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DMC1 attenuates RAD51-mediated recombination in Arabidopsis

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Short title: RAD51 and DMC1 in meiotic recombination in Arabidopsis

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Abstract

Ensuring balanced distribution of chromosomes in gametes, meiotic recombination is essential for fertility in most sexually reproducing organisms. The repair of the programmed DNA double strand breaks that initiate meiotic recombination requires two DNA strand-exchange proteins, RAD51 and DMC1, to search for and invade an intact DNA molecule on the homologous chromosome. DMC1 is meiosis-specific, while RAD51 is essential for both mitotic and meiotic homologous recombination. DMC1 is the main catalytically active strand-exchange protein during meiosis, while this activity of RAD51 is downregulated. RAD51 is however an essential cofactor in meiosis, supporting the function of DMC1. This work presents a study of the mechanism(s) involved in this and our results point to DMC1 being, at least, a major actor in the meiotic suppression of the RAD51 strand-exchange activity in plants. Ectopic expression of DMC1 in somatic cells renders plants hypersensitive to DNA damage and specifically impairs RAD51-dependent homologous recombination. DNA damage-induced RAD51 focus formation in somatic cells is not however suppressed by ectopic expression of DMC1. Interestingly, DMC1 also forms damage-induced foci in these cells and we further show that the ability of DMC1 to prevent RAD51-mediated recombination is associated with local assembly of DMC1 at DNA breaks. In support of our hypothesis, expression of a dominant negative DMC1 protein in meiosis impairs RAD51-mediated DSB repair. We propose that DMC1 acts to prevent RAD51-mediated recombination in Arabidopsis and that this down-regulation requires local assembly of DMC1 nucleofilaments.

Author Summary

Essential for fertility and responsible for a major part of genetic variation in sexually reproducing species, meiotic recombination establishes the physical linkages between homologous chromosomes which ensure their balanced segregation in the production of gametes. These linkages, or chiasmata, result from DNA strand exchange catalyzed by the RAD51 and DMC1 recombinases and their numbers and distribution are tightly regulated. Essential for maintaining chromosomal integrity in mitotic cells, the strand-exchange activity of RAD51 is downregulated in meiosis, where it plays a supporting role to the activity of DMC1. Notwithstanding considerable attention from the genetics community, precisely why this is done and the mechanisms involved are far from being fully understood. We show here in the plant Arabidopsis that DMC1 can downregulate RAD51 strand-exchange activity and propose that this may be a general mechanism for suppression of RAD51-mediated recombination in meiosis.

58 ## Introduction

59 To maintain stable ploidy across generations, sexually reproducing eukaryotes must
60 halve the number of chromosomes in gametes with respect to their mother cells. This is
61 accomplished by meiosis, a specialized cellular division in which DNA replication is followed
62 by two sequential rounds of chromosome segregation [1, 2]. Proper chromosome segregation
63 in mitosis, in which a single division follows DNA replication, is ensured by sister-chromatid
64 cohesion established during S-Phase. This however only works once and an additional
65 mechanism is needed in meiosis. Balanced segregation of chromosomes at the first meiotic
66 division relies upon the establishment of physical connections between the two homologous
67 chromosomes. In most species, these connections are realized by reciprocal recombination
68 events called cross-overs (COs). This process also produces novel combinations of alleles
69 from the two chromosomes, recombining the parental genetic contributions in the production
70 of the new generation. Meiotic recombination is thus both essential for fertility and to reshuffle
71 genetic information, with important consequences for genome evolution [1, 2].
72 Meiotic recombination is initiated by the programmed induction of DNA double strand breaks
73 (DSB) by the SPO11 DNA topoisomerase VI-like complex [3-5] in the chromosomes and their
74 repair by homologous recombination [6-11]. The pair of DNA ends formed are resected by
75 nucleases [12], which remove SPO11 and generate long 3' single-stranded DNA overhangs
76 (ssDNA), which are bound by the heterotrimeric protein RPA to protect the ssDNA and prevent
77 formation of secondary structures [13-15]. The specialized RAD51 and DMC1 recombinases
78 are then loaded onto the ssDNA to form right-handed helical nucleoprotein filaments [16, 17].
79 Earlier studies have proposed that recombinase loading occurs asymmetrically, with RAD51
80 and DMC1 making separate homofilaments on opposite ends of a break [18-20]. However,
81 more recent work in budding yeast and mouse suggested that RAD51 and DMC1
82 homofilaments do load on both DSB ends resulting in mixed recombinase filaments [21-23].
83 This mixed recombinase filament organization has been reconstituted *in vitro* [24] and has thus
84 become the favored model for recombinase loading [16, 17, 23-27]. The RAD51/DMC1-DNA

85 nucleoprotein filament is the active molecular species in searching for and invading a
86 homologous DNA template by the 3'-ended DNA strand(s) [16, 17, 26, 27]. This process is the
87 core of the meiotic recombination pathway and results in the formation of a joint recombination
88 intermediate, which can be resolved to yield non-crossover (NCO) or crossover (CO) products
89 [1, 2, 28]. CO between non-sister-chromatids, in combination with sister chromatid cohesion,
90 establish the physical linkages (chiasmata) necessary to ensure proper chromosomal
91 segregation at the first meiotic anaphase [1, 2, 28].

92 Only CO between non-sister chromatids physically link homologous chromosome pairs
93 and the choice of the sister or non-sister chromatid template for repair of meiotic DSB is thus
94 a key determinant for the outcome of recombination. This must be tightly regulated to favor
95 interhomolog recombination for coordinated chromosomal disjunction at the first meiotic
96 anaphase [29]. With a few exceptions, this crucial step of meiotic recombination requires the
97 co-operation of the two strand exchange proteins, RAD51 and DMC1 (reviewed by [16, 17,
98 30]). RAD51 and DMC1 emerged following a gene duplication event that occurred in the
99 common ancestor of all eukaryotes [31]. They retain more than 50% amino acid identity and
100 closely related structural and biochemical properties [16, 26, 32-34]. Both enzymes assemble
101 on ssDNA at sites of breaks and promote break repair by searching for and invading an intact
102 homologous DNA template molecule. However, while RAD51 is essential for both mitotic and
103 meiotic homologous recombination, DMC1 is meiosis-specific [16, 27, 35, 36]. Why meiotic
104 recombination requires two strand-exchange proteins is not well understood, but given the
105 similar activities of the two proteins it has been generally assumed that they play
106 complementary roles in catalyzing meiotic DSB repair - with DMC1 promoting interhomolog
107 recombination (IH) and crossing-over, while RAD51 favors sister-chromatid repair of the DSB
108 [18, 37]. This assumption has however been called into question by the characterization of the
109 yeast *rad51-II3A* [38] and Arabidopsis RAD51-GFP proteins [39]. While they retain the ability
110 to form nucleoprotein filaments at DSB, these proteins are unable to catalyze invasion of the
111 homologous template. They do however fully complement the absence of RAD51 in meiosis

112 [38-41]. It is thus the presence of the RAD51 protein and not its strand-exchange activity that
113 is needed in meiosis.

114 DMC1 is the predominant strand-exchange protein during meiosis, catalyzing most
115 strand-exchange events, while RAD51 acts only to support DMC1 and to repair residual DSBs
116 after IH recombination and synapsis are complete [38, 39, 42-44]. Accordingly, data from
117 budding yeast indicate that RAD51 activity is downregulated during meiosis so that DMC1
118 catalyzes DNA strand-exchange using the homologous chromosome as a template [38, 45-
119 47]. In budding yeast, down-regulation of Rad51 activity is mediated through inhibition of
120 Rad51-Rad54 complex formation by the coordinated phosphorylation of Hed1 and the Rad51
121 accessory factor Rad54 by the meiosis-specific kinase Mek1 [18, 45, 46, 48-51]. Hed1 is a
122 meiosis-specific factor that binds to Rad51, blocking access of Rad54 and thereby restricting
123 activity of the Rad51 nucleofilaments in meiosis [48, 49, 51, 52]. Second, phosphorylation of
124 threonine 132 of Rad54 by Mek1 reduces the affinity of Rad54 for Rad51 [47, 50]. Both
125 mechanisms affect Rad51-Rad54 complex formation and this in turn attenuates the activity of
126 Rad51, presumably to minimize the use of sister chromatid templates and hence favor DMC1-
127 dependent inter-homolog recombination.

128 Apart from budding yeast, the mechanisms suppressing RAD51 activity during meiosis have
129 not been determined. In plants, bioinformatics searches fail to identify homologs of Hed1 and
130 Mek1. Arabidopsis *rad51* mutants are sterile and *dmc1* mutants have severely reduced fertility.
131 They both show defects in pairing and synapsis, but *rad51* and *rad51 dmc1* mutants exhibit
132 extensive SPO11-dependent chromosome fragmentation in meiotic prophase I, while *dmc1*
133 mutants are characterized by the presence of intact univalent chromosomes in meiotic
134 metaphase I [53-55]. Thus, in the absence of DMC1, meiotic DSB are repaired in a RAD51-
135 dependent manner [53, 55-57] which does not generate chiasmata. This has led to the
136 hypothesis that DMC1 could be involved in down-regulation of RAD51 in Arabidopsis [57, 58].
137 Here, we sought further insights into the mechanism by which RAD51 is downregulated during
138 meiosis in plants and our results support the hypothesis that DMC1 ssDNA binding attenuates
139 RAD51 strand invasion activity. Ectopic expression of DMC1 in vegetative cells blocks RAD51-

140 dependent homologous recombination and renders plants hypersensitive to DNA damage.
141 DNA damage-induced RAD51 focus formation in vegetative cells is not however impaired by
142 ectopic expression of DMC1 and DMC1 also forms damage-induced foci in these cells. The
143 blocking of RAD51 function in vegetative cells thus occurs through inhibition of its activity rather
144 than via inhibition of nucleofilament formation, reminiscent of the situation in meiosis. This
145 conclusion is further supported by the observation that expression of a dominant negative
146 DMC1-GFP fusion protein in meiosis impairs RAD51-mediated meiotic DSB repair.
147 Interestingly, expression of a dominant negative, non-nucleofilament-forming, DMC1-II3A
148 variant did not prevent RAD51-mediated recombination in meiosis or in mitosis, concordant
149 with the argument that the ability of DMC1 to prevent RAD51-mediated recombination requires
150 assembly of DMC1 nucleofilaments at DSB sites.
151 Our data thus suggest that DMC1 triggers down-regulation of RAD51 strand-exchange activity
152 and that local assembly of DMC1 nucleofilaments - but not this strand-exchange activity – may
153 be sufficient to mediate this down-regulation in Arabidopsis.
154
155
156

157 # Results

158 **Ectopic expression of DMC1 in vegetative cells**

159 We expressed *DMC1* in vegetative cells, in which RAD51 is normally the only active
160 recombinase, to test the intrinsic ability of DMC1 to downregulate RAD51. The Arabidopsis
161 *DMC1* genomic sequence (*DMC1g*) was cloned and placed under the control of the *RAD51*
162 promoter to drive its expression in somatic cells (Figure 1A). The complementation of the
163 meiotic defects of *dmc1* mutants by this transgene confirms correct expression of functional
164 DMC1 protein (Figure 1B and C). We selected 3 independent *dmc1-/- pRAD51::DMC1g*
165 transgenic lines and show that all were fertile with a number of seeds per silique very similar
166 to that of wild-type plants (Figure 1C and S1 Data). Chromosome spreads of pollen mother
167 cells confirm that the *pRAD51::DMC1g* transgene restores normal meiosis in *dmc1* mutants
168 (S1 Fig). We note that a similar experiment using the *DMC1* coding sequence ($DMC1_{CDS}$)
169 instead of the genomic sequence, did not rescue the fertility of *dmc1* mutants, presumably
170 indicating the importance of an intron sequence and/or correct splicing for proper *DMC1*
171 expression (Figure 1B). Knowing that the chimeric *pRAD51::DMC1g* transgene allows high
172 expression of *DMC1* we then tested whether it could drive expression of *DMC1* in somatic cells
173 and whether or not it showed DNA damage-inducible expression, as does *RAD51.* We
174 generated wild-type plants homozygous for the transgene insertion and performed RT-PCR
175 on RNA from one-week-old seedlings. *RAD51* expression is relatively low in wild-type
176 seedlings grown under standard conditions but its expression is strongly induced in seedlings
177 treated with 20 μM Mitomycin C (MMC) for 8 hours (Figure 1D). In contrast (and in accordance
178 with its meiosis-specific function), *DMC1* expression is not induced after DNA damage
179 treatment in WT plants. As expected, we observed strong induction of *DMC1* expression in all
180 3 independent *pRAD51::DMC1g* transgenic lines treated with 20 μM MMC for 8 hours (Figure
181 1D). We also tested expression of *HOP2*, *MND1* and *RAD54*, which are HR genes involved in
182 the strand invasion process and known to be induced in somatic cells after treatment with DNA
183 damaging agents [59-64]. Expression of all three genes was induced in both the transgenic

184 plants and the wild-type controls (Figure 1D). Similar results were obtained when seedlings
185 were treated with γ-rays (S2 Fig). Together, these data show that the *pRAD51::DMC1g*
186 transgene allows high expression and induction of *DMC1* in both meiosis and mitosis and that
187 this ectopic expression of *DMC1* does not appear to affect the expression of the other HR
188 genes.
189
190 **Ectopic expression of DMC1 in somatic cells blocks RAD51-mediated DSB repair**
191 We next analyzed whether RAD51-mediated DNA repair in somatic cells is affected by the
192 presence of DMC1. We tested sensitivity of transgenic plants expressing *DMC1* in vegetative
193 cells to the DNA damaging agent Mitomycin C (MMC). MMC forms DNA interstrand cross-link
194 adducts, thereby producing DNA strand breaks that must be repaired by homologous
195 recombination. In Arabidopsis, this is seen in the hypersensitivity of *rad51* mutants to MMC
196 [39, 65]. Progeny of wild-type plants and *rad51* heterozygotes (*RAD51+/-*) carrying the
197 *pRAD51::DMC1g* transgene were grown on solid media containing, or not, 20 μM Mitomycin
198 C and growth was scored after 2 weeks (Figure 2 and S3 Fig). We tested the progeny of
199 *RAD51+/-* heterozygous mutant plants as this permits analysis of the effect of vegetative
200 expression of *DMC1* in both presence (*RAD51+/+* and *RAD51+/-*) or absence (*rad51-/-*) of
201 RAD51. Plants grown under standard conditions did not exhibit any visible phenotypical
202 differences indicating that ectopic expression of *DMC1* does not affect normal plant growth
203 (Figure 2A). However, when grown in medium supplemented with MMC, transgenic plants
204 showed strong hypersensitivity (Figure 2B and C), with 87% to 98% sensitive plants in the
205 progeny of 3 independent *RAD51+/-* transformants (named T1-1, T1-2, and T1-3) carrying the
206 *pRAD51::DMC1g* transgene (Figure 2B and C). This is comparable to the full sensitivity of
207 *RAD51-GFP* plants (which behave as *rad51* mutants, [39, 66]) and in strong contrast to the
208 29% sensitive plants (corresponding to the segregating *rad51* homozygous mutants) observed
209 for the progeny of *RAD51+/-* plants that do not carry the *pRAD51::DMC1g* transgene (Figure
210 2C). To confirm this, we selected *RAD51+/+* plants expressing the *pRAD51::DMC1g* transgene
211 from the progeny of the three independent *RAD51+/-* transgenic lines tested above and

212 examined their sensitivity to MMC. As expected, the three lines exhibited strong sensitivity
213 (S3A-E Fig). Ectopic expression of *DMC1* in somatic cells thus has a dominant negative effect
214 and we hypothesize that this occurs through precluding DSB repair mediated by RAD51.
215

216 **DMC1 does not substitute for RAD51 in DSB repair in somatic cells**

217 An interesting observation from the above data is that DMC1 is not able to substitute for RAD51
218 in somatic cell DSB repair. Segregating *rad51* homozygotes are sensitive to MMC, even
219 though they express *DMC1* (Figure 3B and C). This result might be surprising given the similar
220 biochemical activities of the two proteins [16, 26, 32-34, 67]. Given that meiotic DMC1 activity
221 requires the presence of a RAD51 nucleofilament [38, 39], a possible explanation for this is
222 that if DMC1 also requires a RAD51 filament to be functional in somatic cells, then DMC1 will
223 not repair DSB induced by MMC when expressed in the *rad51* mutant. To check this, we tested
224 whether DMC1 would repair MMC-induced DSB when expressed in *RAD51-GFP* plants. The
225 RAD51-GFP fusion protein forms a nucleofilament which supports the activity of DMC1 in
226 meiosis but is not proficient for DSB repair in somatic cells [39]. All three *RAD51-*
227 *GFP_pRAD51::DMC1g* independent lines tested were strongly sensitive to MMC (S3F-G Fig).
228 Thus, in contrast to meiosis, the presence of the (inactive) RAD51-GFP filament is not
229 sufficient to support DMC1 activity in somatic cells. This suggests that DMC1 activity requires
230 the presence of meiosis-specific co-factors or, alternatively, that DMC1 activity is blocked in
231 somatic cells by unknown factors.
232

233 **DMC1 specifically impairs RAD51-dependent recombination in somatic cells**

234 Our results show that ectopic expression of *DMC1* impairs DSB repair in somatic cells and we
235 hypothesize that this occurs through inhibition of RAD51 activity. However, another possible
236 explanation for this hypersensitivity to DNA damage conferred by *DMC1* mitotic
237 overexpression is that it might cause accumulation of nonproductive DMC1 nucleofilaments,
238 which would block both RAD51-dependent and RAD51-independent recombinational repair,
239 leading to genome instability and thus hypersensitivity to DNA damage. To check this, we

240 tested the effect of ectopic expression of *DMC1* on somatic homologous recombination. If DNA
241 damage hypersensitivity is caused by nonproductive DMC1 nucleofilaments, both RAD51-
242 dependent and RAD51-independent recombination should be affected, while specifically
243 blocking RAD51 should impact only RAD51-dependent recombination. We used the well-
244 characterized *IU-GUS* recombination tester locus that specifically scores RAD51-dependent
245 SDSA/GC recombination (Figure 3A) [68, 69]. This locus consists of a *β-glucuronidase* (*GUS*)
246 gene with a 34-bp linker insertion, rendering it non-functional. Productive GUS recombination
247 at the *IU.GUS* locus involves the use of an internal fragment of the *GUS* gene (5' and 3'
248 deleted) placed in inverted orientation as donor sequence to restore the complete *GUS* gene
249 (Figure 3A). The resulting functional *GUS* gene is scored histochemically as blue tissue sectors
250 (Figure 3A). Spontaneous SDSA/GC recombination at *IU.GUS* was monitored in WT seedlings
251 expressing or not *DMC1* (Figure 3B and S1 Data). We observed a dramatic reduction (4-fold)
252 of SDSA/GC events in two independent transgenic lines (T1-1 and T1-2) compared to WT
253 plants (Figure 3B and S1 Data). This reduction was confirmed after DNA damage induced by
254 MMC treatment (Figure 3C and S1 Data). Seedlings were treated with 20 µM MMC and further
255 grown for 3 days before monitoring recombination events. In wild-type, 30 to 35 SDSA/GC
256 events/seedling were detected after treatment while seedlings ectopically expressing *DMC1*
257 only showed 9-10 recombination events, corresponding to a 3.5-fold reduction (Figure 3C and
258 S1 Data). Thus, ectopic expression of *DMC1* strongly impairs RAD51-dependent
259 recombination.
260 To confirm that DMC1 specifically perturbs RAD51-dependent recombination, we also tested
261 RAD51-independent recombination using the *DGU.US* recombination reporter locus (Figure
262 3D). This reporter consists of an I-SceI restriction site flanked by 3' and 5' truncated copies of
263 the *GUS* gene in direct orientation and with a sequence homology overlap [68, 69]. Restoration
264 of a functional *GUS* gene occurs through SSA recombination which is known to be RAD51-
265 independent [66, 69]. As with the SDSA/GC assay, we measured SSA recombination
266 efficiency in WT seedlings expressing or not *DMC1* (Figure 3D). Data from three independent
267 lines (T1-1, T1-2, and T1-3) clearly showed that *DMC1* ectopic expression does not affect

268 somatic *DGU.US* recombination (Figure 3D and S1 data). We concluded that somatic
269 expression of DMC1 specifically impairs RAD51 activity, leading to inhibition of RAD51-
270 mediated recombination and DSB repair in somatic cells.
271
272 **_DMC1_ expression in somatic cells does not block RAD51 focus formation**
273 We next tested whether expression of *DMC1* in somatic cells blocks RAD51 nucleofilament
274 formation at DNA damage sites. The nucleofilament is the active molecular species that
275 performs the homology search and strand exchange. Accordingly, defects in nucleofilament
276 formation affect RAD51 activity. We analyzed RAD51 focus formation upon DNA damage in
277 somatic cells as a proxy for RAD51 nucleofilament formation. We performed RAD51
278 immunofluorescence staining in root tip nuclei of 5-day-old seedlings treated or not with
279 Mitomycin C (30 µM; Figure 4). As expected, no or very few foci were detected in root tip nuclei
280 of non-treated seedlings (Figure 4A and D). In contrast, numerous RAD51 foci were detected
281 in nuclei from root tips fixed 2 h or 8h after treatment with MMC (Figure 4B-D). In wild-type
282 plants we observed an average of 2.3 foci (± 0.3; n=228) 2h after MMC treatment and this
283 increases up to 15.8 foci (± 1.3; n=229) after 8h of MMC treatment (Figure 4 and S1 Data).
284 Strikingly, similar distributions of RAD51 foci were observed in the root tips of two independent
285 transgenic lines expressing *pRAD51::DMC1g* (T1-1 and T1-2; Figure 4B to D and S1 Data).
286 Thus, *DMC1* expression in somatic cells does not affect RAD51 focus formation and thus
287 probably RAD51 nucleofilament formation.
288
289 **DMC1 focus formation in somatic cells upon DNA damage**
290 Expression of *DMC1* in somatic cells impairs RAD51-mediated DSB repair and recombination,
291 without blocking assembly of RAD51 filaments at DNA breaks. We thus hypothesized that
292 DMC1 inhibits the strand invasion activity of the RAD51 nucleofilament in somatic cells. This
293 conclusion prompted us to verify the ability of DMC1 to assemble at DNA breaks when
294 expressed in somatic cells. We performed RAD51 and DMC1 immunofluorescence staining in
295 root tip nuclei of 5-day-old seedlings treated with 30 µM Mitomycin C for 8 hours (Figure 5). As

296 for RAD51, no DMC1 foci were detected without treatment, while numerous foci were observed
297 after MMC treatment (Figure 5A and B). Interestingly, double immunolocalization of RAD51
298 and DMC1 shows that >90% of RAD51-positive cells were positive for DMC1 (n = 122 cells)
299 and most, if not all, DMC1 positive cells were also positive for RAD51 (96%, n = 115 cells). We
300 also observed strong overlap between RAD51 and DMC1 foci (Figure 5), with 65% of RAD51
301 foci overlapping with DMC1 foci and 82% of DMC1 foci overlapping with RAD51 foci (n = 221
302 foci analyzed). Scanning through overlapping regions in optical sections in several nuclei
303 revealed that the signal intensities for RAD51 and DMC1 are very highly correlated (Fig 5C).
304 This is confirmed by the Pearson correlation coefficient calculated from analysis of 100
305 randomly chosen foci (from 24 different cells), which ranges from 0.31 to 0.92 with a mean
306 value of 0.71 (Figure 5D). RAD51 and DMC1 thus strongly co-localize, supporting the
307 conclusion that they assemble together at DSB sites.
308 DMC1 can thus assemble at DNA breaks in somatic cells. The assembled DMC1 filament is
309 non-productive but appears competent to block RAD51-dependent recombination.
310
311 **Downregulation of RAD51 recombinogenic activity requires DMC1 assembly at DSB**
312 **sites**
313 We next sought to test the capacity of non-productive DMC1 nucleofilaments to block RAD51-
314 dependent recombination through expression of a mutant DMC1 protein, which forms
315 nucleofilaments but is unable to catalyze DNA strand-transfer. We generated a *DMC1-II3A*
316 variant with the rationale that mutating three conserved positively charged residues (R136,
317 R308, and K318, S4 Fig) in the low-affinity DNA binding site (site II) of DMC1 will affect its
318 recombinogenic activity without affecting ssDNA binding, as previously shown in yeast [38].
319 The DMC1 genomic coding sequence was synthesized with mutations to convert the three site
320 II conserved residues (R136, R308, and K318, S4 Fig) to alanine. The Arabidopsis *DMC1-II3A*
321 sequence was ten placed under the control of the *RAD51* promoter and the resulting construct
322 was introduced in wild-type plants. Transgenic plants expressing *pRAD51::DMC1-II3A* were
323 selected and presence of the protein examined (Figure 6A). Western blots confirmed that both

324 the DMC1g and DMC1-II3A proteins are well translated in transgenic seedlings after Mitomycin
325 C treatment (Figures 6A and S5). We then analyzed the presence of DMC1 foci in somatic
326 cells through RAD51 and DMC1 immunofluorescence staining in root tip nuclei of 5-day-old
327 seedlings treated with 30 µM Mitomycin C for 8 hours (Figure 6B). As seen above (Figure 5),
328 numerous RAD51 and DMC1 foci are observed in transgenic seedlings expressing wild-type
329 *DMC1g*. On the contrary, only RAD51 foci could be observed in transgenic plants expressing
330 *pRAD51::DMC1-II3A* (Figure 6B). The inability of our DMC1-II3A to form foci was then
331 confirmed in meiotic cells. The DMC1-II3A sequence was placed under the control of the
332 *DMC1* promoter and the construct was introduced into *DMC1/dmc1* heterozygous plants.
333 Homozygous *dmc1/dmc1* transformants expressing the *DMC1-II3A* transgene were selected.
334 As anticipated, all 13 *dmc1/dmc1* mutant plants expressing the *DMC1-II3A* that we obtained
335 were almost sterile. Counting the number of seeds per silique in two independent
336 transformants revealed that these plants exhibit a *dmc1-like* phenotype (not the expected
337 *rad51*-like fragmentation phenotype), with 2-3 seeds per silique (S6 Fig). Interestingly, we also
338 observed a strong dominant negative phenotype in WT plants showing strongly reduced fertility
339 (S6 Fig). Immunolocalization confirmed the absence of DMC1 foci in *dmc1* mutants expressing
340 *DMC1-II3A* (S7 Fig). Accordingly, immunolocalization of the synaptonemal complex transverse
341 filament protein ZYP1 revealed defective synapsis (*dmc1* phenotype) in these plants (S7 Fig).
342 Thus, in contrast to the analogous protein in budding yeast [38], the Arabidopsis DMC1-II3A
343 does not appear to stably associate and/or polymerize on ssDNA and thus does not support
344 DMC1 presynaptic nucleofilament formation.
345 We tested impacts of DMC1-II3A on MMC hypersensitivity and RAD51-mediated
346 recombination using the *IU-GUS* recombination tester locus described above. As expected by
347 the absence of DMC1 foci, transgenic seedlings expressing the *DMC1-II3A* transgene exhibit
348 neither MMC hypersensitivity (Figure 6C) nor impairment of RAD51-dependent recombination
349 (Figure 6D and S1 Data). The absence of mitotic phenotype of this non-nucleofilament forming
350 mutant DMC1-II3A protein concords with DMC1-mediated downregulation of RAD51
351 recombinogenic activity through DMC1 assembly at DSB sites.

352

353 **Expression of dominant negative DMC1-GFP blocks RAD51 activity in meiosis**

354 Expression of a nucleofilament-forming DMC1 in mitotic cells blocks RAD51 activity, in accord
355 with the model of inhibition of RAD51 recombinogenic activity by the DMC1 nucleofilament. In
356 meiotic cells, blocking RAD51-mediated recombination in the absence of DMC1-mediated
357 recombination should lead to meiotic chromosomal fragmentation, in contrast to the intact
358 univalent chromosomes observed in the *dmc1* null mutant. Unfortunately, the DMC1-II3A
359 protein cannot be used to test the model as it appears not to form nucleofilaments in
360 Arabidopsis. We thus generated a *DMC1-GFP* translational fusion (that we should be able to
361 visualize) with the rationale that the DMC1-GFP protein will be inactive for DNA strand-transfer,
362 while retaining the capacity to form nucleofilaments (analogously to RAD51-GFP in
363 Arabidopsis, mammals and yeast [39, 41, 70-72] and DMC1-GFP in fission yeast [73]).

364 We amplified the DMC1 genomic coding sequence (including introns but without the stop
365 codon) and 2.7 kb of upstream sequence from DNA of wild-type Arabidopsis and fused the
366 *GFP* coding sequence to the 3' end of the DMC1 open reading frame. The fusion construct
367 was introduced into *DMC1/dmc1* heterozygous plants and transformants expressing the
368 *DMC1-GFP* translational fusion protein were selected. As anticipated, all 8 *dmc1/dmc1* plants
369 expressing the *DMC1-GFP* that we obtained were sterile, confirming that the fusion protein is
370 not able to complement meiosis of the Arabidopsis d*mc1* mutant and is thus not functional (S8
371 Fig). Analogously to the RAD51-GFP in Arabidopsis, DMC1-GFP acts as a dominant negative,
372 with more than 80% of wild-type plants (WT) expressing DMC1-GFP (59 of 71) being sterile
373 or showed strongly reduced fertility (S8 Fig).

374 To directly verify whether the DMC1-GFP fusion protein interferes with RAD51 activity we next
375 investigated male meiosis by staining chromosomes with 4',6-diamidino-2-phenylindole (DAPI)
376 (Figure 7). Presence of the *DMC1-GFP* leads to clear chromosome fragmentation (*rad51*-like
377 phenotype) in *DMC1-GFP dmc1-/- RAD51+/-* (compare with *dmc1-/- RAD51+/-* alone in which
378 no fragmentation is observed; Figure 7B). Fragmentation was also observed in *DMC1-GFP*
379 *DMC1+/+ RAD51+/-* plants (Figure 7A and B). The presence of the DMC1-GFP protein thus

380 blocks meiotic RAD51 activity. We note that the penetrance of this phenotype is incomplete
381 and appears to depend upon the presence of wild-type DMC1 protein as well as being
382 impacted by the dosage of RAD51. In contrast to *DMC1-GFP dmc1-/- RAD51+/-, DMC1-GFP*
383 *dmc1-/- RAD51+/+* mutants show a *dmc1-like* phenotype with impaired pairing and synapsis,
384 intact Metaphase I univalents and random chromosome segregation during Anaphase I (Figure
385 7A and B). Likewise, *DMC1-GFP DMC1+/+ RAD51+/+* plants also have a *dmc1-like* phenotype
386 (Figure 7 and S8 Fig).

387 These analyses were extended through immunolocalization of DMC1 to examine the
388 presence of DMC1-GFP foci in meiotic cells (S9 Fig). Using a DMC1-specific antibody,
389 numerous DMC1 foci were observed in wild-type plants expressing DMC1-GFP while none
390 were detected in *dmc1-/-* expressing *DMC1-GFP* (S9 Fig). Yet, the phenotype observed in the
391 DMC1-GFP transgenic plants indicates that the fusion protein is well-expressed and this is
392 supported by observation of GFP fluorescence in nucleus of live meiocytes of wild-type and
393 *dmc1* plants expressing *DMC1-GFP* (S9 Fig). We thus conclude that DMC1-GFP is well-
394 expressed and its nucleofilament-forming ability depends upon the presence of WT DMC1
395 protein, or alternatively that it impedes DMC1 nucleofilament formation. Thus, in DMC1 +
396 DMC1-GFP meiosis, the DMC1-GFP-containing nucleofilaments block RAD51 activity (*rad51*-
397 like phenotype), while no DMC1-GFP nucleofilaments form in the absence of DMC1 and this
398 results in a *dmc1-like* phenotype. These effects on the penetrance of the DMC1-GFP
399 phenotype point both to a particularity of the DMC1-GFP protein and to the importance of a
400 tightly controlled stoichiometry of both recombinases in meiosis, but do not affect the
401 conclusion that DMC1-GFP impedes DMC1-mediated recombination and triggers
402 downregulation of meiotic RAD51-mediated recombination.

403

404 ## Discussion

405 **DMC1 potentially prevents RAD51-mediated recombination**

406 The data presented here support the argument that DMC1 plays a key role in preventing the

407 activity of RAD51 in driving meiotic recombination in Arabidopsis. We present our conclusions
408 in the context of a model on the formation and activity of RAD51 and DMC1 nucleofilaments
409 in Figure 8. In most studied eukaryotes, efficient meiotic recombination requires the presence
410 of both RAD51 and DMC1 [16] (Figure 8A). In our model, RAD51 and DMC1 loading is
411 represented according to the symmetrical loading model with RAD51 and DMC1 homotypic
412 filaments on both DSB ends of a break. This is the most recently supported model and has
413 been shown to better explain regulation of RAD51 and DMC1 activity [16, 17, 21-24, 26, 27].
414 We note however that asymmetric loading has also been previously suggested (in particular
415 in Arabidopsis) and is thus also possible [19]. The nucleofilament is then the active molecular
416 species and data from budding yeast, mouse and Arabidopsis have demonstrated that DMC1
417 is the active recombinase during meiosis with RAD51 being an essential co-factor for DMC1,
418 but its strand exchange activity being inhibited [21, 38-40, 46, 47, 57, 74] (Figure 8A). Thus,
419 absence of RAD51 leads to strong defects in DMC1 loading and therefore defects in meiotic
420 DSB repair [19, 38, 58, 75, 76] (Figure 8B). In budding yeast, down-regulation of Rad51 is
421 achieved by at least two mechanisms involving the meiosis-specific kinase Mek1 : (i) Mek1
422 phosphorylates and stabilizes the meiosis-specific Hed1 which competes with Rad54 for
423 binding to Rad51 [45, 49, 51, 52], and (ii) Mek1 phosphorylates Rad54, reducing its affinity for
424 Rad51 and thereby its activity [50]. However, homologs of Mek1 and Hed1 have not been
425 identified, nor has the mechanism by which RAD51 is downregulated been described in
426 organisms other than budding yeast. We recently confirmed that Arabidopsis RAD54 is
427 essential for RAD51-mediated recombination and that this includes meiotic DSB repair in *dmc1*
428 mutant plants [74] (Figure 8C and E). This study thus further emphasized that the RAD51-
429 RAD54 pathway is down-regulated or plays a minor role in wild-type Arabidopsis meiosis.
430 This work in Arabidopsis suggests that meiotic RAD51-downregulation is mediated by DMC1
431 (Figure 8D and F). In Arabidopsis, previous reports have already suggested that DMC1 may
432 inhibit RAD51, based on observations that deletion of DMC1 in some mutant backgrounds
433 leads to activation of RAD51 [56-58, 74] (Figure 8C). In Arabidopsis *hop2* or *mnd1* mutants,
434 extensive DNA fragmentation occurs in meiosis as a result of defective recombination and

435 absence of DMC1 in these mutants alleviates this DNA fragmentation, presumably through
436 reactivation of RAD51 [57, 58]. That DMC1 is able to inhibit RAD51 recombinogenic activity
437 has also been suggested in budding yeast [18, 46]. Dmc1 is known to favor inter-homolog
438 recombination while Rad51 favors sister chromatid recombination. Accordingly, *hed1* mutants
439 exhibit defects in homolog bias as a result of Rad51 activation, and this effect is stronger in
440 the *dmc1 hed1* double mutant than in the *hed1* single mutant [18, 46] suggesting that Rad51
441 is even more active in absence of Dmc1. Similar conclusions have been drawn from
442 comparisons of *mnd1 hed1* and *dmc1 hed1* mutants [46]. In the *mnd1* mutant, both DMC1 and
443 RAD51 are present but strand-exchange is inhibited, resulting in meiotic arrest, unrepaired
444 DSB and the virtual absence of crossovers. These defects are alleviated by mutating HED1,
445 but to a lesser extent than in *dmc1 hed1* double, and *mnd1 dmc1 hed1* triple mutants [46]. As
446 in Arabidopsis, this is however indirect evidence and whether this DMC1 inhibitory effect
447 occurs in a wild-type context or only in certain mutant backgrounds is not clear.
448 We have expressed a non-functional *DMC1-GFP* allele in meiosis and DMC1 in somatic cells
449 and show that they block RAD51 activity, strongly supporting the hypothesis that DMC1 has
450 the potential to prevent RAD51-mediated recombination (Figure 8D and F). We note however
451 that whether this DMC1-dependent down-regulation of RAD51 also holds true in WT meiotic
452 cells is indirectly inferred here through our data in somatic cells (Figure 8F), the meiotic
453 phenotypes of several mutants described above, and the meiotic phenotype of the DMC1-GFP
454 (Figure 8D). The dominant negative nature of the DMC1-GFP on DMC1 does not allow us to
455 unequivocally conclude whether the meiotic RAD51 inhibition is the result of an intrinsic activity
456 of DMC1 itself, or whether it comes from the presence of the GFP domain. We sought to
457 directly test this though expression of a non-recombinogenic *DMC1-II3A* allele in meiosis but
458 unfortunately failed to do so due to the inability of Arabidopsis DMC1-II3A to form DMC1 foci.
459 We do note however, that this inability of the Arabidopsis protein to form foci is in striking
460 contrast with budding yeast Dmc1-II3A and underlines the existence of differences in behavior
461 and/or regulation of DMC1 between these species.
462

463 **Mechanistic insights into DMC1-dependent down-regulation of RAD51 recombinogenic**
464 **activity**

465 How DMC1 could prevent RAD51 activity is intriguing since RAD51 is an essential co-factor
466 for DMC1 and DMC1 must thus couple RAD51 inhibition with the supporting role of RAD51 on
467 its own activity. Previous work has shown that the meiotic nucleofilament-forming ability, not
468 the strand-exchange activity of RAD51 is needed for supporting DMC1 activity in meiosis and
469 that meiotic RAD51 nucleofilaments form normally in the absence of DMC1 [19, 38-40, 58, 74,
470 76, 77].

471 RAD51 recombination can be downregulated on several levels by affecting various RAD51
472 activities. This includes RAD51 loading on RPA-coated ssDNA, stabilization of the nascent
473 nucleoprotein filament by controlling its assembly/disassembly, RAD51 ATPase activity,
474 reduction of RAD51's capacity to bind dsDNA or even remodeling of the RAD51 nucleoprotein
475 filament into a conformation that is optimally permissive for DNA strand exchange. The work
476 presented here confirms that somatic RAD51 focus formation is not impaired by ectopic
477 expression of *DMC1*, even though the presence of DMC1 blocks their activity in recombination.
478 Thus, the prevention of RAD51-dependent recombination by DMC1 is mediated through down-
479 regulation of activities of RAD51 nucleofilaments, not of their formation. This interpretation is
480 supported by (i) the presence of inactive DNA damage-induced DMC1 foci in somatic cells, (ii)
481 the down-regulation of RAD51 DSB repair in meiosis by a non-functional dominant negative
482 *DMC1-GFP* allele and (iii) it concords with the characterization of several Arabidopsis null
483 mutants (e.g. *hop2* and *mnd1*), in which meiotic DSB repair defects lead to chromosome
484 fragmentation even though both RAD51 and DMC1 form foci at wild-type levels [56-58, 78,
485 79]. The Arabidopsis *sds* mutant (a meiosis-specific cyclin-like protein) has a *dmc1-like* meiotic
486 phenotype with an absence of DMC1 focus formation. Meiotic DSB are however fully repaired
487 by RAD51, most probably using sister chromatid templates [56, 79-82]. Interestingly, deletion
488 of the AAA-ATPase FIDGETIN-LIKE-1 (FIGL1) or its partner FLIP partially restores DMC1
489 focus formation in *sds* mutants but this is associated with important chromosome
490 fragmentation. That this is linked to the presence of DMC1 foci is confirmed by the presence

491 of intact chromosomes in a triple *sds figl1 dmc1* mutant [56, 79].

492 Remarkably, RAD51 is not the only protein inhibited by DMC1. In budding yeast, DMC1 is able
493 to specifically inhibit the ATPase activity (hence the motor activity) of the antirecombinase
494 Srs2, even though Srs2 retains its ability to bind DMC1-ssDNA filaments [83]. The RAD51-
495 GFP protein that is proficient for supporting DMC1 in meiotic recombination exhibits normal
496 ATP binding and hydrolysis *in vitro* arguing against regulation of RAD51 ATPase activity [41].
497 Several other non-exclusive scenarios can be envisioned. For instance, DMC1 may inhibit
498 RAD51 through effects on RAD51's interaction with essential co-factors. It is tempting to
499 speculate that one such co-factor could be RAD54, which could be functionally analogous to
500 the RAD51-RAD54 regulation of budding yeast (Figure 10). Arabidopsis RAD54 is required for
501 RAD51 function in somatic cells [63, 84-86] and we have recently shown that it is also essential
502 for RAD51 recombinogenic activity in meiotic cells in absence of DMC1 [74].

503 Another possibility is that DMC1 affects the properties of the RAD51 nucleofilament. The
504 nucleofilament is the active RAD51 species and filaments with distinct properties have been
505 described. Super-resolution microscopy, single molecule imaging and chromatin
506 immunoprecipitation approaches have revealed that RAD51 and DMC1 form separate
507 homotypic filaments on both ends of a DSB [21-24]. Furthermore, meiotic DMC1 function
508 depends upon the presence of RAD51 nucleofilaments (not their strand-invasion activity) and
509 RAD51 foci form normally in *dmc1* mutants of Arabidopsis, yeast and mice, while numbers of
510 DMC1 foci are significantly reduced in *rad51* mutants [19, 38, 58, 76, 77]. Thus, DMC1 may
511 cap RAD51 filaments and block their activity. In agreement with this, our results show strong
512 overlap of RAD51 and DMC1 foci in somatic cells.

513 Additionally, DMC1 may change the conformation of the RAD51 filaments. RAD51
514 nucleofilaments with different conformational states have been described by crystallography :
515 one with an “extended” conformation and another with a “condensed” conformation [87]. The
516 “extended” conformation is presumed to be the open, active state of the filament and the
517 “condensed” conformation to represent a closed state of the filament. Dynamic arrangement

518 of the nucleofilament is central to its function and it is thus possible that binding of DMC1
519 blocks RAD51 in a closed state.
520 An interesting additional conclusion from our data is that DMC1-dependent down-regulation of
521 RAD51 requires a controlled stoichiometry of the two recombinases. Expression of the
522 dominant negative *DMC1-GFP* allele in meiosis inhibits RAD51 repair only in a *RAD51+/-* (not
523 *RAD51+/+)* context. This suggests that RAD51 down-regulation requires sufficient amounts of
524 DMC1 and presumably DMC1-nucleofilaments. In accordance we found that inhibition of
525 RAD51 activity was stronger in presence of both DMC1-GFP and endogenous DMC1, than by
526 DMC1-GFP alone (compare fragmentation *in DMC1-GFP DMC1+/+ RAD51+/-* vs. *DMC1-GFP*
527 *dmc1-/- RAD51+/-* plants in Figure 6). We speculate that DMC1-GFP alone does not form
528 sufficient stable and/or abundant filaments while mixed DMC1 endogenous/DMC1-GFP
529 filaments are more stable and/or abundant and thus more prone to inhibit RAD51. In support
530 of this, we observed DMC1 foci in *DMC1-GFP DMC1+/+* but not in *DMC1-GFP dmc1-/-* plants,
531 underlining the importance of tight regulation in meiotic cells allowing sufficient and timely
532 expression of *DMC1* versus *RAD51*.
533
534 **Replacement of RAD51 in somatic cells with DMC1 : mitotic HR versus meiotic HR**
535 To investigate whether DMC1 inhibits RAD51, we took the approach of ectopically expressing
536 *DMC1* in somatic cells in which normally only RAD51 is present. RAD51 and DMC1 share
537 similar biochemical properties and catalyze essentially the same DNA strand exchange
538 reaction [16, 26, 33, 34, 67], although DMC1 fibres are able to accommodate unpaired base
539 mismatches [88-92] and results from fission yeast indicate opposite polarities of nucleofilament
540 polymerization of the two proteins [16, 93]. It is not yet clear whether functional differences
541 between RAD51 and DMC1 result from distinct biochemical properties or influence of different
542 co-factors. Our data may provide new insights into this important question. First,
543 immunolocalization studies showed that DMC1 can form DNA damage-induced foci in somatic
544 nuclei, suggesting that DMC1 assembly on ssDNA does not require meiosis-specific co-
545 factors. Although recruitment of DMC1 to sites of DNA breaks requires meiosis-specific co-

546 factors in budding yeast, such as Mei5-Sae3 complex, no such factors have been identified in
547 Arabidopsis and our data suggest that DMC1 may not require any such factors for loading at
548 DNA breaks. We further show that expression of *DMC1* rescues neither somatic HR, nor DNA
549 damage hypersensitivity of *rad51* mutants. Thus, notwithstanding its ability to form
550 nucleofilaments, DMC1 is not able to replace RAD51 for repair in somatic cells. This indicates
551 either that DMC1 strand-exchange activity does require meiosis-specific co-factors and/or
552 chromosomal features or alternatively that DMC1 strand-exchange is inhibited in somatic cells
553 by some yet unknown factors. One evident candidate meiosis-specific co-factor would be the
554 HOP2/MND1 complex, an essential co-factor for DMC1 in meiosis [57, 58, 94-97]. This would
555 fit with an earlier study on the different meiotic phenotypes of the null and hypomorphic
556 Arabidopsis *hop2* mutants, which proposed a model in which transient inhibition of RAD51 by
557 DMC1 is alleviated by interaction between DMC1 and the HOP2/MND1 complex. However, a
558 role for HOP2/MND1 in mitotic RAD51-mediated recombination has been described in
559 mammals and Arabidopsis [58, 62, 98, 99]. HOP2/MND1 expression is strongly responsive to
560 DNA damage in Arabidopsis somatic cells and Arabidopsis *mnd1* mutants have also been
561 shown to be sensitive to DNA damage [59-64]. HOP2/MND1 is thus present and functional in
562 somatic cells and is unlikely to be the "missing" meiotic DMC1 co-factor. In this context, our
563 system also offers a unique opportunity to investigate RAD51-mediated HR vs. DMC1-
564 mediated HR. It would be interesting to see how adding known meiotic specific factors (e.g.
565 REC8,…) to the DMC1 reaction affects somatic recombination. This would provide important
566 information concerning the evolution of the meiotic program and the specificities provided by
567 DMC1 [100].

569 # Materials and Methods

570 **Plant Material and Growth Conditions**

571 The lines used in this study were *rad51-1* [54], *dmc1-1* [53] and *RAD51-GFP* [39]. Seeds were
572 stratified in water at 4°C for 2 days and grown on soil or *in vitro* on 0.8% agar plates with 1%
573 sucrose and half-strength Murashige and Skoog salts medium (M0255; Duchefa Biochemie).
574 Plants were then cultivated in a growth chamber with a 16/8-hour light/dark cycle, temperature
575 23°C and 60% relative humidity.

576

577 **Cloning of DMC1 and plant transformation**

578 For expression of *DMC1* in vegetative cells (here called *DMC1g*), the complete genomic region
579 from the ATG to the stop codon (2793 bp) of *DMC1* was amplified (forward primer
580 ATGATGGCTTCTCTTAAGTAAGT and reverse primer CTAATCCTTCGCGTCAGC), inserted
581 into pDONR-Zeo and verified by sequencing. The fragment was then cloned into a GATEWAY
582 destination vector pMDC32 [101] in which the *35S* promoter was replaced by the Arabidopsis
583 *RAD51* promoter as described in [39].

584 For *DMC1-II3A*, the complete genomic region from the ATG to the stop codon of *DMC1* (as
585 above) was synthesized with mutation to convert R136, R308 and K318 into Alanine (S4 Fig).
586 For expression in meiosis, the synthesized *DMC1-II3A* was cloned into the GATEWAY
587 destination vector pMDC32 in which the 35S promoter was replaced by the DMC1 promoter
588 (2.7kb 5' upstream sequence of *DMC1*) using an AscI/SbfI digest. The DMC1 promoter was
589 amplified from *Landsberg erecta* (*Ler-0*) DNA using forward primer
590 GCGTGCCTGCAGGCACACCTAATCGGTGATTGCC and reverse primer
591 GCTTGGCGCGCCtttctcgctctaagagtctctaa).

592 For translational GFP fusions, the DMC1 genomic region without stop codon and a 2.7kb 5'
593 upstream sequence of *DMC1* was amplified from Landsberg DNA (forward primer
594 CCACACCTAATCGGTGATTG and reverse primer ATCCTTCGCGTCAGCAATG) and cloned
595 into a GATEWAY destination vector using the strategy described in [39].

596 Plasmids were then inserted in an *Agrobacterium tumefaciens* C58C1 strain, which was
597 subsequently used to transform Arabidopsis plants by the floral dip method [102]. Seeds from
598 the Agrobacterium-treated plants were sown on soil and transformants were selected for
599 Hygromycin or Basta resistance.
600
601 **Semi-quantitative RT-PCR analyses**
602 For semi-quantitative RT–PCR, total RNA was extracted from one-week-old seedlings using
603 TRIzol Reagent (ThermoFisher Scientific), following the manufacturer's instructions. After
604 treatment of two µg RNA with RQ1 RNase-free DNase (Promega) and reverse transcription
605 using M-MLV Reverse Transcriptase (Promega) according to the manufacturer's instructions,
606 16 ng was used for each PCR amplification. Primer pairs used for PCR and their sequences
607 are listed in Supplemental Table S1.
608
609 **Mitomycin C sensitivity assay**
610 For the Mitomycin C sensitivity assay, seeds were surface-sterilized and sown onto solid
611 medium containing half-strength Murashige and Skoog salts, 1% sucrose, 0.8% agar and
612 supplemented or not with 20 µM Mitomycin C (SIGMA). After stratification for 2 days at 4°C,
613 plants were grown for two weeks and sensitivity analyzed by counting the number of true
614 leaves as previously described [39, 103]. Plants with three true leaves or less were considered
615 as sensitive [39, 103].
616
617 **Histochemical GUS staining for somatic homologous recombination assay**
618 The well-characterized IU.GUS-8 and DGU-US-1 *in planta* recombination tester lines
619 consisting of an interrupted *ß-glucuronidase* (*GUS*) gene were used to determine the
620 frequency of somatic homologous recombination [68, 69].
621 For the SDSA/GC assay, transgenic plants carrying the transgene were crossed with the
622 recombination tester IU.GUS-8 line [68]. Plants homozygous for the IU.GUS substrate loci and
623 either wild-type or homozygous for the transgene were identified in the F2. Seeds from the F2

624 were used for recombination tests. For the SSA assay, wild-type lines homozygous for
625 DGU.US substrate were transformed with the *pRAD51::DMC1g* expression cassette. T2 plants
626 homozygous for both the DGU.US substrate and the *pRAD51::DMC1g* transgene were
627 selected and used for the GUS assay. Wild-type control plants were also selected from the
628 same transformation batch.
629 For histochemical GUS staining, seeds were surface-sterilized, stratified at 4°C for 2 days and
630 grown in petri dishes on solid medium for 2 weeks. Seedlings were then harvested and
631 incubated in staining buffer (0.2% Triton X-100, 50 mM sodium phosphate buffer (pH 7.2), 2
632 mM X-Gluc (5-bromo-4-chloro-3-indolyl-ß-D-glucuronic acid; Biosynth), dissolved in N,N-
633 dimethylformamide). Plants were infiltrated under vacuum for 15 min and incubated at 37°C
634 for 24 hours. Staining solution was then replaced with 70% ethanol to remove leaf pigments
635 and blue spots were counted under a binocular microscope.
636 For MMC induction, 11-day-old seedlings were transferred on liquid medium supplemented
637 with 20 µM MMC and further grown for 3 days before GUS staining.
638
639 **Meiotic chromosome spreads**
640 Meiotic chromosome spreads were prepared according to [104]. Whole inflorescences were
641 fixed in ice-cold ethanol/glacial acetic acid (3:1) and stored at -20°C until further use. Immature
642 flower buds of appropriate size were selected under a binocular microscope and incubated for
643 75-90 min on a slide in 100µl of enzyme mixture (0.3% w/v cellulase (Sigma), 0.3% w/v
644 pectolyase (Sigma) and 0.3% cytohelicase (Sigma)) in a moist chamber at 37°C. Each bud
645 was then softened for 1 minute in 20 µl 60% acetic acid on a microscope slide at 45°C, fixed
646 with ice-cold ethanol/glacial acetic acid (3:1) and air dried. Slide were mounted in Vectashield
647 mounting medium with DAPI (1.5 $\mu g.ml^{-1}$; Vector Laboratories Inc.).
648
649 **Immunocytological analyses**
650 Slide preparation and immunostaining in root tip nuclei were performed as previously
651 described [105], except that slides were incubated with RAD51 antibody (diluted 1:500) for 24

652 hours at 4°C and 48 hours in case of RAD51 / DMC1 (1:500; 1:20) double immunostaining.
653 Immunolocalisation of proteins in pollen mother cells has been performed as described
654 previously [106]. The anti-ASY1 raised in Guinea Pig was used at a dilution of 1:250 [107], the
655 anti-ZYP1 raised in rabbit [108] was used at a dilution of 1:500, the anti-RAD51 raised in rat
656 [19] was used at a dilution of 1:500, and the anti-DMC1 raised in rabbit [109] was used at a
657 dilution of 1:20.
658
659 **Microscopy**
660 All observations were made with a motorized Zeiss AxioImager.Z1 epifluorescence
661 microscope using a PL Apochromat 100X/1.40 oil objective. Photographs were taken with an
662 AxioCam Mrm camera and appropriate Zeiss filter sets. Image Z-stacks were captured in three
663 dimensions (x, y, z) and further deconvoluted with the deconvolution module (theoretical PSF,
664 iterative algorithm) of the Zeiss ZEN lite software. For presentation, pictures are collapsed Z-
665 stack projections obtained using the Extended-focus module of the ZEN lite software.
666 The pixel intensity profile was analyzed via the colocalization module of the ZEN software
667 using uncollapsed Z-stack files. The pixel intensity through a region of interest was extracted
668 using the ZEN software and plotted against the X dimension. The quantitative co-localization
669 analysis and calculation of Pearson's correlation coefficient was performed using ImageJ
670 modules Coloc2.
671
672 **Western blot analyses**
673 Seven-day-old seedlings grown *in vitro* on 0.8% agar plates were transferred to liquid medium
674 (1% sucrose and half-strength Murashige and Skoog salts medium) supplemented or not with
675 30 µM MMC and incubated for 8 hours. For total protein extraction, about 30 seedlings were
676 frozen in liquid nitrogen and ground in 100 µL extraction buffer (0.1 M Tris-HCl pH 7.5, 20%
677 Glycerol, 2 mM EDTA, 1 mM DTT, 0.2% NP40 Nonidet, 1X Complete Mini Protease inhibitor
678 cocktail (Roche)) and centrifuged at 4°C for 30 min at 20 000 g. Total protein content in the
679 supernatant was carefully determined using the Bradford assay (Bio-Rad). 30 µg of proteins

680 were denatured in the presence of 4X Laemmli Buffer at 95°C for 5 min [110]. Proteins were
681 electrophoresed on 10% SDS-polyacrylamide gels [110] and transferred to nitrocellulose
682 membrane. Equal protein loading was carefully controlled by reversible ponceau staining of
683 the nitrocellulose membrane. DMC1 proteins were detected with a rabbit DMC1 antibody at a
684 dilution of 1:800 (diluted antibody in 3% w/v nonfat dry milk, 1XPBS, 0.1% Tween-20; overnight
685 incubation at 4°C) followed by goat anti-rabbit HRP-conjugated secondary antibody (1:5000
686 dilution in 3% w/v nonfat dry milk, 1XPBS, 0.1% Tween-20 ; 1h incubation at room
687 temperature) and electrochemiluminescence detection (Clarity Max ECL; Bio-Rad). Membrane
688 was eventually imaged using ChemiDoc MP imaging system (Bio-Rad).
689
690
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1089

1091 ## Figure Legends

1092 **Figure 1. *pRAD51::DMC1g* restores fertility of the Arabidopsis *dmc1* mutant and induces**
1093 **expression of DMC1 in somatic cells.**

1094 **(A)** Schematic representation of the *pRAD51::DMC1g* construct. *DMC1g* indicates *DMC1*
1095 genomic sequence. Exons are shown as blue rectangles. **(B)** Wild-type plants have long
1096 siliques full of seeds (mean ± S.D : 54.1 ± 0.6 seeds per silique), while *dmc1* mutants have
1097 very low fertility (mean ± S.D : 3.5 ± 0.25 seeds per silique). Expression of the
1098 *pRAD51::DMC1g* genomic sequence in *dmc1* mutants restores fertility. Mean ± S.D is shown;
1099 n = 4 plants, N = 10 siliques per plant. **(C)** number of seeds per silique in DMC1, *dmc1-/-,* and
1100 three *dmc1-/-* + *pRAD51::DMC1g* independent transformants (T1-1, T1-2, and T1-3) showing
1101 restored fertility. **(D)** RT-PCR expression analysis of *RAD51*, *DMC1*, *RAD54*, *HOP2* and *MND1*
1102 in 7-day-old, untreated and MMC-treated (8 h, 20 µM) seedlings expressing or not the
1103 *pRAD51::DMC1g* transgene. Expression was analyzed in wild-type plants and three
1104 independent transgenic lines (T1-1, T1-2, and T1-3). Actin is used as a loading control.

1105

1106 **Figure 2. Mitomycin C hypersensitivity of transgenic seedlings expressing**
1107 ***pRAD51::DMC1g*.**

1108 **(A)** No growth difference is observed between WT, *RAD51-GFP*, *RAD51+/-* heterozygous and
1109 *RAD51+/-* expressing *pRAD51::DMC1g* seedlings (two-week-old seedlings grown without
1110 MMC). **(B)** Pictures of two-week-old seedlings grown with 20 µM MMC. WT seedlings
1111 (depicted with red square) do not exhibit MMC sensitivity, in contrast to *RAD51-GFP* seedlings
1112 (blue square) and progeny of *RAD51+/-* transgenic lines expressing *pRAD51::DMC1g*.
1113 Pictures of progeny of two independent *RAD51+/-* transgenic lines expressing
1114 *pRAD51::DMC1g* are shown (T1-1 and T1-2). As control, WT (red square) and *RAD51-GFP*
1115 plants (blue square) were also grown on each plate. **(C)** Sensitivity of the seedlings was scored
1116 after 2 weeks and the fractions of sensitive plants (plants with less than 4 true leaves) are
1117 shown (3 biological repeats, each with N > 45 seedlings). Three independent *RAD51+/-*

1118 heterozygous lines expressing *pRAD51::DMC1g* (T1-1,T1-2 and T1-3) were tested and all
1119 showed strong hypersensitivity to MMC.
1120
1121 **Figure 3. DMC1 inhibits RAD51-dependent somatic homologous recombination.**
1122 **(A)** Schematic map of the *IU.GUS* chromosomal recombination reporter locus. Restoration of
1123 the functional *GUS* gene is visualized as blue spots in seedlings. Both spontaneous **(B)** and
1124 MMC induced **(C)** *IU.GUS* recombination rates are strongly reduced in wild-type seedlings
1125 expressing *pRAD51::DMC1g*. Mean numbers of GUS+ recombinant spots per plant ± standard
1126 errors of the means (SEM) are indicated for each genotype (n = 3, N > 45 seedlings). **** p <
1127 0.0001 (Mann-Whitney test). The wild-type plants with and without *pRAD51::DMC1g* are sister
1128 plants from a transgenic line segregating for the *pRAD51::DMC1g* transgene. Analysis were
1129 performed using segregating plants from two independent transgenic lines (T1-2 and T1-3).
1130 **(D)** Schematic map of the *GU.US* chromosomal recombination assay and graph showing mean
1131 numbers of GUS+ recombinant spots per plant ± SEM. Three independent *GU-US*
1132 transformants expressing *pRAD51::DMC1g* (T1-1, T1-2, T1-3) and 2 WT (sister plants from a
1133 population segregating the *pRAD51::DMC1g* transgene) were analyzed (WT for T1-3 is the
1134 same as for T1-2). Three biological replicates, each containing at least 50 plants, were
1135 performed, except for the WT T1-1 for which only two replicates were performed. p > 0.05
1136 (Mann-Whitney test).
1137
1138 **Figure 4. RAD51 foci in somatic cells of *DMC1* overexpressing seedlings.**
1139 **(A-C)** Immunolocalization of RAD51 in root tip nuclei of wild-type plants expressing
1140 *pRAD51::DMC1g*, fixed just before **(A)**, and 2 **(B)** or 8 **(C)** hours after treatment with 30 µM
1141 MMC. T1-1 and T1-2 are two independent transgenic lines expressing *pRAD51::DMC1g*.
1142 MMC-induced RAD51 foci are clearly visible in nuclei of the treated plants. DNA is stained with
1143 DAPI (blue) and RAD51 foci (detected using an antibody against RAD51) are colored in red.
1144 Images are collapsed Z-stack projections of a deconvoluted 3D image stack. (Scale Bars : 5
1145 µm). **(D)** Quantification of RAD51 foci in root tip nuclei of wild-type and two independent

1146 transgenic lines expressing *pRAD51::DMC1g* (T1-1 and T1-2) before and after MMC
1147 treatment. Means ± s.e.m are indicated for each genotype. More than 200 nuclei from at least
1148 3 seedlings were analyzed per genotype.
1149
1150 **Figure 5. DMC1 foci are formed in somatic cells upon DNA damage.**
1151 **(A-B)** Immunolocalization of RAD51 and DMC1 in root tip nuclei of wild-type plants expressing
1152 *pRAD51::DMC1g* fixed 8 hours after treatment with 30 µM Mitomycin C. **(B)** is a magnification
1153 of the area framed in **(A)**. DNA is stained with DAPI (blue), RAD51 foci are colored in red and
1154 DMC1 in green. Images are collapsed Z-stack projections of a deconvoluted 3D image stack.
1155 (Scale bars: 20 µm **(A)** and 5 µm **(B)**).
1156 **(C)** Example of RAD51 and DMC1 signal intensity distribution of the total amount of pixels
1157 through a section of the depicted stained nucleus (red line). RAD51 is shown in red and DMC1
1158 in green. (Scale bar: 5 µm). **(D)** Range of Pearson correlation coefficients (PCCs) of
1159 RAD51/DMC1-positive foci formed after 8 h of MMC treatment. RAD51/DMC1 partially co-
1160 localized in foci with a mean value of PCCs = 0.63 (n = 40).
1161
1162 **Figure 6. DMC1-II3A variant does not form focus nor prevent RAD51-mediated**
1163 **recombination in somatic cells**
1164 **(A)** DMC1g and DMC1-II3A protein are induced by MMC treatment. Total proteins were
1165 extracted from 1-week-old seedlings treated or not with 30 µM MMC for 8 hours and DMC1
1166 abundance measured. No DMC1-specific band (37 kDa) is observed in untreated plants in
1167 both wild-type and transgenic plants. In contrast, while DMC1 is still absent in wild-type plants
1168 after DNA damage treatment, it becomes clearly visible in transgenic plants expressing either
1169 *DMC1g* or *DMC1-II3A*. **(B)** Immunolocalization of RAD51 and DMC1 in root tip nuclei of wild-
1170 type plants, transgenic plants expressing *pRAD51::DMC1g* or *pRAD51::DMC1-II3A* fixed 8
1171 hours after treatment with 30 µM Mitomycin C. DNA is counterstained with DAPI (blue). Images
1172 are collapsed Z-stack projections of a deconvoluted 3D image stack. (Scale bar : 20 µm). **(C)**
1173 Expression of DMC1-II3A does not render plant hypersensitive to MMC nor **(D)** prevent

1174 RAD51-dependent somatic homologous recombination. **(C)** Sensitivity of seedlings was
1175 scored after 2 weeks of growth with 20 μM MMC and the fractions of sensitive plants (plants
1176 with less than 4 true leaves) are shown (3 biological repeats, each with N > 45 seedlings). Two
1177 independent transgenic lines expressing *pRAD51::DMC1-II3A* (T1-1 and T1-2) were tested
1178 and none exhibit MMC hypersensitivity. **(D)** Mean numbers of GUS+ recombinant spots per
1179 plant ± standard errors of the means (SEM) are indicated for each genotype. ($p < 0.05$; Mann-
1180 Whitney test). The wild-type plants with and without *pRAD51::DMC1-II3A* are sister plants from
1181 a transgenic line segregating for the *pRAD51::DMC1-II3A* transgene.
1182
1183 **Figure 7. Expression of DMC1-GFP fusion protein inhibits RAD51-mediated repair of**
1184 **meiotic DBS.**
1185 **(A)** DAPI staining of chromosomes during meiosis. Wild-type cells show pairing and synapsis
1186 of homologous chromosomes in pachytene, five bivalents at metaphase I and two groups of
1187 five chromosomes at anaphase I. *rad51* mutants exhibit defective synapsis and extensive
1188 chromosome fragmentation. Meiotic spreads in *dmc1-/- RAD51+/+* plants expressing *DMC1-*
1189 *GFP* revealed a *dmc1-like* phenotype with defective synapsis but ten intact univalents at
1190 metaphase I, which randomly segregate at anaphase I. In contrast, extensive fragmentation is
1191 observed in both *dmc1-/- RAD51+/-* and *DMC1+/+ RAD51+/-* plants expressing *DMC1-GFP*.
1192 (Scale Bar: 10 μm).
1193 **(B)** Quantification of chromosome fragmentation. The genotype of the plants is indicated below
1194 each bar and number of cells analyzed is listed above.
1195
1196 **Figure 8. Hypothetical model for the activity of RAD51 and DMC1 nucleofilaments.**
1197 **(A)** During meiotic recombination, RAD51 and DMC1 polymerize on ssDNA to form
1198 nucleofilaments. DMC1 is the active nucleofilament but requires the presence of RAD51
1199 nucleofilaments and additional co-factors. Presence of the DMC1 nucleofilament inactivates
1200 the RAD51 nucleofilament either through an unknown signaling pathway, through inhibiting
1201 interaction with co-factors or through nucleofilament remodeling. **(B)** In absence of RAD51,

1202 DMC1 does not form nucleofilaments and DSB repair is defective. **(C)** Absence of DMC1
1203 allows normal polymerization of RAD51 and interaction with co-factors. RAD51
1204 nucleofilaments are thus proficient for DSB repair using sister chromatid templates. This is
1205 reminiscent of the situation in mitosis **(E)** where RAD51 is the sole recombinase. Its
1206 polymerization and interaction with co-factors such as RAD54 lead to an active nucleofilament
1207 proficient for DSB repair**. (D and F)** Addition of non-functional DMC1 (e.g. *DMC1-GFP*) in **(D)**
1208 meiosis or **(F)** mitosis, leads to RAD51 down-regulation. In mitosis, DMC1 may be kept inactive
1209 presumably through absence of meiosis-specific co-factors or chromosomal features.
1210 We note that the spatial organization of recombinases and accessory factors depicted in the
1211 model is hypothetical and thus other organizations are possible (reviewer in [16, 17, 25]). In
1212 particular, although the symmetrical loading of RAD51 and DMC1 is currently favored, it has
1213 been formally demonstrated in yeast only and it is not yet clear whether it applies for other
1214 organisms. This is particularly true for Arabidopsis where asymmetric loading has previously
1215 been suggested [19].
1216
1217

1218 ## Supporting information

1219

1220 **Supplemental Figure 1. DAPI staining of pollen mother cells (PMC) during meiosis in**
1221 **WT, *dmc1* and *dmc1* plants expressing the *pRAD51::DMC1g* transgene.**
1222 In WT meiosis, full pairing and synapsis is observed during Pachytene. Chromosomes
1223 condense and five bivalents linked by chiasmata are visualized during Metaphase I.
1224 Homologous chromosomes segregate in Anaphase I and Meiosis II proceeds leading to the
1225 formation of four balanced nuclei. In contrast, *dmc1* mutants exhibit defects in pairing and
1226 synapsis in pachytene, and 10 univalents are visible at Metaphase I due to absence of
1227 chiasmata. Univalents segregate randomly in Anaphase I and this leads, after Meiosis II, to
1228 polyads. The meiotic defects of the *dmc1* mutant are complemented by the presence of
1229 *pRAD51::DMC1g*. (Scale Bar: 10 µm).

1230

1231 **Supplemental Figure 2. RT-PCR analysis of Homologous Recombination genes in**
1232 **seedlings after irradiation.**
1233 RT-PCR expression analysis of *RAD51*, *DMC1*, *RAD54* and *HOP2/MND1* in 7-day-old
1234 untreated or gamma-irradiated seedlings expressing or not the *pRAD51::DMC1* transgene.
1235 Seedlings were irradiated at 100 Gy and expression was analyzed 2 hours after irradiation.
1236 Actin is used as a loading control.

1237

1238 **Supplemental Figure 3. Expression of *DMC1* in somatic cells has a dominant negative**
1239 **effect and does not substitute for RAD51 in DSB repair in somatic cells.**
1240 **(A to E) Mitomycin C hypersensitivity of transgenic wild-type seedlings overexpressing**
1241 ***DMC1*.** Pictures of two-week-old seedlings grown without (**A**) or with 20 µM MMC **(B to D)** are
1242 shown. **(E**) Sensitivity of the seedlings was scored after 2 weeks and the fractions of sensitive
1243 plants (plants with less than 4 true leaves) are shown (3 biological repeats, each with N > 45
1244 seedlings). Three independent WT lines expressing the *pRAD51::DMC1* transgene were

1245 tested (T1-1, T1-2 and T1-3).

1246 **(F-G) MMC sensitivity of *RAD51-GFP* lines overexpressing *DMC1*. (F)** Pictures of two-
1247 week-old seedlings grown with 20 µM MMC. **(G)** Sensitivity of the seedlings was scored after
1248 2 weeks and the fractions of sensitive plants (plants with less than 4 true leaves) are shown.
1249 Three independent *RAD51-GFP* lines overexpressing *DMC1* were tested (N > 45) and all three
1250 lines showed strong hypersensitivity to MMC. The RAD51-GFP fusion protein forms RAD51
1251 filaments that support the activity of DMC1 in meiosis [39] but this is not sufficient for DMC1
1252 mitotic activity.

1253

1254 **Supplemental Figure 4. Sequence alignment of DMC1 proteins** from *Saccharomyces*
1255 *cerevisiae* (ScDMC1) and *Arabidopsis thaliana* (AtDMC1) and the corresponding mutated
1256 AtDMC1-II3A variant. The alignment was generated using ClustalW. Numbers indicate amino
1257 acid positions. Red letters indicate the three conserved amino acids (R136A, R308A, K318A
1258 in Arabidopsis) that have been mutated in yeast to generated *dmc1-II3A*. Under the
1259 sequences, asterisks, colons and full stops indicate identical, conserved and semi-conserved
1260 residues respectively.

1261

1262 **Supplemental Figure 5. DMC1 protein expression in transgenic seedlings**
1263 **(A)** DMC1g and DMC1-II3A protein are induced by MMC treatment. Total proteins were
1264 extracted from 1-week-old seedlings treated or not with 30 µM MMC for 8 hours and DMC1
1265 abundance measured. No DMC1-specific band (37 kDa) is observed in untreated plants in
1266 both wild-type and transgenic plants. In contrast, while DMC1 is still absent in wild-type plants
1267 after DNA damage treatment, it becomes clearly visible in transgenic plants expressing either
1268 *DMC1g* or *DMC1-II3A*. **(B)** Ponceau Red staining of the above membrane before incubation
1269 with anti-DMC1 antibody, showing equal loading.

1270

1271 **Supplemental Figure 6. DMC1-II3A variant does not complement meiosis of *dmc1***
1272 **mutant and does not interfere with RAD51-mediated repair of meiotic DBS.**

1273 **(A)** Schematic representation of the *pDMC1::DMC1-II3A* construct. Exons are shown as blue
1274 rectangles. **(B)** Expression of the *pDMC1::DMC1II3A* in *dmc1* mutants do not restore fertility.
1275 Left panel : Wild-type plants have long siliques full of seeds, while *dmc1* mutants have very
1276 low fertility. Right panel shows the number of seeds per silique in DMC1, *dmc1* mutant and
1277 two *dmc1* + *pDMC1::DMC1-II3A* independent transformants. Mean is shown as black
1278 horizontal bar; N = 20 to 25 siliques per plant.
1279 **(C)** DAPI staining of chromosomes during meiosis. Wild-type cells show pairing and synapsis
1280 of homologous chromosomes in pachytene, five bivalents at metaphase I and two groups of
1281 five chromosomes at anaphase I. *dmc1* mutants exhibit defective synapsis but ten intact
1282 univalent at metaphase I, which randomly segregate at anaphase I. A similar phenotype is
1283 observed in *dmc1* plants expressing *pDMC1::DMC1-II3A.* (Scale Bar: 10 µm).
1284
1285 **Supplemental Figure 7. Absence of DMC1 focus in transgenic plants expressing DMC1-**
1286 **II3A and impaired synapsis**
1287 **(A)** Male meiocytes stained with ASY1 antibody (red) and DMC1 antibody (green). In wild-type
1288 meiocyte, numerous DMC1 foci are visible on chromosome axes. In contrast, *dmc1* plants
1289 expressing DMC1-II3A lack DMC1 focus. (Scale bar: 5µm). **(B)** Male meiocytes stained with
1290 the ASY1 antibody (red) and the ZYP1 antibody (green). In wild-type pachytene, ZYP1 extends
1291 along the entire length of the chromosomes. In *dmc1* plants expressing DMC1-II3A, ZYP1
1292 staining is restricted to a few foci and short stretches. (Scale bar: 5µm).
1293
1294 **Supplemental Figure 8. Expression of DMC1-GFP fusion protein has a dominant**
1295 **negative effect and impairs RAD51-mediated repair of meiotic DBS.**
1296 **(A)** Schematic representation of the *pDMC1::DMC1-GFP* construct. **(B)** Wild-type plants have
1297 long siliques, full of seeds, while *dmc1* mutants are nearly sterile. Expression of
1298 *pDMC1::DMC1-GFP* in *dmc1* mutants does not restore fertility and even strongly reduces
1299 fertility when expressed in *rad51+/-* or WT plants. **(C)** DAPI staining of chromosomes during
1300 meiosis. Wild-type cells show pairing and synapsis of homologous chromosomes in

1301 pachytene, five bivalents at metaphase I and two groups of five chromosomes at anaphase I.
1302 *rad51* mutants exhibit defective synapsis and extensive chromosome fragmentation. Meiotic
1303 spreads in DMC1+/+ RAD51+/+ plants expressing *DMC1-GFP* revealed a *dmc1-like*
1304 phenotype with defective synapsis but ten intact univalents at metaphase I, which randomly
1305 segregate at anaphase I. In contrast, extensive fragmentation is observed in DMC1+/+
1306 RAD51+/- plants expressing *DMC1-GFP*. (Scale Bar: 10 μm).
1307
1308 **Supplemental Figure 9. Expression of DMC1-GFP fusion protein in meiocytes**
1309 **(A) Immunolocalisation of DMC1 in *DMC1-GFP* plants.** Male meiocytes stained with DAPI
1310 (blue), anti-AtASY1 antibody (red), and anti-DMC1 antibody (green). Numerous DMC1 foci are
1311 visible on the chromosomes in WT plants expressing *DMC1-GFP* but not in *dmc1-/-*_DMC1-
1312 GFP. (Scale bar = 10 μm).
1313 **(B-C) *In vivo* observation of DMC1-GFP fluorescence in meiocytes.**
1314 DMC1-GFP fluorescence is observed in nuclei of DMC1-GFP transgenic plants. (Scale bar =
1315 50 μm in B and 10 μM for close-up view in C).
1316
1317 **S1 Table. List of primers used in this study**
1318
1319 **S1 Data. Raw data for fertility, recombination test and RAD51 foci countings.** These are
1320 numerical data used for figures and that support the findings of this study.