

KAI2-dependent signaling controls vegetative reproduction in *Marchantia polymorpha* through activation of LOG-mediated cytokinin synthesis (14)

Aino Komatsu¹, Mizuki Fujibayashi¹, Kazato Kumagai¹, Hidemasa Suzuki¹, Yuki Hata¹, Yumiko Takebayashi², Mikiko Kojima², Hitoshi Sakakibara^{2,3} and Junko Kyojuka^{1*}

¹ Graduate School of Life Sciences, Tohoku University, Sendai, 980-8577, Japan

²RIKEN Center for Sustainable Resource Science, Tsurumi, Yokohama 230-0045, Japan

³Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya, 464-8601, Japan

7 Abstract (197)

8 *Marchantia polymorpha* reproduces vegetatively (asexually) by producing propagules known as
 9 gemmae within gemma cups and sexually through spores. We previously reported that
 10 KARRIKIN INSENSITIVE 2 (KAI2)-dependent signaling promotes gemma cup and gemma
 11 formation. KAI2A perceives unidentified endogenous ligand(s), tentatively referred to as KAI2
 12 ligands (KL). Perception of KL by KAI2 triggers MAX2-dependent proteolysis of MpSMXL. In
 13 this study, we identified genes working downstream of KAI2-dependent signaling in *M.*
 14 *polymorpha*. We found that KAI2-dependent signaling positively controls the expression of
 15 MpLONLEY GUY (MpLOG), encoding a cytokinin biosynthesis enzyme. Disruption of the
 16 MpLOG function decreased endogenous cytokinin levels and caused defects similar to KAI2-
 17 dependent signaling mutants. Moreover, supplying exogenous cytokinins rescued the defects of
 18 Mplog and KAI2-dependent signaling mutants, implying that cytokinins work downstream of
 19 KAI2-dependent signaling. Activation of MpLOG by KAI2-dependent signaling occurs in a
 20 highly cell-type-specific manner, leading to cell-specific induction of *GEMMA CUP-*
 21 *ASSOCIATED MYB1* (*GCAMI*), the master regulator of vegetative reproduction of *M.*
 22 *polymorpha*. We propose a genetic cascade, starting from KAI2-dependent signaling, that
 23 promotes vegetative reproduction through the induction of MpLOG and *GCAMI*. The interaction
 24 between KAI2-dependent signaling and cytokinin in *M. polymorpha* provides a novel insight into
 25 the function and evolution of KAI2-dependent signaling.

26 Introduction

27 A wide range of plants uses vegetative or asexual reproduction for propagation as their
 28 predominant mechanisms of reproduction¹. In vegetative reproduction, new plants are produced
 29 via propagules, such as adventitious shoots, bulbs, tubers, and rhizome buds². In addition, new
 30 individuals can be formed through fragmentation and clonal growth of the original plant^{3, 4}.
 31 Vegetative reproduction is less affected by seasonal changes than sexual reproduction, which is
 32 often influenced by seasonal factors such as light. Consequently, vegetative reproduction allows
 33 efficient propagation, potentially yielding a huge number of individuals. At the same time,
 34 vegetative reproduction has a clear disadvantage in that it limits the potential for increasing
 35 genetic diversity. Plants regulate the degree of vegetative reproduction in response to
 36 environmental conditions to optimize proliferation, and the molecular mechanisms underlying
 37 this regulation are mostly unknown. In bryophytes, new individuals are generated non-sexually
 38 from propagules and caducous organs of diverse morphology^{2, 5, 6}. In addition, the high
 39 regeneration capacity of bryophytes enables the generation of new individuals from fragments or
 40 parts of gametophytes, such as leaves, shoots, and thalli. *Marchantia polymorpha* proliferates
 41 both through sexual and vegetative reproduction³. For vegetative reproduction, propagules called
 42 gemmae are formed on the base of the gemma cup serving as the primary source for extensive
 43 propagation⁷.

44 Plant hormones are key regulators of almost all aspects of plant growth and development as well
 45 as responses to environmental conditions^{8, 9}. Fine-tuning of synthesis and signaling is pivotal for
 46 controlling plant hormone function. The hormonal actions are intricately interconnected, and the
 47 crosstalks among plant hormones are crucial to maximize their effects¹⁰. We previously reported
 48 that gemma cup and gemma formation are positively controlled by a KARRIKIN INSENSITIVE
 49 2 (KAI2)-dependent signaling pathway in *M. polymorpha*^{11, 12}. KAI2 was identified as the
 50 receptor for karrikins (KARs), molecules derived from smoke from burning vegetation^{13, 14}.
 51 Subsequent genetic analyses have suggested that KAI2 most likely perceives endogenous
 52 ligand(s), currently unidentified and provisionally termed KAI2 ligands (KL)¹⁵. The KAR/KL
 53 signal is transduced through the MORE AXILLARY GROWTH 2 (MAX2)-mediated
 54 proteolysis-dependent signaling pathway. In this pathway, ligand perception by KAI2 triggers the
 55 interaction of MAX2 with suppressor proteins, resulting in the degradation of these suppressor
 56 proteins by the 26S proteasome pathway^{14, 16, 17, 18, 19}. In *Arabidopsis*, SUPPRESSOR OF MAX2
 57 1 (SMAX1) and SMAX1 LIKE2 (SMXL2), encoding proteins with weak homology to ClpB-type
 58 chaperonins, work as the suppressors of KAI2-dependent signaling^{16, 20, 21}. SMXL6, SMXL7, and
 59 SMXL8, paralogs of SMAX1, function as suppressors of the strigolactone signaling pathway^{16, 21}.
 60 ²². All SMXL proteins contain an ethylene-responsive element binding factor-associated

amphiphilic repressor (EAR) motif that interacts with transcriptional corepressors, including TOPLESS (TPL). Consequently, SMXLs are presumed to work as transcription repressors, engaging with transcription factors and transcriptional corepressors^{23, 24}. In addition, SMXL6 works as a transcription factor that suppresses SMXL 6, 7, and 8 transcription through direct binding to their promoters²³. Downstream targets of transcriptional repression mediated by SMXL corepressors, such as *BRANCHIED1*, *TCPI*, and *PRODUCTION OF ANTHOCYANIN PIGMENT1*, have been identified^{23, 24}. A few transcription factors that directly interact with SMXLs have been identified, however, the comprehensive understanding of gene expression regulation by SMXLs is yet to be revealed^{25, 26}.

The KAI2-dependent signaling pathway in seed plants controls diverse aspects of growth and development, including seed germination, seedling growth, leaf development, root growth, root hair development, stress tolerance, and arbuscular mycorrhizal symbiosis^{20, 21, 27, 28, 29, 30, 31}. In addition, crosstalks of KAI2-dependent signaling with other hormones allow for an elaborate fine-tuning of growth and development and responses to the ever-changing environment³². In *Arabidopsis* and *Lotus japonica*, KAI2-dependent signaling controls root and root hair growth in response to low P conditions through modulation of ethylene biosynthesis and signaling^{33, 34}. This leads to the modulation of spatial accumulation of PIN2 and AUX1, leading to changes in auxin accumulation patterns. Shade avoidance in *Arabidopsis* is also mediated by KAR/KL-auxin crosstalk^{28, 35}. Interaction of KAI2-dependent signaling and ABA contributes to the control of drought tolerance and seed germination³⁶. KAI2-dependent signaling interacts with strigolactone biosynthesis, which leads to the regulation of arbuscular mycorrhizal (AM) fungi colonization in rice³¹.

All the core components of KAI2-dependent signaling exist in bryophytes^{37, 38}. *M. polymorpha* contains two KAI2 paralogues (MpKAI2A and MpKAI2B), one MAX2 gene (MpMAX2), and one SMXL (MpSMXL). We previously reported that MpKAI2A, MpMAX2, and MpSMXL work in a single signaling pathway, which depends on the degradation of MpSMXL¹². Although MpKAI2B shows extensive sequence similarity to MpKAI2A, it is unlikely that MpKAI2B works as the KAR/KL signaling receptor. The conservation of the KAI2-dependent signaling pathway in bryophytes was demonstrated in the moss *Physcomitrium patens*, and the liverworts *M. polymorpha* and *Marchantia paleacea*, indicating that KAI2-dependent signaling was established in the common ancestor of land plants^{12, 39, 40}. Later, in the common ancestor of seed plants, the KAI2 gene was duplicated, which led to the evolution of D14, a strigolactone receptor^{14, 41}. Strigolactone signals are transduced via a parallel signaling pathway that shares MAX2 with KAI2-dependent signaling^{16, 17, 18, 42}. Other than the occurrence of D14, the increase in the number

of *KAI2* paralogs occurred independently in some lineages, such as the root parasitic plants and *P. patens*^{41, 42, 43, 44, 45}.

We are interested in uncovering the mechanisms by which KAI2-dependent signaling controls vegetative reproduction in *M. polymorpha*. In this study we identified genes working downstream of KAI2-dependent signaling in *M. polymorpha*. Our analysis indicates that KAI2-dependent signaling positively controls the expression of *MpLOG*, encoding a cytokinin biosynthesis enzyme⁴⁶. We present evidence that cytokinin works downstream of KAI2-dependent signaling. We also demonstrate that activation of *MpLOG* by KAI2-dependent signaling occurs in a highly cell-type-specific manner, leading to cell-specific induction of *GEMMA CUP-ASSOCIATED MYB1 (GCAMI)*, the master regulator of vegetative reproduction^{11, 47}. The cross-regulation between KAI2-dependent signaling and cytokinin in *M. polymorpha* provides a novel insight into the function and evolution of KAI2-dependent signaling.

Results

MpLOG works downstream of KAI2-dependent signaling

We first aimed at identifying genes working downstream of KAI2-dependent signaling by analyzing transcriptome changes upon the induction of MpMAX2-dependent degradation of MpSMXL. For this purpose, we took advantage of the fact that *M. polymorpha* does not respond to strigolactones due to the absence of D14, the cognate receptor of strigolactones. We previously reported that *M. polymorpha* and its close relative *M. paleacea* respond to (–)-GR24, but not to (+)-GR24, synthetic compounds mimicking KL and strigolactones, respectively. However the introduction of *AtD14* enabled *M. polymorpha* and *M. paleacea* to respond to both (+)-GR24 and (–)-GR24 with the signals transmitted through MpMAX2-dependent degradation of MpSMXL³⁹. In *35S:AtD14* transgenic lines of *M. polymorpha* (*MpAtD14ox*), MpSMXL degradation occurs upon perception of endogenous KL and applied (–)-GR24 by MpKAI2A, and perception of applied (+)-GR24 by the introduced AtD14. In addition, since *AtD14* is driven by the strong and constitutive 35S promoter, and AtD14-dependent signaling occurs only after the addition of *rac*-GR24 containing both (+)-GR24 and (–)-GR24, we anticipate that gene expression changes would be amplified in the *MpAtD14ox* line, making it suitable for RNAseq analysis. We applied *rac*-GR24 to the *MpAtD14ox* line and compared changes in transcriptomes 3 and 6 hours after application (Fig. 1a). We identified 283 and 402 genes that were up-regulated and 182 and 278 genes that were down-regulated, 3 and 6 hours respectively, after the application of *rac*-GR24. Among the differentially expressed genes (DEGs), 76 genes were up-regulated and 43 genes were down-regulated, at both 3 and 6 hours (Fig. 1b and Supplementary Fig. 1a).

GO analysis identified that synthesis and metabolism of small compounds were enriched in DEGs up-regulated after 3 hours. In contrast, GO terms related to transcription and replication were enriched in DEGs down-regulated after 3 hours. On the other hand, GO terms related to growth and differentiation were enriched in DEGs down-regulated after 6 hours, while no specific GO term was identified in DEGs up-regulated after 6 hours (Supplementary Fig. 1c, d).

In our previous study, we identified genes whose expression changed in *Mpkai2a* and *Mpmax2*, mutants lacking KAI2-dependent signaling¹². However, due to advancements in gene annotation of *M. polymorpha* progressed, we re-analyzed the RNAseq data and identified additional genes as DEGs. We integrated the data from two comparisons: WT versus mutants and non-treated *MpAtD14ox* versus *rac*-GR24-treated *MpAtD14ox*. We found that the expression levels of 13 genes was increased by *rac*-GR24 treatment after 3 and 6 hours and decreased in the *Mpkai2a* and *Mpmax2* mutants (Fig. 1c, Supplementary Fig. 1b), suggesting that these genes are likely positively regulated by KAI2-dependent signaling.

First, we confirmed that the expression of 12 out of the 13 DEGs is enhanced in WT plants after application of (–)-GR24 (Fig. 1d, Supplementary Fig. 2). We picked three genes, *MpDIENELACTONE HYDROLASE LIKE PROTEIN1* (*MpDLPI*), *MpKAI2B*, and *MpLOG*, from the 13 DEGs for further analysis (Supplementary Table1). The expression of genes controlled by transcription factors that directly interact with MpSMXL is expected to change without the need for a translation step. Indeed, *MpKAI2B*, but not the other two genes, was activated by (–)-GR24 in the presence of cycloheximide (CHX), a translation repressor, suggesting that *MpKAI2B* might be the direct target of KAI2-dependent signaling (Fig. 1e).

In this study, we focused on *MpLOG*, an ortholog of *LONELY GUY* encoding the final enzyme in the active cytokinin biosynthesis pathway^{46, 48, 49}. *MpLOG*, a single-copy gene in *M. polymorpha*, shows high sequence similarity to *LOG* genes in other plant species across the entire coding sequence. We confirmed that *MpLOG* expression is reduced in the *Mpkai2a*, *Mpmax2* mutants, as well as in transgenic lines overexpressing *MpSMXL*^{d53}, which encodes a degradation-resistant version of the MpSMXL suppressor protein (Fig. 1f)¹². Moreover, induction of *MpLOG* expression by (–)-GR24 observed in WT was abolished in lines with the defective KAI2-dependent signaling (Fig. 1g). These results suggest that KAI2-dependent signaling positively controls *MpLOG* expression.

MpLOG catalyzes the final steps of cytokinin biosynthesis in *M. polymorpha*

The cytokinin biosynthesis pathways in plants have been elucidated through studies in angiosperms (Fig. 2a)^{48, 49}. *LOG* encodes a cytokinin riboside 5'-monophosphate

phosphoribohydrolase that releases cytokinin nucleobase and ribose 5'-monophosphate from iPRMP, tZRMP, DZRMP, and cZRMP, leading to the production of N⁶-isopentenyl adenine (iP), *trans*-zeatin (tZ), dihydro-zeatin (DZ), and *cis*-zeatin (cZ), all active cytokinins⁴⁶. Phylogenetic studies demonstrated that *M. polymorpha* contains a single *LOG* gene (Supplementary Fig. 3) while it does not contain genes for canonical adenosine phosphate-isopentenyltransferase (IPT) and CYP735A. It was shown that DZ content is below the detectable level in *M. polymorpha*⁵⁰.

To examine if Mp*LOG* works in the biosynthesis of cytokinins in *M. polymorpha*, we generated loss-of-function mutants of Mp*LOG* using the CRISPR/Cas9 genome editing system with two different gRNAs (Supplementary Fig. 4a) and subsequently measured the cytokinin contents of the resulting plants. We found that cZ was the most abundant cytokinin in WT *M. polymorpha* plants. iP and tZ were also detected (Fig. 2b). The content of tZ was reduced to undetectable levels in the Mp*log* mutants. The levels of iP and cZ were also significantly decreased; however, low levels of iP and cZ were detected in the Mp*log* mutants. In contrast to the decrease in active cytokinins, their ribotide precursors, iPRP, tZRP, and cZRP, were dramatically increased. The riboside precursors, iPR, tZR, and cZR, were also increased, but the amount was two orders of magnitude less than the ribotide precursors. These results support the notion that Mp*LOG* catalyzes the final two steps in cytokinin biosynthesis. In addition, the presence of active cytokinins even in Mp*log* mutants strongly suggests the existence of a Mp*LOG*-independent cytokinin synthesis pathway.

Cytokinins synthesized by Mp*LOG* control gemma cup and gemma formation, as well as thallus growth

We previously reported that KAI2-dependent signaling controls gemma cup and gemma formation, flat thallus growth, and gemma growth arrest in the dark^{11, 12, 39}. We analyzed the phenotype of Mp*log* loss-of-function mutants to investigate whether Mp*LOG* is involved in the regulation of these traits under KAI2-dependent signaling. We reported that gemma cup formation in KAI2-dependent signaling mutants is affected by culture conditions. Specifically, gemma cup formation appears normal on media, whereas it is significantly suppressed when plants are grown on vermiculite^{11, 12}. Therefore, we analyzed gemma cup formation in Mp*log* mutant plants grown on vermiculite. Gemma cup formation was severely suppressed in these plants (Fig. 3a, b), and these defects were rescued by adding 6-benzylaminopurine (BA), a synthetic cytokinin. On the other hand, when Mp*log* mutant plants were grown on medium, gemma cups and gemmae were formed, but the number of gemmae formed per cup was much lower than that in the WT, similar to what is observed in KAI2 signaling mutants (Fig. 3c, d)^{11, 12}. These results suggest that Mp*LOG*-derived cytokinin positively controls gemma cup and gemma formation, resembling the

function of genes in the KAI2-dependent signaling pathway. Gemmae of *Mplog* loss-of-function mutants did not show morphological differences with WT gemmae (Supplementary Fig. 4b). However, the gemmae of *Mplog* mutants showed upward growth of the thallus (Supplementary Fig. 4b, 5a). This defect of *Mplog* mutants was more substantial than that observed in KAI2-dependent signaling mutants. The application of BA rescued the defects (Supplementary Fig. 5b).

Cytokinin works downstream of KAI2-dependent signaling

We showed that *MpLOG* expression is positively regulated by KL signaling, and *MpLOG* catalyzes cytokinin synthesis. The defects in gemma cup and gemma formation observed in the *Mplog* mutants resembled those seen in KAI2-dependent signaling mutants, and *Mplog* defects were rescued by exogenous application of cytokinin (Fig. 3a, b). These results suggest that KAI2-dependent signaling controls gemma cup and gemma formation by controlling cytokinin synthesis. To test this hypothesis, we analyzed if defects in the *Mpkai2a* and *Mpmax2* mutants are rescued by cytokinin. We found that defects in gemma cup formation in *Mpkai2a* and *Mpmax2* were restored by addition of BA in a dose-dependent manner (Fig. 4a, b). The phenotype of upward growth of the thallus of *Mpkai2a* and *Mpmax2* mutants was alleviated by the addition of BA in a dose-dependent manner (Supplementary Fig. 5c, d).

To further confirm that cytokinin acts downstream of KAI2-dependent signaling, which is dependent on the degradation of *MpSMXL*, we measured the contents of endogenous cytokinins (iP, tZ, cZ) in *MpSMXL*^{d53} overexpressing degradation-resistant *MpSMXL*, and in the *Mpsmxl* loss of function mutants (Fig. 4c). The levels of cytokinins were decreased in the *MpSMXL*^{d53} mutants, in which KAI2-dependent signaling is disrupted. In particular, tZ declined to below quantification levels. On the other hand, the contents of tZ and cZ were not affected in *Mpsmxl* mutants, for which the KAI2-dependent signaling is supposed to be enhanced. The level of iP was decreased in *Mpsmxl* mutants for unknown reasons. Overall, our results support the hypothesis that KAI2-dependent signaling positively regulates cytokinin content.

Mpsmxl loss-of-function mutants exhibit phenotypes that are opposite to those of *Mpkai2a* and *Mpmax2*¹¹. More gemmae per cup are formed in *Mpsmxl*. To test the genetic relationship between *MpSMXL* and *MpLOG*, we generated *Mpsmxl Mplog* double mutants. As expected if *MpLOG* is downstream of *MpSMXL*, the *Mpsmxl* mutant phenotypes were alleviated in the double mutants: the *Mpsmxl Mplog* double mutants closely resembled the *Mplog* single mutants (Fig. 4d, f, Supplementary Fig. 5e). Gemma cups were normally formed in the *Mpsmxl* mutants even grown on vermiculite, while it was severely suppressed in *Mplog* and in the double mutant (Fig. 4d, e). *Mpsmxl* mutants produced an increased number of gemmae per cup when grown on medium, whereas this number was reduced in the *Mpsmxl Mplog* double mutant (Fig. 4f, g), suggesting

that the function of *MpLOG* is required for the expression of the phenotypic traits exhibited by *Mpsmx1*. Genetic analysis indicated that *MpLOG* works downstream of the KAI2-dependent signaling pathway for gemma cup and gemma formation. In addition, the angle of the thallus relative to the horizontal line is smaller in *Mpsmx1* and the thallus grew upward in the double mutant as in *Mplog* (Supplementary Fig. 5e, f), indicating that these traits are also under the control of *MpLOG*.

Control of *MpLOG* expression by KAI2-dependent signaling is cell-type specific

To understand the roles of cytokinins in the control of vegetative reproduction in *M. polymorpha*, we analyzed the spatial localization of *MpLOG* expression. The 5 kb and 1 kb promoter regions of the *MpLOG* gene were fused with the *EGFP* gene and a nuclear localization signal sequence (*proMpLOG-EGFPNLS* and *proMpLOG5kb-EGFPNLS*) and introduced into WT. *Mpkai2a* mutation was introduced into *proMpLOG-EGFPNLS* (*proMpLOG-EGFPNLS/Mpkai2a-7*). No difference was observed in the fluorescence pattern between the lines containing the 5 kb and 1 kb promoter regions (Fig. 5a, e, Supplementary Fig. 6). We used the plants containing *proMpLOG-EGFPNLS* with the 1 kb promoter region in the subsequent observations. EGFP fluorescence was observed in the meristem region at the notch and rhizoid initial cells of the gemma (Fig. 5a). The fluorescence in the meristem was not observed in the *Mpkai2a* mutant (Fig. 5b), indicating that the expression of *MpLOG* in the meristem cells is under the control of KAI2-dependent signaling. In contrast, EGFP fluorescence in the rhizoid initial cells was observed in *Mpkai2a*. During gemma cup initiation, EGFP fluorescence was observed in the initiating cups in WT but not in *Mpkai2*, which is consistent with the absence of gemma cups in the *Mpkai2a* mutant (Fig. 5c, d). We further analyzed *MpLOG* expression in the gemma cups and initiating gemmae using confocal microscopy. We observed that *MpLOG* is expressed on the upper surface of the thallus in WT and *Mpkai2a*, indicating that the expression of *MpLOG* in this region is not regulated by KAI2 signaling (Fig. 5e, f). Intense fluorescence was detected in the basal part of the gemma cup in WT (Fig. 5e). At the bottom of the gemma cup, fluorescence localized in gemma initial cells, initiating gemmae, slime papillae, and the gemma cup floor cells in WT (Fig. 5g). On the other hand, in *Mpkai2a* mutants grown on medium, EGFP fluorescence was observed in the slime papillae and gemma cup floor cells, but not in gemma initial cells, and initiating gemmae (Fig. 5h). Therefore, expression of *MpLOG* in the meristem region in the gemmae, gemma initial cells and initiating gemmae is regulated by KAI2-dependent signaling while *MpLOG* expression in the epidermis on the thallus, slime papillae, and gemma cup floor cells is not. We conclude that KAI2-dependent signaling controls gemma cup and gemma formation by enhancing cytokinin synthesis through the promotion of *MpLOG* expression. Although their number is reduced, gemmae are formed in the *Mpkai2a* mutants when plants are grown on medium. However, *MpLOG* expression was not

observed in gemma initial cells and initiating gemmae in *Mpkai2a*. This suggests that either gemmae formed in the *Mpkai2a* mutants do not depend on *MpLOG* or that residual expression levels of *MpLOG* are sufficient for the generation of a small number of gemmae. It is also possible that the initiation of gemmae in *Mpkai2a* on the medium depends solely on nutrient conditions and is independent of cytokinin.

GEMMA CUP-ASSOCIATED MYB1 (GCAMI)* expression is promoted by cytokinin synthesized by *MpLOG

GCAMI is an ortholog of *Arabidopsis* *REGULATOR OF AXILLARY MERISTEMS (RAX)* genes, encoding the R2R3-MYB transcription factors^{47, 51}. *GCAMI* was first identified as the gene responsible for gemma cup formation. Subsequently, we demonstrated that *GCAMI* is also involved in gemma initiation and works downstream of KAI2-dependent signaling¹¹. *GCAMI* expression is known to be positively regulated by cytokinin⁵². Hence, we postulated that *GCAMI* expression is downstream of cytokinin synthesis regulated by *MpLOG*. We first confirmed that *GCAMI* expression is enhanced by cytokinin application (Fig. 6a). In a prior study, we introduced *proGCAMI-EGFPNLS*, containing approximately 5 kb of the of *GCAMI* promoter region, into WT plants. Fluorescence localized in gemma initial cells, initiating gemmae and slime papillae but not in gemma cup floor cells¹¹. Here, we confirmed this expression pattern of *GCAMI* in WT plants (Fig. 6b). The localization of *GCAMI* expression observed in our studies differs slightly from the reported ones⁴⁷. We believe that *GCAMI* expression in the gemma cup floor cells described in Yasui *et al.* (2019) is likely the signal in the slime papillae. EGFP fluorescence was absent from the gemma initial cells and initiating gemmae in the *Mplog* mutant (*proMpGCAMI-EGFPNLS/Mplog*), indicating that *GCAMI* expression in these cells depends on *MpLOG* activity (Fig. 6c). Comparisons of *MpLOG* expression between WT and *Mpkai2a* mutants and *GCAMI* expression between WT and *Mplog* mutants support the idea that *GCAMI* expression in the gemma initial cells, initiating gemmae and possibly in slime papillae is promoted by cytokinins synthesized by *MpLOG* activity regulated by KAI2-dependent signaling (Fig. 7a).

Discussion

In this study, we demonstrated that KAI2-dependent signaling positively controls the expression of *MpLOG*, leading to increased cytokinin levels. Defects in the *Mplog* mutants resembled those of KAI2-dependent signaling mutants. In addition, *Mplog* and KAI2-dependent signaling mutants were rescued by the exogenous supply of cytokinin, supporting the idea that cytokinin synthesis by *MpLOG* works as a downstream regulator of KAI2-dependent signaling. We also showed that

GCAMI, the primary regulator of gemma cup and gemma formation, is activated by cytokinin produced by *MpLOG*. We propose a genetic cascade, starting from KAI2-dependent signaling, leading to vegetative reproduction through the induction of *MpLOG* and *GCAMI* (Fig. 7b).

Plant growth and development rely on the continuous activity of the shoot apical meristem (SAM), where stem cell proliferation promoted by cytokinin is pivotal⁵³. *LOGs* genes, expressed in the stem cell zone of the SAM, play crucial roles in maintaining the stem cell population in the SAM⁴⁶. In *Arabidopsis*, cytokinins synthesized in the tip of the meristem, facilitated by specific localization of *LOG4* expression, serves as the source of a gradient of cytokinin activity, which acts as the spatial cue in the SAM⁵⁴. *LOG* expressed at the tip of meristems controls stem cell proliferation in rice panicle⁴⁶. We showed that *MpLOG* is expressed in the meristem region, gemma initial cells, and initiating gemmae that contain undifferentiated meristematic cells. The *LOG* activity localized in these regions likely contributes to the initiation and growth of gemma cups and gemmae, facilitating vegetative reproduction. Spatially regulated *LOG* expression may be crucial for the proliferation of undifferentiated cells during vegetative reproduction in *M. polymorpha*. This indicates a possibility that the function of *LOG* as a regulator of the undifferentiated cells was established in the common ancestor of land plants. Considering that adenosine phosphate-IPTs and CYP735A are of later origin, the specification of undifferentiated meristematic cells by localized cytokinin synthesis achieved by spatiotemporal regulation of *LOG* activity might be an ancient and fundamental mechanism^{55, 56, 57}. *LOG* genes are present in all major lineages of prokaryotes and plants⁵⁸. *LOG*-containing microbial pathogens cause infection in a broad range of hosts, including plants, insects, and animals, implying the importance of cytokinins as communication molecules in cross-kingdom biological interactions. In *Mycobacterium tuberculosis*, which exclusively infects animal cells, the *LOG* protein levels are tightly controlled through 26S proteasome-dependent degradation, possibly to fine-tune cytokinin production⁵⁹. The control of cytokinin synthesis by *LOG* may be a more universal and widespread system than previously thought and achieved in diverse ways. Further studies on *LOG*'s functions, diversity, and evolution will uncover new insights into the role of cytokinins.

Both gemma cup formation and gemma formation are critical steps for vegetative reproduction; they are distinct developmental processes that occur in different cells at different developmental stages⁷. We previously reported that *GCAM1* promotes gemma formation in addition to gemma cup formation^{11, 47}. In this study, we showed that the KAI2 signaling-*LOG* module controls these two steps by activating *GCAM1*. The control of cytokinin synthesis by KAI2-dependent signaling is highly cell-type specific (Fig. 7a, b). In the gemma, *MpLOG* is expressed in the meristem region and the rhizoid initial cells; however, the expression in the meristem region but not the rhizoid initial cells is KAI2 signaling-dependent. Similarly, among *MpLOG* expression in four types of

cells at the bottom of the gemma cup: gemma initial cells, initiating gemmae, slime papillae, and gemma cup floor cells, expression in slime papillae and floor cells is KAI2 signaling-independent. This indicates that KAI2-dependent signaling is responsible for only a subset of MpLOG expression (Fig. 7a). Coincidentally, *GCAMI* is expressed in cells where MpLOG is activated by KAI2-dependent signaling, suggesting that KL signaling may be profoundly involved in controlling vegetative reproduction. The underlying mechanisms for the cell-type specificity of KAI2-dependent signaling are unknown. Considering that the receptor MpKAI2A localizes in wider regions, a possible explanation is that the cell type-specificity may be regulated at the level of KL biosynthesis¹¹.

GCAMI orthologs control axillary meristem formation in *Arabidopsis*, tomato, and pepper^{51, 60, 61, 62}. This suggests a deep homology between shoot branching in angiosperms and gemma formation in *M. polymorpha*. However, although cytokinin is the upstream regulator of *GCAMI*, *RAX* genes work upstream of cytokinin and promote axillary meristem formation by enhancing cytokinin synthesis and signaling. We further demonstrated that the cytokinin-GCAM1 module is controlled by KAI2-dependent signaling in *M. polymorpha*. However, control of shoot branching by KAI2-dependent signaling is not known, and the cross-regulation of KAI2 signaling and cytokinin has not been demonstrated in angiosperms. In angiosperms, strigolactones control shoot branching at the step of axillary bud outgrowth, through interaction with cytokinin and auxin^{63, 64, 65}. Strigolactones acquired the role of a class of plant hormones by gene duplication of KAI2 in seed plants^{39, 41, 66, 67}. Subsequently, the increase in the number and functional diversification of SMXL genes brought about the specificity of the two signaling pathways⁶⁷. An intriguing possibility is that the link between KAI2-dependent signaling and cytokinin was taken over by strigolactone signaling in angiosperms. We cannot rule out the possibility that KAI2 signaling-cytokinin cross-regulation was acquired in the liverwort lineage.

Cytokinins are present in all land plants, algae, and some fungi and bacteria. Cytokinin biosynthesis and signaling pathways in plants have been mainly studied in angiosperms, however, some reports can be found outside angiosperms^{48, 68, 69}. In angiosperms, cytokinins are synthesized through adenosine phosphate-IPTs and tRNA-IPT. Phylogenetic analysis revealed that adenosine phosphate-IPTs are derived from tRNA-IPT^{55, 56}. Although the exact timing remains controversial, the duplication of tRNA-IPT leading to the adenosine phosphate-IPTs occurred later than the divergence of bryophytes^{55, 56}. Currently, the mechanism of cytokinin biosynthesis in bryophytes is not known, as cognate orthologs of angiosperm adenosine phosphate-IPTs are not present⁵⁷. Bryophytes do not contain orthologs of CYP735A either^{37, 70}. The function of LOG is also enigmatic. Severe reduction of active cytokinins and extensive increases in their precursors in Mplog mutants support the conserved function of LOG in *M. polymorpha*. However, despite a

single *LOG* gene in the genome of *M. polymorpha*, active cytokinins are still present in complete loss of function mutants of *MpLOG*. This suggests the presence of LOG-independent pathways of cytokinin biosynthesis in *M. polymorpha*. Angiosperms contain a small family of LOG genes, but cytokinin levels in plants that lost the function of all *LOG* genes have yet to be reported⁷¹. Therefore, the generality of the LOG-independent cytokinin synthesis pathway is unclear. Recently, the cytokinin/purine riboside nucleosidase1 (*CPN1*), which catalyzes the deribosylation of cytokinin nucleoside precursors and other purine nucleosides, was discovered in rice, supporting the existence of LOG-independent cytokinin synthesis⁷². The ortholog of *CPN1* exists in hornworts, the basal lineage of bryophytes, but not in *M. polymorpha* and *P. patens*, indicating that *CPN1* occurred in the common ancestor of land plants but was lost in *M. polymorpha* and *P. patens*. While *CPN1* contains double Ribo hydro-like domains, *CPN1* homologs with the single Ribo hydro-like domain of unknown function exist in land plants, including *M. polymorpha* (Supplementary Fig. 7). Further understanding of cytokinin synthesis in land plants in general and *M. polymorpha* specifically will help better understand the significance of KL- cytokinin cross-regulation in controlling vegetative reproduction and plant evolution.

Methods

Plant Materials and Growth Conditions

Strain Takaragaike-1 (Tak-1; Japanese male line) was used as the *M. polymorpha* wild-type (WT) in this study. *MpAtD14ox*, *Mpkai2a-2*, *Mpmax2-1*, *Mpsmxl-1*, *Mpsmxl-2*, *proSMXL-SMXL*^{d53}#9, *proSMXL-SMXL*^{d53}#3, and *proGCAMI-EGFPNLS* were described previously^{11, 12}. Plants were generally cultured on half-strength Gamborg's B5 medium with 1% agar, under continuous light (50 ~ 60 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$) at 22°C.

RNA extraction and expression analysis

RNA extraction, cDNA synthesis, and quantitative PCR were performed as previously described^{11, 12}. Primers used are listed in Table S3.

RNA-seq analysis

Gemmae of *MpAtD14ox* were incubated on growth medium for 24 hours at 22 °C under continuous light before being transferred to a liquid medium containing either acetone as the control or 1 μM *rac*-GR24, and then incubated for 3 or 6 hours. Total RNA was extracted from the plants using the NucleoSpin RNA Plant Kit (Macherey-Nagel). Seven biological replicates were prepared for each condition. Total RNA extraction, mRNA isolation, and RNA-seq library

synthesis were performed using the MGISEQ-2000 sequencing platform (MGI Tech). The resultant reads were mapped to the *M. polymorpha* genome version 5.1r1 assembly using HISAT2(v2.1.0) with the default parameters. Mapped reads were counted using the featureCounts function of the Rsubread package (v2.2.6) in R. Gene expression between the samples treated with acetone- and 1 μ M *rac*-GR24 was compared using DESeq2⁷³. Genes with a *p*_{adj} < 0.05 were considered differentially expressed. GO enrichment analysis was conducted as previously described^{3, 74}. The groups of genes that were upregulated or downregulated in the 3-hour and 6-hour treatments are summarized in Supplementary Table 1.

Mutagenesis by CRISPR

CRISPR/Cas9-based genome editing of *MpLOG* was performed as previously described⁷⁵. The vector, pMpGE011-MpLOG containing the target sequences (gRNA1 and gRNA2 of *MpLOG*), was introduced into WT. The vector including gRNA1 of *MpLOG* was also introduced into *Mpsmxl-1* strain. Respective target sites, genomic sequence changes, and amino acid sequence changes of *Mplog-1* and *Mplog-2* are shown in Supplementary Figure 4a. About *Mpsmxl-1*/Mplog #2 and #7, genomic sequence changes, and amino acid sequence changes are shown in Supplementary Table 2. pMpGE010-MpKAI2A-gRNA1 described previously⁷⁵ was introduced into the *proMpLOG-EGFPNLS* strain described below and *proMpLOG-EGFPNLS*/Mpkai2a-7 was selected.

Quantification of cytokinins

WT, *Mplog-1*, *Mplog-2*, *Mpsmxl-1*, *Mpsmxl-2*, *proSMXL-SMXL*^{d53} #9, and *proSMXL-SMXL*^{d53} #3 were sown on growth medium and cultivated for seven to ten days. Approximately 100 mg of fresh weight of each plant was used for the hormone extraction. Analysis of cytokinins was performed as previously described^{50, 76}. Approximately 100 mg of each sample was used for measurement of cytokinins by ultra-performance liquid chromatography (UPLC)-tandem mass spectrometry (AQUITY UPLC System/XEVO-TQXS; Waters, USA) with an ODScolumn (AQUITY UPLC HSS T3, 1.8 mm, 2.1 x 100 mm; Waters).

Phenotypic analysis

Analysis of the thallus phenotypes, gemma-cup formation, and the number of gemmae in a gemma-cup were performed as described previously¹¹. For the comparison of the gemma formation among WT, *Mplog-1*, *Mpsmxl-1*, *Mpsmxl-1*/Mplog#2, and *Mpsmxl-1*/Mplog#7, the apical parts, which had not yet bifurcated and formed a gemma cup, were incubated on new growth medium for 14 days. Gemmae of WT, *Mplog-1*, *Mpsmxl-1*, *Mpsmxl-1*/Mplog#2, and

Mpsmxl-1/Mplog#7 were transferred to vermiculite after 6 days of incubation on growth medium and observed from the side after another 14 days of incubation.

Promoter-reporter analysis

About 5 kb and 1 kb of the promoter regions of MpLOG were amplified from WT genomic DNA by PCR using the primer pairs, proMpLOG-5kb_F/proMpLOG_R, and proMpLOG1kb_F/proMpLOG_R, and cloned into pENTR/D-TOPO (Thermo Fisher Scientific). The generated entry vectors, pENTR-*proMpLOG5kb*, and pENTR-*proMpLOG* were transferred into the binary vectors pMpGWB315-EGFPmodified. The transformants generated by using the resulting vectors, pMpGWB315-EGFPmodified-*proMpLOG5kb* and pMpGWB315-EGFPmodified-*proMpLOG*, were observed as described previously¹¹.

Quantification and statistical analysis

Statistical analyses were performed in R version 4.1.2 (R Development Core Team) using the multcomp R package. Significant differences ($p < 0.05$) were tested using Tukey's honestly significant difference (HSD) test for multiple comparisons. Data are presented as the mean $\pm$ SD.

Data availability

Raw RNA sequencing datasets generated in this study were deposited to Short Read Archive at DNA Data Bank of Japan (DDBJ) under DRA019200 (BioProject: PRJDB18746). Source Data for Fig. 1d-g, 2b, 3b, 3d, 4b-c, 4e, 4g, 6a, and Supplementary Fig. 2a-b, 5b, 5d, 5f are provided with this paper.

References

1. Steeves T. A. & Sussex I. M. Patterns in Plant Development. (1989).
2. Frey W. & Kürschner H. Asexual reproduction, habitat colonization and habitat maintenance in bryophytes. *Flora - Morphology, Distribution, Functional Ecology of Plants* 206, 173-184 (2011).
3. Ishida S. et al. Diminished auxin signaling triggers cellular reprogramming by inducing a regeneration factor in the liverwort *Marchantia polymorpha*. *Plant Cell Physiol* 63, 384-400 (2022).
4. Ikeuchi M. et al. Molecular mechanisms of plant regeneration. *Annu Rev Plant Biol* 70, 377-406 (2019).
5. Shimamura M. *Marchantia polymorpha*: taxonomy, phylogeny and morphology of a model system. *Plant Cell Physiol* 57, 230-256 (2016).
6. Burgeff H. Genetische studien an *Marchantia*. Einführung einer neuen Pflanzenfamilie in die genetische Wissenschaft. Fischer, (1943).
7. Kato H., Yasui Y. & Ishizaki K. Gemma cup and gemma development in *Marchantia polymorpha*. *New Phytol* 228, 459-465 (2020).
8. Waadt R. et al. Plant hormone regulation of abiotic stress responses. *Nat Rev Mol Cell Biol* 23, 680-694 (2022).
9. Gray W. M. Hormonal regulation of plant growth and development. *PLoS Biol* 2, E311 (2004).
10. Vanstraelen M. & Benkova E. Hormonal interactions in the regulation of plant development. *Annu Rev Cell Dev Biol* 28, 463-487 (2012).
11. Komatsu A. et al. Control of vegetative reproduction in *Marchantia polymorpha* by the KAI2-ligand signaling pathway. *Curr Biol* 33, 1196-1210 e1194 (2023).
12. Mizuno Y. et al. Major components of the KARRIKIN INSENSITIVE2-dependent signaling pathway are conserved in the liverwort *Marchantia polymorpha*. *Plant Cell* 33, 2395-2411 (2021).
13. Nelson D. C. et al. F-box protein MAX2 has dual roles in karrikin and strigolactone signaling in *Arabidopsis thaliana*. *Proc Natl Acad Sci USA* 108, 8897-8902 (2011).

14. Waters M. T. et al. Specialisation within the DWARF14 protein family confers distinct responses to karrikins and strigolactones in *Arabidopsis*. *Development* 139, 1285-1295 (2012).
15. Conn C. E. & Nelson D. C. Evidence that KARRIKIN-INSENSITIVE2 (KAI2) receptors may perceive an unknown signal that is not karrikin or strigolactone. *Front Plant Sci* 6, 1219 (2015).
16. Soundappan I. et al. SMAX1-LIKE/D53 family members enable distinct MAX2-dependent responses to strigolactones and karrikins in *Arabidopsis*. *Plant Cell* 27, 3143-3159 (2015).
17. Waters M. T. & Nelson D. C. Karrikin perception and signalling. *New Phytol* 237, 1525-1541 (2023).
18. Zheng J. et al. Karrikin signaling acts parallel to and additively with strigolactone signaling to regulate rice mesocotyl elongation in darkness. *Plant Cell* 32, 2780-2805 (2020).
19. Wang L. et al. Strigolactone and karrikin signaling pathways elicit ubiquitination and proteolysis of SMXL2 to regulate hypocotyl elongation in *Arabidopsis*. *Plant Cell* 32, 2251-2270 (2020).
20. Stanga J. P. et al. SUPPRESSOR OF MORE AXILLARY GROWTH2 1 controls seed germination and seedling development in *Arabidopsis*. *Plant Physiol* 163, 318-330 (2013).
21. Stanga J.P., Morffy N. & Nelson D. C. Functional redundancy in the control of seedling growth by the karrikin signaling pathway. *Planta* 243, 1397-1406 (2016).
22. Wang L. et al. Strigolactone signaling in *Arabidopsis* regulates shoot development by targeting D53-Like SMXL repressor proteins for ubiquitination and degradation. *Plant Cell* 27, 3128-3142 (2015).
23. Wang L. et al. Transcriptional regulation of strigolactone signalling in *Arabidopsis*. *Nature* 583, 277-281 (2020).
24. Song X. et al. IPA1 functions as a downstream transcription factor repressed by D53 in strigolactone signaling in rice. *Cell Res* 27, 1128-1141 (2017).
25. Hu J. et al. BES1 functions as the co-regulator of D53-like SMXLs to inhibit *BRC1* expression in strigolactone-regulated shoot branching in *Arabidopsis*. *Plant Commun* 1, 100014 (2019).
26. Xie, Y. et al. *Arabidopsis* FHY3 and FAR1 integrate light and strigolactone signaling to regulate branching. *Nat Commun* 11, 1955. (2020).
27. Wang L., Waters M. T. & Smith S. M. Karrikin-KAI2 signalling provides *Arabidopsis* seeds with tolerance to abiotic stress and inhibits germination under conditions unfavourable to seedling establishment. *New Phytol* 219, 605-618 (2018).
28. Xu P., Jinbo H. & Cai W. Karrikin signaling regulates hypocotyl shade avoidance response by modulating auxin homeostasis in *Arabidopsis*. *New Phytol* 236, 1748-1761 (2022).

29. Li W. et al. The karrikin receptor KAI2 promotes drought resistance in *Arabidopsis thaliana*. *PLoS Genet* 13, e1007076 (2017).
30. Nelson D. C. et al. Karrikins enhance light responses during germination and seedling development in *Arabidopsis thaliana*. *Proc Natl Acad Sci USA* 107, 7095-7100 (2010).
31. Choi J. et al. The negative regulator SMAX1 controls mycorrhizal symbiosis and strigolactone biosynthesis in rice. *Nat Commun* 11, 2114 (2020).
32. Varshney K. & Gutjahr C. KAI2 can do: karrikin receptor function in plant development and response to abiotic and Biotic Factors. *Plant Cell Physiol* 64, 984-995 (2023).
33. Villacija-Aguilar J. A. et al. KAI2 promotes *Arabidopsis* root hair elongation at low external phosphate by controlling local accumulation of AUX1 and PIN2. *Curr Biol* 32, 228-236 e223 (2022).
34. Carbonnel S. et al. The karrikin signaling regulator SMAX1 controls *Lotus japonicus* root and root hair development by suppressing ethylene biosynthesis. *Proc Natl Acad Sci USA* 117, 21757-21765 (2020).
35. Hamon-Josse M. et al. KAI2 regulates seedling development by mediating light-induced remodelling of auxin transport. *New Phytol* 235, 126-140 (2022).
36. Brun G. et al. CYP707As are effectors of karrikin and strigolactone signalling pathways in *Arabidopsis thaliana* and parasitic plants. *Plant Cell Environ* 42, 2612-2626 (2019).
37. Bowman J. L. et al. Insights into land plant evolution garnered from the *Marchantia polymorpha* genome. *Cell* 171, 287-304 e215 (2017).
38. Bonhomme S. & Guillory A. Synthesis and signalling of strigolactone and KAI2-ligand signals in bryophytes. *J Exp Bot* 73, 4487-4495 (2022).
39. Kodama K. et al. An ancestral function of strigolactones as symbiotic rhizosphere signals. *Nat Commun* 13, 3974 (2022).
40. Lopez-Obando M. et al. *Physcomitrella patens* MAX2 characterization suggests an ancient role for this F-box protein in photomorphogenesis rather than strigolactone signalling. *New Phytol* 219, 743-756 (2018).
41. Bythell-Douglas R. et al. Evolution of strigolactone receptors by gradual neo-functionalization of KAI2 paralogues. *BMC Biol* 15, 52 (2017).
42. Kodama K., Xie, X. & Kyoizuka J. The D14 and KAI2 orthologs of gymnosperms sense strigolactones and KL mimics, respectively, and the signals are transduced to control downstream genes. *Plant Cell Physiol* 64, 1057–1065 (2023)
43. Conn C. E. et al. PLANT EVOLUTION. Convergent evolution of strigolactone perception enabled host detection in parasitic plants. *Science* 349, 540-543 (2015).
44. Toh S. et al. Structure-function analysis identifies highly sensitive strigolactone receptors in *Striga*. *Science* 350, 203-207 (2015).

45. Lopez-Obando M. et al. The Physcomitrium (Physcomitrella) patens PpKAI2L receptors for strigolactones and related compounds function via MAX2-dependent and -independent pathways. *Plant Cell* 33, 3487-3512 (2021).
46. Kurakawa T. et al. Direct control of shoot meristem activity by a cytokinin-activating enzyme. *Nature* 445, 652-655 (2007).
47. Yasui Y. et al. GEMMA CUP-ASSOCIATED MYB1, an ortholog of axillary meristem regulators, is essential in vegetative reproduction in *Marchantia polymorpha*. *Curr Biol* 29, 3987-3995.e3985 (2019).
48. Hirose N. et al. Regulation of cytokinin biosynthesis, compartmentalization and translocation. *J Exp Bot* 59, 75-83 (2008).
49. Kamada-Nobusada T. & Sakakibara H. Molecular basis for cytokinin biosynthesis. *Phytochemistry* 70, 444-449 (2009).
50. Aki S. S. et al. Cytokinin signaling is essential for organ formation in *Marchantia polymorpha*. *Plant Cell Physiol* 60, 1842-1854 (2019).
51. Muller D., Schmitz G. & Theres K. Blind homologous R2R3 Myb genes control the pattern of lateral meristem initiation in *Arabidopsis*. *Plant Cell* 18, 586-597 (2006).
52. Aki S.S. et al. R2R3-MYB transcription factor GEMMA CUP-ASSOCIATED MYB1 mediates the cytokinin signal to achieve proper organ development in *Marchantia polymorpha*. *Sci Rep* 12, 21123 (2022).
53. Hong L. & Fletcher J. C. Stem cells: engines of plant growth and development. *Int J Mol Sci* 24, (2023).
54. Chickarmane V. S. et al. Cytokinin signaling as a positional cue for patterning the apical-basal axis of the growing *Arabidopsis* shoot meristem. *Proc Natl Acad Sci USA* 109, 4002-4007 (2012).
55. Wang X. et al. Evolution and roles of cytokinin genes in angiosperms 1: Do ancient IPTs play housekeeping while non-ancient IPTs play regulatory roles? *Hortic Res* 7, 28 (2020).
56. Nishii K. et al. Tangled history of a multigene family: The evolution of ISOPENTENYLTRANSFERASE genes. *PLoS One* 13, e0201198 (2018).
57. Lindner A. C. et al. Isopentenyltransferase-1 (IPT1) knockout in *Physcomitrella* together with phylogenetic analyses of IPTs provide insights into evolution of plant cytokinin biosynthesis. *J Exp Bot* 65, 2533-2543 (2014).
58. Naseem M., Sarukhanyan E. & Dandekar T. LONELY-GUY knocks every door: crosskingdom microbial pathogenesis. *Trends Plant Sci* 20, 781-783 (2015).
59. Samanovic M. I. et al. Proteasomal control of cytokinin synthesis protects *Mycobacterium tuberculosis* against nitric oxide. *Mol Cell* 57, 984-994 (2015).

- 589 60. Busch B. L. et al. Shoot branching and leaf dissection in tomato are regulated by
590 homologous gene modules. *Plant Cell* 23, 3595-3609 (2011).
- 591 61. Keller T. et al. Arabidopsis REGULATOR OF AXILLARY MERISTEMS1 controls a leaf
592 axil stem cell niche and modulates vegetative development. *Plant Cell* 18, 598-611 (2006).
- 593 62. Schmitz G. et al. The tomato Blind gene encodes a MYB transcription factor that controls
594 the formation of lateral meristems. *Proc Natl Acad Sci USA* 99, 1064-1069 (2002).
- 595 63. Umehara M. et al. Inhibition of shoot branching by new terpenoid plant hormones. *Nature*
596 455, 195-200 (2008).
- 597 64. Gomez-Roldan V. et al. Strigolactone inhibition of shoot branching. *Nature* 455, 189-194
598 (2008).
- 599 65. Dun E. A. et al. Strigolactones and shoot branching: what is the real hormone and how does
600 it work? *Plant Cell Physiol* 64, 967-983 (2023).
- 601 66. Waters M. T. et al. Strigolactone signaling and evolution. *Annu Rev Plant Biol* 68, 291-322
602 (2017).
- 603 67. Walker C.H. et al. Strigolactone synthesis is ancestral in land plants, but canonical
604 strigolactone signalling is a flowering plant innovation. *BMC Biol* 17, 70 (2019).
- 605 68. von Schwanberg K. Moss biology and phytohormones--cytokinins in
606 *Physcomitrella*. *Plant Biol* 8, 382-388 (2006).
- 607 69. Guillory A. & Bonhomme S. Phytohormone biosynthesis and signaling pathways of
608 mosses. *Plant Mol Biol* 107, 245-277 (2021).
- 609 70. Li L. et al. Molecular regulation and evolution of cytokinin signaling in plant abiotic
610 stresses. *Plant Cell Physiol* 63, 1787-1805 (2022).
- 611 71. Tokunaga H. et al. Arabidopsis lonely guy (LOG) multiple mutants reveal a central role of
612 the LOG-dependent pathway in cytokinin activation. *Plant J* 69, 355-365 (2012).
- 613 72. Kojima M. et al. A cell wall-localized cytokinin/purine riboside nucleosidase is involved in
614 apoplastic cytokinin metabolism in *Oryza sativa*. *Proc Natl Acad Sci USA* 120,
615 e2217708120 (2023).
- 616 73. Love M. I., Huber W. & Anders S. Moderated estimation of fold change and dispersion for
617 RNA-seq data with DESeq2. *Genome Biol* 15, 550 (2014).
- 618 74. Yu G. et al. clusterProfiler: an R package for comparing biological themes among gene
619 clusters. *OMICS* 16, 284-287 (2012).
- 620 75. Sugano S. S. et al. Efficient CRISPR/Cas9-based genome editing and its application to
621 conditional genetic analysis in *Marchantia polymorpha*. *PLoS One* 13, e0205117 (2018).
- 622 76. Kojima M. et al. Highly sensitive and high-throughput analysis of plant hormones using
623 MS-probe modification and liquid chromatography-tandem mass spectrometry: an
624 application for hormone profiling in *Oryza sativa*. *Plant Cell Physiol* 50, 1201-1214 (2009).

625

626 **Acknowledgments**

627 We thank Dr. Yoshiya Seto and Dr. Xiaonan Xie for providing (–)-GR24. This research was
628 supported by a Grants-in-Aid from the Ministry of Education, Culture, Sports, Science, and
629 Technology, Japan (23H05409, 20H05684, and 17H06475 to J.K., JP21K15116 to A.K.) and
630 Canon Foundation to J.K.

631

632 **Author contributions**

633 J.K. designed the research and wrote the article. A.K., M.F., K. K., H.S., and Y.H. produced
634 mutants, analyzed phenotypes, and examined gene expression. Y.T., M.K., and H.S. quantified
635 cytokinins.

636

637 **Competing interests**

638 The authors declare no competing interests.

639

Figure legends

Figure 1. MpLOG works downstream of KAI2-dependent signaling.

a MA plots showing the differential gene expression in 1 μ M *rac*-GR24-treated *MpAtD14ox* plants compared to mock-treated *MpAtD14ox* plants, 3 hours (top) and 6 hours (bottom) after the start of treatment.

b Venn diagram showing the number of up-regulated genes after 3 and 6 hours of 1 μ M *rac*-GR24 treatment in *MpAtD14ox* plants.

c Venn diagram showing the number of up-regulated genes after 1 μ M *rac*-GR24 treatment in *MpAtD14ox* plants and the down-regulated genes in *Mpmax2-2* and *Mpkai2a-1* mutants.

d Induction of three up-regulated genes (*MpDLP1*, *MpKAI2B*, and *MpLOG*) in WT by 1 μ M (–)-GR24 treatment for 6 hours examined by quantitative PCR (qPCR) analysis.

e Induction of *MpDLP1*, *MpKAI2B*, and *MpLOG* expression by 1 μ M (–)-GR24 treatment for 6 hours in the presence of 1 μ M Cycloheximide (CHX) examined by qPCR analysis in WT.

f Expression level of *MpLOG* in WT, *Mpkai2a-2*, *Mpmax2-1*, *proSMXL-SMXL^{d53}#3* and *proSMXL-SMXL^{d53}#9*.

g Induction of *MpLOG* expression by 1 μ M (–)-GR24 treatment for 6 hours in WT, *Mpkai2a-2*, *Mpmax2-1*, *proSMXL-SMXL^{d53}#3*, and *proSMXL-SMXL^{d53}#9* examined by qPCR analysis.

Expression levels relative to *ACTIN* (*MpACTIN*) are shown (**d-g**). Bars represent means $\pm$ SD (n=3). The p values were calculated by using the Student's t-tests (**d, e, g**). The Tukey's HSD test was used for multiple comparisons in **f** and statistical differences (p<0.05) are indicated by different letters.

Figure 2. MpLOG catalyzes the final steps of cytokinin biosynthesis in *M. polymorpha*.

a Cytokinin biosynthesis pathway in angiosperms. The addition of an isoprenoid side chain to the N⁶-amino group of an adenine nucleotide by isopentenyltransferases (IPTs) is the rate-limiting step in cytokinin synthesis³³. Two different forms of IPTs, adenosine phosphate-IPTs and tRNA-IPTs, are known in plants. Adenosine phosphate-IPTs use ATP, ADP, or AMP as substrates for prenylation. Subsequently, with the function of CYP735A and an unidentified zeatin reductase, iP riboside 5'-monophosphate (iPRMP), *trans*-zeatin 5'-monophosphate (tZRMP), and dihydrozeatin 5'-monophosphate (DZRMP) are synthesized. tRNA-IPTs prenylate UNN-decoding tRNAs at an adenine (A₃₇), and the release of cytokinin nucleotides through tRNA degradation produces *cis*-zeatin 5'-monophosphate (cZRMP). LOG encodes a cytokinin riboside 5'-monophosphate phosphoribohydrolase that releases cytokinin nucleobase and ribose 5'-monophosphate from iPRMP, tZRMP, DZRMP, and cZRMP, leading to the production of N⁶-isopentenyl adenine (iP), *trans*-zeatin (tZ), dihydro-zeatin (DZ), and *cis*-zeatin (cZ), active

cytokinins. Cytokinin/purine riboside nucleosidase1 (CPN1) catalyzes the deribosylation of cytokinin riboside precursors and other purine ribosides.

b Quantification of cytokinins in WT, *Mplog-1*, and *Mplog-2*. Bars represent means $\pm$ SD (n=3). The Tukey's HSD test was used for multiple comparisons and statistical differences ($p < 0.05$) are indicated by different letters. u.q. indicates under quantification limits. iPRPs, tZRP, and cZRP; ribotide precursors, iPR, tZR, and cZR; riboside precursors.

Figure 3. Cytokinins synthesized by MpLOG control gemma cup and gemma formation.

a Effects of cytokinin application on gemma cup formation in WT, *Mplog-1*, and *Mplog-2*. Plants were grown on vermiculite with 6-benzyl adenine (BA) application to the vermiculite once a week. Yellow arrows indicate gemma cups. Scale bars: 1 cm.

b Effect of cytokinin on the frequency of gemma cup formation in WT, *Mplog-1*, and *Mplog-2* plants. Plants were grown on 1/2 B5 medium for 6 days, then transferred to vermiculite and grown for 3 weeks or more with BA application to the vermiculite once a week. The frequency of gemma cup formation was scored at 8 points after the second bifurcation of the thallus tip. Bars represent mean $\pm$ SD (n=3).

c The top view of gemma cups at day 14 stage in WT, *Mplog-1*, and *Mplog-2* plants grown on 1/2 B5 medium. Scale bars: 1 mm.

d The number of gemmae in gemma cups at day 14 stage in WT, *Mplog-1*, and *Mplog-2* plants grown on 1/2 B5 medium. Bars represent mean $\pm$ SD (n=5). The Tukey's HSD test was used for multiple comparisons and statistical differences ($p < 0.05$) are indicated by different letters.

Figure 4. Cytokinin works downstream of KAI2-dependent signaling.

a Effects of cytokinin application to gemma cup formation in WT, *Mpkai2a-2*, and *Mpmax2-1*. Plants were grown on vermiculite with BA application to the vermiculite once a week. Yellow arrows indicate gemma cups. Scale bars: 1 cm.

b Effect of cytokinin on the frequency of gemma cup formation in WT, *Mpkai2a-2*, and *Mpmax2-1*. Plants were grown on 1/2 B5 medium for 6 days, then transferred to vermiculite and grown for 3 weeks or more with BA application to the vermiculite once a week. The frequency of gemma cup formation was scored at 8 points after the second bifurcation of the thallus tip. Bars represent mean $\pm$ SD (n=3).

c Quantification of active cytokinins (iP, tZ, and cZ) in WT, *Mpsmxl-1*, *Mpsmxl-2*, *proSMXL-SMXL^{d53}#3*, and *proSMXL-SMXL^{d53}#9* plants. Bars represent means $\pm$ SD (n=3). u.q. indicates under quantification limits. iPRPs, tZRP, and cZRP; ribotide precursors, iPR, tZR, and cZR; riboside precursors.

d, e Genetic interaction between MpLOG and MpSMAXL in gemma cup formation. Thalli of WT, *Mplog-1*, *Mpsmxl-1*, *Mpsmxl-1/Mplog#2*, and *Mpsmxl-1/Mplog#7* plants grown on vermiculite (d). Yellow arrows indicate gemma cups. Scale bars: 1 cm. Quantification of gemma cup formation frequency (e). Bars represent mean $\pm$ SD (n=4).

f, g Genetic interaction between MpLOG and MpSMAXL in gemma cup formation in the gemma number phenotype. Top view of day14 gemma cups of WT, *Mplog-1*, *Mpsmxl-1*, *Mpsmxl-1/Mplog#2*, and *Mpsmxl-1/Mplog#7* grown on 1/2 B5 medium (f). Scale bars: 1 mm. Quantification of the number of gemmae per cup (g). Bars represent the mean $\pm$ SD (n=5).

The Tukey's HSD test was used for multiple comparisons and statistical differences ($p < 0.05$) are indicated by different letters in (c), (e) and (f).

Figure 5. Control of MpLOG expression by KAI2-dependent signaling.

a, b Localization of EGFP fluorescence in the gemma of *proMpLOG-EGFPNLS/WT* (a), and *proMpLOG-EGFPNLS/kai2a-7* (b) grown on 1/2 B5 medium, observed by confocal laser scanning microscopy. From the left: EGFP fluorescence, EGFP merged with blight field image, enlargement of the region in yellow square.

c, d Localization of EGFP fluorescence in the thallus of *proMpLOG-EGFPNLS/WT* (c), and *proMpLOG-EGFPNLS/kai2a-7* (d) grown on vermiculite, observed by stereo microscopy. Left: EGFP fluorescence, Right: blight field image.

e, f Localization of EGFP fluorescence in the cross-sections of the midrib of *proMpLOG-EGFPNLS/WT* (e), and *proMpLOG-EGFPNLS/kai2a-7* (f) grown on vermiculite, observed by confocal laser scanning microscopy. Top panels: EGFP fluorescence, Bottom panels: EGFP merged with blight field image.

g, h Localization of EGFP fluorescence in initiating gemmae, slime papilla, and gemma cup floor cells at the bottom of gemma cup in *proMpLOG-EGFPNLS/WT* (g), and *proMpLOG-EGFPNLS/kai2a-7* (h) grown on 1/2 B5 medium, observed by confocal laser scanning microscopy. White arrowheads: gemma initial cells, blue arrowheads: initiating gemmae, yellow arrows: slime papillae, red arrows: gemma cup floor cells. Scale bars: 100 μ m.

Figure 6. GEMMA CUP-ASSOCIATED MYB1 (GCAM1) expression is promoted by cytokinin synthesized by MpLOG.

a The induction of *GCAM* expression by cytokinin was examined using qPCR analysis. Expression levels relative to *MpACTIN* are shown. Bars represent means $\pm$ SD (n=3). The p values were calculated by using the Student's t-tests.

b, c Localization of EGFP fluorescence in initiating gemmae at the bottom of gemma cup in *proGCAM1-EGFPNLS/WT* (b) and *proGCAM1-EGFPNLS/Mplog* (c) grown on 1/2 B5 medium and observed by confocal laser scanning microscopy. White arrowheads: gemma initial cells, blue arrowheads: initiating gemmae, yellow arrows: slime papillae. Scale bars: 100 μ m.

Figure 7. Model of cytokinin-mediated control of gemma cup and gemma formation by KAI2-dependent signaling in *M. polymorpha*.

a Schematic diagram of the *MpLOG* and *GCAM1* expression in WT, *Mpkai2a*, and *Mplog* mutant plants. Green circles indicate EGFP fluorescence. White arrowheads: gemma initial cells, blue arrowheads: initiating gemmae, yellow arrows: slime papillae, red arrows: gemma cup floor cells.

b The model for gemma cup and gemma formation by KAI2-dependent signaling. The activation of *MpLOG* expression by KAI2-dependent signaling in gemma initial cells and initiating gemmae leads to the induction of *GCAM1* expression, resulting in gemma-cup and gemma formation. *MpLOG* expression in slime papillae and gemma cup floor cells is KAI2 signaling independent. *MpLOG* expression induces *GCAM1* expression in slime papillae but not in gemma cup floor cells. The functional significance of *GCAM1* in slime papillae and *MpLOG* in gemma cup floor cells is unknown.

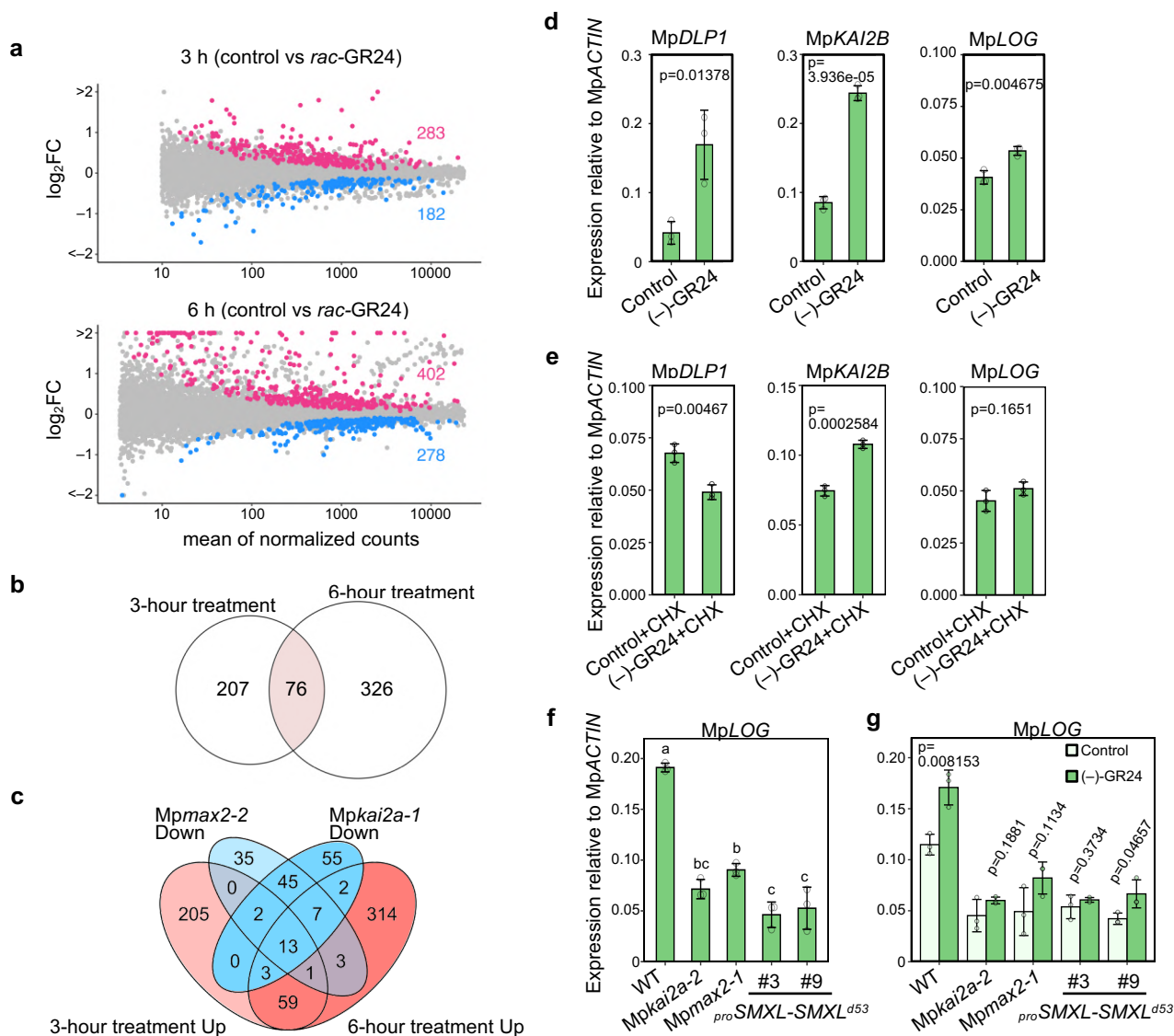


Figure 1. MpLOG works downstream of KAI2-dependent signaling.

a MA plots showing the differential gene expression in 1 μ M *rac*-GR24-treated *MpAtD14ox* plants compared to mock-treated *MpAtD14ox* plants, 3 hours (top) and 6 hours (bottom) after the start of treatment. **b** Venn diagram showing the number of up-regulated genes after 3 and 6 hours of 1 μ M *rac*-GR24 treatment in *MpAtD14ox* plants. **c** Venn diagram showing the number of up-regulated genes after 1 μ M *rac*-GR24 treatment in *MpAtD14ox* plants and the down-regulated genes in *Mpmax2-2* and *Mpkai2a-1* mutants. **d** Induction of three up-regulated genes (*MpDLP1*, *MpKAI2B*, and *MpLOG*) in WT by 1 μ M (-)-GR24 treatment for 6 hours examined by quantitative PCR (qPCR) analysis. **e** Induction of *MpDLP1*, *MpKAI2B*, and *MpLOG* expression by 1 μ M (-)-GR24 treatment for 6 hours in the presence of 1 μ M Cycloheximide (CHX) examined by qPCR analysis in WT. **f** Expression level of *MpLOG* in WT, *Mpkai2a-2*, *Mpmax2-1*, *proSMXL-SMXL*^{d53}#3 and *proSMXL-SMXL*^{d53}#9. **g** Induction of *MpLOG* expression by 1 μ M (-)-GR24 treatment for 6 hours in WT, *Mpkai2a-2*, *Mpmax2-1*, *proSMXL-SMXL*^{d53}#3, and *proSMXL-SMXL*^{d53}#9 examined by qPCR analysis. Expression levels relative to *ACTIN* (*MpACTIN*) are shown (**d-g**). Bars represent means $\pm$ SD (n=3). The p values were calculated by using the Student's t-tests (**d**, **e**, **g**). The Tukey's HSD test was used for multiple comparisons in **f** and statistical differences (p<0.05) are indicated by different letters.

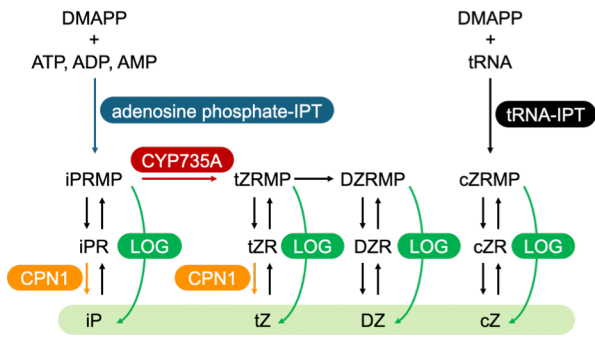
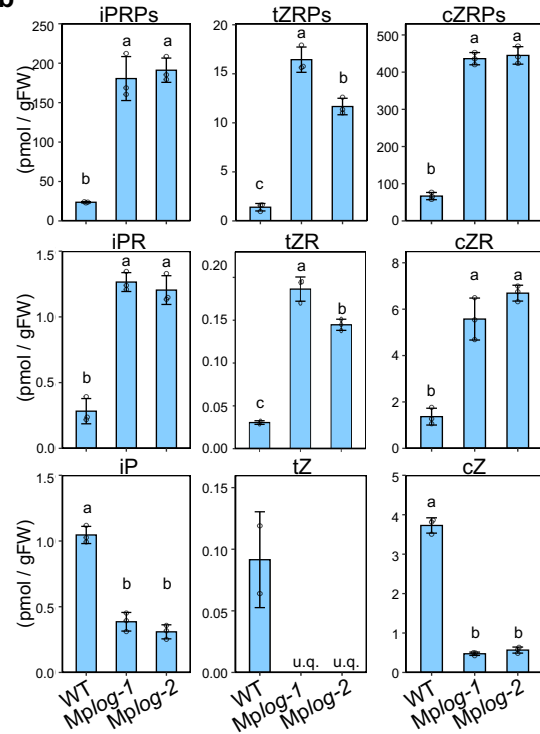
a**b**

Figure 2. MpLOG catalyzes the final steps of cytokinin biosynthesis in *M. polymorpha*.

a Cytokinin biosynthesis pathway in angiosperms. The addition of an isoprenoid side chain to the N⁶-amino group of an adenine nucleotide by isopentenyltransferases (IPTs) is the rate-limiting step in cytokinin synthesis³³. Two different forms of IPTs, adenosine phosphate-IPTs and tRNA-IPTs, are known in plants. Adenosine phosphate-IPTs use ATP, ADP, or AMP as substrates for prenylation. Subsequently, with the function of CYP735A and an unidentified zeatin reductase, iP riboside 5'-monophosphate (iPRMP), *trans*-zeatin 5'-monophosphate (tZRMP), and dihydro zeatin 5'-monophosphate (DZRMP) are synthesized. tRNA-IPTs prenylate UNN-decoding tRNAs at an adenine (A₃₇), and the release of cytokinin nucleotides through tRNA degradation produces *cis*-zeatin 5'-monophosphate (cZRMP). LOG encodes a cytokinin riboside 5'-monophosphate phosphoribohydrolase that releases cytokinin nucleobase and ribose 5'-monophosphate from iPRMP, tZRMP, DZRMP, and cZRMP, leading to the production of N⁶-isopentenyl adenine (iP), *trans*-zeatin (tZ), dihydro-zeatin (DZ), and *cis*-zeatin (cZ), active cytokinins. Cytokinin/purine riboside nucleosidase1 (CPN1) catalyzes the deribosylation of cytokinin riboside precursors and other purine ribosides.

b Quantification of cytokinins in WT, *Mplog-1*, and *Mplog-2*. Bars represent means $\pm$ SD (n=3). The Tukey's HSD test was used for multiple comparisons and statistical differences (p<0.05) are indicated by different letters. u.q. indicates under quantification limits. iPRPs, tZRP, and cZRP; riboside precursors, iPR, tZR, and cZR; riboside precursors.

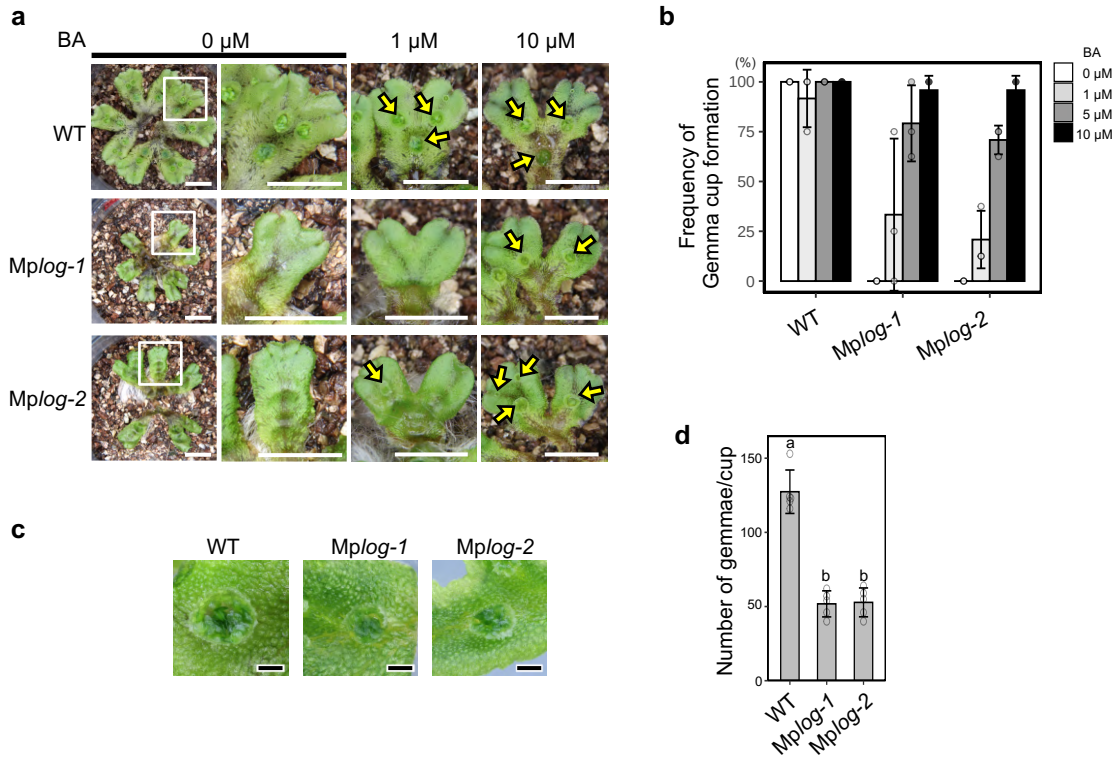


Figure 3. Cytokinins synthesized by MpLOG control gemma cup and gemma formation.

a Effects of cytokinin application on gemma cup formation in WT, *Mplog-1*, and *Mplog-2*. Plants were grown on vermiculite with 6-benzyl adenine (BA) application to the vermiculite once a week. Yellow arrows indicate gemma cups. Scale bars: 1cm.

b Effect of cytokinin on the frequency of gemma cup formation in WT, *Mplog-1*, and *Mplog-2* plants. Plants were grown on 1/2 B5 medium for 6 days, then transferred to vermiculite and grown for 3 weeks or more with BA application to the vermiculite once a week. The frequency of gemma cup formation was scored at 8 points after the second bifurcation of the thallus tip. Bars represent mean $\pm$ SD (n=3).

c The top view of gemma cups at day 14 stage in WT, *Mplog-1*, and *Mplog-2* plants grown on 1/2 B5 medium. Scale bars: 1 mm.

d The number of gemmae in gemma cups at day 14 stage in WT, *Mplog-1*, and *Mplog-2* plants grown on 1/2 B5 medium. Bars represent mean $\pm$ SD (n=5). The Tukey's HSD test was used for multiple comparisons and statistical differences ($p < 0.05$) are indicated by different letters.

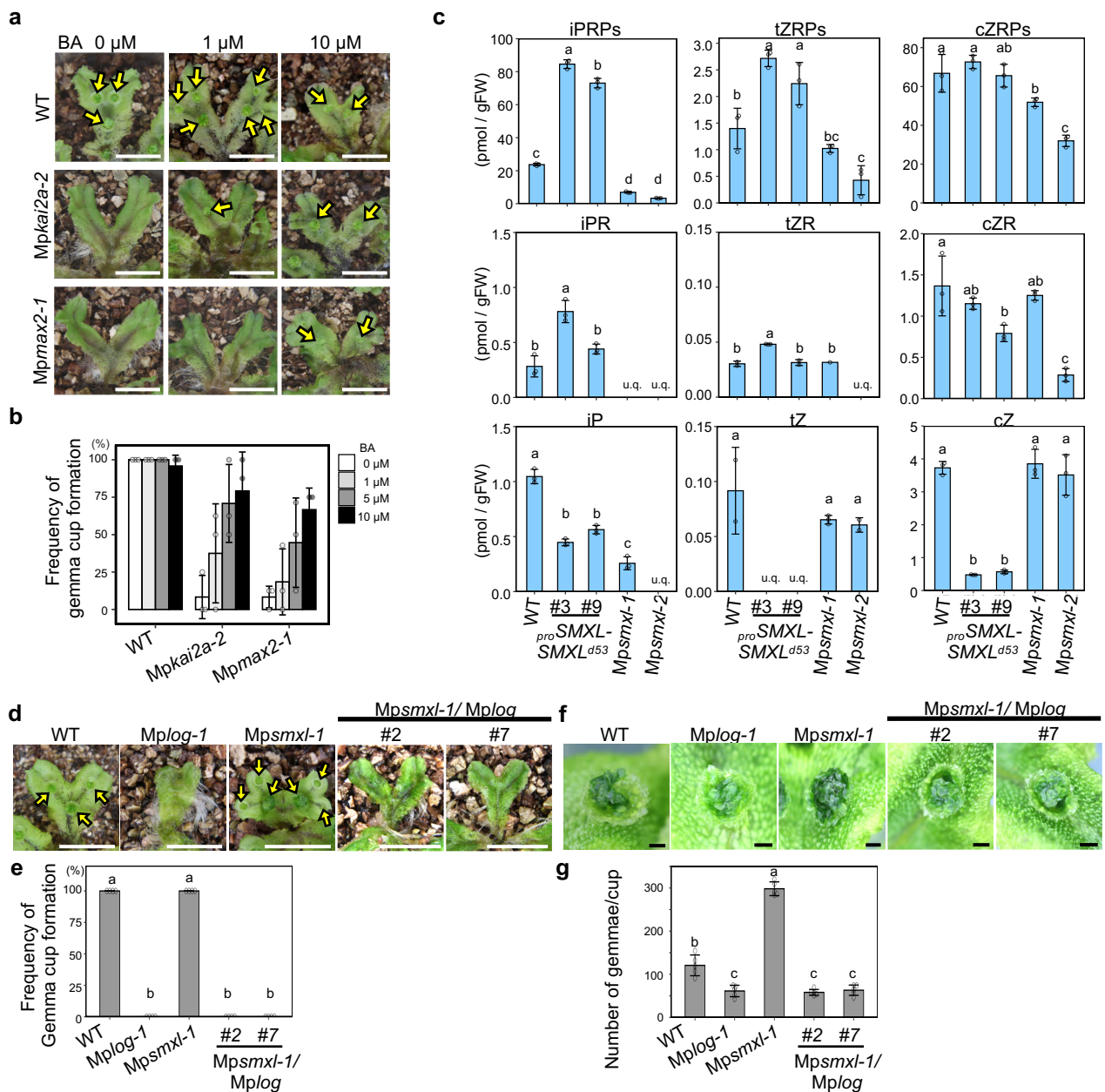


Figure 4. Cytokinin works downstream of KAI2-dependent signaling.

a Effects of cytokinin application to gemma cup formation in WT, *Mpkai2a-2*, and *Mpmx2-1*. Plants were grown on vermiculite with BA application to the vermiculite once a week. Yellow arrows indicate gemma cups. Scale bars: 1 cm. **b** Effect of cytokinin on the frequency of gemma cup formation in WT, *Mpkai2a-2*, and *Mpmx2-1*. Plants were grown on 1/2 B5 medium for 6 days, then transferred to vermiculite and grown for 3 weeks or more with BA application to the vermiculite once a week. The frequency of gemma cup formation was scored at 8 points after the second bifurcation of the thallus tip. Bars represent mean $\pm$ SD ($n=3$). **c** Quantification of active cytokinins (iP, tZ, and cZ) in WT, *Mpsmx1-1*, *Mpsmx1-2*, *proSMXL-SMXL^{d53}#3*, and *proSMXL-SMXL^{d53}#9* plants. Bars represent means $\pm$ SD ($n=3$). u.q. indicates under quantification limits. iPRs, tZRP, and cZRP; ribotide precursors, iPR, tZR, and cZR; riboside precursors. **d**, **e** Genetic interaction between MpLOG and MpSMAXL in gemma cup formation. Thalli of WT, *Mplg-1*, *Mpsmx1-1*, *Mpsmx1-1/Mplg#2*, and *Mpsmx1-1/Mplg#7* plants grown on vermiculite (**d**). Yellow arrows indicate gemma cups. Scale bars: 1 cm. Quantification of gemma cup formation frequency (**e**). Bars represent mean $\pm$ SD ($n=4$). **f**, **g** Genetic interaction between MpLOG and MpSMAXL in gemma cup formation in the gemma number phenotype. Top view of day 14 gemma cups of WT, *Mplg-1*, *Mpsmx1-1*, *Mpsmx1-1/Mplg#2*, and *Mpsmx1-1/Mplg#7* grown on 1/2 B5 medium (**f**). Scale bars: 1 mm. Quantification of the number of gemmae per cup (**g**). Bars represent the mean $\pm$ SD ($n=5$). The Tukey's HSD test was used for multiple comparisons and statistical differences ($p<0.05$) are indicated by different letters in (**c**), (**e**) and (**f**).

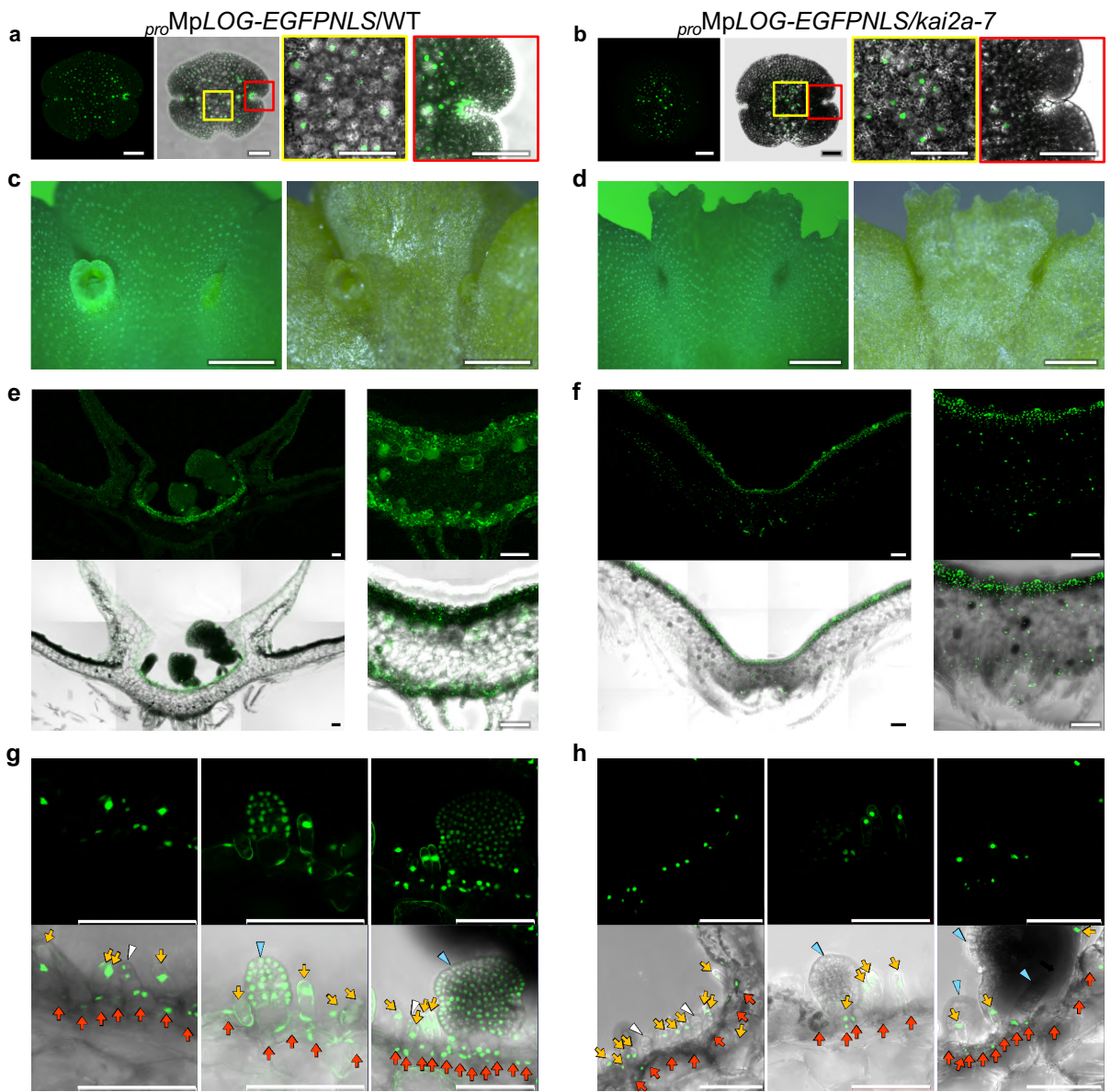


Figure 5. Control of MpLOG expression by KAI2-dependent signaling.

a, b Localization of EGFP fluorescence in the gemma of *proMpLOG-EGFPNLS/WT* (a), and *proMpLOG-EGFPNLS/kai2a-7* (b) grown on 1/2 B5 medium, observed by confocal laser scanning microscopy. From the left: EGFP fluorescence, EGFP merged with bright field image, enlargement of the region in yellow square. **c, d** Localization of EGFP fluorescence in the thallus of *proMpLOG-EGFPNLS/WT* (c), and *proMpLOG-EGFPNLS/kai2a-7* (d) grown on vermiculite, observed by stereo microscopy. Left: EGFP fluorescence, Right: bright field image. **e, f** Localization of EGFP fluorescence in the cross-sections of the midrib of *proMpLOG-EGFPNLS/WT* (e), and *proMpLOG-EGFPNLS/kai2a-7* (f) grown on vermiculite, observed by confocal laser scanning microscopy. Top panels: EGFP fluorescence, Bottom panels: EGFP merged with bright field image. **g, h** Localization of EGFP fluorescence in initiating gemmae, slime papilla, and gemma cup floor cells at the bottom of gemma cup in *proMpLOG-EGFPNLS/WT* (g), and *proMpLOG-EGFPNLS/kai2a-7* (h) grown on 1/2 B5 medium, observed by confocal laser scanning microscopy. White arrowheads: gemma initial cells, blue arrowheads: initiating gemmae, yellow arrows: slime papillae, red arrows: gemma cup floor cells. Scale bars: 100 μ m.

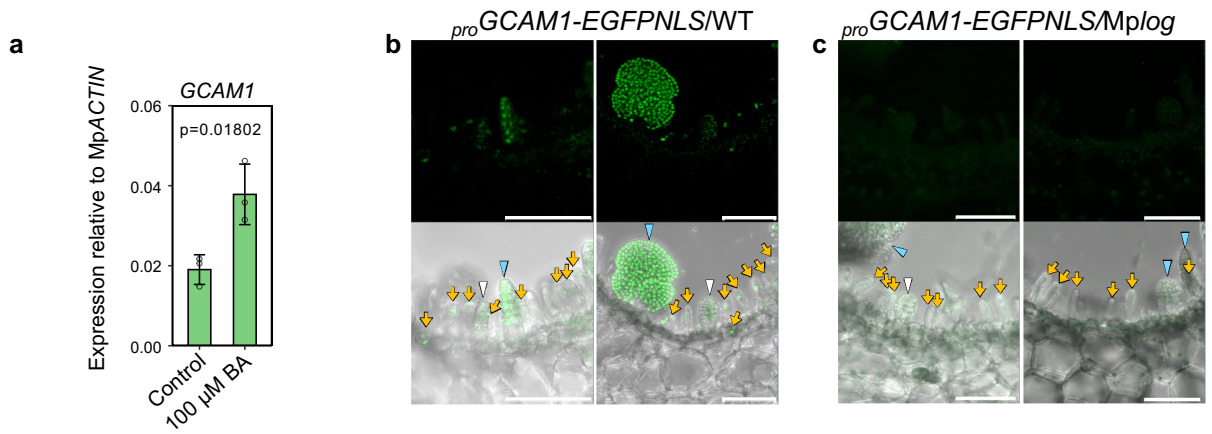


Figure 6. *GEMMA CUP-ASSOCIATED MYB1 (GCAM1)* expression is promoted by cytokinin synthesized by MpLOG.

a The induction of *GCAM* expression by cytokinin was examined using qPCR analysis. Expression levels relative to *MpACTIN* are shown. Bars represent means $\pm$ SD ($n=3$). The p values were calculated by using the Student's t -tests. **b, c** Localization of EGFP fluorescence in initiating gemmae at the bottom of gemma cup in *proGCAM1-EGFPNLS/WT* (**b**) and *proGCAM1-EGFPNLS/Mplog* (**c**) grown on 1/2 B5 medium and observed by confocal laser scanning microscopy. White arrowheads: gemma initial cells, blue arrowheads: initiating gemmae, yellow arrows: slime papillae. Scale bars: 100 μ m.

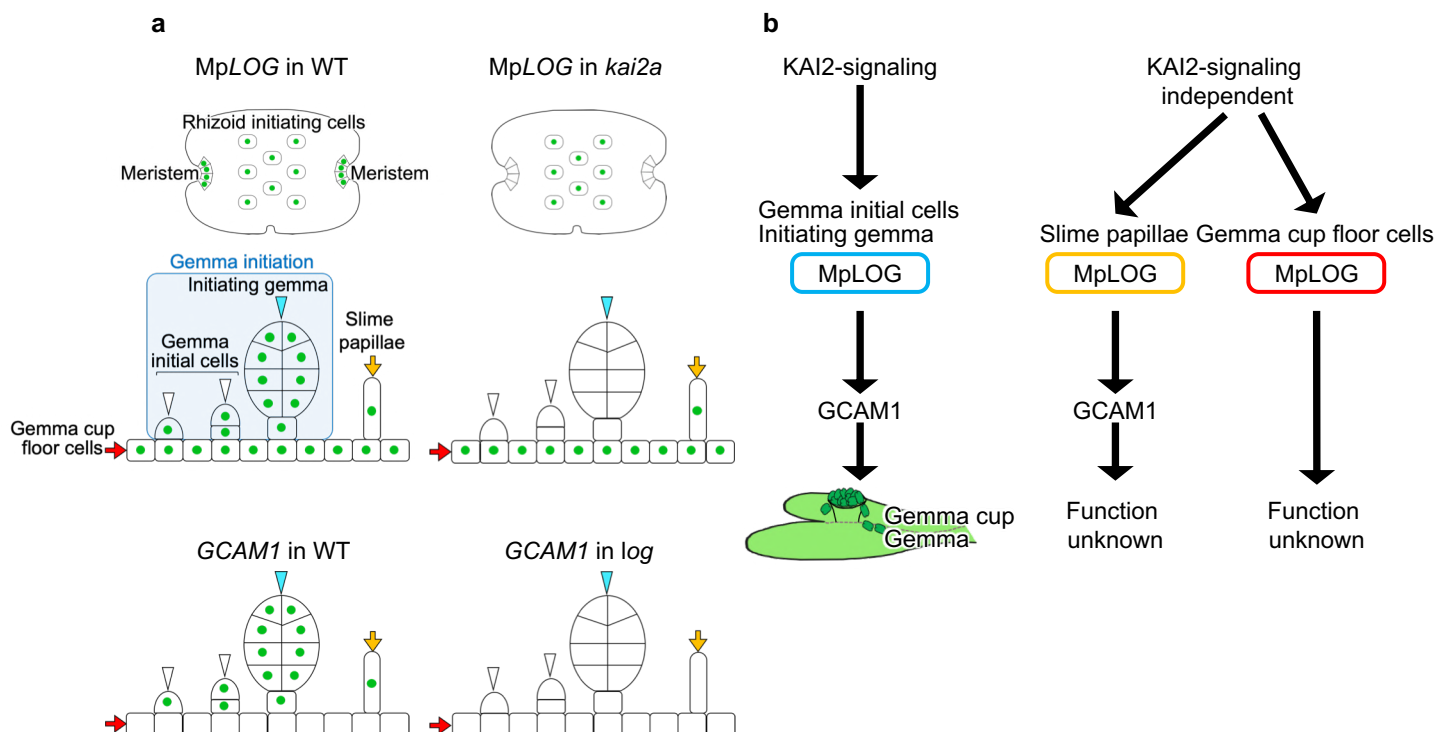
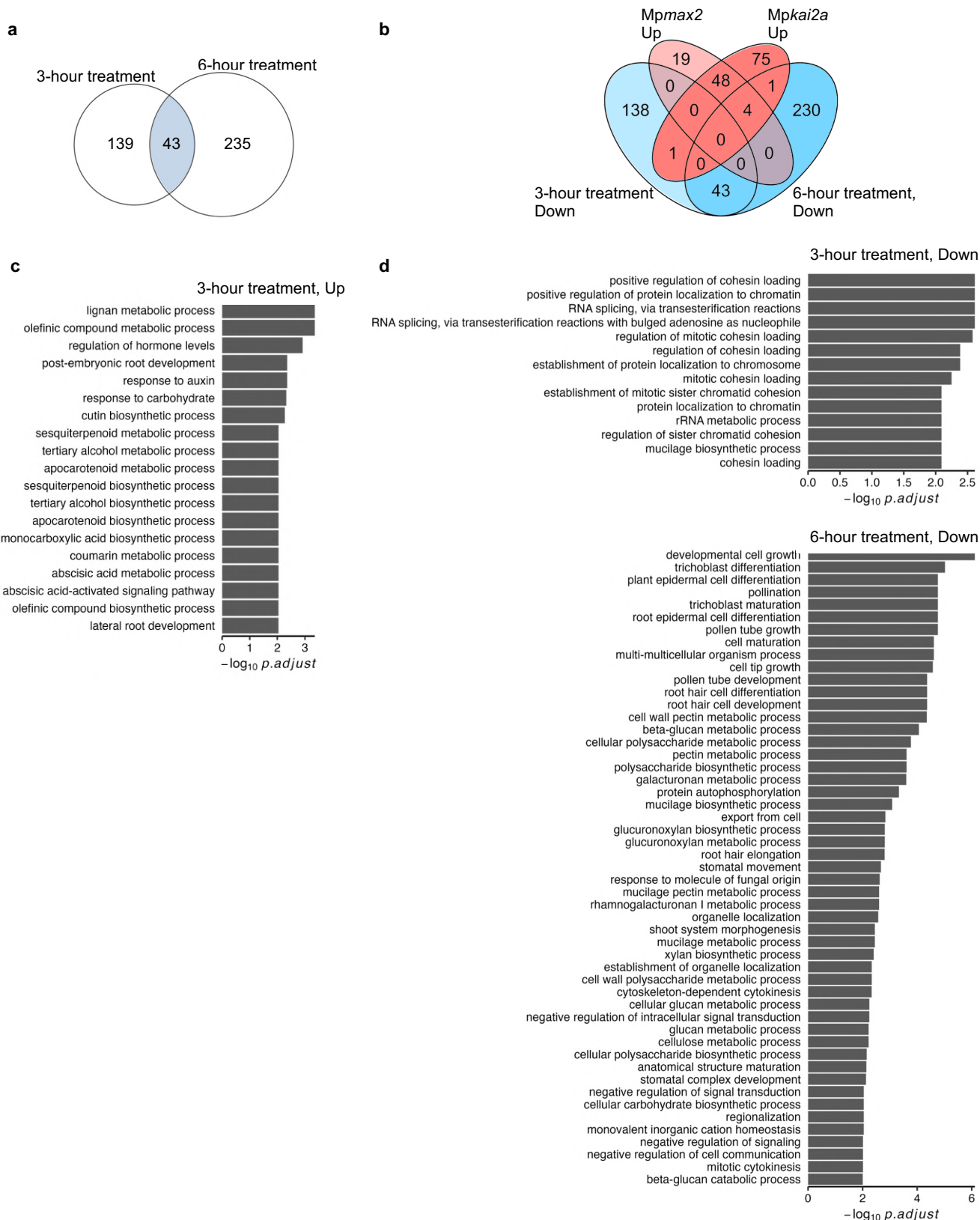


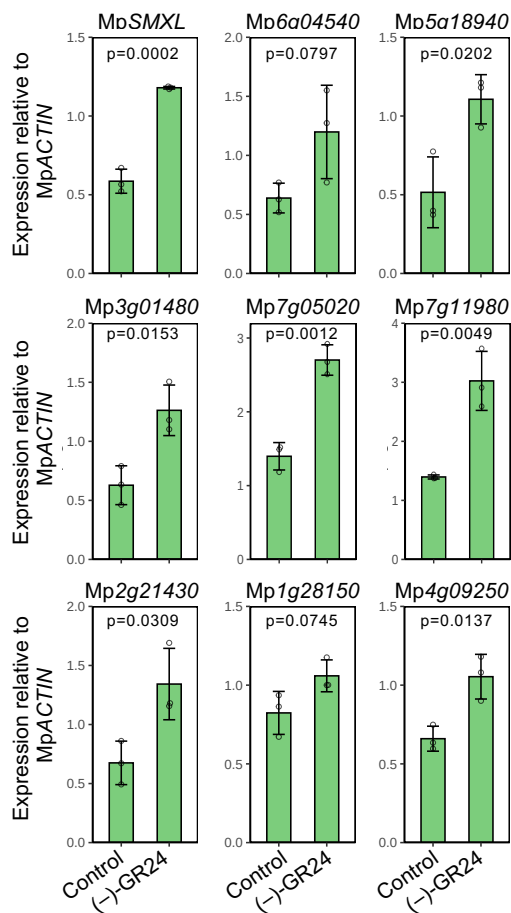
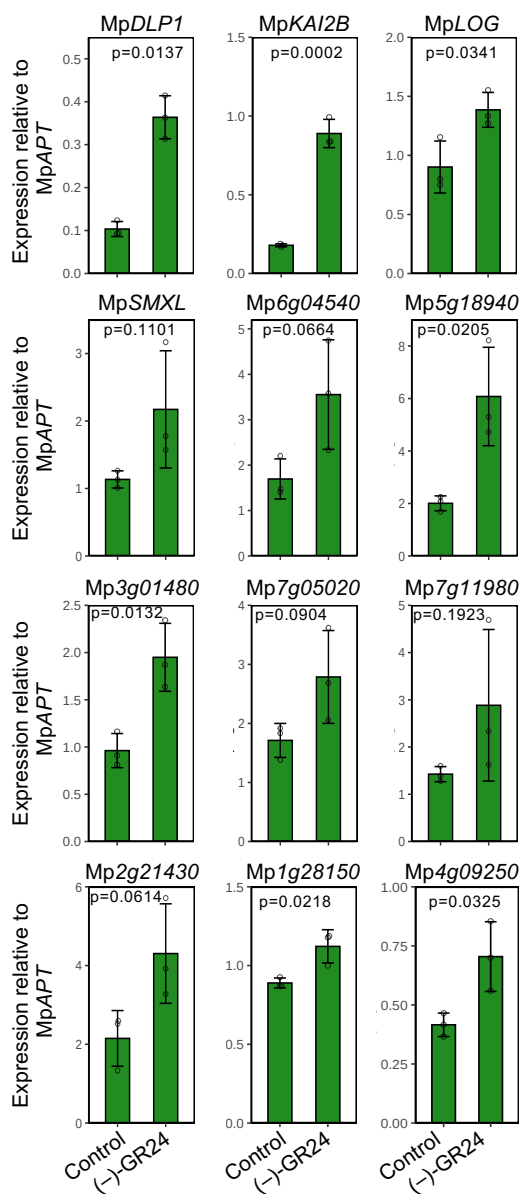
Figure 7. Model of cytokinin-mediated control of gemma cup and gemma formation by KAI2-dependent signaling in *M. polymorpha*.

a Schematic diagram of the MpLOG and GCAM1 expression in WT, *Mpkai2a*, and *Mplog* mutant plants. Green circles indicate EGFP fluorescence. White arrowheads: gemma initial cells, blue arrowheads: initiating gemmae, yellow arrows: slime papillae, red arrows: gemma cup floor cells. **b** The model for gemma cup and gemma formation by KAI2-dependent signaling. The activation of MpLOG expression by KAI2-dependent signaling in gemma initial cells and initiating gemmae leads to the induction of GCAM1 expression, resulting in gemma-cup and gemma formation. MpLOG expression in slime papillae and gemma cup floor cells is KAI2 signaling independent. MpLOG expression induces GCAM1 expression in slime papillae but not in gemma cup floor cells. The functional significance of GCAM1 in slime papillae and MpLOG in gemma cup floor cells is unknown.



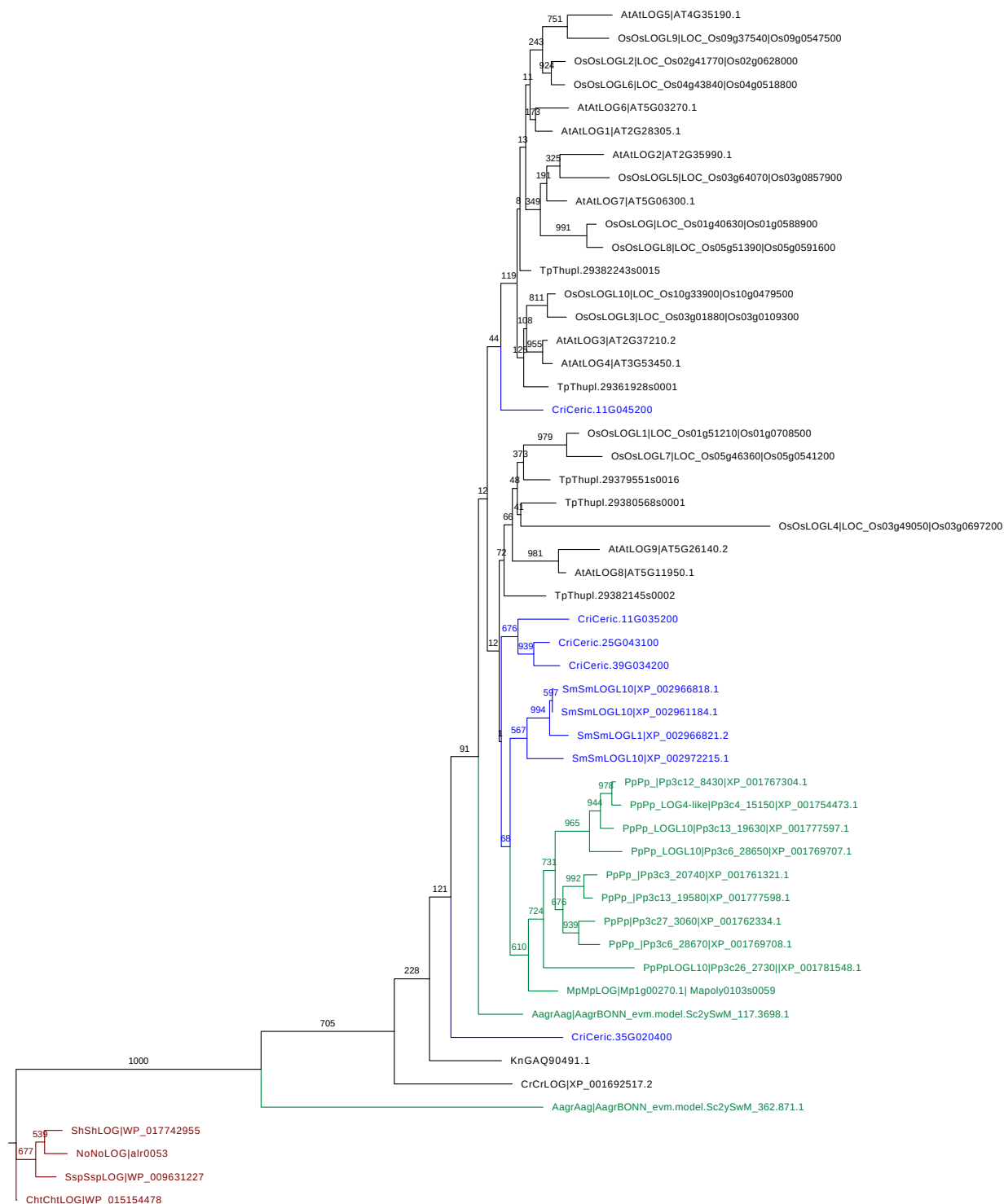
Supplementary Figure 1. RNA-seq analysis of *rac*-GR24-treated plants compared to control plants after 3 or 6 hours.

a Venn diagram showing the number of down-regulated genes after 1 μ M *rac*-GR24 treatment for 3 and 6 hours. **b** Venn diagram showing the number of genes down-regulated after 1 μ M *rac*-GR24 treatment, and up-regulated in *Mpmx2-2* and *Mpkai2a-1* mutants. **c** Gene ontology (GO) analysis of the up-regulated genes after 3 hours of treatment. **d** GO analyses of the down-regulated genes after 3 hours (top) and 6 hour of treatment (bottom).

a**b**

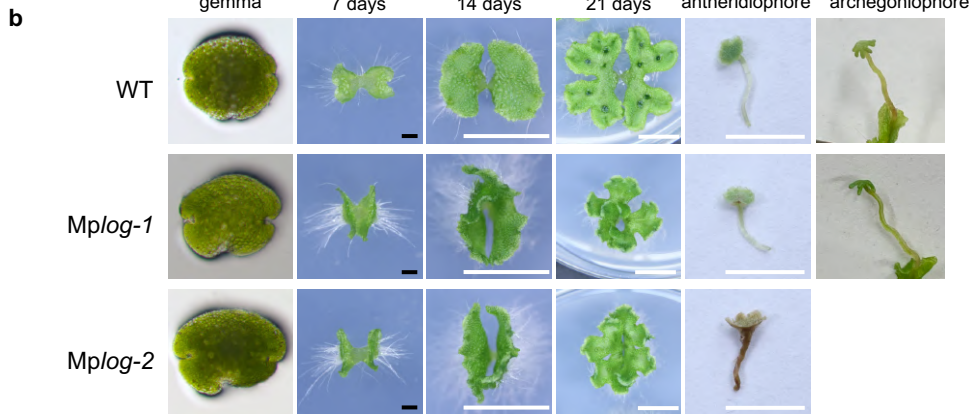
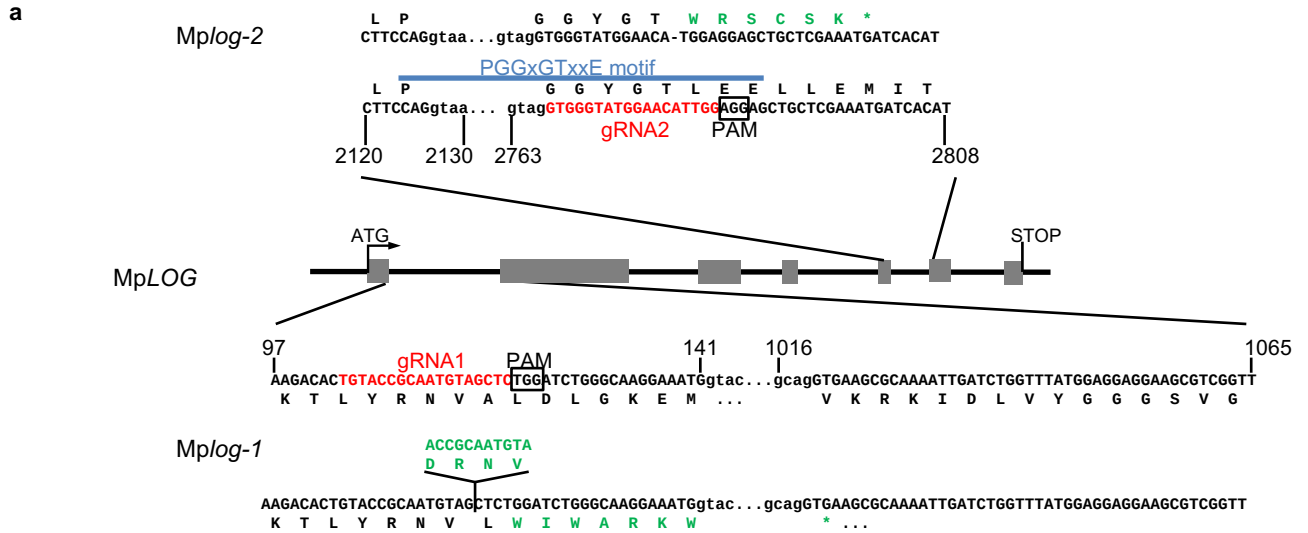
Supplementary Figure 2. Expression levels of genes controlled by KAI2-dependent signaling.

a Induction of 9 up-regulated genes by treatment with (-)-GR24 at 1 μ M for 6 hours examined by quantitative PCR (qPCR) analysis in WT. *MpACTIN* was used as a reference gene. **b** Induction of 12 up-regulated genes by treatment with (-)-GR24 at 1 μ M for 6 hours examined by quantitative PCR (qPCR) analysis in WT. *ADENINE PHOSPHORIBOSYL TRANSFERASE* gene (*MpAPT*) was used as a reference gene. The p values were calculated by using the Student's t-tests.



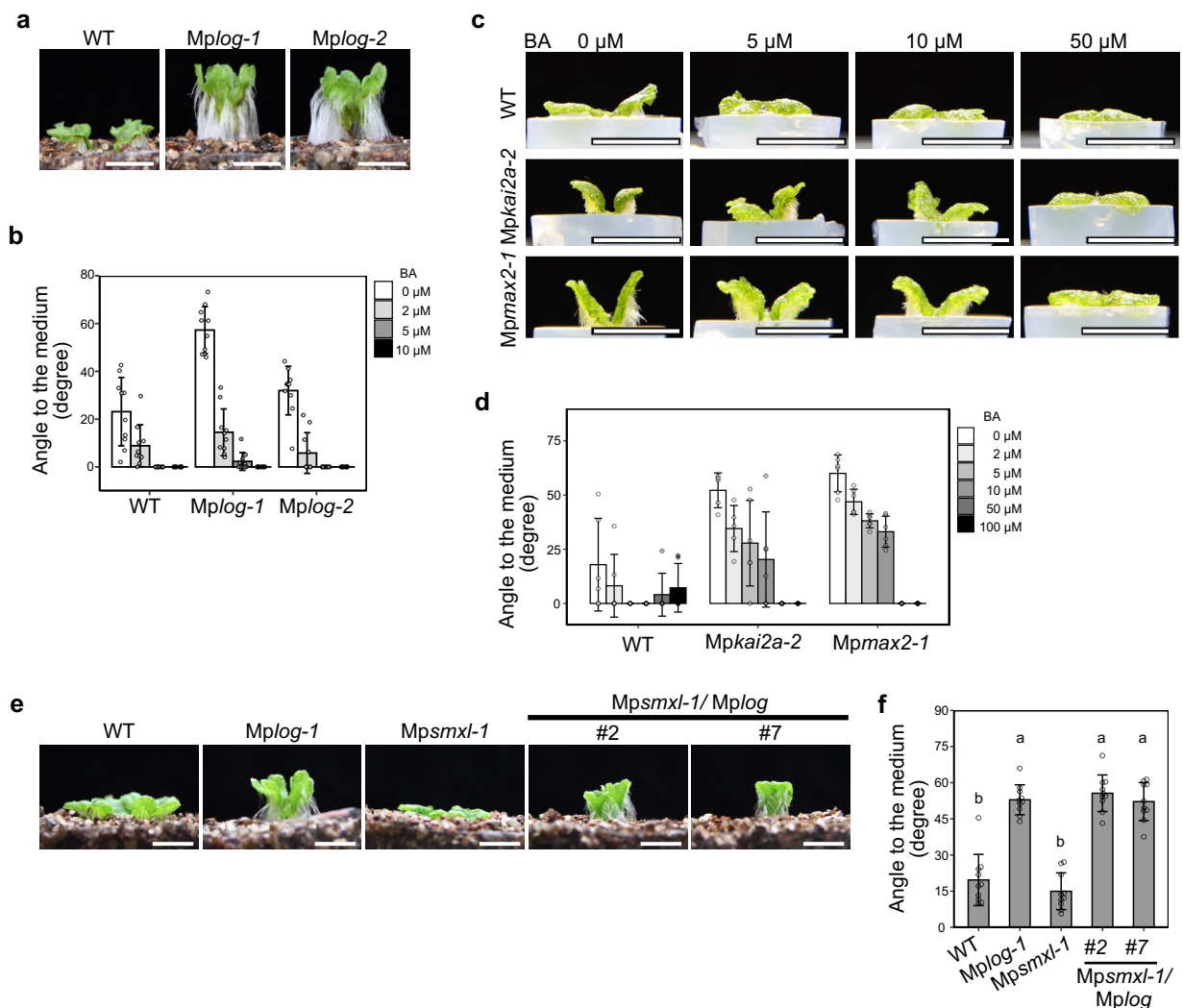
Supplementary Figure 3. Phylogenetic tree of LOG genes.

The phylogenetic analysis was performed using PhyML (<http://www.atgc-montpellier.fr/phyml/>), based on the maximum likelihood method with 1000 times bootstrap. Branches are colored according to plant lineages. Terminals are labeled with the abbreviations of the species name and gene IDs. Os, *Oryza sativa*; At, *Arabidopsis thaliana*; Cri, *Ceratopteris richardii*; Sm, *Selaginella moellendorffii*; Pp, *Physcomitrium patens*; Mp, *Marchantia polymorpha*; Aag, *Anthoceros agrestis*; Kn, *Klebsormidium nitens*; Cr, *Chlamydomonas reinhardtii*; Sh, *Scytonema hofmannii*; No, *Nostoc*; Ssp, *Synechocystis*; Cht, *Chroococcidiopsis*. The bootstrap values are indicated at the branch point.



Supplementary Figure 4. Production of *Mplog* loss of function mutants by CRISPR and their phenotypes.

a Structure of the *MpLOG* gene, the position of guide RNAs, PAM sequences, and resultant mutations are shown. The predicted amino acid sequences of the wild type and mutant LOG protein are shown in black and green letters, respectively. Asterisks indicate stop codons. **b** Morphological phenotypes of *Mplog* mutants, *Mplog-1*, *Mplog-2*. Thalli of *Mplog* mutants are smaller than those of WT and grow upwards. Sexual organs do not show obvious morphological defects and produce spores normally. Scale bars: 1 cm (white), 1 mm (black).

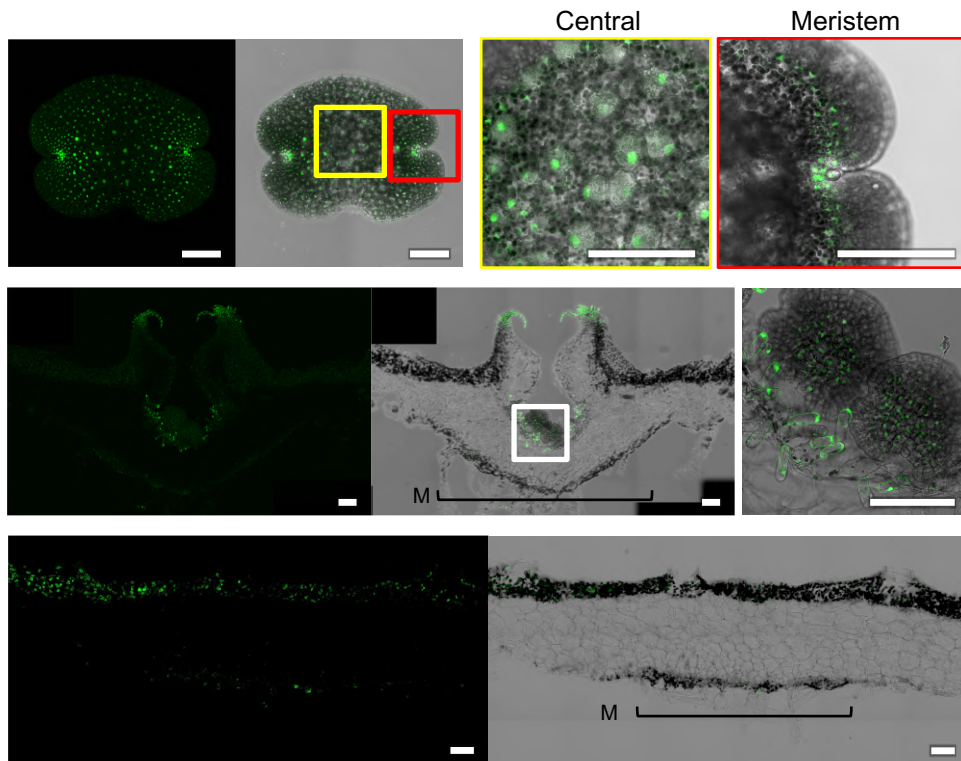


Supplementary Figure 5. Cytokinin positively controls the flat growth of thallus.

a Side view of 14-day-old thalli in WT, *Mplog-1*, and *Mplog-2* grown on vermiculite.

b Effect of cytokinin on thallus angle to horizontal in WT, *Mplog-1*, and *Mplog-2*. Plants were grown on 1/2 B5 medium containing 0, 2, 5, or 10 μ M 6-benzyl adenine (BA). Data are the mean $\pm$ SD (n=10). **c** Side view of 14-day-old thalli in WT, *kai2a-2*, and *Mpmax2-1*. Plants were grown on 1/2 B5 medium containing 0, 5, 10 or 50 μ M BA. **d** Effect of cytokinin on thallus angle to horizontal in WT, *Mpkai2a-2*, and *Mpmax2-1* grown on 1/2 B5 medium containing 0, 2, 5, 10, 50 or 100 μ M BA. Data are the mean $\pm$ SD (n=6).

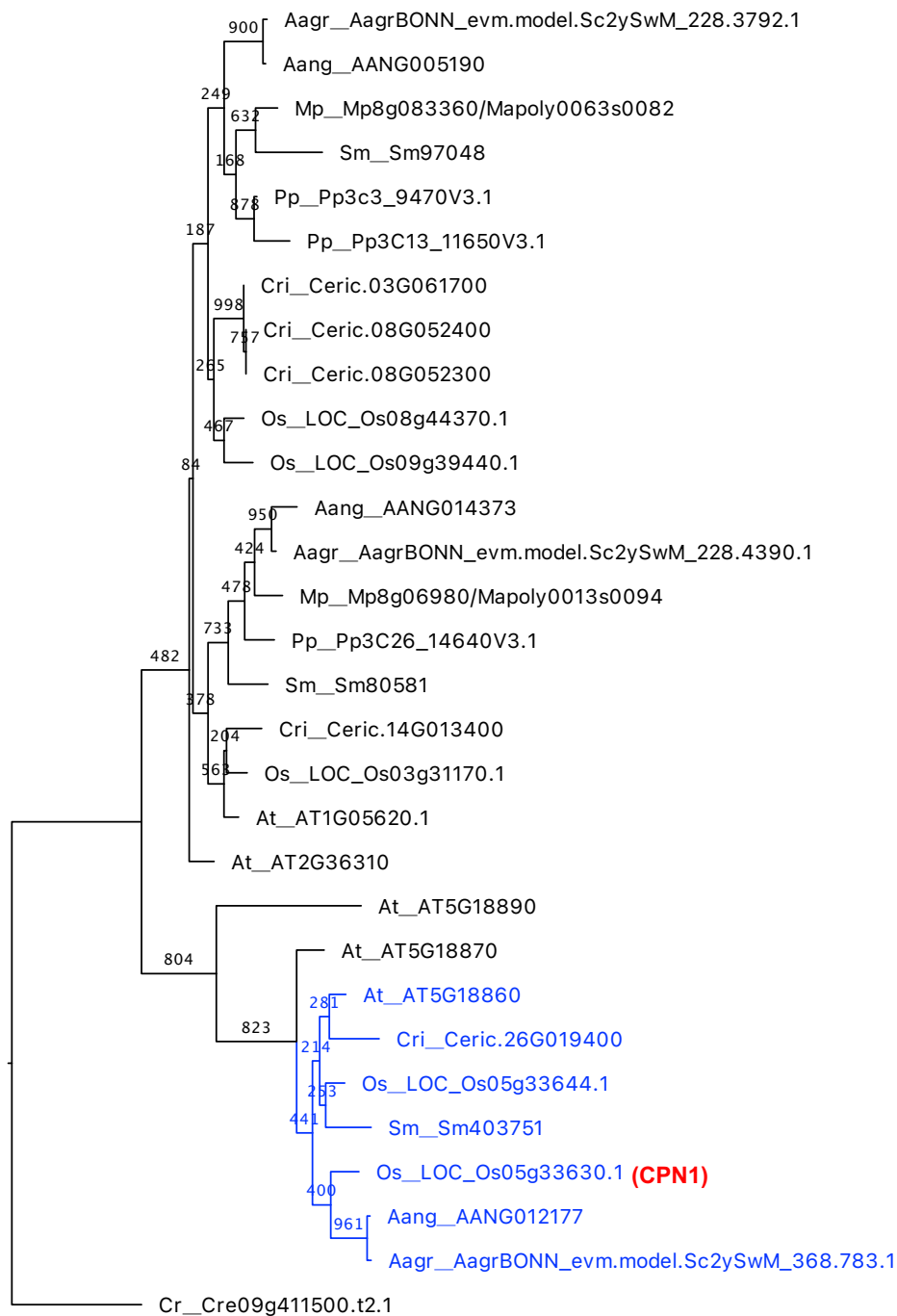
e Side view of thalli in WT, *Mplog-1*, *Mpsmxl-1*, *Mpsmxl-1/Mplog* #2, and *Mpsmxl-1/Mplog* #7 grown on vermiculite. **f** Angles between the 14-d-old thalli and the growth media in WT, *Mplog-1*, *Mpsmxl-1*, *Mpsmxl-1/Mplog* #2, and *Mpsmxl-1/Mplog* #7 grown on 1/2 B5 medium. The Tukey's HSD test was used for multiple comparisons and statistical differences (p<0.05) are indicated by different letters. Scale bars: 1 cm.



Supplementary Figure 6. The expression pattern of MpLOG.

Localization of EGFP fluorescence in gemma (top), in the cross-sections of the gemma cup (middle) and in the midrib (bottom) of *pro*MpLOG5kb-EGFPNLS containing the 5 kb region from the translation start codon. M: midrib region.

Scale bars: 100 μ m.



Supplementary Figure 7. Phylogenetic tree of CPN1 genes.

The phylogenetic analysis was performed using PhyML (<http://www.atgc-montpellier.fr/phyml/>), based on the maximum likelihood method with 1000 times bootstrap. Terminals are labeled with the abbreviations of the species name and transcript ID. Os, *Oryza sativa*; At, *Arabidopsis thaliana*; Cri, *Ceratopteris richardii*; Sm, *Selaginella moellendorffii*; Pp, *Physcomitrium patens*; Mp, *Marchantia polymorpha*; Agr, *Anthoceros agrestis*; Aang, *Anthoceros angustus*. Genes containing double tandem Ribo hydro-like domains are shown in blue. The bootstrap values are indicated at the branch point.

Supplementary Table 1. List of genes up-regulated in 3-hour and 6-hour treatment, and down-regulated in *Mpkai2a* and *Mpmax2*.

id		kai2a_ log2FC	kai2a_ padj	max2_ log2FC	max2_ padj	3h_ log2FC	3h_ padj	6h_ log2FC	6h_ padj	Gene symbol	At gene ID	At gene symbol	At gene Synonym
Mp5g09160	Mapoly 0095s0043	-7.07	1.32E-41	-7.08	1.16E-32	1.83	5.33E-06	2.69	5.45E-64	MpDLP1	AT3G23570	NA	NA
Mp2g04930	Mapoly 0031s0148	-4.79	1.27E-145	-6.11	3.30E-159	2.02	5.83E-83	2.24	7.37E-98	MpKAI2B	AT4G37470	KAI2	NA
Mp6g04540	Mapoly 0034s0062	-2.91	2.73E-12	-3.04	5.26E-13	1.03	1.58E-20	1.39	3.21E-42	NA	AT1G67480	NA	NA
Mp5g18940	Mapoly 0073s0049	-2.64	1.02E-26	-2.90	2.46E-26	0.65	3.29E-02	1.49	1.24E-08	MpMTPSL- like	AT3G45770	NA	NA
Mp3g01480	Mapoly 0007s0140	-1.97	1.49E-36	-2.25	5.97E-39	0.69	8.98E-11	0.75	1.58E-06		NA	NA	NA
Mp7g05020	Mapoly 0062s0024	-1.97	4.12E-06	-1.93	2.29E-05	1.16	5.09E-08	0.53	1.07E-02	MpEXL9	AT5G64260	EXL2	NA
Mp8g02280	Mapoly 0012s0025	-1.94	6.95E-09	-1.82	2.85E-09	0.64	3.94E-04	1.04	5.68E-13	NA	AT1G78860	NA	NA
Mp7g11980	Mapoly 0003s0211	-1.90	1.81E-13	-2.18	1.19E-16	0.60	3.62E-04	0.65	5.55E-04	MpIAMT1	AT5G55250	IAMT1	AtIAMT1
Mp2g21430	Mapoly 0040s0071	-1.67	3.91E-15	-2.28	1.10E-23	0.86	6.97E-10	0.80	2.54E-07	MpABCB9	AT3G28345	ABCB15	MDR13, multi-drug resistance 13
Mp3g06310	Mapoly 0006s0101	-1.58	1.76E-28	-2.06	1.16E-32	0.75	2.90E-21	0.71	5.16E-15	MpSMXL	AT5G57130	NA	SMXL5, SMAX1-like 5
Mp1g28150	Mapoly 0002s0063	-1.37	1.75E-02	-1.15	5.07E-02	0.69	2.66E-02	0.68	2.78E-03	MpOMT4	AT5G54160	OMT1	OMT3, COMT1, caffeaate O- methyltransferase 1, O- methyltransferase 1, O- methyltransferase 1, O- methyltransferase 3
Mp1g00270	Mapoly 0103s0059	-1.27	1.13E-19	-1.04	2.15E-10	0.71	6.16E-11	0.75	3.16E-07	MpLOG	AT2G37210	LOG3	NA
Mp4g09250	Mapoly 0112s0025	-1.26	2.29E-12	-1.60	5.04E-20	0.73	1.58E-04	1.13	4.46E-13	MpCupin57	AT1G02335	GL22	PLASMODESMAL GERMIN- LIKE PROTEIN 2, PDGLP2

Supplementary Table 2. Production of CRISPR mutants.

Allele	Gene	gRNA sequence	Mutatuin site	Amino acid
<i>Mplog-1</i>	<i>MpLOG</i>	TGTACCGCAATGTAGCTC	g.118_119insACCGCAAT GTA	p.V39fs52X
<i>Mplog-2</i>	<i>MpLOG</i>	GTGGGTATGGAACATTGG	g.2781del	p.T128fs135X
<i>proMpLOG-EGFPNLS / Mpkai2a-7</i>	<i>MpKAI2A</i>	GGAGATCAGCTCGTGGTACT	g.59del	p.V20fs21X
<i>Mpsmxl-1 /Mplog #2</i>	<i>MpLOG</i>	TGTACCGCAATGTAGCTC	g.118_119insTACATTGCG GTACAGTGTCCAGTTGA TCCAAT	p.V39fs48X
<i>Mpsmxl-1 /Mplog #7</i>	<i>MpLOG</i>	TGTACCGCAATGTAGCTC	g.118del	p.V39fs48X
<i>proGCAM1-EGFPNLS / Mplog</i>	<i>MpLOG</i>	TGTACCGCAATGTAGCTC	g.118_119insA	p.V39fs53X

※ Notations were followed den Dunnen JT and Antonarakis SE (2000). Hum.mutat. 15:7-12

Supplementary Table 3. Primers used in this study.

Name	Sequence (5'→3')	Usage
MpACT-qPCR-F	AGGCATCTGGTATCCACGAG	qRT-PCR control
MpACT-qPCR-R	ACATGGTCGTTCTCCTCCAGAC	qRT-PCR control
MpKAI2B-qPCR-F	CTGGCATCTCTGGAAAGACC	qRT-PCR
MpKAI2B-qPCR-R	GCATTGCCTCAAACAACTGA	qRT-PCR
MpDLP1-qPCR-F	GGTGTGAAGAAAGTTGGAGTTATGG	qRT-PCR
MpDLP1-qPCR-R	GTGTGAGGAATGAGGGATGGTT	qRT-PCR
MpLOG-qPCR-F	GCACGTGAGCTCCTCGAGAA	qRT-PCR
MpLOG-qPCR-R	CATCTCCCCGTGCATGTCCA	qRT-PCR
Mp6g04540-qPCR-F1	GAGTCGAGATTTAATGGTGTGAATG	qRT-PCR
Mp6g04540-qPCR-R1	CCCAGAAAGAGAAGAGAAGAGAAGA	qRT-PCR
Mp5g18940-qPCR-F1	TGCGTGTATAAGAGCGATCAGAG	qRT-PCR
Mp5g18940-qPCR-R1	GTGATGTATGGCGGTGAGGA	qRT-PCR
Mp3g01480-qPCR-F1	CGAGACTGGCAGCAAGAGAG	qRT-PCR
Mp3g01480-qPCR-R1	GGAAGACCCCGTAGACAAAGATAG	qRT-PCR
Mp7g05020-qPCR-4F	ATTACTCCCGAGGCAAGGCG	qRT-PCR
Mp7g05020-qPCR-4R	ACTCGTTCCGGCAGAAGTCC	qRT-PCR
Mp7g11980-qPCR-4F	TGCCAATCCGTTACGAAGC	qRT-PCR
Mp7g11980-qPCR-4R	AGGCTGCGACAGATTTTGGC	qRT-PCR
Mp2g21430-qPCR-1F	GGCATGCTCCACAACGATCG	qRT-PCR
Mp2g21430-qPCR-1R	ATTCTCCGAGCCCAGAACG	qRT-PCR
MpSMXL-qPCR4_F	TGGGATGTCAGGTCGGAAC	qRT-PCR
MpSMXL-qPCR4_R	AAAAGTTCTGCTGAGCTGCG	qRT-PCR
Mp1g28150-qPCR-4F	TGGCAAGCTCCTCAACCTCG	qRT-PCR
Mp1g28150-qPCR-4R	AAGGAGGTCTTGGTTGCGGG	qRT-PCR
Mp4g09250-qPCR-1F	AACTCCCCGCTCTCACAGG	qRT-PCR
Mp4g09250-qPCR-1R	CCACATCGACGCATCCTTGC	qRT-PCR
MpAPT-qPCR-F	CGAAAGCCCAAGAAGCTACC	qRT-PCR control
MpAPT-qPCR-R	GTACCCCCGGTTGCAATAAG	qRT-PCR control
MpLOG-gRNA1-F	ctcgTGTAACGCAATGTAGCTC	Construction of the genome editing vector for <i>Mplog-1</i> , <i>Mpsmxl-1</i> / <i>Mplog #2</i> , <i>#7</i> , <i>proGCAM1-EGFPNLS</i> / <i>Mplog</i>
MpLOG-gRNA1-R	aaacGAGCTACATTGCGGTACA	Construction of the genome editing vector for <i>Mplog1</i> , <i>Mpsmxl-1</i> / <i>Mplog #2</i> , <i>#7</i> , <i>proGCAM1-EGFPNLS</i> / <i>Mplog</i>
MpLOG-gRNA2-F	ctcgGTGGGTATGGAACATTGG	Construction of the genome editing vector for <i>Mplog-2</i>
MpLOG-gRNA2-R	aaacCCAATGTTCCATACCCAC	Construction of the genome editing vector for <i>Mplog-2</i>
MpLOG-genotype-F1	GAGGTGGAAGAGGAGGAGGA	For checking of the edited site for <i>Mplog-1</i> , <i>Mpsmxl-1</i> / <i>Mplog #2</i> , <i>#7</i> , <i>proGCAM1-EGFPNLS</i> / <i>Mplog</i>
MpLOG-genotype-R1	CTCCCTTATCCACCGACTGC	For checking of the edited site for <i>Mplog-1</i> , <i>Mpsmxl-1</i> / <i>Mplog #2</i> , <i>#7</i> , <i>proGCAM1-EGFPNLS</i> / <i>Mplog</i>
MpLOG-genotype-F2	GCTTTCATAGCGCTTCCAGG	For checking of the edited site for <i>Mplog-2</i>
MpLOG-genotype-R2	GTCTAGTCTACACGCGCACC	For checking of the edited site for <i>Mplog-2</i>
proMpLOG_5kb-F	caccCACGCGACGTACTAGCATCT	Construction of <i>proMpLOG5kb-EGFPNLS</i>
proMpLOG_1kb-F	caccCAGCAGAGGACGGGGGAG	Construction of <i>proMpLOG-EGFPNLS</i>
proMpLOG_R	GAAATCTACAGAGACTTGCCGG	Construction of the genome editing vector for <i>proMpLOG5kb-EGFPNLS</i> and <i>proMpLOG-EGFPNLS</i>
MpKAI2Agenotypefor	CCTGCTTGGCTGGAATGACGAGACGGTGTT	For checking of the edited site for <i>MpKAI2A</i>
MpKAI2Agenotype2rev	GAGATAAGGCCTCTCGATAGATGCAAGGC	For checking of the edited site for <i>MpKAI2A</i>

Supplementary Table 4. Commonly up-regulated DEGs at 3 or 6 hours treatments.

id	up	3h_log2FC	3h_baseMean	3h_padj	6h_log2FC	6h_baseMean	6h_padj
Mp1g00270	3h & 6h	0.71199529	1256.64508	6.16E-11	0.75342512	1721.00723	3.16E-07
Mp1g05220	3h & 6h	0.32430663	457.77033	0.04779049	0.26343079	676.94094	0.00219654
Mp1g06800	3h & 6h	0.44440401	580.588506	0.01521182	0.41205929	1095.12956	2.87E-05
Mp1g09420	3h & 6h	0.55068758	65.1325424	0.03246633	0.47329122	109.101509	0.01050891
Mp1g12030	3h & 6h	0.20795894	1323.42039	0.0124496	0.21626408	2287.16219	0.01576324
Mp1g14990	3h & 6h	0.37467467	849.265843	0.00561349	0.29408657	1176.74244	0.00330979
Mp1g16800	3h & 6h	0.24529377	933.39775	0.00375365	0.38153065	1206.00578	2.12E-14
Mp1g19760	3h & 6h	0.42194621	709.881082	1.34E-06	0.28379791	1011.91202	0.00096514
Mp1g20260	3h & 6h	0.57442506	614.044222	0.00196296	0.41296514	1263.58154	0.00088551
Mp1g20860	3h & 6h	0.55025425	107.994477	0.00192375	0.65203389	181.946758	7.85E-13
Mp1g23210	3h & 6h	0.43255343	1607.15253	0.00065748	0.52256502	1473.46927	0.00010732
Mp1g23650	3h & 6h	0.38805047	2068.35951	0.00022488	0.42834882	3058.86667	3.43E-07
Mp1g26440	3h & 6h	0.29896178	2106.2672	0.02444225	0.27502534	2974.70928	0.00286682
Mp1g27950	3h & 6h	1.36343192	63.3525101	7.87E-12	1.05034731	87.9358105	5.99E-15
Mp1g27970	3h & 6h	0.68183383	34.0187216	0.02049996	0.60769779	52.6973643	0.00314307
Mp1g28150	3h & 6h	0.69169619	129.871274	0.02656506	0.68122185	187.161874	0.00277994
Mp2g04920	3h & 6h	0.6525162	238.402617	0.00786532	0.66689265	452.590131	5.61E-12
Mp2g04930	3h & 6h	2.02145468	2539.26241	5.83E-83	2.24479205	5023.76135	7.37E-98
Mp2g08260	3h & 6h	0.90443304	42.2678393	8.58E-05	1.18137378	50.81393	4.57E-10
Mp2g11820	3h & 6h	0.36774641	267.245838	0.04785183	0.40397554	340.732918	0.00918403
Mp2g11860	3h & 6h	0.20642892	2943.97609	0.03334266	0.20173009	3929.04504	0.00082924
Mp2g12920	3h & 6h	0.38251776	3617.3099	0.00718084	0.20021153	5611.11669	0.02705655
Mp2g20430	3h & 6h	1.55173848	1005.92128	0.00076099	1.19034491	1372.49661	5.57E-08
Mp2g21430	3h & 6h	0.86070609	754.995099	6.97E-10	0.80044571	1095.74232	2.54E-07
Mp2g23680	3h & 6h	0.7005815	1358.28972	1.06E-11	0.49269332	2199.47654	0.00456028
Mp3g01480	3h & 6h	0.69310861	302.597866	8.98E-11	0.74735789	829.597279	1.58E-06
Mp3g06310	3h & 6h	0.7513378	1835.89488	2.90E-21	0.71411366	2625.65948	5.16E-15
Mp3g10080	3h & 6h	0.69077845	269.948286	3.11E-13	0.64467314	445.111716	9.14E-12
Mp3g12930	3h & 6h	0.65882645	463.191219	0.00223322	0.46602591	802.369413	1.14E-07
Mp3g14120	3h & 6h	0.70885018	245.739077	1.13E-08	0.68373982	320.316212	2.30E-08
Mp3g16340	3h & 6h	0.66042476	1675.97226	0.00555718	0.43684369	3259.77295	0.00808643
Mp3g18590	3h & 6h	0.42627975	361.854958	0.00409009	0.49897105	577.383823	0.01006989
Mp4g04360	3h & 6h	0.36127223	1043.91776	1.75E-11	0.22069807	1708.0943	9.26E-05
Mp4g07180	3h & 6h	0.6186193	291.765832	0.00284636	0.47844873	368.729709	0.0031432
Mp4g09250	3h & 6h	0.73163317	341.044842	0.00015778	1.13246099	895.642026	4.46E-13
Mp4g10620	3h & 6h	1.22760428	63.8126992	1.17E-11	0.92980761	111.798411	3.21E-09
Mp4g15090	3h & 6h	0.74310198	352.225917	9.11E-17	0.59018248	566.340487	1.75E-10
Mp4g17760	3h & 6h	0.34905429	474.375869	0.00081921	0.28902801	707.456109	0.03357311
Mp4g23700	3h & 6h	0.80511059	2407.1577	0.00178954	0.42409764	3428.18882	0.01006989
Mp5g02800	3h & 6h	0.91009912	352.702567	8.48E-05	0.7665525	470.564821	2.58E-12
Mp5g09160	3h & 6h	1.83276982	2228.24644	5.33E-06	2.69106213	3907.35782	5.45E-64
Mp5g13870	3h & 6h	0.74615607	5707.83333	0.00231483	1.2853932	9607.16369	8.41E-13
Mp5g18940	3h & 6h	0.65203153	271.800019	0.03285904	1.49107755	307.841107	1.24E-08
Mp5g21020	3h & 6h	0.37349604	452.418094	0.03697847	0.35520359	614.011007	0.00669434
Mp5g24030	3h & 6h	0.29005312	321.017017	0.00629628	0.59631715	537.816535	1.18E-06
Mp6g04540	3h & 6h	1.02928114	127.872924	1.58E-20	1.39009291	244.328557	3.21E-42
Mp6g05000	3h & 6h	0.51241285	5270.17741	0.00030862	0.48788794	9136.97942	0.00015082
Mp6g09290	3h & 6h	0.72953826	2227.06827	6.01E-26	0.87299305	4632.55725	5.03E-20
Mp6g09300	3h & 6h	1.79497269	557.666424	1.00E-43	1.83902871	773.837138	1.16E-51
Mp6g10520	3h & 6h	1.15679446	1325.19115	2.31E-06	0.67450752	2166.74575	1.93E-07
Mp6g16970	3h & 6h	0.98569918	36.4469729	0.00014177	0.62057366	92.7364342	0.01832329
Mp6g17270	3h & 6h	0.46802243	718.693599	9.86E-05	0.33072751	1188.18874	4.00E-05
Mp7g00770	3h & 6h	0.46670705	552.655686	0.00024196	0.44415537	527.834293	0.00430972
Mp7g02090	3h & 6h	0.26584056	445.00001	0.00115205	0.36544247	796.678807	8.90E-09
Mp7g03280	3h & 6h	0.15017403	6658.39585	0.00106976	0.1557989	9666.96612	0.00078739
Mp7g05020	3h & 6h	1.15624989	224.312854	5.09E-08	0.52585241	396.322755	0.0107156
Mp7g05040	3h & 6h	0.38193268	1002.42753	0.00010909	0.26556569	1351.89649	0.00250252
Mp7g06170	3h & 6h	0.41777761	1539.04423	0.03460412	0.32471072	2778.47281	0.01757562
Mp7g06960	3h & 6h	0.24023941	611.2978	0.01946749	0.25942147	965.651725	1.79E-06
Mp7g10170	3h & 6h	0.71906914	433.444427	0.00029243	0.33534545	601.181451	0.00411053
Mp7g11980	3h & 6h	0.59849091	244.782877	0.00036222	0.65215072	519.034127	0.00055537
Mp7g12790	3h & 6h	0.47675327	643.597742	4.49E-05	0.56346513	1500.6234	1.19E-09
Mp7g15820	3h & 6h	0.45370888	211.36092	0.00099343	0.31242873	233.252618	0.03668743
Mp7g17880	3h & 6h	0.63342812	322.633469	0.00136036	0.5163001	458.748319	0.03219593
Mp7g19700	3h & 6h	0.29593891	300.868389	0.02560661	0.29757352	632.310843	0.00038149
Mp8g02280	3h & 6h	0.64121613	138.831002	0.00039438	1.03876754	134.832233	5.68E-13
Mp8g02500	3h & 6h	0.6556798	61.5528148	3.66E-05	0.37690322	115.295472	0.0259067
Mp8g04780	3h & 6h	0.46581611	2993.91415	5.87E-05	0.46257829	3181.2575	9.68E-23
Mp8g04910	3h & 6h	0.31366805	523.976168	2.80E-05	0.48308021	895.365066	4.55E-08
Mp8g04950	3h & 6h	0.30984732	706.877142	0.00254352	0.25439954	1235.69319	0.00063011
Mp8g06260	3h & 6h	0.56969138	163.541687	0.00784358	0.78941303	292.470683	2.48E-13
Mp8g06760	3h & 6h	0.57411042	230.303056	0.00118944	0.34436101	338.2014	0.04759426
Mp8g08780	3h & 6h	0.45675556	356.245186	0.00480768	0.42412736	482.935267	7.83E-10
Mp8g10420	3h & 6h	0.24221534	369.267014	0.0143674	0.25348626	532.024784	0.00087073
Mp8g14310	3h & 6h	0.18747246	668.221672	0.02535938	0.20937797	1224.15478	0.04056111
Mp8g19020	3h & 6h	0.56776246	488.970528	8.13E-07	0.60704447	552.199446	1.53E-08