

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

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1	Apical Dominance Control by TAR-YUC-Mediated Auxin Biosynthesis is a
2	Deep Homology of Land Plants
3	
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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 or outgrowth of distant lateral buds, is a major regulatory mechanism of plant form¹. Nearly 26 27 a century of studies in the sporophyte of flowering plants have established the phytohormone auxin as a front-runner in the search for key factors controlling apical dominance^{2,3}, 28 29 identifying critical roles for long-range polar auxin transport and local auxin biosynthesis in modulating shoot branching⁴⁻¹⁰. A capacity for lateral branching evolved by convergence in 30 31 the gametophytic shoot of mosses and primed its diversification¹¹; however, polar auxin transport is relatively unimportant in this developmental process¹², the contribution of auxin 32 biosynthesis genes has not been assessed and, more generally, the extent of conservation 33 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 dominance through massive and rapid transcriptional reprogramming of auxin-responsive 37 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 show that they are essential for coordinating branch initiation and outgrowth. Our results 41 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.

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 apical dominance^{12,13}. In the model species, *Physcomitrium patens*, gametophore branches 51 arise de novo from epidermal cells in leaf axils, and initiation and outgrowth have been 52 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 (Tables S1 and S2, Figure S1 and S2). In an attempt to specifically address the contribution 65 of auxin-related genes in apical dominance, we then compared the effect of auxin and 66 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 < 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-TRANSFERASE (TAA/TAR) enzymes, that is then converted to auxin by YUCCA FLAVIN 91 MONOOXYGENASE-LIKE (YUC) enzymes¹⁷. Previously published works in the flowering 92 plant Arabidopsis thaliana have proposed that auxin depletion from the leaf axil is needed to 93 promote axillary meristem formation^{9,10}. In contrast, the subsequent inhibition of axillary bud 94 outgrowth depends on expression of AtTAA1/WEI8, AtTAR2, AtYUC1, AtYUC4 and AtYUC6 95 auxin biosynthesis genes in partially overlapping domains of the shoot apical meristem^{6,7}. 96 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 is shared between A. thaliana and P. patens. Auxin metabolite profiling in various genetic 101 backgrounds has shown that both steps of TRP-to-IAA conversion occur in *P. patens*^{15,16}. To 102 103 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 two TAR and three YUC genes that were significantly differentially expressed after 106 107 decapitation (Figure 1D and 1E). Specifically, TARA, TARC and YUCF were induced as early as 2 h.a.d., YUCC was induced from 12 h.a.d., and YUCB was repressed. We also found that 108 109 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 *PpSHI1* and *PpSHI2*¹⁸ (Figure 2P and 2Q). Expression of TAR and YUC genes in isolated gametophore apices was confirmed by 125 126 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 indicates that the expression of TARA and TARC is more restricted in space than that of 128 129 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 promoter expression associated with the formation of new branches. The activity of TARA 140 141 and TARC promoters was detected from the earliest stage of branch formation, throughout the branch initium (Figures 2B, 2C, 2E and 2F). In contrast, YUCB, YUCC and YUCF promoter 142 143 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 TARs). Expression of TAR and YUC genes in newly emerged branches taken from decapitated 148 149 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 branching, we grew corresponding single and double knock-out mutants for 5-8 weeks and 163 164 quantified their branching patterns, as done previously¹² (Figure 3, Figure S6). We found that tara and tarc single mutants had overall similar phenotypes to control plants (Figure S7), and 165 166 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 tips were significantly weaker in auxin biosynthesis mutants (Figure 3A-3H, 3K-3L and 3M-170 3N). These data provide the first direct evidence that auxin biosynthesis genes active in 171 gametophore apices coordinate branch patterning at a distance, thus demonstrating the 172 173 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 30). 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 suppress branching at the gametophore base^{12,19}, and *in vitro* characterization of PpCCD8 181 182 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 yuccf 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 dominance revealed in gene ontology enrichment analyses, such as trehalose-6-phosphate 207 metabolic enzymes²²⁻²⁵ and APETALA2/ETHYLENE RESPONSIVE ELEMENT BINDING 208 209 PROTEIN transcription factors^{26–29} (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 instance, the genome of the liverwort Marchantia polymorpha contains one functional MpTAA 214 gene and two MpYUC genes, named MpYUC1 and MpYUC2³². Similar to our observations 215 216 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 sporophytes³³. In contrast with the classical definition of homology which implies a historical 235 236 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 phylogenetically disparate features' applies here^{41,42}. We conclude that the involvement of 238 TAR-YUC-mediated auxin biosynthesis to regulate apical dominance and shoot branching 239 240 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 Reute⁴³ strain that is more fertile in laboratory conditions was used as the wild-type (WT) 244 245 background for generating all the transgenic lines. Moss colonies were initiated from 1 mm² spot cultures and cultivated for 4-5 weeks (unless otherwise stated) in sterile Magenta GA-7 246 247 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 AIK(SO₄)₂.12H₂O, 0.055 mg/L CuSO₄.5H₂O, 0.028 mg/L KBr, 0.028 mg/L LiCl, 0.389 mg/L MnCl₂.4H₂O, 0.055 mg/L 250 CoCl₂.6H₂O, 0.055 mg/L ZnSO₄.7H₂O, 0.028 mg/L KI and 0.028 mg/L SnCl₂.2H₂O), 0.92 g/L 251 252 di-ammonium tartrate ($C_4H_{12}N_2O_6$) 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 growth cabinets (PHCbi, Etten-Leur, The Netherlands). Genetic transformation was 254 255 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, gametophores were teased apart from moss colonies with thin tip tweezers, planted in an 263 264 upright position on BCDAT petri dishes and their apical portion was cut off with microscissors, as described in Coudert, Palubicki et al. (2015)¹². For pharmacological treatments 265 266 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 concentration of 1 μ M IAA. A 95% ethanol solution was diluted 1,000 times in water and used 268 269 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

All the transgenic lines used in this study are listed in Table S4. Generation and confirmation
of the transcriptional reporter lines *TARA::GFP-GUS*, *TARC::GFP-GUS*, and *YUCF::GFP-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 BamHI/Ncol. The resulting fragment was cloned between the BamHI/Ncol 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 BamHI/BspHI, and cloned between the same two sites in pMT211. Both reporter constructs were linearized with 283 284 Sfil 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 the method presented in Thelander *et al.*⁴⁵. Double knockout lines were confirmed by PCRs 289 290 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*

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

306

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

For each biological replicate and condition, RNA were pooled from 5-10 gametophores
 (Gransden⁴⁷ strain). Three independent biological replicates were produced. Total RNA was
 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-seg 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 NexSeg500 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 CATdb database⁴⁸ (<u>http://tools.ips2.u-psud.fr/CATdb/</u>, ProjectID NGS2017_09_Moss1) 318 319 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 were made based on the article by Rigaill *et al*⁵¹. Genes which did not have at least one read 332 after a counts per million (CPM) normalization in at least one half of the samples were 333 334 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 edgeR method⁵² (version 1.12.0) in the statistical software 'R' (http://www.R-project.org, 336 337 version 2.15.0). Expression differences were compared between "0 h.a.d." and "2 h.a.d.", "6 h.a.d.", "12 h.a.d." or "24 h.a.d." conditions for the "decapitation experiment", and between 338 339 samples "mock" and "10µM 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 gene was considered as differentially expressed if its adjusted p-value was lower than 0.05. 341 Venn diagrams were generated using InteractiVenn⁵³. Gene Ontology (GO) term enrichment 342 analyses were performed with ShinyGO (version 0.66)⁵⁴. 343

344 RNA-seq data availability

RNA-seq datasets are available in the international repository GEO (Gene Expression
Omnibus⁵⁵, <u>http://www.ncbi.nlm.nih.gov/geo</u>) 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 genome⁵⁶. Identifiers of *PpIAA* and *PpARF* genes (Figure 1) are : *PpIAA1B*, *Pp3c8_14720*; 350 PpIAA2, Pp3c24_6610; PpARFa1, Pp3c1_14480; PpARFa3, Pp3c2_25890; PpARFa4, 351 352 Pp3c13_4720 ; PpARFa5, Pp3c26_11550 ; PpARFa6, Pp3c17_19900 ; PpARFa7, 353 Pp3c14_16990; PpARFa8, Pp3c1_40270; PpARFb1, Pp3c27_60; PpARFb2, Pp3c16_6100 ; PpARFb3, Pp3c5_9420 ; PpARFb4, Pp3c6_21370 ; PpARFc1B, Pp3c4_13010 ; PpARFc2, 354 Pp3c6_26890 ; PpARFd1, Pp3c9_21330 and PpARFd2, Pp3c15_9710. Identifiers of PpTAR 355 356 and PpYUC genes (Figure 1) are : PpTARA, Pp3c21 15370 ; PpTARB, Pp3c18 15140 ; PpTARC, Pp3c17_6500 ; PpTARD, Pp3c26_12520 ; PpYUCA, Pp3c3_18590 ; PpYUCB, 357 Pp3c11_11790 ; PpYUCC, Pp3c1_11500 ; PpYUCD, Pp3c2_27740 ; PpYUCE, 358 359 *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 emerged branches (same stages as in Figure 2H-I, K-L, N-O) were dissected out with micro-362 363 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 DNase treated with ezDNase[™] enzyme prior to reverse transcription with SuperScript[™] IV 365 VILO™ Master Mix (Ref: 11766050) following manufacturer's guidelines. qRT-PCR was 366 performed using a ROCHE Fast Start Universal SYBR Green master mix and a ThermoFisher 367 368 StepOnePlus Real-Time PCR System. Thermocycling conditions were: 95°C 10 min; 95°C 10 sec, 58°C 30 sec (40 cycles). Specificity of PCR amplification was checked by melt curve 369 370 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 comparative Ct method⁵⁷. 377

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 in panels M, N and P could be calculated only for gametophores with more than one branch. 388 389 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 received support from the experimental data. A mutant was considered different from WT if 404 405 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 AER⁵⁸. 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



417 Figure 1. Decapitation and auxin treatment have opposite effects on auxin-responsive and auxin signalling gene expression, and affect auxin biosynthesis gene activity in 418 419 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 µM 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 decapitation. The colour code shown at the bottom of the figure is used in panels B-E. "n.d." 428 429 means not detected in the differential gene expression analysis. 430



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, YUCC and YUCF are expressed in the main apex (black arrowheads) and in the basal region 441 442 (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 μ m in I, L, and O, 100 μ m in 452 B, C, E, F, H, K, P and Q, and 50 µm in N and R.



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 increasing size in *tarac* and *yuccf* double mutants, and corresponding wild-type (WT) controls. 457 458 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 yuccf (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 the main panels and 100 μ m in the insets. (K-L) Gametophore length is represented as the 465 466 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 over469 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). (**O**) There was very strong evidence that the position of the lowermost branch was closer to 486 487 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

494



□2 h.a.d. ■6 h.a.d. ■12 h.a.d. ■24 h.a.d.

495

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



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

- 501 decapitation.



505 Figure S3. PCR verification of *PpYUCB and PpYUCC* transcriptional reporter lines in WT background (A) Schematic view of the *PpYUCB::GFPGUS* reporter construct pMT244 506 507 and the *Pp108* locus to which it was targeted. Arrows mark the approximate annealing sites of primers used for PCR verification in B. (B) PCR verification of 5' and 3' junctions to confirm 508 509 correct integration of the *PpYUCB::GFPGUS* reporter construct into the *Pp108* locus. (C) Schematic view of the *PpYUCC::GFPGUS* reporter construct pMT251 and the *Pp108* locus 510 511 to which it was targeted. Arrows mark the approximate annealing sites of primers used for PCR verification in D. (D) PCR verification of 5' and 3' junctions to confirm correct integration 512 513 of the *PpYUCC::GFPGUS* 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



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 ± SD).
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- 523
- 524





Figure S5. Absolute expression levels of TAR and YUC genes in P. patens 528 529 gametophores retrieved from Ortiz-Ramirez et al. (2016)⁶⁰. Correspondence between 530 gene names and identifiers: PpTARA (Pp3c21_15370V3.1, Pp1s167_103V6.1), PpTARB (Pp3c18_15140V3.1, Pp1s3_273V6.1), PpTARC (Pp3c17_6500V3.1, Pp1s26_28V6.1), 531 PpTARD (Pp3c26 12520V3.1, Pp1s6_329V6.1), **PpYUCA** (Pp3c3 18590V1.1, 532 533 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).



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Figure S6. PCR genotyping of *yuccf* double knockout lines produced by a sexual cross 540 541 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



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 indicate the three lowermost metamers. (D-F) WT (D), tara (E) and tarc (F) gametophores 552 553 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 versus *tara*, p-value = 0.35; WT versus *tarc*, p-value = 0.07). (H) The distance to the closest 556 557 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



574 Figure S8. yucc and yucf mutant gametophores have only minor perturbations in their 575 branching patterns. (A-C) Branching patterns in WT, vucc and vucf mutants. Brackets indicate the three lowermost metamers. (D-F) WT (E), yucc (F) and yucf (G) gametophores 576 577 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 over-dispersed, and so Poisson regression was used to test whether and how the 589 590 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, $\log(\mathbb{E}(B|L, Y)) = -5.28 + 3.17Y + (0.20 - 10.002)$ 594 (0.10Y)L, where Y = 1 for yucc; in (K) p = 0.03, $\log(\mathbb{E}(B|L, Y)) = -5.28 + 2.25Y + (0.20 - 100)$ 595 (0.07Y)L, where Y = 1 for yucf). Data for WT were replicated from Figure 3. 596



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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 µM 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(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 by auxin and gametophore decapitation. Bar plots show log2 fold-change in expression of 626 T6P synthase (PpTPS), T6P phosphatase (PpTPP) and trehalase (PpTRE) coding genes in 627 628 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), PpTPPAlike-2 (Pp3c4_20080V3.1), PpTPPAlike-3 (Pp3c4_11080V3.1), PpTPPAlike-4 630 (Pp3c10_13170V3.1), PpTPPAlike-5 (Pp3c3_25080V3.1), PpTPPAlike-6 (Pp3c3_22990V3.1), 631 632 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), PpTRE1like-3 (Pp3c16_7830V3.1), PpTRE1like-4 (Pp3c24_9748V3.1), PpTRE1like-5 636 637 (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 alucose by TREHALASES (TRE). Transgenic Arabidopsis thaliana plants with increased TPP activity in axillary buds have 645 646 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* (RA3) and its paralog ZmTPP4 that both encode TPP enzymes, lead to increased 648 649 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 upregulated after decapitation (Table S3), consistent with TPP and TRE expression profiles 2 654 655 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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- 659



661 Figure S11.





Figure S11 (continued). Expression of numerous *AP2/EREBP* genes is strongly affected by auxin and decapitation. Bar plots show *log2* fold-change in expression of genes associated with PFAM domain PF00847 (AP2-domain) in wild-type gametophores after auxin treatment (red bars) or decapitation (blue bars). GO analyses revealed a significant enrichment of the "DNA binding transcription factor activity" term in gene sets up- and downregulated 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.

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.
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- 683

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