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Apical dominance control by TAR-YUC-mediated auxin biosynthesis is a deep homology of land plants

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23 **Abstract**

24 A key aim in biology is to identify which genetic changes contributed to the evolution of form
25 through time. Apical dominance, the inhibitory effect exerted by shoot apices on the initiation
26 or outgrowth of distant lateral buds, is a major regulatory mechanism of plant form¹. Nearly
27 a century of studies in the sporophyte of flowering plants have established the phytohormone
28 auxin as a front-runner in the search for key factors controlling apical dominance^{2,3},
29 identifying critical roles for long-range polar auxin transport and local auxin biosynthesis in
30 modulating shoot branching⁴⁻¹⁰. A capacity for lateral branching evolved by convergence in
31 the gametophytic shoot of mosses and primed its diversification¹¹; however, polar auxin
32 transport is relatively unimportant in this developmental process¹², the contribution of auxin
33 biosynthesis genes has not been assessed and, more generally, the extent of conservation
34 in apical dominance regulation within the land plants remains largely unknown. To fill this
35 knowledge gap, we sought to identify genetic determinants of apical dominance in the moss
36 *Physcomitrium patens*. Here, we show that leafy shoot apex decapitation releases apical
37 dominance through massive and rapid transcriptional reprogramming of auxin-responsive
38 genes, and altering auxin biosynthesis gene activity. We pinpoint a subset of *P. patens*
39 *TRYPTOPHAN AMINO-TRANSFERASE (TAR)* and *YUCCA FLAVIN MONOOXYGENASE-*
40 *LIKE (YUC)* auxin biosynthesis genes expressed in the main and lateral shoot apices, and
41 show that they are essential for coordinating branch initiation and outgrowth. Our results
42 demonstrate that local auxin biosynthesis acts as a pivotal regulator of apical dominance in
43 moss and constitutes a shared mechanism underpinning shoot architecture control in land
44 plants.

45

46 **Results and discussion**

47 **Massive and rapid reprogramming of auxin-responsive genes follows gametophore** 48 **decapitation**

49 Previous reports have shown that decapitation of the moss gametophore (*i.e.*, the
50 gametophytic leafy shoot) tip promotes lateral branch formation, indicating conservation of
51 apical dominance^{12,13}. In the model species, *Physcomitrium patens*, gametophore branches
52 arise *de novo* from epidermal cells in leaf axils, and initiation and outgrowth have been
53 described as a single continuous development process¹². To assess the dynamics of branch
54 emergence following decapitation, we isolated *P. patens* gametophores from 4-week-old
55 wild-type colonies, cut off their main apices and followed branch development over 48 hours.
56 The first emerged branches were clearly visible 24 hours after decapitation (h.a.d.) and
57 branches with well-developed leaves were observed 48 h.a.d., suggesting that the earliest
58 molecular events leading to branching occur within a day (Figure 1A). To capture a
59 comprehensive overview of the transcriptional changes associated with the release of apical
60 dominance, we therefore analyzed the transcriptome of whole gametophores collected 2, 6,
61 12 and 24 h.a.d., and used gametophores collected immediately after decapitation (0 h.a.d.)
62 as a reference. The proportion of genes whose transcription was affected increased up to 12
63 h.a.d., leading to a maximum of 5028 and 4734 genes down- and up-regulated, respectively,
64 suggesting that major transcriptional changes occur before the first branches become visible
65 (Tables S1 and S2, Figure S1 and S2). In an attempt to specifically address the contribution
66 of auxin-related genes in apical dominance, we then compared the effect of auxin and
67 decapitation at the transcriptional level. We sequenced the transcriptome of 4-week-old wild-
68 type gametophores treated with indole-3-acetic-acid (IAA) for 30 minutes and used mock-
69 treated plants as a reference to identify early auxin-responsive genes. We found that 3652
70 and 4524 genes were down- and up-regulated by IAA, respectively (Tables S1 and S3). By
71 cross-referencing decapitation and IAA-regulated gene sets, we observed a striking global
72 anti-correlation between both treatments (Figure 1B). Sixty-four percent of genes down-
73 regulated 2 h.a.d. were IAA-induced, whilst only 5% were IAA-repressed. A similar trend was
74 observed for “6 h.a.d.” and “12 h.a.d.” gene sets. For example, both *ARF* and *AUX/IAA* auxin
75 signalling genes, previously identified as early auxin-responsive genes¹⁴, responded
76 oppositely in that most genes were induced by IAA and repressed after decapitation (Figure
77 1C). Inversely, 46% of genes up-regulated 2 h.a.d. were IAA-repressed, whilst only 14% were
78 IAA-induced, with a similar trend for “6 h.a.d.” and “12 h.a.d.” gene sets (Figure 1B). Chi-
79 squared tests revealed a significant association between IAA repression/induction and
80 decapitation repression/induction at all four times, and the response was stronger at early

81 time points ($\chi_1^2 = 1915, p < 0.001$ at 2 h.a.d.; $\chi_1^2 = 2731, p < 0.001$ at 6 h.a.d.; $\chi_1^2 = 2007, p <$
82 0.001 at 12 h.a.d.; $\chi_1^2 = 6.855, p = 0.009$ at 24 h.a.d.). Thus, the largely antagonistic
83 transcriptional responses of decapitated and auxin-treated gametophores suggest a major
84 contribution of auxin and its targets in apical dominance control in moss. Moreover, these
85 data support the notion that the main gametophore apex is an auxin source with long-range
86 effects^{12,15}.

87

88 **Decapitation rapidly alters *TAR* and *YUC* auxin biosynthesis gene expression**

89 Auxin is mainly produced from a tryptophan (TRP)-dependent biosynthetic pathway in land
90 plants¹⁶. TRP is converted to indole-3-pyruvic acid (IPyA) by TRYPTOPHAN AMINO-
91 TRANSFERASE (TAA/TAR) enzymes, that is then converted to auxin by YUCCA FLAVIN
92 MONOOXYGENASE-LIKE (YUC) enzymes¹⁷. Previously published works in the flowering
93 plant *Arabidopsis thaliana* have proposed that auxin depletion from the leaf axil is needed to
94 promote axillary meristem formation^{9,10}. In contrast, the subsequent inhibition of axillary bud
95 outgrowth depends on expression of *AtTAA1/WEI8*, *AtTAR2*, *AtYUC1*, *AtYUC4* and *AtYUC6*
96 auxin biosynthesis genes in partially overlapping domains of the shoot apical meristem^{6,7}.
97 Double and triple mutants in these genes, including *yuc1yuc4*, *yuc1yuc4yuc6* and *taa1tar2*
98 allele combinations, show various degrees of decreased apical dominance reflected by an
99 enhanced development of branches. We reasoned that our dataset would provide a reference
100 to test the extent to which the role of auxin biosynthesis genes in apical dominance control
101 is shared between *A. thaliana* and *P. patens*. Auxin metabolite profiling in various genetic
102 backgrounds has shown that both steps of TRP-to-IAA conversion occur in *P. patens*^{15,16}. To
103 determine the changes in auxin biosynthesis gene activity during the release of apical
104 dominance, we therefore retrieved *PpTAR* (named *TAR* hereafter for the sake of readability)
105 and *PpYUC* (named *YUC* hereafter) expression profiles from our RNA-seq data. We identified
106 two *TAR* and three *YUC* genes that were significantly differentially expressed after
107 decapitation (Figure 1D and 1E). Specifically, *TARA*, *TARC* and *YUCF* were induced as early
108 as 2 h.a.d., *YUCC* was induced from 12 h.a.d., and *YUCB* was repressed. We also found that
109 the expression of *TARA*, *YUCE* and *YUCF*, but not *TARC*, *YUCB* and *YUCC*, was affected by
110 exogenous auxin, suggesting that auxin may to some extent regulate its own biosynthesis.
111 Thus, our data suggest that decapitation alters auxin biosynthesis elsewhere in the
112 gametophore.

113

114 ***TAR* and *YUC* genes are mainly active in the apical and basal portions of gametophores**

115 To determine the overall expression patterns of *TARA*, *TARC*, *YUCB*, *YUCC* and *YUCF* genes,
116 we analysed the activity of transcriptional fusions between corresponding promoter regions
117 and a GFP-GUS chimeric protein transformed in a wild-type background (Figure S3). We
118 found that the **spatial expression domains of *TAR* and *YUC* promoters** largely overlapped in
119 whole gametophores. Beta-glucuronidase (GUS) activity was detected at the main apex, at
120 the base, notably in rhizoid cells, and in discrete regions of the stem corresponding to
121 initiating branch apices (Figure 2A, 2D, 2G, 2J and 2M). At the main apex, ***TAR* promoters** are
122 active in the apical cell and emerging leaves¹⁵, and both *TAR* and *YUC* promoter activity were
123 also observed in axillary hair cells surrounding the gametophore apical cell, reminiscent of
124 the expression pattern of auxin-related genes like *PpSH11* and *PpSH12*¹⁸ (Figure 2P and 2Q).
125 Expression of *TAR* and *YUC* genes in isolated gametophore apices was confirmed by
126 quantitative RT-PCR (Figure S4). **Promoter activity of *YUCF***, and to a lesser extent *YUCB* and
127 *YUCC*, was also detected in axillary hair cells elsewhere on the stem (Figure 2M, 2R). This
128 indicates that the expression of *TARA* and *TARC* is more restricted in space than that of
129 *YUCB*, *YUCC* and *YUCF*, and it is unlikely that auxin biosynthesis occurs in leaf axils in the
130 absence of *TAR* activity.

131

132 **Activation of *TAR* and *YUC* gene expression accompanies branch formation**

133 To determine how decapitation affected the activity of selected transcriptional reporters,
134 gametophores were collected 24 hours after apex excision and stained for GUS activity,
135 thereby leaving enough time for new branches to initiate and grow out. The intensity of GUS
136 accumulation patterns remained overall similar to intact controls, except for the ***YUCB***
137 **promoter** that seemed more weakly expressed after decapitation, likely reflecting its
138 transcriptional repression (Figure 1E). The spatial distribution of these patterns was changed
139 in leaf axils where we observed an activation of ***TARA*, *TARC*, *YUCB*, *YUCC* and *YUCF***
140 **promoter expression** associated with the formation of new branches. The activity of ***TARA***
141 **and *TARC* promoters** was detected from the earliest stage of branch formation, throughout
142 the branch initium (Figures 2B, 2C, 2E and 2F). In contrast, ***YUCB*, *YUCC* and *YUCF* promoter**
143 **activity** were first detected at a later stage when the first leaves were clearly visible, and
144 mostly in hair cells of the branch apical region (Figures 2H, 2K, 2N). At a more mature stage
145 of branch development, *i.e.* when leaves and brown rhizoids were well-developed, ***TAR* and**
146 ***YUC* promoters** were active in overlapping spatial domains mirroring those described
147 previously in whole gametophores (Figures 2I, 2L and 2O, data not shown for *TAR*s).
148 Expression of *TAR* and *YUC* genes in newly emerged branches taken from decapitated
149 gametophores was further validated by quantitative RT-PCR (Figure S4). The temporal delay

150 between **TAR and YUC promoter activation** and the differences in their spatial expression
151 domains at early stages of branch initiation suggest that IPyA might be transported from the
152 meristematic cells where it is synthesized to adjacent hair cells to be converted to auxin by
153 YUC enzymes. Alternatively, these differences might be explained by the short-range
154 movement of *TAR* or *YUC* mRNA, or corresponding proteins; the involvement of *YUC* genes
155 expressed in the gametophore but not detected in our differential gene expression analysis,
156 such as *YUCE* (Figure S5); or unmask a putative YUC-independent function of IPyA. Together,
157 the above data suggest that TRP-dependent auxin biosynthesis occurs primarily in the main
158 apex and the basal portion of gametophores, and resumes locally in initiating branches during
159 development.

160

161 **TAR and YUC genes have dual roles in branch initiation and outgrowth**

162 To investigate the function of decapitation-induced *TAR* and *YUC* genes in gametophore
163 branching, we grew corresponding single and double knock-out mutants for 5-8 weeks and
164 quantified their branching patterns, as done previously¹² (Figure 3, Figure S6). We found that
165 *tara* and *tarc* single mutants had overall similar phenotypes to control plants (Figure S7), and
166 observed only minor perturbations of the branch distribution patterns in *yucc* and *yucf* single
167 mutants (Figure S8). In contrast, both *tarac* and *yuccf* double mutants had a shorter apical
168 inhibition zone and inter-branch distance, and an increased branch density with respect to
169 corresponding controls, suggesting that **apical dominance and lateral inhibition from branch**
170 **tips were significantly weaker in auxin biosynthesis mutants** (Figure 3A-3H, 3K-3L and 3M-
171 3N). **These data provide the first direct evidence that auxin biosynthesis genes active in**
172 **gametophore apices coordinate branch patterning at a distance,** thus demonstrating the
173 central hypothesis of a previously published model of branching control¹². We also observed
174 that the position of the lowermost branch was closer to the gametophore base in *tarac* and
175 *yuccf* mutants than in corresponding wild-type controls (Figure 3O). Moreover, the number
176 of branches in the three most basal metamers was much higher in *tarac* (n = 23 branches)
177 and *yuccf* gametophores (n = 15) than in wild-type controls (n = 5 and 3, respectively),
178 suggesting that local auxin biosynthesis prevents branching at the gametophore base
179 (Figures 3A-3D). A CAROTENOID CLEAVAGE DIOXYGENASE 8 (CCD8)-derived cue
180 belonging to the strigolactone family of compounds has previously been proposed to
181 suppress branching at the gametophore base^{12,19}, and *in vitro* characterization of PpCCD8
182 enzymatic activity indicates that this molecule may be the strigolactone precursor
183 carlactone²⁰. These data suggest that auxin could act concomitantly with carlactone to fulfil
184 this inhibitory role, and their mode of interaction remains to be seen. Despite the overall

185 increase in branch number in *tarac* mutants, we observed strong disparities in the branch
186 distribution pattern between gametophores. Whilst some gametophores bore branches on
187 up to six consecutive metamers, others had few or no visible branches at all, which was
188 rather counter-intuitive (Figure 3B). However, on closer inspection of *tarac* gametophores,
189 we noticed that tiny arrested branches were nested in most leaf axils lacking a visible branch
190 (Figure 3I-3J), reminiscent of dormant buds observed in other plants. We found that branch
191 formation was suppressed by exogenous auxin application in *tarac* mutants, as well as *TARA*
192 overexpression (Figure S9). This suggests that in normal conditions, low *TAR* activity triggers
193 branch formation, whilst relatively higher *TAR* activity is necessary to promote branch
194 development at later stages. Thus, branch initiation and outgrowth are separable processes
195 that are coupled during development through controlled spatio-temporal changes in auxin
196 levels, similar to the mechanism coordinating axillary meristem formation and outgrowth in
197 flowering plants²¹.

198
199 This work aimed to research genetic determinants involved in the regulation of apical
200 dominance in the gametophytic shoot of a moss. We have taken a comparative transcriptome
201 approach, showing that early auxin-responsive genes dominate in the response of
202 gametophores to decapitation, and identified *TAR* and *YUC* auxin biosynthesis genes acting
203 redundantly in the main gametophore apex and branch apices. **Perturbations of branching**
204 **patterns measured in *tarac* and *yucf* mutants are consistent with a role of corresponding**
205 **genes in apical dominance control.** In the future, our dataset should enable to explore further
206 the mode of auxin action and, notably, the interplay with putative shared regulators of apical
207 dominance revealed in gene ontology enrichment analyses, such as trehalose-6-phosphate
208 metabolic enzymes²²⁻²⁵ and APETALA2/ETHYLENE RESPONSIVE ELEMENT BINDING
209 PROTEIN transcription factors²⁶⁻²⁹ (Tables S2 and S3, Figures S10 and S11). Our dataset also
210 contains genes of uncharacterized function whose transcription was not affected by auxin,
211 representing an untapped reservoir of putative regulators of shoot architecture.

212 From an evolutionary perspective, the *TAR-YUC*-dependent auxin biosynthesis pathway
213 originated in streptophyte algae and is conserved in all extant land plant lineages^{30,31}. For
214 instance, the genome of the liverwort *Marchantia polymorpha* contains one functional *MpTAA*
215 gene and two *MpYUC* genes, named *MpYUC1* and *MpYUC2*³². Similar to our observations
216 in the moss gametophore, *MpYUC2* expression domain is broader than that of *MpTAA* in the
217 liverwort thallus (*i.e.*, the leafless gametophytic body), and both domains overlap in
218 meristematic notches that contain the apical stem cells at the origin of thallus tissues. Whilst
219 thallus branching occurs by apical notch dichotomy that is developmentally distinct from

220 lateral branching in the moss gametophore^{33,34}, *M. polymorpha* *taa* and *yuc* knock-down
221 plants also display hyperbranching phenotypes. Together with data previously collected in *A.*
222 *thaliana* and our novel findings in *P. patens*, this suggests that a conserved molecular
223 mechanism driving auxin biosynthesis operates locally, in morphologically distinct
224 meristematic structures, and coordinates various branching modes in bryophytes and
225 vascular plants. Although this is still disputed, the most recent molecular phylogenies have
226 resolved bryophytes (including *P. patens* and *M. polymorpha*) as a monophyletic lineage,
227 sister to the vascular plants (including *A. thaliana*), and both lineages could have diverged
228 from their last common ancestor in the Cambrian period (541-485 My ago)³⁵⁻³⁷. The exact
229 nature and morphology of this ancestor remains largely inaccessible, but combined evidence
230 from phylogeny and the fossil record points towards a leafless body with dichotomously
231 branched gametophytic axes bearing nutritionally-dependent and unbranched
232 sporophytes³⁸⁻⁴⁰. Lateral branching, where branches form on the side of growing axes in
233 association with leaves or phyllids (*i.e.*, the leaf-like organs of bryophytes), is therefore a
234 derived trait which arose by convergence in moss gametophytes and vascular plant
235 sporophytes³³. In contrast with the classical definition of homology which implies a historical
236 continuity in the evolution of morphological traits, the notion of deep homology that defines
237 'the sharing of the genetic regulatory apparatus used to build morphologically and
238 phylogenetically disparate features' applies here^{41,42}. We conclude that the involvement of
239 *TAR-YUC*-mediated auxin biosynthesis to regulate apical dominance and shoot branching
240 represents a remarkable case of deep homology in land plants.

241 **Material and methods**

242 ***Physcomitrium patens* plant growth and transformation**

243 The Gransden strain of *Physcomitrium patens* was used for RNA-seq experiments, and the
244 Reute⁴³ strain that is more fertile in laboratory conditions was used as the wild-type (WT)
245 background for generating all the transgenic lines. Moss colonies were initiated from 1 mm²
246 spot cultures and cultivated for 4-5 weeks (unless otherwise stated) in sterile Magenta GA-7
247 tissue culture vessels (Bioworld, Dublin, OH, USA) on BCDAT medium (250 mg/L
248 MgSO₄·7H₂O, 250 mg/L KH₂PO₄ (pH 6.5), 1010 mg/L KNO₃, 12.5 mg/L, FeSO₄·7H₂O, 0.001%
249 Trace Element Solution (0.614 mg/L H₃BO₃, 0.055 mg/L AlK(SO₄)₂·12H₂O, 0.055 mg/L
250 CuSO₄·5H₂O, 0.028 mg/L KBr, 0.028 mg/L LiCl, 0.389 mg/L MnCl₂·4H₂O, 0.055 mg/L
251 CoCl₂·6H₂O, 0.055 mg/L ZnSO₄·7H₂O, 0.028 mg/L KI and 0.028 mg/L SnCl₂·2H₂O), 0.92 g/L
252 di-ammonium tartrate (C₄H₁₂N₂O₆) and 8 g/L agar with CaCl₂ added to a 1 mM concentration
253 after autoclaving, at 23°C under a 16h light/8h dark cycle, at 50-150 μmol.m⁻².s⁻¹ in MLR-352
254 growth cabinets (PHCbi, Etten-Leur, The Netherlands). Genetic transformation was
255 performed as described in Coudert *et al.* (2019)⁴⁴.

256

257 **Decapitation and auxin treatments**

258 For pharmacological treatments of wild-type gametophores, a 70% ethanol solution
259 containing 100 mM indole-3-acetic acid (IAA) (Merck KGaA, Darmstadt, Germany) was
260 diluted 10,000 times in water to reach a final concentration of 10 μM IAA. A 70% ethanol
261 solution was diluted 10,000 times in water and used as a mock control. Moss cultures were
262 soaked in diluted solutions for 30 minutes before tissue harvest. For decapitation,
263 gametophores were teased apart from moss colonies with thin tip tweezers, planted in an
264 upright position on BCDAT petri dishes and their apical portion was cut off with micro-
265 scissors, as described in Coudert, Palubicki *et al.* (2015)¹². For pharmacological treatments
266 of *tarac* mutants, a 95% ethanol solution containing 1 mM indole-3-acetic acid (IAA) (Duchefa
267 Farma B.V., Haarlem, Netherlands) was diluted 1,000 times in water to reach a final
268 concentration of 1 μM IAA. A 95% ethanol solution was diluted 1,000 times in water and used
269 as a mock control. Moss cultures were soaked in diluted solutions for 24 hours 3, 5 and 7
270 weeks after protonema inoculation.

271

272 **Generation of *YUC* transcriptional reporters and *yuc* mutants**

273 All the transgenic lines used in this study are listed in Table S4. Generation and confirmation
274 of the transcriptional reporter lines *TARA::GFP-GUS*, *TARC::GFP-GUS*, and *YUCF::GFP-*
275 *GUS*, as well as the knockout lines *tara*, *tarc*, *tarac*, *yucc*, and *yucf* have been previously

276 described¹⁵. To produce the *YUCB::GFPGUS* reporter construct pMT244 (Figure S3), a 2090
277 bp *YUCB* promoter fragment was amplified from gDNA with primers SS235/SS236 and
278 trimmed with *Bam*HI/*Nco*I. The resulting fragment was cloned between the *Bam*HI/*Nco*I sites
279 of pMT211, a vector allowing promoters to be cloned ahead of a GFP-GUS reporter gene for
280 subsequent integration into the neutral *Pp108* locus⁴⁵. Similarly, to produce the
281 *YUCC::GFPGUS* reporter construct pMT251 (Figure S3), a 1767 bp *YUCC* promoter fragment
282 was amplified from gDNA with primers SS237/SS238, trimmed with *Bam*HI/*Bsp*HI, and
283 cloned between the same two sites in pMT211. Both reporter constructs were linearized with
284 *Sfi*I before they were transformed into WT protoplast as previously described⁴⁶. Stable
285 transformants were selected in the presence of 50 µg.ml⁻¹ hygromycin (Duchefa H0192;
286 Haarlem, the Netherlands). Correct integration was confirmed by PCR (Figure S3). For primer
287 sequences, see Table S5. The *yuccf* double knockout lines were produced by a sexual cross
288 of the confirmed single mutants *Ppyucc-2* and *Ppyucf-1* described previously¹⁵, according to
289 the method presented in Thelander *et al.*⁴⁵. Double knockout lines were confirmed by PCRs
290 demonstrating the loss of internal gene sequences (Figure S5).

291

292 **GUS staining and plant imaging**

293 *Physcomitrium patens* gametophores were isolated from colonies grown on BCDAT and
294 incubated at 37°C in a 100 mM phosphate buffer (pH 7) with 10 mM Tris (pH 8), 1 mM EDTA
295 (pH 8), 0.05% Triton X-100, 5mM potassium ferricyanide, 5mM potassium ferrocyanide and
296 2 mM X-GlcA (5-Bromo-4-chloro-3-indolyl-β-D-glucuronic acid), using times indicated in
297 Figure 2. Tissues were de-stained in 70% ethanol and imaged with a Keyence VHX-900F
298 digital microscope with a 5-50 X or a 50-200 X objective. Two to three independent transgenic
299 lines were observed for each genetic construct (Table S4). Plants not subject to GUS staining
300 were imaged in the same conditions.

301

302 **Definition of branches in *Physcomitrium patens***

303 According to Coudert *et al.*¹¹, a module is defined as “a portion of gametophore arising from
304 a single apical cell”. Here, we consider that the main gametophore axis corresponds to a
305 Class I module, and gametophore branches correspond to Class II lateral modules.

306

307 **RNA-seq data production and analysis**

308 For each biological replicate and condition, RNA were pooled from 5-10 gametophores
309 (Gransden⁴⁷ strain). Three independent biological replicates were produced. Total RNA was
310 extracted using a RNeasy Plant Mini Kit (Qiagen, Hilden, Germany) and treated with DNase

311 according to the supplier's instructions. RNA-seq libraries were made using the
312 TruSeq_Stranded_mRNA_SamplePrep_Guide_15031047_D protocol (Illumina, California,
313 U.S.A.). The RNA-seq samples have been sequenced in paired-end (PE) with a sizing of 260
314 bp and a read length of 75 bases, using an Illumina NexSeq500 technology (IPS2, POPS
315 platform, Gif-sur-Yvette). 24 samples were processed by lane of NextSeq500 using individual
316 bar-coded adapters, generating approximately 30 million of PE reads per sample. All
317 experimental steps, from growth conditions to bioinformatic analyses, were deposited in the
318 CATdb database⁴⁸ (<http://tools.ips2.u-psud.fr/CATdb/>, ProjectID NGS2017_09_Moss1)
319 according to the MINSEQE 'minimum information about a high-throughput sequencing
320 experiment' (<https://doi.org/10.25504/FAIRsharing.a55z32>). To facilitate comparisons, all
321 samples were processed similarly from trimming to count. RNA-Seq pre-processing included
322 trimming library adapters and performing quality controls. Raw data (fastq) were trimmed
323 with fastx toolkit for Phred Quality Score Qscore >20, read length >30 bases, and ribosome
324 sequences were removed with sortMeRNA⁴⁹. The mapper Bowtie⁵⁰ (version 2) was used to
325 align reads against the *Physcomitrium* (*Physcomitrella*) *patens* transcriptome (with local
326 option and other default parameters). The 32926 genes were extracted from Phytozome
327 database (*Physcomitrella patens* transcripts v3.1 gene model). The abundance of each gene
328 was calculated by a local script which parses SAM files and counts only paired-end reads for
329 which both reads map unambiguously one gene, removing multi-hits. According to these
330 rules, around 82,7% of PE reads were associated to a gene, 5-6% PE reads were unmapped
331 and 12-13% of PE reads with multi-hits were removed. Choices for the differential analysis
332 were made based on the article by Rigai et al⁵¹. Genes which did not have at least one read
333 after a counts per million (CPM) normalization in at least one half of the samples were
334 discarded. Library size was normalized using the TMM method and count distribution was
335 modeled with a negative binomial generalized linear model. Dispersion was estimated by the
336 edgeR method⁵² (version 1.12.0) in the statistical software 'R' (<http://www.R-project.org>,
337 version 2.15.0). Expression differences were compared between "0 h.a.d." and "2 h.a.d.", "6
338 h.a.d.", "12 h.a.d." or "24 h.a.d." conditions for the "decapitation experiment", and between
339 samples "mock" and "10 μ M IAA" conditions for the "auxin experiment", using likelihood ratio
340 test and p-values were adjusted by the Benjamini-Hochberg procedure to control FDR. A
341 gene was considered as differentially expressed if its adjusted p-value was lower than 0.05.
342 Venn diagrams were generated using InteractiVenn⁵³. Gene Ontology (GO) term enrichment
343 analyses were performed with ShinyGO (version 0.66)⁵⁴.

344 **RNA-seq data availability**

345 RNA-seq datasets are available in the international repository GEO (Gene Expression
346 Omnibus⁵⁵, <http://www.ncbi.nlm.nih.gov/geo>) under the project identifier GSE188843.

347

348 **Gene identifiers**

349 Gene identifiers were obtained from the most recent annotation (version 3.3) of *P. patens*
350 genome⁵⁶. Identifiers of *PpIAA* and *PpARF* genes (Figure 1) are : *PpIAA1B*, *Pp3c8_14720* ;
351 *PpIAA2*, *Pp3c24_6610* ; *PpARFa1*, *Pp3c1_14480* ; *PpARFa3*, *Pp3c2_25890* ; *PpARFa4*,
352 *Pp3c13_4720* ; *PpARFa5*, *Pp3c26_11550* ; *PpARFa6*, *Pp3c17_19900* ; *PpARFa7*,
353 *Pp3c14_16990* ; *PpARFa8*, *Pp3c1_40270* ; *PpARFb1*, *Pp3c27_60* ; *PpARFb2*, *Pp3c16_6100*
354 ; *PpARFb3*, *Pp3c5_9420* ; *PpARFb4*, *Pp3c6_21370* ; *PpARFc1B*, *Pp3c4_13010* ; *PpARFc2*,
355 *Pp3c6_26890* ; *PpARFd1*, *Pp3c9_21330* and *PpARFd2*, *Pp3c15_9710*. Identifiers of *PpTAR*
356 and *PpYUC* genes (Figure 1) are : *PpTARA*, *Pp3c21_15370* ; *PpTARB*, *Pp3c18_15140* ;
357 *PpTARC*, *Pp3c17_6500* ; *PpTARD*, *Pp3c26_12520* ; *PpYUCA*, *Pp3c3_18590* ; *PpYUCB*,
358 *Pp3c11_11790* ; *PpYUCC*, *Pp3c1_11500* ; *PpYUCD*, *Pp3c2_27740* ; *PpYUCE*,
359 *Pp3c13_21970* and *PpYUCF*, *Pp3c3_20490*.

360 **Quantitative RT-PCR**

361 For expression analyses, apices (including the apical cell and a few phyllids) and newly
362 emerged branches (same stages as in Figure 2H-I, K-L, N-O) were dissected out with micro-
363 scissors and thin tweezers from intact and decapitated gametophores, respectively. Total
364 RNA was isolated using the INVITROGEN PureLink™ RNA Mini Kit (Ref: 12183025). RNA was
365 DNase treated with ezDNase™ enzyme prior to reverse transcription with SuperScript™ IV
366 VILO™ Master Mix (Ref: 11766050) following manufacturer's guidelines. qRT-PCR was
367 performed using a ROCHE Fast Start Universal SYBR Green master mix and a ThermoFisher
368 StepOnePlus Real-Time PCR System. Thermocycling conditions were: 95°C 10 min; 95°C 10
369 sec, 58°C 30 sec (40 cycles). Specificity of PCR amplification was checked by melt curve
370 analysis at each run. *PpTARA*, *PpTARC*, *PpYUCB*, *PpYUCC*, *PpYUCF* and *PpUBI* genes were
371 respectively amplified using the following primer pairs: YCL-R1-TARA-144F, YCL-R1-TARA-
372 144R, YCL-R1-TARC-145F, YCL-R1-TARC-145R, YCL-R1-YUCB-146F, YCL-R1-YUCB-
373 146R, YCL-R1-YUCC-147F, YCL-R1-YUCC-147R, YCL-R1-YUCF-148F, YCL-R1-YUCF-
374 148R, YCL-12F-R1-UBI and YCL-12R-R1-UBI. Expression level of *PpUBI* (Pp1s56_52V6.1,
375 Pp3c12_5760V3.1) was used as a reference to normalize gene expression level between
376 conditions, and Ct values were converted into relative expression values using the
377 comparative Ct method⁵⁷.

378

379 **Sample-size estimation and replicates**

380 For data shown in Figure 1, three independent biological replicates were produced for each
381 condition, and each replicate corresponded to RNA extracted from 5 to 10 gametophores.
382 For Figure 2, two or three independent transgenic lines were analysed for *TAR* and *YUC*
383 transcriptional reporters. Independent lines transformed with the same genetic construct
384 showed similar GUS staining patterns. Numbers in panels A, D, G, J and M indicate the
385 proportion of gametophores with a GUS staining pattern similar to the picture. For Figure 3,
386 data shown in panels A-D were obtained from 60 gametophores collected 5-7 weeks after
387 protonema inoculation, data in panels K and L correspond to all gametophores, and metrics
388 in panels M, N and P could be calculated only for gametophores with more than one branch.
389 For Figure S9, data shown in panels A-C, E-F, I-J were obtained from 60 gametophores
390 collected 5-9 weeks after protonema inoculation.

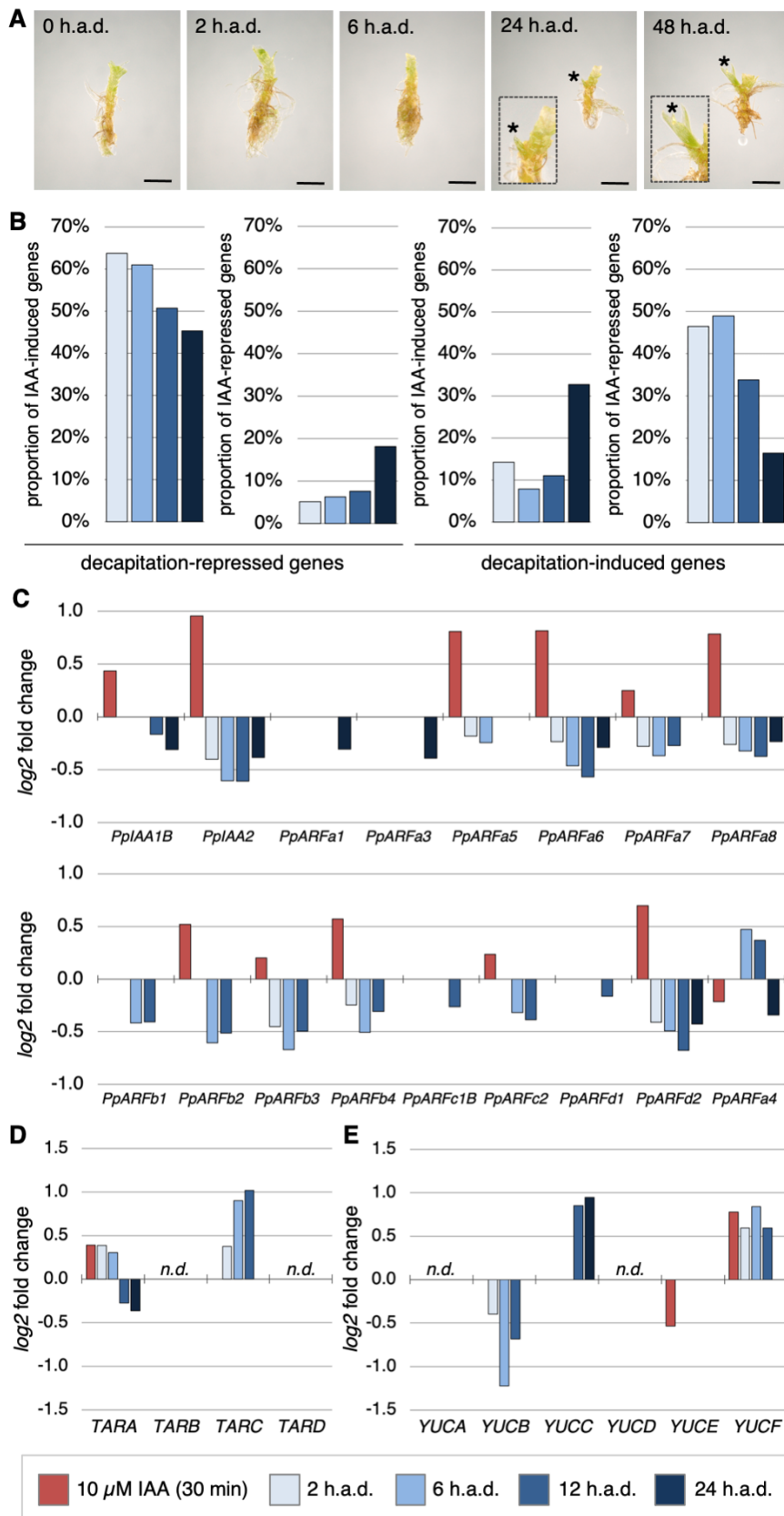
391

392 **Statistical analyses**

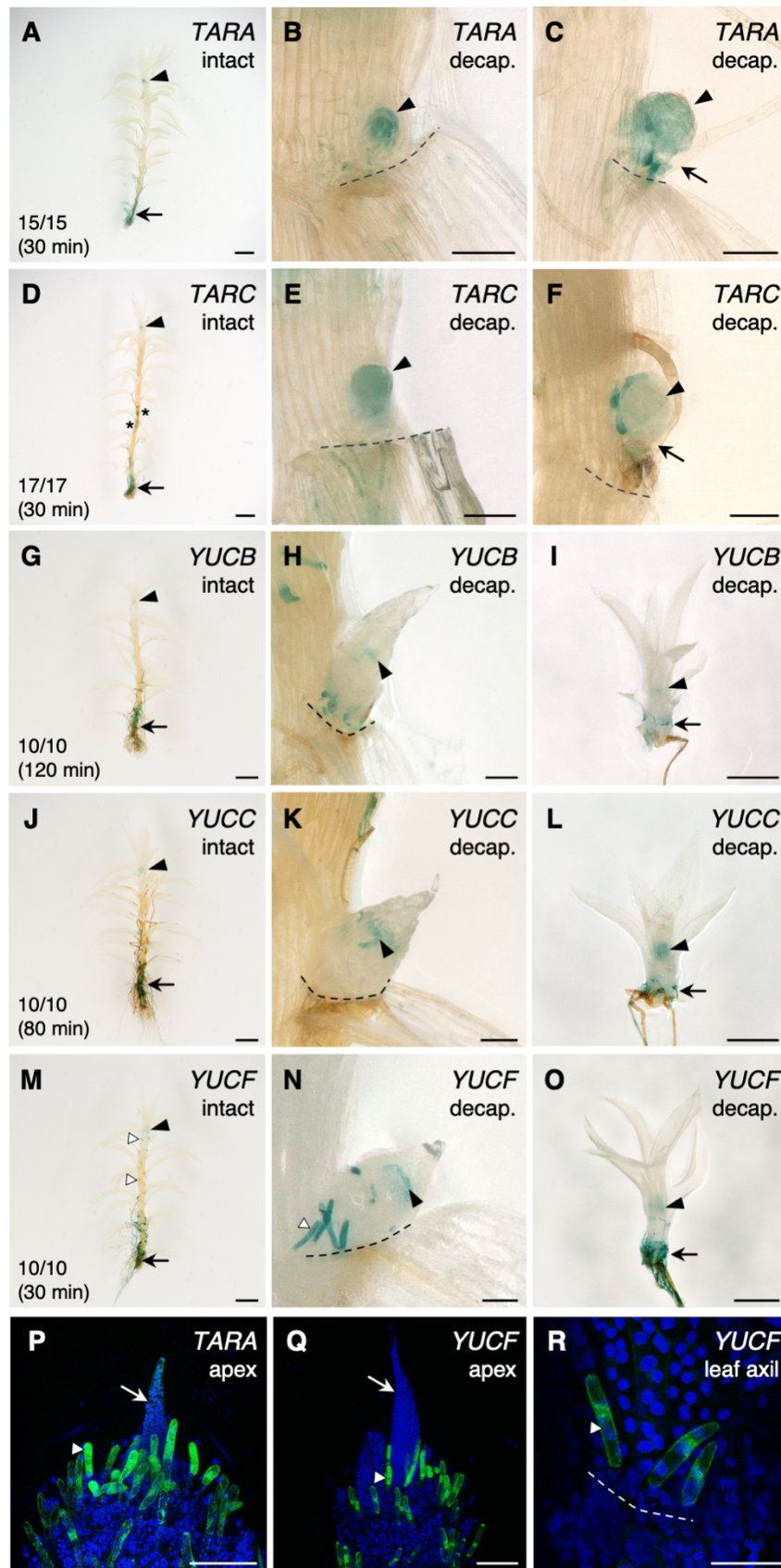
393 Significance of association between IAA repression/induction and decapitation
394 repression/induction was assessed via chi-squared tests. Generalised linear modelling was
395 used to test whether the relationship between branch number and gametophore length
396 depended on genotype (File S1). Poisson or negative binomial regression was used,
397 depending on whether the data were over-dispersed, *i.e.* whether the data were more
398 variable than would be expected under a Poisson model. The most complex fitted models
399 were of the form $\log(\mathbb{E}(B|L, X)) = a_0 + a_1X + (a_2 + a_3X)L$, in which a_0 , a_1 , a_2 and a_3 are
400 coefficients, L is the length, X is an indicator variable depending on genotype (*i.e.* X took
401 values of either 0 or 1 depending upon whether the mutant or WT was considered), and
402 $\mathbb{E}(B|L, X)$ is the expected branch number (B) at any given pair of values of L and X . Analysis
403 of deviance and backwards stepwise elimination was used to find the minimal model that
404 received support from the experimental data. A mutant was considered different from WT if
405 either the interaction term (a_3) or the term corresponding to genotype (a_1) remained in the
406 minimal model, *i.e.* if the expected branch number was affected by the value of X . The choice
407 of whether to use a Poisson and negative binomial model was made via a formal test of over-
408 dispersion for the Poisson version of the model with an interaction, using the function
409 `overdispersion()` in the package `AER`⁵⁸. The terms “weak”, “moderate”, “strong” and “very
410 strong evidence” reflected the translation of p-values into the language of evidence proposed
411 by Muff *et al.*⁵⁹

412

413



417 **Figure 1. Decapitation and auxin treatment have opposite effects on auxin-responsive**
418 **and auxin signalling gene expression, and affect auxin biosynthesis gene activity in**
419 **moss gametophores. (A)** New branches (indicated with asterisks) emerged within 24 hours
420 after gametophore decapitation. Leaves were removed before imaging to observe
421 gametophore branches. Insets show magnifications of emerging branches. Scale bar = 1 mm.
422 **(B)** Cross-analysis of transcriptomes of decapitated and auxin-treated gametophores
423 showed that about two thirds of the genes repressed 2 h.a.d. were induced by a 30-minute
424 treatment with 10 μ M indole-3-acetic acid (auxin), and conversely nearly half of the genes
425 induced 2 h.a.d. were repressed by auxin. **(C)** Bar plots showed that most *PpARF* and *PpIAA*
426 auxin signalling genes identified were repressed after decapitation and induced by auxin. **(D-**
427 **E)** Expression of two *TAR* and three *YUC* auxin biosynthesis genes was affected by
428 decapitation. The colour code shown at the bottom of the figure is used in panels B-E. “*n.d.*”
429 means not detected in the differential gene expression analysis.
430

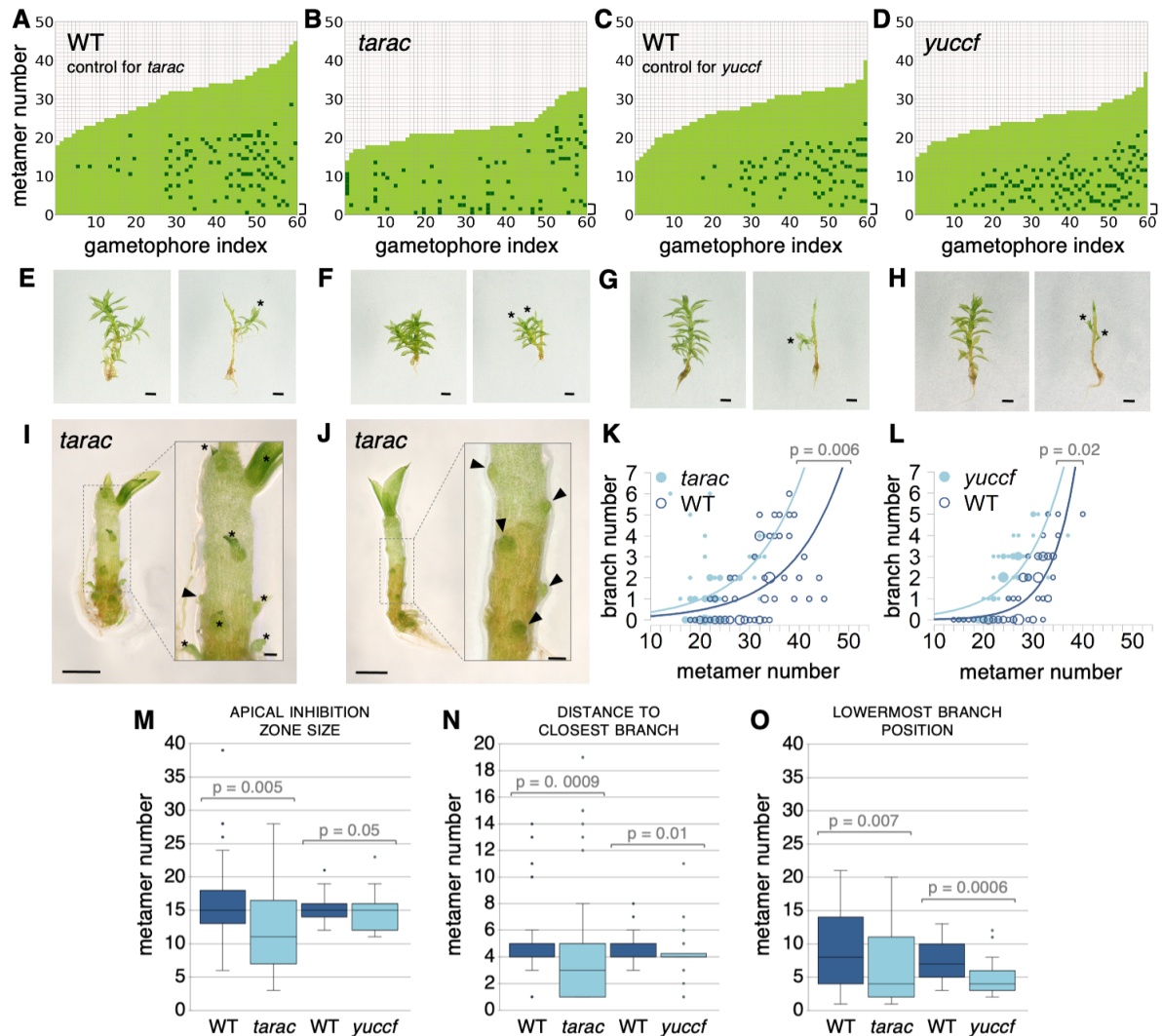


431

432 **Figure 2. *TAR* and *YUC* genes are expressed in meristematic and basal regions of *P.***

433 ***patens* gametophores. (A-O) GUS staining of *TARA::GFP-GUS* (A-C), *TARC::GFP-GUS* (D-**

434 F), *YUCB::GFP-GUS* (G-I), *YUCC::GFP-GUS* (J-L) and *YUCF::GFP-GUS* (M-O) transgenic
435 lines revealed that *TAR* and *YUC* genes are active in largely overlapping expression domains.
436 Gametophore were either observed before (“intact”) or after (“decap.”) apex excision. In
437 intact gametophores, *TARA* and *TARC* are expressed in the main apex (black arrowheads),
438 in emerging branches (asterisks) and in the basal region (black arrows) (A, D). Following
439 decapitation, *TARA* and *TARC* expression is detected in initiating lateral branches (black
440 arrowheads) but not in rhizoids (black arrows) (B, C, E, F). In intact gametophores, *YUCB*,
441 *YUCC* and *YUCF* are expressed in the main apex (black arrowheads) and in the basal region
442 (black arrows). *YUCF* expression is also observed in axillary hairs in leaf axils (white
443 arrowheads) (G, J, M). Following decapitation, *YUCB*, *YUCC* and *YUCF* expression is first
444 detected at a later stage than *TARA* and *TARC*. GUS staining was found in axillary hairs at
445 the apex of newly formed branches (black arrowheads) and, at a later stage, in branch
446 rhizoids (black arrows) (H, I, K, L, N, O). **(P-R)** GFP signal in *TARA::GFP-GUS* and
447 *YUCF::GFP-GUS* lines was strongest in hairs (white arrowheads) located at the gametophore
448 apex (P-Q), and/or in leaf axils (R). Emerging leaves are indicated with white arrows. Dashed
449 lines mark the boundary between the stem and the detached leaf (B, C, E, F, H, K, N, R).
450 Gametophores were soaked in GUS staining solution for times specified in panels A, D, G, J
451 and M. The scale bars represent 1 mm in A, D, G, J and M, 500 μm in I, L, and O, 100 μm in
452 B, C, E, F, H, K, P and Q, and 50 μm in N and R.
453



454

455 **Figure 3. *TAR* and *YUC* genes inhibit branch initiation, but *TAR* genes promote branch**

456 **outgrowth. (A-D)** Plots show the branching patterns of 60 gametophores ordered by

457 increasing size in *tarac* and *yucgf* double mutants, and corresponding wild-type (WT) controls.

458 Each gametophore is represented as a series of metamers (light green squares) and lateral

459 branch position is indicated in dark green. Brackets indicate the three lowermost metamers.

460 **(E-H)** WT (E), *tarac* (F), WT (G) and *yucgf* (H) gametophores before (left) and after (right)

461 removing leaves, with asterisks marking branches. Scale bars represent 1 mm. **(I-J)** *tarac*

462 gametophores with leaves removed revealed dormant or arrested buds with no visible leaves

463 (arrowheads) and tiny branches with few visible leaves (asterisks), which is never observed

464 in WT in which branches initiate and immediately grow out. Scale bars represent 500 μ m in

465 the main panels and 100 μ m in the insets. **(K-L)** Gametophore length is represented as the

466 number of metamers and the bubble area is proportional to the number of gametophores

467 with the same branch number (*B*) at a given length (*L*). Bubble plots showed that branch

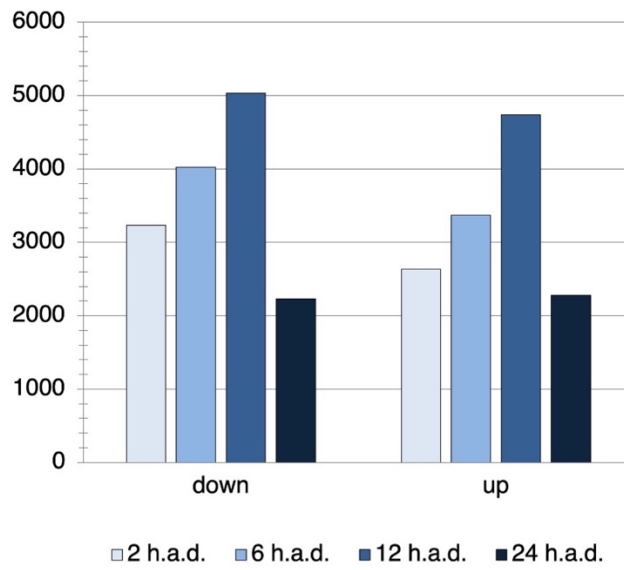
468 number at a given length is increased in *tarac* compared with WT. The data were over-

469 dispersed, and so negative binomial regression was used to test whether and how the
470 relationship between branch number and gametophore length differed between mutants and
471 WT (see ‘Material and methods’). For (K) the best-fitting relationship indicated WT was
472 smaller than *tarac* at all lengths (p-value=0.006; $\log(\mathbb{E}(B|L, W)) = -1.94 - 0.73W + 0.095L$,
473 where $W = 1$ for WT). For *yuccf* mutants, the data were not over-dispersed, and so Poisson
474 regression was used to test whether and how the relationship between branch number and
475 gametophore length depended on treatment (see ‘Material and methods’ and SI data). The
476 best-fitting relationship included an interaction, indicating the nature of the difference in the
477 number of branches between WT and mutants depended upon the length (in (L), $p = 0.02$,
478 $\log(\mathbb{E}(B|L, Y)) = -5.28 + 3.25Y + (0.20 - 0.08Y)L$, where $Y = 1$ for *yuccf*). **(M)** There was
479 strong and moderate evidence that the apical inhibition zone was reduced in *tarac* and *yuccf*
480 double mutants, respectively, compared with corresponding wild-type controls (Wilcoxon
481 rank sum test with continuity correction; WT versus *tarac*, p-value = 0.005; WT versus *yuccf*,
482 p-value = 0.05). **(N)** There was very strong and strong evidence that the distance to the
483 closest branch, a measurement of branch spacing, was reduced in *tarac* and *yuccf* double
484 mutants, respectively, compared with wild-type controls (Wilcoxon rank sum test with
485 continuity correction; WT versus *tarac*, p-value = 0.0009; WT versus *yuccf*, p-value = 0.01).
486 **(O)** There was very strong evidence that the position of the lowermost branch was closer to
487 the gametophore base in *tarac* and *yuccf* double mutants than in WT (Wilcoxon rank sum test
488 with continuity correction; WT versus *tarac*, p-value = 0.007; WT versus *yuccf*, p-value =
489 0.0006). The terms “weak”, “moderate”, “strong” and “very strong evidence” reflected the
490 translation of p-values into the language of evidence proposed by Muff *et al.*⁵⁹
491

492 **Supplementary data**

493

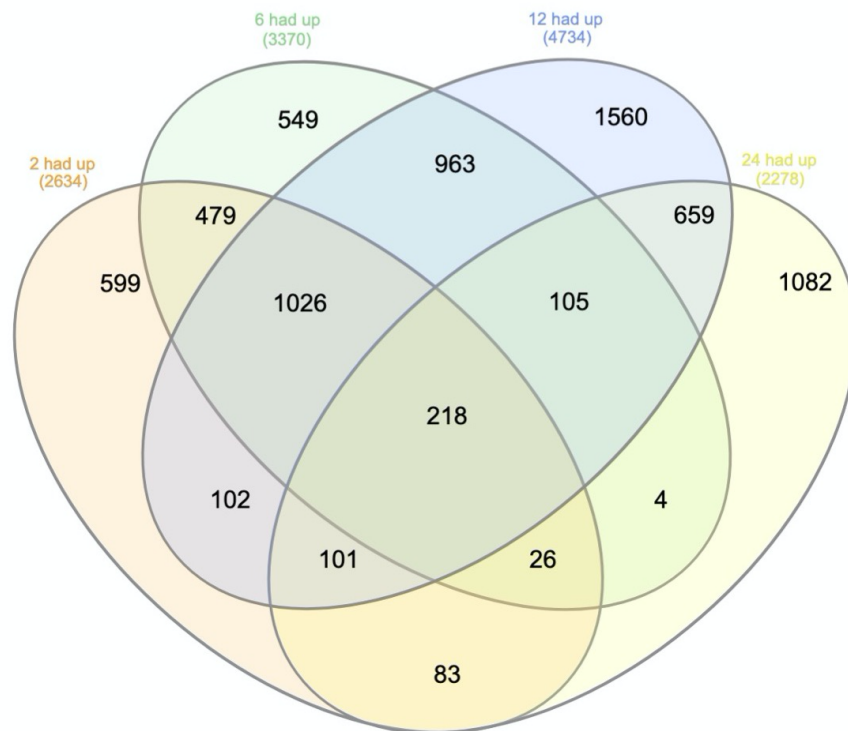
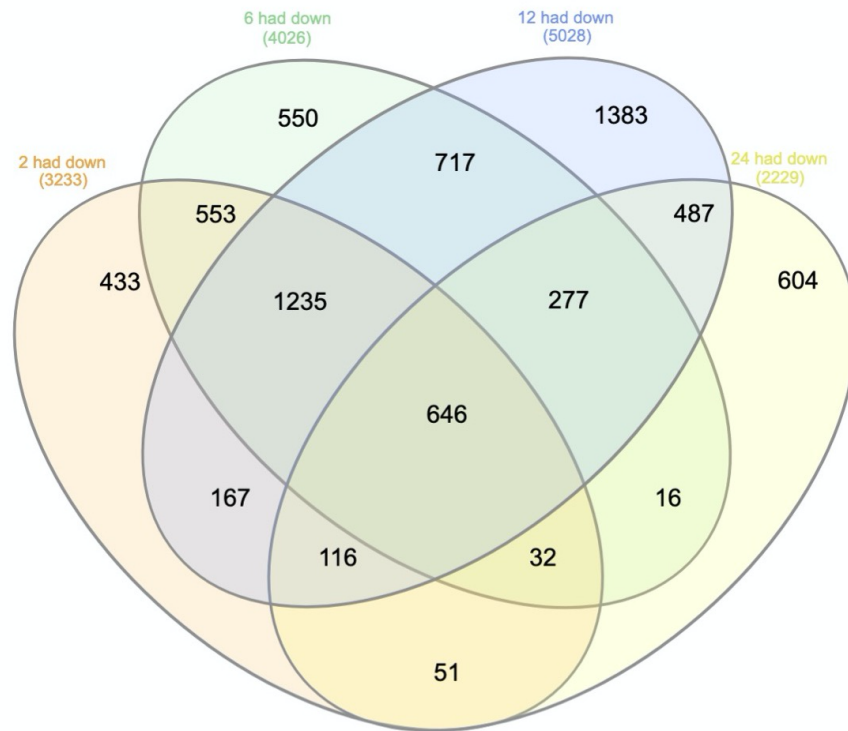
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496 **Figure S1. Number of genes repressed or induced 2, 6, 12 and 24 hours after**

497 **decapitation.**

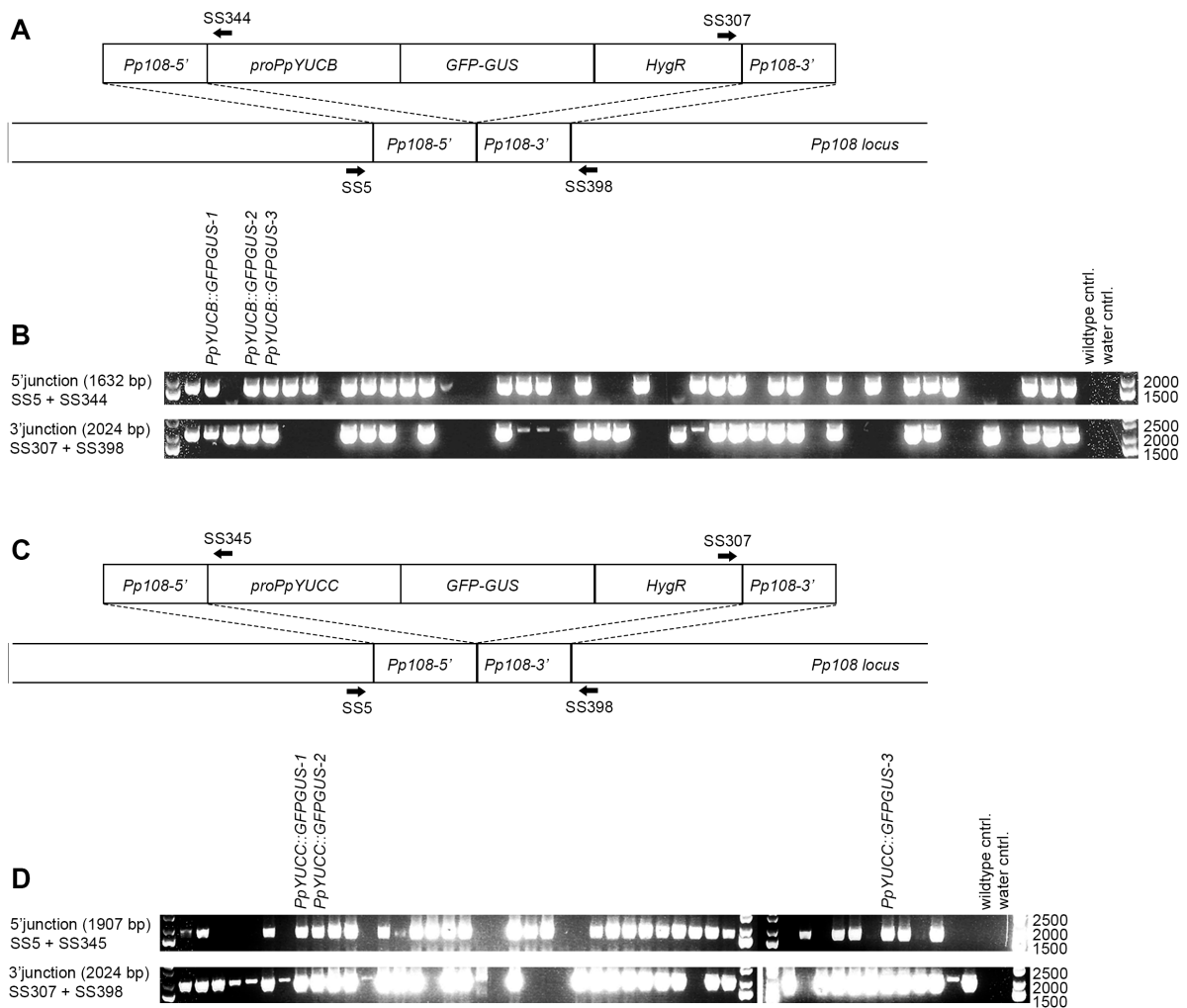


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500 **Figure S2. Venn diagrams for all genes repressed or induced 2, 6, 12 and 24 hours after**
 501 **decapitation.**

502



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504

505 **Figure S3. PCR verification of *PpYUCB* and *PpYUCC* transcriptional reporter lines in**

506 **WT background (A) Schematic view of the *PpYUCB::GFP-GUS* reporter construct pMT244**

507 **and the *Pp108* locus to which it was targeted. Arrows mark the approximate annealing sites**

508 **of primers used for PCR verification in B. (B) PCR verification of 5' and 3' junctions to confirm**

509 **correct integration of the *PpYUCB::GFP-GUS* reporter construct into the *Pp108* locus. (C)**

510 **Schematic view of the *PpYUCC::GFP-GUS* reporter construct pMT251 and the *Pp108* locus**

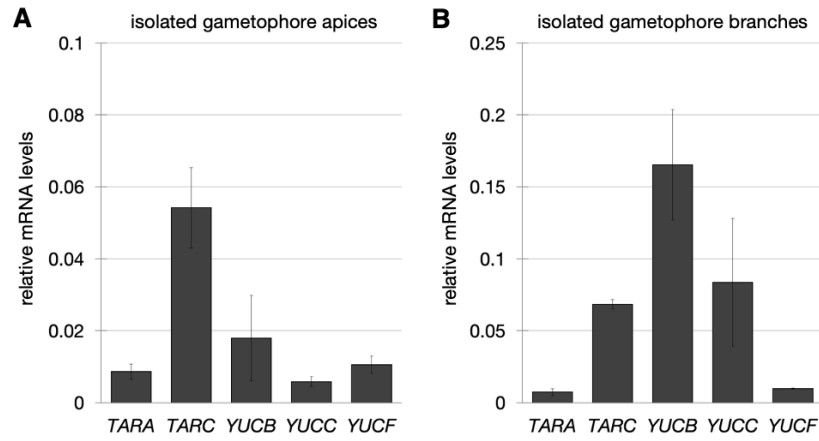
511 **to which it was targeted. Arrows mark the approximate annealing sites of primers used for**

512 **PCR verification in D. (D) PCR verification of 5' and 3' junctions to confirm correct integration**

513 **of the *PpYUCC::GFP-GUS* reporter construct into the *Pp108* locus. In both B and D, expected**

514 **product sizes are indicated within parentheses. For primer sequences, see Table S5.**

515

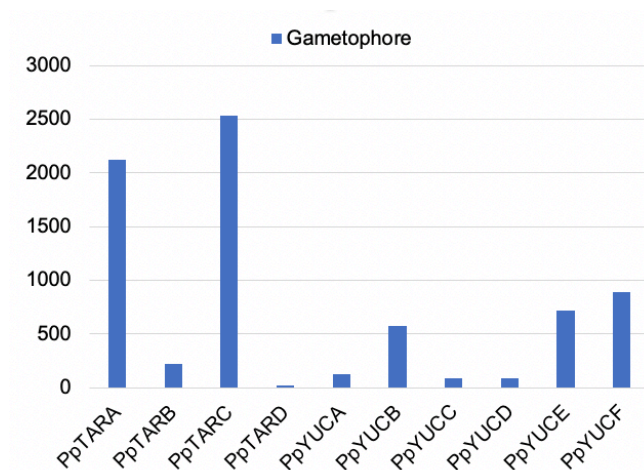


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518 **Figure S4. Quantitative RT-PCR analysis of *TARA*, *TARC*, *YUCB*, *YUCC* and *YUCF***
 519 **mRNA accumulation levels relative to *UBI* internal control in *Physcomitrium patens***
 520 **gametophore apices (A) and branches (B) (mean level of three independent biological**
 521 **replicates \pm SD).**

522
523
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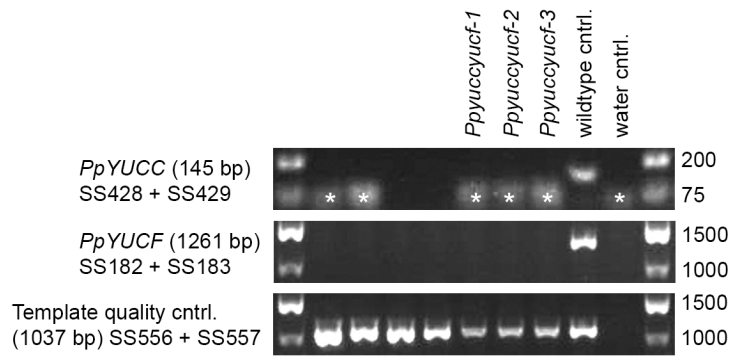
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528 **Figure S5. Absolute expression levels of *TAR* and *YUC* genes in *P. patens***
529 **gametophores retrieved from Ortiz-Ramirez *et al.* (2016)⁶⁰.** Correspondence between
530 gene names and identifiers: *PpTARA* (Pp3c21_15370V3.1, Pp1s167_103V6.1), *PpTARB*
531 (Pp3c18_15140V3.1, Pp1s3_273V6.1), *PpTARC* (Pp3c17_6500V3.1, Pp1s26_28V6.1),
532 *PpTARD* (Pp3c26_12520V3.1, Pp1s6_329V6.1), *PpYUCA* (Pp3c3_18590V1.1,
533 Pp1s312_60V6.1), *PpYUCB* (Pp3c11_11790V3.1, Pp1s11_6V6.1), *PpYUCC*
534 (Pp3c1_11500V3.1, Pp1s139_131V6.1), *PpYUCD* (Pp3c2_27740V3.1, Pp1s22_291V6.1),
535 *PpYUCE* (Pp3c13_21970V3.1, Pp1s37_90V6.1), *PpYUCF* (Pp3c3_20490V3.1,
536 Pp1s204_126V6.1).

537



538

539

540 **Figure S6. PCR genotyping of *yuccf* double knockout lines produced by a sexual cross**

541 **of the single knockout lines *Ppyucc-2* and *Ppyucf-1* (Landberg et al., 2020)¹⁵.** The upper

542 panel shows the result for a PCR confirming the loss of an internal *PpYUCC* gene sequence.

543 Asterisks mark primer dimers. The middle panel shows the result for a PCR confirming the

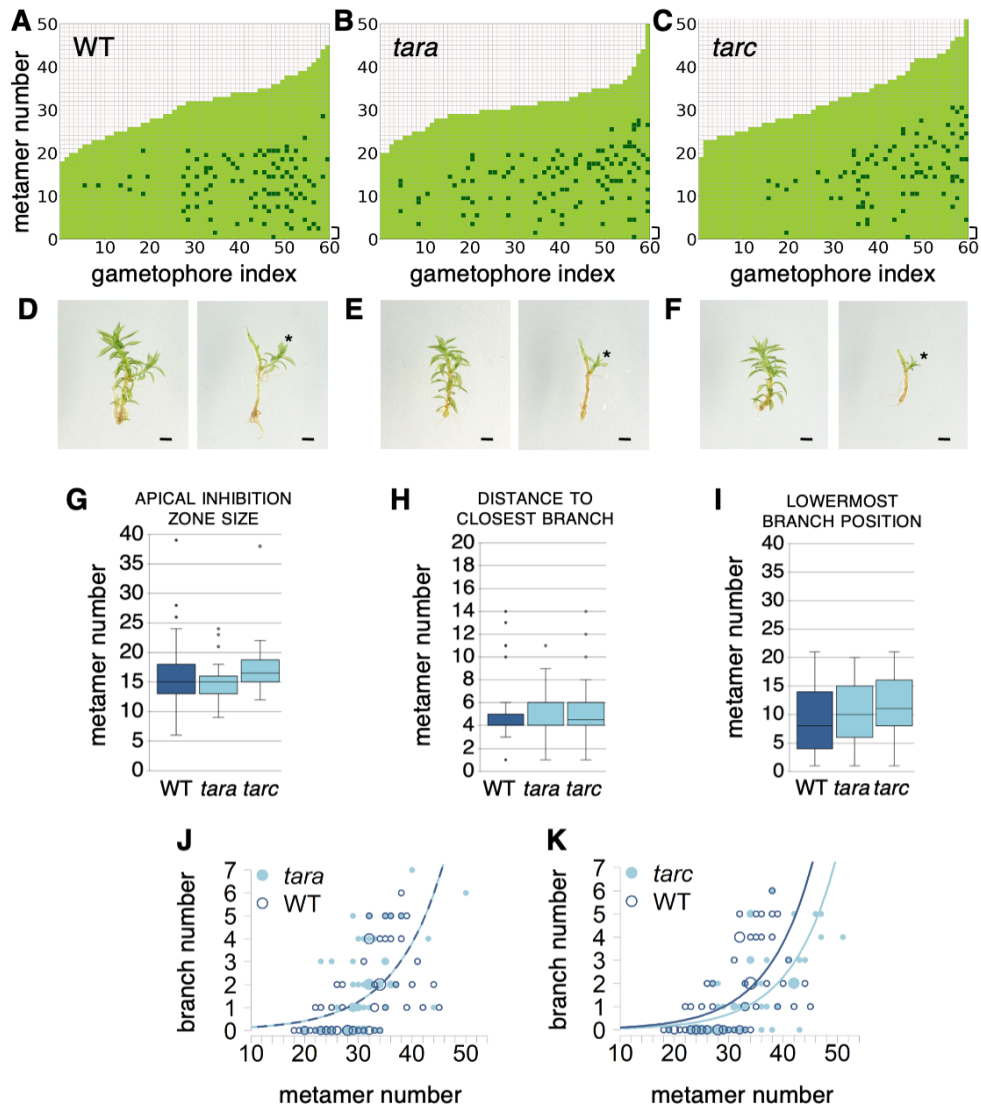
544 loss of an internal *PpYUCF* gene sequence. The bottom panel shows amplification of an

545 unrelated locus to confirm integrity of the gDNA used as template in all three panels. Next to

546 each panel, the primers used and the expected product size are noted. For primer sequences,

547 see Table S5.

548



549

550 **Figure S7. *tara* and *tarc* mutants have similar gametophore branching patterns than**

551 **wild-type. (A-C)** Branching patterns in wild-type (WT), *tara* and *tarc* gametophores. Brackets

552 indicate the three lowermost metamers. **(D-F)** WT (D), *tara* (E) and *tarc* (F) gametophores

553 before (left) and after (right) removing leaves, with asterisks marking branches. Scale bars

554 represent 1 mm. **(G)** The apical inhibition zone was not different in *tara* or *tarc* single mutants,

555 compared with wild-type control (Wilcoxon rank sum test with continuity correction; WT

556 versus *tara*, p-value = 0.35 ; WT versus *tarc*, p-value = 0.07). **(H)** The distance to the closest

557 branch was not different in *tara* or *tarc* single mutants, compared with wild-type control

558 (Wilcoxon rank sum test with continuity correction; WT versus *tara*, p-value = 0.92 ; WT

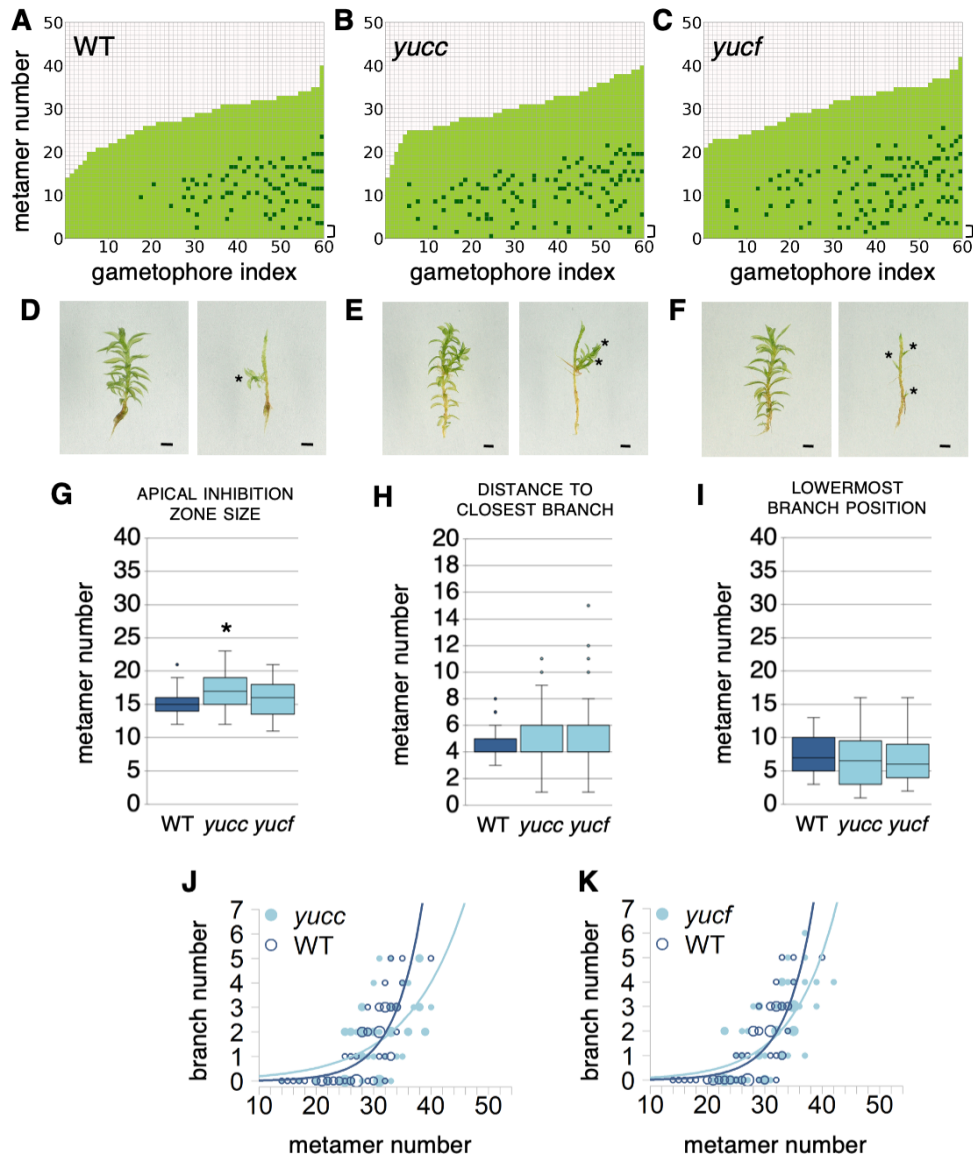
559 versus *tarc*, p-value = 0.14). **(I)** The position of the lowermost branch was similar in *tara*, *tarc*

560 and WT (Wilcoxon rank sum test with continuity correction ; WT versus *tara*, p-value = 0.30 ;

561 WT versus *tarc*, p-value = 0.12). **(J-K)** Bubble plots showed that branch number at a given

562 length slightly decreased in older *tarc* gametophores and unchanged in *tara*, compared with

563 WT. Gametophore length is represented as the number of metamers and the bubble area is
564 proportional to the number of gametophores with the same branch number (B) at a given
565 length (L). The data were over-dispersed, and so negative binomial regression was used to
566 test whether and how the relationship between branch number and gametophore length
567 differed between mutants and WT. For (J) the best-fitting relationship indicated no difference
568 between *tara* and WT (p-value = 0.39; $\log(\mathbb{E}(B|L)) = -3.11 + 0.11L$ for both treatments). For
569 (K) the best-fitting relationship indicated WT was larger than *tarc* at all lengths (p-value =
570 0.01; $\log(\mathbb{E}(B|L, W)) = -4.02 + 0.50W + 0.12L$, where $W = 1$ for WT). Data for WT were
571 replicated from Figure 3.
572



573

574 **Figure S8. *yucc* and *yucf* mutant gametophores have only minor perturbations in their**

575 **branching patterns. (A-C)** Branching patterns in WT, *yucc* and *yucf* mutants. Brackets

576 indicate the three lowermost metamers. **(D-F)** WT (E), *yucc* (F) and *yucf* (G) gametophores

577 before (left) and after (right) removing leaves, with asterisks marking branches. Scale bars

578 represent 1 mm. **(G)** The apical inhibition zone was increased in *yucc* mutants compared with

579 wild-type control (Wilcoxon rank sum test with continuity correction different from WT, *p-

580 value ≤ 0.05 ; WT versus *yucc*, p-value = 0.003 ; WT versus *yucf*, p-value = 0.82). **(H)** The

581 distance to the closest branch was similar in *yucc* and *yucf* single mutants, compared with

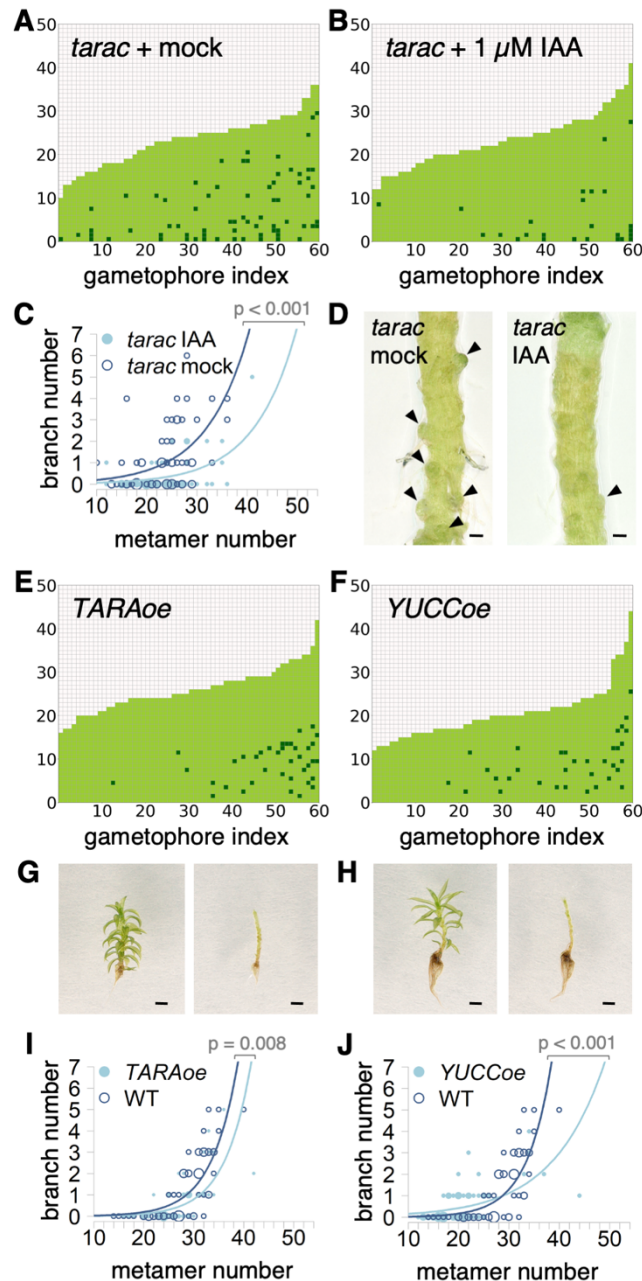
582 wild-type control (Wilcoxon rank sum test with continuity correction; WT versus *yuc*, p-value

583 = 0.77 ; WT versus *yucf*, p-value = 0.76). **(I)** The position of the lowermost branch was similar

584 in *yucc*, *yucf* and WT (Wilcoxon rank sum test with continuity correction; WT versus *yucc*, p-

585 value = 0.49 ; WT versus *yucf*, p-value = 0.34). **(J-K)** Bubble plots showed that branch number

586 responded differently to length in all mutants compared with WT. Gametophore length is
587 represented as the number of metamers and the bubble area is proportional to the number
588 of gametophores with the same branch number (B) at a given length (L). The data were not
589 over-dispersed, and so Poisson regression was used to test whether and how the
590 relationship between branch number and gametophore length depended on treatment (see
591 'Material and methods'). The best-fitting relationship included an interaction for all three
592 mutants, indicating the nature of the difference in the number of branches between WT and
593 mutant depended upon the length (in (J), $p = 0.002$, $\log(\mathbb{E}(B|L, Y)) = -5.28 + 3.17Y + (0.20 -$
594 $0.10Y)L$, where $Y = 1$ for *yucc*; in (K) $p = 0.03$, $\log(\mathbb{E}(B|L, Y)) = -5.28 + 2.25Y + (0.20 -$
595 $0.07Y)L$, where $Y = 1$ for *yucf*). Data for WT were replicated from Figure 3.
596



597

598

599 **Figure S9. *tarac* double mutants treated with auxin, and *TARA* and *YUCC***

600 **overexpression lines have reduced gametophore branching. (A-B) Plots show the**

601 **branching patterns of 60 gametophores in *tarac* double mutants treated with solvent control**

602 **(A) or 1 μ M IAA (B). Each gametophore is represented as a series of metamers (light green**

603 **squares) and lateral branch position is indicated in dark green. (C) Gametophore length is**

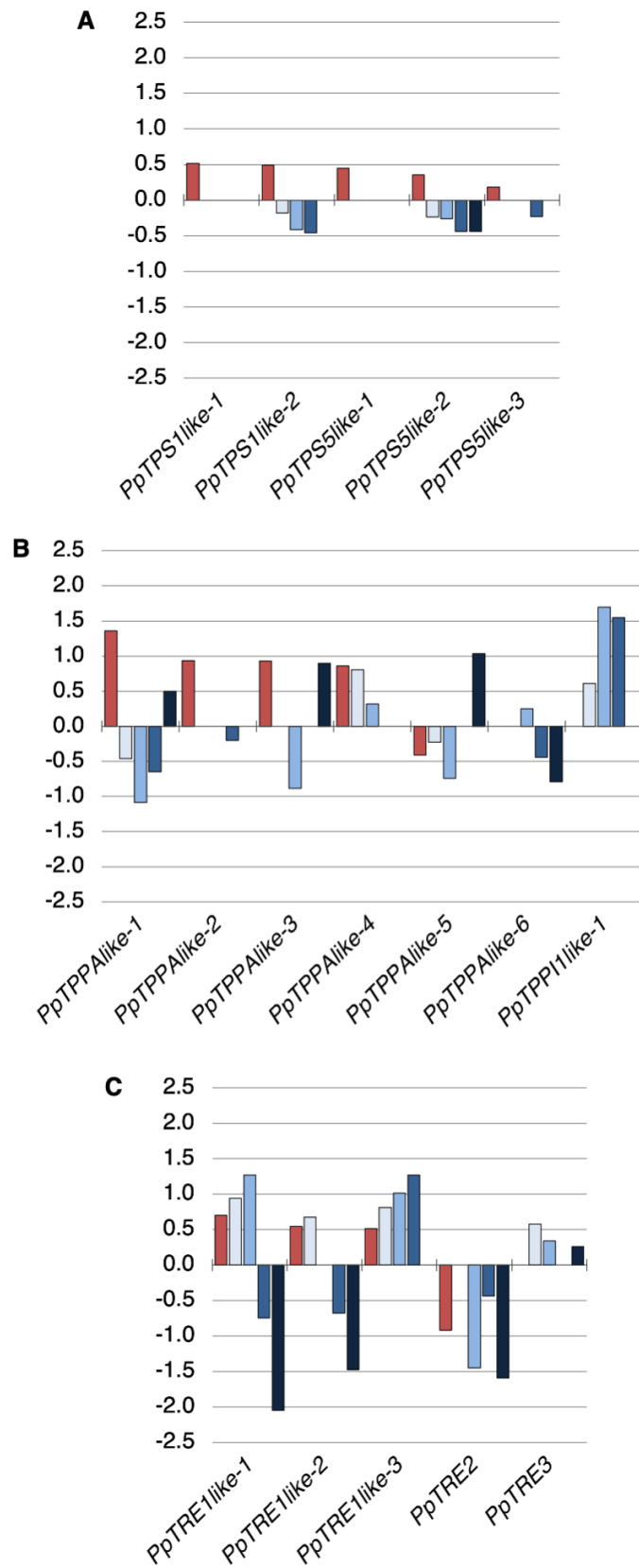
604 **represented as the number of metamers and the bubble area is proportional to the number**

605 **of gametophores with the same branch number (*B*) at a given length (*L*). Data were not over-**

606 **dispersed, and so Poisson regression was used to test whether and how the relationship**

607 **between branch number and gametophore length depended on treatment. For *tarac*, the**

608 best-fitting relationship indicated *tarac* + mock was larger than *tarac* + IAA at all lengths (p-
609 value < 0.001; $\log(\mathbb{E}(B|L, W)) = -3.93 + 1.11M + 0.19L$, where $M = 1$ for *tarac* + mock). **(D)**
610 Mock-treated *tarac* gametophores had numerous arrested axillary buds (arrowheads), this
611 phenotype was largely suppressed by IAA treatment. Leaves were removed prior to imaging.
612 Scale bars represent 100 μm . **(E-F)** Plots show the branching patterns of 60 gametophores
613 ordered by increasing size in *TARA* (E) and *YUCC* (F) overexpression (oe) lines, corresponding
614 WT is shown in Figure 3C. **(G-H)** *TARAOe* (G) and *YUCCoe* (H) gametophores before (left) and
615 after (right) removing leaves. Scale bars represent 1 mm. **(I-J)** Data were not over-dispersed,
616 and so Poisson regression was used to test whether and how the relationship between
617 branch number and gametophore length depended on treatment. For *TARAOe*, the best-
618 fitting relationship indicated *TARAOe* was smaller than WT at all lengths (p-value =
619 0.008, $\log(\mathbb{E}(B|L, W)) = -5.98 + 0.51W + 0.19L$, where $W = 1$ for WT). For *YUCCoe*, the best-
620 fitting relationship included an interaction, indicating the nature of the difference in the
621 number of branches between WT and mutants depended on length (p-value <
622 0.001, $\log(\mathbb{E}(B|L, Y)) = -5.82 + 3.00Y + (0.20 - 0.10Y)L$, where $Y = 1$ for *YUCCoe*).
623

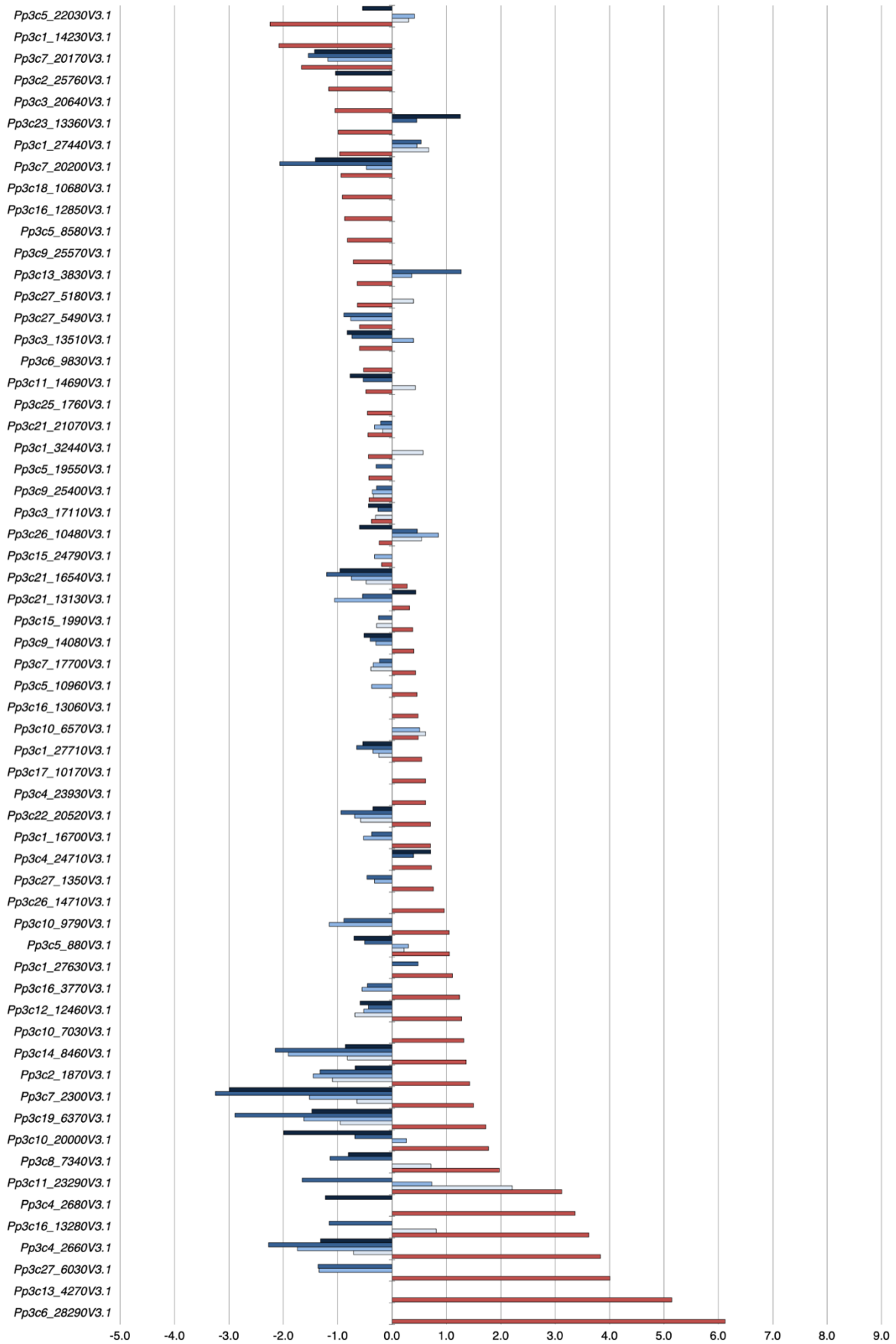


625 **Figure S10. Expression of trehalose-6-phosphate (T6P) metabolism genes is affected**
626 **by auxin and gametophore decapitation.** Bar plots show \log_2 fold-change in expression of
627 *T6P synthase (PpTPS)*, *T6P phosphatase (PpTPP)* and *trehalase (PpTRE)* coding genes in
628 wild-type gametophores after treatment with auxin (red bars) or decapitation (blue bars).
629 Correspondence between gene names and identifiers: *PpTPPAlike-1* (Pp3c12_20050V3.1),
630 *PpTPPAlike-2* (Pp3c4_20080V3.1), *PpTPPAlike-3* (Pp3c4_11080V3.1), *PpTPPAlike-4*
631 (Pp3c10_13170V3.1), *PpTPPAlike-5* (Pp3c3_25080V3.1), *PpTPPAlike-6* (Pp3c3_22990V3.1),
632 *PpTPPC1like-1* (Pp3c12_7510V3.1), *PpTPPE1like-1* (Pp3c12_7530V3.1), *PpTPPI1like-1*
633 (Pp3c10_23130V3.1), *PpTPS1like-1* (Pp3c5_17730V3.1), *PpTPS1like-2* (Pp3c6_16450V3.1),
634 *PpTPS5like-1* (Pp3c25_6990V3.1), *PpTPS5like-2* (Pp3c11_17560V3.1), *PpTPS5like-3*
635 (Pp3c7_15250V3.1), *PpTRE1like-1* (Pp3c5_16470V3.1), *PpTRE1like-2* (Pp3c23_11240V3.1),
636 *PpTRE1like-3* (Pp3c16_7830V3.1), *PpTRE1like-4* (Pp3c24_9748V3.1), *PpTRE1like-5*
637 (Pp3c24_9750V3.1), *PpTRE2* (Pp3c6_4940V3.1), *PpTRE3* (Pp3c10_5310V3.1). GO terms
638 related to “trehalose metabolism” were significantly enriched in the “up-regulated 2 h.a.d.”
639 gene set (Table S2). Trehalose 6-phosphate (T6P) metabolic genes regulate branching and
640 apical dominance in flowering plants. For example, shoot decapitation in *Pisum sativum* (pea)
641 triggers an increase of T6P levels in a few hours, which contributes to promoting branch
642 outgrowth²². T6P is synthesized from uridine diphosphate-glucose and glucose-6-phosphate
643 by the activity of T6P SYNTHASES (TPS) and converted to trehalose by T6P
644 PHOSPHATASES (TPP), which is then hydrolysed into glucose by TREHALASES (TRE).
645 Transgenic *Arabidopsis thaliana* plants with increased TPP activity in axillary buds have
646 reduced T6P levels and delayed branch outgrowth, whilst plants with increased levels of T6P
647 in the vasculature have enhanced branching²³. Moreover, mutations in *Zea mays RAMOSA3*
648 (*RA3*) and its paralog *ZmTPP4* that both encode TPP enzymes, lead to increased
649 inflorescence branching^{24,25}. Here, we found that all *PpTPS* genes were repressed after
650 decapitation, whilst *PpTPP* and *PpTRE* genes were either repressed or induced, suggesting
651 that T6P levels are dynamically regulated during the release of apical dominance. Besides,
652 we found that *TPS* genes were IAA-induced and decapitation-repressed, and GO terms
653 related to trehalose metabolism were associated with genes both induced by auxin and up-
654 regulated after decapitation (Table S3), consistent with *TPP* and *TRE* expression profiles 2
655 and 6 h.a.d.. Thus, our data suggest that apically-produced auxin regulates the activity of
656 T6P metabolic genes in the gametophore.

657

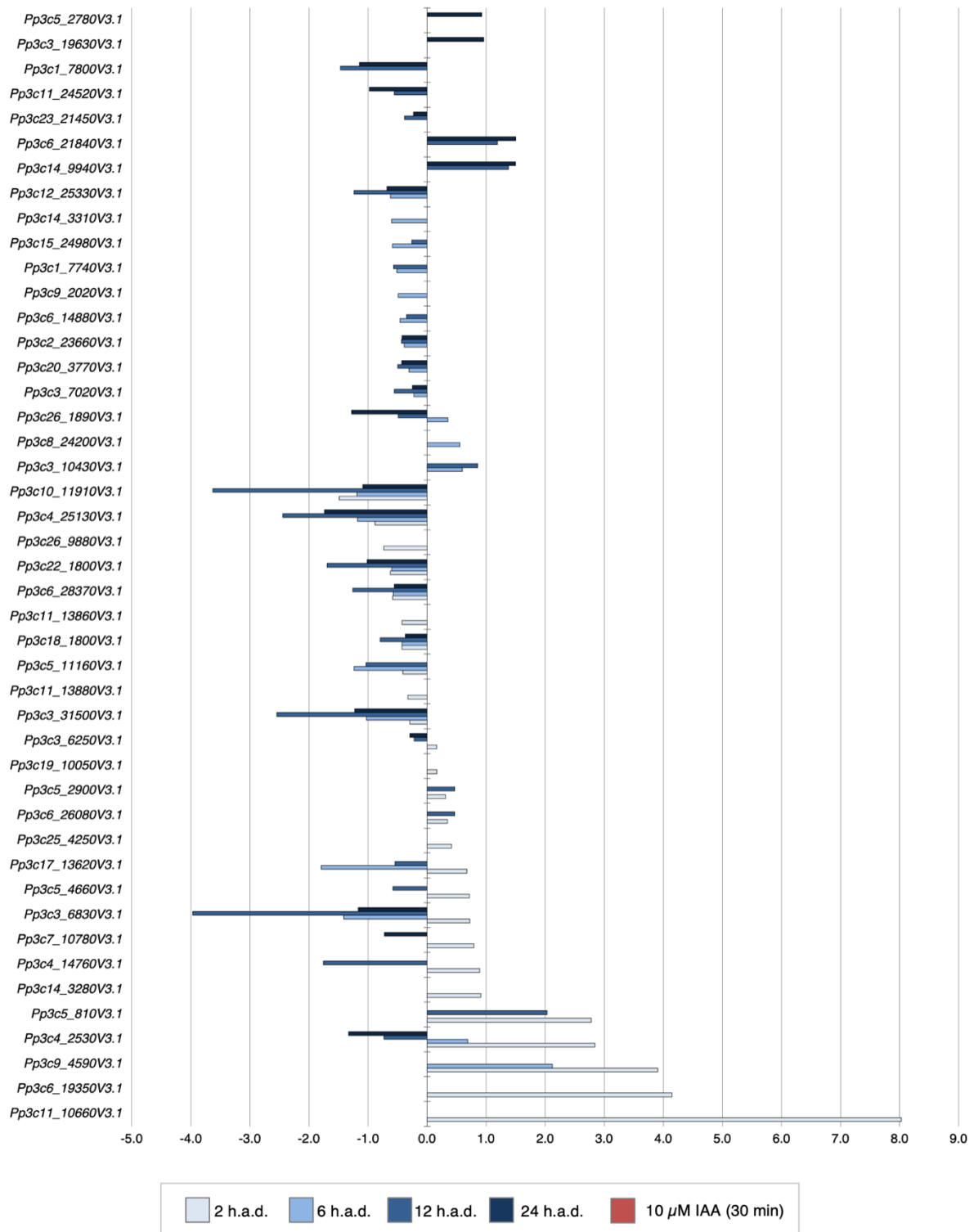
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660

661 **Figure S11.**



662

663 **Figure S11 (continued). Expression of numerous AP2/EREBP genes is strongly affected**
 664 **by auxin and decapitation.** Bar plots show \log_2 fold-change in expression of genes
 665 associated with PFAM domain PF00847 (AP2-domain) in wild-type gametophores after auxin
 666 treatment (red bars) or decapitation (blue bars). GO analyses revealed a significant
 667 enrichment of the “DNA binding transcription factor activity” term in gene sets up- and down-
 668 regulated after decapitation (Table S2). Members of the *APETALA2/ETHYLENE-*

669 *RESPONSIVE ELEMENT BINDING PROTEIN (AP2/EREBP)* family represented about two
670 thirds of enriched DNA binding transcription factor coding genes and included putative
671 shared regulators of meristematic function, such as *STEMIN1 (Pp3c1_27440v3.1)*, known to
672 promote stem cell formation through local depletion of a repressive chromatin mark, and
673 *Pp3c26_9880v3.1*, the closest homologue to *Solanum lycopersicum LEAFLESS* and *A.*
674 *thaliana PUCHI* and *DORNRÖSCHEN-LIKE*, which are regulated by auxin and involved in
675 meristem identity and lateral organ initiation control²⁶⁻²⁹.
676

677 **Author contributions**

678 MT and KL designed molecular constructs, generated transgenic lines and analyzed data ;
679 SH, LST and VB produced RNA seq data and performed differential analyses ; NC performed
680 statistical analyses ; YC designed the study, performed biological experiments with help from
681 AM and GC, analyzed data and wrote the manuscript with input from all co-authors.

682

683

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