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# 1 The molecular signatures of compatible and 2 incompatible pollination

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14

15

## 16 **Abstract:**

17 Fertilization in flowering plants depends on the early contact and recognition of pollen grains by  
18 the receptive papilla cells of the stigma. To identify the associated molecular pathways, we  
19 developed a transcriptomic analysis based on single nucleotide polymorphisms (SNPs) present in  
20 two *Arabidopsis thaliana* accessions, one used as female and the other as male. We succeeded in  
21 distinguishing 80 % of transcripts according to their parental origins and drew up a catalog of genes  
22 whose expression is modified after pollen-stigma interaction. Global analysis of our data reveals  
23 that pattern-triggered immunity (PTI)-associated transcripts are upregulated after compatible  
24 pollination. From our analysis, we predicted the activation of the Mitogen-activated Protein Kinase  
25 3 on the female side after compatible pollination, which we confirmed through expression and  
26 mutant analysis. Our work defines the molecular signatures of compatible and incompatible  
27 pollination, highlights the active status of incompatible stigmas, and unravels a new MPK3-  
28 dependent cell wall feature associated with stigma-pollen interaction.

29

## 30 **Introduction:**

31 In flowering plants, the early interaction between the tip of the female organ (stigma) and the  
32 male gametophyte (pollen grain) act as a checkpoint for fertilization. This first step of the female-  
33 male interaction includes recognition by the female tissues of the male partner. In the  
34 Brassicaceae, sophisticated mechanisms have evolved that allow the papilla cells of the stigma to  
35 reject self pollen while accepting non-self pollen. These self/non-self recognition mechanisms are  
36 underlie self-incompatibility and promote genetic variability within species. Following  
37 compatible pollination, the dry pollen grain starts to hydrate on the stigma papilla and ultimately  
38 germinates, producing a tube that penetrates the wall of stigmatic cells and grows down to  
39 convey the male gamete towards the ovules for fertilization<sup>1,2</sup>. By contrast, when a pollen grain is  
40 recognized as incompatible, it fails to hydrate properly and shows defective germination. This  
41 rejection mechanism is initiated by a ligand-receptor interaction and is genetically controlled by a  
42 single polymorphic locus called the S-locus<sup>3</sup>. The S-locus Cysteine Rich protein (SCR)/ S-locus  
43 protein 11 (SP11) located on the pollen surface interacts with its cognate S-locus Receptor  
44 Kinase (SRK) localized to the plasma membrane of the papilla cells<sup>4,5</sup>. This interaction leads to  
45 the phosphorylation of SRK that triggers the downstream cascade leading to pollen rejection<sup>1,5,6</sup>.  
46 Cellular events triggered in the stigma papillae by these two pathways, compatible and  
47 incompatible, have started to be more clearly defined. Compatible pollination induces actin  
48 network orientation, calcium export and polarized secretion towards the pollen grain<sup>7-11</sup>.  
49 Incompatible pollen leads to inhibition of actin polymerization and vesicular trafficking  
50 accompanied by a strong calcium influx within the stigmatic cell<sup>7,12,13</sup>. Stigmatic calcium fluxes  
51 were reported to involve the autoinhibited Ca<sup>2+</sup>-ATPase13 (ACA13) for pollen acceptance<sup>8</sup> and a  
52 glutamate receptor-like channel (GLR) for pollen rejection<sup>12</sup>. In addition, the stigmatic EXO70A1  
53 protein was identified as a factor required for polarized secretion during compatible pollination,  
54 which is negatively regulated in incompatible reaction<sup>13,14</sup>.

55 Whereas these results help us understand specific downstream pathways, they are dependent on  
56 strong *a priori*. To obtain a global picture of the early fertilization events with no *a priori*,  
57 transcriptome approaches were also conducted. The main goal was to draw up catalogues of  
58 genes modulated during pollination so as to unravel the stigmatic response to compatible or

59 incompatible pollen<sup>8,15-18</sup>. However, the main drawback of these approaches was the difficulty in  
60 accurately distinguishing between pollen and stigma derived transcripts. To address that issue,  
61 transcriptome analysis<sup>19</sup> has recently been applied to identify sex-specific genes expressed during  
62 pollination, but this strategy needs great amounts of tissues from the specific transgenic line and  
63 fine techniques of biochemistry.

64 Here, inspired by previous RNA-seq analysis<sup>20</sup>, we developed a new experimental procedure  
65 coupled with a bioinformatic analysis of sequencing data to comprehensively unravel the  
66 dynamic events that occur both in the stigma and pollen grain following compatible and  
67 incompatible pollinations. We took advantage of the SNPs existing between two distinct  
68 *Arabidopsis thaliana* accessions, one used as female (Col-0) and the other as male (C24), to  
69 differentiate male and female transcripts based on a new statistical methodology, ASE-TIGAR<sup>21</sup>  
70 which can take all sequenced reads in account even those without SNPs while previous study  
71 used only reads with SNPs<sup>20</sup>. Our analysis allowed the identification of 80 % of mRNAs  
72 according to their parental origin and revealed transcriptional changes occurring specifically in  
73 either the stigma or pollen grain/tube. Gene Ontology and signaling pathway prediction of up-  
74 regulated genes showed that pattern-triggered immunity (PTI) pathways, including induction of  
75 the Mitogen-activated Protein Kinase 3 (MPK3), were activated on the female side after  
76 compatible pollination. Mutant analysis then confirmed that MPK3 is implicated in the growth of  
77 pollen tubes in papilla cells.

78

## 79 **Results:**

### 80 **Experimental setup to isolate transcripts from compatible and** 81 **incompatible pollination in *A. thaliana***

82 To compare self-compatible and self-incompatible pollination, we needed to have the same  
83 genetic background, albeit with different stigmatic responses to pollen. *Arabidopsis thaliana* has  
84 a high level of self-fertilization due to mutations that disrupt the self-incompatibility system  
85 present in its outbreeding ancestor<sup>22</sup>. To obtain a self-incompatible *Arabidopsis thaliana*  
86 background, we transformed *A. thaliana* with a functional *SRK-SCR* gene pair isolated from its

87 close self-incompatible relative *A. lyrata*<sup>23,24</sup>. To analyze both compatible and incompatible  
88 reactions and take advantage of nucleotide polymorphisms between *A. thaliana* accessions, we  
89 generated two transgenic lines that restored the incompatible response: one in the Col-0  
90 background expressing the *SRK* gene from the *A. lyrata* S14 haplotype (Col-0/*SRK14*) and the  
91 other in C24 background expressing the *SCR* gene from the same *S*-haplotype (C24/*SCR14*) (Fig.  
92 1a, Supplementary Fig. 1a). To express the SRK protein in stigmatic cells, we used the *SLRI*  
93 promoter that displays a strong stigma-specific activity in *Brassicaceae*<sup>25,26</sup>. Expression of the  
94 *SCR* gene was controlled by its own promoter. We selected one transgenic line for Col-0/*SRK14*  
95 and one for C24/*SCR14*. Both lines were self-fertile.

96 Next, we tested the fertilization response in crosses between these lines. As expected, stigmas of  
97 Col-0/*SRK14* pollinated with wild-type C24 pollen showed a clear compatible reaction with  
98 hydration of pollen grains and more than 55 pollen tubes elongation on papilla cells (Fig. 1a  
99 upper part, Supplementary Fig. 1bd). By contrast, when Col-0/*SRK14* stigmas were pollinated  
100 with C24/*SCR14* pollen, a strong incompatible reaction was observed as deduced from poor  
101 pollen hydration and the absence of pollen tube germination (Fig. 1a lower part, Supplementary  
102 Fig. 1cd).

103 Building on these experimental validations, we performed a time-course experiment focusing on  
104 two time-points of the interaction to identify genes whose expression is modified following  
105 pollen-stigma interaction. We selected an early stage (10 minutes after pollen deposition), which  
106 corresponds to the start of pollen grain hydration, and a later stage (one hour after pollination)  
107 when pollen tubes reach the base of the stigma. We sequenced mRNAs extracted from pollinated  
108 stigmas at 10, and 60 min after compatible (Col-0/*SRK14* x C24) pollination (C10, C60,  
109 respectively) and for incompatible (Col-0/*SRK14* x C24/*SCR14*) pollination (I10, I60,  
110 respectively) (Fig. 1a). We also extracted mRNA right after pollination, 0 min (Col-0/*SRK14* x  
111 C24), as a control for both compatible and incompatible pollination (C0).

112  
113 **SNP-based transcriptome analysis using variants between Col-0 and**  
114 **C24**

115 To distinguish the parental origin of the transcripts, we developed a method based on the  
116 detection of small genomic variations, including SNPs and small insertions and deletions (indels)  
117 between Col-0 and C24 accessions. The pipeline includes three main steps (Fig. 1b,  
118 Supplementary Fig. 2). In the first step, variations between Col-0/*SRK14* and C24 genomes were  
119 identified by whole-genome sequencing of the two strains with the read depth of 9.3 X for Col-  
120 0/*SRK14* and 14.2 X for C24 (Supplementary Table 1). After aligning clean reads against the  
121 reference genome (TAIR10, accession Col-0), SNPs and short indels between the mapped reads  
122 and the reference genome sequence were identified. Reads from Col0/*SRK14* and C24 covered  
123 95.8% and 90.6% of TAIR10 genome sequence, respectively. When compared to the reference,  
124 the line Col-0/*SRK14* had 2,032 variants, while C24/*SCR14* had 732,767 variants, respectively. In  
125 a second step, we introduced previously identified variants in the sequence of the TAIR10  
126 template to produce two new reference genome sequences, one for Col-0/*SRK14* and one for C24  
127 (Fig. 1b, Supplementary Fig.2). At the end of this process, between the new genomes of Col-  
128 0/*SRK14* and C24, we identified 616,781 SNPs and 446,999 indels (Supplementary Table 2).  
129 24 % and 31 % of genomic polymorphism were found in UTRs and CDSs for Col-0/*SRK14* and  
130 C24, respectively, and led to sequence variations in predicted mRNAs (Supplementary Table 2).  
131 We then annotated these two new genome sequences by projecting gene models from TAIR10.  
132 We predicted 39,205 gene models in Col-0/*SRK14* and 39,206 in C24. We then extracted  
133 predicted mRNA sequences for each gene model from these annotated Col-0/*SRK14* and C24  
134 genome sequences to produce maternal and paternal reference transcripts. We obtained a list of  
135 39,204 predicted gene models that were shared between maternal and paternal reference. The  
136 number of predicted mRNAs with at least one SNP between Col-0/*SRK14* and C24 was 31,271  
137 among the total common predicted mRNAs (39,204) (Fig. 1c). This result allowed us to  
138 distinguish the origin of about 80 % ( $31,271/39,204 = 79.8\%$ ) of mRNAs with SNP-based  
139 analysis. The last step included RNA read mapping and the estimation of sex-specific isoform  
140 abundances using ASE-TIGAR. The total length of raw reads from each condition was more than  
141 6,400 Mb (Supplementary Table 3). ASE-TIGAR uses a Bayesian approach to estimate allele-  
142 specific expression<sup>21</sup>. This approach allowed us to use all sequenced reads even those without  
143 SNPs. After obtaining the estimated read counts from ASE-TIGAR, we used DESeq2<sup>27</sup> to  
144 normalize counts, for female and male transcripts, respectively (Fig. 1b, Supplementary Fig. 2  
145 Supplementary Table. 4).

146 From this large dataset, we first determined the contribution of each tissue (stigma vs pollen) in  
147 mixed samples harvested immediately after compatible pollination (C0). We found that among  
148 the 47.0 million RNA reads, 69 % were estimated as derived from Col-0/*SRK14* (stigma), and  
149 31 % were from C24 (pollen) (Fig. 1d) although 15 % of reads were assigned to the genes  
150 without using SNPs. This proportion is stable over time (0, 10 min, 60 min) and independent of  
151 the pollination type (compatible, incompatible), thus suggesting that these proportions are  
152 reflecting the relative abundance of stigmatic cells compared with pollen grains collected by our  
153 manipulation.

154 Then, we analyzed the relative abundance of each transcript between stigma and pollen. To do so,  
155 transcript abundance was computed by ASE-TIGAR and was expressed as Fragments Per Kilo  
156 base of exon per Million reads mapped (FPKM), which accounts for sequencing depth and gene  
157 length. We then normalized FPKM (nFPKM) by dividing the FPKM of each transcript by the  
158 ratio of the transcript counts from stigma [nFPKM(stigma)] or pollen [nFPKM(pollen)] at C0  
159 (Fig. 1d, Supplementary Table 4). Values of nFPKM were displayed as a hexbin<sup>28</sup> plot to  
160 visualize the distribution of gene expression levels. Genes without SNPs did not show any  
161 particular pattern (Fig. 1e, bottom line). Gene expression levels were widely distributed and  
162 genes highly expressed in female or in male were clearly separated, suggesting the existence of  
163 distinct transcript signatures between stigma and pollen (Fig. 1e).

164

## 165 **Post-validation of the SNP-based analysis**

166 To have a global view of expressed genes in stigma and pollen, we constructed three classes of  
167 genes from calculated nFPKM: expressed genes, sex-preferentially and sex-specifically  
168 expressed genes (see methods for precise criteria of gene selection) (Supplementary Table 5).

169 Briefly, we defined genes that showed nFPKM >1 as expressed genes, those that were expressed  
170 at least a ten-fold higher in stigma than in pollen as stigma-preferentially expressed genes, and  
171 those that were at least one hundred-fold higher expressed in stigma than in pollen as stigma-  
172 specific expressed genes (and vice versa for pollen preferentially and specifically expressed  
173 genes) (Fig. 2a).

174 To evaluate the accuracy of our SNP-based analysis, we first focused on sex-specific genes and  
175 analysed the top 20 highly expressed genes (Supplementary Table 5) using the ThaleMine  
176 database (<https://apps.araport.org/thalemine/begin.do>). Heat maps of gene expression levels  
177 based on Cheng et al., 2016 were generated (Supplementary Fig. 3). Interestingly, stigma specific  
178 and highly expressed genes were clearly excluded from pollen even though many of them were  
179 also expressed in various tissues. By contrast, most pollen specific and highly expressed genes  
180 showed expression restricted to pollen and stage 12-inflorescences, which contain mature pollens  
181 (Supplementary Fig.3) Moreover, within the list of stigma-specific genes, we found *AtSI*  
182 (*AT3G12000.1*), which has been described as specifically expressed in *Arabidopsis* stigmas<sup>29,30</sup>,  
183 and in the list of pollen-specific genes, we found *CPK34* (*AT5G19360.1*), the protein product of  
184 which is involved in pollen tube regulation<sup>31</sup>. Finally, to confirm the specific expression of these  
185 genes in stigma or pollen, we carried out RT-PCR and sequence the cDNAs of SNP-containing  
186 regions using C0 sample as template. From the top 20 highly expressed genes, we selected genes  
187 whose location of SNPs permitted designing of primers. We found that, as expected, mRNAs of  
188 genes in the stigma-specific genes had SNPs only from Col-0/*SRK14* (stigma), whereas mRNAs  
189 of genes in the pollen-specific genes had SNPs only from C24 (pollen) SNPs (Fig. 2b).

190 To assess the global consistency of our data sets with already published studies, we further  
191 analyzed our SNP-based data by correlating them tissue-specific transcripts reported from  
192 microarray experiments<sup>32-34</sup>. Stigma associated transcripts from our analysis showed poor  
193 correlation with male transcriptomes from mature pollen or growing pollen tube, whereas the  
194 highest correlation was observed with transcriptome from unpollinated stigmas. Conversely, our  
195 pollen associated transcriptome showed a high correlation with male transcriptomes and only  
196 poorly correlated with female transcriptomes or transcriptomes from vegetative tissues (Fig. 2c).  
197 Correlations between another recent SNP-based analysis, which identified pistil- and pollen tube-  
198 specific transcripts 8 hour after pollination<sup>20</sup> and the tissue/organ-specific transcripts showed  
199 similar trends to our stigma- and pollen-transcripts (Fig. 2c).

200 To further characterize the pollen vs. stigma associated transcripts, we looked for Gene Ontology  
201 (GO) term enrichment at C0 within the list of sex-preferentially expressed genes (FDR < 0.05).  
202 Although GO terms may be somewhat subjective or not fully consolidated by functional tests,  
203 with the current annotation, we observed a clear difference between the two sets of transcripts



204 (Fig. 2d). From GO term enrichment of the top 1000 highly expressed genes among stigma or  
205 pollen -preferentially expressed genes (Supplementary Table 5, Supplementary Table 6), our  
206 analysis revealed high enrichment of several GO terms associated with metabolism in stigmas  
207 (such as “photosynthesis”, “mitochondrial-”, and “-metabolic process”) suggesting an active  
208 metabolic state of stigmatic cells (Fig. 2d left). Conversely, GO terms on the pollen side were  
209 specific to pollen functions such as “pollen tube growth”, “pollen sperm cell differentiation” and  
210 “cell tip growth” (Fig. 2d right).

211 Osaka et al.<sup>35</sup> previously reported the transcriptome of unpollinated stigmas using laser  
212 microdissection of *Arabidopsis* stigmatic cells. Among the top 100 expressed genes in this latter  
213 analysis, 44 were common with the top 100 expressed genes in stigma of our analysis at C0<sup>35</sup>.

214 Altogether, these results validate our SNP-based workflow, which allows identification of  
215 female- and male- derived transcripts from a combination of tissues following pollination.

216

## 217 **Gene expression dynamics triggered after pollination**

218 To examine the transcriptomic response of pollen and stigma following compatible or  
219 incompatible pollination, we first performed a principal component analysis (PCA)<sup>36</sup> using the  
220 gene expression levels of each biological replicate in all conditions (Fig. 3a). Data from stigma-  
221 and pollen-transcripts were treated separately (Fig. 3a left and right, respectively). The total  
222 explained variance of the first two principal components (PC1 and PC2) is around 39% (28 +11)  
223 for stigma and 35% (24 + 11) for pollen. These relatively low percentages are explained by the  
224 fact we took all genes and not only those that are specific for compatible or incompatible  
225 reaction. It also explain the fact that the different conditions (C0, C10, C60, I10 & I60) were not  
226 always well separated along those axis.

227 Nevertheless, comparing the PCA of stigma and of pollen transcripts suggested different  
228 dynamics in each tissue. On the stigma side, the PCA showed that both compatible and  
229 incompatible pollinations triggered a transcriptional response. Along the PC1 axis, capturing  
230 almost 30% of the explained variance, samples are temporally sorted from 0 min to 60 minutes,  
231 thus suggesting that PC1 can be interpreted as a time-axis. For the second axis, explaining 14%  
232 of the observed variability, compatible samples are located in the lower part when incompatible

233 samples are located in the upper part, close to those of the starting point (C0). This seems to  
234 indicate that this axis is oriented by the compatible/incompatible effect. Meanwhile, on the  
235 pollen side, all the conditions except C60 were clustered together. Again PC1 captures the  
236 temporality of the compatible response, however we cannot conclude that PC2 is related to the  
237 compatible response effect since early compatible and incompatible clusters overlap.

238 The clear response pattern of pollen at 1 hour is coherent with the massive changes displayed by  
239 compatible pollen, which hydrated and germinated a pollen tube growing in the stigmatic tissue  
240 (Fig. 3a).

241 After performing differentially expressed gene (DEG) analysis, we found more up regulated  
242 genes than down regulated genes, and more DEGs in stigma than in pollen (1841 up in stigma vs.  
243 595 in pollen, 513 down in stigma vs. 113 down in pollen) (Supplementary Table 7). To get a  
244 clearer picture on the number of genes whose expressions were modified during the course of  
245 pollination, we then used a Venn diagram representation (Fig. 3b) only with up regulated genes  
246 ( $\text{padj} < 0.1$ ,  $\text{FC} > 2$ ) to study. The Venn diagram of up-regulated genes in stigma (Fig. 3b left)  
247 showed similar dynamics to those observed in PCA, with a moderate alteration of gene  
248 expression in incompatible reactions, with only 45 genes at I10 and 344 genes at I60. By contrast,  
249 following compatible pollination, a massive and progressive change of gene expression was  
250 detected with 414 up-regulated genes at C10 and 1038 at C60 (Fig. 3b left). On the pollen side  
251 (Fig. 3b right), very few genes showed altered expression, except in condition C60, where 551  
252 genes were up-regulated (6 common in C10 and C60, 533 exclusively in C60) (Supplementary  
253 Table 8). Again this correlates perfectly with the PCA. Although the pollen is completely  
254 changing its physiology during compatible pollination, the stigma does not remain passive and  
255 undergoes a massive molecular reprogramming.

## 256 **PTI pathways induced after compatible pollination in stigma**

257 The rapid and global transcriptomic changes in stigma after compatible pollination motivated us  
258 to investigate the molecular events occurring at this stage. The 944 genes (158 + 163 + 623)  
259 induced only after compatible reaction (Fig. 3b left) showed dramatically different GO terms and  
260 enrichment from those preferentially expressed at C0 in the stigma (Fig. 2d left, Fig. 3c). There  
261 were 126 terms with high confidence ( $\text{FDR} < 0.05$ ) and 85 terms with  $\text{FDR} < 0.005$

262 (Supplementary Table 9). In particular, we found many GO terms including “response” and  
263 “signalling”, thus suggesting the prompt activation of signalling pathways in response to the  
264 pollination event. We also applied the enrichment analysis for 108 genes (104 + 4) that were  
265 induced only after incompatible pollination in stigma. But this was not informative when  
266 compared with compatible reaction as only 9 terms showed a FDR < 0.05 with the minimum  
267 FDR = 0.006.

268 To predict induced pathways after pollination, we then mapped the 944 up-regulated genes after  
269 compatible pollination to KEGG pathways<sup>37</sup>. We found many metabolism related pathways,  
270 signaling pathways including hormone-signaling (with 20 genes), plant-pathogen interaction  
271 (with 22 genes) and MAPK pathways (with 11 genes). Interestingly, two plant-pathogen  
272 interaction pathways known as pattern-triggered immunity (PTI) induced by bacterial flg22 and  
273 EF-Tu<sup>38</sup> were clearly distinguishable with 5 up-regulated genes in compatible situation (Fig. 4,  
274 green). On the other hand, among the 108 (4+104) exclusively up-regulated genes after  
275 incompatible pollination, we could not distinguish clear induction of PTI pathways as only one  
276 gene, *EFR*, was up-regulated (Fig. 4, gray).

277 In the compatible pollination, *SERK4*, *BAK1*, *EFR* and *FLS2* - all genes known to encode  
278 receptors of bacterial components - were expressed in stigma at C0 and *SERK4* was up regulated  
279 at C60 (FC = 2.3). Although *MKK6* and the transcription factor-encoding genes *WRKY22* and  
280 *WRKY25* were not expressed at C0, the first two were already induced at C10 and induction was  
281 maintained at C60, while *WRKY25* was induced only at C60 (all FCs were between 2 and 2.8).  
282 *MPK4* and *MPK6* were highly expressed from C0 to C60 compared with other components of the  
283 pathway but did not exhibit significant expression change. Interestingly, even though *MPK3*,  
284 *WRKY33* and *WRKY53* did not have SNPs, their high induction following compatible (FC = 6.3,  
285 10.0, 11.0 respectively) compared with incompatible (FC = 1.5, 2.8, 5.2) reaction suggests that  
286 this PTI pathway is a hallmark of compatible pollination (Fig. 5, Supplementary Table. 10).  
287 Altogether, these analyses indicate that the PTI pathway is rapidly induced in stigma only after  
288 compatible pollination.

289

290 **Stigma-pollen interaction activates and requires MPK3**

291 The predicted mRNA of *MPK3* did not have SNPs between Col-0/*SRK14* and C24, preventing  
292 the accurate assignment of the increased expression to stigma tissues. To check whether  
293 activation of MPK3-associated pathways occurs in stigma, as predicted from our analysis, we  
294 derived the list of genes known to be co-expressed with MPK3 from ATTED-II  
295 (<http://atted.jp/>)<sup>39</sup>. Genes in the list were ordered based on Pearson's correlation coefficient with  
296 MPK3 expression (from high to low) (Supplementary Table 11). After removing the genes  
297 without SNPs, we checked the expression of the top 50 genes using our SNP-based analysis in  
298 stigma (Supplementary Fig. 4, upper plot) and pollen (Supplementary Fig. 4, lower plot). At C0,  
299 most of the genes were moderately expressed in stigma but not in pollen. After compatible  
300 pollination, they were induced only in stigma, supporting our hypothesis of a stigma-specific  
301 MPK3 pathway induction. Subsequently, we took advantage of the MPK3-knockout line *mpk3*-  
302 *DG*<sup>40-42</sup> to monitor the expression and activation of MPK3 protein *in vivo*.

303 Immunoblotting was performed on protein extracts of pollinated stigmas from the following  
304 combinations of crosses: Col-0 x *mpk3* (i.e., MPK3 protein only present in stigma), *mpk3* x Col-0  
305 (i.e., MPK3 only in pollen), Col-0 x Col-0 (MPK3 in both), and *mpk3* x *mpk3* as a negative  
306 control. Pollinated stigmas were harvested immediately or 60 min after pollination,  
307 corresponding to C0 and C60, respectively. The absence of a specific band for MPK3 from the  
308 *mpk3* x *mpk3* sample confirmed the complete knockout of the mutant (Fig. 6a). MPK3 protein  
309 was detected in both stigma (Col-0 x *mpk3*) and pollen (*mpk3* x Col-0) but with a higher  
310 abundance in the stigma (Fig. 6ab). MPK3 was slightly accumulated 60 min after pollination in  
311 stigma and in pollen (FC = 1.6 and 2.4, respectively). Subsequently, we checked whether MPK3  
312 was activated through phosphorylation using an anti-Phospho MPK antibody. At C0, the band  
313 corresponding to phosphorylated MPK3 was very weak, but its intensity clearly increased after  
314 compatible pollination in stigma but not in pollen (FC = 5.2 and 0.9 respectively) (Fig. 6cd).  
315 Altogether these results demonstrate that MPK3 is both transcriptionally and post-translationally  
316 activated in the stigma during compatible pollination.

317 To elucidate whether the loss of MPK3 function might have any effect on pollen-stigma  
318 interaction, we pollinated *mpk3* stigmas with Col-0 pollen and observed the pollinated stigmas 60  
319 min after pollination by scanning electron microscopy. Col-0 x Col-0 pollination was used as a  
320 control. Before pollination, there were no apparent differences between stigmas of *mpk3* and Col-

321 0 (Supplementary Fig. 5). Hydration of pollen grains and elongation of pollen tubes occurred  
322 normally on *mpk3* stigmas compared to the control. However, when we measured the width of  
323 the contact sites between the pollen tube and stigma (white arrows on Fig. 7a), even if the  
324 distributions of the values were partly overlapping we found that contact sites were significantly  
325 wider on *mpk3* stigmas compared with Col-0 (Fig. 7b). This demonstrates that MPK3 contributes  
326 to the stigma-pollen interaction and structure.

327

## 328 **Discussion:**

329 To decipher the mechanisms that control reproduction in flowering plants, we extracted the  
330 global transcriptomic signatures associated with both pollen and stigmatic cells following their  
331 interaction during compatible or incompatible pollination. Although transcriptomes of pollinated  
332 stigmas have been recently published<sup>17,18</sup>, these analyses did not try to distinguish between pollen  
333 and stigma transcripts. In the present work, we provide a comprehensive transcriptomic dataset  
334 for stigma-pollen interactions using a novel SNP-based analysis. We took advantage of a recently  
335 developed statistical tool called ASE-TIGAR, which is based on a Bayesian approach to estimate  
336 allele-specific expression in diploid cells<sup>21</sup>. Nariai et al. showed an accurate estimation of gene  
337 expression from RNA-seq with ASE-TIGAR and identified some autosomal genes as allele-  
338 specific genes in a human reference lymphoblastoid cell line. We applied this new tool to  
339 discriminate transcripts from female-male mixed tissues during the first steps of the fertilization  
340 process. Our experimental design and bioinformatic pipeline allowed us to unveil the  
341 transcriptomic response of the pollen/pollen tube and that of the stigma following compatible or  
342 incompatible pollination at two early (10 min and 60 min) time points of the male-female  
343 interaction (Fig. 1). It is worth noting that another SNP-based pipeline has recently been  
344 published<sup>20</sup>, identifying transcripts derived from the pistil and pollen tubes collected 8h after  
345 compatible pollination. Although we cannot compare the two analyses directly, as pollination  
346 time points were different and only compatibility was studied by Leydon et al., both analyses  
347 succeeded in distinguishing male and female transcripts as presented in Fig. 2c. However, while  
348 Leydon et al., 2017 used 12 % of reads that had SNPs among the total sequenced reads, in our  
349 analysis we took all the RNA-seq reads into account, including those without SNPs<sup>20,21</sup>. This  
350 difference in the approaches has likely contributed to making our analysis more exhaustive, as

351 exemplified by the remarkable segregation we found between male transcripts and those derived  
352 from female and vegetative tissues (Fig.2 c). The sex preferentially- or specific-expressed gene  
353 lists we drew up contain many of the already identified female or male specific genes and  
354 reported GO term enrichment<sup>8,13,14</sup> (Fig.2). Our analysis also clarified the expression of genes that  
355 have been reported to have function during pollination. The exocyst complex, including the  
356 EXO70A1 protein, has been shown to play a role in pollen acceptance, probably through its  
357 function in the secretion of factors required for pollen hydration and pollen tube growth<sup>13,14</sup>. We  
358 found *EXO70A1* to be stably expressed in stigmas over time (nFPKM(stigma) = 27.1 at C0, with  
359 no expression change following pollination). *ACA13* which was shown to be involved in calcium  
360 fluxes was up-regulated after both compatible (FC=3.2 at C60) and incompatible (FC=2.4 at I60)  
361 pollinations; similar up-regulation after compatible pollination was also reported by Iwano et al.,  
362 2014.

363 Together with the correlation analysis (Fig. 2c) and experimental evaluations (Fig. 2b) we can  
364 conclude that our female- / male-transcripts globally represent stigma-/pollen-transcripts.  
365 Transcriptomic dynamics shown by PCA and Venn diagrams were not substantial in terms of  
366 variance and number of DEGs (Fig. 3ab). These results are in line with those of Matsuda et al.<sup>17</sup>  
367 who reported only a few percent of difference between transcripts before and 60 min after  
368 pollination for both compatible and incompatible interactions.

369 While our data are rather consistent with other transcriptomic analyses of pollinated stigmas<sup>8,17,35</sup>  
370 (Fig. 2, 3), the clear distinction we made between male and female transcripts also reveals  
371 surprises. The Venn diagram suggested a rapid transcriptomic response in the stigma for  
372 compatible (414 up-regulated genes at C10) but not for incompatible pollination (45 up-regulated  
373 genes at I10) (Fig. 3b left). Since almost no specific transcriptional induction was detected at I10  
374 (only 4 up-regulated genes), this suggests that key molecules required for the incompatibility  
375 reaction are already present in the stigma (Fig.3b left, I10up). This may be consistent with the  
376 observation that the pollen rejection response in *A. thaliana* lines exhibiting a restored  
377 incompatibility system is extremely rapid and occurs within minutes<sup>12</sup>. Astonishingly, while no  
378 cellular changes were detectable by SEM on stigmas one hour after incompatible pollination (Fig.  
379 1a), we identified more than one hundred up-regulated genes in stigmas and only 6 in pollen at  
380 I60 (Fig. 3b). This demonstrates that the stigma undergoes major molecular changes following

381 incompatible pollination, i.e. that incompatibility is actively maintained through a global  
382 physiological modification of the stigmatic cells. The stigma up-regulated genes can be  
383 assimilated to the stigmatic response to self-incompatible pollen. As the incompatibility reaction  
384 is maintained for several days, at least as long as SRK is properly expressed in the stigma, we  
385 may propose that among the stigma up-regulated genes there are key factors required for the  
386 maintenance of SI. Since components of the signaling pathway downstream of the SRK-  
387 SP11/SCR receptor-ligand complex are still not fully unveiled, our data provide a key resource to  
388 identify new genes involved in the incompatibility response and its maintenance over time.

389 Our results revealed a molecular signature of compatible pollination in stigma involving the  
390 activation of stress response genes and PTI pathways (Figs. 3, 4, 5). The similarity between the  
391 stigma-pollen interaction and plant-pathogen interaction has been discussed and has drawn  
392 researchers' attention for a long time<sup>10,43-45</sup>. Several transcriptomic analyses of pollinated  
393 stigmas/pistils reported enrichment in transcripts linked to stress or defense response in female  
394 tissues<sup>18,20,34,45,46</sup>. Tung et al. and Leydon et al. suggested that such defense-related genes could  
395 have functions in pollination. Moreover, although incompatible response has been considered to  
396 share common mechanism with plant-pathogen interaction as it is a response to block the  
397 invader<sup>18,45</sup>, in our analysis, GO terms associated with defense responses did not clearly appear  
398 after incompatible pollination but rather were mainly associated with the compatible response  
399 (Fig. 3c).

400 Recently, Zhang et al.<sup>18</sup> performed a time course transcriptome analysis of compatible and  
401 incompatible reaction in *Brassica napus*. They speculated that compatible responses had close  
402 parallels with plant-pathogen interactions, mainly with effector-triggered susceptibility, while  
403 incompatible response rather resembled effector-triggered immunity, and that PTI could be  
404 common to both compatible and incompatible responses. Although we found up-regulated  
405 defense related genes in the incompatible reaction, no clear defense pathways were identified by  
406 KEGG analysis. By contrast, our study clearly indicates that two PTI pathways were induced  
407 during compatible response (Fig.4). Indeed, the SNP-based analysis demonstrated the stigma  
408 specific expression and induction of genes involved in these pathways (Fig. 5). This result was  
409 not completely unexpected since pollen tube growth resembles pathogen attack from several  
410 angles, for example invasive growth recognition of an external invader, cell wall digestion, and

411 calcium burst<sup>8,47,48</sup>. We suggest that plant responses to pollen and pathogens share conserved  
412 molecular mechanisms at early stages of the interactions but that later downstream deviations  
413 could lead to different reactions, one inhibiting pathogen invasion, while the other promotes  
414 pollen tube growth in the pistil. Because links between symbiotic and pathogen interactions have  
415 been already been uncovered<sup>49,50</sup>, we can also speculate that the response of plant cells to  
416 symbiotic organisms could share common mechanisms with the response of the stigma to pollen.  
417 How these shared modules evolved together whilst maintaining highly specific responses (i.e.  
418 pollination, PTI, symbiosis) is an intriguing question for future studies.

419 MPK3 is one of the key components of PTI signaling pathways<sup>38</sup>, and we confirmed both the  
420 induction of genes associated with MPK3 expression, and MPK3 activation itself, in compatible  
421 pollinated stigmas (Supplementary Fig. 4, and Fig. 6). The increased pollen-stigma contact sites  
422 we observed in *mpk3* mutant suggest that the structural properties of the cell wall in the mutant  
423 could somehow be impaired (Fig. 7). Although the role of mechanical signals and cell wall  
424 reorganization has been studied in growing pollen tubes *in vitro*<sup>51-53</sup>, very little is known about  
425 components involved in pollen tube penetration and elongation within the stigma. It seems likely  
426 that major cell wall modifications are necessary to allow pollen tube growth through softening  
427 and loosening of the cell matrix<sup>48</sup>. How the pollen tube and stigma papilla contribute to these  
428 changes is still unknown even though both male and female partners are likely to contribute. The  
429 identification of a MPK3-dependent stigma-pollen interaction structure provides an interesting  
430 phenotypic indicator for such future studies.

431 MPK3 is involved in sensing cell wall modifications and reorganization during development as  
432 well as following the perception of external signals like pathogen attacks and wounding by  
433 receptor-like Wall-Associated Kinases (WAKs)<sup>54,55</sup>. WAKs bind pectin fragments and  
434 oligogalacturonic acids that are generated by pathogen attacks and wounding. In stigmas, two of  
435 the WAK family members (*AT2G23450* and *AT1G79670*) were stably expressed (nFPKM(stigma)  
436 = 11.7 and 12.2, respectively at C0 with no expression change following pollination), and another  
437 member, *AT1G21210*, was up-regulated after compatible pollination (FC = 2.3 at C60).  
438 Interestingly, Glycine-Rich Protein3 (GRP3), a possible ligand for WAKs that is known to bind  
439 to WAK1<sup>56</sup> was highly expressed (nFPKM(stigma) = 303.0 at C0 and no expression change  
440 following pollination) in stigmas. In addition, we found that several pectin related enzymes were



441 up-regulated in stigmas after compatible pollination. Taken together, we propose that cell wall  
442 reorganization that allows pollen tube growth in the stigma could be dependent on MPK3-based  
443 pathways (Fig. 8).

444 Our study elucidates the molecular signatures of pollen and stigma responses following pollen-  
445 stigma interaction in compatible and incompatible situations. Most remarkably, we reveal that the  
446 acceptance of pollen grains involves the activation of PTI pathways and we confirm the long-  
447 suspected idea that fertilization and plant pathogen responses share common molecular signaling  
448 components.

449

## 450 **Materials and Methods:**

### 451 ***A. lyrata* SCR14 and SRK14 gene cloning and plasmid construction**

452 We used Gateway® vectors<sup>57</sup> (Life Technologies, USA) for expression of transgenes in  
453 *Arabidopsis thaliana*. The *AlSRK14* genomic sequence spanning the coding region from the start  
454 to the stop codons (3,620 kb) was amplified from genomic DNA of an *Arabidopsis lyrata*  
455 individual containing the *SI4*-locus with specific AttB-containing primers  
456 (5'GGGGACAAGTTTGTACAAAAAAGCAGGCTACCATGAGAGGTGTAATACCAAAGT  
457 ACC3' and  
458 5'GGGGACCACTTTGTACAAGAAAGCTGGGTTTACCGAGGTTCCACTTCCGTGGTGG3'  
459 ) and subsequently inserted by BP recombination into a pDon207 plasmid. A fragment of 4,081  
460 kb containing the *AlSCR14* gene, 1,844 kb of the 5' upstream region and 817 bp of the 3'  
461 downstream region were amplified from genomic DNA of *Arabidopsis lyrata* individual  
462 containing the *SI4*-locus using the AttB-containing primer  
463 (5'GGGGACAAGTTTGTACAAAAAAGCAGGCTCGGGTAGCTCAACCTAGCTAAG3' and  
464 5' ACCACTTTGTACAAGAAAGCTGGGT CATGATCACCAAAGACAAGATCC3'). This  
465 fragment was subsequently inserted by BP recombination into a pDONR-Zeo plasmid. The DNA  
466 fragment containing the *Brassica oleracea* *SLR1* promoter (1.5 kb upstream of the *SLR1* start  
467 codon<sup>25,26</sup>) was inserted into a the pDONR P4-P1R plasmid<sup>58</sup>. The *SLR1* promoter, the genomic  
468 sequence *AlSRK14* and a 3' mock sequence were inserted in the pK7m34GW destination vectors  
469 by three fragment LR recombination. The genomic sequence *AlSCR14*, a 5' mock sequence and a

470 3'mock sequence were inserted in the pB7m34G. GenBank accession numbers for pg*AlSCR14*  
471 (promoter and gene of *AlSCR14*) and p*SLR1-gAlSRK14* (*SLR1* promoter and *AlSRK14* gene) are  
472 MH680584 and MH680585, respectively.

### 473 **Plant material and growth condition**

474 *Arabidopsis thaliana* C24 and Col-0 transgenic plants were generated using *Agrobacterium*  
475 *tumefaciens*-mediated transformation according to Logemann et al. 2006<sup>59</sup>. *AlSRK14* construct  
476 was introduced in Col-0 (Col-0/*SRK14*). *AlSCR14* construct was introduced in C24 (C24/*SCR14*).  
477 Unique insertion lines homozygotes for the transgene were selected. mpk3-DG<sup>40-42</sup> was kindly  
478 gifted from Marcel Wiermer and Yuelin Zhang. All plants including Col-0 and C24 were grown  
479 in growth chambers under long-day cycles (16h light/8h dark at 21°C/19°C).

### 480 **Aniline blue staining and observation**

481 Buds at the end of stage 12<sup>60</sup> were emasculated and 18 hours later, stigmas which have reached  
482 the stage 13 or Early 14 (14E), were pollinated with mature pollen grains. After 2 h, pollinated  
483 stigmas were fixed in acetic acid 10%, ethanol 50% and stained with Aniline Blue for  
484 epifluorescence microscopy observation. Germinated pollen grains with pollen tubes within the  
485 stigmas were manually counted. We considered that pollination is incompatible when fewer than  
486 5 pollen tubes were able to overcome the stigmatic barrier<sup>61</sup>.

487

### 488 **Genomic DNA and mRNA preparation**

489 For genomic DNA extraction, about 2 mL volume young inflorescences of Col-0/*SRK14* and  
490 C24 were harvested, respectively. After grinding the material in liquid nitrogen, DNA was  
491 extracted as described previously<sup>62</sup>. For RNA extraction, late stage 12<sup>60</sup> Col-0/*SRK14* flowers  
492 were emasculated and 16-20h after emasculation stigmas were pollinated with compatible (C24)  
493 or incompatible pollen (C24/*SCR14*). 0, 10 or 60 minutes after compatible pollination (C0, C10  
494 and C60) or incompatible pollination (I10, I60), 50 stigmas were harvested manually using fine  
495 tweezers, then frozen in liquid nitrogen and stored at - 80 °C until further processing. After  
496 grinding the material in liquid nitrogen, RNA was extracted and purified by using Arcturus®  
497 PicoPure® RNA Isolation Kit (Applied Biosystems / Thermo Fisher Scientific) following the  
498 manufacturer instruction except that we added a DNase treatment (Qiagen, catalog#79254). Five

499 biological replicates of RNA at each point were prepared then four replicates were selected for  
500 sequencing based on their quality and quantity.

### 501 **Whole genome sequencing and variant calling**

502 Library preparation and whole genome sequencing of Col-0/SRK14 and C24 were performed by  
503 HELIXIO (Clermont-Ferrand, France; <http://www.helixio.com/>) with TruSeq® DNA PCR-free  
504 Library Preparation kit (Illumina) and NextSeq500 platform (Illumina) applying paired-end  
505 sequencing (2×150 bp) (Supplementary Table 1). We then proceeded to the analysis of the  
506 sequencing data using GATK3.5 after quality check by FastQC  
507 (<http://www.bioinformatics.babraham.ac.uk/projects/fastqc>), trimming and pairing of the  
508 resulting reads using custom Perl scripts. We aligned the reads to the Col-0 reference genome  
509 (TAIR10) with BWA<sup>63</sup>, applied GATK<sup>64</sup> base quality score recalibration, indel realignment,  
510 duplicate removal, and performed SNP and INDEL discovery across all samples according to  
511 GATK Best Practices recommendations<sup>65,66</sup>. After checking the depth of coverage of two samples  
512 by using GATK DepthOfCoverage (Supplementary Fig. 6), we performed filtering applying  
513 depth 3 for Col-0/SRK14 and depth 6 for C24 and homozygous for both, then obtained the  
514 complete set of variants for each genome as VCF files.

### 515 **Production of new reference genomes**

516 Col-0/SRK14 and C24 genome sequences were derived from TAIR10 genome, by introducing  
517 the called variants in the sequence, by using GATK FastaAlternateReferenceMaker. Gene  
518 annotations from TAIR10 genome were transferred onto the two new genome sequences using  
519 RATT<sup>67</sup> in “Strain” mode and seqret from EMBOSS suite<sup>68</sup>. To characterize polymorphism  
520 (SNPs and short indels) between obtained Col-0/SRK14 and C24 genome sequences, inside and  
521 outside predicted genes, we aligned chromosome sequences using LAST (v. 938)<sup>69</sup>, after training  
522 LAST on chromosomes 1.

### 523 **RNA sequencing and expression analysis**

524 Library preparation and RNA sequencing were performed by HELIXIO with TruSeq® Standard  
525 mRNA sample Preparation kit (Illumina) and NextSeq500 platform (Illumina) applying paired-  
526 end sequencing (2×75 bp). We then proceeded to the analysis of the sequencing data using ASE-  
527 TIGAR after quality check by FastQC, trimming and pairing of the resulting reads using custom

528 Perl scripts. First, we derived mRNA reference sequences from new reference genomes and  
529 merged them in one FASTA file, then mapped the clean reads on the mRNA reference with  
530 Bowtie2<sup>70</sup>. We then run ASE-TIGAR with SAM files produced by mapping and the mRNA  
531 reference<sup>21</sup> (<http://nagasakilab.csml.org/ase-tigar/>). Raw sequencing data is available at NCBI  
532 database (<https://www.ncbi.nlm.nih.gov/sra/SRP154565>) under SRA accession: SRP154565.

### 533 **Comparison with published datasets**

534 To check the consistency of our RNA-seq data with previously published microarray data<sup>32–34</sup>,  
535 for each pair of condition, we computed a Pearson correlation coefficient ( $r$ ) over all genes with  
536 SNPs from our variant analysis, using mean of estimated read counts from three or four  
537 biological replicates for RNA-seq and mean absolute intensity for multiple microarray replicates.  
538 We also computed Pearson correlation coefficients ( $r$ ) between previously published RNA-seq  
539 data<sup>20</sup> and the microarray data sets.

### 540 **Differential gene expression analysis and calculation of nFPKM.**

541 Differential expression analysis on the whole transcriptome was performed using DESeq2<sup>27</sup>.  
542 After performing principal component analysis (PCA) for all biological replicates at each  
543 condition, we removed one replicate at I10 from female samples and one at I60 from male  
544 samples, as they were isolated from their other replicates. All the analyses, including the  
545 presented PCA, were performed with the sample set after the removal.

546 Normalized FPKM (nFPKM) was calculated by dividing stigma- or pollen-FPKM by female or  
547 male transcript proportion at each condition (C0, C10, C60, I10, I60).

548

### 549 **Criteria to select expressed genes, sex-preferentially or specifically expressed genes**

550 To select expressed genes, sex-preferentially or specifically expressed genes at C0 (Fig. 2a,  
551 Supplementary Table 5), we used nFPKM. We defined stigma-expressed genes consisting  
552 [ $\text{nFPKM}(\text{stigma}) > 1 \cap \text{with SNPs}$ ] and stigma-preferentially expressed genes consisting  
553 [ $\text{nFPKM}(\text{stigma}) > 1 \cap \text{nFPKM}(\text{stigma}) \geq 10 \times \text{nFPKM}(\text{pollen})$ ]. Sex-specifically expressed  
554 genes were selected among sex-preferentially expressed genes applying more stringent criteria.  
555 Stigma-specifically expressed genes consist [ $\text{stigma preferentially expressed genes} \cap$

556  $n\text{FPKM}(\text{pollen}) \leq 1 \cap n\text{FPKM}(\text{stigma}) > 100 \cap n\text{FPKM}(\text{stigma}) > 100 \times n\text{FPKM}(\text{pollen})$ ], then  
557 sorted by  $n\text{FPKM}(\text{stigma})$  from the largest to the smallest. Vice-versa for pollen (preferentially /  
558 specifically) expressed genes

### 559 **GO term and enrichment analysis and pathway analysis**

560 GO and gene enrichment analyses were performed using Gene Ontology Consortium website  
561 (<http://www.geneontology.org/>) and PANTHER13.1 software<sup>71,72</sup>. We used “GO biological  
562 process complete” for the classification. GO terms with False Discovery Rate (FDR) < 0.05 were  
563 considered as significantly enriched.

564 Pathway analysis was performed using and KEGG PATHWAY Database using KEGG Mapper  
565 software ([http://www.kegg.jp/kegg/tool/map\\_pathway2.html](http://www.kegg.jp/kegg/tool/map_pathway2.html))<sup>37</sup>.

### 566 **RT-PCR and sequencing**

567 For experimental evaluation of the SNP-based analysis with reverse transcription polymerase  
568 chain reaction (RT-PCR) and sequencing, we selected genes among the top-20 specifically  
569 expressed genes that have SNPs at suitable positions allowing discrimination of parental origin  
570 (stigma vs pollen) (Fig. 2b). RNA at C0 was purified as described above. cDNA was generated  
571 using SuperScript® VILO™ cDNA Synthesis Kit (Invitrogen). Primers were designed by  
572 Primer3web (<http://bioinfo.ut.ee/primer3/>) at the flanking region of SNP including sites. PCR was  
573 performed with GoTaq (Promega). PCR products were purified using PCR clean-up Gel  
574 extraction kit (Macherey-Nagel) and then sequenced.

### 575 **Protein extraction and western blotting**

576 Proteins from around 50 pollinated stigmas at C10 and C60 were extracted in SDG buffer (Tris-  
577 HCl 62.5 mM pH 6.8, Glycerol 10%, DTT 2%, SDS 2.5 %). Proteins were separated on a 10 %  
578 SDS-PAGE gel and immunodetected with Anti-AtMPK3 (Sigma, M8318), anti-phospho p44/p42  
579 MAPK (Cell Signaling, #9101S), or anti-alpha-Tubulin (Sigma, T5168). Band intensities were  
580 normalized to tubulin signal using ImageJ software.

### 581 **Environmental Scanning Electron Microscopy (SEM).**

582 Late stage 12 stigmas were pollinated with mature Col-0 pollen and 60 min after pollination,  
583 stigmas were observed under Hirox SEM (SH-3000 table-top) at -20 °C with an accelerating

584 voltage of 10 kV. The width of each pollen tube–stigma contact site was measured using ImageJ  
585 software. We applied a Student test to the measurements.

586

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738

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748 **Author Contributions:** CK, JJ, MDR developed the data analysis pipeline and performed the  
749 bioinformatics analyses. AL, JL supported the bioinformatics analyses. AL helped with  
750 interpretation of the transcriptomic data. CK is responsible for all experiments and analysis  
751 performed in the study except described below. FR produced and characterized Col-0/*SRK14* and  
752 C24/*SCR14* lines. CK and LR performed the image acquisition by scanning electron microscope.  
753 CK, LR, FR, IFL harvested plant materials and extracted RNA. CK, TG, IFL designed the study  
754 and CK, JJ, TG, IFL wrote the manuscript. All the authors contributed to the discussion.

755

## 756 **Figure legends:**

757 **Fig. 1 | SNP-based RNA-seq analysis.** **a**, Time course of sample collection. Flowers of Col-  
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759 (C24/*SCR14*) pollen grains, respectively. Stigmas were harvested just before the stigma  
760 (dashed line) 0, 10, 60 min after compatible (C0, C10, C60) or incompatible (I10, I60) pollination  
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763 pollination. Scale bar = 20  $\mu$ m. **b**, Three steps of a SNP-based data analysis pipeline. **c**, Number  
764 of predicted mRNAs derived from the reference sequence produced at the second step in b.  
765 Around 80 % of coding regions have SNPs between Col-0/*SRK14* and C24. **d**, Number and ratio  
766 of estimated read counts for Col-0/*SRK14* and C24 at C0 computed by ASE-TIGAR at the third  
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769 without SNPs were plotted outside.

770

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772 specifically) -expressed genes at C0 selected by nFPKM (Supplementary Table 5). **b**, RT-PCR  
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774 selected among the 20 specifically-expressed genes and the rank of the genes according to the  
775 expression level are presented. RNA was extracted from pollinated stigmas at C0 then we  
776 analysed their SNP-information by RT-PCR and sequencing. SNP number corresponds to the  
777 number of SNPs present in the sequenced regions. **c**, Heat map of Pearson's correlation  
778 coefficient between the stigma/pollen transcripts from the SNP-based analysis and the  
779 previously published transcripts. **d**, Top ten gene enrichment categories of stigma or pollen  
780 preferentially-expressed genes at C0 according to the GO term on biological processes.  
781 Enrichment analysis was performed with the one thousand top expressed genes in stigma (left)  
782 or pollen (right) among the list, respectively. Only top 10 first enrichment categories are shown.  
783 Selection criteria for genes analysed in **b** (sex-specific), and **d** (sex-preferential), are described  
784 in the manuscript.

785

786 **Fig. 3 | Gene expression dynamics after pollination.** **a**, PCA of stigma (left) and pollen (right)  
787 transcripts. Biological replicates of all the conditions were analysed. **b**, Venn diagrams showing  
788 the number of up-regulated (FC > 2) genes in stigma (left) and pollen (right), after compatible  
789 (green and violet) or incompatible (grey) pollinations. **c**, Gene enrichment categories of up-

790 regulated genes after compatible pollination in stigma according to GO term on biological  
791 processes. Only genes specifically induced after compatible reaction (b, left; 158+163+623)  
792 were analysed. Only top 20 enrichment categories are shown.

793

794 **Fig. 4 | Induced genes in PTI pathways after pollination in stigma.**

795 KEGG pathway mapping applied for the genes exclusively up-regulated after compatible  
796 pollination (Fig.3 b, left; 158+163+623) and incompatible pollination (Fig.3 b, left; 4+0+104),  
797 respectively. Green colored genes were identified from the compatible gene set and grey were  
798 from the incompatible set. Up-regulated gene in our analysis assigned to *BAK1/BKK1* was  
799 *SERK4*, and to *MKK1/2* was *MKK6* (see Fig. 5 and Supplementary Table 10).

800

801 **Fig. 5 | Induced pathways in stigma after compatible pollination.** Genes in green boxes with  
802 solid line were with SNPs and up-regulated (FC > 2) at 10 min and 60 min (light green), or only  
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807

808 **Fig.6 | Activation of a MPK3-dependent pathway after compatible pollination.**

809 Immunoblotting assays after compatible pollination with *mpk3* mutant line. Pollination between  
810 Col-0 x Col-0, *mpk3* x *mpk3*, Col-0 x *mpk3*, and *mpk3* x Col-0 were performed. Protein was  
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812 for **a**, and anti-phospho-p44/42 MPK antibody was used for **c**. The shown western blots are  
813 representative of three independent biological replicates. All of them showed similar results. **b.**,  
814 **d**. Semi-quantification of the band intensity (b for a, d for c). Green, violet and grey bars indicate  
815 signals from stigma side, from pollen side, and from both sides, respectively. Error bars indicate  
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817 signals from other MPK proteins.

818

819 **Fig.7 | *mpk3* stigma changes the width of pollen tube contact sites.** **a**, Pollen tube contact  
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821 stigma papilla is artificially colorized in green and one pollen grain and its tube in violet,  
822 respectively. Scale bar = 10  $\mu$ m. Double-arrows indicate the width of pollen tube contact site  
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824 Observations were performed three different days; n is the total number of contact sites  
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826 day.\*\*\* indicates p-value < 0.01.

827

828 **Fig.8 | Stigma-pollen interaction involves MPK3-dependent pathway.** Schematic model for  
829 stigma-pollen interaction. i, Signals at the contact site between pollen tube and papilla cell. ii,  
830 Signal transduction involving MPK3 pathways. iii, Gene expression change. iv, Protein  
831 activations without transcriptomic change v, Stigma cell wall reorganization. vi, Effect on pollen  
832 tube by stigma surface property.

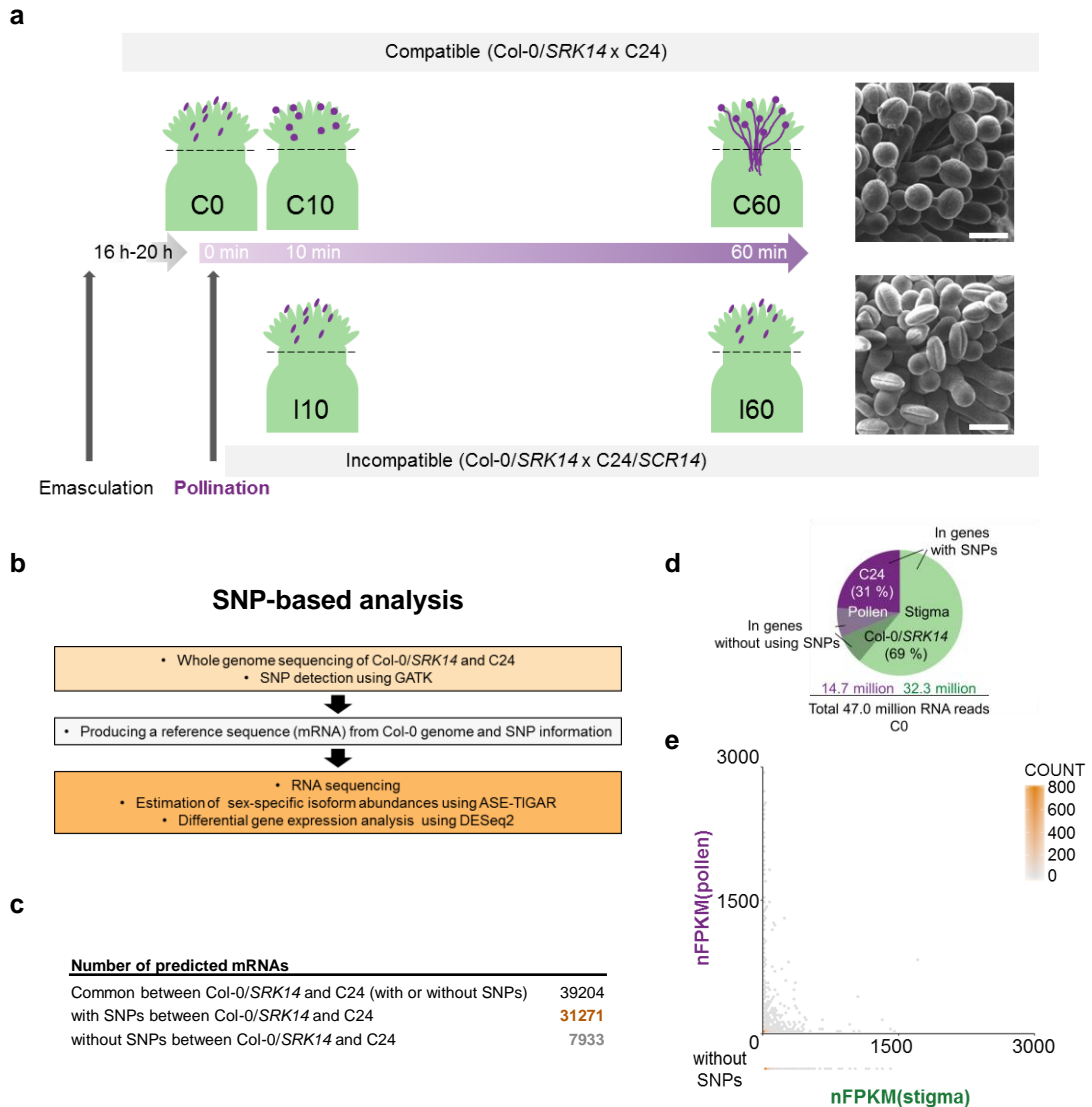
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834 **Additional information:**

835 Supplementary Fig. 1

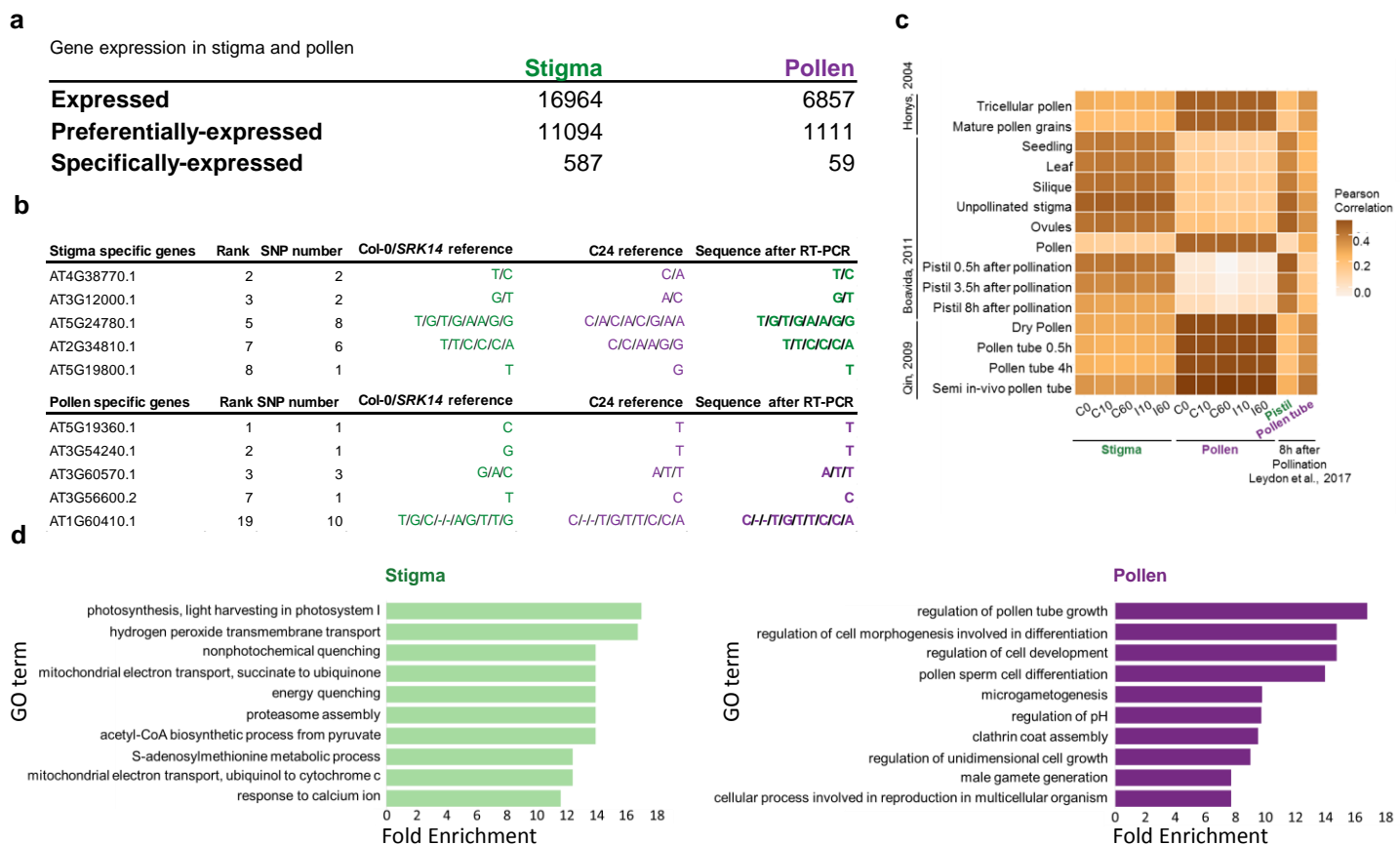
836 Supplementary Fig. 2

- 837 Supplementary Fig. 3
- 838 Supplementary Fig. 4
- 839 Supplementary Fig. 5
- 840 Supplementary Fig. 6
- 841 Supplementary Table. 1
- 842 Supplementary Table. 2
- 843 Supplementary Table. 3
- 844 Supplementary Table. 4
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- 850 Supplementary Table. 10
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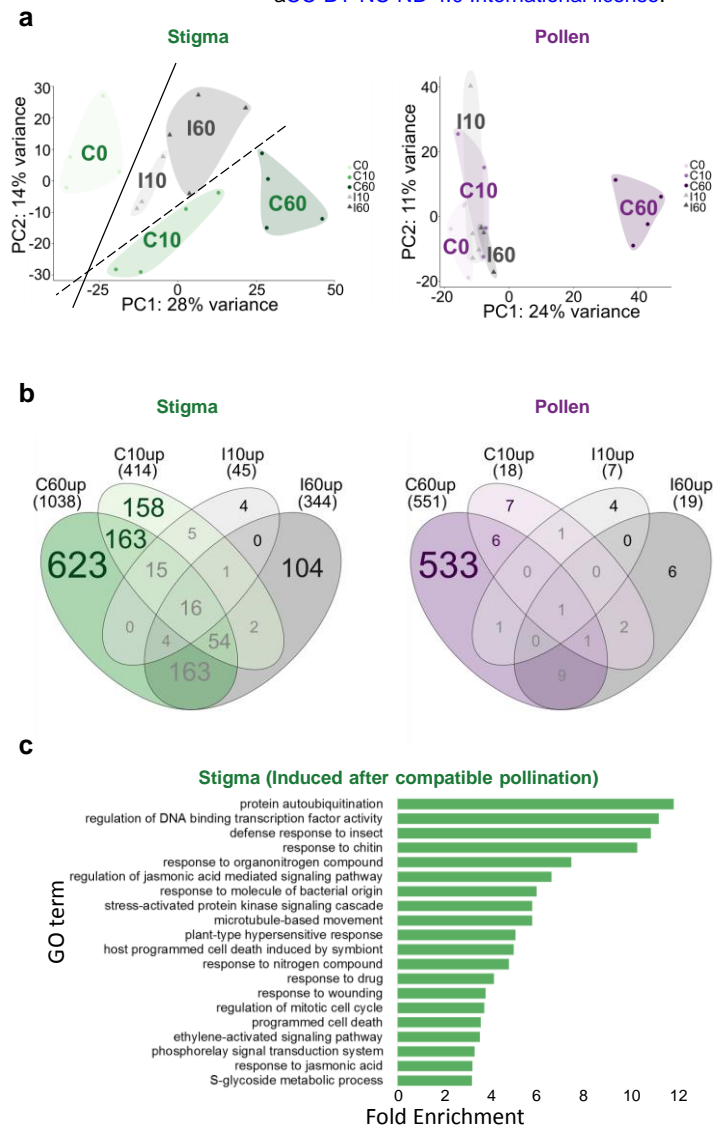


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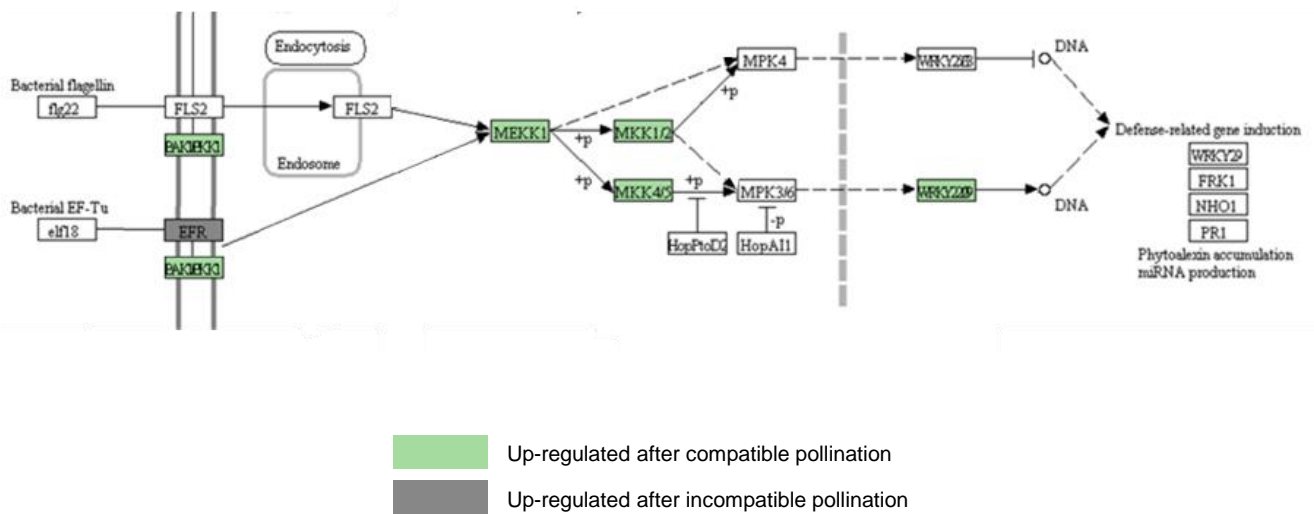




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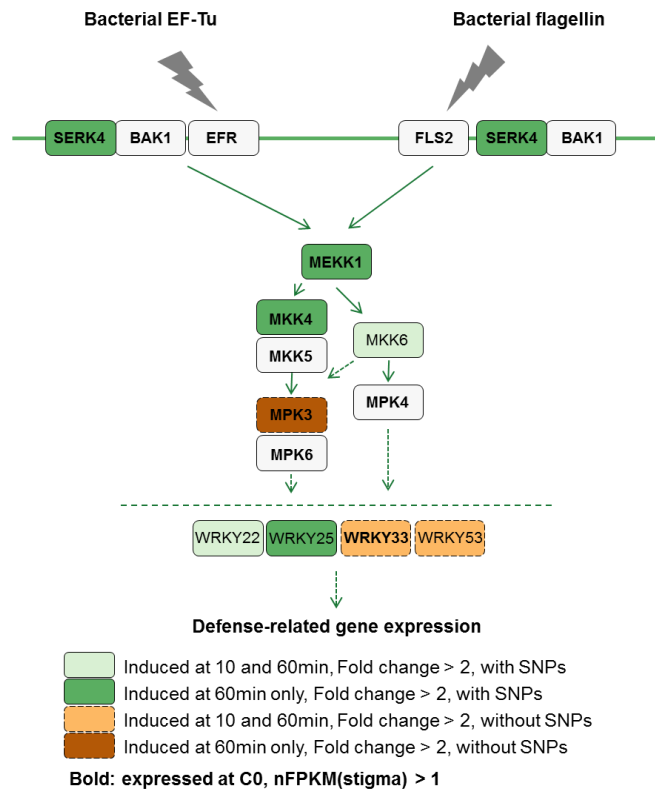


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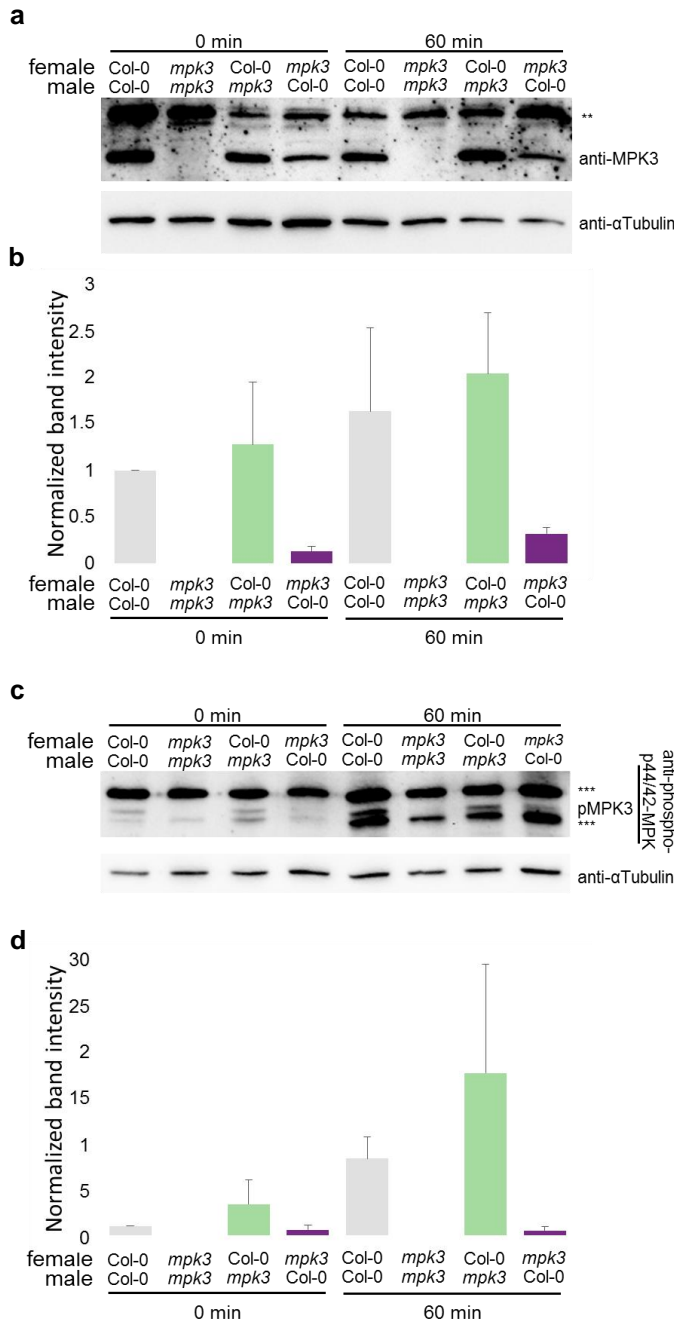


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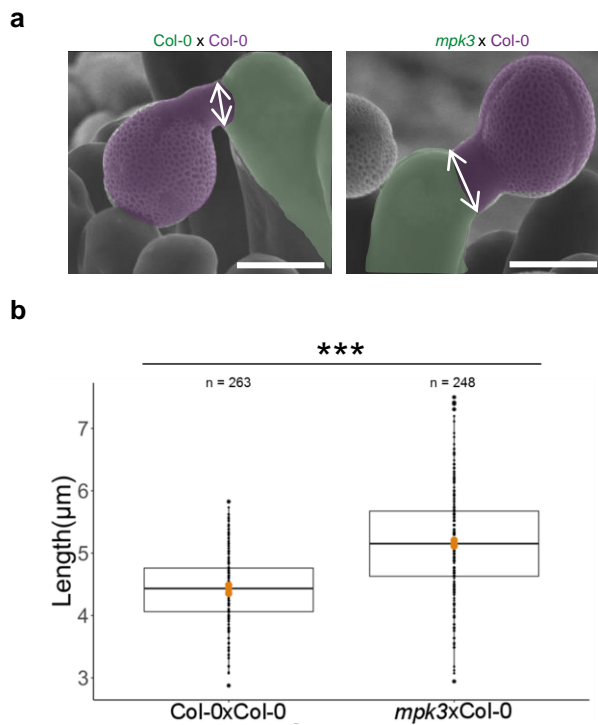
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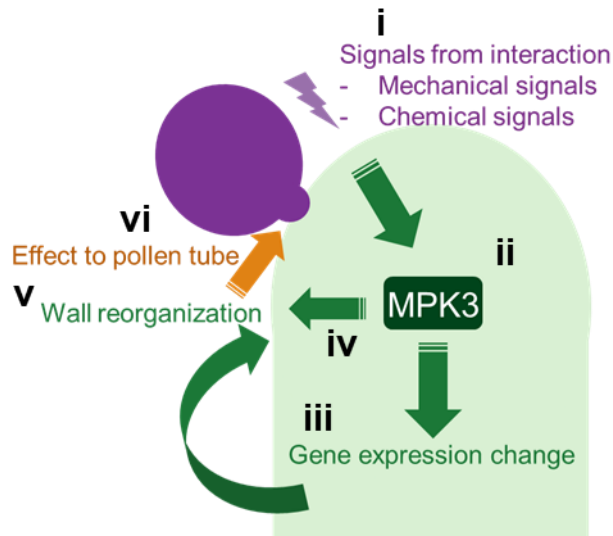
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