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1 **Bread Wheat TaSPO11-1 exhibits evolutionary conserved function in meiotic recombination**  
2 **across distant plant species.**

3

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## 27 **Summary – Abstract (250 words)**

28 The manipulation of meiotic recombination in crops is essential to rapidly develop new plant varieties,  
29 producing more in a sustainable manner. One option is to control the formation and repair of the  
30 meiosis-specific DNA double strand breaks (DSBs) that initiate recombination between the  
31 homologous chromosomes and ultimately lead to crossovers. These DSBs are introduced by the  
32 evolutionarily conserved topoisomerase-like protein SPO11 and associated proteins. Here, we  
33 characterized the homoeologous copies of the SPO11-1 protein in hexaploid bread wheat (*Triticum*  
34 *aestivum* L.). The genome contains three *SPO11-1* gene copies that exhibit 93-95% identity at the  
35 nucleotide level and clearly, the A and D copies originated from the diploid ancestors *Triticum urartu*  
36 and *Aegilops tauschii*, respectively. Further, phylogenetic analysis of 105 plant genomes revealed a  
37 clear partitioning between monocots and dicots, with the seven main motifs being almost fully  
38 conserved even between clades. The functional similarity of the proteins among monocots was  
39 confirmed through complementation analysis of the rice *spo11-1* mutant by the wheat *TaSPO11-1-5D*  
40 coding sequence. Also, remarkably, albeit the wheat and Arabidopsis SPO11-1 proteins share only  
41 55% identity and the partner proteins also differ, the *TaSPO11-1-5D* cDNA significantly restored the  
42 fertility of the Arabidopsis *spo11-1* mutant indicating a robust functional conservation of the SPO11-1  
43 protein activity, across distant plants. These successful heterologous complementation assays, using  
44 the Arabidopsis and rice hosts, are good surrogates to validate the functionality of candidate genes and  
45 cDNA, as well as variant constructs, when the transformation and mutant production in wheat is much  
46 longer and tedious.

## 47 **Significance statement (69 words)**

48 We analysed the three homoeologous copies of *SPO11-1* genes in the bread wheat genome. They are  
49 very similar but not identical, revealing their parental origin from diploid wheats. These SPO11-1  
50 proteins contain the seven essential and evolutionary conserved motifs, now found in 105 plant  
51 species. The coding sequence of the wheat D-copy significantly complemented the rice and

52 *Arabidopsis spo11-1* mutants indicating a strong functional conservation between highly divergent  
53 species.

54 **Keywords**

55 SPO11-1; Meiotic recombination; Protein evolution; Wheat; Grasses.

56

## 57 **Introduction**

58 The improvement of genetic diversity in cultivated species is of utmost importance especially towards  
59 the development of a sustainable agriculture using for example lower amounts of water, fertilizers,  
60 pesticides or fungicides. One way to reach this challenge is explore the tremendous gene resources that  
61 already exist in nature. This can be achieved by crossing the elite lines with related and/or exotic  
62 varieties bearing advantageous alleles of agronomical interest. Such genetic admixture occurs  
63 naturally through meiotic recombination and gamete formation. The mechanisms of meiotic  
64 recombination leading to crossover products (COs) have long been studied in model organisms,  
65 including plants (reviewed by (Mercier *et al.* 2015)). Importantly, crossover recombination not only  
66 generates new combinations of parental alleles, but also ensures the faithful distribution of the  
67 homologous chromosomes during the reductional meiotic division (MI), key to the success of sexual  
68 reproduction and fertility (Hunter 2015, Mercier, *et al.* 2015).

69 Meiotic recombination is initiated by the formation of programmed DNA double-strand breaks (DSBs)  
70 catalysed by the topoisomerase-like protein SPO11 (Bergerat *et al.* 1997, Keeney *et al.* 1997); for  
71 reviews, see (de Massy 2013, Lam and Keeney 2014, Robert *et al.* 2016b)). Resembling the archaeal  
72 topoisomerase VI complex, SPO11 (ortholog of the archaea A subunit) is likely working in a protein  
73 complex with one or several other proteins (Lam and Keeney 2014), in particular with the MTOPVIB  
74 protein (orthologous to the archaea B subunit) to form an active catalytic complex (Figure 1) (Robert  
75 *et al.* 2016a, Vrielynck *et al.* 2016).

76 *SPO11* is evolutionarily conserved among sexually reproducing organisms and is encoded by a single  
77 gene in most organisms (Malik *et al.* 2007). However, there are several *SPO11* genes in plant  
78 genomes. The Arabidopsis genome carries three related genes named *SPO11-1*, *SPO11-2* and *SPO11-*  
79 *3* (Hartung and Puchta 2000, Grelon *et al.* 2001, Hartung and Puchta 2001, Sprink and Hartung 2014).  
80 Both *SPO11-1* and *SPO11-2* are essential for meiotic recombination (Grelon, *et al.* 2001, Hartung and  
81 Puchta 2001, Stacey *et al.* 2006, Hartung *et al.* 2007) while *SPO11-3* is involved in somatic endo-  
82 reduplication (Hartung *et al.* 2002, Sugimoto-Shirasu *et al.* 2002, Yin *et al.* 2002). The situation in rice  
83 (*Oryza sativa*) is even more complex with five *SPO11*-related genes (Jain *et al.* 2006, Yu *et al.* 2010,

84 An *et al.* 2011). Of these rice genes, to date only *OsSPO11-1* and *OsSPO11-2* have been clearly  
85 demonstrated to be required for meiotic recombination (Yu, *et al.* 2010, Fayos *et al.* 2019). The  
86 interactions between SPO11-1, SPO11-2 and MTOPVIB proteins have recently been described in  
87 Arabidopsis, rice and mice (Figure 1) (Fu *et al.* 2016, Robert, *et al.* 2016a, Vrielynck, *et al.* 2016, Xue  
88 *et al.* 2016).

89 Another layer of complexity in meiosis in plants is polyploidy, a common feature of plant kingdom,  
90 which concerns about 30% of existing flowering plants (Wood *et al.* 2009), including most of the  
91 world's important crops. Polyploid species can be divided into autopolyploid species derived from  
92 whole genome duplication, such as potato (*Solanum tuberosum*, 4x), strawberry (*Fragaria ananassa*,  
93 8x), and allopolyploid species originating from crosses between closely related species such as oilseed  
94 rape (*Brassica napus*,  $2n = 4x = 38$ , AACC), cotton (*Gossypium arboreum*,  $2n = 4x = 52$ , AADD) or  
95 tobacco (*Nicotiana tabacum*,  $2n = 4x = 48$ ). Allopolyploid species thus contain different sets of related  
96 but not completely homologous chromosomes called homoeologues. It is now clearly established that  
97 all flowering plants have experienced at least one and usually several rounds of polyploidy (also called  
98 whole genome duplications; WGD) during the course of their evolution (Soltis *et al.* 2009, Van de  
99 Peer *et al.* 2009). Thus, other angiosperms, including Arabidopsis, rice or Brassica crops, are ancient  
100 polyploids (paleo-polyploids) that returned to a functionally diploid state by a massive elimination of  
101 some but not all duplicated genes post-WGD (a process called fractionation; (Doyle *et al.* 2008)).  
102 Given the prevalence of WGD in plants, it is critical to integrate and extend our knowledge of  
103 important biological processes such as meiotic recombination into the field of polyploidy. From this  
104 point of view, (Lloyd *et al.* 2014) showed that meiotic genes return to a single copy more rapidly than  
105 genome-wide average in Angiosperms. Analysis of the presence and expression of meiotic genes in  
106 two recent polyploid species (oilseed rape and bread wheat; ~10,000 years ago) suggested that their  
107 loss is passive and is a long-term process.

108 Among allopolyploid species, hexaploid bread wheat (*Triticum aestivum* L.;  $2n = 6x = 42$ ) is derived  
109 from two successive interspecific crosses (Blake *et al.* 1999, Huang *et al.* 2002). These involved the  
110 three diploid species *T. monococcum* ssp *urartu* (AA), a yet unknown species related to the *Sitopsis*  
111 section (BB; the closest one being *Aegilops speltoides*; S genome) and *Aegilops tauschii* (DD). The

112 recent release of the anchored and annotated wheat whole-genome sequence (IWGSC 2018) thus  
113 revealed that more than 60% of the 39,474 genes are present in triplicates and more than 90% have at  
114 least two homoeologous copies.

115 The large size (17 Gb) and complexity of the hexaploid wheat genome makes it challenging for  
116 functional analyses of gene, in particular for gene involved in complex biological complex such as  
117 meiosis. Until now, only a dozen genes (*TaRAD51*, *TaRAD51C*, *TaRAD51D*, *TaDMC1*, *TaMRE11*,  
118 *TaRAD50*, *TaASY1*, *TaZYP1*, *TaPHS1*, *TaPh1*, *TaREC8*, *TaRECQ-7*), among more than 100 known as  
119 involved in meiotic recombination in plants, have been cloned and significantly analysed in wheat.  
120 These analyses have mainly been limited to comparisons of sequences of homoeologous copies,  
121 expression analyses and immunolocalization (Boden *et al.* 2007, de Bustos *et al.* 2007, Boden *et al.*  
122 2009, Devisetty *et al.* 2010, Perez *et al.* 2011, Khoo *et al.* 2012a, Khoo *et al.* 2012b, Ma *et al.* 2018,  
123 Gardiner *et al.* 2019). *TaPh1*, which controls homoeologous recombination in wheat, remains the best-  
124 defined locus involved in meiosis in wheat (Griffiths *et al.* 2006, Moore 2014, Martin *et al.* 2017).

125 To date, there is no study analysing *SPO11-1* from a polyploid species. *SPO11-1* is a key gene at the  
126 onset of the recombination process. It is therefore essential to understand how it behaves in a  
127 polyploid context such as that of bread wheat. In this work, we wanted to know to what extent wheat  
128 *SPO11-1* homoeologous copies (TaSPO11-1-5A, TaSPO11-1-5B and TaSPO11-1-5D) are conserved  
129 between each other and with those of other plants. We thus sought to identify the hexaploid wheat  
130 *SPO11-1* gene and to assess its functionality during meiosis. We isolated the homoeologous (A, B and  
131 D) copies of *TaSPO11-1* and performed a phylogenetic analysis with *SPO11-1* from different species.  
132 The three copies were compared in detail with those of Arabidopsis and Rice to estimate their  
133 conservation. We extend these comparisons in demonstrating the functionality and evolutionary  
134 conservation of wheat TaSPO11-1 through heterologous complementation of the corresponding  
135 Arabidopsis and Rice mutant lines, i.e. across distant plant species.

136

## 137 **Results**

### 138 ***SPO11-1* is conserved between the three homoeologous genomes of the hexaploid** 139 **wheat.**

140 To identify the orthologous copies of *SPO11-1* within the bread wheat genome, we used the newly  
141 released wheat genome sequence (IWGSC 2018) and the Arabidopsis *SPO11-1* protein sequence as a  
142 query (At3g13170). Three copies were identified, one on each of the three homoeologous genomes  
143 from group 5: TraesCS5A02G391400, TraesCS5B02G396300 and TraesCS5D02G401100 further  
144 named *TaSPO11-1-5A*, *TaSPO11-1-5B* and *TaSPO11-1-5D*, respectively. The length of the loci was  
145 similar with approximately 3.9 kb for the genomic sequences and 1.2 kb for the coding sequences  
146 (CDS) (Table 1). The gene structures, previously described for land plants (Sprink and Hartung 2014)  
147 with 15 exons and 14 introns, are identical and exhibit 93-95% identity including the introns (98% for  
148 the coding sequences). The three coding sequences which share 99% identity between each other  
149 encode proteins of 387, 386 and 387 amino acids, respectively (Table 1; Figure S1). The single Amino  
150 acid Polymorphism (SAP) mapped at position 45 and 74 for the 5A copy, 189 and 291 for the 5B copy  
151 and 64 and 66 for the 5D copy. In addition, the 5B copy carries an in-frame deletion of 3 nucleotides,  
152 removing an Alanine amino acid at position 72 (Figure S1).

153

### 154 **The *SPO11-1* genes are conserved between wheat and wheat ancestors.**

155 We then sought to assess the sequences of the bread wheat *SPO11-1* copies with their ancestors. The  
156 ancestor of the B genome remains unknown but the wheat *SPO11-1-5A* and 5D sequences could be  
157 compared with their respective ancestors *Triticum urartu* and *Aegilops tauschii*, respectively. The  
158 *Triticum urartu* (TRIUR3\_12346) and the *Aegilops tauschii* (LOC109743941) *SPO11-1* gene and the  
159 corresponding CDS sequences are reported in Table 1. The *Triticum urartu* and *Aegilops tauschii*  
160 *SPO11-1* genes share the conserved structure of 15 exons and 14 introns. Then, we re-annotated these  
161 sequences manually using the newly released wheat sequence as a basis for splicing sites and obtained  
162 two newly annotated CDS sequences of 1173 bp for *Triticum urartu* and 1164 bp for *Aegilops tauschii*  
163 encoding two proteins of 390 and 387 aa, respectively (Table 1).



164 Close comparison of the wheat TaSPO11-1-5A and -5D CDS and protein sequences with their  
165 ancestors showed that wheat TaSPO11-1-5D and *Aegilops tauschii*'s are 100% identical while  
166 TaSPO11-1-5A and *Triticum urartu*'s SPO11-1 share 99% identity (Figure S2). Furthermore, the SAP  
167 Cys/Ser at position 64 and Ser/Asp at position 66 between *Triticum urartu* and *Aegilops tauschii*'s  
168 SPO11-1 are conserved between the wheat A and D genomes (see above; Figures S1 and S2). These  
169 results suggest that the wheat SPO11-1 sequence has not changed since the polyploidization event.  
170 The extreme conservation of the homoeologous *SPO11-1* genes and proteins within the Triticeae tribe  
171 suggests that they are all functional and presumably under a strong selection pressure in regard to their  
172 essential role in meiosis. In accordance with this, expression data showed that all three homoeologous  
173 genes are equivalently expressed during meiosis (Martin *et al.* 2018).

174

175 **The SPO11-1 proteins are highly conserved and shares key protein domains**  
176 **throughout plant kingdom.**

177 To get deeper insight into the evolution of SPO11-1 within plants, we first compared the *Arabidopsis*  
178 *thaliana* and wheat SPO11-1 amino acid sequences (Figure S3). We found significant identity (54-  
179 55%) similar to the BLAST-P analysis (e-value = 4e-156) (Table 1; Figure S3). This relies on the  
180 presence of seven highly conserved motifs present on the archaeobacterial subunit A of topoisomerase  
181 VI (Bergerat, *et al.* 1997, Keeney, *et al.* 1997, Diaz *et al.* 2002, Malik, *et al.* 2007), *Arabidopsis*  
182 *thaliana* (Hartung and Puchta 2000, Hartung, *et al.* 2007, Shingu *et al.* 2010), wheat (Figure 2 and  
183 Figure S3), and many other organisms (Malik, *et al.* 2007). We note that the first motif that contains  
184 the catalytic Tyrosine residue for DSB formation (Tyr103 in *Arabidopsis*) (Bergerat, *et al.* 1997) is  
185 located at position 129 in the SPO11-1 A and D homoeologous wheat copies and at position 128 in the  
186 B copy. The second motif contains the invariant Arginine 130 (R156 in the A and D copies and R155  
187 in the B copy) that is essential for the function of SPO11-1 *in vivo* (Diaz, *et al.* 2002, Shingu, *et al.*  
188 2010). The fourth motif contains conserved residues implicated in TopoVI DNA binding activity  
189 (Glycine 215 and Arginine 222, 223 and 226 in *Arabidopsis*) (Shingu, *et al.* 2010). Finally, the  
190 seventh motif (10 amino acids) is fully conserved between *Arabidopsis* and wheat (Figure 2 and

191 Figure S3). This Toprim domain contains two conserved residues invariant in all species examined in  
192 this study (Lysine 332 and Glutamic acid 334 in Arabidopsis) that affect DSB formation in yeast when  
193 mutated (Diaz, *et al.* 2002). Interestingly, a 3D predictive structural modelling of the TaSPO11-1-5D  
194 protein shows that all seven motifs are linked to each other (Figure 2). This also shows that the  
195 essential DNA binding (5Y-CAP) and cleavage (Toprim) domains are clearly physically  
196 distinguishable from one another (Figure 3). Altogether, these analyses indicate that all SPO11 key  
197 residues and domains are conserved in the wheat homoeologous SPO11-1 proteins.

198 Then to extend our analysis to other plant species, we retrieved 155 SPO11-1 protein sequences from  
199 153 plant species including monocots and dicots. A curation step based on the accuracy of the ATG  
200 and STOP codon position, the splicing and the integrity of the sequences allowed to retain and aligned  
201 107 robust sequences from 105 species (Supplementary material S4). The amino acid sequence  
202 similarity ranged from 51% (*Cucumis melo*) to 100% (*Aegilops tauschii*). Compared to the wheat  
203 SPO11-1-5D copy, the mean similarity between these plant SPO11-1 proteins reach 65%. They all  
204 contain the seven most conserved motifs that landmark the SPO11 orthologs. We thus built a  
205 consensus sequence for each motif based on the alignment of the sequences (Figure 4) and calculated  
206 the average identities of each motif from the 107 sequences (Figure 4B). The motifs show very strong  
207 conservation with identities of 90.3, 89.1, 89.6, 93.3, 95.1, 99.1 and 96.5% for motifs 1, 2, 3, 4, 5, 6  
208 and 7, respectively (Figure 4B). In particular, the active residues described above are fully conserved,  
209 except the last arginine in motif 4 (Arg226 in Arabidopsis) that is variant in 11/105 (10.5 %) of the  
210 sequences. Among these, the six variants with a Glycine instead of Arginine, specifically belong to the  
211 Rosids clade and specifically to the Rosales order. Finally, the alignment of the 107 complete  
212 sequences led us to generate a phylogenetic tree which revealed a perfect separation into two groups  
213 corresponding to monocots (blue, Figure 5) and dicots (red, Figure 5). Interestingly, *Amborella*  
214 *trichopoda*, sister of the angiosperms (flowering plants), is at the frontier of the groups, suggesting it  
215 shares properties of both groups. Altogether, our extensive computational analysis of the SPO11-1  
216 protein sequences in plants highlighted extensive conservation of the 7 key protein sequence motifs as  
217 well as limited evolution since the separation of monocots and dicots.

218

219 **Heterologous expression of the Wheat *TaSPO11-1-5D* coding region restores the fertility**  
220 **of the Rice *spo11-1* mutant.**

221 The strong conservation of SPO11-1 protein sequences across plant species does not, in itself, prove  
222 that they are functionally interchangeable between species, especially given that they form parts of a  
223 multi-protein complex. To address this issue, we sought to determine whether or not the expression of  
224 the bread wheat SPO11-1 protein would rescue the meiotic phenotypes and sterility of the *spo11-1*  
225 mutant of another monocotyledonous plant, namely rice. To perform this heterologous  
226 complementation assay, the wheat *TaSPO11-1-5D* coding sequence placed under the maize Ubiquitin  
227 promoter was introduced in our *Oryza sativa* ssp *japonica* var. Kitaake *Osspo11-1-1* mutant line  
228 generated through CRISPR/Cas9 mutagenesis (see Methods; Fayos et al., unpublished). The *Osspo11-*  
229 *1-1* mutation is a frameshift resulting from a single nucleotide (A) insertion in the ATG sequence  
230 (ATAG). The homozygous *Osspo11-1-1* mutant lines fail to form chiasma during meiosis and are  
231 sterile (Yu, et al. 2010, Fayos, et al. 2019). Functional complementation can thus be easily visualized  
232 as an increase in seed production. Three *spo11-1-1* homozygous plants (out of 33 primary  
233 transformants) carrying a single copy of the *UBI::TaSPO11-1-5D* transgene were obtained by  
234 transformation and remarkably, were fertile (Figure 6A). In the following T1 generation, the  
235 restoration of the rice *spo11-1-1* fertility strictly co-segregated with the presence of the  
236 *UBI::TaSPO11-1-5D* transgene. Indeed, comparison of the number of filled spikelets in wild-type  
237 plants and in the progeny of two transformants grown in similar conditions shows that Rice *spo11-1-1*  
238 mutant plants expressing the *UBI::TaSPO11-1-5D* transgene exhibit a fertility comparable to that of  
239 wild-type plants (Figure 6B). Altogether, these results demonstrate that the *TaSPO11-1-5D* coding  
240 sequence is functional and the wheat protein can functionally replace the rice SPO11-1 in its essential  
241 meiotic function.

242

243 **Heterologous expression of the Wheat *TaSPO11-1-5D* coding region restores the fertility**  
244 **of the Arabidopsis *spo11-1* mutant.**

245 The success of the wheat-rice interspecies complementation prompted us to investigate whether or not

246 the wheat *TaSPO11-1-5D* complements the *spo11-1* meiotic defects in Arabidopsis, a more distant  
247 species belonging to a different clade. In Arabidopsis, the *Atspo11-1* mutant fails to form meiotic  
248 DSBs and exhibits a severe reduction in fertility (Grelon, *et al.* 2001). Thus, we placed the *TaSPO11-*  
249 *1-5D* coding sequence under the control of the Arabidopsis *RAD51* promoter and introduced this  
250 construct into the Arabidopsis *SPO11-1/spo11-1-2* heterozygous plants (Figure 7A). The *RAD51*  
251 promoter is well expressed in Arabidopsis meiocytes (Chen *et al.* 2010, Yang *et al.* 2011, Walker *et al.*  
252 2018) and known to drive successful complementation of other Arabidopsis meiotic mutants (Da Ines  
253 *et al.* 2013). PCR genotyping of the *SPO11-1* locus of 41 *TaSPO11-1* primary transformants showed  
254 that 15 were homozygous for the *spo11-1-2* allele (*spo11-1-2/spo11-1-2*), 15 heterozygous (*SPO11-*  
255 *1/spo11-1-2*) and 11 were wild-type (*SPO11-1/SPO11-1*). Remarkably, 14/15 *spo11-1-2* homozygous  
256 plants carrying the *TaSPO11-1-5D* transgene exhibited 15% to 70% fertility, instead of the 5%  
257 residual fertility observed in the absence of the transgene (Figure 7C and Table S1). Then, we  
258 monitored fertility in the progeny (T2 generation) of four randomly selected T1 plants. Consistently,  
259 the restoration of fertility (30 to 80%) strictly co-segregated with the presence of the transgene (Figure  
260 7D and Table S2). Thus, the heterologous expression of the Wheat *TaSPO11-1-5D* protein is able to  
261 restore fertility in the Arabidopsis *spo11-1* mutant.

262

### 263 **Restoration of the wild-type meiotic progression in the Arabidopsis *spo11-1* mutant** 264 **expressing the *TaSPO11-1-5D* transgene.**

265 In wild-type plants, the meiotic chromosomes condense, recombine and synapse during prophase I  
266 (Figure 8A-E). Full synapsis of the homologs is seen at late prophase I (Figure 8B). The chromosomes  
267 then further condense and five bivalents (homologous chromosome pairs attached together by  
268 chiasmata and sister chromatid cohesion) are observed at metaphase I (Figure 8C). Homologous  
269 chromosomes then segregate to opposite poles to give two sets of five chromosomes at metaphase II  
270 (Figure 8D). Meiosis II then proceeds and gives rise to four balanced haploid nuclei (Figure 8E). In  
271 contrast, the *spo11-1* mutants lack DSBs formation, hence recombination, pairing and synapsis of the  
272 homologs (Figure 8F-G), manifested by the presence of 10 univalents instead of bivalents at

273 metaphase I (Figure 8H). Chromosome mis-segregation eventually produces unbalanced metaphase II  
274 (Figure 8I) and polyads (Figure 8J). In sharp contrast, the cytogenetic analysis of pollen mother cells  
275 from the *spo11-1* plants expressing the *TaSPO11-1-5D* transgene revealed the presence of normal  
276 meiotic figures (Figure 8K-O). In particular, 5 bivalents were readily observed at metaphase I (Figure  
277 8M). Subsequent proper homologous chromosome segregation at anaphase I (Figure 8N) followed by  
278 separation of sister chromatids during the second equational division resulted in 4 balanced meiotic  
279 products (Figure 8O). We note however that in accordance with the partial restoration of the fertility in  
280 the complemented plants, most metaphases I exhibited a mixture of bivalents and univalents (Figure  
281 9A). Thus, to examine whether the expression of two copies of the transgene will quantitatively  
282 improve the faithful progression of meiosis, we characterized two independent transgenic lines  
283 homozygous for the *TaSPO11-1-5D* transgene. Their fertility (~ 40%) remained in the average range  
284 of the single copy transgene lines (line 3 and 36, see Figure 7). Cytologically, in the two  
285 complemented lines, we observed a mean of 1.7 (n=57) and 2.6 (n=74) bivalents per cell instead of  
286 0.05 bivalents/cell (n = 19) in the *spo11-1* mutants and 5 (n=36) in the wild-type meiocytes (Figure 9B  
287 and C). We also noted that a majority of bivalents in the complemented lines exhibited a rod-shaped  
288 structure with a single chiasma, although ring-shaped bivalents with at least two chiasmata were also  
289 observed (10 to 30% of bivalents for line 3 and 36, respectively). Accordingly, in both complemented  
290 lines, the number of chiasmata per cell significantly increased reaching a mean of  $2 (\pm 1.7, n = 57)$  and  
291  $3.6 (\pm 2.6, n = 74)$ , respectively. This is a strong increase compared to the *spo11-1* mutant ( $0.05 \pm 0.2,$   
292  $n = 19$ ), yet lower than wild-type plants ( $9.3 \pm 0.8, n = 36$ ).

293 Altogether, these results demonstrate that the wheat SPO11-1 can functionally replace the absence of  
294 the Arabidopsis SPO11-1 ortholog, substantially restoring meiotic recombination and normal meiotic  
295 progression. Beyond the protein sequence homology, these results demonstrated its evolutionary  
296 conserved function.

297

298 **Meiotic DSBs are formed in *Arabidopsis spo11-1* mutants expressing Wheat *TaSPO11-1-***  
299 ***5D* transgene.**

300 The presence of bivalents and chiasmata indicate that meiotic recombination occurs and hence that  
301 meiotic DSBs are formed in plants expressing *TaSPO11-1-5D* transgene. Given that fertility is not  
302 fully restored, this however suggest that less DSBs might be produced in the complemented lines or,  
303 alternatively, that DSBs are repaired without forming COs. We thus sought to analyse the ability of  
304 *TaSPO11-1-5D* to form DSBs in meiotic cells. We performed immunolocalization of the strand-  
305 exchange protein RAD51 as a marker for DSB formation in both wild-type and *spo11-1 + TaSPO11-1*  
306 complemented plants (line 36). As expected, numerous RAD51 foci were observed in early prophase I  
307 cells of wild-type plants (mean of 92 foci per cell, n = 39; Figure 10). RAD51 foci were also observed  
308 in pollen mother cell nuclei of *spo11-1* mutant plants expressing *TaSPO11-1-5D*. However, a strong  
309 two-fold reduction in RAD51 foci formation was detected in these plants (mean of 45 foci per cell, n =  
310 47; Figure 10). This strongly suggests that DSB levels are reduced in the complemented lines and this  
311 may explain the limited complementation by *TaSPO11-1*.

312

313 **Wheat *SPO11-1-5D* functionally interacts with Arabidopsis *SPO11-2* and *MTOPVIB* to induce**  
314 **meiotic recombination.**

315 Current knowledge suggests that *SPO11-1* does not exhibit DNA cleavage activity alone but acts in a  
316 protein complex, physically interacting with the *SPO11-2* and *MTOPVIB* proteins in Arabidopsis and  
317 functionally related orthologs in other organisms, in order to form an active topoisomerase VI-like  
318 complex that catalyses meiotic DSB formation (Figure 1, Robert *et al.*, 2016; Vrielynck *et al.*, 2016).  
319 So, to determine whether *TaSPO11-5D* also needs the presence of the Arabidopsis *SPO11-2* and  
320 *MTOPVIB* proteins to induce meiotic recombination, we crossed our *spo11-1\_TaSPO11-1-5D*  
321 transgenic plants with Arabidopsis *spo11-2* or *mtopVIB* mutant lines and analysed the fertility of the  
322 double mutants. Clearly, as shown in Figure 11, the presence of the *TaSPO11-1-5D* transgene did not  
323 rescue the sterility of the *spo11-1 spo11-2* and *spo11-1 mtopVIB* double mutant plants. This excludes

324 the possibility that TaSPO11-1 induce DSBs independently of SPO11-2 (non-plant organisms have a  
325 single SPO11 protein) and confirms the need for the MTOPVIB to form DSBs.

326

## 327 **Discussion**

328 **TaSPO11-1 homoeologous copies are highly similar between each other and to those from**  
329 **angiosperms.**

330 To identify *SPO11-1* genes from wheat (*T. aestivum*), we exploited the first assembled and annotated  
331 pseudomolecule sequence of the wheat genome (IWGSC 2018). Using *in silico* assignment of the  
332 Arabidopsis SPO11-1 protein (At3g13170), we readily identified the three homoeologous copies,  
333 mapping on chromosomes 5A, 5B and 5D and indicating good conservation of the protein sequence  
334 between the two species. The three homoeologous wheat copies are highly similar with ~95% identity  
335 at the nucleotide level for the genomic sequences. This is consistent with data from expression of  
336 wheat genes (Ramirez-Gonzalez *et al.* 2018) showing an homoeologous SNP diversity ranging from  
337 95.0% to 97.2% within triads (genes present in only three homoeologous copies).

338 We also observed that the D copy (TaSPO11-1-5D) is identical to the copy from *Ae. tauschii*, the  
339 donor of the D genome, while the A copy (TaSPO11-1-5A) is only very slightly different from that of  
340 *T. monococcum ssp urartu*, the donor of the A genome. Divergence between the A and the B genome  
341 lineages occurred ~7 million years ago (MYA; (Marcussen *et al.* 2014)) while the D genome diverged  
342 from the A and B genomes, 1 to 2 million years after. The two successive polyploidization events  
343 giving rise to *T. aestivum* occurred at least 0.58 to 0.82 MYA for the first one and 0.23 to 0.43 MYA  
344 for the second one. In addition, it is suggested that only a few accessions of *Ae. tauschii* contributed to  
345 the D genome of bread wheat (Giles and Brown 2006).

346 SPO11-1 proteins are characterized by the presence of several conserved domains (Bergerat, *et al.*  
347 1997, Keeney, *et al.* 1997). Accordingly, these seven domains are highly conserved in all the plant  
348 sequences that we examined. In particular, the essential residues for DNA cleavage (Tyr103 in  
349 Arabidopsis) or binding (Gly215, Arg222, Arg223 and Arg226 in Arabidopsis) are highly conserved  
350 although their position changed slightly according to the total size of the protein, which indeed varies

351 among species for yet unknown reasons. A previous study using 42 SPO11-1 sequences from land  
352 plants, but not wheat, indicated that SPO11-1 is highly conserved in plants (Sprink and Hartung 2014).  
353 Here, we analysed SPO11-1 sequences from more than 100 plants, including wheat, and show that  
354 SPO11-1 exhibits high sequence identities. In particular, more than 90% identity was observed in the  
355 most broadly evolutionary conserved functional domains. Overall, our extensive phylogenetic analyses  
356 based on sequence comparison of plant SPO11-1 indicates that this protein evolved slowly and  
357 exhibits an evolutionary pattern consistent with known relationships between plant species.

358

359 **Heterologous complementation analyses reveal functional conservation of the SPO11 complex**  
360 **features.**

361 Beyond the computational analyses of the *SPO11-1* genes, we asked to what extent the function of  
362 SPO11-1 is also evolutionary conserved throughout plants, testing the complementation of the rice and  
363 *Arabidopsis spo11-1* mutants with the *SPO11-1-5D* cDNA from bread wheat. Wheat and rice are  
364 monocots while *Arabidopsis thaliana* is a more distantly related dicot. Remarkably, our data show that  
365 expression of the wheat gene was able to complement both rice and *Arabidopsis* mutants, with full  
366 complementation in the former.

367 Furthermore, our analyses in *Arabidopsis* show that the complementation by TaSPO11-1 restores DSB  
368 formation (RAD51 foci) and recombination (chiasmata) and still requires the presence of the wild-type  
369 *Arabidopsis SPO11-2* and *MTOPVIB* genes. Wheat SPO11-1 and *Arabidopsis SPO11-2* and  
370 *MTOPVIB* are able to interact and form a functional inter-species complex, albeit resulting in only a  
371 partial restoration (10 to 70% with most lines showing 20-40% restoration). Immunolocalization of the  
372 RAD51 recombinase indicates that less DSBs are formed in the complemented plants (50% of wild-  
373 type level in the tested line) and this likely explains the partial complementation.

374 Partial complementation in *Arabidopsis* with *Arabidopsis* clones has been frequently observed, as for  
375 *Arabidopsis SPO11-1* (Xue *et al.* 2018). Many factors could influence the efficacy of  
376 complementation: the use of a recipient T-DNA mutant plant, T-DNA integration, the choice of the  
377 promoter or the use of CDS or genomic sequences. Although this cannot be excluded, we do not think  
378 that reduced DSBs formation in our transgenic plants result from lower expression of *TaSPO11-1*



379 gene. Using the same strategy as for TaSPO11-1, we could show that expression of *TaSPO11-2* cDNA  
380 driven by the RAD51 promoter is able to fully restore fertility of the Arabidopsis *spo11-2* mutant  
381 (Benyahya et al., unpublished). This indicates that RAD51 promoter allows sufficient transcription of  
382 *TaSPO11* genes. Conversely, translation may be affected. This is particularly true since codon usage  
383 bias is well known to be different in monocots and dicots (Plotkin and Kudla 2011, Camiolo *et al.*  
384 2015). However, without specific TaSPO11-1 antibodies this hypothesis cannot be tested. In the  
385 present case of expression in an heterologous species, an additional key factor for the incomplete  
386 complementation and the reduced DSB formation is the amino acid sequence divergence of the  
387 transgenic and endogenous proteins that needs to interact in a multi-protein complex. Sufficient  
388 restoration of the mutant phenotypes has been obtained to conclude on the formation of functional  
389 interactions but the incomplete complementation uncovered subtle deficiencies of interest. For  
390 instance, TaSPO11-1 may be less prone to interact with other DSB-associated proteins and to form an  
391 active complex. In this context, it will be interesting to individually assay the other members of the  
392 SPO11 complex and attempt to co-express in Arabidopsis mutants the wheat TaSPO11-1, TaSPO11-2  
393 and TaMTOPVIB once the likely homoeologous genes and coding regions have been well identified.  
394 It will also be interesting to more extensively analyse amino acid sequence divergence and its effect on  
395 interaction with other essential DSB-associated endogenous proteins.  
396 Eventually, working with hypomorphic mutants of the meiotic SPO11/MTOPVIB complex with  
397 reduced DSBs could be very valuable to better understand the relationship between DSB formation  
398 and CO regulation (CO assurance, homeostasis and interference).

399

## 400 **Conclusion**

401 In this study, we isolated the three wheat homoeologous copies for SPO11-1: TaSPO11-1-5A,  
402 TaSPO11-1-5B, TaSPO11-15D. The three copies are highly similar between each other and with those  
403 from diploid ancestors, *T. urartu* and *Ae. tauschii*, suggesting that all three are functional. SPO11-1  
404 protein is very well conserved across angiosperms with conserved domains. Remarkably, due to the  
405 high level of similarity, TaSPO11-1-5D protein was able to restore the fertility of rice and Arabidopsis

406 *spo11-1* mutants. This also showed that the wheat proteins could be used (and hence further studied)  
407 in other more tractable model plants. This is of particular interest in polyploid species in which the  
408 redundancy of function brought by the homoeologous genes and variant alleles adds an additional  
409 level of genetic complexity in the wild type context, and where the construction of appropriate mutants  
410 remains technically difficult and time-consuming due to the polyploidy of the genome (Ramirez-  
411 Gonzalez, *et al.* 2018).

412

## 413 **Experimental Procedures**

### 414 **Plant material and growth conditions**

415 The rice mutant was obtained through CRISPR/Cas9 genome editing as described in Fayos et al.,  
416 (unpublished). Rice plants were cultivated in controlled conditions with a temperature of 28°C during  
417 the day and 24°C during the night, with 60% hygrometry. The natural light is completed by artificial  
418 sodium light (700µmol/m<sup>2</sup>/s). The *Arabidopsis thaliana spo11-1-2*, *spo11-2-3* and *mtopVIB-2* mutants  
419 used in this work have been described previously (Grelon, *et al.* 2001, Hartung, *et al.* 2007, Vrielynck,  
420 *et al.* 2016). *Arabidopsis* plants were grown under the following standard conditions: seeds were  
421 stratified in water at 4°C for 2 days and grown on soil or in vitro on 0.8% agar plates, 1% sucrose and  
422 half-strength Murashige and Skoog salts (M0255; Duchefa Biochemie). Plants were cultivated in a  
423 greenhouse or a growth chamber with a 16/8 hour light/dark cycle, at 23°C and 60% relative humidity.

424

### 425 **Recovery and synthesis of *TaSPO11-1***

426 Wheat genome D *SPO11-1* DNA sequence (TraesCS5A02G391400) was first retrieved through a  
427 BLAST analysis research on the newly annotated wheat genome sequence (IWGSC 2018) using the  
428 *Arabidopsis thaliana SPO11-1* protein sequence (At3g13170) as an input with basic BLAST  
429 parameters. The annotated CDS sequence was determined using Triannot pipeline (Leroy *et al.* 2012).  
430 CDS sequence was synthesized with a short additional sequence at the 5' end of the gene (coding for  
431 the peptide PEFMAMEAPGIR) and flanked with GATEWAY attB sites. Synthesized product was  
432 inserted into pDONR/Zeo and further verified by sequencing.

433

### 434 **3D modelling and rendering**

435 3D structural model of TaSPO11-1-5D protein was generated by homology modelling on PHYRE2  
436 online pipeline ((Kelley *et al.* 2015), <http://www.sbg.bio.ic.ac.uk/~phyre2/html/page.cgi?id=index>)  
437 with intensive modelling mode and TaSPO11-1-5D full sequence as amino acid sequence. Rendering  
438 was made with PyMOL 2.3.3 software.

439 **Recovery of SPO11-1 protein sequences from multiple plant species, alignment,**  
440 **logo and phylogeny**

441 We retrieved all plant SPO11-1 sequences using Wheat and Arabidopsis SPO11-1 sequences as query  
442 on NCBI BLAST website (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) with nr database. The Barley  
443 SPO11-1 sequence was retrieved using IPK Barley BLAST Server website ([https://webblast.ipk-](https://webblast.ipk-gatersleben.de/barley_ibsc/viroblast.php)  
444 [gatersleben.de/barley\\_ibsc/viroblast.php](https://webblast.ipk-gatersleben.de/barley_ibsc/viroblast.php)) and the Barley AA (HC and LC) Morex v2.0 database.  
445 Multiple Sequence Alignment was done using ClustalW with basic parameters. Motifs logo were built  
446 using WebLogo website (<https://weblogo.berkeley.edu/>) (Crooks *et al.* 2004). Phylogeny was done  
447 with the following settings: ClustalW alignment and PHYLIP Neighbor Joining for the construction of  
448 the tree.

449

450 **Cloning of *TaSPO11-1* and plant transformation**

451 For rice complementation, a LR GATEWAY recombination cassette was inserted in a pZmUBI-tNos  
452 vector at MCS location using BamH1 restriction sites to form a pZmUBI-LR-tNos vector. The  
453 complete *TaSPO11-1* CDS fragment was inserted in this vector under the control of the UBI promoter  
454 using GATEWAY cloning sites. Rice seed embryo-derived callus from line segregating the *Osspo11-*  
455 *1-1* mutation were then transformed accordingly to the method described in (Sallaud *et al.* 2003).  
456 Primary transformants expressing the *TaSPO11-1* transgene were selected on hygromycin selection  
457 medium. Mutation in the ATG of *OsSPO11-1* (Loc\_Os03g54091) was ascertain by PCR (primers:  
458 SPO11-R1 ccaaaattcttgggtgct and SPO11-F2 cggaggagcagtagttctgg) and sequencing. Presence and  
459 integrity of the transgene was also verified by PCR (primers: pUBI-F cttgatatacttgatgatggc and  
460 tNOS-R cgcaagaccggcaacagattc) and sequencing.

461 For Arabidopsis complementation, the complete *SPO11-1* CDS fragment was cloned into the  
462 GATEWAY destination vector pMDC32 (Curtis and Grossniklaus 2003) in which the 35S promoter  
463 was replaced with the Arabidopsis *RAD51* promoter (1031bp upstream of the *RAD51* ATG; (Da Ines,  
464 *et al.* 2013) with a HindIII/AscI digest . The plasmid was then inserted in an *Agrobacterium*  
465 *tumefaciens* C58C1 strain which was subsequently used to transform *Atspo11-1-2* heterozygous

466 mutant plants by the floral dip method (Clough and Bent 1998). T1 seeds from the *Agrobacterium*-  
467 transformed plants were sown on soil and T1 transformants were selected for Hygromycin resistance  
468 on 0.5X MS/ 1% sucrose/ 0.8% agar plates containing 15µg/ml Hygromycin B Gold (InvivoGen).  
469 Presence of the transgene and genotypes of transformants were verified by PCR.

470

### 471 **Arabidopsis male meiotic chromosome spreads**

472 Chromosome spreads were prepared according to (Ross *et al.* 1996). Whole inflorescences were fixed  
473 in ice-cold ethanol/glacial acetic acid (3:1) for 3 x 30 min and stored at -20°C until further use.  
474 Immature flower buds were rinsed twice at room temperature in distilled water for 5 min. This was  
475 followed by two washes in citrate buffer for 5 min. Buds of appropriate size were selected under a  
476 binocular microscope and incubated for 75 to 90 minutes on a slide in 100µL of enzyme mixture  
477 (0.3% w/v cellulase (Sigma), 0.3% w/v pectolyase (Sigma) and 0.3% cytohelicase (Sigma)) in a moist  
478 chamber at 37°C. Each bud was then softened for 1 minute in 15µL of acetic acid (60%) on a  
479 microscope slide at 45°C, fixed with ice-cold ethanol/glacial acetic acid (3:1) and air-dried.  
480 Eventually, slides were mounted in Vectashield mounting medium with DAPI (1.5 µg.mL<sup>-1</sup>; Vector  
481 Laboratories Inc.).

482 For chiasma counting, number of chiasmata at metaphase I was estimated based on bivalent  
483 configuration: rod-shaped bivalents were considered to contain a single chiasma and ring-shaped  
484 bivalents, two (one on each arm) (Sanchez Moran *et al.* 2001).

485

### 486 **Immunolocalization of proteins in pollen mother cells (PMCs)**

487 Spreads of PMCs for immunolocalization of RAD51 were performed as described previously  
488 (Armstrong *et al.* 2002). Primary antibodies used for immunostaining were: anti-ASY1 raised in  
489 guinea Pig (1:500) (Higgins *et al.* 2004) and anti-RAD51 raised in rat (1:500) (Kurzbauer *et al.* 2012).

490

### 491 **Microscopy**

492 All observations were made with a motorized Zeiss AxioImager.Z1 epifluorescence microscope  
493 (Zeiss) using a PL Aplanachromat 100X/1.40 oil objective. Photographs were taken with an AxioCam  
494 MRm camera (Zeiss) driven by ZEN Pro software (Zeiss). Captured images were further processed  
495 and adjusted for brightness and contrast on ZEN Pro and ImageJ/FIJI software.

496

### 497 **Statistical analysis**

498 All graphs and statistical analyses were performed using software GraphPad PRISM 6. To determine  
499 whether differences between two groups were statistically significant, groups were compared using  
500 ordinary one-way ANOVA and Holm-Sidak test to account for multiple comparisons. A *P*-value of  
501 0.05 or less was considered to be statistically significant.

502

503

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511

## 512 **Supplementary Materials Legends**

513 **Figure S1. Sequence alignment of Bread wheat SPO11-1 proteins.** Alignment was generated using  
514 ClustalW. Numbers indicate amino acid positions. Red letters indicate single amino acid  
515 polymorphism and blue letters denote amino acid deleted in the B genome. Identical residues are  
516 highlighted in black and asterisks, colons and dots under the sequences, indicate identical, conserved  
517 and semi-conserved residues, respectively.

518

519 **Figure S2. Sequence alignment of SPO11-1 proteins from bread wheat and ancestors.** Alignment  
520 of SPO11-1 from genome A, D, and their ancestors *Triticum urartu* and *Aegilops tauschii* was  
521 generated using ClustalW. Numbers indicate amino acid positions. Amino acid highlighted in cyan  
522 and yellow designate single amino acid polymorphism.

523

524 **Figure S3. Sequence alignment of bread wheat and Arabidopsis proteins.** Alignment was  
525 generated using ClustalW. Numbers indicate amino acid positions. Red squares with roman numerals  
526 indicate the conserved motifs. Identical residues are highlighted in black and asterisks, colons and dots  
527 under the sequences, indicate identical, conserved and semi-conserved residues, respectively.

528

529 **Figure S4. Sequence alignment of 107 plant SPO11-1 proteins.** Alignment was generated using  
530 ClustalW 2.0. Numbers indicate amino acid positions. Asterisks, colons and dots under the sequences,  
531 indicate identical, conserved and semi-conserved residues, respectively.

532

533 **Table S1.** Seed number per silique in wild-type, *Atspo11-1* and *Atspo11-1-TaSPO11-1* primary  
534 transformants. 10 to 12 fruits were counted per plant. (n.d. : not determined). P-value were calculated  
535 using ordinary one-way ANOVA and Holm-Sidak test to account for multiple comparisons.

536

537 **Table S2.** Seed number per silique in wild-type and in the progeny of *Atspo11-1-2-TaSPO11-1*  
538 transformants. Seeds were counted in 4 plants per genotype and 8 fruits per plant. (n.d. : not  
539 determined). P-value were calculated using ordinary one-way ANOVA and Holm-Sidak test to  
540 account for multiple comparisons.

541



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729

730

731 **Table**732 **Table 1:** Wheat, its ancestors and *Arabidopsis thaliana* *SPO11-1* gene characteristics. gDNA:

733 genomic DNA; CDS: coding sequence; bp: base pair; aa: amino acid.

Gene	Species	Gene ID	gDNA (bp)	CDS (bp)	Nb of exons	Protein ID			protein (aa)	Annotation status
						NCBI	Genbank	Uniprot		
<i>SPO11-1-5A</i>	<i>T. aestivum</i>	TraesCS5A02G391	3937	1164	15	-	-	A0A3B6	387	newly
		400						KM14		annotated
<i>SPO11-1-5B</i>	<i>T. aestivum</i>	TraesCS5B02G396	3928	1161	15	-	-	A0A3B6	386	newly
		300						LTD1		annotated
<i>SPO11-1-5D</i>	<i>T. aestivum</i>	TraesCS5D02G401	3970	1164	15	-	-	A0A3B6	387	newly
		100						MY04		annotated
<i>SPO11-1</i>	<i>T. urartu</i>	TRIUR3_12346	3946	1161	15	-	EMS541 33.1	M7YTX6	390	reannotated
<i>SPO11-1</i>	<i>Aegilops tauschii</i>	LOC109743941	3970	1164	15	XP_0201 58624.1	-	-	387	reannotated
<i>AtSPO11-1</i>	<i>A. thaliana</i>	At3G13170	2812	1089	15	NP_1879 23.1	-	Q9M4A2	362	unmodified

734

735

736

## 737 **Figures Legends**

### 738 **Figure 1: Schematic representation of the putative SPO11 meiotic DSB catalytic complex.**

739 This protein complex is suggested to be an heterotetramer composed of one heterodimer (SPO11-1  
740 and SPO11-2; green and blue, respectively) and one homodimer (MTOFVIB; orange) (Robert, *et al.*  
741 2016b).

742

### 743 **Figure 2: Protein sequence alignment of *Triticum aestivum* and *Arabidopsis thaliana* SPO11-1.**

744 Only the seven conserved motifs are shown. Conserved amino acids are highlighted in green and  
745 similar amino acids are highlighted in yellow. Catalytically active tyrosine and conserved glycine and  
746 arginine involved in DNA-binding activity are in red.

747

### 748 **Figure 3: 3D modelling of TaSPO11-1-5D protein.**

749 A 3D structural model of SPO11-1 obtained by sequence homology modelling with PHYRE2 online  
750 pipeline. This SPO11-1 model consists of amino acid residues 1 to 387. 336 residues over 387 (87%)  
751 were modelled with >90% accuracy. A and B are mirror views of the structural model rendered with  
752 PyMol software. The seven conserved motifs are shown in green, the Toprim domain in light pink and  
753 the 5Y-CAP domain in light yellow. Catalytic tyrosine is depicted in magenta and the glycine and  
754 arginine essential for DNA-binding activity are depicted in red and blue, respectively.

755

### 756 **Figure 4: Sequence and conservation level of the seven conserved motifs in SPO11-1 proteins**

757 **from 105 species. (A)** Consensus sequence of the seven conserved motifs extracted from 107 SPO11-  
758 1 sequences. **(B)** Conservation level of the seven motifs within 107 SPO11-1 sequences. White boxes:  
759 lower and upper quartile of conservation rate for each motif. Black lines: mean conservation rate for  
760 each motif. Dashed lines: maximum and minimum conservation rate values. Circles: outliers.

761

### 762 **Figure 5: Phylogenetic tree of plant SPO11-1 proteins from 105 species.**

763 Dicotyledons are shown in red, monocotyledons in blue and *Amborella trichopoda*, sister of the  
764 angiosperms, is shown in purple.

765

766 **Figure 6: Wheat *TaSPO11-1-5D* restores fertility of the Rice *spo11-1* mutant.**

767 (A) Pictures of rice *spo11-1* mutant plants and panicles expressing or not the wheat *TaSPO11-1-5D*  
768 transgene. Rice *spo11-1* plants are sterile and develop panicles with empty spikelets (right panel). In  
769 contrast, *spo11-1* mutant plants expressing wheat *TaSPO11-1-5D* are fertile and develop panicles with  
770 filled spikelets (left panel). (B) Percentage of filled spikelet per panicle in wild-type plants and  
771 progeny of two *spo11-1* transformants expressing wheat *TaSPO11-1-5D* (T2 and T64). In the box and  
772 whiskers, each dot represents the percentage of filled spikelet per panicle in one plant (n = 6 for wild-  
773 type plants, n = 5 for *spo11-1*, and n = 5 for *spo11-1 TaSPO11-1-5D* plants). Means are represented by  
774 a + and horizontal bars denote medians.

775

776 **Figure 7: Wheat *TaSPO11-1-5D* restores fertility of the Arabidopsis *spo11-1* mutant**

777 (A) Schematic representation of the *pRAD51:TaSPO11-1* construct. (B) Wild-type plants have long  
778 siliques full of seeds, while *Atspo11-1* mutants are sterile and exhibit very short siliques. Expression of  
779 the *TaSPO11-1* in *Atspo11-1* mutants restores fertility. (C) number of seeds per silique in Wild-type,  
780 *Atspo11-1*, and 15 *Atspo11-1 + pRAD51:TaSPO11-1* independent primary transformants. Each dot  
781 represents the number of seeds in one silique. (D) number of seeds per silique in Wild-type, and T2  
782 progeny of 4 *Atspo11-1 + pRAD51:TaSPO11-1* independent primary transformants (T3, T12, T13 and  
783 T36 as indicated under graph). Each dot represents the number of seeds in one silique. Blue dots show  
784 number of seeds per silique in wild-type, red dots *Atspo11-1* mutants and black dots represent  
785 *Atspo11-1* mutants expressing *TaSPO11-1*.

786

787 **Figure 8: Meiotic progression in wild-type, *Atspo11-1* mutants and *Atspo11-1* mutants**  
788 **complemented with wheat *TaSPO11-1*.**

789 DAPI staining of chromosomes during meiosis in Arabidopsis (A-E) wild-type, (F-J) *spo11-1* and (K-  
790 O) *spo11-1 + TaSPO11-1* plants. (A, F, K) Early prophase I, (B, G, L) Late prophase I, (C, H, M)

791 Metaphase I, **(D, I, N)** Metaphase II, and **(E, J, O)** Tetrad. In wild-type, cells show pairing and  
792 synapsis of homologous chromosomes at late prophase I **(B)**, five bivalents at metaphase I **(C)**, two  
793 groups of five chromosomes at Metaphase II **(D)** and balanced tetrads **(E)**. *spo11-1* mutants exhibit  
794 defective synapsis **(G)**, univalent in Metaphase I **(H)** and unbalance Metaphase II **(I)** and polyads **(J)**.  
795 In *spo11-1* expressing wheat TaSPO11-1 **(K-O)**, wild-type meiotic figures can be observed. (Scale  
796 Bar: 10  $\mu$ m).

797

798 **Figure 9: Expression of wheat *TaSPO11-1* in *Atspo11-1* mutant promotes bivalent formation**

799 **(A)** Representative images of Metaphase I are shown (Scale Bar: 10  $\mu$ m).

800 **(B)** Mean number of bivalents (dark grey) and pairs of univalent (grey) per meiosis

801 **(C)** Bivalents per cell (in percentage). *Atspo11-1* plants expressing *TaSPO11-1* show a significant  
802 increase of bivalent formation when compared to *Atspo11-1* mutants. Number of cells analyzed is  
803 indicated in parentheses.

804

805 **Figure 10: Reduced numbers of RAD51 foci in *Atspo11-1* mutants complemented with wheat**  
806 ***TaSPO11-1*.**

807 **(A)** Immunolocalization of RAD51 (green) and the chromosome axis protein ASY1 (red) on  
808 leptotene/zygotene meiotic chromosome spreads. (Scale Bars: 5  $\mu$ m). **(B)** Quantification of RAD51  
809 foci per positive cell throughout prophase I in both wild-type and *Atspo11-1* mutants expressing  
810 *TaSPO11-1* (T36). (p-value < 0.0001, Mann-Whitney test).

811

812 **Figure 11: Complementation of *Atspo11-1* by *TaSPO11-1* requires presence of *AtSPO11-2* and**  
813 ***AtMTOFVIB*.**

814 **(A)** Pictures of siliques from wild-type, and mutant plants expressing or not *TaSPO11-1*. Genotype of  
815 the mutants are indicated above pictures. Fertile plants have long siliques while sterile plants have  
816 short siliques. Fertility of *Atspo11-1* + *TaSPO11-1* is lost by deletion of either *AtSPO11-2* or  
817 *AtMTOFVIB*. **(B)**: Mean number of seeds per silique. Each dot represents the number of seeds in one  
818 silique.



