FBXO47 is essential for preventing the synaptonemal complex from premature disassembly in mouse male meiosis.

**Highlights**

- FBXO47 is a stabilizer of the synaptonemal complex during male meiotic prophase.
- FBXO47 KO shows precocious disassembly of the synaptonemal complex.
- FBXO47 may function independently of SCF E3 ligase to maintain homolog synapsis.

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FBXO47 is essential for preventing the synaptonemal complex from premature disassembly in mouse male meiosis

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SUMMARY
Meiotic prophase I is a prolonged G2 phase that ensures the completion of numerous meiosis-specific chromosome events. During meiotic prophase I, homologous chromosomes undergo synopsis to facilitate meiotic recombination yielding crossovers. It remains largely elusive how homolog synopsis is temporally maintained and destabilized during meiotic prophase I. Here we show that FBXO47 is the stabilizer of the synaptonemal complex during male meiotic prophase I. Disruption of FBXO47 shows severe impact on homologous chromosome synopsis, meiotic recombination, and XY body formation, leading to male infertility. Notably, in the absence of FBXO47, although once homologous chromosomes are synapsed, the synaptonemal complex is precociously disassembled before progressing beyond pachytene. Remarkably, Fbxo47 KO spermatocytes remain in an earlier stage of meiotic prophase I and lack crossovers, despite apparently exhibiting diplotene-like chromosome morphology. We propose that FBXO47 plays a crucial role in preventing the synaptonemal complex from premature disassembly during cell cycle progression of meiotic prophase I.

INTRODUCTION
Meiosis consists of a single DNA replication followed by two rounds of chromosome segregation, which halves the chromosome number to ultimately produce haploid gametes. During meiotic prophase I, sister chromatids are organized into proteinaceous structures, termed axial element (AE) or chromosome axis (Zickler and Kleckner, 2015). Homologous chromosomes (homologs) then undergo synopsis, which is promoted by the assembly of synaptonemal complex (SC) (Cahoon and Hawley, 2016). Homolog synopsis facilitates meiotic recombination yielding crossovers, a process that produces physical linkages called chiasmata between the homologs (Baudat et al., 2013; Keeney et al., 2014). Although homolog synopsis persists until meiotic recombination is completed during pachytene, it is dissolved upon diplotene-Metaphase I transition. Thus, homolog synopsis and desynapsis are temporally regulated. However, it remains elusive how homolog synopsis is temporally maintained and destabilized during meiotic prophase I.

SCF (SKP1–Cullin–F-box) E3 ubiquitin ligase is a key regulator of cell cycle (Cardozo and Pagano, 2004; Deshaies, 1999). Accumulating lines of evidence suggest that SCF is involved in homolog synopsis in a wide variety of organisms. In mouse, homologous chromosomes showed premature desynapsis in Skp1 conditional KO spermatocytes (Guan et al., 2020), suggesting that SCF is required for the maintenance of SC during male meiotic prophase I. In Drosophila female, SkpA, a SKP1 homolog, is required for the assembly and/or the maintenance of SC (Barbosa et al., 2021). In budding yeast Saccharomyces cerevisiae, deletion of Cdc53 that encodes Cullin resulted in defects in SC formation (Zhu et al., 2021). Thus, SCF is involved in the process of homolog synopsis during meiotic prophase I in diverse organisms.

Fbox-domain containing proteins act as a substrate recognition subunit in SCF E3 ubiquitin ligase (Jin et al., 2004; Kipreos and Pagano, 2000; Reitsma et al., 2017). It has been shown that Fbox-domain containing proteins are involved in homolog synopsis in a wide variety of organisms. In rice plant, Oryza sativa, mutants of MEIOTIC F-box MOF (He et al., 2016) and another Fbox ZYG O 1 (Zhang et al., 2017) showed defects in DNA double-strand break (DSB) repair and bouquet formation during meiotic prophase I. In budding yeast, temperature-sensitive mutant of Cdc4 that encodes F-box protein showed defective SC...
Figure 1. Identification of the meiosis-specific factor FBXO47
(A) Genomic view of MEIOSIN binding peak over Fbxo47 loci. Genomic coordinates were obtained from Ensembl.
(B) The expression of Fbxo47 in WT and Meiosin KO was examined using RT-PCR. Testis RNA was obtained from WT (3 animals each for P8 and P10) and Meiosin KO (3 animals, P10). The graph shows the expression level of Fbxo47 normalized by that of GAPDH. Data are represented as mean with SD. Expression level of Fbxo47 in P10 WT was set to 1. Statistical significance was determined by one-way ANOVA for all the dataset (p = 0.0221). For pair-wise comparison between P10 WT and Meiosin KO, statistical significance is shown by p value (Two-tailed t-test). *: p < 0.05.
(C) The tissue-specific expression pattern of Fbxo47 was examined by RT-PCR. Testis RNA was obtained from embryonic day 18 (E18), 3-weeks old (3w) and 8-weeks old (8w) male mice. Ovary RNA was obtained from adult 8-weeks old (8w) female mice. RT-indicates control PCR without reverse transcription.
(D) Expression patterns of Fbxo47 and other key developmental genes are reanalyzed using public scRNA-seq data of spermatogenic cells in adult mouse testis (GEO: GSE109033). Expression patterns of Fbxo47 and other key developmental genes are shown in UMAP plots. Key developmental genes include Zbtb16: spermatogonia, Stra8: differentiating spermatogonia and preleptotene spermatocyte, Dmc1: meiotic prophase I spermatocyte, Acrv1: round and elongated spermatid. UMAP of Zbtb16 and Stra8 was adopted from our previous study.
(E) Expression profiles of Fbxo47, Stra8, and Dmc1 in E11.5, E12.5, E13.5, and E15.5 fetal ovaries along pseudotime trajectory of germ cells. Pseudotime analysis was performed by reanalyzing scRNA-seq data (DRA011172). Pseudotime expression profile of Stra8 was adopted from our previous study.
was expressed weakly in meiotic spermatocytes, and highly in spermatids in testes, which is consistent with the expression of Fbxo47. Fbxo47 expression of endogenous FBXO47 that we generated. rabbit M: rabbit anti-FBXO47 middle region, rabbit (C) guinea pig anti-FBXO47 C-terminal region. (H) Western blot showed immunoprecipitates after tandem affinity purifications using anti-FLAG and anti-HA from cytoplasmic and chromatin extracts of WT (untagged control) and Fbxo47-3FH KI mouse testes (P15-18). The same membrane was sequentially reblootted with different antibodies against the endogenous FBXO47 that we generated. rabbit M: rabbit anti-FBXO47 middle region, rabbit (C) guinea pig anti-FBXO47 C-terminal region.

Previously, we identified MEIOSIN that plays an essential role in meiotic initiation both in mouse male and female (Ishiguro et al., 2020). MEIOSIN together with STRA8 (Kojima et al., 2019) activates meiotic genes and directs the switching from mitosis to meiosis. In the present study, we identified the Fbxo47 gene that encodes a Fbox protein, as one of the MEIOSIN/STRA8-target genes. Previous genetic studies suggested Fbxo47 homologs are implicated in the progression of meiotic prophase I in different species. In C. elegans, mutation in prom-1 that encodes putative Fbxo47 homolog, showed reduced homologous chromosome pairing and bivalent formation (Jantsch et al., 2007). In medaka fish, fbxo47 mutant fails to complete meiotic prophase I in females but switches developmental fate from oogenesis into spermatogenesis (Kikuchi et al., 2020). In mouse, the Fbxo47 gene that has previously been identified as a meiotic gene by single cell RNA-seq analysis of testes is essential for mouse spermatogenesis (Chen et al., 2018). FBXO47 interacts with SKP1 in vitro and disruption of FBXO47 led to the failure in completing homolog synapsis (Hua et al., 2019). Although previous studies suggested that FBXO47 homologs and distant meiotic Fbox-domain containing proteins play a role in homologous chromosome pairing/synapsis and meiotic recombination in a wide variety of organisms, the precise mechanisms how these proteins are involved in these processes remained elusive. Furthermore, whether FBXO47 is indeed involved in the function of SCF is unknown.

Here we show that mouse FBXO47 is essential for maintaining homolog synapsis during meiotic prophase I. FBXO47 is a cytoplasmic protein rather than a telomere binding protein. We demonstrate that in Fbxo47 KO spermatocytes, homologous chromosome synapsis is complete, but SC is precociously disassembled. Further, we show that Fbxo47 KO spermatocytes fail to progress beyond pachytene and remain in earlier meiotic prophase I in terms of cell cycle progression, despite the apparent exhibition of diplotene-like morphology of chromosomes. We propose that FBXO47 is essential for preventing SC from premature destruction during cell cycle progression of male meiotic prophase I. Further, we discuss the different observations and interpretations between the present study and the previous study on FBXO47 (Hua et al., 2019).

RESULTS

FBXO47 is expressed in mouse testes

Previously, we demonstrated that MEIOSIN collaborating with STRA8 activates meiotic genes, which are required for numerous meiotic events (Ishiguro et al., 2020). In spermatocytes, we identified Fbxo47 as one of the MEIOSIN/STRA8-bound genes (Figure 1A). Our previous RNA-seq analysis showed that expression of Fbxo47 was significantly downregulated in Meiosin KO testes at postnatal day 10 (P10) when a cohort of spermatocytes should undergo the first wave of meiotic entry (Ishiguro et al., 2020). We confirmed this by RT-qPCR analysis demonstrating that Fbxo47 expression level was indeed downregulated in Meiosin KO testis at P10 (Figure 1B). We further examined the expression pattern of Fbxo47 in different mouse tissues by RT-PCR analysis. Fbxo47 gene showed higher expression levels in adult testis compared to other adult organs that we examined (Figure 1C). Spermatogenic expression of Fbxo47 gene was further confirmed by the reanalysis of previous scRNA-seq data of adult mouse testis (Hermann et al., 2018) (Figure 1D). The result indicated that Fbxo47 was coordinately expressed with the landmark genes of meiotic spermatocyte such as Dmc1, and spermatid at spermiogenesis such as Acrv1, rather than those of spermatogonia such as Zbtb16 (Figure 1D). We noticed that Fbxo47 mRNA was expressed weakly in meiotic spermatocytes, and highly in spermatids in testes, which is consistent
Figure 2. Lack of detection of FBXO47-SKP association in testes by immunoprecipitation

(A) Schematic illustrations of the Skp1-3xFLAG-HA knock-in (Skp1-3FH KI) allele. Blue boxes represent exons. The stop codon in the exon six was replaced with in-frame 3xFLAG-HA and the endogenous 3’UTR.

(B) Chromosome spreads of WT (untagged) and Skp1-3FH KI spermatocytes were immunostained as indicated. Scale bar: 5 µm.

(C) Silver staining of the immunoprecipitates from cytosolic extracts of WT (untagged control) and Skp1-3FH KI mouse testes (14–21-day-old) after tandem affinity purifications using anti-FLAG and anti-HA antibodies. Arrowhead: SKP1-3xFLAG-HA.
Figure 2. Continued

(D) The immunoprecipitates from the cytosolic fraction of the WT (untagged control) and Skp1-3FH KI testis extracts were subjected to liquid chromatography-tandem mass spectrometry (LC-MS/MS) analyses. The Fbox-containing proteins and SCF subunits identified by the LC-MS/MS analysis are presented after excluding the proteins detected in the control mock purification. The proteins are listed with SwissProt accession number, the number of peptide hits, and Mascot scores. Full list of identified proteins are shown in the Data S1. It is worth noting that SC central element components, Six6OS1 and SYCE1, were included in the LC-MS/MS data of SKP1-3xFLAG-HA immunoprecipitates (Data S1). This suggests that SCF E3 ubiquitin ligase may target those SC components using an F box protein listed in the LC-MS/MS data as a substrate recognition subunit.

(E) Western blot showed immunoprecipitates from cytosolic extracts of WT (untagged control), Fbxo47-3FH KI and Skp1-3FH KI (heterozygous) testes (14–21-day-old) after tandem affinity purifications using anti-FLAG and anti-HA antibodies. The same membrane was sequentially rebotted with different antibodies as indicated. Red *: FBXO47-3xFLAG-HA, Green *: SKP1-3xFLAG-HA, Blue *: endogenous SKP1, Black *: nonspecific band. Note that SKP1 was not detected in FBXO47 immunoprecipitate from Fbxo47-3FH KI testis extracts, and reciprocally FBXO47 was not detected in SKP1 immunoprecipitate from Skp1-3FH KI testis extracts.

(F) Chromosome spreads of WT and Fbxo47 KO spermatocytes were immunostained as indicated. Scale bar: 5 μm.

To determine the meiotic stage-specific expression of FBXO47 protein, we generated different antibodies against FBXO47 C-terminal region (aa 271–451) and middle region (aa 173–316). However, we failed to evaluate stage specificity of endogenous FBXO47 protein expression by immunostaining, although it was uncertain whether this was because of the sensitivity of the antibodies, inaccessibility of the antibodies to the epitopes, or low expression level of FBXO47 protein in the target cells.

To circumvent this issue, we generated Fboxo47-3xFLAG-HA knock-in (Fboxo47-3FH KI) mice, which allowed the detection of FBOX47-3xFLAG-HA protein expressed from endogenous Fboxo47 locus (Figures 1G and S1). We examined FBOX47-3xFLAG-HA fusion protein from cytosolic and chromatin extracts of Fboxo47-3FH KI testes. Immunoblotting demonstrated that FBOX47 protein was detected with FLAG antibody only when it was enriched by tandem immunoprecipitations using anti-FLAG and anti-HA antibodies (Figure 1H), suggesting that the expression level of FBXO47 protein was low in testes. We noticed that more FBOX47 protein was detected in the cytosolic fraction compared to the chromatin fraction (Figure 1H), suggesting its predominant localization in the cytoplasm rather than on the chromatin. Sequential rebbloting showed that different antibodies against the endogenous FBOX47 protein that we generated detected the same protein as indicated by anti-FLAG antibody (Figure 1H).

In summary, FBXO47 is expressed presumably at a low level in meiotic prophase I.

Lack of detection of FBXO47-SKP association in testes by immunoprecipitation

FBXO47 possesses a putative Fbox domain, whose biological function has remained elusive. It is well known that Fbox-domain containing proteins confers substrate specificity to SCF (SKP1–Cullin–F-box) E3 ubiquitin ligase (Jin et al., 2004), and 69 different Fbox proteins are estimated to be encoded in the human genome (Reitsma et al., 2017). This prompted us to examine whether SKP1, a major core subunit of SCF, was co-immunoprecipitated with FBXO47 by immunoblot and mass spectrometry analysis (Figures 1H and S2). However, we failed to detect SKP1 in FBXO47 immunoprecipitates.

To further examine whether FBXO47 serves as a subunit of SCF by reciprocal immunoprecipitation of SKP1, we generated Skp1-3xFLAG-HA knock-in (Skp1-3FH KI) mice, which allowed the detection of SKP1-3xFLAG-HA protein expressed from endogenous Skp1 locus and its associated factors (Figure 2A). Although the homozygous Skp1-3xFLAG-HA KI mice were embryonic lethal, heterozygous knock-in mice were fertile and developed normally. Consistent with a previous study (Guan et al., 2020), although SKP1-3xFALG-HA fusion protein localized along the SC in the Skp1-3FH KI spermatocytes (Figure 2B), FBXO47 protein did not show such a specific localization pattern on the chromosome (Figure S3B). SKP1-3xFLAG-HA was enriched by tandem immunoprecipitations using anti-FLAG and anti-HA antibodies from testis cytosolic fraction (Figure 2C). Mass spectrometry analysis demonstrated that total of 45 different Fbox-domain containing proteins and SCF core subunits (SKP1, RBX1, CUL1, and CUL7) were co-immunoprecipitated with SKP1-3xFLAG-HA (Figure 2D, Data S1). However, we failed to detect FBXO47 in the SKP1-3xFLAG-HA immunoprecipitates either by mass spectrometry analysis or by western blotting (Figures 2D and 2E). SKP1 localized along the SC in Fboxo47 KO, suggesting that localization of SKP1 did...
Expression of FBXO47 is limited to early meiotic prophase I in mouse testes

To identify the specific stage in which FBXO47 was expressed, we performed immunostaining using stage specific markers SYCP3 (a component of meiotic chromosome axis), SYCP1 (a marker of homologous chromosome synapsis), and γH2AX (a marker of DSBs). Immunostaining of the Fbxo47-3FH KI testis (P15) indicated that FBXO47 protein was detected by HA antibody in average 21% (n = 3) among total SYCP3 positive seminiferous tubules (Figure 3A). Close inspection of seminiferous tubules showed that FBXO47 protein indicated by the presence of HA staining appeared in the cytosol at leptotene and zygotene (Figures 3B and 3C). Notably, the expression level of FBXO47-3xFLAG-HA fusion protein declined in pachytene, when homologs were fully synapsed (Figure 3C). Testis-specific histone H1t is a specific marker of spermatocytes (Figure 3D). None of H1t positive spermatocytes showed HA+/SYCP3+ in Fbxo47-3FH KI testes (n = 3 animals), whereas none of those was HA+/SYCP3+ in WT (n = 3 animals). Scale bar: 100 μm.

Disruption of Fbxo47 led to severe defect in spermatogenesis

To address the role of Fbxo47 in meiosis, we deleted Exon3-Exon11 of Fbxo47 loci in C57BL/6 fertilized eggs through the CRISPR/Cas9 system (Figure 4A). RT-PCR analysis showed that Fbxo47 mRNA expression level was absent in Fbxo47 KO testis (Figure 4B). Although Fbxo47 KO male mice did not show overt phenotype in somatic tissues, defects in male reproductive organs were evident with smaller-than-normal testes (Figure 4C). Histological analysis revealed that postmeiotic spermatids and spermatooza were absent in eight-week-old Fbxo47 KO seminiferous tubules (Figure 4D). Accordingly, sperm was absent in adult Fbxo47 KO caudal epididymis (Figure 4E). Consistently, seminiferous tubules that contain PNA lectin (a marker of spermatids) positive cells were absent in Fbxo47 KO (Figure 4F). Thus, the later stage of spermatogenesis was severely abolished in Fbxo47 KO seminiferous tubules, resulting in male infertility (Figure 4G). In contrast to male, Fbxo47 KO females exhibited seemingly normal fertility with no apparent defects in adult ovaries (Figure 4H). Consistent with this histological observation of ovaries, metaphase I not depend on FBXO47 (Figure 2F). Altogether, our data suggest that FBXO47 may function independently of SCF in mouse testes. Previous study showed that FBXO47 interacts with SKP1 in yeast two-hybrid assay and in GFP-SKP1 IP using HEK293T cell extract that overexpressed FLAG-FBXO47 and GFP-SKP1 (Hua et al., 2019). We do not know the exact reason for these controversial observations between our present study and the previous one (Hua et al., 2019). This could be because of their detection methodology using yeast and overexpression of FBXO47 in culture cells and/or technically limited sensitivity of our detection by immunoprecipitation, because the expression level of FBXO47 protein was low in spermatocytes.

Expression of FBXO47 was expressed in early meiotic prophase I in the testis

(Figure 3) Testis sections from Fbxo47-3FH KI and control (untagged) mice (P15) were stained for HA, SYCP3, and DAPI. Average 21% of the seminiferous tubules that have SYCP3+ spermatocytes showed HA+/SYCP3+ in Fbxo47-3FH KI testes (n = 3 animals), whereas none of those was HA+/SYCP3+ in WT (n = 3 animals). Scale bar: 100 μm.

(B) Seminiferous tubule sections from Fbxo47-3FH KI and control (untagged) mice (P15) were stained for HA, SYCP3, γH2AX, and DAPI. Lep: leptotene. Scale bar: 25 μm.

(C) Seminiferous tubule sections were stained for HA, SYCP3, SYCP1, and DAPI as in (B). Zyg: zygotene, Pac: pachytene spermatocyte, rS: round spermatid, eS: elongating spermatid. Scale bar: 25 μm.

(D) Testis sections from Fbxo47-3FH KI and control (untagged) mice (n = 3 for each genotype, P18) were stained for HA, H1t, and DAPI as in (A). Number of seminiferous tubules that have HA+/H1t+ cells was counted per the seminiferous tubules that have H1t+ spermatocyte cells (S2, 36, 18 tubules for untagged control; 15, 51, 36 tubules for Fbxo47-3FH KI mice). Scale bar: 100 μm.

(E) Seminiferous tubule sections (P18) were stained for HA, SYCP3, H1t, and DAPI as in (B). Lep: leptotene, Pac: pachytene spermatocyte, rS: round spermatid, eS: elongating spermatid. Scale bar: 25 μm.

(F) Seminiferous tubule sections (8-weeks old) were immunostained as indicated. Scale bar: 25 μm. Note that pachy signals of HA immunostaining were nonspecific, because they were visible in control.

(G) Embryonic ovary sections (E16.5) were immunostained as indicated. Scale bar: 100 μm.

Expression of FBXO47 led to severe defect in spermatogenesis

To address the role of Fbxo47 in meiosis, we deleted Exon3-Exon11 of Fbxo47 loci in C57BL/6 fertilized eggs through the CRISPR/Cas9 system (Figure 4A). RT-PCR analysis showed that Fbxo47 mRNA expression level was absent in Fbxo47 KO testis (Figure 4B). Although Fbxo47 KO male mice did not show overt phenotype in somatic tissues, defects in male reproductive organs were evident with smaller-than-normal testes (Figure 4C). Histological analysis revealed that postmeiotic spermatids and spermatooza were absent in eight-week-old Fbxo47 KO seminiferous tubules (Figure 4D). Accordingly, sperm was absent in adult Fbxo47 KO caudal epididymis (Figure 4E). Consistently, seminiferous tubules that contain PNA lectin (a marker of spermatids) positive cells were absent in Fbxo47 KO (Figure 4F). Thus, the later stage of spermatogenesis was severely abolished in Fbxo47 KO seminiferous tubules, resulting in male infertility (Figure 4G). In contrast to male, Fbxo47 KO females exhibited seemingly normal fertility with no apparent defects in adult ovaries (Figure 4H). Consistent with this histological observation of ovaries, metaphase I...
Figure 4. Spermatogenesis was impaired in Fbxo47 knockout male

(A) The allele with targeted deletion of Exon3-13 in Fbxo47 gene was generated by the introduction of CAS9, the synthetic gRNAs designed to target intron2 and the downstream of Exon11 (arrowheads), and ssODN (green and red boxes) into C57BL/6 fertilized eggs.

(B) Fbxo47 mRNA expression was examined by RT-PCR. Testis RNA was obtained from Fbxo47+/−/C0 and Fbxo47 KO males (P13). RT-indicates control PCR without reverse transcription.

(C) Testes from Fbxo47+/−/C0 and Fbxo47 KO (8-weeks old). Testis/body-weight ratio (mg/g) of Fbxo47+/−/C0 and Fbxo47 KO mice (8-weeks old) is shown on the right (Mean with SD). n: the number of animals examined. Statistical significance is shown by ****: p < 0.0001 (Two-tailed t-test). Scale bar: 5 mm.

(D) H&E staining of the sections from Fbxo47+/−/C0 and Fbxo47 KO testes (8-weeks old). Biologically independent mice for each genotype were examined. Scale bar: 100 μm.

(E) H&E staining of the sections from Fbxo47+/−/C0 and Fbxo47 KO epididymis (8-weeks old). Biologically independent mice for each genotype were examined. Scale bar: 100 μm.

(F) Seminiferous tubule sections (8-weeks old) were stained for SYCP3, PNA lectin, and DAPI. Note that the seminiferous tubule that contained PNA-positive elongated spermatids were not identified in Fbxo47 KO testes. Scale bar: 25 μm.

(G) Number of pups born by mating Fbxo47+/−/ and Fbxo47 KO males with Fbxo47+/−/ or Fbxo47 KO females (N = number of females in the same cage) to examine fertility. Fbxo47 KO male #1 was initially mated with three Fbxo47+/−/ females (all 6-weeks old at the start point of mating). After one month, another Fbxo47 KO male #2 started to cohabit with those females (8-weeks old at the start point of mating). This cage was observed for 3 months from the start of mating.

(H) Open Access
oocytes derived from Fbxo47 KO females processes normal number of bivalent chromosomes with chiasmata, indicating that Fbxo47 KO oocytes had progressed normal meiotic prophase I (Figure 4I). Furthermore, Fbxo47 KO females were fertile (Figures 4G and 4J), although we could not exclude the possibility that more subtle defects might have occurred in the ovaries besides fertility. Thus, the infertility caused by disruption of Fbxo47 was male specific. Therefore, these results suggest that requirement of FBXO47 is sexually different in mouse.

**Synaptonemal complex was prematurely disassembled in Fbxo47 KO spermatocytes**

To further investigate which at stage the primary defect appeared in the Fbxo47 KO, we analyzed the progression of spermatogenesis by immunostaining. Testis-specific histone H1t is a marker of spermatocytes later than mid pachytene and round spermatids (Cobb et al., 1999; Drabent et al., 1996). Close inspection of the seminiferous tubules (3 weeks) by immunostaining with antibodies against H1t along with SYCP3 (a component of meiotic chromosome axis) indicated that Fbxo47 KO spermatocytes failed to reach mid pachytene, whereas spermatocytes in age-matched control passed beyond mid pachytene as indicated by the presence of H1t staining (Figure 5A). This suggests that progression of meiotic prophase I was blocked in Fbxo47 KO spermatocytes. Immunostaining analysis of spread chromosome with antibodies against SYCP3 along with SYCP1 (a marker of homolog synapsis) demonstrated that Fbxo47 KO spermatocytes underwent homologous chromosome synapsis and seemingly reached pachytene stage as in age-matched control (Figure 5B).

Curiously, however, Fbxo47 KO spermatocytes exhibited apparent diplotene-like chromosome morphology (Figure 5B), despite the failure in reaching H1t positive mid pachytene (Figure 5A). It should be mentioned that in the diplotene-like Fbxo47 KO spermatocytes, SYCP3-stained axes do not show thickening at the telomere ends, although it should typically be observed in late pachytene and diplotene chromosomes of spermatocytes.

It is known that homolog synapsis is initiated at interstitial regions on the chromosome arm at zygote, and that desynapsis of homologs first starts at interstitial regions on the chromosome arm, whereas telomere regions are prone to be the last place of desynapsis at diplotene (Hisig et al., 2012; Qiao et al., 2012). This cytological difference readily distinguishes desynapsed chromosomes at diplotene from unsynapsed ones at zygote. Indeed, those Fbxo47 KO spermatocytes with diplotene-like chromosome morphology apparently showed a typical feature of desynapsis of homologs, wherein telomere regions retained homolog synapsis while interstitial regions were free from synapsis. To solve the paradox that Fbxo47 KO spermatocytes showed diplotene-like chromosome morphology despite the failure of progressing beyond H1t-positive pachytene stage, we further analyzed the meiotic prophase I population at P15 and P18 in the first wave of spermatogenesis of Fbxo47 KO testes. It should be mentioned that more zygote and reciprocally less pachytene populations were observed in Fbxo47 KO spermatocytes compared to WT at P15 and P18 (Figure 5C). This implies that the process of homolog synapsis, at least in part, may be delayed in Fbxo47 KO spermatocytes as has been shown previously (Hua et al., 2019).

Notably, “diplotene-like” cells (6.7%) appeared in Fbxo47 KO spermatocytes as early as P15, whereas the first wave of spermatogenesis was yet to pass beyond pachytene stage in the age-matched WT (Figure 5C). HORMAD1 localizes along unsynapsed chromosomes before pachytene and desynapsed chromosomes at diplotene, but dissociates from synapsed chromosomes (Daniel et al., 2011; Shin et al., 2010; Wojtasz et al., 2009). In Fbxo47 KO spermatocytes, HORMAD1 dissociated from synapsed chromosomes at pachytene and re-localizes on desynapsed chromosomes at diplotene-like stage as in those of WT (Figure 5D), suggesting that localization of HORMAD1 on chromosomes was normally regulated. Histone H3 Ser10 phosphorylation (H3S10p) by Aurora B kinase of the chromosome passenger complex marks the centromeric region at diplotene and the whole chromosome at metaphase I (Parra et al., 2003, 2009). In the control spermatocytes, the centromeric regions at diplotene were indicated by immunostaining of H3S10p (Figure 5E). In contrast, H3S10p-positive centromeric regions were not observed in Fbxo47 KO diplotene-like spermatocytes (Figure 5E). This observation indicated that Fbxo47 KO spermatocytes failed...
Figure 5. Premature disassembly of SC in Fbxo47 KO spermatocytes

For a Figure360 author presentation of this figure, see https://doi.org/10.1016/j.isci.2022.104008.

(A) Seminiferous tubule sections (P18 and 8-weeks old) were stained for SYCP3, H1t, and DAPI. Pa: pachytene spermatocyte, rS: round spermatid, eS: elongating spermatid. Shown on the right is the quantification of the seminiferous tubules that have H1t+/SYCP3+ cells per the seminiferous tubules that have SYCP3+ spermatocyte cells in WT and...

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Figure 5. Continued

(F) Spermatocytes isolated from the control Fbxo47+/− and Fbxo47 KO testes (P18) were cultured in vitro in the presence or absence of OA for 3 h. Quantification of meiotic prophase I stage is shown on the right. n: the number of cells examined. Note that the control spermatocytes showed a typical feature of diakinesis/Meta I with condensed chromosomes and remaining SYCP3 at centromeres.

(G) Seminiferous tubule sections from 8-weeks old mice were subjected to TUNEL assay with immunostaining for SYCP3. Shown on the right is the quantification of the seminiferous tubules that have TUNEL + cells counted. Note that centromeric regions are positively stained for H3S10P in the control diplotene spermatocyte but not in diplotene-like spermatocyte in Fbxo47 KO spermatocytes.

(H) Schematic illustration of the precocious SC disassembly observed in Fbxo47 KO spermatocytes. The expression timing of H11 and H3S10P markers is shown.

to reach bona fide diplotene stage of meiotic prophase I, albeit exhibiting apparent homolog desynapsis. Thus, we reasoned that even though homolog synapsis once occurred, it was destabilized during pachytene in Fbxo47 KO spermatocytes. Altogether, FBXO47 plays a negative role in desynapsis or FBXO47 is required for stability of synopsis.

Mid-late pachytene spermatocytes acquire competency for meiotic prophase-Metaphase I transition induced by the response to phosphatase inhibitor okadaic acid (OA) (Cobb et al., 1999). In vitro culture of isolated spermatocytes in the absence or presence of OA demonstrated that although the control spermatocytes progressed to diakinesis/metaphase I in the presence of OA, Fbxo47 KO spermatocytes did not (Figure 5F). Because Fbxo47 KO spermatocytes were yet to acquire competency for OA-induced progression into metaphase I, even the most advanced Fbxo47 KO spermatocytes remained in an earlier cell cycle stage compared to the control. These results suggested that the primary defect occurred at zygotene or early pachytene stage in Fbxo47 KO spermatocytes. Notably, TUNEL positive cells were observed in ~21% of Fbxo47 KO seminiferous tubules (Figure 5G), suggesting that Fbxo47 KO spermatocytes were consequently eliminated by apoptosis. Altogether, these results suggested that SC was prematurely disassembled in Fbxo47 KO spermatocytes (Figure 5H).

**Fbxo47 KO spermatocytes show defects in meiotic recombination**

Aforementioned results suggested that FBXO47 protein was required for stable maintenance of SC (Figure 5). SC facilitates meiotic recombination that is executed by DSB formation and repair steps. Then SC is disassembled after the completion of crossover formation. Given that SC was prematurely destabilized in Fbxo47 KO spermatocytes, we assumed two possibilities: (1) premature SC disassembly could be a result of early completion of meiotic recombination. (2) premature SC disassembly abolished the processes of meiotic recombination. To address these issues, we examined DSB formation, repair events, and meiotic silencing of unsynapsed chromatin (MSUC) by immunostaining of γH2AX. The first wave of γH2AX is mediated by ATM after DSB formation at leptotene (Mahadevaiah et al., 2001) and disappears during DSB repair. The second wave of γH2AX at zygotene is mediated by ATR that targets unsynapsed chromosomes (Royo et al., 2013). At zygotene, γH2AX signal appeared in Fbxo47 KO spermatocytes in the same manner as WT (Figure 6A), indicating that DSB formation normally occurred in Fbxo47 KO spermatocytes. However, γH2AX signals largely persisted throughout the nuclei until pachytene-like and diplotene-like stages in Fbxo47 KO spermatocytes, whereas they overall disappeared in WT pachytene spermatocytes except for retaining on the XY body (Figure 6A). This observation suggested that DSBs
were still not repaired and/or newly generated in Fbxo47 KO spermatocytes. Alternatively, γH2AX signals newly appeared in response to MSUC in Fbxo47 KO spermatocytes. This may imply at least in part that homolog synapsis was partly incomplete in Fbxo47 KO spermatocytes, as suggested by more zygotene

Figure 6. Fbxo47 KO spermatocytes show defects in meiotic recombination

(A) Chromosome spreads of WT and Fbxo47 KO spermatocytes (P18) were immunostained for SYCP3, SYCP1, and γH2AX.

(B) Chromosome spreads of WT and Fbxo47 KO spermatocytes (P18) were immunostained for SYCP3, SYCP1, and BRCA1.

(C) Chromosome spreads of WT and Fbxo47 KO spermatocytes were stained as indicated. Immunostained chromosome spread of pachytene spermatocytes is shown. The number of RAD51 foci is shown in the scatterplot with median (right). Statistical significance is shown by p value (Mann-Whitney U-test). ****: p < 0.0001. ***: p < 0.001. **: p < 0.01. Lep.: leptotene, Zyg.: Zygotene, Pac.: Pachytene, Z-like: Zygotene-like, P-like: Pachytene-like, D-like: Diplotene-like. n: the number of cells examined. Data were pooled from two to three animals at P18.

(D) Chromosome spreads of WT and Fbxo47 KO spermatocytes were stained as indicated. The number of MSH4 foci is shown in the scatterplot with median (right). Statistical significance is shown by p value (Mann-Whitney U-test). *: p < 0.05. Data were pooled from two to three animals at P18.

(E) Chromosome spreads of Fbxo47+/− and Fbxo47 KO spermatocytes (P18) were stained as indicated. The number of MLH1 foci is shown in the scatterplot with median (right). Statistical significance is shown by p value (Mann-Whitney U-test). ***: p < 0.0001. Scale bars: 5 μm.
and reciprocally less pachytene populations in Fbxo47 KO (Figure 5C). Notably, XY body was not detectable in Fbxo47 KO pachytene and diplotene-like spermatocytes, suggesting that FBXO47 is directly or indirectly required for XY body formation. Furthermore, BRCA1, a marker of asynapsis (Broering et al., 2014; Scully et al., 1997; Turner et al., 2004), appeared along unsynapsed autosomal axes in zygotene Fbxo47 KO spermatocytes as in those of WT (Figure 6B). This suggests that MSUC was normally activated in Fbxo47 KO spermatocytes. Crucially, in contrast to unsynapsed chromosomes in zygotene, BRCA1 was not observed along precociously desynapsed chromosomes in Fbxo47 KO diplotene-like spermatocytes (Figure 6B). This suggests that MSUC was canceled in Fbxo47 KO diplotene-like spermatocytes, presumably once homolog synapsis had successfully been achieved.

RAD51 facilitates the invasion of 3'-extended strand into the duplex of homolog at DSBs (Cloud et al., 2012; Shinohara and Shinohara, 2004). In Fbxo47 KO, the number of RAD51 foci was significantly increased in Fbxo47 KO spermatocytes at leptotene and zygotene (Figure 6C). Although we do not know the exact reason for the increase of RAD51 foci at leptotene in Fbxo47 KO, this may imply that more DSBs were generated. Reciprocally, the number of MSH4 foci was decreased in Fbxo47 KO zygotene spermatocytes (Figure 6D). These observations suggest that although RAD51 was normally loaded onto DSBs, the processes of homologous recombination-mediated repair were delayed in the absence of FBXO47. Accordingly, the number of MLH1 foci, a marker of crossover (CO) that should appear in mid to late pachytene spermatocytes, was significantly reduced in Fbxo47 KO pachytene-like spermatocytes (Figure 6E). This further implies that spermatocytes were yet to reach bona fide mid to late pachytene in the absence of FBXO47. Altogether, precocious disassembly of SC was a cause of the defect in meiotic recombination rather than a result of early completion of meiotic recombination.

Previous study showed that mouse FBXO47 interacts with SKP1 and telomere binding proteins, TRF1 and TRF2, and Fbxo47 KO spermatocytes showed defects in telomere bouquet formation (Hua et al., 2019), which were different to the observations in the present study (Figures S3A–S3C). This could be in part because of detection sensitivity or different epitope recognition of different antibodies. Because the frequency of bouquet formation was quite low even in WT spermatocytes in mouse (Figure S3D), as shown in our previous study (Ishiguro et al., 2014), the potential defect in bouquet formation in Fbxo47 KO spermatocytes further needs to be evaluated. Although we do not know the exact reason for the different observations in Fbxo47 KO testes, subtle differences in the detection and assay conditions or mice that were used could account for the differences in the observations.

**DISCUSSION**

**FBXO47 stabilizes homolog synapsis in mouse**

We have shown that FBXO47 is required for the maintenance of homolog synapsis during prolonged meiotic prophase I in male. Previous study found that any spermatocytes that progressed beyond zygotene were not found in Fbxo47 KO (Hua et al., 2019). Although partly agreeing with their interpretation, in the present study, Fbxo47 KO spermatocytes failed to progress beyond the bona fide pachytene stage of meiotic prophase I in terms of cell cycle status. The present study rather suggest that homologs were prematurely desynapsed in Fbxo47 KO spermatocytes, albeit the existence of Fbxo47 KO spermatocytes exhibiting apparent pachytene and diplotene-like chromosome morphologies (Figure 5).

Fbxo47 KO spermatocytes showed precocious desynapsis, albeit exhibiting apparently “diplotene-like” morphology (Figure 5B). Although this phenomenon in Fbxo47 KO spermatocytes was partly similar to that observed in conditional Skp1 KO (Guan et al., 2020), marked phenotypic differences were observed between Fbxo47 KO and Skp1 KO spermatocytes. In Skp1 KO testis, late pachytene spermatocytes are absent and concurrently diplotene spermatocytes are increased. Skp1 KO spermatocytes at least reach H1t positive mid-pachytene in terms of cell cycle, but most of them contain desynapsed chromosomes at pericentric end termed “Y pachynema”. Thus, Skp1 KO spermatocytes show precocious desynapsis and pachytene exit. In contrast, Fbxo47 KO spermatocytes failed to reach H1t positive mid-pachytene (Figure 5A). Although apparent diplotene-like morphology of homologous chromosomes appeared in Fbxo47 KO spermatocytes (Figure 5B), “Y pachynema” was not observed in Fbxo47 KO, unlike in Skp1 KO spermatocytes. Thus, Fbxo47 KO spermatocytes show precocious desynapsis despite the
failure of progression beyond pachytene. These results suggested that the primary defect in Fbxo47 KO spermatocytes occurred at an earlier cell cycle stage than Skp1 KO spermatocytes. HORMAD1 localizes along unsynapsed and desynapsed chromosomes during meiotic prophase (Daniel et al., 2011; Shin et al., 2010), and dissociates from synapsed chromosomes by the action of TRIP13 AAA ATPase (Wojtasz et al., 2009). Although HORMAD1 persists both in synapsed and desynapsed chromosomes in Skp1 KO spermatocyte, localization of HORMAD1 on chromosomes was normally regulated in Fb xo47 KO (Figure 3D). Thus, precocious desynthesis could be derived at least in part from failure of HORMAD1 removal in Skp1 KO and from a different mechanism in Fb xo47 KO. Moreover, although the DSB repair process indicated by γH2AX staining (Figure 6A) was impaired both in Fb xo47 KO and in Skp1 KO spermatocytes, the extent of crossover formation was different between them. Although significant number of MLH1 foci were observed in mid-late pachytene and diplotene spermatocytes in Skp1 KO; however, MLH1 foci were rarely observed in pachytene and diplotene-like spermatocytes in Fb xo47 KO (Figure 6E). Thus, meiotic recombination and crossover formation were more progressed in Skp1 KO than in Fb xo47 KO.

We showed sex specific difference in the phenotype of Fb xo47 KO mice. Although Fb xo47 is expressed in embryonic ovaries (Figures 1E and 1F), Fb xo47 KO oocytes had progressed through meiotic prophase I and the Fb xo47 KO females showed fertility comparable to WT (Figures 4H–4J), which is similar to male-specific meiotic defects observed in previous studies (Takemoto et al., 2020) (Horisawa-Takada et al., 2021). Although we could not exclude the possibility that more subtle defects might have occurred in the Fb xo47 KO ovaries, this may be at least in part because of strict requirement of FBXO47 for XY body formation during male meiotic prophase I (Figure 6A). Alternatively, because FBXO47 expression was hardly detected at protein level in the embryonic ovaries albeit the expression of Fb xo47 mRNA (Figure 3G), FBXO47 protein may have a negligible role in females.

**Distinct functions of FBXO47 homologs in diverse organisms**

Fb xo47 homologues and other distant F box proteins have been implicated in meiotic prophase progression in various species. Although defects accompanying DSB repair and crossover are similarly observed in mouse and C. elegans Fb xo47 mutants, the primary causes are assumed to be different. In C. elegans, PROM-1 encodes Fb xo47 homolog. In C. elegans, organization of gonadal germ line is divided into mitotic/meiotic entry zone, transition zone corresponding to zygote, and pachytene zone. Prom-1 mutant showed delayed and asynchronous initiation of homolog pairing, so that distinct transition zone was missing and meiotic entry zone was rather extended (Jantsch et al., 2007) with attenuating CHK-2 activity (Antoine et al., 2021; Mohammad et al., 2018). Further, PROM-1 was proposed to down regulate mitotic cell cycle proteins such as Cyclin E homolog CYE-1 at meiotic entry, independently of promoting homolog pairing as a positive regulator of CHK-2 kinase (Mohammad et al., 2018). Thus, PROM-1 functions very early in meiotic prophase in C. elegans, which is similar to our observation in mice (Figure 5). In prom-1 meiocytes, however, homolog pairing was defective and nonhomologous synopsis was consequently pronounced in autosomes but not in X chromosome. Thus, PROM-1 is implicated in promoting autosome homolog pairing. This is a contrast to our observation, in which homolog synopsis once took place normally, followed by premature desynthesis in Fb xo47 KO spermatocytes (Figure 5B).

In the teleost fish medaka, fb xo47 mutant XX germ cells exhibit abnormally condensed chromosomes in ovaries and fail to undergo oogenesis after diplotene, showing that the sexual fate of XX germ cells turns into spermatogenesis (Kikuchi et al., 2020). Thus, fb xo47 is involved in the regulation of cell division in ovaries, and in turn the suppression of spermatogenesis in female germ cells in medaka. The germline feminization under fb xo47 is mediated at least by two downstream transcription factors lhx8b and fgl/a during early meiotic prophase in medaka. Despite the phenotypic similarities and differences observed in the mutants of FBXO47 homologs in diverse organisms, FBXO47 homologs commonly act during meiotic prophase, although at different time points.

**Distant F box proteins are involved in homolog synopsis**

SCF and F box proteins are involved in the process of homolog synopsis during meiotic prophase I in diverse organisms. In plants, although no Fb xo47 homologs exist, distant F box proteins are involved in homolog synopsis. In rice plant (O. sativa), MEIOTIC F BOX (MOF) encodes an F BOX protein, and interacts
with OSK1, a homolog of SKP1 (He et al., 2016). MOF acts as a subunit of SCF and localizes on the chromosome during meiotic prophase I. In mof mutant male meiocytes, telomeres were not clustered and homolog synapsis was lost as indicated by complete absence of ZEP1, a transverse filament of SC. Thus, MOF plays a role in telomere bouquet formation during homolog pairing in male meiocyte. In rice plant, ZYGOTENE1 (ZYGO1) encodes another F box protein that has a limited similarity to mouse FBL12 (Zhang et al., 2017). In zygo1 mutant, polarized enrichment of OsSAD1, a SUN-domain containing protein, along nuclear envelope was lost and full-length homolog pairing was consequently impaired. This led to defective DSB repair of meiotic recombination, causing both male and female sterility in zygo1 mutant. Thus, ZYGO1 also plays a role in telomere bouquet formation during homolog pairing in rice plant. These studies suggest that rice F box proteins MOF and ZYGO1 act as an SCF component, and play a role in bouquet formation rather than in the process of SC formation, which is different from the role of mouse FBXO47 in SC maintenance.

In budding yeast, an F box protein Cdc4 acts as a substrate subunit of SCF during meiotic prophase I. SCF Cdc4 is assumed to regulate SC assembly by counteracting the Pch2 (TRIP13 in mammals)-dependent negative action that induces SC disassembly (Zhu et al., 2021). It is proposed that SCF CDC4 targets the putative negative regulator of SC assembly toward degradation, and in turn stabilizes SC. Although how Pch2 itself or its downstream factors are counteracted by SCF CDC4 remains elusive, F box protein Cdc4 acts for the maintenance of SC in budding yeast.

In Drosophila female, knockdown of SkpA, a Skp1 homolog, caused premature disassembly of SC (Barbosa et al., 2021). Depletion of F box proteins, Fboxo42 and Slmb/βTrcp, showed incomplete formation and precocious disassembly of SC, which was similar to the observation in Fbxo47 KO mouse. PP2A catalytic (C) subunit and structural (A) subunit were identified as a candidate substrate of Fboxo42. Because overexpression of a PP2A subunit Wrd (B56) phenocopied Fbxo42 knockdown, the SCF Fboxo42 is assumed to stabilize SC by restricting PP2A-Wrd (B56) association. In these regards, Drosophila Fboxo42 and budding yeast Cdc4 share a similar role to mouse FBXO47 in maintaining SC stability.

Previous studies showed PLK1 mediated-phosphorylation regulate SC disassembly in mouse (Jordan et al., 2012), and PP2A phosphatase inhibitor OA promotes premature exit from pachytene and SC disassembly (Cobb et al., 1999). Thus, phosphorylation level of SC regulates its stability during meiotic cell cycle. Given that FBXO47 exists in the cytosol rather than localizing to the chromatin (Figure 1H), it is possible that FBXO47 may protect the SC directly or indirectly from a putative destabilizer that regulates the phosphorylation level of SC during early meiotic prophase I (Figure 7). It is still a large enigma how FBXO47 acts for preventing premature SC disassembly, and further investigation is required for understanding the precise mechanism of FBXO47 function.

**Limitations of study**

Although our data showed that FBXO47 was not co-immunoprecipitated with SCF, we cannot exclude the possibility that this was because of the sensitivity of the antibodies, inaccessibility of the antibodies to the epitopes, or low expression level of FBXO47 protein in the spermatocytes. Further, we observed the differences in Fboxo47 KO phenotypes between our current study and the previous one (Hua et al., 2019), subtle differences in the detection and assay conditions or mice that were used could account for the differences in the observations.
STAR METHODS

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SUPPLEMENTAL INFORMATION

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AUTHOR CONTRIBUTIONS

N.Tanno and K.T. performed the cytological and biochemical analyses. R.S. performed reanalysis of scRNA-seq data. N.Tani performed MS analyses. Y.T.H. performed the RT-PCR. K.A. designed the knockout mice. S.F. performed histological analyses. N.Takeda assisted oocyte experiments. K.I. supervised experiments, conducted the study, and wrote the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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Shinohara, A., and Shinohara, M. (2004). Roles of recA homologues Rad51 and Dmc1 during meiotic recombination. Genes Dev. 18, 265–275. https://doi.org/10.1101/gad.219477.113.
**STAR METHODS**

**KEY RESOURCES TABLE**

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| Antibodies          |        |            |
| Mouse anti-FLAG M2 (IB, 1:1000) | Sigma-Aldrich | Cat# F1804 RRID: AB_262044 |
| Rabbit anti-HA (WB, IF, 1:1000) | Abcam | Cat# ab9110 RRID: AB_307019 |
| Rabbit anti-Actin (IF, 1:1000) | Sigma-Aldrich | Cat# A2066 RRID: AB_476693 |
| Mouse anti-MLH1 (IF, 1:500) | BD Biosciences | Cat# 551092 RRID: AB_394041 |
| Rabbit anti-H3S10P (IF, 1:2000) | Abcam | Cat# ab1791 RRID: AB_302613 |
| Rabbit anti-SYCP1 (IF, 1:1000) | Abcam | Cat# ab15090 RRID: AB_301636 |
| Mouse anti-γH2AX (IF, 1:1000) | Abcam | Cat# ab26350 RRID: AB_470861 |
| Rabbit anti-RAD51 (IF, 1:500) | Santa Cruz | Cat# SC-8349 RRID: AB_2253533 |
| Rabbit anti-MSH4 (IF, 1:500) | Santa Cruz | Cat# ab58666 RRID: AB_881394 |
| Rabbit anti-SKP1 (IB, 1:1000) | Abcam | Cat# ab10546 RRID: AB_297285 |
| Rabbit anti-TRF2 (IB, 1:1000) | Novus Biologicals | Cat# NB110-57130 RRID: AB_644199 |
| Rabbit anti-Lamin B (IF, 1:1000) | Santa Cruz | Cat# SC-6216 RRID: AB_648156 |
| Mouse anti-TRF1 (IF, 1:1000) | Shibuya et al., 2014 | N/A |
| Rabbit anti-TRF1 (IB, 1:1000) | Shibuya et al., 2014 | N/A |
| Guinea pig anti-SYCP3 (IF, 1:2000) | Ishiguro et al., 2020 | N/A |
| Rat anti-SYCP3 (IF, 1:1000) | Ishiguro et al., 2020 | N/A |
| Mouse anti-SYCP1 (IF, 1:1000) | Ishiguro et al., 2020 | N/A |
| Rabbit anti-FBXO47 M (WB, 1:1000) | This paper | N/A |
| Rabbit anti-FBXO47 C (WB, 1:1000) | This paper | N/A |
| Guinea pig anti-FBXO47 C (WB, 1:1000) | This paper | N/A |
| Guinea pig anti-H1t (IF, 1:2000) | provided by Mary Ann Handel | N/A |
| Rabbit anti-BRCA1 (IB, 1:500) | provided by Satoshi Namekawa | N/A |
| Mouse anti-HA SD8 monoclonal antibody-coupled Magnet agarose | MBL | Cat# M132-10 RRID: AB_11142502 |
| anti-FLAG M2 monoclonal antibody agarose affinity gel (IP, 50μl per IP of testes extract) | Sigma-Aldrich | Cat# M8823 RRID: AB_2637089 |
| Rabbit anti-HORMAD1 | ProteinTech | Cat# 13917-1-AP RRID: AB_2120844 |

**Bacterial and virus strains**

| E. coli strain BL21-CodonPlus(DE3)-RIPL | Agilent | Cat# 230280 |

(Continued on next page)
| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| Chemicals, peptides, and recombinant proteins | | |
| Ni-NTA agarose | QIAGEN | Cat# 30210 |
| Tissue-Tek O.C.T. compound | Sakura Finetek | Cat# 4583 |
| Vectashield mounting medium containing DAPI | Vector Laboratory | Cat# H-1200 |
| Superscript III Reverse Transcriptase | Thermo Fisher Scientific | Cat# 18080044 |
| TRIzol reagent | Thermo Fisher Scientific | Cat# 15596018 |
| Ex-Taq polymerase | Takara | Cat# RR001B |
| ECL prime | GE healthcare | Cat# RPN2232 |
| NuPAGE 4-12 %Bis-Tris Protein Gel | Thermo-Fisher Scientific | Cat# NP0322 |
| CNBr-activated Sepharose | GE healthcare | Cat# 17043001 |
| Pronase | MERCK | Cat# 10165921001 |
| M16 medium | Sigma-Aldrich | Cat# MR016 |
| M2 medium | Sigma-Aldrich | Cat# MR015 |
| 3xFLAG peptide | Sigma-Aldrich | Cat# F4799 |
| SimplyBlue | Thermo-Fisher | Cat# LC6065 |
| Pierce DTT, No-Weight Format (48x7.7mg) | Thermo Fisher Scientific | Cat# 20291 |
| Pierce Iodoacetamide, Single-Use (24x9 3mg) | Thermo Fisher Scientific | Cat# 90034 |
| Trypsin/Lys-C Mix, Mass Spec Grad (5x20µg) | Promega | Cat# V5073 |
| cOmplete, EDTA-free | Roche | Cat# 4 693 132 |
| RIP buffer | Thermo | Cat# 89900 |
| collagenase | Sigma-Aldrich | Cat# C0130 |
| DNase II | Sigma-Aldrich | Cat# 8764 |
| Proteinase inhibitor cocktail | Sigma-Aldrich | Cat# P8340 |
| H&E Staining System | Leica | Cat# 3801698 |
| Cas9 protein | NIPPON GENE | Cat# 317-08441 |
| Glycogen | Wako | Cat# 077-05311 |
| Triton X-100 | Sigma-Aldrich | Cat# T9284 |
| PNA lectin | Sigma-Aldrich | Cat# L7381 |
| Opti-MEM I Reduced Serum Medium | Thermo Fisher Scientific | Cat# 31985062 |
| RNasin® Plus Ribonuclease Inhibitors | Promega | Cat# N2611 |
| Giemsa | Wako | Cat# 079-04391 |
| Critical Commercial Assays | | |
| MEBSTAIN Apoptosis TUNEL Kit Direct | MBL | Cat# 8445 |
| TB Green Premix Ex Taq II (Tli RNaseH Plus) | Takara | Cat# RR820A |
| Deposited data | | |
| ChIP-seq data of MEIOSIN and STRA8 data (DDBJ Sequence Read Archive) | Ishiguro et al. 2020 | DRA007066, DRA007778, DRA009056 |
| scRNA-seq data of fetal ovaries | Shimada et al. 2021 | DRA 011172 |
| Original data | Mendeley Data | https://doi.org/10.17632/ct84bbsw5.2 |
| Experimental models: Organisms/strains | | |
| Mouse: Fbxo47 knockout | This paper | CARD ID 2777 |
| Mouse: Fbxo47-3xFLAG-HA knock-in | This paper | CARD ID 2972 |
| Mouse: Skp1-3xFLAG-HA knock-in | This paper | CARD ID 2638 |
| Mouse: C57BL/6N | SLC | N/A |

(Continued on next page)
RESOURCE AVAILABILITY

Lead contact
Further information and requests for the resources and reagents should be directed to and will be fulfilled by the Lead Contact, Kei-ichiro Ishiguro (ishiguro@kumamoto-u.ac.jp).

Materials availability
The ChIP-seq data of MEIOSIN and STRA8 are described in our previous study (Ishiguro et al., 2020) and available in the DDBJ Sequence Read Archive (DRA) under accession number DRA007066, DRA007778, DRA009056. Mouse lines generated in this study have been deposited to Center for Animal Resources and Development (CARD), Fbxo47 Ex3-11Δ knockout mouse (ID 2777), Fbxo47-3xFLAG-HA knock-in mouse (ID 2638), and Skp1-3xFLAG-HA knock-in mouse (ID 2638). Plasmid expression vectors generated in this study have been deposited to RIKEN BRC: pET28c-Fbxo47-C (aa272-451) (ID RDB19263) and pET19b-Fbxo47-M (aa174-316) (ID RDB19264). The antibodies are available upon request. There are restrictions to the availability of antibodies due to the lack of an external centralized repository for their distribution and our need to maintain the stock. We are glad to share antibodies with reasonable compensation by the requestor for its processing and shipping. All unique/stable reagents generated in this study are available from the Lead Contact with a completed Materials Transfer Agreement.

Data and code availability
All data supporting the conclusions are present in the paper and the supplementary materials. The source data (for Figures 1B, 1F, 4C, 4G, 4I, 4J, 5A, 5C, 5F, 5G, 6C–6E, and S3D) are provided in Data S2. The original images for all of the figures in this paper are deposited in public depository Mendeley Data, V1, https://doi.org/10.17632/ct84bbswv5.2. The ChIP-seq data of MEIOSIN and STRA8 (Ishiguro et al., 2020) have been deposited in the DDBJ Sequence Read Archive (DRA) and available under accession number DRA007066, DRA007778, DRA009056. There is no code used in this study.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Animals
Fbxo47 Ex3-11Δ knockout and Fbxo47-3xFLAG-HA knock-in mice were C57BL/6 background (age: 13–21 days old, 8-weeks old). Skp1-3xFLAG-HA knock-in mouse was congenic with C57BL/6 background (age: 14–21 days old). Male mice were used for immunoprecipitation of testis extracts, histological analysis of testes, immunostaining of testes, and RT-PCR experiments. Female mice were used for histological

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### REAGENT or RESOURCE SOURCE IDENTIFIER

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| Oligonucleotides    |        |            |
| tracrRNA            | FASMAC | GE-002     |
| Primers for RT-PCR, genotyping, genome editing | See Table S1 | N/A |
| Recombinant DNA     |        |            |
| pET28c-Fbxo47-C (aa272-451) | This paper | RDB19263 |
| pET19b-Fbxo47-M (aa174-316) | This paper | RDB19264 |
| Software and algorithms |        |            |
| genome browser IGV  | Thorvalsdottir et al., 2013 | http://meme-suite.org/tools/meme-chip |
| SoftWorx            | GE Healthcare | N/A |
| CellSens            | OLYMPUS | N/A |
| B2-X                | KEYENCE | N/A |
| Proteome Discoverer version 1.4 | Thermo Fisher Scientific | N/A |
| Mascot search engine version 2.5 | Matrix Science | N/A |
| Xcalibur            | Thermo Fisher Scientific | N/A |
| Thermal Cycler Dice Real Time System Software | Takara | Ver.5.11B for TP850 |
| Prisni8             | Graphpad | RRID:SCR_002798 |
analysis of the ovaries, and immunostaining experiments (age: 8-weeks old). Whenever possible, each knockout animal was compared to littermates or age-matched non-littermates from the same colony, unless otherwise described. Animal experiments were approved by the Institutional Animal Care and Use Committee (approval F28–078, A30–001, A28–026, A2020-006).

METHOD DETAILS

Generation of \textit{Fbxo47} knockout mice and genotyping

\textit{Fbxo47} knockout mouse was generated by introducing Cas9 protein (317–08441; NIPPON GENE, Toyama, Japan), tracrRNA (GE-002; FASMAC, Kanagawa, Japan), synthetic crRNA (FASMAC), and ssODN into C57BL/6N fertilized eggs using electroporation. For generating \textit{Fbxo47} Exon3-11 deletion (Ex3-11\textDelta) allele, the synthetic crRNAs were designed to direct TACACCTAGTGATAGCACTT(GGG) of the \textit{Fbxo47} intron 2 and AGAGCACTAGTCAGTGAATG(CGG) in the 3’-neighboring region of the Exon11. ssODN: 5’-GCTCAAAGTAAGCAGCAACAGAGCAGCAGTGTATATTATCATTGGGATGCTGAGGAGGCAAATTTGCGAGGGTTTGAAGC-3’ was used as a homologous recombination template.

The electroporation solutions contained (10 \textmu M of tracrRNA, 10 \textmu M of synthetic crRNA, 0.1 \textmu g/mL of Cas9 protein, 1 \textmu g/\textmu L of ssODN) for \textit{Fbxo47} knockout in Opti-MEM I Reduced Serum Medium (31985062; Thermo Fisher Scientific). Electroporation was carried out using the Super Electroporator NEPA 21 (NEPA GENE, Chiba, Japan) on Glass Microslides with round wire electrodes, 1.0 mm gap (45–0104; BTX, Holliston, MA). Four steps of square pulses were applied (1, three times of 3 mS poring pulses with 97 mS intervals at 30 V; 2, three times of 3 mS polarity-changed poring pulses with 97 mS intervals at 30 V; 3, five times of 50 mS transfer pulses with 50 mS intervals at 4 V with 40% decay of voltage per each pulse; 4, five times of 50 mS polarity-changed transfer pulses with 50 mS intervals at 4 V with 40% decay of voltage per each pulse).

The targeted \textit{Fbxo47} Ex3-11\textDelta allele in F0 mice were identified by PCR using the following primers:

Fbxo47-F1: 5’-TCCTCTCCTGTGTTCTATCAAACAG-3’ and Fbxo47-R1: 5’- TGCTAAGAGGTGTTAAAGAATGGAC-3’ for the knockout allele (825 bp). Fbxo47-F3: 5’-TCTGACCATGACGCTATCTCCTCC-3’ and Fbxo47-R1 for wild-type allele (503 bp).

The PCR amplicons were verified by sequencing. Primer sequences are listed in Table S1.

Generation of \textit{Fbxo47-3xFLAG-HA} knock-in mice and genotyping

\textit{Fbxo47-3xFLAG-HA} knock-in mouse was generated by introducing Cas9 protein, tracrRNA, synthetic crRNA, and ssODN into C57BL/6N fertilized eggs using electroporation as described above. The synthetic crRNA was designed to direct AGCCATCTCCTTCTAAGTC(AGG) of the \textit{Fbxo47}. ssODN: 5’-GAACCTCATAAAGGAGGTGCTGTATGACCATGAACTCAAAAGACCAGCTGGTG ATTTAAAAAGATCATGACACGTATTCAAAGGAGTGA CGATGAAAGGATACGGTTACGCTGCCCGACGTCCGATGTCCGATGGTTGATGCTGC-3’ was used as a homologous recombination template.

The targeted \textit{Fbxo47-3xFLAG-HA} knock-in allele in F0 mice were identified by PCR using the following primers:

Fbxo47-F4: 5’-TCTGTTCCATCTTCTCCATGCTCAGGC-3’ and Fbxo47-R3: 5’- TGAAGAGCCAGAATTTTCCAG-3’ for the knock-in allele (396 bp), and for wild-type allele (294 bp). The PCR amplicons were verified by sequencing. Primer sequences are listed in Table S1.

Generation of \textit{Skp1-3xFLAG-HA} knock-in mouse and genotyping

The targeting vector was designed to insert 3xFLAG-HA-3’UTR in frame with the coding sequence into the Exon 6 of the \textit{Skp1} genomic locus. Targeting arms of 1225bp and 1481bp fragments, 5’ and 3’ of the Exon 6 of \textit{Skp1} gene respectively, were generated by PCR from mouse C57BL/6 genomic DNA and directionally cloned flanking p.GK-Neo-polyA and DT-A cassettes. The 5’ arm was followed by nucleotide sequences encoding 3xFLAG, HA and the 3’UTR of \textit{Skp1} gene. TT2 ES cells were co-transfected with the targeting vector and pX330 plasmids (Addgene) expressing Caspr-gRNAs directing GCTGGCATTGACTCGGGGTTA and CGCCACATCCCGTGATT (tgg), which locate at the 3’ region of the Exon 6 of \textit{Skp1} gene. The G418-resistant ES clones were screened for homologous recombination with the \textit{Skp1} locus by PCR using
primers SKP1_5Arm_F2: 5’- G GTCAGCAACACTGCTG AACAGCTTG-3’ and KI96ES-19814R-HA: 5’-GGGCACGTCGTAGGGGTAT CCCTTG -3’ for the left arm (1909 bp) ; pKO2-3armF: 5’-AGGAACTTCGGA ATAGGAAC-3’ and SKP1_RightArm_R2: 5’-TGCAGTGGAGGCTCAGTCCAGCTTC-3’ for the right arm (1897 bp).

The homologous recombinant cells were isolated and chimeric mice were generated by aggregation (host ICR) of recombinant ES cells. Chimeric males were mated to C57BL/6N females and the progenies were genotyped by PCR using the primers:

SKP1onL2_F2: 5’- ATCATTGTTCCCAGGTGGAG -3’ and SKP1onRight_R1: 5’- GACTAGAACAAGATGACAGG -3’ for the knock-in allele (2078 bp) and the WT allele (1275bp). Primer sequences are listed in Table S1.

Histological analysis
Testes, caudal epididymis and ovaries were fixed in Bouin’s solution, and embedded in paraffin. Sections were prepared on CREST-coated slides (Matsunami) at 6 µm thickness. The slides were deparaffinized and stained with hematoxylin and eosin.

For Immunofluorescence staining, testes were embedded in Tissue-Tek O.C.T. compound (Sakura Finetek) and frozen. Cryosections were prepared on the CREST-coated slides (Matsunami) at 8 µm thickness, and then air-dried. The serial sections of frozen testes were fixed in 4% paraformaldehyde in PBS for 5 min at room temperature and washed briefly in PBS. After washing, the serial sections were permeabilized in 0.1% TritonX100 in PBS for 5 min. The sections were blocked in 3% BSA/PBS, and incubated at room temperature with the primary antibodies in a blocking solution. After three washes in PBS, the sections were incubated for 1 h at room temperature with Alexa-dye-conjugated secondary antibodies (1:1000; Invitrogen) in a blocking solution. PNA lectin staining was done using FITC-conjugated Lectin from Arachis hypo-gaea (IF, 1:1000, Sigma: L7381). TUNEL assay was performed using MEBSTAIN Apoptosis TUNEL Kit Direct (MBL 8445). DNA was counterstained with Vectashield mounting medium containing DAPI (Vector Laboratory).

Immunostaining of spermatocytes
Surface-spread nuclei from spermatocytes were prepared by the dry down method as described (Peters et al., 1997) (Takemoto et al., 2020) with modification. The slides were then air-dried and washed with water containing 0.1% TritonX100 or frozen for longer storage at −30°C. The slides were permeabilized in 0.1% TritonX100 in PBS for 5 min, blocked in 3% BSA/PBS, and incubated at room temperature with the primary antibodies in 3% BSA/PBS. After three washes in PBS, the sections were incubated for 1 h at room temperature with Alexa-dye-conjugated secondary antibodies (1:1000; Invitrogen) in a blocking solution. For bouquet counting, cells were suspended in PBS without hypotonic treatment and structurally preserved nuclei of spermatocytes were prepared by cytospin at 1000 rpm for 5 min (Thermofisher). Cells were fixed with 4% PFA in PBS for 5 min. The slide grasses were washed with PBS containing 0.1% Triton-X100 in PBS. After washing with PBS, immunofluorescence staining was performed immediately. DNA was counterstained with Vectashield mounting medium containing DAPI (Vector Laboratory).

Imaging
Immunostaining images were captured with DeltaVision (GE Healthcare). The projection of the images was processed with the SoftWorx software program version 7.2.1 (GE Healthcare). All images shown were Z-stacked. Bright field images and immunofluorescent images for counting seminiferous tubules, were captured with BIOREVO BZ-X710 (KEYENCE), and processed with BZ-H3A program. XY-stitching capture by 10x objective lens was performed for multiple-point color images using BZ-X Wide Image Viewer. Images were merged over the field using BZ-H3A Analyzer (KEYENCE). If the SYCP3 image was too dim for counting the SYCP3+ seminiferous tubules, the contrast of the color channel used for SYCP3 was enhanced in the XY-stitched image.

In vitro oocyte culture and Giemsa staining of metaphase chromosome spread
Ovaries collected from 4-week-old female mice were used after 46 to 48 h of treatment with 5 IU of pregnant mare serum gonadotropin. GV oocytes were isolated by puncturing the follicles in M2 medium (Sigma MR-015). The GV oocytes were cultured in M16 medium (Sigma MR-016) in a 5% CO2 atmosphere.
at 37°C for 6 h. For Giemsa staining of metaphase chromosome spread, oocytes were exposed to 0.5% Pronase (MERCK 10165921001) to remove the zona pellucida, and treated in hypotonic buffer containing 1% sodium citrate/0.1% PVA for 15 min. The oocytes and oocyte-like cells were placed on the slides, fixed in the Carnoy’s Fixative (75% Methanol, 25% Acetic Acid), and stained in 3% Giemsa solution for 30 min.

**Culture of OA-induced meta I spermatocyte**

Culture of OA-induced Meta I spermatocytes were performed as described (Wiltshire et al., 1995). The isolated spermatocytes were cultured in the presence or absence of 5 μM okadica acid (OA) for 3 h.

**Antibodies**

The following antibodies were used for immunoblot (IB) and immunofluorescence (IF) studies: mouse anti-FLAG M2 (Sigma-Aldrich F1804), rabbit anti-HA (IB, IF, 1:1000, Abcam: ab9110), rabbit anti-Actin (IB, 1:1000, Sigma-Aldrich A20666), mouse anti-MLH1 (IF, 1:500, BD Biosciences: 551,092), rabbit anti-H3S10p (IF, 1:2000, Abcam ab5176), rabbit anti-Histone H3 (IB, 1:1000, Abcam ab1791), rabbit anti-SYCP1 (IF, 1:1000, Abcam ab15090), rabbit anti-HORMAD1 (IF, 1:1000, ProteinTech 13917-1-AP), goat anti-Lamin B (IF, 1:1000, Santa Cruz: SC-8349), rabbit anti-MSH4 (IF, 1:500, Abcam ab58666), rabbit anti-SKp1 (IB, 1:1000, Abcam ab10546), rabbit anti-RAD51 (IF, 1:500, Santa Cruz: SC-6216), mouse anti-TRF1 (Shibuya et al., 2014) (IF, 1:1000), rabbit anti-TRF1 (Shibuya et al., 2014) (IB, 1:1000), rabbit anti-TRF2 (IB, 1:1000, NB110-57130), mouse anti-SYCP1 (IF, 1:1000) (Ishiguro et al., 2011), rat anti-SYCP3 (Ishiguro et al., 2020) (IF, 1:1000), rabbit anti-BRCA1 (IF, 1:500, kindly provided by Satoshi Namekawa), guinea pig anti-H1t (IF, 1:2000, kindly provided by Mary Ann Handel).

**Production of antibodies against FBXO47**

Polyclonal antibodies against mouse FBXO47 C-terminal (aa272-451) were generated by immunizing rabbits and a guinea pig. FBXO47 middle region (aa174-316) were generated by immunizing a rabbit. His-tagged recombinant proteins of FBXO47 middle region (aa174-316) and C-terminal (aa272-451) were produced by inserting cDNA fragments in-frame with pET19b and pET28c (Novagen) respectively in E. coli strain BL21-CodonPlus (DE3)-RIPL (Agilent), solubilized in a denaturing buffer (6 M HCl-Guanidine, 20 mM Tris-HCl pH 7.5) and purified by Ni-NTA (QIAGEN) under denaturing conditions. The antibodies were affinity-purified from the immunized serum with immobilized antigen peptides on CNBr-activated Sepharose (GE healthcare).

**PCR with reverse transcription**

Total RNA was isolated from tissues and embryonic gonads using TRIzol (Thermo Fisher). cDNA was generated from total RNA using Superscript III (Thermo Fisher) followed by PCR amplification using Ex-Taq polymerase (Takara) and template cDNA.

For RT-qPCR, total RNA was isolated from WT (n = 3) and Meiosin KO (n = 3) testes, and cDNA was generated as described previously (Ishiguro et al., 2020). Fbxo47 cDNA was quantified by ΔCT method using TB Green Premix Ex Taq II (Tli RNaseH Plus) and Thermal cycler Dice (Takara), and normalized by GAPDH expression level.

qPCR was performed in duplicates, and the average ddCt value was calculated for each cDNA sample. The expression level of Fbxo47 was divided by that of GAPDH to give the relative expression level of Fbxo47 to GAPDH. Relative expression level of Fbxo47 to GAPDH was normalized to 1 for a given P10 WT sample.

Sequences of primers used for RT-PCR were as follows:

- **GAPDH-F:** 5’-TTCACCACCATGGAGAAGGC-3’
- **GAPDH-R:** 5’-GGCATGACTGTTGCTGATGA-3’
- **Gapdh_F2:** 5’-ACCACAGTCCATGCGCATC-3’
- **Gapdh_R2:** 5’-TCCACACCCCTGTTGTGAT-3’
Gapdh_Ex6F: 5’-GGTTGTCTCCTGCAGACTTCA-3’

Gapdh_mRNAR: 5’-GCCGTATTCATTGTCATACCAGG-3’

Fbxo47-F 1443F: 5’-GCATAGCAAATGCTTTTGCCTGTG-3’

Fbxo47-R 1605R: 5’-GAGATAGCGTTCAATGCGGTCAGATAC-3’

Primer sequences are listed in Table S1.

**Preparation of testis extracts and immunoprecipitation**

Testis chromatin-bound and -unbound extracts were prepared as described previously (Ishiguro et al., 2014). Briefly, testicular cells collected in PBS containing 1 μM MG132, and suspended in low salt extraction buffer (20 mM Tris-HCl pH 7.5, 100 mM KCl, 0.4 mM EDTA, 0.1% Triton X-100, 10% glycerol, 1 mM β-mercaptoethanol) supplemented with Complete Protease Inhibitor (Roche). After homogenization, the soluble chromatin-unbound fraction was separated after centrifugation at 100,000 g for 10 min at 4°C. The chromatin bound fraction was extracted from the insoluble pellet by high salt extraction buffer (20 mM HEPES-KOH pH 7.0, 400 mM KCl, 5 mM MgCl₂, 0.1% Tween 20, 10% glycerol, 1 mM β-mercaptoethanol) supplemented with Complete Protease Inhibitor. The solubilized chromatin fraction was collected after centrifugation at 100,000 g for 10 min at 4°C.

**Immuno-affinity purification**

Immuno-affinity purification was performed with anti-FLAG M2 monoclonal antibody-coupled magnetic beads (Sigma-Aldrich M8823) from the testis chromatin-bound and -unbound fractions of Fbxo47-3xFLAG-HA knock-in mice and Skp1-3xFLAG-HA knock-in mice (14–21-day-old). For negative control, mock immuno-affinity purification was done from the age-matched wild type mice. The beads were washed with high salt extraction buffer for chromatin-bound proteins and low salt extraction buffer for chromatin-unbound proteins. The anti-FLAG-bound proteins were eluted by 3xFLAG peptide (Sigma-Aldrich). The second immuno-affinity purification was performed anti-HA 5D8 monoclonal antibody-coupled Magnet agarose (MBL M132-10). The bead-bound proteins were eluted with 40 μl of elution buffer (100 mM Glycine-HCl pH 2.5, 150 mM NaCl), and then neutralized with 4 μl of 1 M Tris-HCl pH 8.0.

The immunoprecipitated proteins were run on 4–12% NuPAGE (Thermo-Fisher) in MOPS-SDS buffer and silver-stained with Silver Quest (Thermo-Fisher), immunoblotted or analyzed by LC-MS/MS. For the immunoblot of whole testes extracts from WT, Fbxo47 KO, and Fbxo47-3FH Kl mice, lysates were prepared in RIPA buffer and run on 8% Laemmli SDS-PAGE in Tris-Glycine-SDS buffer. Immunoblot images were developed using ECL prime (GE healthcare) and captured by FUSION Solo (VILBER).

**Mass spectrometry**

The immunoprecipitated proteins were run on 4–12% NuPAGE (Thermo Fisher) by 1 cm from the well and stained with SimplyBlue (Thermo Fisher) for in-gel digestion. The gel containing proteins was excised, cut into approximately 1mm sized pieces. Proteins in the gel pieces were reduced with DTT (Thermo Fisher), alkylated with iodoacetamide (Thermo Fisher), and digested with trypsin and Lysyl endopeptidase (Promega) in a buffer containing 40 mM ammonium bicarbonate, pH 8.0, overnight at 37°C. The resultant peptides were analyzed on an Advance UHPLC system (ABRME1ichrom Bioscience) connected to a Q Exactive mass spectrometer (Thermo Fisher) processing the raw mass spectrum using Xcalibur (Thermo Fisher Scientific). The raw LC-MS/MS data was analyzed against the NCBI non-redundant protein/translated nucleotide database restricted to Mus musculus using Proteome Discoverer version 1.4 (Thermo Fisher) with the Mascot search engine version 2.5 (Matrix Science). A decoy database comprised of either randomized or reversed sequences in the target database was used for false discovery rate (FDR) estimation, and Percolator algorithm was used to evaluate false positives. Search results were filtered against 1% global FDR for high confidence level. All full lists of LC-MS/MS data are shown in Supplementary Data S1 (Excel file).
ChIP-seq data and public RNA-seq data analysis
MEIOSIN ChIP-seq data described in our previous study (Ishiguro et al., 2020) was analyzed for the Fbxo47 locus. MEIOSIN binding site was shown along with genomic loci from Ensembl on the genome browser IGV.

Single cell RNA-seq data analysis
The scRNA-seq data of fetal ovaries was derived from DRA 011172 (Shimada et al., 2021). The scRNA-seq data of mouse adult testis was derived from GEO: GSE109033 (Hermann et al., 2018). Reanalyses of scRNA-seq data were conducted using the Seurat package for R (v.3.1.3) (Stuart et al., 2019) and pseudotime analyses were conducted using monocle package for R: R (ver. 3.6.2), RStudio (ver.1.2.1335), and monocle (ver. 2.14.0) (Qiu et al., 2017) following developer’s tutorial.

QUANTIFICATION AND STATISTICAL ANALYSIS
Statistical analyses, and production of graphs and plots were done using GraphPad Prism8 (version 8.4.3) or Microsoft Excel (version 16.48).

Figure 1B Testis RNA was obtained from P8 WT (3 animals), P10 WT (3 animals), P10 Meiosin KO (3 animals). qPCR was performed in duplicates, and the average ddCt value was calculated for each cDNA sample. The expression level of Fbxo47 was divided by that of GAPDH to give the relative expression level of Fbxo47 to GAPDH. Relative expression level of Fbxo47 to GAPDH was normalized to 1 for a given P10 WT sample. Bar graph indicates mean with SD. Statistical significance was determined by one-way ANOVA for all the dataset (p = 0.0221). For pairwise comparison between P10 WT and Meiosin KO, statistical significance was determined by t test. p < 0.05. See Data S2.

Figure 1F RNA was obtained from WT Embryonic ovaries (E12.5 to E18.5). qPCR was performed in triplicates or quadruplicates, and the average ddCt value was calculated for each cDNA sample. The expression level of Fbxo47 was divided by that of GAPDH to give the relative expression level of Fbxo47 to GAPDH. Relative expression level of Fbxo47 to GAPDH was normalized to 1 for a given E12.5 WT sample. Bar graph indicates mean with SD. See Data S2.

Figure 3A Testis sections (P15) were obtained from non-tagged control (3 animals) and Fbxo47-3FH KI (3 animals). Number of seminiferous tubules that have HA+/SYCP3+ cells was counted per the seminiferous tubules that have SYCP3+ spermatocyte cells (84, 85, 45 tubules for non-tagged control, 123, 90, 79 tubules for Fbxo47-3FH KI).

Figure 3D Testis sections (P18) were obtained from non-tagged control (3 animals) and Fbxo47-3FH KI (3 animals). Number of seminiferous tubules that have HA+/H1t+ cells was counted per the seminiferous tubules that have H1t+ spermatocyte cells (52, 36, 18 tubules for non-tagged control; 15, 51, 36 tubules for Fbxo47-3FH KI).

Figure 4C Quantification of testes/body-weight ratio (mg/g) in Fbxo47+/− (8w; n = 4) and Fbxo47 KO (8w; n = 10) mice. n: the number of animals examined for each genotype. Bar graph indicates mean with SD. Statistical significance was determined by t-test. p < 0.0001. See Data S2.

Figure 4J Cumulative number of pups born from Fbxo47+/− (n = 4, all 6-week old at the start point of mating) and Fbxo47 KO (n = 4, all 6-week old at the start point of mating) females was counted for 18 weeks of breeding. See Data S2.

Figure 5A Quantification of the seminiferous tubules that have H1t+/SYCP3+ cells per the seminiferous tubules that have SYCP3+ spermatocyte cells in Fbxo47 heterozygous (p18: 62, 61, 29 tubules/animal were counted from n = 3 animals; 8w: 135, 143, 45 tubules/animal were counted from n = 3 animals) and Fbxo47 KO (p18: 105, 59, 141 tubules/animal were counted from n = 3 animals; 8w: 36, 55, 63, 64, 88, 69, 108 tubules/animal were counted from n = 7 animals) testes. n: the number of animals examined for each genotype. Bar graph indicates mean with SD. Statistical significance was determined by unpaired t-test. p = 0.0012 for Fbxo47 heterozygous versus Fbxo47 KO at P18. See Data S2.
Figure 5C Spermatocytes in the four developmental stages (leptotene, zygotene, pachytene, and diplotene(-like)) per total cells in meiotic prophase I were quantified in WT (n = 727 from one animal) and Fbxo47 KO (n = 659 from one animal) at P15, and in WT (n = 561 from one animal) and Fbxo47 KO (n = 516 from one animal) at P18. See Data S2.

Figure 5F Spermatocytes in the four developmental stages (leptotene or zygotene, pachytene, and diplotene(-like)) per total cells in meiotic prophase I were quantified in Fbxo47+/−/C0 (n = 105 for OA−, 117 for OA+) and Fbxo47 KO (n = 140 for OA−, 117 for OA+). See Data S2.

Figure 5G Quantification of the seminiferous tubules that have TUNEL + cells per total tubules in Fbxo47+/− (8w: n = 3) and Fbxo47 KO (8w: n = 3) testes. Bar graph indicates mean with SD. Statistical significance was determined by t-test. p = 0.0072. See Data S2.

Figure 6C Numbers of RAD51 foci on SYCP3 axes were counted in WT and Fbxo47 KO. Number of foci was indicated in the scatterplot with median. Statistical significance was determined by Mann-Whitney U-test. ****: p < 0.0001. ***: p < 0.001. **: p < 0.01. See Data S2.

Figure 6D Numbers of MSH4 foci on SYCP3 axes were counted in WT and Fbxo47 KO. Number of foci was indicated in the scatterplot with median. Statistical significance was determined by Mann-Whitney U-test. p < 0.05. See Data S2.

Figure 6E Numbers of MLH1 foci on SYCP3 axes were counted in Fbxo47+/− pachyene (n = 15), or Fbxo47 KO pachyene (n = 13) and diplotene-like (n = 13). Number of foci was indicated in the scatterplot with median. Statistical significance was determined by Mann-Whitney U-test. p < 0.0001. See Data S2.

Figure S3D The frequency of bouquet stage spermatocytes nuclei in wild-type (n = 355) and Fbxo47 KO (n = 342) was scored at 12 day post-partum. Statistical significance was determined by Chi square-test. p = 0.5025. See Data S2.