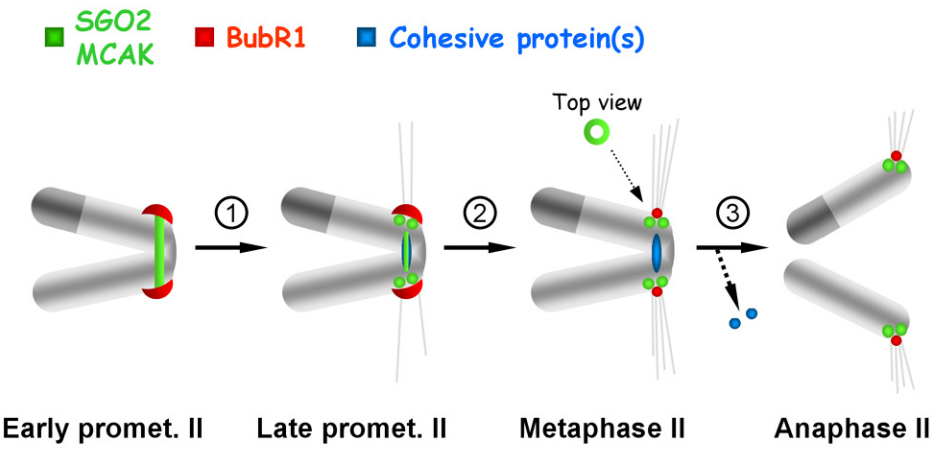


Supplementary Fig 3 SGO2 redistributes at the inner centromere domain of prometaphase 3T3 cells. Double immunolabelling of SGO2 (green) with kinetochores (ACA) (red), and counterstaining of chromatin with DAPI (blue). (**A-D**) Prometaphase. SGO2 appears either as a band that traverses the entire inner centromere domain and joins sister kinetochores (upper inset in **D**) or as pairs of dots below each kinetochore (lower inset in **D**). The inset in **B** and **C** shows a selected prometaphase chromosome (arrowed in **A**) presenting a pair of SGO2 signals below each sister kinetochore. (**E-H**) Metaphase. SGO2 appears as a pair of dots below each kinetochore (upper inset in **H**). In top-view, SGO2 is observed as a ring surrounding the kinetochore (lower inset in **H**). (**I-L**) Early anaphase. Faint SGO2 signals are still present at centromeres. (**M-P**) Telophase. SGO2 has disappeared from centromeres.



Supplementary Fig 4 Schematic representations summarizing the codistributions of SGO2 with the cohesin subunits RAD21 and REC8 from metaphase I up to late interkinesis. One chromosome is depicted in light grey and its homologue is darker grey. Chromosomes are telocentric, and the metaphase I bivalent shows a single interstitial chiasma. Trilaminar kinetochores are indicated in brown, kMTs in light grey, SGO2 in green, RAD21 in red, and REC8 in dark blue. Regions of overlap are in yellow and blue, respectively. In metaphase I bivalents and segregating anaphase I chromosomes, SGO2 and RAD21 colocalise as a 'double cornet'-like 3D structure at the inner centromeric domain below the closely associated sister kinetochores, when centromeres are side-viewed. By contrast, REC8 only colocalises with SGO2 and RAD21 in the vertical region of the double cornet structure. In top-viewed metaphase I centromeres, SGO2 and RAD21 appear as two closely associated

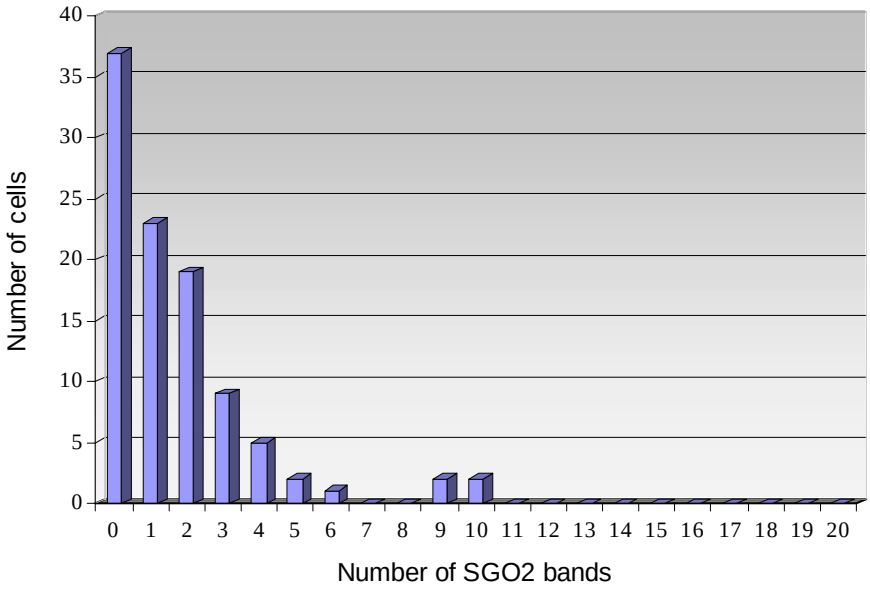
rings, while REC8 is observed as a round spot at the region of contact between the rings. During the metaphase I/anaphase I transition cohesin complexes with either RAD21 or REC8 are released from chromosome arms (step 1), while sister kinetochores separate during the anaphase I/telophase I transition (step 2). SGO2 persists at telophase I centromeres colocalising with REC8 only at the vertical region of the T-shaped structure at the inner centromeric domain, while RAD21 redistributes and appears as small bars. During the telophase I/early interkinesis transition, SGO2 and REC8 are released from centromeres, while RAD21 persists as elongated bars. In late interkinesis nuclei, RAD21 is present as elongated bars, and SGO2 reappears as large centromeric signals.



Supplementary Fig 5 Schematic representation summarizing the distributions of SGO2, MCAK, and the spindle checkpoint protein BubR1 from early prometaphase II up to anaphase II. Chromosomes are telocentric. Kinetochore MTs are indicated in light grey, SGO2 and MCAK in green, BubR1 in red, and putative proteins implied in centromere cohesion in blue. In early prometaphase II chromosomes, SGO2 and MCAK colocalise at the inner centromeric domain as a band between sister kinetochores that are brightly labelled with BubR1. In late prometaphase II chromosomes, the interaction of spindle MTs from opposite poles with sister kinetochores triggers the redistribution of SGO2 and MCAK (step 1). The subsequent capture and stabilization of kMTs by sister kinetochores promotes the release of BubR1, and tension across the metaphase II centromere, promoting the complete redistribution of SGO2 and MCAK below sister kinetochores (step 2). SGO2 and MCAK appear as a ring below each kinetochore in top-views of the centromere. The putative cohesive proteins at the inner centromere domain would thus be unmasked and released or cleaved by separase (step 3) to allow chromatid separation.

Supplementary Table

Number of SGO2 bands	0	1	2	3	4	5	6	7	8	9	10	11-20
Number of cells (N=100)	37	23	19	9	5	2	1	0	0	2	2	0



Supplementary Table 1 SGO2 redistributes at the inner centromere domain during chromosome congression to the metaphase II plate. The Table shows the number of centromeres where SGO2 appears as a band between sister kinetochores in 100 prometaphase II/metaphase II spermatocytes. Note that mouse prometaphase II/metaphase II spermatocytes present twenty chromosomes. The histogram is the schematic representation of the data shown in the Table.