SMC5/6 is required for the formation of segregation-competent bivalent chromosomes during meiosis I in mouse oocytes

Grace Hwang a, Fengyun Sun b, Marilyn O’Brien b, John J. Eppig b, Mary Ann Handel b, Philip W. Jordan a,b

a Department of Biochemistry and Molecular Biology, Johns Hopkins University Bloomberg School of Public Health, Baltimore, MD 21205, USA
b The Jackson Laboratory, Bar Harbor, ME 04609, USA

Correspondence: pjordan8@jhu.edu

Summary statement:
The structural maintenance of chromosomes complex, SMC5/6, is essential for female fertility and required for chromosome condensation and segregation during meiosis I in mouse oocytes

Keywords:
SMC5/6, cohesin, condensin, meiosis, chromosome segregation, aneuploidy

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**Abstract:**

SMC complexes include three major classes: cohesin, condensin, and SMC5/6. However, the localization pattern and genetic requirements for the SMC5/6 complex during mammalian oogenesis had not previously been examined. In mouse oocytes, the SMC5/6 complex is enriched at the pericentromeric heterochromatin, and also localizes along chromosome arms during meiosis. The infertility phenotypes of females with a Zp3-Cre-driven conditional knockout (cKO) of Smc5 demonstrated that maternally expressed SMC5 protein is essential for early embryogenesis. Interestingly, protein levels of SMC5/6 complex components in oocytes decline as wild-type females age. When SMC5/6 complexes were completely absent in oocytes during meiotic resumption, homologous chromosomes failed to segregate accurately during meiosis I. Despite what appears to be an inability to resolve concatenation between chromosomes during meiosis, localization of topoisomerase II alpha to bivalents was not affected; however, localization of condensin along the chromosome axes was perturbed. Taken together, these data demonstrate that the SMC5/6 complex is essential for the formation of segregation-competent bivalents during meiosis I, and findings suggest that age-dependent depletion of the SMC5/6 complex in oocytes could contribute to increased incidence of oocyte aneuploidy and spontaneous abortion in aging females.
Introduction:

Meiosis is a specialized cell division required for the formation of haploid gametes. Following pre-meiotic DNA replication, homologous chromosomes pair and recombine. DNA recombination occurs within the context of a proteinaceous scaffold known as the synaptonemal complex (SC), which ensures close juxtaposition of homologs (Handel and Schimenti, 2010). After desynapsis, homologous chromosomes remain linked via chiasmata, which are a visible manifestation of crossover recombination. Chiasmata are biologically essential as they ensure that homologous chromosomes bi-orient and thus segregate from each other during the first meiotic division (meiosis I). Subsequently, sister chromatids segregate during meiosis II, resulting in the formation of haploid gametes.

Regulation of meiosis is sexually dimorphic in mammals. Research using the mouse as a model has helped to delineate the dimorphic features that are also observed in humans. In most male mammals, meiosis is initiated postnatally, with continual production of spermatocytes undergoing meiosis throughout life. In female mice, meiosis is initiated during fetal development but arrests in a prolonged diplotene, or dictyate, stage of prophase I. Cohorts of dictyate stage oocytes begin growth shortly after birth and meiosis does not resume in vivo until after the preovulatory surge of luteinizing hormone (LH) in post-pubescent mice. However, fully-grown oocytes undergo spontaneous, LH-independent, resumption of meiosis after isolation and culture under supportive conditions (Pincus and Enzmann, 1935). Meiosis, whether occurring in vivo or in vitro, becomes arrested again after progression to metaphase II and is completed only after fertilization or parthenogenic activation.

Cohorts of oocytes resume meiosis throughout the reproductive life span and therefore can reflect aging effects. As women age, their oocytes become more susceptible to chromosome missegregation, which can lead to infertility and developmental abnormalities (Hassold and Hunt, 2001). Therefore, it is important to determine molecular pathways that are prone to error in oocytes, especially the proteins required for monitoring and facilitating chromosome segregation (MacLennan et al., 2015).

The structural maintenance of chromosomes (SMC) complexes are important regulators of chromosome dynamics and structure during mitosis and meiosis. Each member of the SMC family, which includes cohesin, condensin, and SMC5/6, is comprised of a V-shaped SMC protein heterodimer. The SMC proteins each have a hinge domain that is flanked by long coiled-coil domains, which allows the proteins to fold back on themselves. The C and N
globular heads interact with each other, forming an ATP-binding and ATP hydrolysis site. The ATPase domains are bridged together by non-SMC elements (Nasmyth and Haering, 2005).

Cohesin is a SMC1/3 heterodimer that is linked by an α-kleisin and a stromal antigen protein. During mitosis, cohesin is required to maintain sister chromatid cohesion before the metaphase-to-anaphase transition (Remeseiro and Losada, 2013). However, to ensure that sister chromatids segregate together during meiosis I, centromeric cohesin is maintained until meiosis II (Petronczki et al., 2003). In addition, cohesin complexes are required for accurate recombination and synapsis between homologous chromosomes (Rankin, 2015). Meiosis-specific cohesin components, including SMC1β, two α-kleisins (REC8 and RAD21L) and a stromal antigen protein (STAG3), are important for these additional requirements of cohesins during meiosis (Bannister et al., 2004; Fukuda et al., 2014; Herran et al., 2011; Hopkins et al., 2014; Llano et al., 2014; Revenkova et al., 2004; Winters et al., 2014; Xu et al., 2005). Mutation of meiosis-specific cohesin components in female mice results in an increased frequency of oocyte aneuploidy and premature ovarian failure (Herran et al., 2011; Hodges et al., 2005; Murdoch et al., 2013).

The two condensin complexes (I and II) are composed of the SMC2 and SMC4 heterodimers, but their kleisin subunit, and pair of HEAT repeat elements are unique (Hirano, 2015). Condensins localize to the longitudinal axes of bivalents following meiotic resumption in mouse oocytes, and both complexes are required for chromosome compaction before meiosis I (Houlard et al., 2015; Lee et al., 2011). However, only condensin II is essential for disentanglement of chromosomes prior to their segregation.

SMC5/6 heterodimers are linked by NSMCE4, a kleisin subunit (Verver et al., 2015). Two additional subunits, NSMCE1 and NSMCE3, interact with one another and with NSMCE4 (Palecek et al., 2006; Pebernard et al., 2008). NSMCE1 contains a RING-finger domain, common to E3 ubiquitin ligases, and NSMCE3 contains a MAGE (meleanoma-associated antigen gene) domain. NSMCE3 enhances the E3 ubiquitin ligase activity of NSMCE1 (Doyle et al., 2010). NSMCE2, which contains an SP-RING domain, binds to the coiled-coil region of SMC5 and can function as an E3 SUMO ligase (Andrews et al., 2005; Potts and Yu, 2007; Zhao and Blobel, 2005).

Studies assessing the SMC5/6 complex in mammalian germ cells have been limited to analyses of its localization pattern during mammalian spermatogenesis (Gómez et al., 2013; Verver et al., 2014; Verver et al., 2013). Because the regulation of meiosis is sexually dimorphic, there
may be temporal and functional differences in the roles of SMC5/6 in females versus males. This study demonstrates that the SMC5/6 complex is enriched at the pericentromeric regions and is also detected along chromosome arms during female meiosis. To determine the function of the SMC5/6 complex following meiotic resumption in mouse oocytes, an oocyte-specific conditional knockout (cKO) mouse was created, deleting a floxed Smc5 allele using the Zp3-Cre transgene, which is expressed in growing oocytes before meiotic resumption (Lan et al., 2004; Lewandoski et al., 1997). Analysis of the female Smc5 cKO mutants led to two major findings: 1) Maternal expression of SMC5 before meiotic resumption is essential for embryogenesis; and 2) absence of SMC5/6 during meiotic resumption results in oocyte aneuploidy due to an inability to resolve chromosomes during meiosis I. Furthermore, protein levels of SMC5/6 components in oocytes decline as wild-type females age, implicating the SMC5/6 complex as a potential contributor to oocyte aneuploidy and infertility in aging females.

Results:

SMC5/6 is enriched at oocyte pericentromeric heterochromatin during meiosis

Chromatin spreads were prepared to assess the localization of the SMC5/6 complex during female meiosis via immunofluorescence microscopy with antibodies raised against SMC5, SMC6 and NSMCE1 (Fig. 1; Fig. S1). Meiotic prophase sub-stages were determined by assessing chromosome axis morphology (synaptonemal complex protein, SYCP3) and centromere pairing (anti-centromere autoantibody, CEN; also known as ACA and CREST). During leptotene SMC6 localized throughout the spread chromatin (Fig. 1A). By early zygonema, SMC6 was enriched at pericentromeric heterochromatin. At pachynema, SMC6 remained enriched at pericentromeric heterochromatin, and was also evident at lower intensity along the arms of chromosomes. These localization patterns were partially resistant to DNase treatment (Fig. S2). Additionally, SMC6 was observed as foci along chromosome axes and chromosome ends (Fig. 1B). SMC6 foci were not always evident on pachytene stage chromatin spreads, and did not overlap with MLH1 foci (Fig. S3), suggesting that they may be transient and stage-specific. At early diplonema, SMC6 remained enriched at the pericentromeric heterochromatin, however this enrichment was decreased by late diplonema. Analysis of SMC5, NSMCE1 and an additional antibody raised against SMC6 resulted in similar localization patterns (Fig. S1). Differences in localization patterns are likely due to epitope accessibility, as is the case with mouse prophase spermatocytes (Gómez et al., 2013), SMC6
localization to the pericentromeric heterochromatin in oocytes overlaps with that observed for TOP2A (Fig. 1C).

Following meiotic resumption, SMC6 was enriched at the pericentromeric heterochromatin during meiosis I and remained present at metaphase II (MII), when oocytes arrest (Fig. 1D). Chromosome spread preparations of metaphase I (MI) oocytes demonstrated that there was also some SMC6 protein along chromosome arms (Fig. 1E).

Contrasting data have been reported on whether mutation of cohesin component, REC8, affects Smc5/6 axis loading during meiosis in budding yeast (Copsey et al., 2013; Lilienthal et al., 2013). Localization of SMC6 was assessed using a Rec8 mouse mutant (Bannister et al., 2004). The enrichment of SMC6 to the pericentromeric heterochromatin and localization to chromosome arms was not affected in Rec8 mutants (Fig. 1F), demonstrating that REC8 was not required for SMC6 localization. This finding is supported by observations made using mouse spermatocytes, where mutation of Smc1β did not affect SMC5/6 localization (Gómez et al., 2013).

**Oocyte-specific conditional mutation of Smc5 results in infertility**

Mice that harbored a conditional knockout (cKO) allele of Smc5 were used to assess the requirement of the SMC5/6 complex for the meiotic divisions and formation of blastocysts (Fig. 2A,B, see Materials and Methods). Exon 4 of Smc5 was flanked by loxP Cre recombinase target sequences and this allele was termed Smc5 flox (Fig. 2A). Breeding heterozygous Smc5 flox mice to mice expressing the Cre recombinase transgene generated a KO allele termed Smc5 del. The heterozygous Smc5 del mice exhibited no gross morphological abnormalities during development and adult life. No offspring homozygous for the Smc5 del mice allele were produced, indicating that homozygosity for the deletion allele is lethal. Therefore, to determine if Smc5 is essential for oogenic meiotic divisions, a hemizygous Cre recombinase transgene under the control of the promoter for the zona-pelucida protein 3 gene (Zp3-Cre tg/0) was used. This transgene is expressed exclusively in growing dictyate oocytes before resumption of the first meiotic division (Lan et al., 2004; Lewandoski et al., 1997). Breeding Smc5 +/flox, Zp3-Cre tg/0 (control) females to wild-type males showed that mutation of the Smc5 flox allele mediated by Zp3-Cre was 100% efficient (Fig. 2C). The Smc5 flox/del, Zp3-Cre tg/0 (Smc5 cKO) females failed to produce litters (N=5), despite having normal ovarian morphology and equivalent oocyte numbers (Fig. S4A,B).
**Smc5 cKO oocytes are incapable of mature blastocyst formation following IVF**

In vitro oocyte maturation (IVM) and fertilization (IVF) was used to determine whether blastocysts could be obtained from Smc5 cKO oocytes. Fully-grown germinal vesicle (GV) oocytes were isolated from the large antral follicles of Smc5 cKO (Smc5 flox/del, Zp3-Cre tg/0) and control (Smc5 +/flox, Zp3-Cre tg/0) female ovaries aged between 4 and 12 weeks, and cultured in media that supported meiotic resumption in vitro (IVM). There was no observable delay in GV break down (GVBD), indicative of meiotic resumption (Fig. S4C), and likewise no reduction in frequency of oocytes that underwent polar body extrusion (PBE) and metaphase II (MII) arrest (Fig 3A,B). However, following IVF using sperm from a wild-type mouse, fertilized oocytes from Smc5 cKO females failed to form mature blastocysts, with many embryos arresting at the 4 to 16 cell stages (Fig 3A-C). Intriguingly, there was a difference in IVF results between oocytes from mice that were 4 weeks of age (considered as the “juvenile” cohort), and mice that were between 12 and 16 weeks of age (considered the “adult” cohort). In the “juvenile” cohort, fertilized oocytes progressed to the 2-cell stage at levels comparable to their littermate controls (Fig. 3A). In contrast, the cohort of “adult” fertilized oocytes displayed a significant decrease in 2-cell stage embryos following IVF (Fig. 3B). In addition, although there was a significant decrease in embryos progressing beyond the 2-cell stage compared to the littermate control, the “juvenile” cohort of embryos collectively progressed further than the “adult” cohort (Fig. 3A,B). Embryos from the “juvenile” cohort were assessed via light and immunofluorescence microscopy. Cells and nuclei from the control embryos displayed similar shape and size, and the nuclei harbored an SMC6 signal (Fig. 3C,D; Fig. S5). In contrast, embryos from the Smc5 cKO embryos contained low or undetectable levels of SMC6 protein, and nuclei were irregular in size, which is consistent with defects during mitosis and imbalanced chromosome segregation during cell division.

**Only the “adult” Smc5 cKO oocytes display aneuploidy at metaphase II**

To determine whether the observed failure to form blastocysts was due to defects in chromosome segregation during meiosis, the number and morphology of chromosomes in oocytes arrested at MII were assessed. Due to the age-related differences observed in the IVF studies, MII oocytes from “juvenile” and “adult” mice were assessed separately. MII chromosome spread preparations of the “juvenile” Smc5 cKO oocytes did not exhibit significant increases of aneuploidy or chromosome abnormalities (Fig. 4A,B). In contrast, chromosome spread preparations from the “adult” Smc5 cKO females displayed abnormal
chromosome number and morphology, and separated sister chromatids were observed (Fig. 4A,B). Chromosome number and morphology was also assessed within the confines of the cell by treating the oocytes with monastrol. Monastrol binds to and disrupts the function of kinesin protein, KIF11, resulting in monopolar spindles making it easier to distinguish each sister chromatid pair (Stein and Schindler, 2011). Centromere number was counted using an anti-centromere autoantibody (CEN). In addition, the presence of the SMC5/6 complex was determined using an SMC6 antibody. Complementary to the chromosome spread preparations (Fig. 4A,B), the monastrol treated MII oocytes from the “juvenile” Smc5 cKO cohort did not exhibit significant differences compared to the control oocytes with respect to centromere counts or chromatin morphology (Fig. 4C-E). Furthermore, most (83%) of the oocytes from the “juvenile” Smc5 cKO harbored SMC6 protein signal. In contrast, the majority (61%) of monastrol treated oocytes from the “adult” Smc5 cKO cohort lacked SMC6 signal, and presented significant differences with regards to centromere counts compared to littermate controls (Fig. 4C,D; Fig. S6A). Additionally, it was not possible to obtain centromere counts from more than 50% of the monastrol treated Smc5 cKO oocytes from the “adult” mice, because the chromatin was grossly abnormal, demonstrating stretched morphology, and indistinguishable sister chromatid pairs (Fig. 4C,E; Fig. S6A). Furthermore, 5% of Smc5 cKO MII oocytes displayed abnormal morphology indicative of oocyte degeneration (Fig. S6B,C).

Oocyte SMC5/6 protein levels decrease in aging females

Excision of the floxed 4th exon of Smc5 driven by the Zp3-Cre transgene was shown to be 100% efficient based on mating tests, PCR analysis and the IVF data (Fig. 2B,C; Fig. 3A). However, data from monastrol treated MII oocytes demonstrated that the SMC6 protein was still present in most oocytes of the “juvenile” Smc5 cKO cohort (Fig. 4C). These data suggest that SMC5/6 protein levels present before Cre-mediated deletion of Smc5 are sufficient to support proficient meiosis, but not embryogenesis. Furthermore, the majority of oocytes from the “adult” cohort do not harbor residual SMC6 protein, and fail to form chromosomally normal MII oocytes (Fig. 4). As fertility and genome integrity are negatively correlated with age, it can be postulated that SMC5/6 levels within GV oocytes of wild-type mice may decrease with age. To test this hypothesis oocyte protein extracts from three groups of C57BL6/J wild-type mice aged 4, 12 and 24 weeks were assessed for SMC5, SMC6, NSMCE1 and NSMCE2 protein levels (Fig. 5A,B). From this analysis it was determined that protein levels for all four SMC5/6 components decreased significantly in oocytes isolated from older mice.
**Smc5 is a maternal-effect gene**

As there were residual levels of SMC6 detected in the oocytes isolated from “juvenile” Smc5 cKO mice, it was hypothesized that SMC5/6 levels were adequate to facilitate chromosome segregation during meiosis, but was insufficient for sustaining proper mitotic segregation during the early embryogenesis. To further assess the relationship between Smc5 mutation and the capacity to form mature blastocysts, wild-type, heterozygous Smc5 del male and female mice were used for IVF to test effects of paternal versus maternal inheritance of the mutant allele. The oocytes used in these assays were from 4 week old mice, and therefore equivalent to the designated “juvenile” age group. In addition, Smc5 cKO male mice (Smc5 flox/del, Hspa2-Cre tg/0), which are fertile and produce sperm that almost exclusively carry the Smc5 del allele, were used for IVF. Based on mating tests with C57BL6/J wild-type females, 98% of progeny from the Smc5 flox/del, Hspa2-Cre males carry the Smc5 del allele (Fig. 5C). When sperm from the heterozygous Smc5 del and Smc5 flox/del, Hspa2-Cre males were combined with wild-type oocytes the levels of mature blastocysts obtained were equivalent to the wild-type IVF (Fig. 5D), showing that presence of the paternally inherited Smc5 del allele does not affect early embryogenesis. When female heterozygous Smc5 del oocytes were fertilized with wild-type sperm, levels of mature blastocysts were equivalent to the wild-type IVF results, suggesting that the expression of Smc5 during oocyte growth is essential for supporting early stages of embryogenesis. When the heterozygous Smc5 del oocytes were fertilized with sperm from the Smc5 flox/del, Hspa2-Cre males, the level of blastocysts obtained reduced by approximately half, which supports the fact that early stages of embryonic development are affected in embryos homozygous for mutation of Smc5. Homozygous mutation of other components of the SMC5/6 complex, Smc6 and Nsmce2, have also been shown to cause embryonic lethality (Jacome et al., 2015; Ju et al., 2013). Taken together with the IVF and MII data obtained for the “juvenile” Smc5 cKO females (Fig. 3; Fig. 4; Fig. S5), these results suggest that Smc5 expression during oocyte growth, before meiotic resumption, is critical for embryogenesis, and therefore, Smc5 is a maternal-effect gene.

**Oocyte-specific cKO of Smc5 causes chromosome stretching during meiosis I**

Because abnormal chromosome morphology was observed in oocytes from the “adult” Smc5 cKO group at metaphase II arrest, it is possible that chromosome morphology and segregation earlier, during meiosis I, was perturbed. The localization of SMC5/6 components in the Smc5 cKO and control oocytes during meiosis I was assessed. SMC5/6 components SMC5, SMC6
and NSMCE1 were enriched at the pericentromeric heterochromatin during the metaphase to anaphase I transition in control oocytes, but were absent in the Smc5 cKO oocytes (Fig. 6A). Oocytes were assessed during the metaphase to anaphase I transition (Fig. 6B-D). In the majority (95%, N = 144) of the control oocytes proficient segregation of homologous chromosomes was observed. In sharp contrast, the majority (62%, N = 220) of Smc5 cKO experimental oocytes displayed chromosome stretching and lagging chromosomes. The severe chromatin stretching observed between homologous chromosomes (Fig. 6C) suggests that deletion of Smc5 prevented decatenation of homologous chromosomes.

Given the meiotic abnormalities described above, the spindle assembly checkpoint (SAC) was assessed in the Smc5 cKO oocytes. SAC satisfaction during the metaphase to anaphase I transition was indirectly determined by assessing the SAC protein MAD2, which normally localizes to kinetochores during prometaphase, and remains there until ubiquitous bipolar microtubule-kinetochore attachment satisfies the SAC (Lara-Gonzalez et al., 2012). MAD2 staining was present at the kinetochores at prometaphase in both control and Smc5 cKO oocytes (Fig. 6F). MAD2 signal at the kinetochore was absent in both control and Smc5 cKO oocytes undergoing the metaphase to anaphase I transition (Fig. 6G). These observations suggest that mutation of Smc5 does not affect the temporal pattern of MAD2 localization, and therefore may not affect SAC function, consistent with the lack of MI oocyte arrest. Additionally, cell cycle kinase, PLK1, localized to kinetochores in control and Smc5 cKO oocytes (Fig. S7).

Absence of the SMC5/6 complex causes aberrant localization of condensin

Premature depletion of REC8 before the meiosis I division in oocytes is associated with chromosome missegregation (Chiang et al., 2010; Tachibana-Konwalski et al., 2010). Therefore, localization of REC8 was assessed using metaphase I chromosome spreads. REC8 was present along the axes of the bivalents in control and Smc5 cKO oocytes from “adult” mice, with no apparent difference between them (Fig. 7A). These results suggest that mutation of Smc5 before meiotic resumption does not significantly affect localization of REC8-containing cohesins.

SMC5/6 colocalizes with TOP2A in mouse oocytes (Fig. 1C), and similar to the Smc5 cKO oocytes from the “adult” cohort, inhibition of TOP2A function results in severe defects in chromosome condensation and homologous chromosome separation (Li et al., 2013). Therefore, the effect of Smc5 cKO on the localization of TOP2A during meiosis I was determined using “adult” mice. TOP2A was enriched at the pericentromeric regions in control
oocytes, and was also detected along chromosome arms (Fig. 7B). No detectable change in TOP2A localization was observed in the Smc5 cKO oocytes (Fig. 7B).

Condensins are required to ensure chromosome segregation during meiosis I in mouse oocytes (Houlard et al., 2015). Similar to the results presented here for Smc5 cKO oocytes, conditional mutation of a condensin II component, Ncaph2, resulted in chromosome stretching during meiosis I due to an inability to disentangle chromosomes. To determine whether condensin localization is affected in the absence of the SMC5/6 complex, localization of the condensin I and II subunit SMC4 was assessed using “adult” cohorts of mice. In control metaphase I chromosome spread preparations, SMC4 was present along the longitudinal axes of each bivalent (Fig. 7C). In contrast, in chromosome spread preparations from Smc5 cKO metaphase I oocytes, there was a significant reduction in SMC4 signal along chromosome arms (Fig. 7C,D). In addition, the SMC4 signal on chromosome arms was discontinuous and the normal linear pattern along chromosomes axes was difficult to distinguish. However, there was no apparent reduction in condensin signal that colocalized with the kinetochore/centromeric regions in Smc5 cKO metaphase I chromosome spread preparations compared to control (Fig. 7E).

Discussion:

This study of a genetic model for oocyte depletion of SMC5 has demonstrated that the SMC5/6 complex is essential for ensuring accurate chromosome segregation following meiotic resumption and during early embryogenesis. Furthermore, the data suggest that SMC5/6 complex protein levels diminish as mice age, and Smc5 is a maternal-effect gene.

Smc5/6 localization pattern implicates multiple-functions during meiosis

SMC5/6 is enriched at the pericentromeric heterochromatin regions throughout meiosis in mouse oocytes, which is consistent with what was found in mouse spermatocytes (Gomez et al., 2013; Verver et al., 2013). The pericentromeric heterochromatin region consists of densely packed repetitive sequences and is at high risk of aberrant recombination events when double-strand breaks within these regions are repaired via homologous recombination (HR) (Goodarzi and Jeggo, 2012). SMC5/6 prevents HR within repetitive sequences such as rDNA in yeast, and heterochromatin in Drosophila mitotic cells (Torres-Rosell et al., 2007; Chiolo et al., 2011). Taken together, studies using mouse spermatocytes and oocytes suggest that SMC5/6 performs a similar function at the pericentromeric heterochromatin during meiosis (Gomez et al., 2013; Verver et al., 2013).
Although lower in signal intensity, SMC5/6 also localized throughout the chromatin during meiosis. This is consistent to what has been reported for mouse spermatocytes (Gomez et al., 2013, Verver et al., 2013). SMC5/6 was also visible along chromosome axes at pachynema in oocytes, which was also detected in mouse spermatocytes (Gomez et al., 2013). In addition, transient foci of SMC6 were detected along the chromosome arms in female germ cells during pachynema, suggesting a role during meiotic recombination, which has previously been reported using budding yeast and Caenorhabditis elegans (Bickel et al., 2010; Hong et al., 2016; Checchi et al., 2014; Hong et al., 2016; Copsey et al., 2013; Lilienthal et al., 2013; Xaver et al., 2013). In mammals, every chromosome pair obtains many recombination sites but generally yields only one to two crossover sites (Kauppi et al., 2004). Designations of which recombination sites become crossovers involve antagonistic roles between ubiquitin E3 ligase HEI10 and SUMO E3 ligase RNF212 (Reynolds et al., 2012; Qiao et al., 2014; Rao et al., 2017; Ahuja et al., 2017). It is possible that the SMC5/6 complex is a substrate of HEI10 and RNF212. Therefore, these SMC6 foci could indicate that SMC5/6 plays a role in regulating recombination during mammalian meiosis.

**Differences between the Smc5 cKO oocytes isolated from “juvenile” and “adult” mice**

SMC6 protein was detected in the majority of oocytes in the “juvenile” Smc5 cKO cohort. However, SMC6 was not detected in the majority of “adult” Smc5 cKO oocytes. As a consequence of this difference, oocytes from “juvenile” Smc5 cKO mice progress to MII without aberrant chromosome configurations (Fig. 4), whereas oocytes from “adult” Smc5 cKO mice fail to accurately segregate chromosomes during meiosis I (Fig. 6). Despite evidence for proficient meiosis from analysis of MII ploidy and chromosome morphology in oocytes from the “juvenile” Smc5 cKO cohort, these oocytes failed to form mature blastocysts when fertilized with sperm bearing a wild-type Smc5 gene. This failure to form mature blastocysts is attributed to aberrant chromosome segregation during mitosis (Fig. 3; Fig. S5). This phenotype is reminiscent to the mitotic catastrophe observed in Smc5 cKO mouse embryonic stem cells (Pryzhkova and Jordan, 2016).

The phenotypes observed and differences between “juvenile” and “adult” Smc5 cKO mice implies the following hypotheses. Firstly, SMC5/6 protein levels before oocyte growth are important for proficient chromosome segregation during meiotic resumption (Fig. 4; Fig. 6). Secondly, SMC5/6 protein levels present in oocytes diminish as mice age (Fig. 5). Thirdly, there is a critical level of SMC5 protein that is required for proficient chromosome segregation.
during oocyte meiosis (Fig. 4). Fourthly, expression of Smc5 during the oocyte growth phase is critical during early embryogenesis (Fig. 3; Fig. 4).

**SMC5/6 protein levels are diminished in aging oocytes**

Frequency of meiotic segregation errors increases as women age, especially after the age of ~35, resulting in dramatically increased incidence of miscarriage and birth defects (Hassold and Hunt, 2001). During the long prophase arrest that precedes meiosis I in female mammals, cohesin declines gradually and in aged oocytes the reduction of cohesin causes destabilization of chiasmata and separation of sister centromeres, which can result in chromosome missegregation during meiosis I (Lister et al., 2010; Tachibana-Konwalski et al., 2010; Tsutsumi et al., 2014). This current study determined that SMC5/6 protein levels decrease in oocytes isolated from older mice, and by correlation of phenotypes, this could also contribute to age-related aneuploidy and infertility (Fig. 8). Using an inducible transgene of Rec8, it was recently shown that cohesin is established in fetal oocytes during DNA replication, and there is no detectable turnover of cohesin in arrested oocytes, or during meiotic resumption (Burkhardt et al., 2016). Development of inducible, tagged version of an SMC5/6 component could be used to determine whether the SMC5/6 complex is replenished during meiotic resumption, or it remains stably associated with the chromatin for months following meiotic arrest.

Heterozygous mutants of cohesin components lead to age-related increases in oocyte aneuploidy (Murdoch et al., 2013). Therefore, it is possible that a heterozygous mutation of a SMC5/6 component could lead to age-related errors during oogenesis too. Supporting this notion, it has been shown that heterozygous mutation of Nsme2 results in increased incidences of micronuclei and polynucleation in MEFs (Jacome et al., 2015).

**Smc5 is a maternal-effect gene**

Early stages of embryogenesis are almost entirely dependent on the oocyte for subcellular organelles and proteins before the robust activation of the embryonic genome at cleavage-stage development (Fig. 8A). These maternal proteins are encoded by maternal-effect genes (Li et al., 2010). Approximately, 45-50 maternal-effect genes have been identified in mammals, and many of these are involved in chromatin structure, modification and genome integrity (Zhang and Smith, 2015). Reduced levels of maternal-effect genes have been associated with reduced oocyte developmental competence characteristic of ovarian aging (Guglielmino et al., 2011; Hamatani et al., 2004; Pan et al., 2008; Zhang and Smith, 2015). The IVF experiments
presented in this study showed that embryogenesis was aberrant only when Smc5 was mutated during the oocyte growth phase, and provision of a functional Smc5 gene from sperm was insufficient to facilitate embryogenesis. These data suggest that Smc5 is a maternal-effect gene in mouse. Recently, it was reported that smc5 and smc6 of Drosophila melanogaster are also maternal-effect genes (Tran et al., 2016), suggesting that this feature is conserved in many sexually reproducing organisms.

**SMC5/6 may be required to assist condensin functions and TOP2A-dependent decatenation**

Inhibition of TOP2A function in mouse oocytes and RNAi-mediated depletion in fly oocytes during meiosis I cause similar chromosome segregation defects observed in the Smc5 conditional knockout mouse oocytes (Hughes and Hawley, 2014; Li et al., 2013). Components of the SMC5/6 complex colocalize with TOP2A during prophase and following meiotic resumption in mouse oocytes. This is supported by previous observations made using mouse spermatocytes (Gómez et al., 2013). RNAi knockdown of SMC5 and SMC6 in human RPE-1 cells alters chromosomal localization properties of TOP2A (Gallego-Paez et al., 2013). Therefore, it was hypothesized that mutation of Smc5 would affect TOP2A localization in mouse oocytes. However, no defects in TOP2A localization were observed, which aligns with what was reported for Smc5 cKO in mouse embryonic stem cells (Pryzhkova and Jordan, 2016). Studies of yeast SMC5/6 have shown that the complex is linked with TopoII-dependent catenation/decatenation functions (Jeppsson et al., 2014; Kanno et al., 2015; Kegel et al., 2011). Furthermore, meiotic depletion of Top2 in budding yeast affects Smc5 localization (Copsey et al., 2013). While TOP2A localization is unaffected by mutation of Smc5 in mouse oocytes, the functionality of TOP2A may still be affected.

Analysis of metaphase I chromosome spreads revealed that SMC5/6 is required for normal localization of condensin along chromosome arms. The phenotypes observed here for the Smc5 cKO mutant are reminiscent of the Ncaph2 condensin II cKO mutant (Houlard et al., 2015), as both display abnormal chromosome morphology, similar stretching of chromosomes and chromosome segregation defects during meiosis I. There is mounting evidence for a functional link between SMC5/6 and condensin. RNAi depletion of SMC5 and SMC6 in human RPE-1 cells resulted in defective axial localization of condensin (Gallego-Paez et al., 2013). Abnormal condensin localization was also observed using Smc5 cKO mouse embryonic stem cells (Pryzhkova and Jordan, 2016). Furthermore, mutation of smc-5 in C. elegans leads to abnormal
distribution of condensin along bivalents during meiosis I (Hong et al., 2016). However, previous studies were not able to determine whether the defects in condensin localization were specific to the prophase to metaphase transition. Utilizing the Zp3-Cre transgene to mutate Smc5 suggests that there is a functional relationship between condensin and SMC5/6 that is specific to meiotic resumption.

It has been shown that condensin and TOP2A activities are coordinated to ensure efficient chromosome condensation, sister chromatid decatenation and subsequent segregation in budding yeast (Charbin et al., 2014; Leonard et al., 2015). Based on the collective observations made using human and mouse systems it is proposed that the aberrant localization of condensin observed in Smc5 mutant oocytes results in the loss of coordination between condensin and TOP2A, leading to an inhibition of chromosome resolution during meiosis (Fig. 8B).

**Conclusion**

The data demonstrate that SMC5/6 levels diminish in oocytes as mice age, leading to increased incidence of chromosome missegregation during meiosis (Fig. 8A). Furthermore, Smc5 is a maternal-effect gene and its expression during oocyte maturation is critical for early stages of embryogenesis (Fig. 8A). The SMC5/6 complex ensures that chromosomes are accurately resolved and segregated during female meiosis (Fig. 8B), and influences the localization of condensin, and based on published work this likely affects the function of TOP2A. Like cohesin and condensin, the SMC5/6 complex is critical to chromosome integrity in oocytes following their long arrested state. Protein levels of SMC5/6 components in oocytes are diminished in aging mice, suggesting that SMC5/6 levels are correlated with age-related oocyte and embryo chromosomal abnormalities. These data present the possibility that genetic and expression variations of SMC5/6 components are linked with fertility differences between individuals and defects may cause premature ovarian failure.
Materials and methods:

Ethics statement

All mice were bred at The Jackson Laboratory (JAX, Bar Harbor, ME) and Johns Hopkins University (JHU, Baltimore, MD) in accordance with the National Institutes of Health and U.S. Department of Agriculture criteria and protocols for their care and use were approved by the Institutional Animal Care and Use Committees (IACUC) of JAX and JHU.

Mice

Mice harboring Smc5 with a floxed exon 4 (designated Smc5\textsuperscript{flox}) and deleted exon 4 (designated Smc5\textsuperscript{del}) were previously described (Pryzhkova and Jordan, 2016). Heterozygous Smc5\textsuperscript{del} mice were bred to mice harboring the Zp3-Cre transgene (C57BL/6-Tg(Zp3-cre)93Knw/J), which resulted in progeny heterozygous for the Smc5\textsuperscript{del} allele and hemizygous for the Zp3-Cre transgene (Smc5\textsuperscript{+/del}, Zp3-Cre tg/0). Male Smc5\textsuperscript{+/del}, Zp3-Cre tg/0 mice were bred to homozygous Smc5\textsuperscript{flox} female mice to derive Smc5 cKO (Smc5\textsuperscript{flox/del}, Zp3-Cre tg/0) and control (Smc5\textsuperscript{+/flox}, Zp3-Cre tg/0) genotypes. The Smc5\textsuperscript{flox/del} genotype was used as an additional control. The same mating strategy was employed to create the male Smc5\textsuperscript{flox/del}, Hspa2-Cre tg/0 cKO mice, using mice harboring the Hspa2-Cre transgene (C57BL/6-Tg(Hspa2-cre)1Eddy/J).

PCR genotyping

Primers used are described in Figure 2 and Table S1. PCR conditions: 90°C for 2 min; 30 cycles of 90°C for 20 sec, 58°C for annealing, 72°C for 1 min.

Oocyte harvesting, culture and IVF

Female mice were injected intraperitoneally with 5 IU of equine chorionic (eCG; Sigma) to stimulate ovarian follicle development. GV-staged oocytes were harvested from ovaries 44 to 48 hrs later. Oocytes were cultured in MEM\textalpha medium supplemented with 5% fetal bovine serum (FBS; Gibco), and 3 mg/ml bovine serum albumin (BSA; Sigma-Aldrich). To harvest oocytes at metaphase II (MII) stage, mice were injected intraperitoneally with 5 IU of eCG (Sigma) and then with human chorionic gonadotropin (hCG; Sigma) 44-48 hrs later. After 15-16 hrs, MII oocytes were harvested from the ampulla of the oviduct. Ovulated oocyte-cumulus cell complexes were exposed to 300 IU/mL of hyaluronidase (Sigma) in MEM\textalpha medium supplemented with 3 mg/ml BSA to denude oocytes of surrounding cumulus cells.
For GVBD analysis, oocytes were harvested into MEMα medium supplemented with 5% FBS, 3 mg/ml BSA and 200 µM IBMX (Sigma-Aldrich). The oocytes were then washed and cultured in MEMα medium supplemented with 5% FBS, 3 mg/ml BSA and assessed for GVBD.

For IVF studies, eCG primed oocytes were first cultured in MEMα medium supplemented with 5% FBS, 3 mg/ml BSA and 2.5 µl EGF (10ng/ml; Epidermal Growth Factor) overnight. Following hyaluronidase treatment (Sigma), oocytes with a polar body indicative of progression to MII were counted. Oocytes were washed and cultured in MEMα medium supplemented with 3 mg/ml BSA and 10 µl of sperm extracted from an adult male mouse epididymis. Following IVF oocytes were washed and cultured in KSOM media and observed each day to assess embryogenesis.

For monastrol treatment MII oocytes were incubated in 10 mM monastrol (Sigma-Aldrich) in MEMα medium for 1.5 hrs at 37°C. Oocytes were washed in MEMα medium prior to fixation.

All cultures were incubated at 37°C in a 5% CO₂, 5% O₂ and 90% N₂ atmosphere.

Microscopy

Prophase-stage oocyte chromatin spreads, whole-oocyte and embryo mounts, MII chromosome spreads for chromosome number counts, as well as MI and MII chromosome spreads for immunofluorescence microscopy analyses were performed using techniques previously described (Susiarjo et al., 2009). Primary antibodies used and dilutions are listed in Table S2. Secondary antibodies against mouse, rabbit, and human IgG and conjugated to Alexa 488, 568 or 633 (Life Technologies) were used at 1:500 dilution. Oocytes were then mounted with Vectashield + DAPI medium (Vector Laboratories) or Clearmount (Invitrogen). DNase I treatment, chromatin spreads were treated with 100 U/ml of DNase I in DNaseI buffer (1% BSA, 10 mM MnCl₂, 1 mM CaCl₂, 50 mM Tris pH 7.5) for 1 hour at 37°C prior to staining.

Images were captured using a Zeiss Cell Observer Z1 linked to an ORCA-Flash 4.0 CMOS camera (Hamamatsu) and analyzed with the Zeiss ZEN 2012 blue edition image software. Photoshop (Adobe) was used to prepare figure images.

Western blot analyses

Protein lysate from eCG primed oocytes were isolated from C57BL6/J mice using methods previously described (Marangos, 2016). Protein extracts containing 150 oocytes were run on 4-15% gradient SDS PAGE gels (Bio-Rad) and transferred to PVDF membranes. Primary
antibodies and dilutions used are presented in Table S2. At a 1:10,000 dilution, goat anti-mouse and goat anti-rabbit horseradish peroxidase-conjugated antibodies (Invitrogen) were used as secondary antibodies. Antibody signal was detected via treatment with Bio-Rad ECL western blotting substrate and captured using Syngene XR5 system. Protein levels were assessed using Image J (NIH).

Acknowledgments:

We thank John Schimenti for providing the Rec8 mutant mouse, Karen Schindler for the REC8 antibody and Tatsuya Hirano for the NCAPH1 and 2 antibodies. We thank Marina Pryzhkova for technical assistance.

Author Contributions:

G.H, J.J.E, M-A.H and P.W.J. conceived the project and wrote the manuscript. Experiments performed by G.H., F.S., M.O-B and P.W.J.
References:


Figure 1. SMC5/6 localization during female meiosis.

(A-C) Chromatin spread preparations of wild-type ovarian germ cells at different stages of meiotic prophase I. (A-B) Immunolabeled with antibodies against CEN (blue, kinetochore/centromere marker), the SC lateral element protein SYCP3 (red), and SMC6 (green, ab18039). (B) SMC6 localization on the pericentromeric heterochromatin, along chromosome arms and foci on chromosome axes during pachynema. (C) TOP2A (green) localization at pachynema. (D) Wild-type metaphase I whole oocyte preparation, DAPI (DNA, blue), SMC6 (green, ab18039), α-tubulin (red) and CEN (red). (E) Wild-type metaphase I chromatin spread, DAPI (blue), SMC6 (red, ab18039) and CEN (green). (F) Chromatin spread of an embryonic ovarian germ cell from a Rec8 mutant, SYCP3 (red) and SMC6 (green, ab18039). Boxed regions are magnified 3x. Complementary data using additional SMC5/6 antibodies in Figure S1. Scale bar: 10µm
Figure 2. Conditional mutation of Smc5 using the Zp3-Cre recombinase results in female infertility.

(A) Schematic of mouse Smc5 floxed allele containing loxP sites, flanking exon 4, and the resulting Smc5 deleted allele after excision of exon 4 by Cre recombinase. Arrows represent primers for PCR genotyping of mice. (B) DNA agarose gel image of PCR products for genotyping. Lane 1 and 2 represent a control genotype (Smc5 +/-; Zp3-cre tg/0). Lane 1 (Smc5 +/-; flox allele): 410bp wt allele, 563bp and 644bp flox allele. Lane 2 (Zp3-cre tg/0): 420bp internal control and 281bp Zp3-Cre transgene. Lane 3 and 4 represent Smc5 cKO (Smc5 flox/del; Zp3-cre tg/0). Lane 3 (Smc5 flox/del): 563 bp and 644 bp flox allele, and 763 bp del allele. Lane 4 (Zp3-cre tg/0): Same as Lane 2. (C) Fertility tests and offspring genotyping results for Smc5 mutant and control mice. At least five mice were tested for each group.
Figure 3. Smc5 cKO oocytes fail to form mature blastocysts.

(A-C) Assessment of PBE and blastocyst formation following IVF. (A) PBE and IVF data obtained for “juvenile” (4 weeks of age) control (Smc5 +/flox, Zp3-Cre tg/0) and Smc5 cKO (Smc5 flox/del, Zp3-Cre tg/0). Oocytes used for control, N=123 and Smc5 cKO, N=150. The P values (one-tailed paired t-test) for the indicated comparisons are P=0.0771 (n.s.), P=0.1085 (n.s.), P=0.0003 (**), P<0.0002 (***) and P<0.0001 (***) for MII, 2 cells, >2 cells, early blastocysts and mature blastocysts, respectively. (B) PBE and IVF data obtained for the “adult” (≥ 12 weeks of age) control and Smc5 cKO. The total number of oocytes used for control, N=105 and Smc5 cKO, N=134. The error bars represent the variation between three independent experiments. The P values (one-tailed paired t-test) for the indicated comparisons are P=0.4287 (n.s.), P=0.0282 (*), P=0.0004 (**), P<0.0001 (***) and P<0.0001 (***) for MII, 2 cells, >2 cells early blastocysts and mature blastocysts, respectively. (C and D) Example images of cell morphology following IVF for control and Smc5 cKO. (D) Embryos stained
with DAPI (blue, DNA), SMC6 (red) and CEN (green). Boxed regions are magnified 3x. Arrow points to a nucleus with irregular size. Collectively, IVF was performed six times using a total of 10 mice for each control and Smc5 cKO cohort. Scale bar: 10µm
Figure 4. Metaphase II oocytes from “adult” Smc5 cKO have aneuploidy and abnormal chromosome morphology.

Control (Smc5 +/flo x, Zp3-Cre tg/0) and Smc5 cKO (Smc5 flo x/del, Zp3-Cre tg/0) oocytes arrested at MII were assessed for chromosome number, centromere number and chromosome morphology using two separate age groups (“juvenile” = 4 weeks old, and “adult” ≥ 12 weeks old). (A) Table summarizing chromosome count data from chromosome spreads. (B) Examples of chromosome spreads of control and Smc5 cKO MII oocytes. Red arrows point to single chromatids and yellow arrow shows an example of abnormal chromosome morphology. (C) MII oocytes treated with monastrol, DAPI (blue, DNA), SMC6 (red), and CEN (green). (D) Scatter dot-plot graph of centromere counts from monastrol treated MII oocytes obtained from “juvenile” cohorts of control (average = 39.6, N = 50) and Smc5 cKO mice (average=39.4, N=50) and “adult” cohorts of control (average = 39.1, N = 50) and Smc5 cKO mice (average=36.7, N=25). Mean and standard deviation of the columns of each graph are represented by the black bars and P values are given for indicated comparisons (Mann-Whitney, two-tailed). (E) Bar graph of percentage of oocytes with abnormal chromosome morphology from “juvenile” cohorts of control (average=4.84%, N=62) and Smc5 cKO mice (average=17.24%, N=50), and “adult” cohorts of control (average=13.25%, N=83) and Smc5
cKO mice (average=53.49%, N=86). Mean and standard error measurement of the columns of each graph are represented by the black bars and P values are given for indicated comparisons (chi-square test). Collectively, at least 10 mice for each group were used to obtain the data. Scale bar: 10µm
Figure 5. SMC5/6 protein levels decrease in oocytes as mice age and Smc5 is essential for embryogenesis.

(A and B) Protein was extracted from oocytes of wild-type C57BL6/J mice that were 4, 12 and 24 weeks of age. (A) Protein extracts from 150 oocytes from wild-type C57BL/6J mice were loaded onto each lane of a 4-15% SDS PAGE gradient gel and assessed for SMC5, SMC6, NSMCE1 and NSMCE2 protein levels. Nexilin and α-Tubulin were loading controls. (B) Bar graph of the average relative protein levels for each SMC5/6 complex component. Protein band signal intensities were normalized against the Nexilin loading control. Error bars represent standard deviation. Two-tailed paired t-tests were performed to compare each group and P values were defined as P < 0.05 (*), P < 0.005 (**) and P < 0.0005 (***)). The data is based on five sets of 150 oocytes isolated from three separate rounds of oocyte harvest. (C) Mating test and genotyping data obtained for the Smc5 flox/del (control) and Smc5 flox/del, Hspa2-Cre cKO males mated to C57BL6/J females. N=5 males tested, 3 litters. (D) Mature blastocyst counts following IVF of MII oocytes obtained from wild-type, heterozygous Smc5 mutant and Smc5 flox/del, Zp3-cre tg/0 mice fertilized with sperm from wild-type, heterozygous Smc5 del mutants or Smc5, Hspa2-Cre cKO males.
Figure 6. Smc5 cKO oocytes display lagging and stretched chromosomes during meiosis I.

(A) Metaphase I oocytes from control (Smc5 +/floox, Zp3-Cre tg/0) and Smc5 cKO (Smc5 flox/del, Zp3-Cre tg/0) mice, DAPI (blue, DNA), α-tubulin (green, α-TUB), CEN (green), and a subunit of the SMC5/6 complex (SMC5, SMC6, NSMCE1, red). (B) Oocytes transitioning from metaphase I to anaphase I, DAPI (blue, DNA), α-TUB (green) and CEN (red). (C) Smc5
cKO oocyte undergoing metaphase I to anaphase I transition displaying chromatin stretching, DAPI (purple, DNA) and CEN (green). Images with a 3x magnification of chromosome stretches on the right. (D) Bar graph of percentages of oocytes (N=104 for control, and N=167 for Smc5 cKO) showing even metaphase I to anaphase I chromosome segregation (MI to AI even), or chromosome stretching (MI to AI stretch). Bar graph displays percentages of oocytes in anaphase I (N=40 for control and N=53 for Smc5 cKO), showing no lagging chromosomes (AI no lagging), and lagging chromosomes (AI lagging). The P values (one-tailed paired t-test) for the indicated comparisons are P=0.004 (***) and P=0.0078 (***) for the MI to AI and AI quantification respectively. (F) Pro-metaphase I and (G) metaphase I oocytes were stained with DAPI (blue, DNA), MAD2 (green) and CEN (purple). Collectively, at least 10 mice for each group were used to obtain the data. Scale bar: 10µm
Figure 7. Condensin signal is reduced along chromosome arms in Smc5 cKO oocytes during meiosis I.

Chromatin spreads of metaphase I oocytes from control (Smc5 +/- flox, Zp3-Cre tg/0) and Smc5 cKO (Smc5 flox/del, Zp3-Cre tg/0) mice. Metaphase I chromosomes were stained with DAPI (blue, DNA), CEN (green) and either (A) cohesin component, REC8, (B) Topoisomerase IIα (TOP2A), or (C) condensin component, SMC4, in red. Representative chromosomes with a 3x magnification are present to the right of each chromosome spread. (D) Quantification of signal intensity of condensin (SMC4) signal along chromosome arms and associated with the centromere (overlapping with CEN). P values are given for indicated comparisons (Mann-Whitney, one-tailed) (N =20). Scale bar: 10µm
Figure 8. *Smc5* is a maternal-effect gene, and SMC5/6 is required for the formation of bivalent chromosomes capable of segregation during meiosis I in mouse oocytes.

(A) SMC5/6 levels diminish in oocytes as mice age (red bars), leading to increased incidence of chromosome missegregation during meiosis. Regardless of age, maternal expression of SMC5 during oocyte maturation and early embryogenesis (blue bars), prior to activation of the embryonic genome (green bar), is essential for the formation of a functional blastocyst. (B) The SMC5/6 complex ensures that homologous chromosomes are accurately resolved and segregated during female meiosis I. Depletion of the SMC5/6 in aging oocytes may be a source of chromosome segregation error.
Supplemental Figure S1. SMC5/6 is enriched on pericentromeric heterochromatin during meiosis I. (A-C) Chromatin spread preparations of embryonic ovarian germ cells of 15.5 and 17.5 days post-coitum and 0.5 days post-partum from wild-type mice at different stages of meiotic prophase I. Chromatin spreads were immunolabeled with antibodies against CEN (blue, kinetochore/centromere marker), the SC lateral element protein SYCP3 (red), and an SMC5/6 (green) component. (A) Localization of SMC5 during zygotene, pachytene and diplotene stages of meiosis. The lower panels display SMC5 immunostaining as a greyscale image. The boxed regions are magnified 3× below the whole chromatin spread image to demonstrate the enrichment at the pericentromeric heterochromatin and along chromosome arms. (B) A pachytene stage oocyte spread showing NSMCE1 localization on the pericentromeric heterochromatin. (C) A pachytene stage oocyte spread showing SMC6 localization on the pericentromeric heterochromatin and along chromosome arms using an additional antibody raised against SMC6 (ab155495). Scale bar: 10µm
Supplemental Figure S2. SMC5/6 association to chromosome axes is resistant to DNase I treatment. Representative chromatin spread preparation of embryonic ovarian germ cells of 18.5 days post-coitum. Chromatin spreads were treated with DNase I and immunolabeled with antibodies against CEN (purple, kinetochore/centromere marker), the SC lateral element protein SYCP3 (red), and SMC6 (ab18039, green). Negative DAPI staining for DNA confirms successful DNase I treatment. Scale bar: 10µm
Supplemental Figure S3. SMC6 foci do not colocalize with MLH1 foci. (A-C) Chromatin spread preparations of embryonic ovarian germ cells of 18.5 days post-coitum. Assessment of MLH1 (green), SYCP3 (blue) and SMC6 (ab18039, red) localization during pachynema. (D) Table summary of MLH1 and SMC6 foci observations. Scale bar: 10µm
Supplemental Figure S4. *Smc5* cKO ovaries do not display any defects in primary or secondary oocyte number and oocytes do not have a delay in GVBD.

(A) Periodic acid-Schiff staining of 5 micron cross sections of control and *Smc5* cKO ovaries. Scale bar = 200 microns. (B) Proportion of primary, pre-antral, early antral and pre-ovulatory oocyte counts per mm² for control and *Smc5* cKO ovaries, 3 mice were used for each genotype, 12 weeks of age. Mean and standard deviation of the columns of each graph are represented by the black bars. No significant difference between the control and *Smc5* cKO was observed according to two-way ANOVA test. (C) GVBD kinetics of control and *Smc5* cKO oocytes isolated from mice 12 weeks of age. GV oocytes were harvested and cultured in MEMα/BSA + 5% FBS supplemented with IBMX (3-isobutyl-l-methyl-xanthine) to inhibit germinal vesicle breakdown (GVBD). The oocytes were then washed in MEMα/BSA + 5% FBS to remove the IBMX and assessed every 20 minutes for GVBD. The experiment was performed using three mice for the control and *Smc5* cKO groups. The error bars represent the variation between three independent experiments. The total number of oocytes used for control, N = 86 and *Smc5* cKO, N = 79.
Supplemental Figure S5. Smc5 cKO oocytes fail to form mature blastocysts.

Oocytes from control and Smc5 cKO “juvenile” mice were fertilized with C57BL6/J wild-type sperm and resultant embryos were fixed and stained for DAPI (blue, DNA), SMC6 (red), and CEN (green). The boxed regions are magnified 3x to the right of the whole embryo images. Scale bar: 10µm
Supplemental Figure S6. Smc5 cKO oocytes at MII.

(A) MII oocytes from “adult” Smc5 cKO mice treated with monastrol, DAPI (blue, DNA), SMC6 (red), and CEN (green). The purpose of each panel is to demonstrate the differences in chromatin morphology, centromere/kinetochore signal and SMC6 signal observed in these experiments. (B and C) Immunofluorescence images of oocytes from control and Smc5 cKO mice. Oocytes were stained with DAPI (blue, DNA) and immunolabeled with antibodies against α-TUB (green) and CEN (red). (B) Example of an oocyte from the control mice that has reached MII arrest. The MII spindle and polar body are magnified 3x to the right of the whole oocyte image. (C) Example of an oocyte from the Smc5 cKO mice that demonstrate degeneration. The abnormal spindle is magnified 3x to the right of the whole oocyte image. 5% of oocytes from the “adult” cohort of Smc5 cKO mice harbored this defective morphology (N = 11/220). Scale bar: 10µm
Supplemental Figure S7. Localization of PLK1 in control and Smc5 cKO MI oocytes.

(A) Chromatin spread preparations of MI-stage oocytes from “adult” control and Smc5 cKO, DAPI (blue, DNA), PLK1 (red), CEN (green). (B) Whole oocyte preparations of MI-stage oocytes from “adult” control and Smc5 cKO, DAPI (blue, DNA), Aurora B (red), CEN (green). Scale bar: 10µm
**Supplemental Table S1: Primers used in this study**

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**Supplemental Table S2: Antibodies used in this study**

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