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Supplementary Materials for

SPO16 binds SHOC1 to promote homologous recombination and crossing-over in meiotic prophase I

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This PDF file includes:

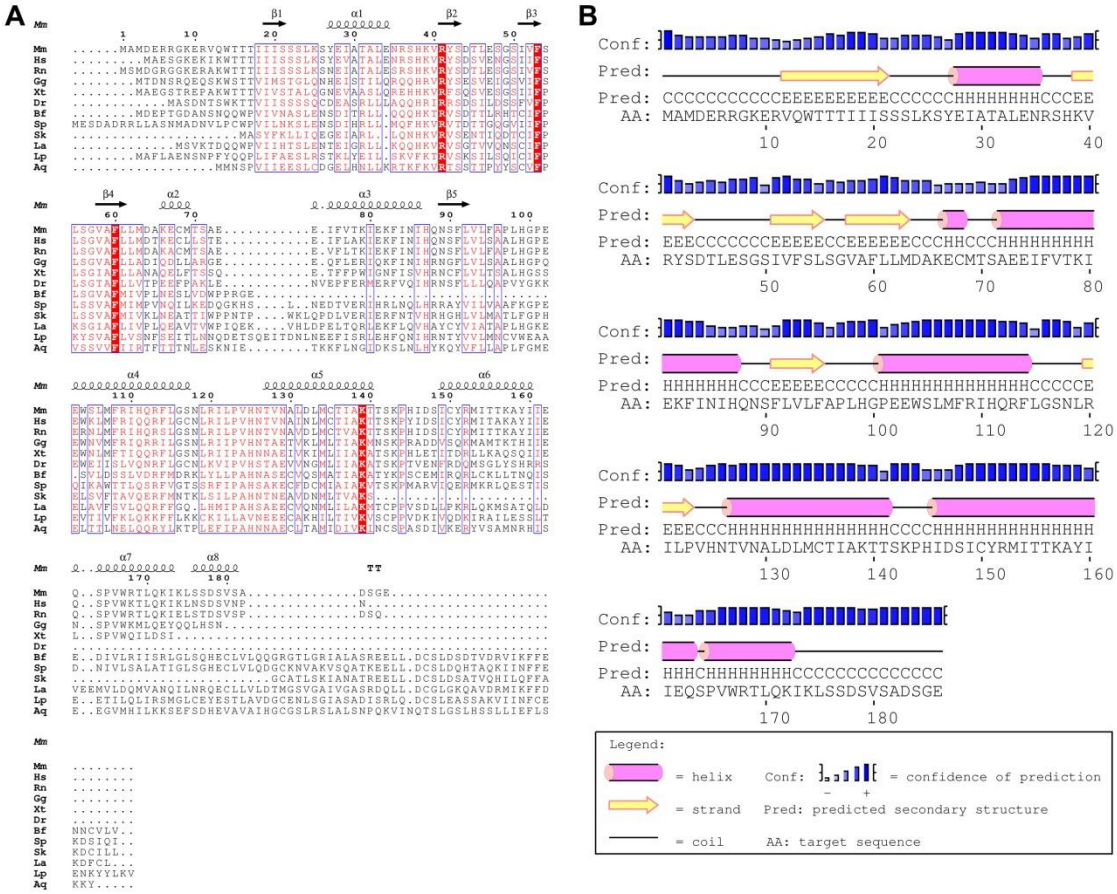
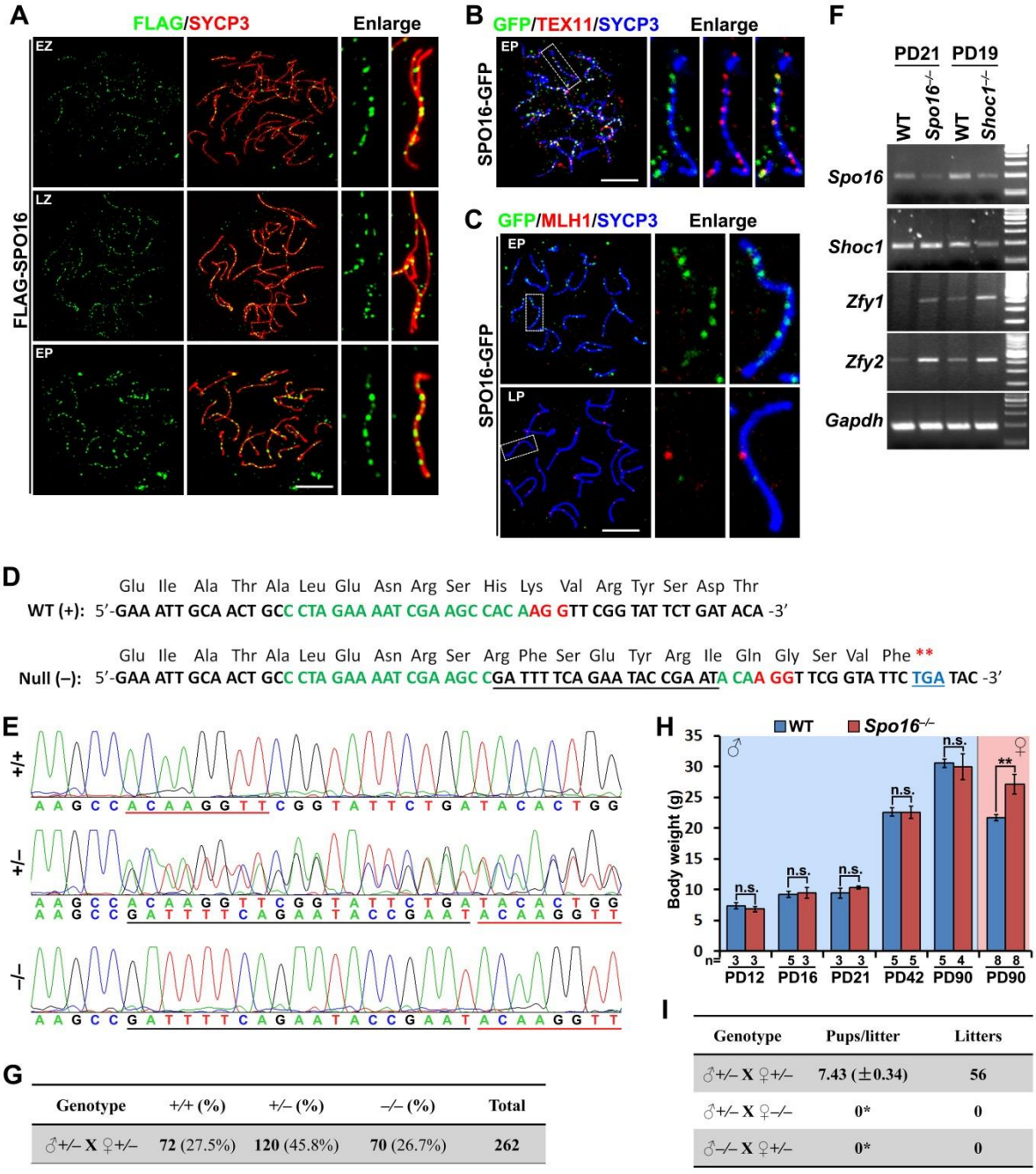

A
B
Conf:
Pred:
AA:
Legend:
= helix
= strand
= coil
= confidence of prediction
Pred: predicted secondary structure
AA: target sequence
C
MmSPO16
ApXPF
MERGE
D
MmSPO16
ScSpo16
MERGE

Fig. S1. *Mm*SPO16 has a conserved XPF-like domain. (**A**) Alignment of SPO16 members showing structural conservation among species. Mm, *Mus musculus*; Hs, *Homo sapiens*; Rn, *Rattus norvegicus*; Gg, *Gallus gallus*; Xt, *Xenopus tropicalis*; Dr, *Danio rerio*; Bf, *Branchiostoma floridae*; Sp, *Stylophora pistillata*; Sk, *Saccoglossus kowalevskii*; La, *Lingula anatina*; Lp, *Limulus polyphemus*; Aq, *Amphimedon queenslandica*. The predicted secondary structural elements of *Mm*SPO16 are shown above the alignment. Fully conserved amino acid residues are shown in white on a red background, whereas partially conserved residues are shown within blue boxes. (**B**) PSIPRED secondary structure prediction for *Mm*SPO16. (**C**) FATCAT analysis of the modeled *Mm*SPO16 (left) compared with the structure of *Ap*XPF (PDB: 2bgwA, middle). Both proteins are colored with the rainbow graduation (blue to red from N terminus to C-terminus). In the merged panel (right), *Mm*SPO16 is shown in grey, whereas *Ap*XPF is shown in red. (**D**) FATCAT analysis of the modeled *Mm*SPO16 (left) compared with the structure of *Sc*SPO16 (PDB: 6bzgA, middle). Both proteins are colored with the rainbow graduation (blue to red from N terminus to C-terminus). In the merged panel (right), *Mm*SPO16 is shown in grey, whereas *Sc*SPO16 is shown in different colors (seperated by twists).



Genotype	+/+ (%)	+/– (%)	–/– (%)	Total
♂+/– X ♀+/–	72 (27.5%)	120 (45.8%)	70 (26.7%)	262

Genotype	Pups/litter	Litters
♂+/– X ♀+/–	7.43 (±0.34)	56
♂+/– X ♀–/–	0*	0
♂–/– X ♀+/–	0*	0

Fig. S2. Localization of SPO16 and generation of knockout mice. (**A**) Immunofluorescent staining of exogenous expressed-, FLAG tagged-SPO16 protein (green) and SYCP3 (red) on the nuclear surface spreads of wild-type testes electroporated with plasmids encoding FLAG-SPO16. SYCP3 marked the meiotic chromosome axes. Enlarged images showed the partially or fully synapsed homologous pairs. EZ, early zygonema; LZ, late zygonema; EP, early pachynema.

Scale bar , 10 μm. (**B**) Co-staining of SPO16-GFP (green) with middle recombination marker TEX11 (red) on the nuclear surface spreads of wild-type testes electroporated with plasmids encoding FLAG-SPO16. Scale bars, 10 μm. (**C**) Co-staining of SPO16-GFP (green) with the late recombination marker MLH1 (red) on the nuclear surface spreads of wild-type testes electroporated with plasmids encoding SPO16-GFP. LP, late pachynema. Scale bars, 10 μm. (**D**) Schematic diagram of the null allele for *Spo16*, which contains a 19-bp insertion within the selected sgRNA and introduces a premature stop codon (**). The insertion was underlined with black color. (**E**) Sequencing results of mice with different genotypes: $Spo16^{+/+}$, $Spo16^{+/-}$ and $Spo16^{-/-}$. (**F**) Semi-quantitative PCR showing the relative mRNA level of indicated genes in WT, $Spo16^{-/-}$ and $Shoc1^{-/-}$ testes. (**G**) Numbers and percentages of $Spo16^{+/+}$, $Spo16^{+/-}$ and $Spo16^{-/-}$ pubs from $Spo16^{+/-}$ to $Spo16^{+/-}$ breeding. (**H**) Growth of WT and $Spo16^{-/-}$ males as measured by body weight. Numbers of testes analyzed (n) are indicated. Error bars indicate S.E.M. *** $P < 0.001$ by two-tailed Student's *t* tests. n.s., not significant. (**I**) Pups/litter and litter numbers analyzed for indicated breeding. *, no pups or pregnancies were observed through a 3-month breeding. n>6 for each breeding set.

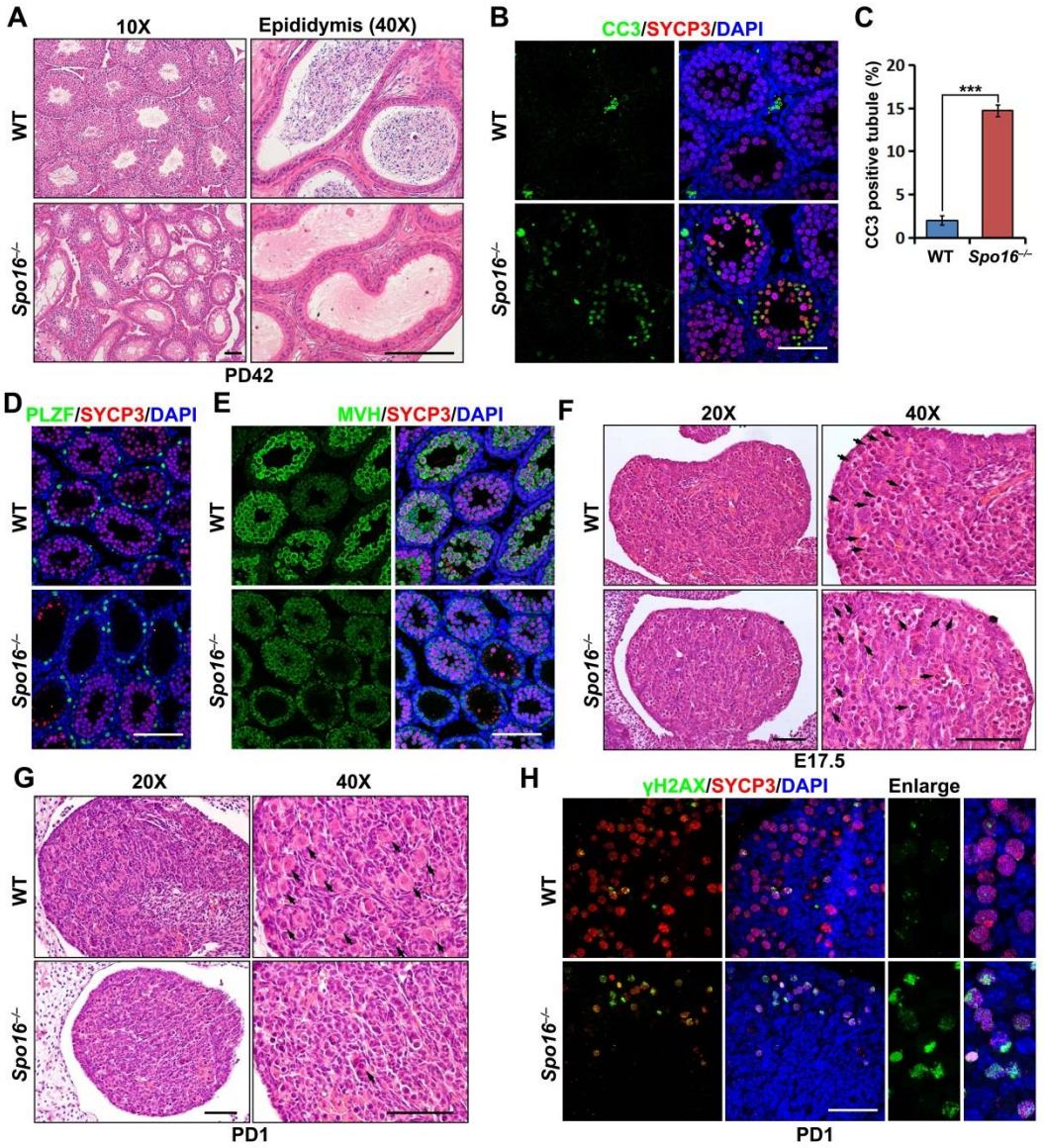


Fig. S3. SPO16 deletion leads to massive germline loss. (**A**) H&E staining showing the histology of testes and epididymis derived from WT and *Spo16*$^{-/-}$ males at PD42. Scale bars, 50 μm. (**B**–**C**) Massive apoptosis in *Spo16*$^{-/-}$ testes as shown by cleaved caspase 3 (CC3, green) immunofluorescent staining. Scale bar, 50 μm. Error bars indicate S.E.M. *** $P < 0.001$ by two-tailed Student's *t* tests. (**D**–**E**) PLZF (marker of undifferentiated spermatogonia, D) and MVH (marker of germ cells, E) staining on testes sections at PD21. Scale bars, 100 μm. (**F**–**G**) H&E staining of ovaries derived from WT and *Spo16*$^{-/-}$ females at E17.5 and PD1. Scale bars, 50 μm. Arrows indicate oocytes. (**H**) γH2AX (green) staining showing the defects in DSB repair in *Spo16*$^{-/-}$ PGCs at E17.5. Scale bar, 50 μm.

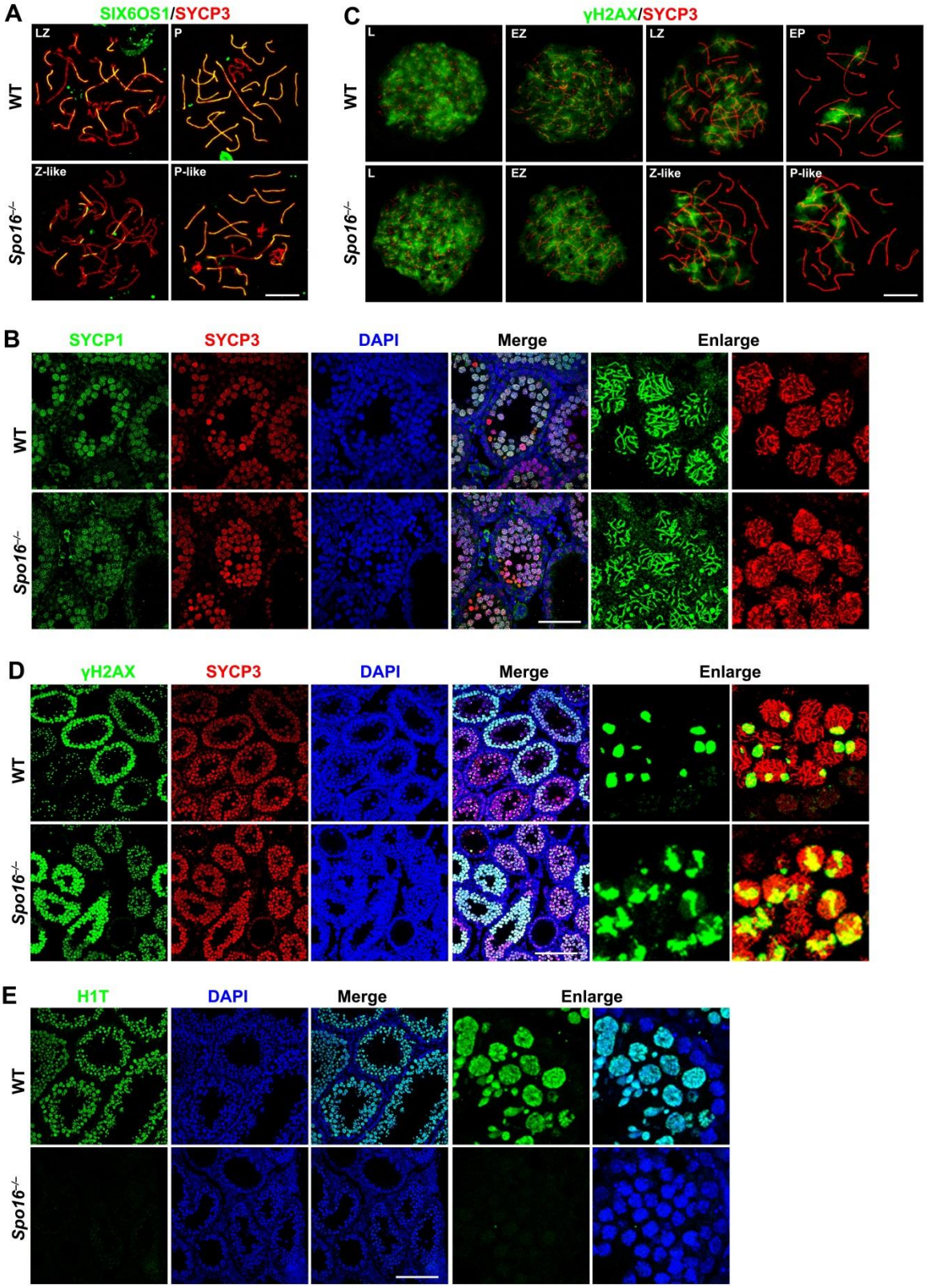

A
SIX6OS1/SYCP3
LZ
P
WT
Z-like
P-like
Spo16−/−
C
γH2AX/SYCP3
L
EZ
LZ
EP
WT
L
EZ
Z-like
P-like
Spo16−/−
B
SYCP1
SYCP3
DAPI
Merge
Enlarge
WT
Spo16−/−
D
γH2AX
SYCP3
DAPI
Merge
Enlarge
WT
Spo16−/−
E
H1T
DAPI
Merge
Enlarge
WT
Spo16−/−

Fig. S4. Insufficient meiotic prophase progression in *Spo16*$^{-/-}$ testes. (**A**) Staining of SIX6OS1 (marker of synapsis, green) with SYCP3 (red) on the nuclear surface spreads of spermatocytes derived from WT and *Spo16*$^{-/-}$ males at PD42. Z-like, zygonema-like; P-like, pachytenema-like. Scale bar, 10 μm. (**B**) Staining of SYCP1 with SYCP3 on the sections of WT and *Spo16*$^{-/-}$ males at PD21. Scale bar, 50 μm. (**C**) γH2AX (green) staining on the nuclear surface spreads showing the generation and repair of DSBs during meiotic prophase. Scale bar, 10 μm. (**D**) γH2AX (green) staining on the sections of WT and *Spo16*$^{-/-}$ testes at PD21. Scale bar, 100 μm. (**E**) H1T staining on the sections of WT and *Spo16*$^{-/-}$ testes at PD42. Scale bar, 100 μm.

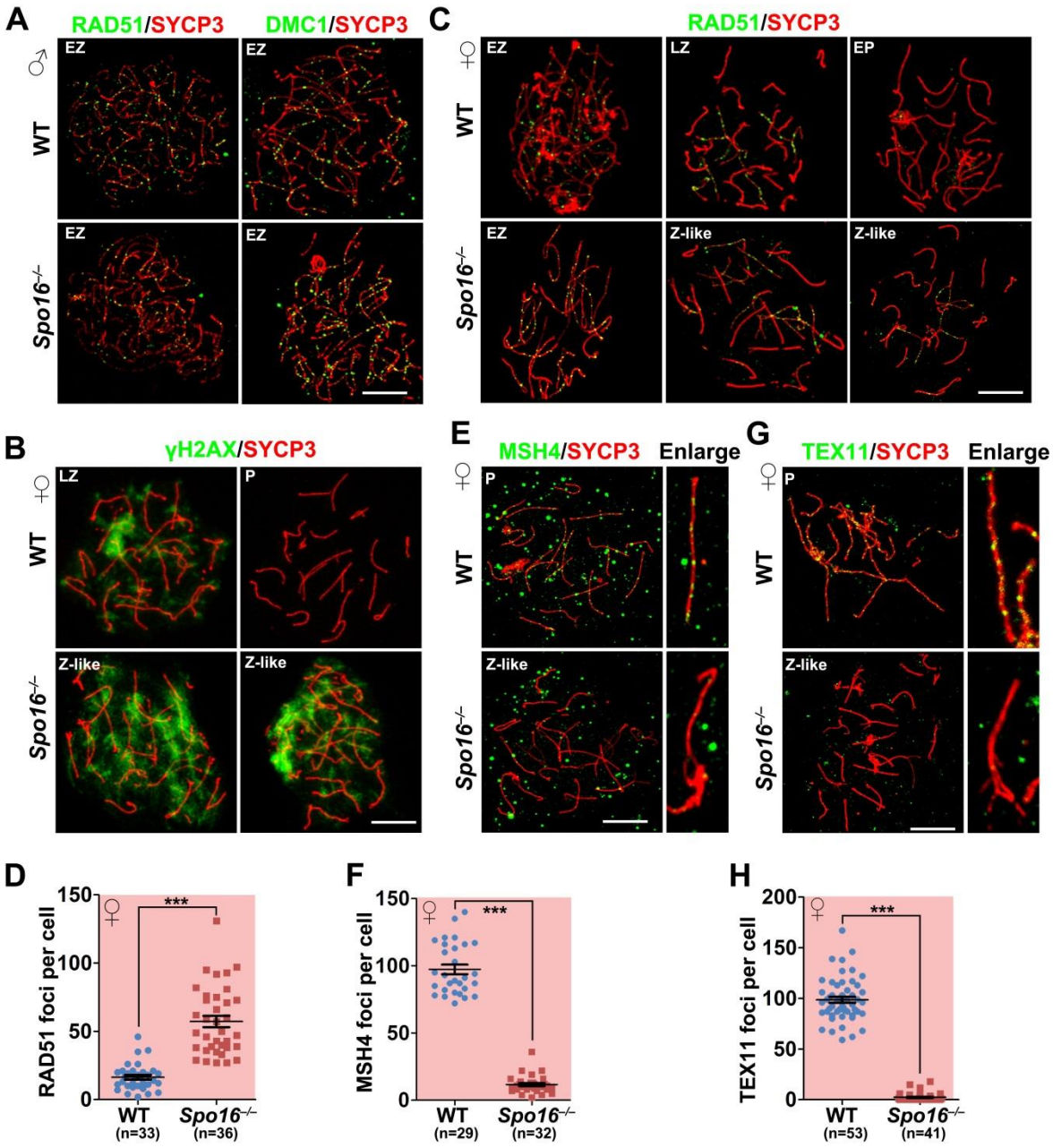


Fig. S5. Insufficient meiotic recombination in SPO16-deleted spermatocytes and oocytes. (**A**) Immunostaining of indicated proteins on WT and *Spo16*$^{-/-}$spermatocytes at early zygotene stage. Scale bar, 10 μm. (**B**) γH2AX (green) staining showing the unrepaired regions in female PGCs derived from WT and *Spo16*$^{-/-}$ovaries at E17.5. Scale bar, 10 μm. (**C**–**D**) RAD51 (green, C) was detected on the nuclear surface spreads of WT and *Spo16*$^{-/-}$ PGCs and the quantification of RAD51 foci was shown in (D). Scale bar, 10 μm. Numbers of PGCs analyzed (n) were indicated.

Median focus numbers were marked. Error bars indicated S.E.M. *** $P < 0.001$ by two-tailed Student's *t* tests. (A–D) MSH4 (A) and TEX11 (B) were detected on the nuclear surface spreads of WT and *Spo16*$^{-/-}$ PGCs, and the quantification of their foci was shown in (C) and (D), respectively. Scale bars, 10 μm.

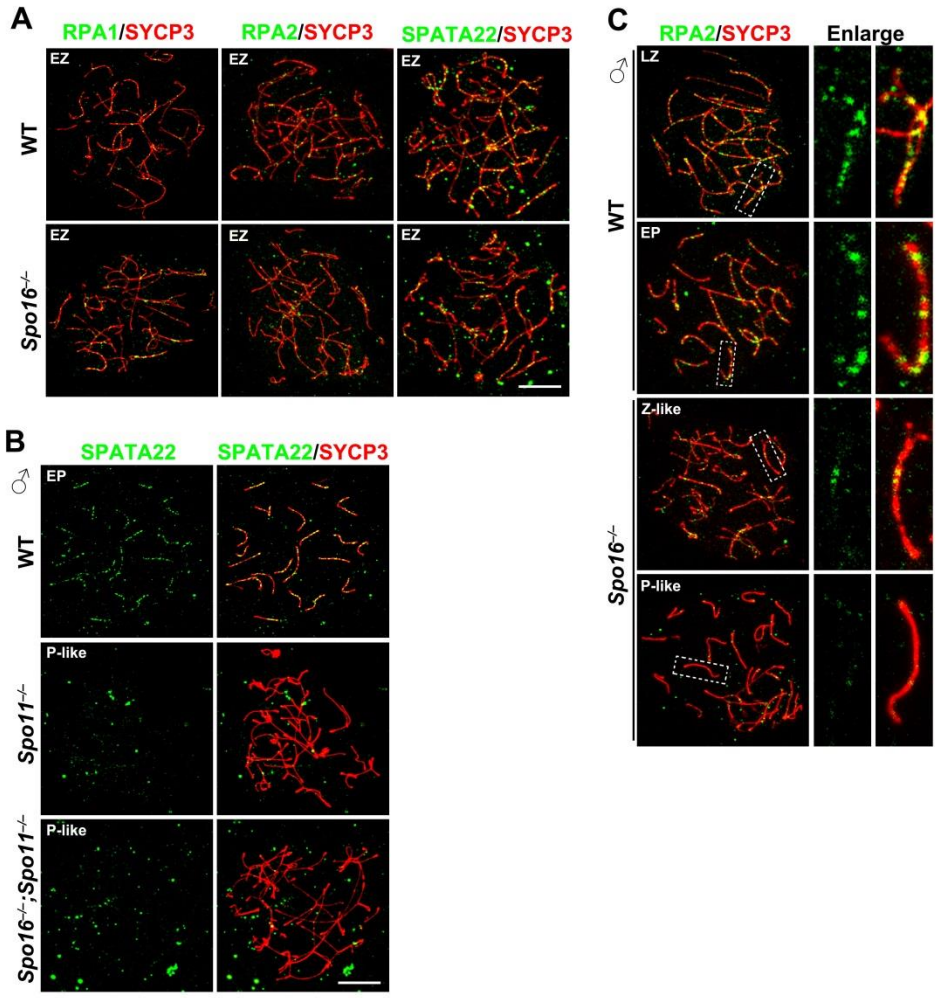


Fig. S6. Detection of RPA complex in WT and SPO16-deleted spermatocytes. (**A**) Immunostaining of indicated proteins on WT and *Spo16*$^{-/-}$ spermatocytes at early zygotene stage. Scale bar, 10 μm. (**B**) SPATA22 (green) was detected on the nuclear surface spreads of WT, *Spo11*$^{-/-}$ and *Spo16*$^{-/-}$*;Spo11*$^{-/-}$ spermatocytes. Scale bar, 10 μm. (**C**) RPA2 was detected on the nuclear surface spreads of WT and *Spo16*$^{-/-}$ spermatocytes. Scale bar, 10 μm.

Table S1. Genes specifically expressed in meiotic prophase I.

Number	**Human gene symbol**	**Mouse gene symbol**	**Known in meiosis** (Reference)
1	*ACSS2*	*Acss2*	
2	*ADAD1*	*Adad1*	Y
3	*C9orf84*	*Ai481877, Mzip2*	Y
4	*AK9*	*Ak9*	
5	*ALG13*	*Alg13*	
6	*ANKRD31*	*Ankrd31*	
7	*ANKRD7*	*Ankrd7*	
8	*APTR*	*A630072m18rik*	
9	*ARHGAP9*	*Arhgap9*	
10	*ARIH2*	*Arih2*	
11	*ART3*	*Art3*	
12	*ASF1B*	*Asf1b*	Y
13	*ASZ1*	*Asz1*	Y
14	*ATP6AP1*	*Atp6ap1*	
15	*BBS4*	*Bbs4*	
16	*BCAP31*	*Bcap31*	
17	*BIN2*	*Bin2*	
18	*BRD1*	*Brd1*	Y
19	*BSCL2*	*Bscl2*	
20	*BTG3*	*Btg3*	
21	*C11orf24*	*1810055g02rik*	
22	*C11orf48*	*Lbhd1*	
23	*C11orf65*	*4930550c14rik*	
24	*C11orf70*	*9230110c19rik*	
25	*C11orf85*	*Majin*	Y
26	*C14orf164*	*Rnf212b*	Y
27	*C14orf39*	*Six6os1*	Y
28	*C15orf43*	*4933406j08rik, Terb2*	Y
29	*C15orf48*	*Aa467197*	
30	*C16orf70*	*D230025d16rik*	
31	*C16orf95*	*1700018b08rik*	
32	*C17orf104*	*Gm1564,meioc*	Y
33	*C17orf75*	*5730455p16rik*	
34	*C18orf63*	*Gm17266*	
35	*C19orf38*	*Ab124611*	
36	*C19orf57*	*4930432k21rik*	
37	*C1orf105*	*4930558k02rik*	
38	*C1orf146*	*1700028k03rik*	*Mspo16*, this study

Number	**Human gene symbol**	**Mouse gene symbol**	**Known in meiosis** (Reference)
39	*C1orf35*	*2310033p09rik*	
40	*C20orf194*	*4930402h24rik*	
41	*C5orf45*	*3010026o09rik*	
42	*C5orf47*	*4930524b15rik*	
43	*C9orf43*	*4933430i17rik*	
44	*CAMSAP1*	*Camsap1*	
45	*CAPN3*	*Capn3*	
46	*CCDC11*	*Ccdc11*	
47	*CCDC150*	*Ccdc150*	
48	*CCDC151*	*Ccdc151*	
49	*CCDC152*	*Ccdc152*	
50	*CCDC155*	*Ccdc155*	Y
51	*CCDC172*	*Ccdc172*	
52	*CCDC173*	*Ccdc173*	
53	*CCDC176*	*Ccdc176*	
54	*CCDC181*	*Ccdc181*	
55	*CCDC42*	*Ccdc42*	
56	*CCDC7*	*Ccdc7*	
57	*CCDC73*	*Ccdc73*	
58	*CCDC88A*	*Ccdc88A*	
59	*CCDC93*	*Ccdc93*	
60	*CCNB3*	*Ccnb3*	Y
61	*CCNE1*	*Ccne1*	Y
62	*CCNE2*	*Ccne2*	Y
63	*CCT6B*	*Cct6b*	
64	*CDC45*	*Cdc45*	
65	*CDK16*	*Cdk16*	Y
66	*CDKL3*	*Cdkl3*	
67	*CENPU*	*Cenpu*	
68	*CHAF1A*	*Chaf1a*	
69	*CHIC2*	*Chic2*	
70	*CIAPIN1*	*Ciapin1*	
71	*CIPC*	*Cipc*	
72	*CLNS1A*	*Clns1a*	
73	*CMTR1*	*Cmtr1*	
74	*CNTD1*	*Cntd1*	Y
75	*CNTRL*	*Cntrl*	
76	*COL17A1*	*Col17a1*	
77	*CRAT*	*Crat*	
78	*CTCFL*	*Ctcfl*	Y

Number	**Human gene symbol**	**Mouse gene symbol**	**Known in meiosis** (Reference)
79	*CTU2*	*Ctu2*	
80	*CXorf40A*	*1110012l19rik*	
81	*CXorf58*	*Loc102640868*	
82	*DDX28*	*Ddx28*	
83	*DDX39A*	*Ddx39a*	
84	*DERL3*	*Derl3*	
85	*DFNB59*	*Dfnb59*	
86	*DGAT1*	*Dgat1*	
87	*DMRTB1*	*Dmrtb1*	Y
88	*DMRTC2*	*Dmrtc2*	Y
89	*DNMT1*	*Dnmt1*	Y
90	*DNTTIP1*	*Dnttip1*	
91	*DPEP3*	*Dpep3*	
92	*DUS1L*	*Dus1l*	
93	*DYDC1*	*Dydc1*	
94	*DZIP3*	*Dzip3*	
95	*EAF2*	*Eaf2*	
96	*EFCAB7*	*Efcab7*	
97	*EID3*	*Eid3*	
98	*ENKD1*	*Enkd1*	
99	*ERAS*	*Eras*	
100	*ESCO2*	*Esco2*	
101	*EYA3*	*Eya3*	
102	*FAM117A*	*Fam117a*	
103	*FAM122A*	*Fam122a*	
104	*FAM204A*	*Fam204a*	
105	*FAM217A*	*Fam217a*	
106	*FAM228A*	*Fam228a*	
107	*FAM71E1*	*Fam71e1*	
108	*FANCA*	*Fanca*	Y
109	*FANCL*	*Fancl*	Y
110	*FARP2*	*Farp2*	
111	*FBXO15*	*Fbxo15*	
112	*FBXO25*	*Fbxo25*	
113	*FBXO4*	*Fbxo4*	
114	*FBXW7*	*Fbxw7*	
115	*FH*	*Fh*	
116	*FKBP6*	*Fkbp6*	Y
117	*FLYWCH2*	*Flywch2*	
118	*FOXR1*	*Foxr1*	

Number	**Human gene symbol**	**Mouse gene symbol**	**Known in meiosis** (Reference)
119	*FUK*	*Fuk*	
120	*GABPB2*	*Gabpb2*	
121	*GDF9*	*Gdf9*	
122	*GDPD4*	*Gdpd4*	
123	*GINS2*	*Gins2*	
124	*GLCCI1*	*Glcci1*	
125	*GLE1*	*Gle1*	
126	*GPAA1*	*Gpaa1*	
127	*GPAT2*	*Gpat2*	
128	*GSAP*	*Gsap*	
129	*GTF2A1L*	*Gtf2a1l*	
130	*HAUS7*	*Haus7*	
131	*HCCS*	*Hccs*	
132	*HDAC6*	*Hdac6*	
133	*HESX1*	*Hesx1*	
134	*HFM1*	*Hfm1*	Y
135	*HINFP*	*Hinfp*	
136	*HIPK1*	*Hipk1*	
137	*HIRIP3*	*Hirip3*	
138	*HIST1H3A*	*Hist1h3a*	
139	*HIST3H3*	*Hist3h3*	
140	*HMCES*	*Hmces*	
141	*HMOX2*	*Hmox2*	
142	*HORMAD1*	*Hormad1*	Y
143	*HORMAD2*	*Hormad2*	Y
144	*HPX*	*Hpx*	
145	*HSF2BP*	*Hsf2bp*	
146	*HUS1B*	*Hus1b*	
147	*IFT122*	*Ift122*	
148	*IFT81*	*Ift81*	
149	*IL13RA2*	*Il13ra2*	
150	*IL23A*	*Il23a*	
151	*INCA1*	*Inca1*	
152	*IQCB1*	*Iqcb1*	
153	*JADE3*	*Jade3*	
154	*JOSD2*	*Josd2*	
155	*KCTD19*	*Kctd19*	
156	*KHDC1*	*Khdc1*	
157	*KIAA1715*	*Kiaa1715*	
158	*KIF18A*	*Kif18a*	Y

Number	**Human gene symbol**	**Mouse gene symbol**	**Known in meiosis** (Reference)
159	*KLHL20*	*Klhl20*	
160	*L3MBTL1*	*L3mbtl1*	
161	*LACC1*	*Lacc1*	
162	*LCA5L*	*Lca5l*	
163	*LCMT2*	*Lcmt2*	
164	*LDHC*	*Ldhc*	Y
165	*LEAP2*	*Leap2*	
166	*LIMK2*	*Limk2*	Y
167	*LOC728637*	*Meikin*	Y
168	*LOC81691*	*2610020h08rik*	
169	*LRRC34*	*Lrrc34*	
170	*LRRC46*	*Lrrc46*	
171	*LSS*	*Lss*	
172	*LY6G5C*	*Ly6g5c*	
173	*LY6K*	*Ly6k*	Y
174	*LYPD4*	*Lypd4*	
175	*LYRM9*	*Lyrm9*	
176	*LYST*	*Lyst*	
177	*MACF1*	*Macf1*	
178	*MAD2L1*	*Mad2l1*	Y
179	*MAGEB3*	*Mageb3*	
180	*MAN1B1*	*Man1b1*	
181	*MAP3K3*	*Map3k3*	
182	*MCMDC2*	*Mcmdc2*	Y
183	*MDH1B*	*Mdh1b*	
184	*MED14*	*Med14*	
185	*MEI1*	*Mei1*	Y
186	*MEIOB*	*Meiob*	Y
187	*MLF1*	*Mlf1*	
188	*MORN2*	*Morn2*	
189	*MOV10L1*	*Mov10l1*	Y
190	*MPP2*	*Mpp2*	
191	*MSH5*	*Msh5*	Y
192	*MTL5*	*Mtl5*	
193	*NCAPH2*	*Ncaph2*	
194	*NCOA5*	*Ncoa5*	
195	*NGLY1*	*Ngly1*	
196	*NIPBL*	*Nipbl*	
197	*NKAP*	*Nkap*	
198	*NKIRAS1*	*Nkiras1*	

Number	**Human gene symbol**	**Mouse gene symbol**	**Known in meiosis** (Reference)
199	*NOL8*	*Nol8*	
200	*NRIP3*	*Nrip3*	
201	*NSRP1*	*Nsrp1*	
202	*NSUN7*	*Nsun7*	
203	*NUP210L*	*Nup210l*	
204	*OSCP1*	*Oscp1*	
205	*PANK3*	*Pank3*	
206	*PARP12*	*Parp12*	
207	*PARP6*	*Parp6*	
208	*PCID2*	*Pcid2*	
209	*PDHA1*	*Pdha1*	
210	*PDIA3*	*Pdia3*	
211	*PDPK1*	*Pdpk1*	
212	*PEX12*	*Pex12*	
213	*PEX5*	*Pex5*	
214	*PHF7*	*Phf7*	
215	*PHF8*	*Phf8*	
216	*PHLPP1*	*Phlpp1*	
217	*PHYHIPL*	*Phyhipl*	
218	*PIGP*	*Pigp*	
219	*PITX2*	*Pitx2*	
220	*PKMYT1*	*Pkmyt1*	
221	*PLS1*	*Pls1*	
222	*POLG2*	*Polg2*	
223	*POLN*	*Poln*	
224	*POMT1*	*Pomt1*	
225	*PON3*	*Pon3*	
226	*PRDM9*	*Prdm9*	Y
227	*PRKACB*	*Prkacb*	
228	*PRSS21*	*Prss21*	Y
229	*PRSS50*	*Prss50*	
230	*PSMA8*	*Psma8*	
231	*PSMC3IP*	*Psmc3ip*	Y
232	*PSMD10*	*Psmd10*	
233	*PTBP2*	*Ptbp2*	Y
234	*PXT1*	*Pxt1*	
235	*RAD21L1*	*Rad21l1*	
236	*RAD51AP2*	*Rad51ap2*	
237	*RAD9B*	*Rad9b*	Y
238	*RBP4*	*Rbp4*	Y

Number	**Human gene symbol**	**Mouse gene symbol**	**Known in meiosis** (Reference)
239	*REC114*	*Rec114*	Y
240	*RIBC2*	*Ribc2*	
241	*RNF182*	*Rnf182*	
242	*RNF19B*	*Rnf19b*	
243	*RNF212*	*Rnf212*	Y
244	*RNF32*	*Rnf32*	
245	*RPS6KA3*	*Rps6ka3*	
246	*RPS6KA6*	*Rps6ka6*	
247	*RRAGB*	*Rragb*	
248	*RSPH3*	*Rsph3*	
249	*RUNDC3B*	*Rundc3b*	
250	*RWDD2B*	*Rwdd2b*	
251	*SAP30*	*Sap30*	
252	*SAPCD1*	*Sapcd1*	
253	*SCML1*	*Scml1*	
254	*SCML2*	*Scml2*	Y
255	*SERHL*	*Serhl*	
256	*SH3BGR*	*Sh3bgr*	
257	*SHCBP1L*	*Shcbp1l*	Y
258	*SIRT3*	*Sirt3*	
259	*SKAP1*	*Skap1*	
260	*SLC25A31*	*Slc25a31*	Y
261	*SLC25A42*	*Slc25a42*	
262	*SLC35G2*	*Slc35g2*	
263	*SLC4A1AP*	*Slc4a1ap*	
264	*SMAP2*	*Smap2*	
265	*SMC1B*	*Smc1b*	Y
266	*SNRNP40*	*Snrnp40*	
267	*SOCS7*	*Socs7*	
268	*SPACA1*	*Spaca1*	Y
269	*SPAG6*	*Spag6*	
270	*SPATA22*	*Spata22*	Y
271	*SPDL1*	*Spdl1*	
272	*SPDYA*	*Spdya*	Y
273	*SPO11*	*Spo11*	Y
274	*SPOPL*	*Spopl*	
275	*SRR*	*Srr*	
276	*STAG3*	*Stag3*	Y
277	*STRIP2*	*Strip2*	
278	*SUGP1*	*Sugp1*	

Number	**Human gene symbol**	**Mouse gene symbol**	**Known in meiosis** (Reference)
279	*SUGP2*	*Sugp2*	
280	*SYCE1*	*Syce1*	Y
281	*SYCE2*	*Syce2*	Y
282	*SYCE3*	*Syce3*	Y
283	*SYCP1*	*Sycp1*	Y
284	*SYCP2*	*Sycp2*	Y
285	*SYCP3*	*Sycp3*	Y
286	*SYNGR4*	*Syngr4*	
287	*SYNJ1*	*Synj1*	
288	*TAF12*	*Taf12*	
289	*TARSL2*	*Tarsl2*	
290	*TAZ*	*Taz*	
291	*TBC1D2*	*Tbc1d2*	
292	*TCP11*	*Tcp11*	
293	*TCTEX1D2*	*Tctex1d2*	
294	*TCTN1*	*Tctn1*	
295	*TDRD9*	*Tdrd9*	Y
296	*TEX101*	*Tex101*	Y
297	*TEX12*	*Tex12*	Y
298	*TEX14*	*Tex14*	Y
299	*TEX15*	*Tex15*	Y
300	*TEX264*	*Tex264*	
301	*TEX30*	*Tex30*	
302	*TMEM116*	*Tmem116*	
303	*TNFAIP8L3*	*Tnfaip8L3*	
304	*TOPAZ1*	*Topaz1*	Y
305	*TPTE*	*Tpte*	
306	*TRAFD1*	*Trafd1*	
307	*TRIP13*	*Trip13*	Y
308	*TRMU*	*Trmu*	
309	*TSGA10*	*Tsga10*	
310	*TTC18*	*Ttc18*	
311	*TTC39A*	*Ttc39a*	
312	*UBA5*	*Uba5*	
313	*UFD1L*	*Ufd1*	
314	*UIMC1*	*Uimc1*	
315	*UPB1*	*Upb1*	
316	*USP37*	*Usp37*	
317	*USP6*	*Usp6*	
318	*WARS*	*Wars*	

Number	**Human gene symbol**	**Mouse gene symbol**	**Known in meiosis** (Reference)
319	*WBSCR22*	*Bud23*	
320	*WDR3*	*Wdr3*	
321	*WDR91*	*Wdr91*	
322	*VRK1*	*Vrk1*	Y
323	*YEATS4*	*Yeats4*	
324	*YY2*	*Yy2*	
325	*ZCCHC17*	*Zcchc17*	
326	*ZCCHC7*	*Zcchc7*	
327	*ZCCHC9*	*Zcchc9*	
328	*ZCWPW1*	*Zcwpw1*	
329	*ZDHHC11*	*Zdhhc11*	
330	*ZMAT1*	*Zmat1*	
331	*ZMYND12*	*Zmynd12*	
332	*ZNF143*	*Znf143*	
333	*ZNF280B*	*Znf280b*	
334	*ZNF280C*	*Znf280c*	
335	*ZNF318*	*Znf318*	
336	*ZNF346*	*Znf346*	
337	*ZNF398*	*Znf398*	
338	*ZNF541*	*Znf541*	
339	*ZNF563*	*Znf563*	
340	*ZNF622*	*Znf622*	
341	*ZNF711*	*Znf711*	
342	*ZNF76*	*Znf76*	
343	*ZPBP2*	*Zpbp2*	Y

Table S2. Homology of *Mm*SPO16 to known proteins.

Number	Hit	Name	Probability	E-value	SS	Cols	Target Length
1	2M9M_A	Fanconi anemia-associated protein of 24; Fanconi anemia, FAAP24, ERCC4 domain; NMR {Homo sapiens}	95.99	0.02	11.7	118	147
2	2BGW_B	XPF ENDONUCLEASE (E.C.2.7.7.-); HYDROLASE, STRUCTURE SPECIFIC ENDONUCLEASE, NUCLEOTIDE; HET: SO4; 2.8A {AEROPYRUM PERNIX} SCOP: c.52.1.20, a.60.2.5	94.85	0.55	13.4	130	219
3	2A1I_A	DNA excision repair protein ERCC-1; ERCC1, XPF, NER, central domain; 1.9A {Homo sapiens} SCOP: c.52.1.20	94.1	0.54	11.4	136	146
4	4BXO_A	FANCONI ANEMIA GROUP M PROTEIN; HYDROLASE-DNA COMPLEX, DNA BINDING, PSEUDO-NUCLEASE; 2.15A {HOMO SAPIENS}	92.14	4.3	13.3	137	254
5	2ZIU_B	Mus81 protein, Crossover junction endonuclease; Helix-hairpin-Helix, Alternative splicing, DNA damage; 2.7A {Danio rerio}	91.94	5	13.5	128	341
6	4M6W_A	Fanconi anemia group M protein; FANCM, FAAP24, XPF/Mus81, Fanconi anemia; HET: SO4; 2.9A {Homo	91.33	4.8	12.9	131	221

Number	Hit	Name	Probability	E-value	SS	Cols	Target Length
		sapiens}					
7	4BXO_B	FANCONI ANEMIA GROUP M PROTEIN; HYDROLASE-DNA COMPLEX, DNA BINDING, PSEUDO-NUCLEASE; 2.15A {HOMO SAPIENS}	89.1	8.7	14.5	136	217
8	6BZG_B	Protein ZIP2, Sporulation-specific protein 16; XPF-ERCC1, Meiosis, Recombination, DNA BINDING; HET: SO4, P6G; 2.13A {Saccharomyces cerevisiae}	88.28	13	14	132	206
9	5UJ7_C	Origin recognition complex subunit 1; Replication, DNA-binding, AAA+ ATPase, DNA; HET: ATP; 3.394A {Homo sapiens}	82.19	20	9.6	124	436
10	4P0P_B	Crossover junction endonuclease MUS81, Crossover; resolvase, Hydroase-DNA complex, HYDROLASE-DNA complex; 2.8A {Homo sapiens}	79.86	51	14	133	393
11	4P0P_A	Crossover junction endonuclease MUS81, Crossover; resolvase, Hydroase-DNA complex, HYDROLASE-DNA complex; 2.8A {Homo sapiens}	79.5	39	13.3	134	306

Number	Hit	Name	Probability	E-value	SS	Cols	Target Length
12	2ZIV_A	Mus81 protein, Crossover junction endonuclease; Helix-hairpin-Helix, Alternative splicing, DNA damage; 2.7A {Danio rerio}	70.98	72	14.2	144	311
13	1J24_A	ATP-dependent RNA helicase, putative; structure-specific endonuclease, HYDROLASE; 1.78A {Pyrococcus furiosus} SCOP: c.52.1.20	69.25	56	11.6	136	143

Table S3. Primer sequences.

Primer name	Genes targeted	Application	Sequences (5'-3')
P1	*Spo16*	Genotyping (435bp for WT)	5'-TCAGTTTTTAGTTCTTTGACACTGC-3'
P2			5'-GTGCATCCACATATTTTACCAAAC-3'
P3	*Spo16*	Genotyping (with P2; 209bp/228bp for WT/null allele)	5'-TTCAAGCATGTCTTATGTTCGTG-3'
S522	*Dmc1*	Genotyping (233bp/147bp for WT/null allele)	5'-CCGGCCAGATTACATTTCTT-3'
S523			5'-AAAGGGACTGCTGAGGCATA-3'
S524			5'-GCCAGAGGCCACTTGTGTAG-3'
S519	*Spo11*	Genotyping (165bp/200bp for WT/null allele)	5'-CTGCTCAGGGAGGAGAACAC-3'
S520			5'-TCAGGACAGGGCATAGCAGT-3'
S521			5'-GCCAGAGGCCACTTGTGTAG-3'
S317	*Shoc1*	Common primer	5'-TTGCATTACTTTCAAGTGGTGG-3'
S517		Genotyping (with S317; 240bp for WT allele)	5'-CGTTCTCTATTCCAGGTGGAGA-3'
S518		Genotyping (with S317; 240bp for null allele)	5'-TTATCGTTCTCTATTCCAGGTTTG-3'
S067	*Spo16*	RT-PCR (186bp)	5'-CTGAAGAATGGAGTCTCATGTTCA-3'
S068			5'-GCTCTATGATGTACGCTTTAGTTGT-3'
Z531	*Gapdh*	RT-PCR (181bp)	5'-ACACTGAGGACCAGGTTGTCTC-3'
Z532			5'-TACTCCTTGGAGGCCATGTAG-3'
S642	*Zfy1*	RT-PCR (365bp)	5'-GCCAGTGCTCTCTTAAACCAA-3'
S643			5'-TGAGTACACAAAGTCCCAGCA-3'
S644	*Zfy2*	RT-PCR (385bp)	5'-GCCAGTGCTATGTTACACCAT-3'
S645			5'-TCTGTATGCATTGTCCCAGCA-3'

Table S4. Antibody information.

Protein name	Manufacture (catalogue number)	Origin	Applications (working dilution)	Website Link
SYCP1	Abcam (ab15087)	Rabbit	IF (1:200)	https://www.citeab.com/antibodies/771942-ab15087-anti-scp1-antibody
SYCP3	homemade	Rat	IF (1:500)	Immunogen: Full length of mouse SYCP3
SIX6OS1	homemade	Rabbit	IF (1:200)	Immunogen: aa1-200 of mouse SIX6OS1; Immunized by Capra Science Antibodies AB.
SHOC1/ MZIP2	homemade	Rabbit	WB (1:500); IF (1:100)	Immunogen: aa474-635 of mouse SHOC1/MZIP2; Immunized by Core facilities, Zhejiang University School of Medicine.
GFP	ThermoFisher (A-11122)	Rabbit	IF (1: 500)	https://www.thermofisher.com/antibody/product/GFP-Tag-Antibody-Polyclonal/A-11122
FLAG	Sigma-aldrich (F3165)	Mouse	IF (1: 100)	http://www.sigmaaldrich.com/catalog/product/sigma/f3165?lang=en&region=SE
RAD51	Abcam (ab176458)	Rabbit	IF (1: 200)	http://www.abcam.com/rad51-antibody-chip-grade-ab176458.html
DMC1	Abcam (ab11054)	Mouse	IF (1: 100)	http://www.abcam.com/dmc1-antibody-2h124-ab11054.html
RPA1	Abcam (ab87272)	Rabbit	IF (1: 50)	http://www.abcam.com/rpa70-antibody-ab87272.html
RPA2	Cell Signaling (2208T)	Rat	IF (1: 100)	https://www.cellsignal.com/products/primary-antibodies/rpa32-rpa2-4e4-rat-mab/2208
SPATA22	Proteintech (16989-1-AP)	Rabbit	IF (1: 100)	http://www.ptglab.com/products/SPATA22-Antibody-16989-1-AP.htm

MLH1	BD (551092)	Mouse	IF (1: 200)	http://www.bdbiosciences.com/us/applications/research/apoptosis/purified-antibodies/purified-mouse-anti-mlh-1-with-control/p/551092
MSH4	Abcam (ab58666)	Rabbit	IF (1: 50)	http://www.abcam.com/msh4-antibody-ab58666.html
MVH	Abcam (ab13840)	Rabbit	IF (1: 200); IHC (1: 400)	http://www.abcam.com/ddx4--mvh-antibody-ab13840.html
γH2AX	Cell Signaling (9718S)	Rabbit	IF (1: 400)	https://www.cellsignal.com/products/primary-antibodies/phospho-histone-h2a-x-ser139-20e3-rabbit-mab/9718?N=4294956287&Ntt=h2a.x&fromPage=plp
Cleaved caspase 3	Cell Signaling (9664S)	Rabbit	IF (1: 200)	https://www.cellsignal.com/products/primary-antibodies/cleaved-caspase-3-asp175-5a1e-rabbit-mab/9664
TRF1	homemade	Mouse	IF (1:200)	Immunogen: Full length of mouse TRF1
HORMAD 1	Abcam (ab155176)	Rabbit	IF (1: 200)	http://www.abcam.com/hormad1-antibody-ab155176.html
CREST	Fitzgerald Industries (70R-21494)	Human	IF (1: 200)	https://www.fitzgerald-fii.com/crest-antibody-70r-21494.html
PLZF	Santa Cruz (sc-22839)	Mouse	IF (1:200)	https://www.scbt.com/scbt/sv/product/plzf-antibody-h-300?requestFrom=search
H1T	Gifted by Mary Ann Handel and Ewelina Bolcun-Filas	Guinea pig	IF (1:200)	