

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

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15

16 **Abstract**

17 **Meiotic prophase is a prolonged G2 phase that ensures the completion of numerous**
18 **meiosis-specific chromosome events. During meiotic prophase, homologous chromosomes**
19 **undergo synapsis to facilitate meiotic recombination yielding crossovers. It remains**
20 **largely elusive how homolog synapsis is temporally maintained and destabilized during**
21 **meiotic prophase. Here we show that FBXO47 is the stabilizer of synaptonemal complex**
22 **during male meiotic prophase. Disruption of FBXO47 shows severe impact on homologous**
23 **chromosome synapsis and DSB repair processes, leading to male infertility. Notably, in the**
24 **absence of FBXO47, although once homologous chromosomes are synapsed, the**
25 **synaptonemal complex is precociously disassembled before progressing beyond pachytene.**
26 **Remarkably, *Fbxo47* KO spermatocytes remain in earlier stage of meiotic prophase and**
27 **lack crossovers, despite apparently exhibiting diplotene-like chromosome morphology. We**
28 **propose that FBXO47 functions independently of SCF E3 ligase, and plays a crucial role**
29 **in preventing synaptonemal complex from premature disassembly during cell cycle**
30 **progression of meiotic prophase.**

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32

33 **Introduction**

34 Meiosis consists of a single DNA replication followed by two rounds of chromosome
35 segregation, which halves the chromosome number to ultimately produce haploid gametes.

36 During meiotic prophase I, sister chromatids are organized into proteinaceous structures, termed
37 axial element (AE) or chromosome axis (Zickler and Kleckner, 2015). Homologous
38 chromosomes (homologs) then undergo synapsis, which is promoted by the assembly of
39 synaptonemal complex (SC) (Cahoon and Hawley, 2016). Homolog synapsis facilitates meiotic
40 recombination yielding crossovers, a process that produces physical linkages called chiasmata
41 between the homologs (Baudat et al., 2013) (Keeney et al., 2014). While homolog synapsis
42 persists until meiotic recombination is completed during pachytene, it is dissolved upon
43 diplotene-Metaphase I transition. Thus, homolog synapsis and de-synapsis is temporally
44 regulated. However, it remains elusive how homolog synapsis is temporally maintained and
45 destabilized during meiotic prophase.

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

55 Fbox-domain containing proteins act as a substrate recognition subunit in SCF E3 ubiquitin
56 ligase (Kipreos and Pagano, 2000) (Jin et al., 2004) (Reitsma et al., 2017). It has been shown
57 that Fbox-domain containing proteins are involved in homolog synapsis in a wide variety of
58 organisms. In rice plant *Oryza sativa*, mutants of MEIOTIC F-box *MOF* (He et al., 2016) and
59 another Fbox *ZYGO1* (Zhang et al., 2017) showed defects in DNA double-strand break (DSB)
60 repair and bouquet formation during meiotic prophase. In budding yeast, temperature sensitive
61 mutant of *Cdc4* that encodes F-box protein showed defective SC formation and DSB repair
62 (Zhu et al., 2021). In *Drosophila* female, depletion of Slmb (β Trcp) and CG6758 (Fbxo42)
63 caused impaired assembly and/or premature disassembly of SC (Barbosa et al., 2021). Although
64 the substrates are yet to be indentified in most of the cases, Fbox-domain containing proteins
65 directly or indirectly regulate the assembly and disassembly of SC.

66
67 Previously, we identified MEIOSIN that plays an essential role in meiotic initiation both in
68 mouse male and female (Ishiguro et al., 2020). MEIOSIN together with STRA8 (Kojima et al.,
69 2019) activates meiotic genes and directs the switching from mitosis to meiosis. In the present
70 study, we identified *Fbxo47* gene that encodes a Fbox protein, as one of the
71 MEIOSIN/STRA8-target genes. Previous genetic studies suggested FBXO47 homologs are
72 implicated in the progression of meiotic prophase in different species. In *C. elegance*, mutation
73 in *prom-1* that encodes putative *Fbxo47* homolog, showed reduced homologous chromosome
74 pairing and bivalent formation (Jantsch et al., 2007). In medaka fish, *fbxo47* mutant fails to
75 complete meiotic prophase in female but switches developmental fate from oogenesis into
76 spermatogenesis (Kikuchi et al., 2020). In mouse, *Fbxo47* gene that has previously been

77 identified as a meiotic gene by single cell RNA-seq analysis of testes, is essential for mouse
78 spermatogenesis (Chen et al., 2018) (Hua et al., 2019). Although previous studies suggest that
79 FBXO47 homologs and distant meiotic Fbox-domain containing proteins play a role in
80 homologous chromosome pairing/synapsis and meiotic recombination in a wide variety of
81 organisms, the precise mechanisms how these proteins are involved in these processes remained
82 elusive. Furthermore, whether FBXO47 is indeed involved in the function of SCF is unknown.
83 Here we show that mouse FBXO47 is essential for maintaining homolog synapsis during
84 meiotic prophase. FBXO47 is a cytoplasmic protein rather than a telomere binding protein, and
85 functions independently of SCF. We demonstrate that in *Fbxo47* KO spermatocytes,
86 homologous chromosome synapsis is complete, but SC is precociously disassembled. Further,
87 we show that *Fbxo47* KO spermatocytes fail to progress beyond pachytene and remain in earlier
88 meiotic prophase in terms of cell cycle progression, despite the apparent exhibition of
89 diplotene-like morphology of chromosomes. We propose that FBXO47 is essential for
90 preventing SC from premature destruction during cell cycle progression of male meiotic
91 prophase. Further, we discuss the different observations and interpretations between the present
92 study and the previous study on FBXO47 (Hua *et al.*, 2019).

93

94 **Results**

95 **FBXO47 is expressed in mouse testes**

96 Previously, we demonstrated that MEIOSIN collaborating with STRA8 activates meiotic genes,
97 which are required for numerous meiotic events (Ishiguro *et al.*, 2020). In spermatocytes, we
98 identified *Fbxo47* as one of the MEIOSIN/STRA8-bound genes (Fig. 1A). Our previous
99 RNA-seq analysis showed that expression of *Fbxo47* was significantly downregulated in
100 *Meiosin* KO testes at postnatal day 10 (P10) when a cohort of spermatocytes should undergo the
101 first wave of meiotic entry (Ishiguro *et al.*, 2020). We confirmed this by RT-qPCR analysis
102 demonstrating that *Fbxo47* expression level was indeed downregulated in *Meiosin* KO testis at
103 P10 (Fig. 1B). We further examined the expression patterns of *Fbxo47* in different mouse
104 tissues by RT-PCR analysis. *Fbxo47* gene showed higher expression levels in adult testis
105 compared to other adult organs that we examined (Fig. 1C). Spermatogenic expression of
106 *Fbxo47* gene was further confirmed by the reanalysis of previous scRNA-seq data of adult
107 mouse testis (Hermann *et al.*, 2018) (Fig. 1D). The result indicated that *Fbxo47* was
108 coordinately expressed with the landmark genes of meiotic spermatocyte such as *Dmc1*, and
109 spermatid at spermiogenesis such as *Acrv1*, rather than those of spermatogonia such as *Zbtb16*
110 (Fig. 1D). We noticed that *Fbxo47* mRNA was expressed weakly in meiotic spermatocytes, and
111 highly in spermatids in testes, which is consistent with a previous study (Chen *et al.*, 2018). In
112 females, expression of *Fbxo47* mRNA was examined by the reanalysis of previous scRNA-seq
113 data of fetal ovaries (Shimada *et al.*, 2021). We found that *Fbxo47* was coordinately expressed
114 during meiotic prophase, such as *Dmc1* (Fig. 1E). Expression of *Fbxo47* mRNA culminated
115 at E16.5 and declined afterward in the ovary (Fig. 1F).

116 To determine the meiotic stage-specific expression of FBXO47 protein, we generated different
117 antibodies against FBXO47 C-terminal region (aa 271- 451) and middle region (aa173-316).

118 However, we failed to evaluate stage specificity of endogenous FBXO47 protein expression by
119 immunostaining, although it was uncertain whether this was due to the sensitivity of the
120 antibodies, inaccessibility of the antibodies to the epitopes, or low expression level of FBXO47
121 protein in the target cells.
122 To circumvent this issue, we generated *Fbxo47-3xFLAG-HA* knock-in (*Fbxo47-3FH-GFP* KI)
123 mice, which allowed the detection of FBXO47-3xFLAG-HA protein expressed from
124 endogenous *Fbxo47* locus (Fig. 1G, Fig. S1). We examined FBXO47-3xFLAG-HA fusion
125 protein from cytosolic and chromatin extracts of *Fbxo47-3FH-GFP* KI testes. Immunoblotting
126 demonstrated that FBXO47 protein was detected with FLAG antibody only when it was
127 enriched by tandem immunoprecipitations using anti-FLAG and anti-HA antibodies (Fig. 1H),
128 suggesting that the expression level of FBXO47 protein was low in testes. We noticed that more
129 FBXO47 protein was detected in the cytosolic fraction compared to the chromatin fraction (Fig.
130 1H), suggesting its predominant localization in the cytoplasm rather than on the chromatin.
131 Sequential reblotting showed that different antibodies against the endogenous FBXO47 protein
132 that we generated detected the same protein as indicated by anti-FLAG antibody (Fig. 1H).
133 Previous study showed that FBXO47 binds to telomeric proteins TRF1 and TRF2 (Hua *et al.*,
134 2019). However, we failed to detect neither TRF1 nor TRF2 in FBXO47 immunoprecipitates
135 from testis chromatin fraction (Fig. S3A), which was a sharp contrast to the previous study (Hua
136 *et al.*, 2019).

137

138 **FBXO47 may function independently of SCF in spermatocytes.**

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

146 To further examine whether FBXO47 serves as a subunit of SCF by reciprocal
147 immunoprecipitation of SKP1, we generated *Skp1-3xFLAG-HA* knock-in (*Skp1-3FH-GFP* KI)
148 mice, which allowed the detection of SKP1-3xFLAG-HA protein expressed from endogenous
149 *Skp1* locus and its associated factors (Fig. 2A). Although the homozygous *Skp1-3xFLAG-HA* KI
150 mice were embryonic lethal, heterozygous knock-in mice were fertile and developed normally.
151 Consistent with a previous study (Guan *et al.*, 2020), SKP1-3xFLAG-HA fusion protein
152 localized along the SC in the *Skp1-3FH-GFP* KI spermatocytes (Fig. 2B). SKP1-3xFLAG-HA
153 was enriched by tandem immunoprecipitations using anti-FLAG and anti-HA antibodies from
154 testis cytosolic fraction (Fig. 2C). Mass spectrometry analysis demonstrated that total of 45
155 different Fbox-domain containing proteins and SCF core subunits (SKP1, RBX1, CUL1, CUL7)
156 were co-immunoprecipitated with SKP1-3xFLAG-HA (Fig. 2D, Supplementary Data1).

157 However, we failed to detect FBXO47 in the SKP1-3xFLAG-HA immunoprecipitates either by
158 mass spectrometry analysis or by western blotting (Fig. 2D, E). SKP1 localized along the SC in

159 *Fbxo47* KO, suggesting that localization of SKP1 did not depend on FBXO47 (Fig. 2F).
160 Altogether, our data suggest that FBXO47 may function independently of SCF in mouse testes.
161 Previous study showed that FBXO47 interacts with SKP1 in yeast two hybrid assay and in
162 GFP-SKP1 IP using HEK293T cell extract that overexpressed FLAG-FBXO47 and
163 GFP-SKP1 (Hua *et al.*, 2019). Although we do not know the exact reason for these controversial
164 observations between our present study and the previous one (Hua *et al.*, 2019), this could be
165 due to their detection methodology using yeast and overexpression of FBXO47 in culture cells.
166

167 **Expression of FBXO47 is limited to early meiotic prophase in mouse testes**

168 To identify the specific stage in which FBXO47 was expressed, we performed immunostaining
169 using stage specific markers SYCP3 (a component of meiotic chromosome axis), SYCP1 (a
170 marker of homologous chromosome synapsis), and γ H2AX (a marker of DSBs).
171 Immunostaining of the *Fbxo47-3FH-GFP* KI testis (P15) indicated that FBXO47 protein was
172 detected by HA antibody in average 21% (n = 3) among total SYCP3 positive seminiferous
173 tubules (Fig. 3A). Close inspection of seminiferous tubules showed that FBXO47 protein
174 indicated by the presence of HA staining appeared in the cytosol at leptotene and zygotene (Fig.
175 3B, C). Notably, the expression level of FBXO47-3xFLAG-HA fusion protein declined in
176 pachytene, when homologs were fully synapsed (Fig. 3C). Testis-specific histone H1t is a
177 marker of spermatocytes later than mid pachytene (Cobb *et al.*, 1999) (Drabent *et al.*, 1996).
178 Immunostaining of seminiferous tubules by testis-specific histone H1t indicated that FBXO47
179 protein was expressed only in H1t negative stage (Fig. 3D). None of H1t positive spermatocytes
180 showed FBXO47 immunostaining (Fig. 3E), suggesting that FBXO47 expression had declined
181 by mid-pachytene. Thus, the expression of FBXO47 protein was limited to a narrow window of
182 early meiotic prophase. Although the expression of *Fbxo47* mRNA was upregulated in
183 spermatids, immunostaining of FBXO47 protein detected no more than background levels in
184 spermatids (Fig. 3F). This suggested that the expression of FBXO47 might be
185 post-transcriptionally suppressed after post-meiotic spermatids to have the expression
186 specifically limited to early meiotic prophase.
187
188

189 **Disruption of *Fbxo47* led to severe defect in spermatogenesis**

190 In order to address the role of *Fbxo47* in meiosis, we deleted Exon3-Exon11 of *Fbxo47* loci in
191 C57BL/6 fertilized eggs through the CRISPR/Cas9 system (Fig. 4A). RT-PCR analysis showed
192 that *Fbxo47* mRNA expression level was absent in *Fbxo47* KO testis (Fig. 4B). Although
193 *Fbxo47* KO male mice did not show overt phenotype in somatic tissues, defects in male
194 reproductive organs were evident with smaller-than-normal testes (Fig. 4C). Histological
195 analysis revealed that post-meiotic spermatids and spermatozoa were absent in eight-week-old
196 *Fbxo47* KO seminiferous tubules (Fig. 4D). Accordingly, sperm was absent in adult *Fbxo47* KO
197 caudal epididymis (Fig. 4E). Consistently, seminiferous tubules that contain PNA lectin (a
198 marker of spermatids) positive cells were absent in *Fbxo47* KO (Fig. 4F). Thus, the later stage
199 of spermatogenesis was severely abolished in *Fbxo47* KO seminiferous tubules, resulting in

200 male infertility (Fig. 4G). In contrast to male, *Fbxo47* KO females exhibited seemingly normal
201 fertility with no apparent defects in adult ovaries (Fig. 4H). Consistent with this histological
202 observation of ovaries, metaphase I oocytes derived from *Fbxo47* KO females processes normal
203 number of bivalent chromosomes with chiasmata, indicating that *Fbxo47* KO oocytes had
204 progressed normal meiotic prophase (Fig. 4I). Furthermore, *Fbxo47* KO females were fertile
205 (Fig. 4G, J), although we could not exclude the possibility that more subtle defects might have
206 occurred in the ovaries besides fertility. Thus, the infertility caused by disruption of *Fbxo47* was
207 male specific. Therefore, these results suggest that requirement of FBXO47 is sexually different
208 in mouse.

209

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

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

223 Curiously, however, *Fbxo47* KO spermatocytes exhibited apparent diplotene-like chromosome
224 morphology, despite the failure in reaching H1t positive mid pachytene (Fig. 5A). It is known
225 that homolog synapsis is initiated at interstitial regions on the chromosome arm at zygotene, and
226 that de-synapsis of homologs first starts at interstitial regions on the chromosome arm, while
227 telomere regions are prone to be the last place of de-synapsis at diplotene (Bisig *et al.*, 2012)
228 (Qiao *et al.*, 2012). This cytological difference readily distinguishes de-synapsed chromosomes
229 at diplotene from un-synapsed ones at zygotene. Indeed, those *Fbxo47* KO spermatocytes with
230 diplotene-like chromosome morphology apparently showed a typical feature of de-synapsis of
231 homologs, wherein telomere regions retained homolog synapsis while interstitial regions were
232 free from synapsis. To solve the paradox that *Fbxo47* KO spermatocytes showed diplotene-like
233 chromosome morphology despite the failure of progressing beyond H1t-positive pachytene
234 stage, we further analyzed the meiotic prophase population at P15 and P18 in the first wave of
235 spermatogenesis of *Fbxo47* KO testes. Notably, diplotene-like cells (6.7 %) appeared in *Fbxo47*
236 KO spermatocytes as early as P15, whereas the first wave of spermatogenesis was yet to pass
237 beyond pachytene stage in the age-matched WT (Fig. 5C). HORMAD1 localizes along
238 un-synapsed chromosomes before pachytene and de-synapsed chromosomes at diplotene, but
239 dissociates from synapsed chromosomes (Shin *et al.*, 2010) (Daniel *et al.*, 2011) (Wojtasz *et al.*,
240 2009). In *Fbxo47* KO spermatocytes, HORMAD1 dissociated from synapsed chromosomes at

241 pachytene and re-localizes on de-synapsed chromosomes at diplotene-like stage as in those of
242 WT (Fig. 5D), suggesting that localization of HORMAD1 on chromosomes was normally
243 regulated. Histone H3 Ser10 phosphorylation (H3S10p) by Aurora B kinase of the chromosome
244 passenger complex marks the centromeric region at diplotene and the whole chromosome at
245 metaphase I (Parra et al., 2009) (Parra et al., 2003). In the control spermatocytes, the
246 centromeric regions at diplotene were indicated by immunostaining of H3S10p (Fig. 5E). In
247 contrast, H3S10p-positive centromeric regions were not observed in *Fbxo47* KO diplotene-like
248 spermatocytes (Fig. 5E). This observation indicated that *Fbxo47* KO spermatocytes failed to
249 reach *bona fide* diplotene stage of meiotic prophase, albeit exhibiting apparent homolog
250 de-synapsis. Thus, we reasoned that even though homolog synapsis once occurred, it was
251 destabilized during pachytene in *Fbxo47* KO spermatocytes. It should be mentioned that more
252 zygotene and reciprocally less pachytene populations were observed in *Fbxo47* KO
253 spermatocytes compared to WT at P15 and P18 (Fig. 5C). This implies that the process of
254 homolog synapsis, at least in part, may be delayed in *Fbxo47* KO spermatocytes.

255
256 Mid-late pachytene spermatocytes acquire competency for meiotic prophase-Metaphase I
257 transition indicated by the response to phosphatase inhibitor okadaic acid (OA) (Cobb *et al.*,
258 1999). *In vitro* culture of isolated spermatocytes in the absence or presence of OA demonstrated
259 that while the control spermatocytes progressed to diakinesis/metaphase I in the presence of OA,
260 *Fbxo47* KO spermatocytes did not (Fig. 5F). Since *Fbxo47* KO spermatocytes were yet to
261 acquire competency for OA-induced progression into metaphase I, even the most advanced
262 *Fbxo47* KO spermatocytes remained in an earlier cell cycle stage compared to the control.
263 These results suggested that the primary defect occurred at zygotene or early pachytene stage in
264 *Fbxo47* KO spermatocytes. Notably, TUNEL positive cells were observed in ~21% of *Fbxo47*
265 KO seminiferous tubules (Fig. 5G), suggesting that *Fbxo47* KO spermatocytes were
266 consequently eliminated by apoptosis. Altogether, these results suggested that SC was
267 prematurely disassembled in *Fbxo47* KO spermatocytes (Fig. 5H).

268 269 ***Fbxo47* KO spermatocytes show defects in meiotic recombination**

270 Aforementioned results suggested that FBXO47 protein was required for stable maintenance of
271 SC (Fig. 5). SC facilitates meiotic recombination that is executed by DSB formation and repair
272 steps. Then SC is disassembled after the completion of crossover formation. Given that SC was
273 prematurely destabilized in *Fbxo47* KO spermatocytes, we assumed two possibilities: (1)
274 premature SC disassembly could be a result of early completion of meiotic recombination. (2)
275 premature SC disassembly abolished the processes of meiotic recombination. To address these
276 issues, we examined DSB formation and repair events by immunostaining of γ H2AX. The first
277 wave of γ H2A is mediated by ATM after DSB formation at leptotene (Mahadevaiah *et al.*,
278 2001), and disappears during DSB repair. The second wave of γ H2A at zygotene is mediated by
279 ATR that targets unsynapsed chromosomes (Royo *et al.*, 2013). At zygotene, γ H2AX signal
280 appeared in *Fbxo47* KO spermatocytes in the same manner as WT (Fig. 6A), indicating that
281 DSB formation normally occurred in *Fbxo47* KO spermatocytes. However, γ H2AX signals

282 largely persisted throughout the nuclei until pachytene-like and diplotene-like stages in *Fbxo47*
283 KO spermatocytes, while they overall disappeared in WT pachytene spermatocytes except for
284 retaining on the XY body (Fig. 6A). This observation suggested that DSB was still not repaired
285 in *Fbxo47* KO diplotene-like spermatocytes. Furthermore, BRCA1, a marker of asynapsis
286 (Scully et al., 1997) (Turner et al., 2004) (Broering et al., 2014), appeared along unsynapsed
287 autosomal axes in zygotene *Fbxo47* KO spermatocytes as in those of WT (Fig. 6B). This
288 suggests that meiotic silencing of unsynapsed chromatin (MUSC) was normally activated in
289 *Fbxo47* KO spermatocytes. Crucially, in contrast to un-synapsed chromosomes in zygotene,
290 BRCA1 was not observed along precociously de-synapsed chromosomes in *Fbxo47* KO
291 diplotene-like spermatocytes (Fig. 6B). This suggests that MUSC was canceled in *Fbxo47* KO
292 diplotene-like spermatocytes, presumably once homolog synapsis had successfully been
293 achieved.

294 RAD51 facilitates the invasion of 3'-extended strand into the duplex of homolog at DSBs
295 (Cloud et al., 2012) (Shinohara and Shinohara, 2004). In accordance with the persistent DSBs in
296 *Fbxo47* KO (Fig. 6A), the number of RAD51 foci was significantly increased in *Fbxo47* KO
297 spermatocytes (Fig. 6C). Reciprocally, the number of MSH4 foci was decreased in *Fbxo47* KO
298 spermatocytes (Fig. 6D). These observations suggest that although RAD51 was normally loaded
299 onto DSBs, the processes of homologous recombination-mediated repair were delayed or
300 blocked in the absence of FBXO47. Accordingly, the number of MLH1 foci, a marker of
301 crossover (CO), was significantly reduced in *Fbxo47* KO pachytene-like spermatocytes
302 compared to WT pachytene spermatocytes (Fig. 6E). This implies that crossover recombination
303 was incomplete in the absence of FBXO47. Altogether, precocious disassembly of SC was a
304 cause of the defect in meiotic recombination rather than a result of early completion of meiotic
305 recombination.

306
307

308 **Discussion**

309 **FBXO47 stabilizes homolog synapsis independently of SCF in mouse**

310 We have shown that FBXO47 is required for the maintenance of homolog synapsis during
311 prolonged meiotic prophase. *Fbxo47* KO spermatocytes showed precocious de-synapsis, albeit
312 exhibiting apparently “diplotene-like” morphology (Fig. 5B). Although this phenomenon in
313 *Fbxo47* KO spermatocytes was partly similar to that observed in conditional *Skp1* KO (Guan *et*
314 *al.*, 2020), marked phenotypic differences were observed between *Fbxo47* KO and *Skp1* KO
315 spermatocytes. In *Skp1* KO testis, late pachytene spermatocytes are absent and concurrently
316 diplotene spermatocytes are increased. *Skp1* KO spermatocytes at least reach H1t positive
317 mid-pachytene in terms of cell cycle, but most of them contain de-synapsed chromosomes at
318 pericentric end termed “Y pachynema”. Thus, *Skp1* KO spermatocytes show precocious
319 de-synapsis and pachytene exit. In contrast, *Fbxo47* KO spermatocytes failed to reach H1t
320 positive mid-pachytene (Fig. 5A). Although apparent diplotene-like morphology of homolog
321 chromosomes appeared in *Fbxo47* KO spermatocytes (Fig. 5B), “Y pachynema” was not
322 observed in *Fbxo47* KO, unlike in *Skp1* KO spermatocytes. Thus, *Fbxo47* KO spermatocytes

323 show precocious desynapsis despite the failure of progression beyond pachytene. These results
324 suggested that primary defect in *Fbxo47* KO spermatocyte occurred at earlier cell cycle stage
325 than *Skp1* KO spermatocytes. HORMAD1 localizes along unsynapsed and de-synapsed
326 chromosomes during meiotic prophase (Shin *et al.*, 2010) (Daniel *et al.*, 2011), and dissociates
327 from synapsed chromosomes by the action of TRIP13 AAA ATPase (Wojtasz *et al.*, 2009).
328 Whereas HORMAD1 persists both in synapsed and desynapsed chromosomes in *Skp1* KO
329 spermatocyte, localization of HORMAD1 on chromosomes was normally regulated in *Fbxo47*
330 KO (Fig. 5D). Thus, precocious desynapsis could be derived at least in part from failure of
331 HORMAD1 removal in *Skp1* KO and from different mechanism in *Fbxo47* KO. Moreover,
332 while DSB repair process indicated by γ H2AX staining (Fig. 6A) was impaired both in *Fbxo47*
333 KO and in *Skp1* KO spermatocytes, the extent of crossover formation was different between
334 them. Whereas significant number of MLH1 foci were observed in mid-late pachytene and
335 diplotene spermatocytes in *Skp1* KO, MLH1 foci were rarely observed in pachytene and
336 diplotene-like spermatocytes in *Fbxo47* KO (Fig. 6E). Thus, meiotic recombination and
337 crossover formation were more progressed in *Skp1* KO than in *Fbxo47* KO.

338
339 Fbox-domain containing proteins confers substrate specificity to SCF E3 ubiquitin ligase (Jin *et al.*
340 *et al.*, 2004) (Reitsma *et al.*, 2017). Although FBXO47 possesses a putative Fbox domain, it was
341 not detected in the SKP1 immunoprecipitates from *Skp1-3xFLAG-HA* KI testes (Fig. 2D,
342 Supplementary Data1). Reciprocally, SKP1 was not detected in the immunoprecipitates from
343 *Fbxo47-3xFLAG-HA* KI testes (Fig. 1H, Fig S2). Furthermore, while SKP1 localized along
344 lateral element (LE) of synapsed chromosomes (Fig. 2B) (Guan *et al.*, 2020), FBXO47 protein
345 did not show such a specific localization pattern on the chromosome (Fig. S3B). Although we
346 cannot formally exclude a possibility that FBXO47 is incorporated as a substrate recognition
347 subunit in SCF under specific regulation, our results suggest that FBXO47 may not be
348 incorporated in the function of SCF, and rather FBXO47 may function independently of SCF
349 in spermatocytes.

350

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

352 *Fbxo47* homologues and other distant F-box proteins have been implicated in meiotic prophase
353 progression in various species. Although defects accompanying DSB repair and crossover are
354 similarly observed in mouse and *C. elegans* *Fbxo47* mutants, the primary causes are assumed to
355 be different. In *C. elegans*, PROM-1 encodes *Fbxo47* homolog. In *C. elegans*, organization of
356 gonadal germline is divided into mitotic/meiotic entry zone, transition zone corresponding to
357 zygotene, and pachytene zone. *Prom-1* mutant showed delayed and asynchronous initiation of
358 homolog pairing, so that distinct transition zone was missing and meiotic entry zone was rather
359 extended (Jantsch *et al.*, 2007) with attenuating CHK-2 activity (Mohammad *et al.*, 2018)
360 (Baudrimont *et al.*, 2021). Further, PROM-1 was proposed to down regulate mitotic cell cycle
361 proteins such as Cyclin E homolog CYE-1 at meiotic entry, independently of promoting
362 homolog pairing as a positive regulator of CHK-2 kinase (Mohammad *et al.*, 2018). Thus,
363 PROM-1 functions very early in meiotic prophase in *C. elegans*, which is similar to our

364 observation in mice (Fig. 5). In *prom-1* meicytes however, homolog pairing was defective and
365 non-homologous synapsis was consequently pronounced in autosomes but not in X
366 chromosome. Thus, PROM-1 is implicated in promoting autosome homolog pairing. This is a
367 contrast to our observation, in which homolog synapsis once took place normally, followed by
368 premature desynapsis in *Fbxo47* KO spermatocytes (Fig. 5B).

369 In the teleost fish medaka, *fbxo47* mutant XX germ cells exhibit abnormally condensed
370 chromosomes in ovaries and fail to undergo oogenesis after diplotene, showing that the sexual
371 fate of XX germ cells turns into spermatogenesis (Kikuchi *et al.*, 2020). Thus, *fbxo47* is
372 involved in the regulation of cell division in ovaies, and in turn the suppression of
373 spermatogenesis in female germ cells in medaka. The germline feminization under *fbxo47* is
374 mediated at least by two downstream transcription factors *lhx8b* and *figla* during early meiotic
375 prophase in medaka. Despite the phenotypical similarities and differences observed in the
376 mutants of *Fbxo47* homologs in diverse organisms, *FBXO47* homologs commonly act during
377 meiotic prophase, although at different time points.

378

379 **Distinct interpretations on the function of FBXO47 in mouse**

380 Previous study showed that mouse *FBXO47* interacts with *SKP1* and telomere binding proteins,
381 *TRF1* and *TRF2* (Hua *et al.*, 2019). According to the study, *FBXO47* was localized to telomeres
382 during meiotic prophase. Furthermore, *TRF2* were destabilized and telomeres were detached
383 from the nuclear envelope in *Fbxo47* KO spermatocytes, causing defects in telomere bouquet
384 formation (Hua *et al.*, 2019). Those observations led to propose that *FBXO47* binds to telomeric
385 proteins *TRF1* and *TRF2*, and plays a role in protecting *TRF2* from destruction (Hua *et al.*,
386 2019). However, we failed to detect either *TRF1* or *TRF2* in *FBXO47* immunoprecipitates from
387 testis chromatin fraction (Fig. S3A). Further, we failed to observe localization of *FBXO47* to
388 telomeres (Fig. S3B) and detachment of telomeres from nuclear envelope in *Fbxo47* KO
389 spermatocytes (Fig. S3C), which contrast to the previous report (Hua *et al.*, 2019). Since the
390 frequency of bouquet formation was quite low even in WT spermatocytes in mouse (Fig. S3D),
391 as shown in our previous study (Ishiguro *et al.*, 2014), the potential defect in bouquet formation
392 in *Fbxo47* KO spermatocytes further needs to be evaluated. Furthermore, our *SKP1*
393 immunoprecipitation from *Skp1-3xFLAG-HA* KI testes (Fig. 2D, Supplementary Data1) and
394 reciprocal *FBXO47* immunoprecipitation from *Fbxo47-3xFLAG-HA* KI testes (Fig. 1H, Fig S2)
395 failed to show supporting evidence that *FBXO47* serves as a subunit of SCF. Although we do
396 not know the exact reason for the discrepancies between the two studies with similar histological
397 phenotype of the seminiferous tubules in *Fbxo47* KO testes, subtle differences in the dection
398 and assay conditions or mice that were used could account for the differences in the
399 observations.

400

401 **Distant F-box proteins are involved in homolog synapsis**

402 SCF and F-box proteins are involved in the process of homolog synapsis during meiotic
403 prophase in diverse organisms. In plants, although no *Fbxo47* homolog exists, distant F-box
404 proteins are involved in homolog synapsis. In rice plant (*Oryza sativa*), MEIOTIC F-BOX

405 (MOF) encodes a F-BOX protein, and interacts with OSK1, a homolog of SKP1 (He *et al.*,
406 2016). MOF acts as a subunit of SCF and localizes on the chromosome during meiotic prophase.
407 In *mof* mutant male meiocytes, telomeres were not clustered and homolog synapsis was lost as
408 indicated by complete absence of ZEP1, a transverse filament of SC. Thus, MOF plays a role in
409 telomere bouquet formation during homolog pairing in male meiocyte. In rice plant,
410 ZYGOTENE1 (ZYG01) encodes another F-box protein that has a limited similarity to mouse
411 FBL12 (Zhang *et al.*, 2017). In *zygo1* mutant, polarized enrichment of OsSAD1, a SUN-domain
412 containing protein, along nuclear envelope was lost and full-length homolog pairing was
413 consequently impaired. This led to defective DSB repair of meiotic recombination, causing both
414 male and female sterility in *zygo1* mutant. Thus, ZYG01 also plays a role in telomere bouquet
415 formation during homolog pairing in rice plant. These studies suggest that rice F-box proteins
416 MOF and ZYG01 act as a SCF component, and play a role in bouquet formation rather than in
417 the process of SC formation, which is different to the role of mouse FBXO47 in SC
418 maintenance.

419
420 In budding yeast, a F-box protein Cdc4 acts as a substrate subunit of SCF during meiotic
421 prophase. SCF^{Cdc4} is assumed to regulate SC assembly by counteracting the Pch2 (TRIP13 in
422 mammals)-dependent negative action that induces SC disassembly (Zhu *et al.*, 2021). It is
423 proposed that SCF^{Cdc4} targets the putative negative regulator of SC assembly toward
424 degradation, and in turn stabilizes SC. Although how Pch2 itself or its downstream factors is
425 counteracted by SCF^{CDC4} remains elusive, F-box protein Cdc4 acts for the maintenance of SC in
426 budding yeast.

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

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

444
445

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456

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

462

463 **Declaration of interests:** The authors declare no competing interests.

464

465

466 **Figure legend**

467 **Figure 1. Identification of the meiosis-specific factor FBXO47**

468 (A) Genomic view of MEIOSIN binding peak over *Fbxo47* loci. Genomic coordinates were
469 obtained from Ensembl.

470 (B) The expression of *Fbxo47* in WT and *Meiosin* KO was examined using RT-PCR. Testis
471 RNA was obtained from WT (3 animals each for P8 and P10) and *Meiosin* KO (3 animals). The
472 graph shows the expression level of *Fbxo47* normalized by that of *GAPDH* with SD. Expression
473 level of *Fbxo47* in P10 WT was set to 1. Statistical significance is shown by *p*-value
474 (Two-tailed t-test). *: $p < 0.05$.

475 (C) The tissue-specific expression pattern of *Fbxo47* was examined by RT-PCR. Testis RNA
476 was obtained from embryonic day 18 (E18), 3-weeks old (3w) and 8-weeks old (8w) male mice.
477 Ovary RNA was obtained from adult 8-weeks old (8w) female mice. RT- indicates control PCR
478 without reverse transcription.

479 (D) Expression patterns of *Fbxo47* and other key developmental genes are reanalyzed using
480 public scRNA-seq data of spermatogenic cells in adult mouse testis (GEO: GSE109033)
481 (Hermann *et al.*, 2018). Expression patterns of *Fbxo47* and other key developmental genes are
482 shown in UMAP plots. Key developmental genes include *Zbtb16*: spermatogonia, *Stra8*:
483 differentiating spermatogonia and pleleptotene spermatocyte, *Dmc1*: meiotic prophase
484 spermatocyte, *Acrv1*: round and elongated spermatid. UMAP of *Zbtb16* and *Stra8* was adopted
485 from our previous study (Horisawa-Takada *et al.*, 2021).

486 (E) Expression profiles of *Fbxo47*, *Stra8* and *Dmc1* in E11.5, E12.5, E13.5, E15.5 fetal ovaries
487 along pseudotime trajectory of germ cells. Pseudotime analysis was performed by reanalyzing
488 scRNA-seq data (DRA011172) (Shimada *et al.*, 2021). Pseudotime expression profile of *Stra8*
489 was adopted from our previous study (Horisawa-Takada *et al.*, 2021).

490 (F) The expression pattern of *Fbxo47* in the embryonic ovary was examined by
491 RT-qPCR. Average values normalized to E12.5 gonad are shown with SD from
492 technical triplicates or quadruplicates. N=1 gonadal sample for each embryo.

493 (G) Schematic illustrations of the *Fbxo47-3xFLAG-HA* knock-in (*Fbxo47-3FH* KI) allele. Blue
494 boxes represent exons. The stop codon in the exon 11 was replaced with in-frame *3xFLAG-HA*
495 and the endogenous 3'UTR.

496 (H) Western blot showed immunoprecipitates after tandem affinity purifications using
497 anti-FLAG and anti-HA from cytoplasmic and chromatin extracts of WT (non-tagged control)
498 and *Fbxo47-3FH* KI mouse testes (P15-18). The same membrane was sequentially reblotted
499 with different antibodies against the endogenous FBXO47 that we generated. rabbit M: rabbit
500 anti-FBXO47 middle region, rabbit C: rabbit anti-FBXO47 C-terminal region, G.pig C: guinea
501 pig anti-FBXO47 C-terminal region.

502

503 **Figure 2. FBXO47 is not involved in the function of SCF.**

504 (A) Schematic illustrations of the *Skp1-3xFLAG-HA* knock-in (*Skp1-3FH* KI) allele. Blue boxes
505 represent exons. The stop codon in the exon 6 was replaced with in-frame *3xFLAG-HA* and the
506 endogenous 3'UTR.

507 (B) Chromosome spreads of WT (non-tagged) and *Skp1-3FH* KI spermatocytes were
508 immunostained as indicated. Scale bar: 5 μ m.

509 (C) Silver staining of the immunoprecipitates from cytosolic extracts of WT (non-tagged
510 control) and *Skp1-3FH* KI mouse testes after tandem affinity purifications using anti-FLAG and
511 anti-HA antibodies. Arrowhead: SKP1-3xFLAG-HA.

512 (D) The immunoprecipitates from the cytosolic fraction of the WT (non-tagged control) and
513 *Skp1-3FH* KI testis extracts were subjected to liquid chromatography tandem-mass
514 spectrometry (LC-MS/MS) analyses. The F-box-containing proteins and SCF subunits identified
515 by the LC-MS/MS analysis are presented after excluding the proteins detected in the control
516 mock purification. The proteins are listed with SwissProt accession number, the number of
517 peptide hits and Mascot scores. Full list of identified proteins are shown in the Supplementary
518 Data1. It is worth noting that SC central element components, Six6OS1 and SYCE1, were
519 included in the LC-MS/MS data of SKP1-3xFLAG-HA immunoprecipitates (Supplementary
520 Data1). This suggests that SCF E3 ubiquitin ligase may target those SC components using an
521 F-box protein listed in the LC-MS/MS data as a substrate recognition subunit.

522 (E) Western blot showed immunoprecipitates from cytosolic extracts of WT (non-tagged
523 control), *Fbxo47-3FH* KI and *Skp1-3FH* KI (heterozygous) testes after tandem affinity
524 purifications using anti-FLAG and anti-HA antibodies. The same membrane was sequentially
525 reblotted with different antibodies as indicated. Red *: FBXO47-3xFLAG-HA, Green *:

526 SKP1-3xFLAG-HA, Blue *: endogenous SKP1, Black *: non-specific band. Note that SKP1
527 was not detected in FBXO47 immunoprecipitate from *Fbxo47-3FH* KI testis extracts, and
528 reciprocally FBXO47 was not detected in SKP1 immunoprecipitate from *Skp1-3FH* KI testis
529 extracts.

530 (F) Chromosome spreads of WT and *Fbxo47* KO spermatocytes were immunostained as
531 indicated. Scale bar: 5 μ m.

532

533 **Figure 3. FBXO47 was expressed in early meiotic prophase**

534 (A) Testis sections from *Fbxo47-3FH* KI and control (non-tagged) mice (P15) were stained for
535 HA, SYCP3 and DAPI. Average 21% of the seminiferous tubules that have SYCP3+
536 spermatocytes showed HA+/SYCP3+ in *Fbxo47-3FH* KI testes (n = 3 animals), while none of
537 those was HA+/SYCP3+ in WT (n = 3 animals). Scale bar: 100 μ m.

538 (B) Seminiferous tubule sections from *Fbxo47-3FH* KI and control (non-tagged) mice (P15)
539 were stained for HA, SYCP3, γ H2AX and DAPI. Lep: leptotene. Scale bar: 25 μ m.

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

543 (D) Testis sections from *Fbxo47-3FH* KI and control (non-tagged) mice (n = 3 for each
544 genotype, P18) were stained for HA, H1t and DAPI as in (A). Number of seminiferous tubules
545 that have HA+/ H1t+ cells was counted per the seminiferous tubules that have H1t+
546 spermatocyte cells (52, 36, 18 tubules for non-tagged control; 15, 51, 36 tubules for
547 *Fbxo47-3FH* KI mice). Scale bar: 100 μ m.

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

551 (F) Seminiferous tubule sections (8-weeks old) were immunostained as in (E). Lep: leptotene,
552 Pac: pachytene spermatocyte, eS: elongating spermatid. Scale bar: 25 μ m. Note that pachy
553 signals of HA immunostaining were nonspecific, since they were visible in control.

554

555 **Figure 4. Spermatogenesis was impaired in *Fbxo47* knockout male**

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

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

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

566 (D) Hematoxylin and eosin staining of the sections from *Fbxo47*^{+/-} and *Fbxo47* KO testes
567 (8-weeks old). Biologically independent mice for each genotype were examined. Scale bar: 100
568 μm .
569 (E) Hematoxylin and eosin staining of the sections from *Fbxo47*^{+/-} and *Fbxo47* KO epididymis
570 (8-weeks old). Biologically independent mice for each genotype were examined. Scale bar: 100
571 μm .
572 (F) Seminiferous tubule sections (8-weeks old) were stained for SYCP3, PNA lectin and DAPI.
573 Note that the seminiferous tubule that contained PNA-positive elongated spermatids were not
574 identified in *Fbxo47* KO testes. Scale bar: 25 μm .
575 (G) Number of pups born by mating *Fbxo47*^{+/-} and *Fbxo47* KO males with *Fbxo47*^{+/-} or
576 *Fbxo47* KO females (N = number of females in the same cage) to examine fertility. *Fbxo47* KO
577 male #1 was initially mated with three *Fbxo47*^{+/-} females (all 6-weeks old at the start point of
578 mating). After one month, another *Fbxo47* KO male #2 was started to cohabit with those
579 females (8-weeks old at the start point of mating). This cage was observed for 3 months from
580 the start of mating.
581 (H) Hematoxylin and Eosin stained sections of *Fbxo47*^{+/-} and *Fbxo47* KO ovaries (8-weeks
582 old). Scale bar: 100 μm .
583 (I) Giemza staining of metaphase I chromosomes from *Fbxo47*^{+/-} (N=20) and *Fbxo47* KO
584 spermatocytes (N=26).
585 (J) Cumulative number of pups born from *Fbxo47*^{+/-} (n=4, all 6-weeks old at the start point of
586 mating) and *Fbxo47* KO (n=4, all 6-weeks old at the start point of mating) females.
587

588 **Figure 5. Premature disassembly of SC in *Fbxo47* KO spermatocytes.**

589 (A) Seminiferous tubule sections (P18 and 8-weeks old) were stained for SYCP3, H1t and
590 DAPI. Pa: pachytene spermatocyte, rS: round spermatid, eS: elongating spermatid. Shown on
591 the right is the quantification of the seminiferous tubules that have H1t⁺/SYCP3⁺ cells per the
592 seminiferous tubules that have SYCP3⁺ spermatocyte cells in WT and *Fbxo47* KO mice (Mean
593 with SD). n: the number of animals examined for each genotype. Statistical significance is
594 shown (Unpaired t-test). ** : $p = 0.0012$ for *Fbxo47* heterozygous versus *Fbxo47* KO at P18.
595 *Fbxo47* heterozygous (p18: 62, 61, 29 tubules/animal were counted from 3 animals; 8w: 135,
596 143, 45 tubules/animal were counted from 3 animals) and *Fbxo47* KO testes (p18: 105, 59,
597 141 tubules/animal were counted from 3 animals; 8w: 36, 55, 63, 64, 88, 69, 108
598 tubules/animal were counted from 7 animals). Scale bar: 25 μm .
599 (B) Chromosome spreads of WT and *Fbxo47* KO spermatocytes (3-4 weeks old) were
600 immunostained as indicated. Enlarged images are shown to highlight de-synapsed chromosomes
601 in diplotene-like *Fbxo47* KO spermatocytes. Scale bar: 5 μm .
602 (C) Quantification of meiotic prophase stage spermatocytes per total SYCP3⁺ spermatocytes in
603 WT and *Fbxo47* KO mice at P15 and P18 is shown. n: the number of cells examined.
604 (D) Chromosome spreads of pachytene and diplotene-like *Fbxo47* KO spermatocyte were
605 immunostained for SYCP3, H3S10P and HORMAD1. Scale bar: 5 μm .

606 (E) Chromosome spreads of diplotene spermatocyte in the control and diplotene-like
607 spermatocyte in *Fbxo47* KO spermatocytes (P18) were immunostained for SYCP3, H3S10P and
608 DAPI. Scale bar: 5 μ m. Note that centromeric regions are positively stained for H3S10P in the
609 control diplotene spermatocyte but not in diplotene-like spermatocyte in *Fbxo47* KO
610 spermatocytes.
611 (F) Spermatocytes isolated from the control *Fbxo47*^{+/-} and *Fbxo47* KO testes were cultured *in*
612 *vitro* in the presence or absence of OA for 3 hours. Quantification of meiotic prophase stage is
613 shown on the right. n: the number of cells examined. Note that the control spermatocytes
614 showed a typical feature of diakinesis/Meta I with condensed chromosomes and remaining
615 SYCP3 at centromeres.
616 (G) Seminiferous tubule sections from 8-weeks old mice were subjected to TUNEL assay with
617 immunostaining for SYCP3. L: leptotene, Pa: pachytene. Shown on the right is the
618 quantification of the seminiferous tubules that have TUNEL⁺ cells per total tubules in
619 *Fbxo47*^{+/-} (8w; n=3) and *Fbxo47* KO (8w; n=3) testes (mean with SD). Statistical significance
620 is shown by ** $p = 0.0072$ (Two-tailed t-test). Scale bar: 25 μ m.
621 (H) Schematic illustration of the precocious SC disassembly observed in *Fbxo47* KO
622 spermatocytes. The expression timing of H1t and H3S10P markers is shown.
623

624 **Figure 6. *Fbxo47* KO spermatocytes show defects in meiotic recombination**

625 (A) Chromosome spreads of WT and *Fbxo47* KO spermatocytes were immunostained for
626 SYCP3, SYCP1 and γ H2AX.
627 (B) Chromosome spreads of WT and *Fbxo47* KO spermatocytes were immunostained for
628 SYCP3, SYCP1 and BRCA1.
629 (C) Chromosome spreads of WT and *Fbxo47* KO spermatocytes were stained as indicated.
630 Immunostained chromosome spread of pachytene spermatocytes are shown.
631 The number of RAD51 foci is shown in the scatter plot with median (right). Statistical
632 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:
633 Pachytene-like, D-like: Diplotene-like. n: the number of cells examined.
634 (D) Chromosome spreads of WT and *Fbxo47* KO spermatocytes were stained as indicated. The
635 number of MSH4 foci is shown in the scatter plot with median (right). Statistical significance is
636 shown by p -value (Mann-Whitney U-test). *: $p < 0.05$.
637 (E) Chromosome spreads of *Fbxo47*^{+/-} and *Fbxo47* KO spermatocytes were stained as
638 indicated. The number of MLH1 foci is shown in the scatter plot with median (right). Statistical
639 significance is shown by p -value (Mann-Whitney U-test). ***: $p < 0.0001$. Scale bars: 5 μ m.
640
641

642 **Figure 7. A model of FBXO47 function to prevent premature SC disassembly**

643 Schematic illustration how FBXO47 may protect SC from a putative destabilizer during early
644 meiotic prophase.
645

646 **Supplementary Figure 1. Generation of *Fbxo47-3xFLAG-HA* knock-in mice**

647 (A) Testes from WT (no-tagged) and the *Fbxo47-3xFLAG-HA* KI homozygous mice (8-weeks
648 old). Scale bar: 5 mm.

649 (B) Hematoxylin and eosin staining of the testes (upper) and epididymis (lower) sections from
650 WT (non-tagged control) and the *Fbxo47-3xFLAG-HA* KI homozygous testes (8-weeks old).
651 Scale bar: 100 μ m.

652 Note that The FBOX47-3xFLAG-HA fusion protein was physiologically functional considering
653 the normal fertility shown in homozygous male and female mice with the KI allele.

654

655 **Supplementary Figure 2. MS analyses of FBXO47 interacting factors in testis extracts**

656 The immunoprecipitates (IP) from the cytosolic fraction of the testis extracts were subjected to
657 liquid chromatography tandem-mass spectrometry (LC-MS/MS) analyses. The proteins
658 identified by the LC-MS/MS analysis of FBXO47-IP are presented after excluding the proteins
659 detected in the control IgG-IP. The proteins with more than 1 different peptide hits are listed
660 with UniProt accession number, the number of peptide hits and Mascot scores.

661

662 **Supplementary Figure 3. FBXO47 do not localize to telomeres**

663 (A) Western blot showed immunoprecipitates from chromatin extracts of WT (non-tagged
664 control) and *Fbxo47-3FH* KI mouse testes (from 139 and 148 animals at P14-19, respectively)
665 after tandem affinity purifications using anti-FLAG and anti-HA antibodies. The same
666 membrane was sequentially reblotted with different antibodies as indicated. *: non-specific
667 band. Arrowhead: TRF2. Note that western blot did not detecte either TRF1 or TRF2 in the
668 FBXO47 immunoprecipitate.

669 (B) Chromosome spreads of *Fbxo47-3FH* KI and control (non-tagged) spermatocytes were
670 immunostained as indicated. Images with enhanced contrast for HA color channel are shown.
671 Scale bar: 5 μ m. Note that FBXO47 did not show specific localization pattern to telomeres. We
672 observed no more than background signals, even though contrast for HA images was enhanced.

673 (C) Structurally-preserved nuclei of spermatocytes were prepared by squashing *Fbxo47* KO
674 testis tubules, and immunostained for LAMIN-B, TRF1 and SYCP3. The image acquired at the
675 equator of the spermatocyte nuclei is shown. Note that telomeres attachment to the nuclear
676 envelope was intact in *Fbxo47* KO spermatocytes.

677 (D) The indicated spermatocyte nuclei were immunostained as indicated (Upper). Telomere
678 clustering in wild-type (n=355) and *Fbxo47* KO (n=342) was scored at 12 day post-partum. The
679 frequency of bouquet stage spermatocytes is shown (Bottom). Statistical significance is shown
680 by N.S. $p = 0.5025$ (chi square-test).

681

682 **Supplementary Figure 4. Uncropped images of gels and blots**

683 Full-length / uncropped images of agarose gel (Fig1C, Fig4B) and immunoblots (Fig1H, Fig2E,
684 Fig S3A) are shown. Immunoblotted membrane was sequentially reprobed with different
685 antibodies. For SKP1, H3, TRF1 immunoblots, the same membrane was stripped, cut according
686 to molecular weight marker and reprobed with different antibody, so that different proteins

687 could be simultaneously probed with different antibodies. For Fig2E, the membrane was first
688 immunoblotted with anti-HA antibody. After stripping, immunoblotted membrane was cut at a
689 height of between 37 kDa and 25 kDa. Upper and lower membrane was immunoblotted with
690 rabbit anti-FBXO47 middle region antibody and rabbit anti-SKP1 antibody, respectively. The
691 membrane was combined and images were acquired sequentially at different exposure time.

692

693

694 **Supplementary Data1 (Excel). Whole list of identified proteins by MS analyses of FBXO47**
695 **IP and SKP1 IP from testis extracts**

696 Coloidal blue stained gel after running the samples is shown in the first tab. Gel was cut into
697 pieces before LC-MS/MS analysis. Full list of proteins identified by the LC-MS/MS analysis
698 are shown in the second tab. The proteins are listed with UniProt accession number, the number
699 of peptide hits and Mascot scores. In the third tab, proteins are presented after excluding the
700 proteins detected in the control mock purification, IgG and keratin.

701

702 **Supplementary Data2 (Excel). The source data for statistics**

703 The source data (for Fig.1B, Fig.1F, Fig.4C, Fig.4G, Fig. 4I, Fig. 4J, Fig. 5A, Fig. 5C, Fig. 5F,
704 Fig.5G, Fig. 6C, Fig. 6D, Fig. 6E) are shown in the tabs.

705

706

707 **STAR Methods**

708 **Lead Contact and Material Availability**

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

711 All data supporting the conclusions are present in the paper and the supplementary materials.

712 The source data (for Fig.1B, Fig.1F, Fig.4C, Fig.4G, Fig. 4I, Fig. 4J, Fig. 5A, Fig. 5C, Fig. 5F,
713 Fig.5G, Fig. 6C, Fig. 6D, Fig. 6E, FigS3D) are provided in Supplementary data2 (Excel). The

714 original images for all of the figures in this paper are deposited in public depository.

715 The CHIP-seq data of MEIOSIN and STRA8 are described in our previous study (Ishiguro *et al.*,

716 2020) and available in the DDBJ Sequence Read Archive (DRA) under accession number

717 DRA007066, DRA007778, DRA009056. Mouse lines generated in this study have been

718 deposited to Center for Animal Resources and Development (CARD), *Fbxo47* Ex3-11Δ

719 knockout mouse (ID 2777), *Fbxo47-3xFLAG-HA* knock-in mouse (ID 2972), and

720 *Skp1-3xFLAG-HA* knock-in mouse (ID 2638). Plasmid expression vectors generated in this

721 study have been deposited to RIKEN BRC: pET28c-*Fbxo47*-C (aa272-451) (ID RDB192639)

722 and pET28c-*Fbxo47*-M (aa174 -316) (ID RDB19264). The antibodies are available upon

723 request. There are restrictions to the availability of antibodies due to the lack of an external

724 centralized repository for their distribution and our need to maintain the stock. We are glad to

725 share antibodies with reasonable compensation by the requestor for its processing and shipping.

726 All unique/stable reagents generated in this study are available from the Lead Contact with a

727 completed Materials Transfer Agreement.

728

729 **Experimental Model and Subject Details**

730 **Animals**

731 *Fbxo47* Ex3-11Δ knockout and *Fbxo47-3xFLAG-HA* knock-in mice were C57BL/6 background.
732 *Skp1-3xFLAG-HA* knock-in mouse was congenic with C57BL/6 background. Male mice were
733 used for immunoprecipitation of testis extracts, histological analysis of testes, immunostaining
734 of testes, and RT-PCR experiments. Female mice were used for histological analysis of the
735 ovaries, and immunostaining experiments. Whenever possible, each knockout animal was
736 compared to littermates or age-matched non-littermates from the same colony, unless otherwise
737 described. Animal experiments were approved by the Institutional Animal Care and Use
738 Committee (approval F28-078, A30-001, A28-026, A2020-006).
739

740 **Method Details**

741 **Generation of *Fbxo47* knockout mice and genotyping**

742 *Fbxo47* knockout mouse was generated by introducing Cas9 protein (317-08441; NIPPON
743 GENE, Toyama, Japan), tracrRNA (GE-002; FASMAG, Kanagawa, Japan), synthetic crRNA
744 (FASMAG), and ssODN into C57BL/6N fertilized eggs using electroporation. For generating
745 *Fbxo47* Exon3-11 deletion (Ex3-11Δ) allele, the synthetic crRNAs were designed to direct
746 TACACCTAGTGATAGCACTT(GGG) of the *Fbxo47* intron 2 and
747 AGAGCACTAGTCACTGAATG(CGG) in the 3'-neighboring region of the Exon11. ssODN:
748 5'-
749 GCTCAAAGTAAGCAAAGCAACAGAGAGCACTAGTCACTGATTATTTATTCAGTTGG
750 GATGCTGAGGAGGCAAATTGCCAGGTGTTTGAAGC
751 -3' was used as a homologous recombination template.
752 The electroporation solutions contained (10μM of tracrRNA, 10μM of synthetic crRNA, 0.1
753 μg/μl of Cas9 protein, 1μg/μl of ssODN) for *Fbxo47* knockout in Opti-MEM I Reduced Serum
754 Medium (31985062; Thermo Fisher Scientific). Electroporation was carried out using the Super
755 Electroporator NEPA 21 (NEPA GENE, Chiba, Japan) on Glass Microslides with round wire
756 electrodes, 1.0 mm gap (45-0104; BTX, Holliston, MA). Four steps of square pulses were
757 applied (1, three times of 3 mS poring pulses with 97 mS intervals at 30 V; 2, three times of 3
758 mS polarity-changed poring pulses with 97 mS intervals at 30 V; 3, five times of 50 mS transfer
759 pulses with 50 mS intervals at 4 V with 40% decay of voltage per each pulse; 4, five times of 50
760 mS polarity-changed transfer pulses with 50 mS intervals at 4 V with 40% decay of voltage per
761 each pulse).
762 The targeted *Fbxo47* Ex3-11Δ allele in F0 mice were identified by PCR using the following
763 primers:

764 Fbxo47-F1: 5'-TCCTCTCTCTGTCTCTTTATTCAACAG-3' and Fbxo47-R1: 5'-
765 TGCTAAGAAGGTGGTAAAGAATGTGAC-3' for the knockout allele (825 bp). Fbxo47-F3:
766 5'- TCTGACCATGAACGCTATCTCTTCC-3' and Fbxo47-R1 for wild-type allele (503 bp).
767 The PCR amplicons were verified by sequencing. Primer sequences are listed in Table S1.
768

769 **Generation of *Fbxo47-3xFLAG-HA* knock-in mice and genotyping**

770 *Fbxo47-3xFLAG-HA* knock-in mouse was generated by introducing Cas9 protein, tracrRNA,
771 synthetic crRNA, and ssODN into C57BL/6N fertilized eggs using electroporation as described
772 above. The synthetic crRNA was designed to direct ACGCTATCTCTTCCTAAGTC(AGG) of
773 the *Fbxo47*.

774 ssODN:

775 5'-GAACTTCCATAAGGAGGTGCTGTATCTGACCATGAACGCTATCTCTTCCGGAGAC
776 TACAAAGACCATGACGGTGATTATAAAGATCATGACATCGATTACAAGGATGACGA
777 TGACAAGGGATAACCCCTACGACGTGCCCGACTACGCCTAAGTCAGGAAGCTTGTGT
778 CCCTCTGGACTGGCATTTCAGGGGAGTGATGCC-3'

779 was used as a homologous recombination template.

780 The targeted *Fbxo47-3xFLAG-HA* knock-in allele in F0 mice were identified by PCR using the
781 following primers:

782 Fbxo47-F4: 5'-TCTGTTCCATCTTCTCCATGCTCAGGC-3' and Fbxo47-R3: 5'-
783 TGAAGAGCCAGAACTTGTTTTCCAG-3' for the knock-in allele (396 bp), and for wild-type
784 allele (294 bp). The PCR amplicons were verified by sequencing. Primer sequences are listed in
785 Table S1.
786

787 **Generation of *Skp1-3xFLAG-HA* knock-in mouse and genotyping**

788 The targeting vector was designed to insert 3xFLAG-HA-3'UTR in frame with the coding
789 sequence into the Exon 6 of the *Skp1* genomic locus. Targeting arms of 1225bp and 1481bp
790 fragments, 5' and 3' of the Exon 6 of *Skp1* gene respectively, were generated by PCR from
791 mouse C57BL/6 genomic DNA and directionally cloned flanking pGK-*Neo*-polyA and *DT-A*
792 cassettes. The 5' arm was followed by nucleotide sequences encoding 3xFLAG, HA and the
793 3'UTR of *Skp1* gene. TT2 ES cells were co-transfected with the targeting vector and pX330
794 plasmids (Addgene) expressing Crispr-gRNAs directing GCTGGCATTGACTCGGGGTA(ggg)
795 and CGCCACCATAACCCGGTGATT (tgg), which locate at the 3' region of the Exon 6 of *Skp1*
796 gene. The G418-resistant ES clones were screened for homologous recombination with the *Skp1*
797 locus by PCR using primers SKP1_5Arm_F2: 5'-

798 GGTCAGCAAACTGCTGAACAGCTTG-3' and

799 KI96ES-19814R-HA: 5'- GGGCACGTCGTAGGGGTATCCCTTG -3' for the left arm (1909
800 bp); pKO2-3armF: 5'-AGGAACTTCGGAATAGGAAC-3' and

801 SKP1_RightArm_R2: 5'-TGCAGTGGAGGCTCAGTCCAGCTTC-3' for the right arm (1897
802 bp).
803

804 The homologous recombinant cells were isolated and chimeric mice were generated by
805 aggregation (host ICR) of recombinant ES cells. Chimeric males were mated to C57BL/6N
806 females and the progenies were genotyped by PCR using the primers:
807 SKP1onL2_F2: 5'- ATCATTGTTCCCAGGTGGAG -3' and
808 SKP1onRight_R1: 5'- GACTAGAACAAGATGACAGG -3'
809 for the knock-in allele (2078 bp) and the WT allele (1275bp). Primer sequences are listed in
810 Table S1.

811

812 **Histological Analysis**

813 Testes, caudal epididymis and ovaries were fixed in Bouin's solution, and embedded in paraffin.
814 Sections were prepared on CREST-coated slides (Matsunami) at 6 µm thickness. The slides
815 were deparaffinized and stained with hematoxylin and eosin.
816 For Immunofluorescence staining, testes were embedded in Tissue-Tek O.C.T. compound
817 (Sakura Finetek) and frozen. Cryosections were prepared on the CREST-coated slides
818 (Matsunami) at 8 µm thickness, and then air-dried. The serial sections of frozen testes were
819 fixed in 4% paraformaldehyde in PBS for 5 min at room temperature and washed briefly in PBS.
820 After washing, the serial sections were permeabilized in 0.1% TritonX100 in PBS for 5 min.
821 The sections were blocked in 3% BSA/PBS, and incubated at room temperature with the
822 primary antibodies in a blocking solution. After three washes in PBS, the sections were
823 incubated for 1 h at room temperature with Alexa-dye-conjugated secondary antibodies (1:1000;
824 Invitrogen) in a blocking solution. PNA lectin staining was done using FITC-conjugated
825 Lectin from *Arachis hypogaea* (IF, 1:1000, Sigma: L7381). TUNEL assay was performed
826 using MEBSTAIN Apoptosis TUNEL Kit Direct (MBL 8445). DNA was counterstained with
827 Vectashield mounting medium containing DAPI (Vector Laboratory).

828

829 **Immunostaining of spermatocytes**

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

843

844 **Imaging**

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

854

855

856

857 ***In vitro* oocyte culture and Giemsa staining of metaphase chromosome spread**

858 Ovaries collected from 4-week-old female mice were used after 46 to 48 h of treatment with 5
859 IU of pregnant mare serum gonadotropin. GV oocytes were isolated by puncturing the follicles
860 in M2 medium (Sigma MR-015). The GV oocytes were cultured in M16 medium (Sigma
861 MR-016) in a 5% CO₂ atmosphere at 37°C for 6hours. For Giemsa staining of metaphase
862 chromosome spread, oocytes were exposed to 0.5% Pronase (MERCK 10165921001) to remove
863 the zona pellucida, and treated in hypotonic buffer containing 1% sodium citrate/0.1% PVA for
864 15min. The oocytes and oocyte-like cells were placed on the slides, fixed in the Carnoy's
865 Fixative (75 % Methanol, 25% Acetic Acid), and stained in 3% Giemsa solution for 30min.

866

867 **Culture of OA-induced Meta I spermatocyte**

868 Culture of OA-induced Meta I spermatocytes were performed as described (Wiltshire et al.,
869 1995). The isolated spermatocytes were cultured in the presence or absence of 5 µM okadaic
870 acid (OA) for 3 h.

871

872 **Antibodies**

873 The following antibodies were used for immunoblot (IB) and immunofluorescence (IF) studies:
874 mouse anti-FLAG M2 (Sigma-Aldrich F1804), rabbit anti-HA (IB, IF, 1:1000, Abcam: ab91110),
875 rabbit anti-Actin (IB, 1:1000, Sigma-Aldrich A2066), mouse anti-MLH1 (IF, 1:500, BD
876 Biosciences: 551092), rabbit anti-H3S10p (IF, 1:2000, Abcam ab5176), rabbit anti-Histone H3
877 (IB, 1:1000, Abcam ab1791), rabbit anti-SYCP1 (IF, 1:1000, Abcam ab15090), mouse
878 anti-gH2AX (IF, 1:1000, Abcam ab26350), rabbit anti-RAD51 (IF, 1:500, Santa Cruz:
879 SC-8349), rabbit anti-MSH4 (IF, 1:500, Abcam ab58666), rabbit anti-SKP1 (IB, 1:1000,
880 Abcam ab10546), rabbit anti-HORMAD1 (IF, 1:1000, ProteinTech 13917-1-AP), goat
881 anti-Lamin B (IF, 1:1000, Santa Cruz: SC-6216), mouse anti-TRF1 (Shibuya et al., 2014) (IF,
882 1:1000), rabbit anti-TRF1 (Shibuya *et al.*, 2014) (IB, 1:1000), rabbit anti-TRF2 (IB, 1:1000,
883 NB110-57130), mouse anti-SYCP1 (IF, 1:1000) (Ishiguro et al., 2011), rat anti-SYCP3
884 (Ishiguro *et al.*, 2020) (IF, 1:1000), guinea pig anti-SYCP3 (Ishiguro *et al.*, 2020) (IF, 1:2000),

885 rabbit anti-BRCA1 (IF, 1:500, kindly provided by Satoshi Namekawa), guinea pig anti-H1t (IF,
886 1:2000, kindly provided by Marry Ann Handel).

887

888 **Production of antibodies against FBXO47**

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

898

899 **PCR with reverse transcription.**

900 Total RNA was isolated from tissues and embryonic gonads using TRIzol (Thermo Fisher).
901 cDNA was generated from total RNA using Superscript III (Thermo Fisher) followed by PCR
902 amplification using Ex-Taq polymerase (Takara) and template cDNA.
903 For RT-qPCR, total RNA was isolated from WT (n = 3) and *Meiosin* KO (n = 3) testes, and
904 cDNA was generated as described previously (Ishiguro *et al.*, 2020). *Fbxo47* cDNA was
905 quantified by Δ CT method using TB Green Premix Ex Taq II (Tli RNaseH Plus) and Thermal
906 cycler Dice (Takara), and normalized by *GAPDH* expression level.
907 qPCR was performed in duplicates, and the average ddCt value was calculated for each cDNA
908 sample. The expression level of *Fbxo47* was divided by that of *GAPDH* to give the relative
909 expression level of *Fbxo47* to *GAPDH*. Relative expression level of *Fbxo47* to *GAPDH* was
910 normalized to 1 for a given P10 WT sample.

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

912 GAPDH-F: 5'-TTCACCACCATGGAGAAGGC-3'

913 GAPDH-R: 5'-GGCATGGACTGTGGTCATGA-3'

914 Gapdh_F2: 5'-ACCACAGTCCATGCCATCAC-3'

915 Gapdh_R2: 5'-TCCACCACCCTGTTGCTGTA-3'

916 Gapdh_Ex6F: 5'-GGTTGTCTCCTGCGACTTCA-3'

917 Gapdh_mRNAR: 5'-GCCGTATTCATTGTCATACCAGG-3'

918 *Fbxo47*-F 1443F: 5'-GCATAGCAAATGCTTTTGCCTGTG-3'

919 *Fbxo47*-R 1605R: 5'-GAGATAGCGTTCATGGTCAGATAC-3'

920 Primer sequences are listed in Table S1.

921

922

923 **Preparation of testis extracts and immunoprecipitation**

924 Testis chromatin-bound and -unbound extracts were prepared as described previously (Ishiguro
925 *et al.*, 2014). Briefly, testicular cells were suspended in low salt extraction buffer (20 mM

926 Tris-HCl pH 7.5, 100 mM KCl, 0.4 mM EDTA, 0.1% TritonX100, 10% glycerol, 1 mM
927 β -mercaptoethanol) supplemented with Complete Protease Inhibitor (Roche). After
928 homogenization, the soluble chromatin-unbound fraction was separated after centrifugation at
929 100,000g for 10 min at 4°C. The chromatin bound fraction was extracted from the insoluble
930 pellet by high salt extraction buffer (20 mM HEPES-KOH pH 7.0, 400 mM KCl, 5 mM MgCl₂,
931 0.1% Tween20, 10% glycerol, 1 mM β -mercaptoethanol) supplemented with Complete Protease
932 Inhibitor. The solubilized chromatin fraction was collected after centrifugation at 100,000g for
933 10 min at 4°C.

934

935 **Immuno-affinity purification**

936 Immuno-affinity purification was performed with anti-FLAG M2 monoclonal antibody-coupled
937 magnetic beads (Sigma-Aldrich M8823) from the testis chromatin-bound and -unbound
938 fractions of *Fbxo47-3xFLAG-HA* knock-in mice and *Skp1-3xFLAG-HA* knock-in mice (14 to
939 21-day old). For negative control, mock immuno-affinity purification was done from the testis
940 chromatin-bound and -unbound fractions from the age-matched wild type mice. The beads were
941 washed with high salt extraction buffer for chromatin-bound proteins and low salt extraction
942 buffer for chromatin-unbound proteins. The anti-FLAG-bound proteins were eluted by 3xFLAG
943 peptide (Sigma-Aldrich). The second immuno-affinity purification was performed anti-HA 5D8
944 monoclonal antibody-coupled Magnet agarose (MBL M132-10). The bead-bound proteins were
945 eluted with 40 μ l of elution buffer (100 mM Glycine-HCl pH 2.5, 150 mM NaCl), and then
946 neutralized with 4 μ l of 1 M Tris-HCl pH 8.0.

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

953

954 **Mass spectrometry**

955 The immunoprecipitated proteins were run on 4-12 % NuPAGE (Thermo Fisher) by 1 cm from
956 the well and stained with SimplyBlue (Thermo Fisher) for in-gel digestion. The gel containing
957 proteins was excised, cut into approximately 1mm sized pieces. Proteins in the gel pieces were
958 reduced with DTT (Thermo Fisher), alkylated with iodoacetamide (Thermo Fisher), and
959 digested with trypsin and Lysyl endopeptidase (Promega) in a buffer containing 40 mM
960 ammonium bicarbonate, pH 8.0, overnight at 37°C. The resultant peptides were analyzed on an
961 Advance UHPLC system (ABRMEIchrom Bioscience) connected to a Q Exactive mass
962 spectrometer (Thermo Fisher) processing the raw mass spectrum using Xcalibur (Thermo Fisher
963 Scientific). The raw LC-MS/MS data was analyzed against the NCBI non-redundant
964 protein/translated nucleotide database restricted to *Mus musculus* using Proteome Discoverer
965 version 1.4 (Thermo Fisher) with the Mascot search engine version 2.5 (Matrix Science). A
966 decoy database comprised of either randomized or reversed sequences in the target database was

967 used for false discovery rate (FDR) estimation, and Percolator algorithm was used to evaluate
968 false positives. Search results were filtered against 1% global FDR for high confidence level.
969 All full lists of LC-MS/MS data are shown in Supplementary Data1 (Excel file).

970

971

972 **ChIP-seq Data and Public RNA-seq data Analysis**

973 MEIOSIN ChIP-seq data described in our previous study (Ishiguro *et al.*, 2020) was analyzed
974 for the *Fbxo47* locus. MEIOSIN binding site was shown along with genomic loci from Ensembl
975 on the genome browser IGV.

976

977 **Single cell RNA-seq Data Analysis**

978 The scRNA-seq data of fetal ovaries was derived from DRA 011172 (Shimada *et al.*, 2021).
979 10xGenomics Drop-seq data of mouse adult testis was derived from GEO: GSE109033
980 (Hermann *et al.*, 2018). Reanalyses of scRNA-seq data were conducted using the Seurat
981 package for R (v.3.1.3) (Stuart *et al.*, 2019) and pseudotime analyses were conducted using
982 monocle package for R: R (ver. 3.6.2), RStudio (ver.1.2.1335), and monocle (ver. 2.14.0) (Qiu
983 *et al.*, 2017) following developer's tutorial.

984

985 **Quantification and Statistical analysis**

986 Statistical analyses, and production of graphs and plots were done using GraphPad Prism8
987 (version 8.4.3) or Microsoft Excel (version 16.48).

988

989 **Figure 1B** Testis RNA was obtained from P8 WT (3 animals), P10 WT (3 animals), *Meiosin*
990 KO (3 animals). qPCR was performed in duplicates, and the average ddCt value was calculated
991 for each cDNA sample. The expression level of *Fbxo47* was divided by that of *GAPDH* to give
992 the relative expression level of *Fbxo47* to *GAPDH*. Relative expression level of *Fbxo47* to
993 *GAPDH* was normalized to 1 for a given P10 WT sample. Bar graph indicates mean with SD.
994 Statistical significance was determined by t-test.

995

996 **Figure 1F** RNA was obtained from WT Embryonic ovaries (E12.5 to E18.5). qPCR was
997 performed in triplicates or quadruplicates, and the average ddCt value was calculated for each
998 cDNA sample. The expression level of *Fbxo47* was divided by that of *GAPDH* to give the
999 relative expression level of *Fbxo47* to *GAPDH*. Relative expression level of *Fbxo47* to *GAPDH*
1000 was normalized to 1 for a given E12.5 WT sample. Bar graph indicates mean with SD.

1001

1002 **Figure 3A** Testis sections (P15) were obtained from non-tagged control (3 animals) and
1003 *Fbxo47-3FH* KI (3 animals). Number of seminiferous tubules that have HA+/ SYCP3+ cells
1004 was counted per the seminiferous tubules that have SYCP3+ spermatocyte cells (84, 85, 45
1005 tubules for non-tagged control, 123, 90, 79 tubules for *Fbxo47-3FH* KI).

1006

1007 **Figure 3D** Testis sections (P18) were obtained from non-tagged control (3 animals) and
1008 *Fbxo47-3FH* KI (3 animals). Number of seminiferous tubules that have HA+/*H1t+* cells was
1009 counted per the seminiferous tubules that have *H1t+* spermatocyte cells (52, 36, 18 tubules for
1010 non-tagged control; 15, 51, 36 tubules for *Fbxo47-3FH* KI).

1011

1012 **Figure 4C** Quantification of testes/body-weight ratio (mg/g) in *Fbxo47*^{+/-} (8w; n=4) and
1013 *Fbxo47* KO (8w; n=10) mice. n: the number of animals examined for each genotype. Bar graph
1014 indicates mean with SD. Statistical significance was determined by t-test.

1015

1016 **Figure 4I** Cumulative number of pups born from *Fbxo47*^{+/-} (n=4, all 6-week old at the start
1017 point of mating) and *Fbxo47* KO (n=4, all 6-week old at the start point of mating) females was
1018 counted for 18 weeks of breeding.

1019

1020 **Figure 5A** Quantification of the seminiferous tubules that have *H1t+*/*SYCP3+* cells per the
1021 seminiferous tubules that have *SYCP3+* spermatocyte cells in *Fbxo47* heterozygous (p18: 62,
1022 61, 29 tubules/animal were counted from n= 3 animals; 8w: 135, 143, 45 tubules/animal
1023 were counted from n= 3 animals) and *Fbxo47* KO (p18: 105, 59, 141 tubules/animal were
1024 counted from n= 3 animals; 8w: 36, 55, 63, 64, 88, 69, 108 tubules/animal were
1025 counted from n= 7 animals) testes. n: the number of animals examined for each genotype. Bar
1026 graph indicates mean with SD. Statistical significance was determined by unpaired t-test. $p =$
1027 0.0012 for *Fbxo47* heterozygous versus *Fbxo47* KO at P18.

1028

1029 **Figure 5C** Spermatocytes in the four developmental stages (leptotene, zygotene, pachytene, and
1030 diplotene(-like)) per total cells in meiotic prophase were quantified in WT (n=727 from one
1031 animal) and *Fbxo47* KO (n=659 from one animal) at P15, and in WT (n=561 from one animal)
1032 and *Fbxo47* KO (n=516 from one animal) at P18.

1033

1034 **Figure 5F** Spermatocytes in the four developmental stages (leptotene or zygotene, pachytene,
1035 and diplotene(-like)) per total cells in meiotic prophase were quantified in *Fbxo47*^{+/-} (n=105
1036 for OA -, 117 for OA+) and *Fbxo47* KO (n=140 for OA -, 117 for OA+).

1037

1038 **Figure 5G** Quantification of the seminiferous tubules that have TUNEL+ cells per total tubules
1039 in *Fbxo47*^{+/-} (8w: n=3) and *Fbxo47* KO (8w: n=3) testes. Bar graph indicates mean with SD.
1040 Statistical significance was determined by t-test.

1041

1042 **Figure 6C** Numbers of RAD51 foci on *SYCP3* axes were counted in WT and *Fbxo47* KO.
1043 Number of foci was indicated in the scatter plot with median. Statistical significance was
1044 determined by Mann-Whitney U-test.

1045

1046 **Figure 6D** Numbers of MSH4 foci on SYCP3 axes were counted in WT and *Fbxo47* KO.
1047 Number of foci was indicated in the scatter plot with median. Statistical significance was
1048 determined by Mann-Whitney U-test.
1049
1050 **Figure 6E** Numbers of MLH1 foci on SYCP3 axes were counted in *Fbxo47*^{+/-} pachyene
1051 (n=15), or *Fbxo47* KO pachyene (n=13) and diplotene-like (n=13). Number of foci was
1052 indicated in the scatter plot with median. Statistical significance was determined by
1053 Mann-Whitney U-test.
1054
1055 **Figure S3D**
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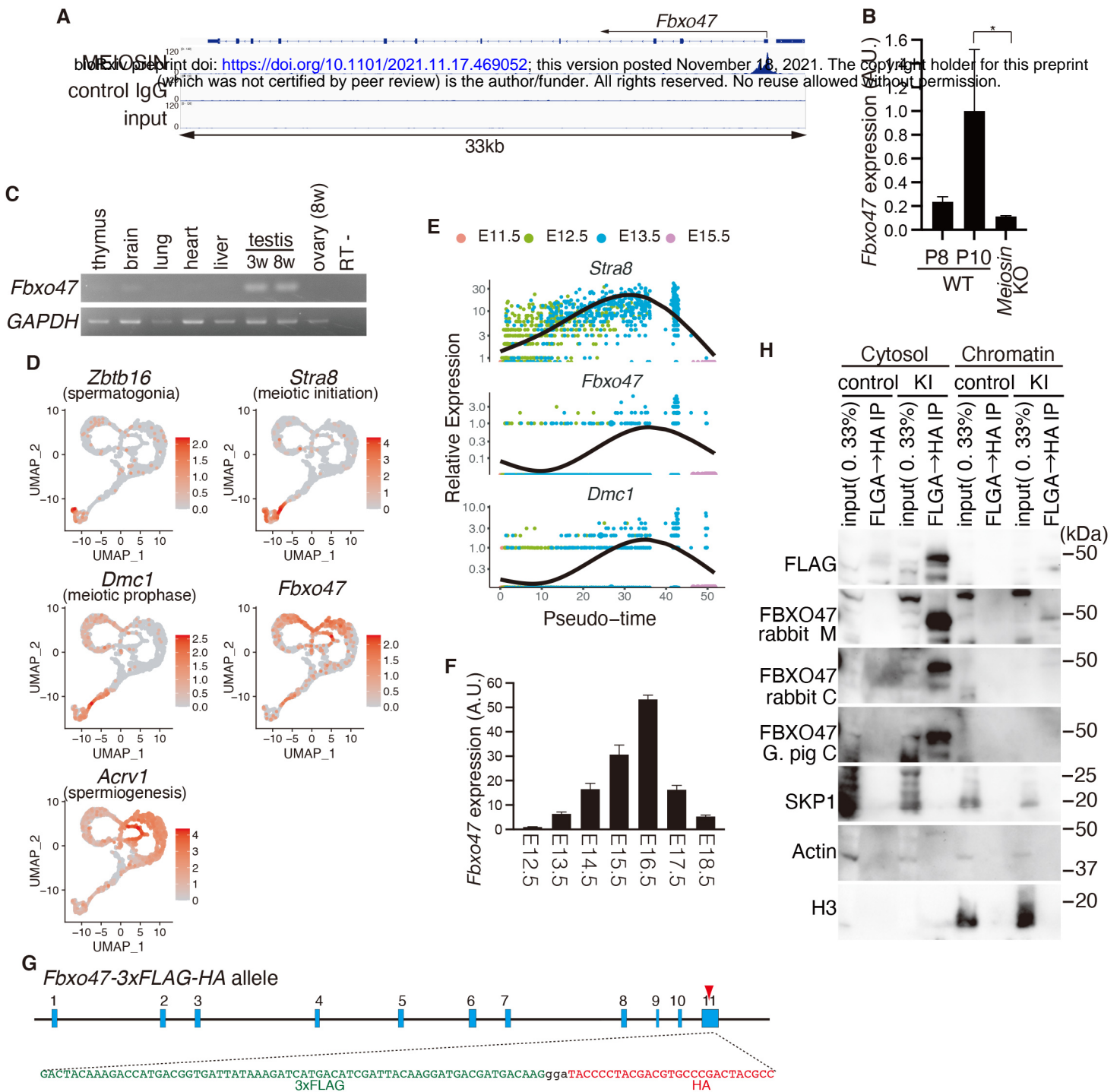
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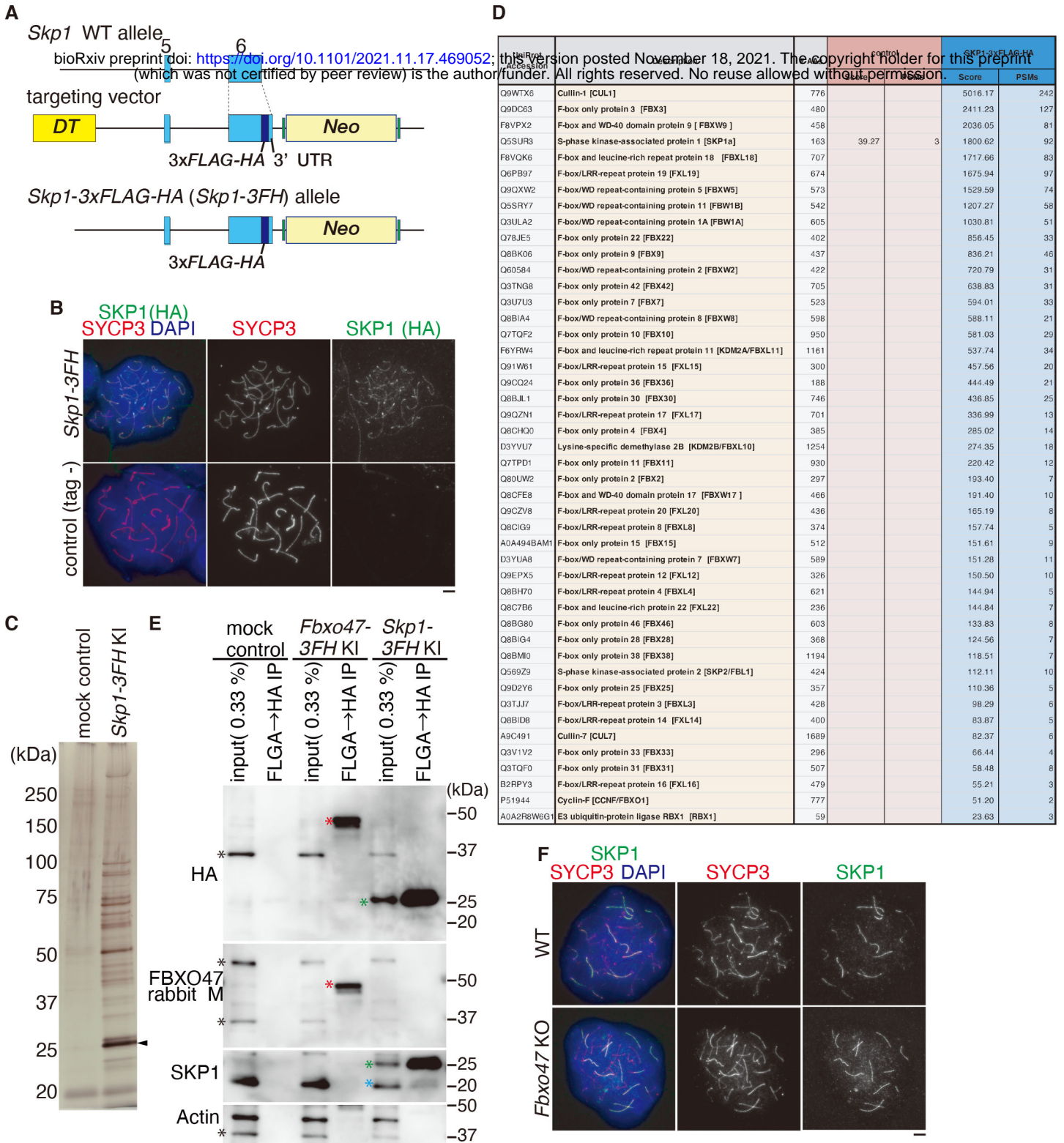
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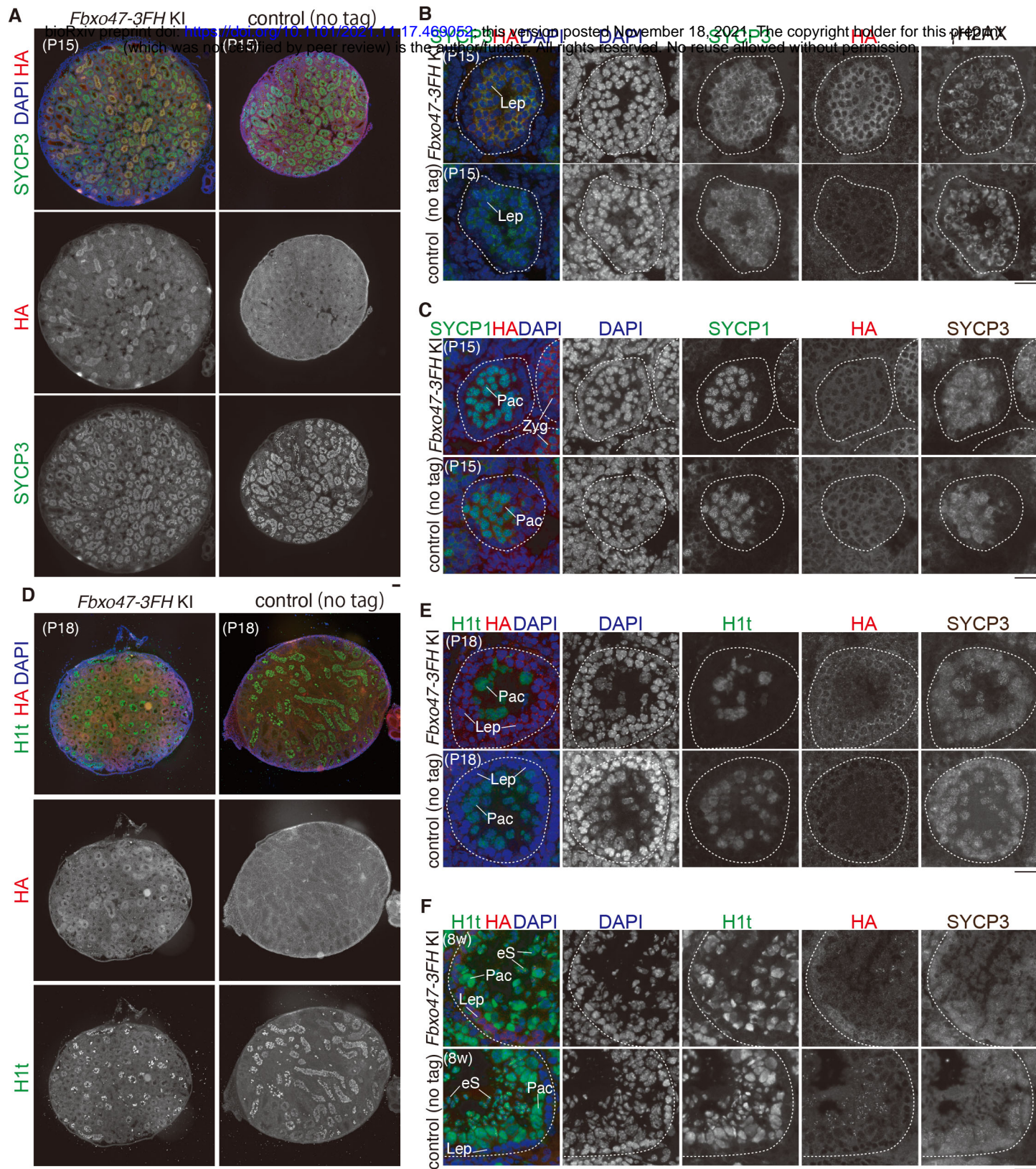
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Figure 1

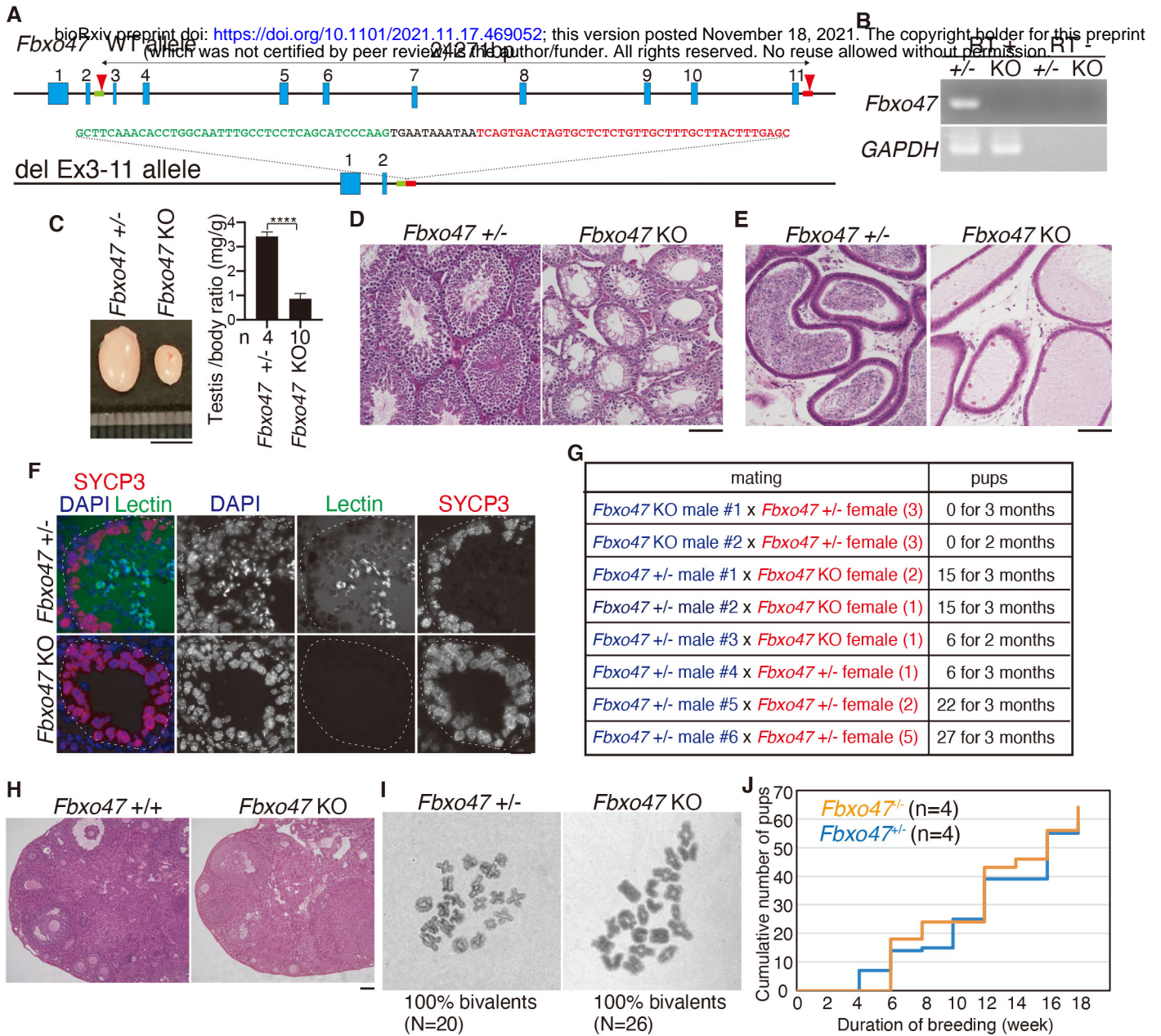


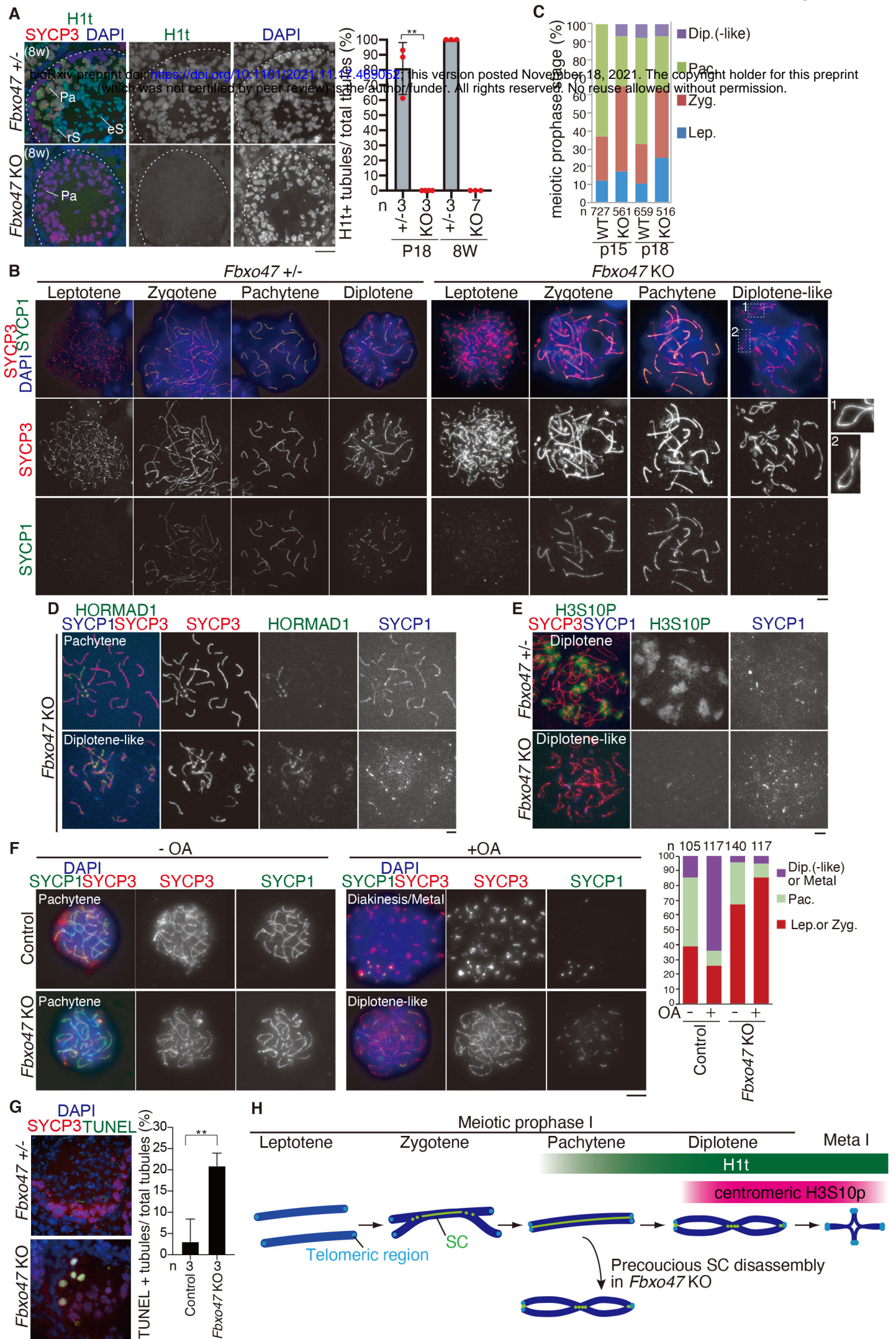
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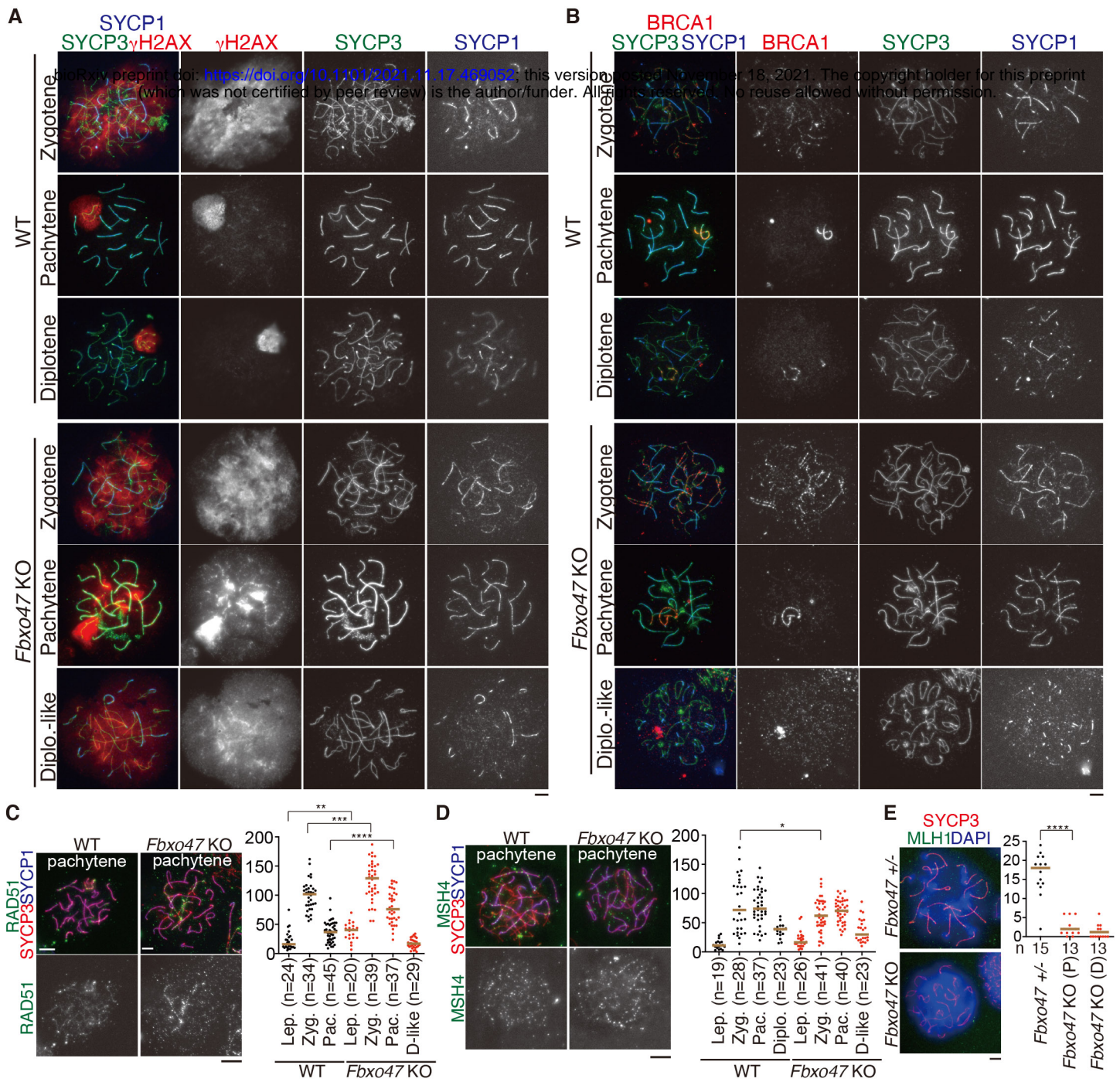




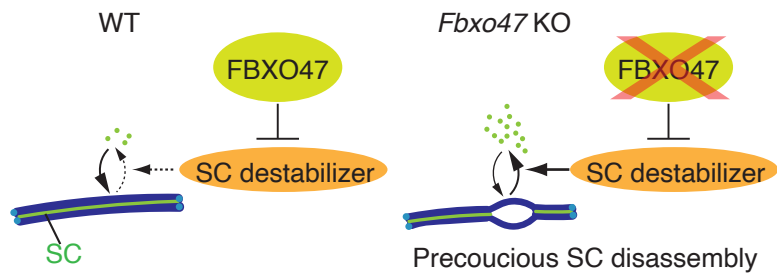
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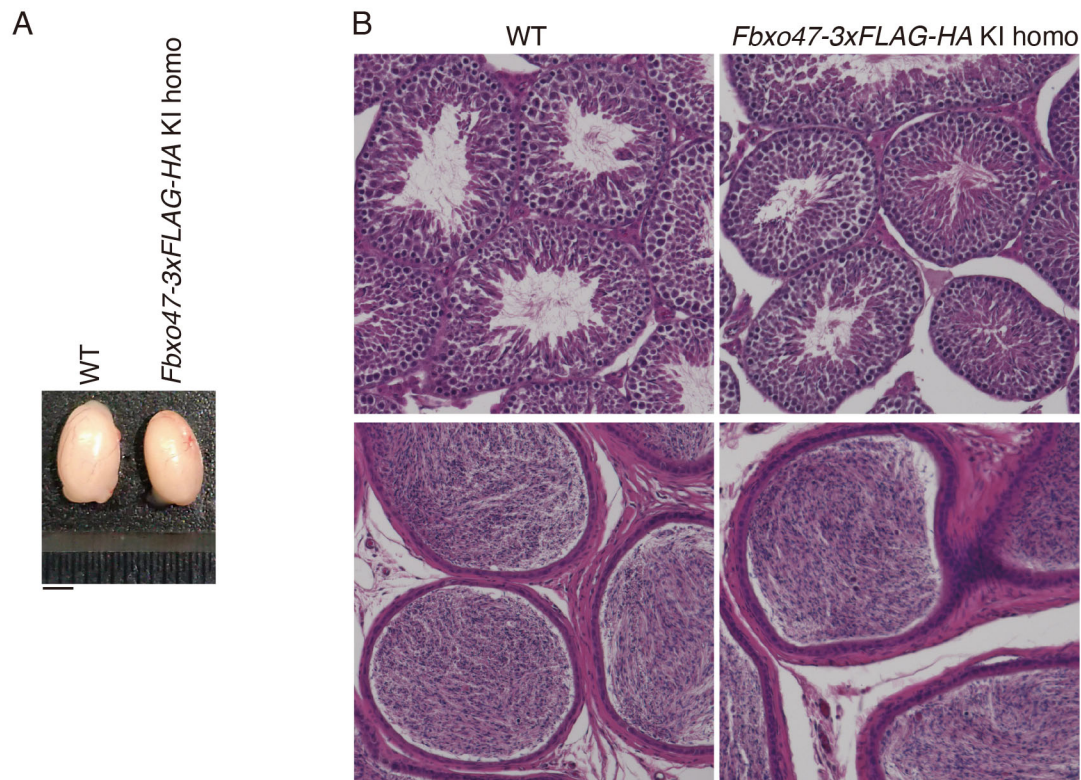




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Supplementary Figure 1. Generation of *Fbxo47-3xFLAG-HA* knock-in mice (related to Figure 1)

(A) Testes from WT (no-tagged) and the *Fbxo47-3xFLAG-HA* KI homozygous mice (8-weeks old). Scale bar: 5 mm.

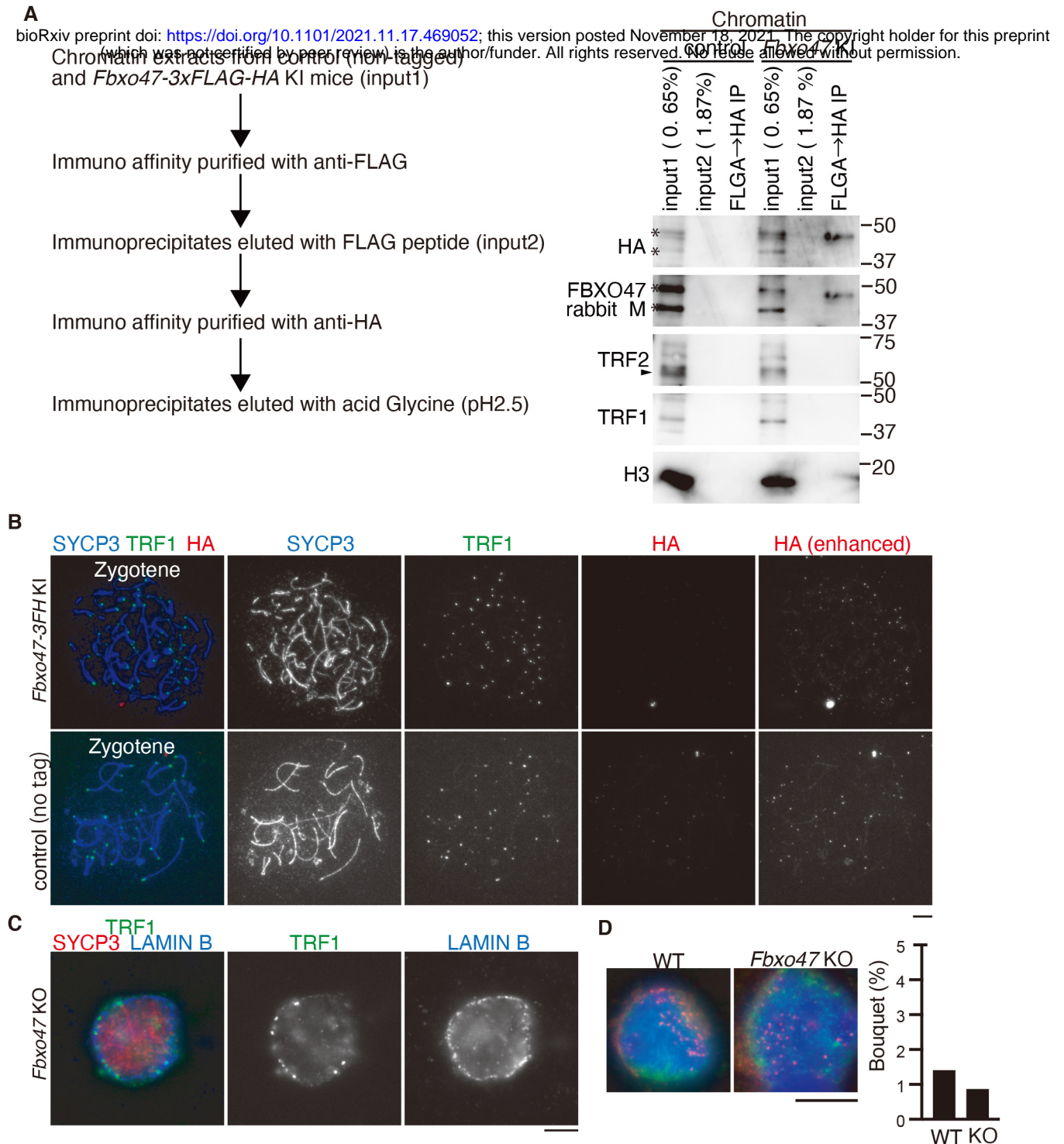
(B) Hematoxylin and eosin staining of the testes (upper) and epididymis (lower) sections from WT (non-tagged control) and the *Fbxo47-3xFLAG-HA* KI homozygous testes (8-weeks old). Scale bar: 100 μ m.

Note that The FBOX47-3xFLAG-HA fusion protein was physiologically functional considering the normal fertility shown in homozygous male and female mice with the KI allele.

Accession	Description	FBXO47-3xFLAG-HA IP from cytosol	
		Score	PSMs
P16546	Spectrin alpha chain (Sptan1)	700.00	34
A2A6H3	F-box only protein 47 (Fbxo47)	396.96	17
Q3UDF8	eukaryotic translation initiation factor 2, subunit 3, structural gene X-linked (Eif2s3x)	139.60	5
Q61749	Translation initiation factor eIF-2B subunit delta (Eif2b4)	112.64	6
Q8VDD5	Myosin-9 (Myh9)	100.59	7
Q8BP92	Reticulocalbin-2 (Rcn2)	94.35	4
A0AUM9	Eif2b3 protein (Eif2b3)	82.91	5
Q3TQP7	acetyl-Coenzyme A acetyltransferase 1 (Acat1)	76.43	3
P43275	Histone H1.1 (Hist1h1a)	71.91	3
Q6ZPE2	Myotubularin-related protein 5 (Sbf1)	66.18	3
Q99L45	Eukaryotic translation initiation factor 2 subunit 2 (Eif2s2)	60.83	3
Q9CSU2	26S proteasome regulatory subunit RPN11 (Psm14)	57.53	3
Q922W7	2900073G15Rik protein (Myl12a)	55.52	2
D6RFB8	DNA polymerase (Pold1)	54.02	4
Q91XU3	Phosphatidylinositol 5-phosphate 4-kinase type-2 gamma (Pip4k2c)	49.04	2
Q99LD9	DnaJ homolog subfamily A member 1 (Dnaja1)	48.43	3
Q7M754	Try10-like trypsinogen (Gm5409)	45.86	6
P63037	DnaJ homolog subfamily A member 1 (Dnaja1)	45.20	3
Q63ZW9	Copa protein (Copa)	43.81	3
Q80VC9	Calmodulin-regulated spectrin-associated protein 3 (Camsap3)	40.34	2
Q8BJY1	26S proteasome non-ATPase regulatory subunit 5 (Psm5)	33.62	2
B2RXC6	DNA-directed RNA polymerase subunit (Polr3a)	31.02	3
Q68FL6	Methionine--tRNA ligase, cytoplasmic (Mars)	29.26	2
Q3U1G4	Sec3-PIP2_bind domain-containing protein (Exoc1)	28.42	2
Q925I1	ATPase family AAA domain-containing protein 3 (Atad3)	23.98	2
A0A3B2WBH9	Tight junction protein ZO-2 (Tjp2)	21.72	2
F6ZQQ3	26S proteasome non-ATPase regulatory subunit 13 (Psm13)	21.36	2

Supplementary Figure 2. MS analyses of FBXO47 interacting factors in testis extracts (related to Figure 1)

The immunoprecipitates (IP) from the cytosolic fraction of the testis extracts were subjected to liquid chromatography tandem-mass spectrometry (LC-MS/MS) analyses. The proteins identified by the LC-MS/MS analysis of FBXO47-IP are presented after excluding the proteins detected in the control IgG-IP. The proteins with more than 1 different peptide hits are listed with UniProt accession number, the number of peptide hits and Mascot scores.



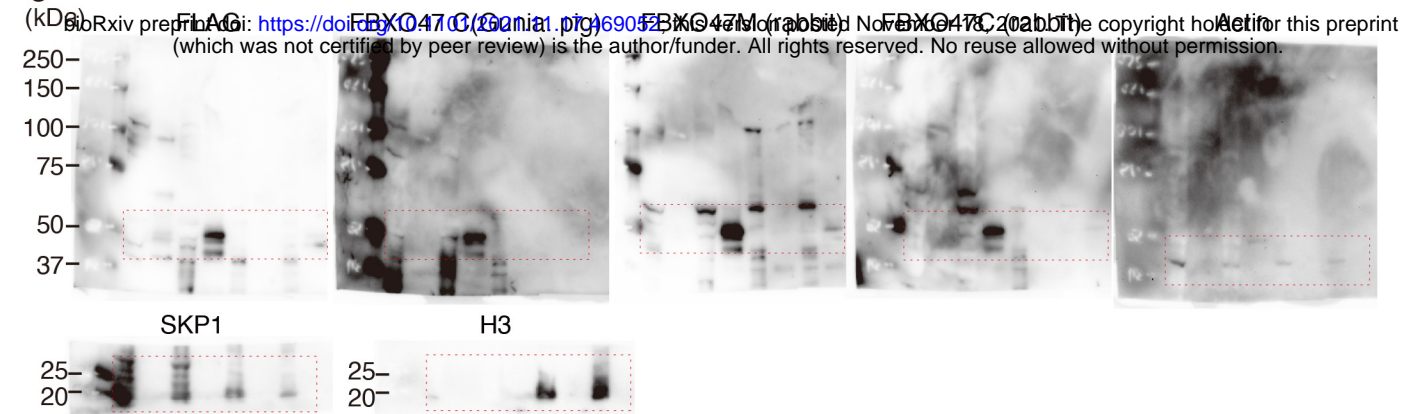
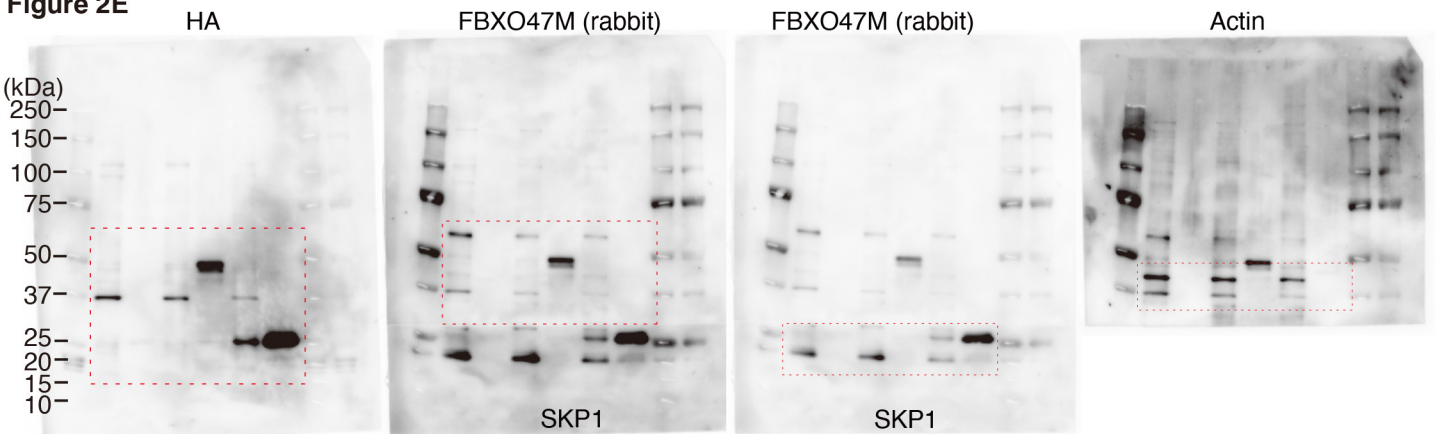
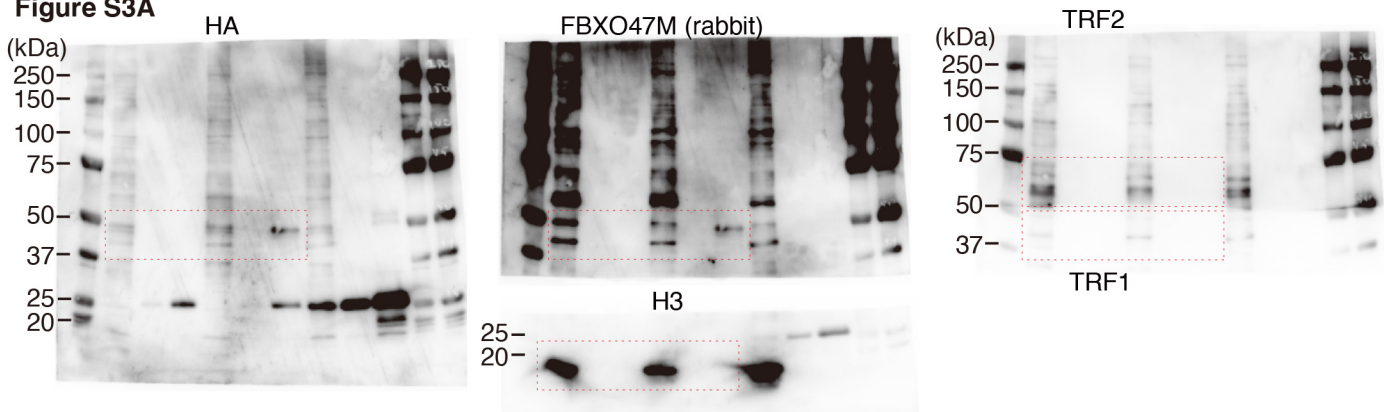
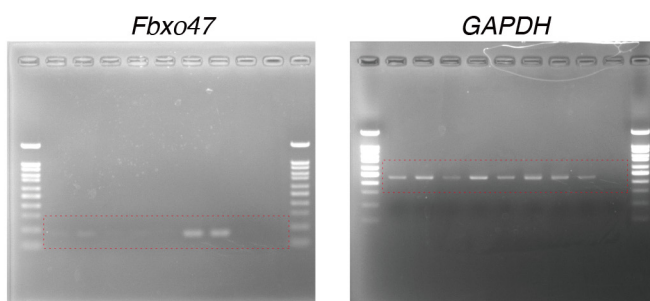
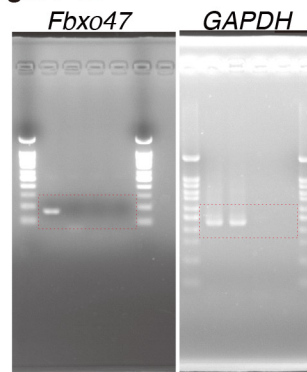
Supplementary Figure 3. FBXO47 do not localize to telomeres (related to Figure 1)

(A) Western blot showed immunoprecipitates from chromatin extracts of WT (non-tagged control) and *Fbxo47-3FH* KI mouse testes (from 139 and 148 animals at P14-19, respectively) after tandem affinity purifications using anti-FLAG and anti-HA antibodies. The same membrane was sequentially reblotted with different antibodies as indicated. *: non-specific band. Arrowhead: TRF2. Note that western blot did not detect either TRF1 or TRF2 in the FBXO47 immunoprecipitate.

(B) Chromosome spreads of *Fbxo47-3FH* KI and control (non-tagged) spermatocytes were immunostained as indicated. Images with enhanced contrast for HA color channel are shown. Note that FBXO47 did not show specific localization pattern to telomeres. We observed no more than background signals, even though contrast for HA images was enhanced.

(C) Structurally-preserved nuclei of spermatocytes were prepared by squashing *Fbxo47* KO testis tubules, and immunostained for LAMIN B, TRF1 and SYCP3. The image acquired at the equator of the spermatocyte nuclei is shown. Note that telomeres attachment to the nuclear envelope was intact in *Fbxo47* KO spermatocytes. Scale bars: 5 μ m.

(D) The indicated spermatocyte nuclei were immunostained as indicated (left). Telomere clustering in wild-type (n=355) and *Fbxo47* KO (n=345) was scored at 12 day post-partum. The frequency of bouquet stage spermatocytes is shown (right). Statistical significance is shown by N.S. $p = 0.5025$ (chi square-test).

Figure 1H**Figure 2E****Figure S3A****Figure 1C****Figure 4B****Supplementary Figure 4. Uncropped images of gels and blots.**

Full-length / uncropped images of agarose gel (Fig1C, Fig4B) and immunoblots (Fig1H, Fig2E, Fig S3A) are shown. Immunoblotted membrane was sequentially reprobbed with different antibodies. For SKP1, H3, TRF1 immunoblots, the same membrane was stripped, cut according to molecular weight marker and reprobbed, so that different proteins could be simultaneously probed with different antibodies.