1

2

3

4   **ATR is a multifunctional regulator of male mouse meiosis**

5

6   Alexander Widger[1], Shantha K Mahadevaiah[1], Julian Lange[2], Elias Ellnati[1], Jasmin
7   Zohren[1], Takayuki Hirota[1], Marcello Stanzione[3], Obah Ojarikre[1], Valdone Maciulyte[1],
8   Dirk de Rooij[4], Attila Tóth[3], Scott Keeney[2] and James MA Turner[1]*

9

10

11   Affiliations:

12

13   [1] Sex Chromosome Biology Lab, the Francis Crick Institute, 1 Midland Road,
14   London, NW1 1AT, UK

15   [2] Molecular Biology Program, Howard Hughes Medical Institute, Memorial Sloan
16   Kettering Cancer Center, New York, New York 10065, USA

17   [3] Institute of Physiological Chemistry, Faculty of Medicine at the TU Dresden,
18   Fetscherstraße 74, 01307 Dresden, Germany

19   [4] Center for Reproductive Medicine, Academic Medical Center, University of
20   Amsterdam, Amsterdam 1105 AZ, The Netherlands

21

22   *Corresponding author: james.turner@crick.ac.uk

23

24

25

Meiotic cells undergo genetic exchange between homologous chromosomes through programmed DNA double-strand break (DSB) formation, recombination and synapsis[1, 2]. In mice, the DNA damage-regulated phosphatidylinositol-3-kinase-like kinase (PIKK) ATM regulates all of these processes[3-6]. However, the meiotic functions of another major PIKK, ATR, have remained elusive, because germ line-specific depletion of this kinase is challenging. Using an efficient conditional strategy, we uncover roles for ATR in male mouse prophase I progression. Deletion of ATR causes chromosome axis fragmentation and germ cell elimination at mid pachynema. ATR is required for homologous synapsis, in a manner genetically dissociable from DSB formation. In addition, ATR regulates loading of recombinases RAD51 and DMC1 to DSBs and maintenance of recombination foci on synapsed and asynapsed chromosomes. Mid pachytene spermatocyte elimination in ATR deficient mice cannot be rescued by deletion of ATM and the third DNA damage-regulated PIKK, PRKDC, consistent with the existence of a PIKK-independent surveillance mechanism in the mammalian germ line. Our studies identify ATR as a multifunctional regulator of mouse meiosis.

ATR is a serine-threonine kinase with ubiquitous functions in somatic genome stability and checkpoint control[7]. Studies on non-mammalian model organisms have revealed that ATR is also essential for meiosis. ATR orthologues regulate meiotic DSB resection[8], stoichiometry of DSB-associated strand-exchange proteins RAD51 and DMC1[9], inter-homologue bias[10, 11] and crossover formation[12]. They are also components of prophase I checkpoints that ensure centromere pairing[13], timely repair of recombination intermediates[14, 15], and correct coupling of DNA replication

2

51 with DSB induction[16, 17]. Determining the functions of ATR in mouse meiosis has

52 been hampered by the fact that ablation of this protein causes embryonic lethality[18,

53 19]. An inducible *Cre-ERT2* approach recently revealed that ATR regulates meiotic

54 sex chromosome inactivation (MSCI), the silencing of the X and Y chromosomes in

55 male meiosis, via serine-139 H2AFX phosphorylation (γH2AFX)[20]. However, this

56 method resulted in partial rather than complete ATR depletion. We therefore sought

57 a superior conditional strategy for dissecting additional meiotic ATR functions.

58

59 For this purpose, we generated male mice carrying one *Atr* floxed (*Atr^fl^*) allele, in

60 which the exon 44 kinase domain of *Atr* is flanked by *loxP* sites[21], and one *Atr* null

61 (*Atr^−^*) allele, in which the first three coding exons of *Atr* are replaced by a neomycin

62 selection cassette[19]. The resulting *Atr^fl/−^* males also carried a transgene expressing

63 *Cre* recombinase under the control of either a *Stra8* or *Ngn3* promoter fragment.

64 *Stra8-Cre* is expressed from P3 (postnatal day 3)[22], while *Ngn3-Cre* is expressed

65 from P7[23, 24]. Testis weights at P30 were reduced three to fourfold in *Atr^fl/−^ Stra8-Cre*

66 males and *Atr^fl/−^ Ngn3-Cre* males relative to *Atr^fl/+^ Cre* carrying controls, while body

67 weights were unaffected (Fig. 1a). Western blotting showed that ATR protein was

68 reduced in *Atr^fl/−^ Stra8-Cre* testes, and even more so in *Atr^fl/−^ Ngn3-Cre* testes (Fig.

69 1b). This finding supports previous evidence that the majority of testis ATR

70 expression occurs in spermatocytes[20, 25]. Testis histology revealed germ cell failure

71 at seminiferous tubule stage IV, corresponding to mid pachynema of meiosis, in both

72 *Cre* models (Fig. 1c), reminiscent of findings in *Atr^fl/−^ Cre-ERT2* mice[20]. However, the

73 stage IV elimination was clearly less robust in *Atr^fl/−^ Stra8-Cre* than *Atr^fl/−^ Ngn3-Cre*

74 males, because in the former model elongating spermatids were observed in some

3

75   testis sections (Fig. 1c inset). We therefore focused on $Atr^{fl/-}$ $Ngn3-Cre$ mice

76   (hereafter $Atr^{fl/-}$), with $Atr^{fl/+}$ $Ngn3-Cre$ (hereafter $Atr^{fl/+}$) serving as controls.

77

78   Combined immunofluorescence for ATR and the axial element protein SYCP3[26]

79   confirmed that the characteristic ATR staining pattern observed in control leptotene,

80   zygotene and pachytene spermatocytes (Fig. 1d) was absent in $Atr^{fl/-}$ males (Fig.

81   1e). Furthermore, MSCI, assayed at early pachynema by acquisition of γH2AFX on

82   the XY bivalent and RNA FISH for expression of the X chromosome gene $Scml2$,

83   was present in control males (Fig. 1f) but abolished in $Atr^{fl/-}$ males (Fig. 1g). Thus, by

84   multiple criteria, $Atr^{fl/-}$ males exhibited efficient ATR depletion.

85

86   At stage IV, when wild type spermatocytes reach mid pachynema, $Atr^{fl/-}$

87   spermatocytes contained highly fragmented chromosome axes and nucleus-wide

88   γH2AFX staining (Supplementary Fig. 1a; see Methods for meiotic staging criteria

89   used throughout this manuscript). These cells were readily distinguishable from $Atr^{fl/-}$

90   cells at leptonema, in which axial elements were shorter and uniform in length, and

91   γH2AFX staining across the nucleus was more heterogeneous (Supplementary Fig.

92   1b). Mid pachytene axis fragmentation and nucleus-wide γH2AFX staining were also

93   noted in $Atm^{-/-}$ males (Supplementary Fig. 1c), as described previously[27]. However,

94   neither phenotype was observed in $Spo11^{-/-}$, $Dmc1^{-/-}$ and $Msh5^{-/-}$ males

95   (Supplementary Fig. 1d-f), which display stage IV arrest. Instead, γH2AFX in $Spo11^{-}$

96    $^{/-}$ spermatocytes was restricted to the transcriptionally inactive pseudosex body

97   (Supplementary Fig. 1d), while in $Dmc1^{-/-}$ and $Msh5^{-/-}$ spermatocytes it formed axis-

98   associated clouds (Supplementary Fig. 1e,f), consistent with published reports[27-29].

99   These findings suggest that mid pachytene axis degeneration and nucleus-wide

4

100   γH2AFX staining are features of ATR and ATM deletion, and not merely a

101   consequence of stage IV germ cell death.

102   We then used *Atr*$^{fl/−}$ males to address whether ATR regulates homologous synapsis.

103   We performed immunostaining for SYCP3 and the asynapsis marker HORMAD2,

104   focusing on *Atr*$^{fl/−}$ cells at early pachynema, i.e. prior to extensive axial element

105   fragmentation (Supplementary Fig. 1a). Synapsis was normal in 86% (n=104) of

106   early pachytene cells from control males. As expected, HORMAD2 was absent on

107   the autosomal bivalents and present on the non-homologous, asynapsed regions of

108   the X and Y chromosome in these instances (Fig. 2a). Furthermore, in these cells,

109   non-homologous synapsis between the sex chromosomes and the autosomes was

110   not observed. However, in *Atr*$^{fl/−}$ males, only 20% (n=107) of early pachytene cells

111   achieved complete homologous synapsis. The remaining cells exhibited varying

112   degrees of asynapsis affecting the XY pair and the autosomes (Fig. 2b; see below

113   for details). In addition, non-homologous synapsis between the sex chromosomes

114   and autosomes occurred in 25% of *Atr*$^{fl/−}$ cells (Fig. 2b). In mice, the XY

115   pseudoautosomal regions (PARs) undergo late synapsis and DSB formation[30], and

116   in yeast the homologue bias of late-forming DSBs is partially dependent on ATR[8].

117   We therefore determined whether asynapsis in *Atr*$^{fl/−}$ males more commonly affects

118   the sex chromosomes than the autosomes. To identify the sex chromosomes, we

119   performed DNA FISH using probes for the X chromosome (*Slx* probe) and Y

120   chromosome (*Sly* probe; Fig. 2a,b; insets). In *Atr*$^{fl/−}$ males 77% of early pachytene

121   cells exhibited XY asynapsis, while 59% exhibited autosomal asynapsis (see legend

122   for further details). In control males, 13% of early pachytene cells exhibited XY

123   asynapsis and 13% autosomal asynapsis. Thus ATR deletion has a more deleterious

124   effect on XY than on autosomal synapsis.

5

125

126   Asynapsis can result from defects in synaptonemal complex (SC) formation or

127   recombination. To address whether ATR can promote SC formation independent of

128   recombination, we used the *Spo11* null mutation, which permits genetic dissociation

129   of synapsis from recombination initiation[31]. *Spo11*$^{-/-}$ males do not form programmed

130   DSBs, yet achieve extensive SC formation between non-homologues[32, 33]. If deletion

131   of ATR impeded SC formation in *Spo11* nulls, then ATR must have a role in SC

132   formation. We therefore compared synapsis between *Spo11*$^{-/-}$ and *Atr*$^{fl/-}$ *Spo11*$^{-/-}$

133   males. Like *Spo11*$^{-/-}$ males, *Atr*$^{fl/-}$ *Spo11*$^{-/-}$ males exhibited stage IV germ cell

134   elimination (Supplementary Fig. 2a,b). We classified SC formation, assessed using

135   SYCP3 and the SC central element component SYCE2[34], into three classes: i)

136   complete SC, encompassing the entire axis length, ii) partial SC, extending along

137   only part of an axis, and iii) focal SC (Fig. 2c-e). Relative to *Spo11*$^{-/-}$ males (Fig. 2c),

138   *Atr*$^{fl/-}$ *Spo11*$^{-/-}$ males (Fig. 2d) exhibited a decrease in complete and partial SC

139   formation, and an increase in focal SC (Fig. 2c-e). These findings suggest that ATR

140   promotes conversion of SC foci into longer SC stretches. Using *Atr*$^{fl/-}$ *Spo11*$^{-/-}$

141   males, we were also able to demonstrate that formation of the pseudosex body in

142   *Spo11*$^{-/-}$ males is ATR-dependent (Supplementary Fig. 2c,d).

143

144   Our data suggested that ATR can promote synapsis independent of recombination.

145   To strengthen these findings, we devised an additional method for higher throughput

146   quantitation of synapsis across multiple genotypes. We triple immunostained cells for

147   SYCP3, SYCE2 and centromeres, and counted the number of centromeres that had

148   achieved synapsis, i.e. that colocalised with SYCE2 signals (centromere-SYCE2, or

149   CS number; Fig. 2f-i). The mean CS number was reduced in *Atr*$^{fl/-}$ relative to control

6

150   males, confirming that ATR is required for normal levels of synapsis (Fig. 2f,i). The

151   mean CS number was also lower in $Spo11^{-/-}$ than in $Atr^{fl/-}$ males, demonstrating that

152   synapsis is more severely impaired by loss of SPO11 than loss of ATR (Fig. 2f,g,i).

153   In $Atr^{fl/-}$ $Spo11^{-/-}$ males, the mean CS number was reduced relative to that in $Spo11^{-}$

154   $^{/-}$ males (Fig. 2g,h,i). Thus, ATR promotes SC formation both in the presence and

155   absence of SPO11-generated DSBs.

156

157   Next, we examined roles of ATR in DSB formation. Orthologues of ATM influence

158   DSB homeostasis by acting as negative regulators of DSB induction[3, 35-38]. In

159   *Saccharomyces cerevisiae* ATR promotes DSB formation indirectly by increasing the

160   length of prophase I[39], but its impact on DSB levels in mammals is unknown. To

161   address this question, we measured abundance of covalent SPO11-oligonucleotide

162   (SPO11 oligo) complexes, by-products of DSB induction, in testis extracts[40].

163   Consistent with previous work[3], in $Atm^{-/-}$ testes SPO11-oligo complex levels were

164   elevated (Fig. 3a,b) and SPO11 oligos were shifted to a size larger than those

165   observed in wild type mice (Fig. 3c,d). However SPO11-oligo levels and size

166   distribution in $Atr^{fl/-}$ testes were not detectably changed relative to controls (Fig. 3a-

167   d). Thus, ATR and ATM have distinct functions with respect to DSB formation.

168

169   During leptonema, resected DSBs are coated with RPA and the recombinases

170   RAD51 and DMC1, which carry out strand invasion and recombinational repair. We

171   used immunostaining to determine whether these early recombination components

172   are influenced by ATR. Leptotene focus counts for RPA subunit 2 (hereafter termed

173   RPA) were similar between $Atr^{fl/-}$ and controls (Fig. 3e-g). This finding supports

174   conclusions from SPO11-oligo complex quantitation that ATR does not influence

7

175   DSB abundance (Fig. 3a,b), and is consistent with RPA acting upstream of ATR[41].

176   Importantly though, in $Atr^{fl/-}$ males, RAD51 counts were reduced by almost half

177   relative to controls (Fig. 3h-j). DMC1 counts were lower by a similar magnitude (Fig.

178   3k-m). Thus, in $Atr^{fl/-}$ males, DSB abundance appears grossly unaffected, but

179   recombinase localization is compromised.

180

181   Next, we quantitated RPA, RAD51 and DMC1 foci during later prophase I.

182   Interestingly, while RPA counts in $Atr^{fl/-}$ males were equivalent to those in controls at

183   late leptonema (Fig. 3e-g), they were reduced at mid zygonema (Fig. 4a-c). RAD51

184   and DMC1 counts were also lower in $Atr^{fl/-}$ than control males at this stage (Fig. 4c;

185   Supplementary Fig. 3a-d). At early pachynema, DSB markers were assayed both on

186   the autosomes and on the X chromosome, focusing initially on cells with normal

187   autosomal synapsis. Relative to control males, in $Atr^{fl/-}$ males RPA, RAD51 and

188   DMC1 counts were reduced on the autosomes (Fig. 4d-f; Supplementary Fig. 3e-h)

189   and on the X chromosome (Fig. 4g,h,j; Supplementary Fig. 3i-l). The recombination

190   defect in $Atr^{fl/-}$ males therefore affects RAD51 and DMC1 at leptonema, and all three

191   DSB markers at mid zygonema and early pachynema.

192

193   In mice, synapsis is dependent upon recombination. Asynapsis in $Atr^{fl/-}$ males could

194   therefore result not only from SC defects (Fig. 2), but also from aberrant

195   recombination. We therefore asked whether early pachytene $Atr^{fl/-}$ spermatocytes

196   with autosomal asynapsis exhibit alterations in DSB marker counts relative to $Atr^{fl/-}$

197   spermatocytes with normal autosomal synapsis. We used RPA as a representative

198   DSB marker, and determined counts on the X chromosome as an indication of

199   recombination intermediate levels. Since it can be obscured by asynapsed

8

200 autosomes, the X chromosome was identified using *Slx* DNA FISH. In *Atr*$^{fl/-}$ cells

201 with autosomal asynapsis (Fig. 4i) X chromosome RPA counts were lower than

202 those in *Atr*$^{fl/-}$ cells exhibiting normal autosomal synapsis (Fig. 4h,j). Thus, greater

203 alterations in recombination intermediates correlate with the asynapsis phenotype in

204 *Atr*$^{fl/-}$ males.

205

206 We then investigated mechanisms driving mid pachytene elimination of *Atr*$^{fl/-}$

207 spermatocytes. In male mice, defective recombination causes germ cell loss via a

208 mechanism promoted by ATM[42]. Since *Atr*$^{fl/-}$ males exhibited a recombination defect

209 (Fig. 3,4), deletion of *Atm* in these mice might be expected to rescue their germ cell

210 loss phenotype. However, *Atr*$^{fl/-}$ males also exhibit defective MSCI, which can

211 independently cause stage IV elimination[20, 43]. We therefore predicted that mid

212 pachytene germ cell loss would be preserved in mice doubly deficient for ATR and

213 ATM.

214

215 To distinguish these possibilities, we examined testis histology in *Atr*$^{fl/-}$ *Atm*$^{-/-}$

216 mutants. As expected, *Atr*$^{fl/-}$ *Atm*$^{-/-}$ testes exhibited efficient reduction in ATR and

217 ATM protein levels (Fig. 5a,b). Leptotene and zygotene H2AFX phosphorylation,

218 which are catalyzed by ATM and ATR, respectively[27, 28, 44, 45], were also absent in

219 *Atr*$^{fl/-}$ *Atm*$^{-/-}$ males, providing additional evidence that both PIKKs had been

220 efficiently depleted in these double mutants (Fig. 5c, left and middle panel).

221 Consistent with our prediction, stage IV elimination was preserved in *Atr*$^{fl/-}$ *Atm*$^{-/-}$

222 testes (Fig. 5d). Furthermore, the mid pachytene nucleus-wide γH2AFX staining

223 observed in *Atr*$^{fl/-}$ males (Supplementary Fig. 1a) and *Atm*$^{-/-}$ males (Supplementary

224 Fig. 1c) was also seen in *Atr*$^{fl/-}$ *Atm*$^{-/-}$ males (Fig. 5c, right panel).

225

226   Our findings were consistent with a checkpoint-independent mechanism, most likely

227 defective MSCI, driving germ cell loss in $Atr^{fl/-}$ $Atm^{-/-}$ males. However, the remaining

228 DNA damage-regulated PIKK, *Prkdc*, was still present in these mutants, and could

229 contribute a checkpoint function in the absence of ATR or ATM. We therefore

230 examined germ cell progression in males deficient in all three DNA damage-

231 regulated PIKKs. For this experiment we used the *Prkdc scid* mutation[46] and an *Atm*

232 *flox* allele[47], because combined homozygosity for *Prkdc scid* and the *Atm* null

233 mutation causes embryonic lethality[48]. In the resulting $Atr^{fl/-}$ $Atm^{fl/-}$ $Prkdc^{scid/scid}$

234 males, testis ATM and ATR levels were depleted (Fig. 5a,b), and consequently ATM

235 and ATR-dependent γH2AFX staining was absent (Fig. 5e, left and middle panels).

236 Interestingly, mid pachytene nucleus-wide γH2AFX staining was also abolished (Fig.

237 5e, right panel). Thus, PRKDC mediates pachytene serine-139 H2AFX

238 phosphorylation in $Atr^{fl/-}$ $Atm^{fl/-}$ males. Nevertheless, in $Atr^{fl/-}$ $Atm^{fl/-}$ $Prkdc^{scid/scid}$

239 testes stage IV germ cell loss still occurred (Fig. 5f). Thus, mid pachytene elimination

240 persists in mice deficient in all three DNA damage-regulated PIKKs.

241

242   The functions of ATR in mammalian meiosis have been unclear. We show here that

243 ATR is required during unperturbed meiosis to regulate chromosome axis integrity,

244 synapsis and recombination. Notably, as is the case in mitosis[7], in meiosis ATR has

245 roles that are both shared and distinct from ATM. Both ATM and ATR are required

246 for synapsis, but they exert different influences on DSB levels and on recombination

247 focus constituents. Such differences are likely explained by the contrasting substrate

248 specificities[7] and meiotic expression profiles[28] of these kinases.

249

10

250 Our findings show that ATR can promote synapsis independently of meiotic DSB

251 formation, presumably through modification of SC proteins. Among the many SC

252 components, HORMAD1/2 and SMC3 are established ATR phosphortargets[20, 49, 50].

253 While additional SC candidates no doubt exist[51, 52], HORMAD1 is of particular

254 interest, because this protein can also promote synapsis in the absence of

255 recombination[31]. We find that in wild type males, asynapsis of the XY pair occurs at

256 similar frequency to that of all autosomal pairs combined. This bias towards XY

257 asynapsis may be attributable to the small length and terminal location of the PAR.

258 Notably however, in *Atr* mutants, the bias is exaggerated, such that asynapsis of the

259 XY bivalent occurs at higher frequency than that of all autosomes combined. The

260 PAR is unusual, undergoing late DSB formation and synapsis[30], and being enriched

261 for repressive chromatin marks not observed at the termini of autosomal bivalents[53,

262 54]. We suggest that ATR promotes one or more of these unique properties to ensure

263 successful XY interactions.

264

265 At leptonema in ATR-deficient males, RAD51 and DMC1 counts are reduced, while

266 RPA counts are unaffected. This finding could indicate a role for ATR in

267 RAD51/DMC1 loading. It has been suggested that ATR phosphorylates RAD51[55].

268 ATR also phosphorylates CHK1 during meiosis[56], and CHK1 in turn promotes

269 RAD51 loading at DSBs[57]. Subsequently, at zygonema and pachynema,

270 spermatocytes lacking ATR exhibit lower counts not only for RAD51/DMC1 but also

271 for RPA. This reduction in all DSB markers could imply additional functions for ATR

272 in later processing of recombination intermediates. Alternatively, it may indicate the

273 presence of fewer unrepaired DSBs. Reduced DSB number could, in turn, result

274 from premature repair using the sister chromatid[11], or from a failure to induce new

275   DSBs on asynapsed chromosomes[58]. Interestingly, the ATR substrates HORMAD1/2

276   are implicated both in inhibiting sister chromatid recombination[59, 60] and in promoting

277   ongoing DSB formation[59, 61]. Our data are less consistent with a defect in generating

278   new DSBs on asynapsed chromosomes, because SPO11-oligo data showed no

279   reduction in DSB formation in ATR mutants. Furthermore, in ATR mutants decreased

280   RPA counts were also observed on synapsed autosomes, and the magnitude of the

281   decrease was similar to that seen for the asynapsed X chromosome. Whether ATR

282   functions in later stages of recombination, e.g. crossover formation, is unclear, since

283   late recombination markers appear after the point of arrest in $Atr^{fl/-}$ germ cells

284   (Supplementary Fig. 4).

285

286   An interesting finding is that mid pachytene elimination persists in mice deficient in

287   all three DNA damage-regulated PIKKs. Multiple lines of evidence suggest that PIKK

288   depletion in these mice is efficient; nevertheless the use of conditional alleles means

289   that residual PIKK activity may be present and sufficient for checkpoint maintenance.

290   Setting aside this caveat, our findings do not exclude a contribution of PIKKs to mid

291   pachytene elimination in synapsis and recombination mutants. However, they do

292   confirm the existence of additional mechanisms that can trigger elimination. Under

293   such circumstances, mid pachytene failure is likely caused by defective MSCI[43]. We

294   suggest that the coexistence of multiple overlapping surveillance mechanisms during

295   prophase I in males explains why checkpoint responses are more robust than those

296   in females[62, 63]. Further insight into this important sexual dimorphism will require the

297   application of alternative conditional strategies for PIKK deletion in the female mouse

298   germ line.

299 **Figure Legends**

300

301 **Figure 1: A conditional strategy for efficient depletion of ATR during male**

302 **mouse meiosis.** P30 testis and body weights (**a**), testis western blots (**b**), and

303 Periodic Acid-Schiff and haemotoxylin / eosin stained stage IV testis sections (**c**) in

304 *Atr$^{fl/+}$ Stra8-Cre* males (n = 9 males), *Atr$^{fl/-}$ Stra8-Cre* males (n = 10 males), *Atr$^{fl/+}$*

305 *Ngn3-Cre* males (n = 13 males) and *Atr$^{fl/-}$ Ngn3-Cre* males (n = 13 males; means

306 and p values for **a** indicated; unpaired t test). Inset in (**c**) shows presence of

307 elongating spermatids in some tubules from *Atr$^{fl/-}$ Stra8-Cre* males. (**d**,**e**) ATR

308 (magenta) and SYCP3 (green) staining in *Atr$^{fl/+}$ Ngn3-Cre* (denoted *Atr$^{fl/+}$*) and *Atr$^{fl/-}$*

309 *Ngn3-Cre* (denoted *Atr$^{fl/-}$*) males. In *Atr$^{fl/+}$* males (n = 2 males) ATR is observed as

310 foci (see insets) in 85% of leptotene cells (n = 20 cells) and 82% of zygotene cells (n

311 = 28 cells), and on the asynapsed region of the XY bivalent in 100% of pachytene

312 cells (n = 30 cells). In *Atr$^{fl/-}$* males (n = 2 males) ATR is observed in no cells at these

313 three stages (n = 21, 30 and 32 cells at leptonema, zygonema and pachynema,

314 respectively). (**f**) Validation of ATR depletion in *Atr$^{fl/-}$* by analysis of MSCI. In *Atr$^{fl/+}$*

315 males, all pachytene cells show coating of the X and Y chromosome (arrowheads)

316 but not the pseuodautosomal regions (PAR, arrowhead) by γH2AFX (magenta; left

317 panels; XY bivalent in box magnified in smaller panels). This coating causes

318 silencing of the X chromosome gene *Scml2* (magenta; right panels) and

319 compartmentalization of the XY bivalent (labeled with HORMAD2; green) in the sex

320 body (n = one male, 29 cells; magnified in smaller panels). (**g**) In *Atr$^{fl/-}$* males, XY

321 chromosome γH2AFX coating and sex body compartmentalization do not occur. As

322 a result, *Scml2* expression (arrow) persists in all early pachytene cells (n = one male;

323 30 cells). Scale bar in (**c**) 20 μm, other scale bars 10 μm.

13

324

325 **Figure 2: ATR is required for homologous synapsis.** Examples of early

326 pachytene synaptic outcomes in (**a**) $Atr^{fl/+}$ males (n = 3 males) and (**b**) $Atr^{fl/-}$ males (n

327 = 3 males) assessed using HORMAD2 (magenta), SYCP3 (green) and subsequent

328 DNA FISH using *Slx* and *Sly* probes (labeled in insets as X chromosome in green

329 and Y chromosome in white). The *Slx* probe hybridizes to a sub-region of the X

330 chromosome (circled), while the *Sly* probe coats the majority of the Y chromosome.

331 In $Atr^{fl/+}$ males (**a**) both the autosomes and the XY PARs are synapsed, while the

332 non-homologous regions of the XY pair are asynapsed. In $Atr^{fl/+}$ males (n=104 cells,

333 2 males), 90 cells had normal synapsis, 12 cells had asynapsis of both the XY and

334 autosomes, 1 cell had asynapsis only of the XY and 1 cell had asynapsis only of the

335 autosomes. (**b**) Three examples of synaptic defects in $Atr^{fl/-}$ males, each described

336 above respective image. In $Atr^{fl/-}$ males (n=107 cells, 2 males), 21 cells had normal

337 synapsis, 59 cells had asynapsis of both the XY and autosomes, 23 cells had

338 asynapsis only of the XY and 4 cells had asynapsis only of the autosomes. (**c**,**d**)

339 Comparison of pachytene synaptic outcomes in (**c**) $Spo11^{-/-}$ and (**d**) $Atr^{fl/-}$ $Spo11^{-/-}$

340 males using immunostaining for SYCE2 (magenta) and SYCP3 (green). (**e**)

341 Quantitation of complete, partial and focal SC in $Spo11^{-/-}$ males (n = 2 males; 88

342 cells) and $Atr^{fl/-}$ $Spo11^{-/-}$ males (n = 2 males; 96 cells). Means and p values

343 (unpaired t test) indicated. (**f**-**h**) Epistasis analysis of SPO11 and ATR in synapsis

344 using the same markers plus immunostaining for centromeres (CENT; white) to

345 determine CS number. For each cell, colocalizing SYCE2-CENT signals are

346 indicated with dashed circles, and resulting CS numbers are shown. (**i**) CS number

347 in $Atr^{fl/+}$ males (n = 2 males; 55 cells), $Atr^{fl/-}$ males (n = 2 males; 37 cells), $Spo11^{-/-}$

14

348   males (n = 2 males; 114 cells) and *Atr*$^{fl/-}$ *Spo11*$^{-/-}$ males (n = 2 males; 64 cells).

349   Mean values and p values (Mann-Whitney test) indicated. Scale bars 10 μm.

350

351   **Figure 3: ATR ablation does not alter DSB levels but leads to reduction in**

352   **leptotene recombinase counts.** (**a-d**) Analysis of SPO11-oligo complexes in P13

353   testes. SPO11 was immunoprecipitated from whole-testis extracts and SPO11-

354   associated oligos were end-labeled with terminal deoxynucleotidyl transferase and

355   [α-^{32}P] dCTP, then either separated on SDS-PAGE gels followed by autoradiography

356   (**a**) and western blotting with anti-SPO11 antibody (**b**) or digested with proteinase K

357   and resolved on denaturing polyacrylamide sequencing gels (**c**; background-

358   subtracted lane traces in **d**). A representative experiment is shown. An additional

359   *Atr*$^{fl/+}$ *Cre* – control is shown to demonstrate that the *Ngn3-Cre* transgene does not

360   influence SPO11-oligo levels. In (**a**) and (**b**), the bar indicates SPO11-oligo

361   complexes, arrowhead indicates the immunoglobulin heavy chain, and asterisk

362   marks non-specific labelling; ND = not determined. Each panel shows lanes from the

363   same exposure of a single western blot or autoradiograph, with intervening lanes

364   omitted. For quantitation, SPO11-oligo complex signals were background-subtracted

365   and normalized to *Atr fl/+ Cre* – (n = 2) controls. SPO11-oligo quantitation: *Atr*$^{fl/+}$

366   (1.05 ± 0.07-fold, mean and s.d., n = 2 males), *Atr*$^{fl/-}$ (1.17 ± 0.22-fold, n = 4 males)

367   and *Atm*$^{-/-}$ (14.58 ± 5.24-fold, n = 3 males). (**e-m**) Analysis of leptotene focus counts

368   using SYCP3 (green) and early recombination markers (magenta): RPA (**e-g**),

369   RAD51 (**h-j**) and DMC1 (**k-m**) in *Atr*$^{fl/+}$ males (n = 2 males; 27 cells for RPA, 19 cells

370   for RAD51, 14 cells for DMC1) and *Atr*$^{fl/-}$ males (n = 2 males; 21 cells for RPA, 19

371   cells for RAD51, 13 cells for DMC1). Mean and p values (Mann-Whitney test)

372   indicated. Scale bars 10 μm.

15

373

374 **Figure 4: ATR regulates DSB marker counts during zygonema and pachynema.**

375 (**a**,**b**) RPA (magenta) and SYCP3 (green) immunostaining in $Atr^{fl/+}$ and $Atr^{fl/-}$ males.

376 (**c**) RPA, RAD51 and DMC1 counts at mid zygonema in $Atr^{fl/+}$ males (n = 2 males; 16

377 cells for RPA, 15 cells for RAD51, 15 cells for DMC1) and $Atr^{fl/-}$ males (n = 2 males;

378 16 cells for RPA, 15 cells for RAD51, 15 cells for DMC1). (**d**,**e**) Examples of early

379 pachytene cells from $Atr^{fl/+}$ and $Atr^{fl/-}$ males with normal autosomal synapsis. Two

380 representative autosomes (aut 1 and 2) are boxed in upper panels and magnified in

381 lower panels. (**f**) autosomal RPA, RAD51 and DMC1 counts at early pachynema in

382 $Atr^{fl/+}$ males (n = 2 males; 49 cells for RPA, 19 cells for RAD51, 33 cells for DMC1)

383 and $Atr^{fl/-}$ males (n = 2 males; 43 cells for RPA, 19 cells for RAD51, 35 cells for

384 DMC1). (**g**,**h**) Early pachytene $Atr^{fl/+}$ and $Atr^{fl/-}$ cells with normal autosomal synapsis

385 and the X chromosome (boxed in upper panels) identified by Slx DNA FISH (dashed

386 circles in lower panels). (**i**) Early pachytene $Atr^{fl/-}$ cell with autosomal asynapsis. (**j**) X

387 chromosome RPA, RAD51 and DMC1 counts at early pachynema in $Atr^{fl/+}$ males (n

388 = 2 males; 64 cells for RPA, 49 cells for RAD51, 59 cells for DMC1) and $Atr^{fl/-}$ males

389 (n = 2 males; 62 cells for RPA in autosomal synapsis category, 35 cells for RPA in

390 autosomal asynapsis category, 36 cells for RAD51, 53 cells for DMC1). Mean and p

391 values (Mann-Whitney test) indicated. Scale bars 10 μm.

392

393 **Figure 5: Mid pachytene germ cell elimination is preserved in mice deficient in**

394 **the PIKKs.** Western blot showing (**a**) ATR and (**b**) ATM depletion in mice with

395 different PIKK mutations. (**c**) γH2AFX (magenta) and SYCP3 (green)

396 immunostaining (n = 2 males, 25 cells for each stage) and (**d**) stage IV elimination in

397 $Atr^{fl/-}$ $Atm^{-/-}$ males. (**e**) γH2AFX and SYCP3 immunostaining (n = 2 males, 25 cells

398   for each stage) and (**f**) stage IV elimination in $Atr^{fl/-} Atm^{fl/-} Prkdc^{scid/scid}$ males. Scale

399   bar 20 μm in (**d,f**) and 10 μm in (**c,e**).

400

401   **Methods**

402

403   **Animal experiments**

404

405   All mice were maintained according to UK Home Office Regulations at the National

406   Institute for Medical Research (NIMR) and the Francis Crick Institute Mill Hill

407   laboratory. Genetically modified models are previously published and are maintained

408   on a predominantly C57BL/6 background: $Ngn3$-Cre^{24}, $Stra8$-Cre^{22}, Atr^{19}, Atr $flox^{64}$,

409   Atm^{65}, Atm $flox^{47}$, $Spo11^{33}$, $Dmc1^{66}$. $Prkdc$ $scid/scid$ mice were obtained from the

410   Jackson Labs and are maintained on an NOD background. Littermate controls were

411   used where possible.

412

413   **Immunofluorescence and western blotting**

414

415   Immunofluorescence experiments on surface spread spermatocytes were carried out

416   as described[44] In brief, cells were permeabilized for 10 min in 0.05 % Triton X-100

417   and fixed for one hour min in 2 % formaldehyde, 0.02 % SDS in phosphate-buffered

418   saline (PBS). Slides were rinsed in distilled water, air dried and blocked in PBT

419   (0.15 % BSA, 0.10 % TWEEN-20 in PBS) for one hour. Slides were incubated with

420   the following antibodies in a humid chamber overnight at 37°C: guinea-pig anti-

421   SYCP3 (made in-house) 1:500, rabbit anti-SYCP3 (Abcam ab-15092) 1:100, rabbit

422   anti-ATR (Cell Signalling #2790) 1:50, mouse anti-γH2AFX (Millipore 05-636) 1:100,

423   rabbit anti-HORMAD2 (Tóth lab) 1:100, guinea-pig anti-SYCE2 (gift from Howard

424   Cooke / Ian Adams) 1:800, human anti-centromere (CREST ab gift from Bill

425   Earnshaw) 1:1000, rabbit anti-RPA (Abcam ab-2175) 1:100 anti-rabbit RAD51

426   (Calbiochem PC130) 1:25, goat anti-DMC1 (Santa Cruz sc-8973) 1:25. Western

427   blotting was carried out as described[67].

428

429   **Meiotic staging**

430

431   The presence of asynapsis can make discrimination between zygotene cells and

432   pachytene cells with asynapsis challenging. We used the following criteria: (1) DAPI

433   staining: at zygonema DAPI staining is bright and centromeres are clustered in a few

434   subdomains, while in pachynema DAPI staining is heterogeneous, with euchromatin

435   stained faintly and centromeres stained brightly and forming multiple subdomains.

436   (2) The length and thickness of axial elements / SCs: axial elements are long and

437   thin in zygonema, but shorter and thicker in pachynema. (3) Chromosomal

438   asynchrony: in contrast to zygotene cells, pachytene cells with asynapsis exhibit

439   asynchrony in synapsis between individual bivalents, i.e. the coexistence of

440   completely asynapsed bivalents and multiple fully synapsed bivalents. We

441   determined that $Atr^{fl/-}$ males cells exhibiting chromosome axis fragmentation were at

442   mid pachytene by analysis of SYCP3 staining in testis sections; where stage IV

443   tubules can be readily identified. For comparison of synapsis between $Spo11^{-/-}$

444   males and $Atr^{fl/-}$ $Spo11^{-/-}$ males, pachytene cells were identified by virtue of having

445   fully developed axial elements.

446

447

448 **RNA, DNA FISH and SPO11-oligo analysis**

449

450 FISH was carried out with digoxigenin-labeled probes as previously described[68].

451 CHORI BAC probe, RP24-204O18 (CHORI) was used for *Scml2* RNA FISH, RP23-

452 470D15 for *Slx* DNA FISH, and RP24-502P5 for *Sly* DNA FISH. Analyses of

453 abundance of SPO11-oligo complexes and sizes of SPO11 oligos were performed

454 as described[3, 40].

455

456 **Microscopy**

457

458 Imaging was performed using an Olympus IX70 inverted microscope with a 100-W

459 mercury arc lamp. For chromosome spread and RNA FISH imaging, an Olympus

460 UPlanApo 100x/1.35 NA oil immersion objective was used. For testis section

461 imaging, an Olympus UPlanApo 40x/0.75 NA objective was used. A Deltavision RT

462 computer-assisted Photometrics CoolsnapHQ CCD camera with an ICX285

463 Progressive scan CCD image sensor was utilized for image capture. 8 or 16-bit

464 (512x512 or 1024x1024 pixels) raw images of each channel were captured and later

465 processed using Fiji software.

466

467 **Statistics**

468

469 Statistical calculations were performed using GraphPad Prism 6.0. For comparison

470 of two genotypes, Mann-Whitney test or t-tests were performed.

471

472

Acknowledgements

Author contributions

A.W, O.O, V.M and J.M.A.T performed animal generation and genotyping, A.W, S.K.M, M.S and J.M.A.T performed immunofluorescence, A.W, E.E and V.M performed western blots, A.W and S.K.M performed RNA FISH, T.H performed DNA FISH, J.L and S.K performed SPO11-oligo blots, A.W. and D. de. R performed histology, A.W and J.Z performed data plotting and statistics, A.T supplied HORMAD2 antibody. J.T wrote the manuscript with critical input from A.T, S.K and Turner lab members.

References

1. Baudat, F., Imai, Y. & de Massy, B. Meiotic recombination in mammals: localization and regulation. *Nat Rev Genet* **14**, 794-806 (2013).

2. Zickler, D. & Kleckner, N. Meiotic chromosomes: integrating structure and function. *Annu Rev Genet* **33**, 603-754 (1999).

3. Lange, J. *et al.* ATM controls meiotic double-strand-break formation. *Nature* **479**, 237-240 (2011).

4. Barchi, M. *et al.* ATM promotes the obligate XY crossover and both crossover control and chromosome axis integrity on autosomes. *PLoS Genet* **4**, e1000076 (2008).

5. Xu, Y. *et al.* Targeted disruption of ATM leads to growth retardation, chromosomal fragmentation during meiosis, immune defects, and thymic lymphoma. *Genes Dev* **10**, 2411-2422 (1996).

6. Barlow, C. *et al.* Atm deficiency results in severe meiotic disruption as early as leptonema of prophase I. *Development* **125**, 4007-4017 (1998).

7. Marechal, A. & Zou, L. DNA damage sensing by the ATM and ATR kinases. *Cold Spring Harb Perspect Biol* **5** (2013).

8. Joshi, N., Brown, M.S., Bishop, D.K. & Borner, G.V. Gradual implementation of the meiotic recombination program via checkpoint pathways controlled by global DSB levels. *Mol Cell* **57**, 797-811 (2015).

9. Kurzbauer, M.T., Uanschou, C., Chen, D. & Schlogelhofer, P. The recombinases DMC1 and RAD51 are functionally and spatially separated during meiosis in Arabidopsis. *The Plant cell* **24**, 2058-2070 (2012).

10. Grushcow, J.M. *et al.* Saccharomyces cerevisiae checkpoint genes MEC1, RAD17 and RAD24 are required for normal meiotic recombination partner choice. *Genetics* **153**, 607-620 (1999).

11. Carballo, J.A., Johnson, A.L., Sedgwick, S.G. & Cha, R.S. Phosphorylation of the axial element protein Hop1 by Mec1/Tel1 ensures meiotic interhomolog recombination. *Cell* **132**, 758-770 (2008).

12. Carpenter, A.T. Recombination nodules and synaptonemal complex in recombination-defective females of Drosophila melanogaster. *Chromosoma* **75**, 259-292 (1979).

13. Falk, J.E., Chan, A.C., Hoffmann, E. & Hochwagen, A. A Mec1- and PP4-dependent checkpoint couples centromere pairing to meiotic recombination. *Dev Cell* **19**, 599-611 (2010).

14. Abdu, U., Brodsky, M. & Schupbach, T. Activation of a meiotic checkpoint during Drosophila oogenesis regulates the translation of Gurken through Chk2/Mnk. *Curr Biol* **12**, 1645-1651 (2002).

15. Lydall, D., Nikolsky, Y., Bishop, D.K. & Weinert, T. A meiotic recombination checkpoint controlled by mitotic checkpoint genes. *Nature* **383**, 840-843 (1996).

16. Murakami, H. & Nurse, P. Meiotic DNA replication checkpoint control in fission yeast. *Genes Dev* **13**, 2581-2593 (1999).

17. Blitzblau, H.G. & Hochwagen, A. ATR/Mec1 prevents lethal meiotic recombination initiation on partially replicated chromosomes in budding yeast. *eLife* **2**, e00844 (2013).

543   18.   de Klein, A. *et al.* Targeted disruption of the cell-cycle checkpoint gene ATR leads to
544   early embryonic lethality in mice. *Curr Biol* **10**, 479-482 (2000).
545   19.   Brown, E.J. & Baltimore, D. ATR disruption leads to chromosomal fragmentation and
546   early embryonic lethality. *Genes Dev* **14**, 397-402 (2000).
547   20.   Royo, H. *et al.* ATR acts stage specifically to regulate multiple aspects of mammalian
548   meiotic silencing. *Genes Dev* **27**, 1484-1494 (2013).
549   21.   Brown, E.J. & Baltimore, D. Essential and dispensable roles of ATR in cell cycle arrest
550   and genome maintenance. *Genes Dev* **17**, 615-628 (2003).
551   22.   Sadate-Ngatchou, P.I., Payne, C.J., Dearth, A.T. & Braun, R.E. Cre recombinase
552   activity specific to postnatal, premeiotic male germ cells in transgenic mice. *Genesis*
553   **46**, 738-742 (2008).
554   23.   Zheng, K. & Wang, P.J. Blockade of pachytene piRNA biogenesis reveals a novel
555   requirement for maintaining post-meiotic germline genome integrity. *PLoS Genet* **8**,
556   e1003038 (2012).
557   24.   Schonhoff, S.E., Giel-Moloney, M. & Leiter, A.B. Neurogenin 3-expressing progenitor
558   cells in the gastrointestinal tract differentiate into both endocrine and non-
559   endocrine cell types. *Dev Biol* **270**, 443-454 (2004).
560   25.   Keegan, K.S. *et al.* The Atr and Atm protein kinases associate with different sites
561   along meiotically pairing chromosomes. *Genes Dev* **10**, 2423-2437 (1996).
562   26.   Lammers, J.H. *et al.* The gene encoding a major component of the lateral elements of
563   synaptonemal complexes of the rat is related to X-linked lymphocyte-regulated
564   genes. *Mol Cell Biol* **14**, 1137-1146 (1994).
565   27.   Barchi, M. *et al.* Surveillance of different recombination defects in mouse
566   spermatocytes yields distinct responses despite elimination at an identical
567   developmental stage. *Mol Cell Biol* **25**, 7203-7215 (2005).
568   28.   Bellani, M.A., Romanienko, P.J., Cairatti, D.A. & Camerini-Otero, R.D. SPO11 is
569   required for sex-body formation, and Spo11 heterozygosity rescues the prophase
570   arrest of Atm-/- spermatocytes. *J Cell Sci* **118**, 3233-3245 (2005).
571   29.   Mahadevaiah, S.K. *et al.* Extensive meiotic asynapsis in mice antagonises meiotic
572   silencing of unsynapsed chromatin and consequently disrupts meiotic sex
573   chromosome inactivation. *J Cell Biol* **182**, 263-276 (2008).
574   30.   Kauppi, L. *et al.* Distinct properties of the XY pseudoautosomal region crucial for
575   male meiosis. *Science* **331**, 916-920 (2011).
576   31.   Daniel, K. *et al.* Meiotic homologue alignment and its quality surveillance are
577   controlled by mouse HORMAD1. *Nat Cell Biol* **13**, 599-610 (2011).
578   32.   Romanienko, P.J. & Camerini-Otero, R.D. The mouse Spo11 gene is required for
579   meiotic chromosome synapsis. *Mol Cell* **6**, 975-987 (2000).
580   33.   Baudat, F., Manova, K., Yuen, J.P., Jasin, M. & Keeney, S. Chromosome synapsis
581   defects and sexually dimorphic meiotic progression in mice lacking Spo11. *Mol Cell* **6**,
582   989-998 (2000).
583   34.   Costa, Y. *et al.* Two novel proteins recruited by synaptonemal complex protein 1
584   (SYCP1) are at the centre of meiosis. *J Cell Sci* **118**, 2755-2762 (2005).
585   35.   Joyce, E.F. *et al.* Drosophila ATM and ATR have distinct activities in the regulation of
586   meiotic DNA damage and repair. *J Cell Biol* **195**, 359-367 (2011).
587   36.   Zhang, L., Kim, K.P., Kleckner, N.E. & Storlazzi, A. Meiotic double-strand breaks occur
588   once per pair of (sister) chromatids and, via Mec1/ATR and Tel1/ATM, once per
589   quartet of chromatids. *Proc Natl Acad Sci U S A* **108**, 20036-20041 (2011).
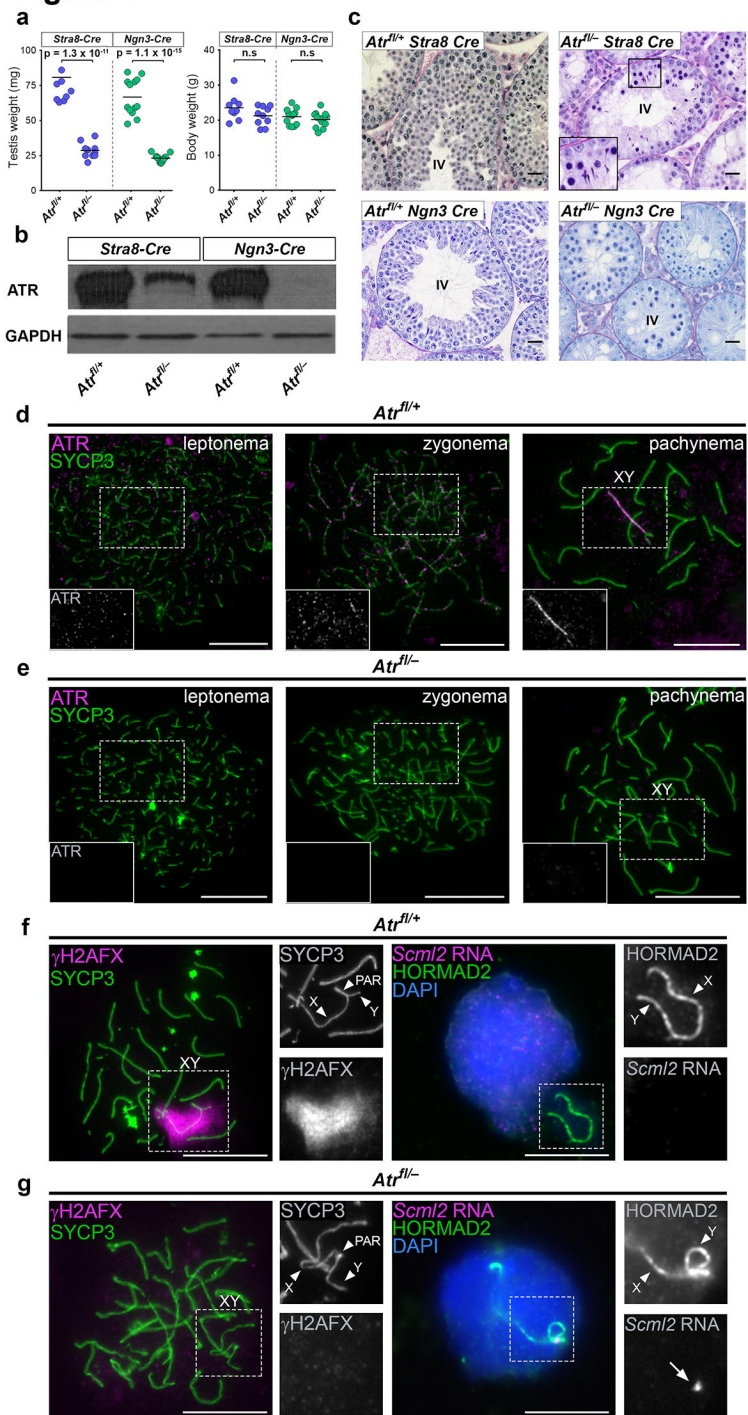
590   37.   Carballo, J.A. *et al.* Budding yeast ATM/ATR control meiotic double-strand break
591   (DSB) levels by down-regulating Rec114, an essential component of the DSB-
592   machinery. *PLoS Genet* **9**, e1003545 (2013).
593   38.   Garcia, V., Gray, S., Allison, R.M., Cooper, T.J. & Neale, M.J. Tel1(ATM)-mediated
594   interference suppresses clustered meiotic double-strand-break formation. *Nature*
595   **520**, 114-118 (2015).
596   39.   Gray, S., Allison, R.M., Garcia, V., Goldman, A.S. & Neale, M.J. Positive regulation of
597   meiotic DNA double-strand break formation by activation of the DNA damage
598   checkpoint kinase Mec1(ATR). *Open biology* **3**, 130019 (2013).
599   40.   Neale, M.J., Pan, J. & Keeney, S. Endonucleolytic processing of covalent protein-
600   linked DNA double-strand breaks. *Nature* **436**, 1053-1057 (2005).
601   41.   Zou, L. & Elledge, S.J. Sensing DNA damage through ATRIP recognition of RPA-ssDNA
602   complexes. *Science* **300**, 1542-1548 (2003).
603   42.   Pacheco, S. *et al.* The ATM signaling cascade promotes recombination-dependent
604   pachytene arrest in mouse spermatocytes. *PLoS Genet* **11**, e1005017 (2015).
605   43.   Royo, H. *et al.* Evidence that meiotic sex chromosome inactivation is essential for
606   male fertility. *Curr Biol* **20**, 2117-2123 (2010).
607   44.   Turner, J.M. *et al.* Silencing of unsynapsed meiotic chromosomes in the mouse. *Nat*
608   *Genet* **37**, 41-47 (2005).
609   45.   Fernandez-Capetillo, O., Liebe, B., Scherthan, H. & Nussenzweig, A. H2AX regulates
610   meiotic telomere clustering. *J Cell Biol* **163**, 15-20 (2003).
611   46.   Araki, R. *et al.* Nonsense mutation at Tyr-4046 in the DNA-dependent protein kinase
612   catalytic subunit of severe combined immune deficiency mice. *Proc Natl Acad Sci U S*
613   *A* **94**, 2438-2443 (1997).
614   47.   Zha, S., Sekiguchi, J., Brush, J.W., Bassing, C.H. & Alt, F.W. Complementary functions
615   of ATM and H2AX in development and suppression of genomic instability. *Proc Natl*
616   *Acad Sci U S A* **105**, 9302-9306 (2008).
617   48.   Gurley, K.E. & Kemp, C.J. Synthetic lethality between mutation in Atm and DNA-
618   PK(cs) during murine embryogenesis. *Curr Biol* **11**, 191-194 (2001).
619   49.   Luo, H. *et al.* Regulation of intra-S phase checkpoint by ionizing radiation (IR)-
620   dependent and IR-independent phosphorylation of SMC3. *J Biol Chem* **283**, 19176-
621   19183 (2008).
622   50.   Fukuda, T. *et al.* Phosphorylation of Chromosome Core Components May Serve as
623   Axis Marks for the Status of Chromosomal Events during Mammalian Meiosis. *PLoS*
624   *Genet* **8**, e1002485 (2012).
625   51.   Traven, A. & Heierhorst, J. SQ/TQ cluster domains: concentrated ATM/ATR kinase
626   phosphorylation site regions in DNA-damage-response proteins. *Bioessays* **27**, 397-
627   407 (2005).
628   52.   Carballo, J.A. & Cha, R.S. Meiotic roles of Mec1, a budding yeast homolog of
629   mammalian ATR/ATM. *Chromosome Res* **15**, 539-550 (2007).
630   53.   Turner, J.M., Burgoyne, P.S. & Singh, P.B. M31 and macroH2A1.2 colocalise at the
631   pseudoautosomal region during mouse meiosis. *J Cell Sci* **114**, 3367-3375 (2001).
632   54.   Peters, A.H. *et al.* Loss of the Suv39h histone methyltransferases impairs mammalian
633   heterochromatin and genome stability. *Cell* **107**, 323-337 (2001).
634   55.   Flott, S. *et al.* Regulation of Rad51 function by phosphorylation. *EMBO Rep* **12**, 833-
635   839 (2011).
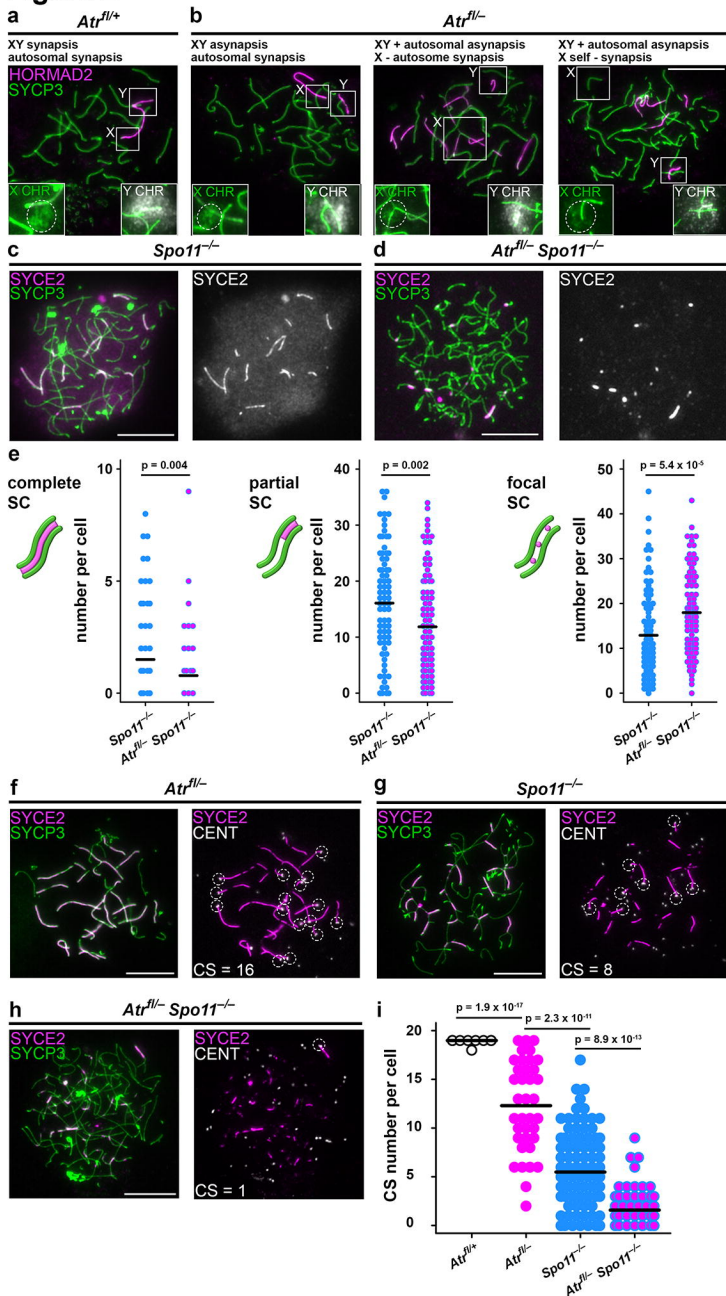
636   56.   Fedoriw, A.M., Menon, D., Kim, Y., Mu, W. & Magnuson, T. Key mediators of somatic
637   ATR signaling localize to unpaired chromosomes in spermatocytes. *Development*
638   **142**, 2972-2980 (2015).
639   57.   Sorensen, C.S. *et al.* The cell-cycle checkpoint kinase Chk1 is required for mammalian
640   homologous recombination repair. *Nat Cell Biol* **7**, 195-201 (2005).
641   58.   Kauppi, L. *et al.* Numerical constraints and feedback control of double-strand breaks
642   in mouse meiosis. *Genes Dev* **27**, 873-886 (2013).
643   59.   Wojtasz, L. *et al.* Mouse HORMAD1 and HORMAD2, two conserved meiotic
644   chromosomal proteins, are depleted from synapsed chromosome axes with the help
645   of TRIP13 AAA-ATPase. *PLoS Genet* **5**, e1000702 (2009).
646   60.   Shin, Y.H., McGuire, M.M. & Rajkovic, A. Mouse HORMAD1 is a meiosis i checkpoint
647   protein that modulates DNA double- strand break repair during female meiosis. *Biol*
648   *Reprod* **89**, 29 (2013).
649   61.   Keeney, S., Lange, J. & Mohibullah, N. Self-organization of meiotic recombination
650   initiation: general principles and molecular pathways. *Annu Rev Genet* **48**, 187-214
651   (2014).
652   62.   Morelli, M.A. & Cohen, P.E. Not all germ cells are created equal: aspects of sexual
653   dimorphism in mammalian meiosis. *Reproduction* **130**, 761-781 (2005).
654   63.   Hunt, P.A. & Hassold, T.J. Sex matters in meiosis. *Science* **296**, 2181-2183 (2002).
655   64.   Ruzankina, Y. *et al.* Deletion of the developmentally essential gene ATR in adult mice
656   leads to age-related phenotypes and stem cell loss. *Cell Stem Cell* **1**, 113-126 (2007).
657   65.   Barlow, C. *et al.* Atm-deficient mice: a paradigm of ataxia telangiectasia. *Cell* **86**, 159-
658   171 (1996).
659   66.   Pittman, D.L. *et al.* Meiotic prophase arrest with failure of chromosome synapsis in
660   mice deficient for Dmc1, a germline-specific RecA homolog. *Mol Cell* **1**, 697-705
661   (1998).
662   67.   Hamer, G., Kal, H.B., Westphal, C.H., Ashley, T. & de Rooij, D.G. Ataxia telangiectasia
663   mutated expression and activation in the testis. *Biol Reprod* **70**, 1206-1212 (2004).
664   68.   Mahadevaiah, S.K., Costa, Y. & Turner, J.M. Using RNA FISH to study gene expression
665   during mammalian meiosis. *Methods Mol Biol* **558**, 433-444 (2009).
666

Figure 1

a

Stra8-Cre	Ngn3-Cre
Testis weight (mg)	

Stra8-Cre: p = 1.3 x 10⁻¹¹ → $p = 1.3 \times 10^{-11}$
Ngn3-Cre: p = 1.1 x 10⁻¹⁵ → $p = 1.1 \times 10^{-15}$

Testis weight (mg): $Atr^{fl/+}$ / $Atr^{fl/-}$ (Stra8-Cre), $Atr^{fl/+}$ / $Atr^{fl/-}$ (Ngn3-Cre)

Body weight (g): n.s / n.s

b Stra8-Cre | Ngn3-Cre

ATR
GAPDH

$Atr^{fl/+}$ $Atr^{fl/-}$ $Atr^{fl/+}$ $Atr^{fl/-}$

c

$Atr^{fl/+}$ Stra8 Cre | $Atr^{fl/-}$ Stra8 Cre
$Atr^{fl/+}$ Ngn3 Cre | $Atr^{fl/-}$ Ngn3 Cre

d $Atr^{fl/+}$
leptonema | zygonema | pachynema
ATR | SYCP3 | XY

e $Atr^{fl/-}$
leptonema | zygonema | pachynema
ATR | SYCP3 | XY

f $Atr^{fl/+}$
γH2AFX | SYCP3 | XY | SYCP3 | PAR | X | Y | γH2AFX
Scml2 RNA | HORMAD2 | DAPI | HORMAD2 | X | Y | Scml2 RNA

g $Atr^{fl/-}$
γH2AFX | SYCP3 | XY | SYCP3 | PAR | X | Y | γH2AFX
Scml2 RNA | HORMAD2 | DAPI | HORMAD2 | Y | X | Scml2 RNA

Figure 2

a *Atr^{fl/+}*
XY synapsis
autosomal synapsis
HORMAD2 (magenta)
SYCP3 (green)
X CHR
Y CHR

b *Atr^{fl/−}*
XY asynapsis
autosomal synapsis
X CHR
Y CHR

XY + autosomal asynapsis
X - autosome synapsis
XY CHR
Y CHR

XY + autosomal asynapsis
X self - synapsis
X CHR
Y CHR

c *Spo11^{−/−}*
SYCE2 (magenta)
SYCP3 (green)
SYCE2

d *Atr^{fl/−} Spo11^{−/−}*
SYCE2 (magenta)
SYCP3 (green)
SYCE2

e

complete SC
number per cell
p = 0.004
Spo11^{−/−}   *Atr^{fl/−} Spo11^{−/−}*

partial SC
number per cell
p = 0.002
Spo11^{−/−}   *Atr^{fl/−} Spo11^{−/−}*

focal SC
number per cell
p = 5.4 x 10^{-5}
Spo11^{−/−}   *Atr^{fl/−} Spo11^{−/−}*

f *Atr^{fl/−}*
SYCE2 (magenta)
SYCP3 (green)
SYCE2
CENT
CS = 16

g *Spo11^{−/−}*
SYCE2 (magenta)
SYCP3 (green)
SYCE2
CENT
CS = 8

h *Atr^{fl/−} Spo11^{−/−}*
SYCE2 (magenta)
SYCP3 (green)
SYCE2
CENT
CS = 1

i
CS number per cell
p = 1.9 x 10^{-17}
p = 2.3 x 10^{-11}
p = 8.9 x 10^{-13}
Atr^{fl/+}   *Atr^{fl/−}*   *Spo11^{−/−}*   *Atr^{fl/−} Spo11^{−/−}*

Figure 3

Figure 4

Figure 5

a

ATR

Tubulin

b

ATM

Tubulin

c $Atr^{fl/-} Atm^{-/-}$

γH2AFX leptonema
SYCP3

γH2AFX zygonema
SYCP3

γH2AFX pachynema
SYCP3

d $Atr^{fl/-} Atm^{-/-}$

IV

e $Atr^{fl/-} Atm^{fl/-} Prkdc^{scid/scid}$

γH2AFX leptonema
SYCP3

γH2AFX zygonema
SYCP3

γH2AFX pachynema
SYCP3

f $Atr^{fl/-} Atm^{fl/-} Prkdc^{scid/scid}$

IV   IV