Title

An evolutionarily conserved mechanism for production of secondary meristems in land plants

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27 **Abstract (145/150 words)**

28 A variety of plants in diverse taxa can reproduce asexually via vegetative propagation, in
 29 which clonal propagules with new meristem(s) are generated directly from vegetative
 30 organs. A basal land plant, *Marchantia polymorpha*, develops clonal propagules, gemmae,
 31 in a specialized receptacle, gemma cup. Here we report an R2R3-MYB transcription factor,
 32 designated GEMMA CUP-ASSOCIATED MYB 1 (GCAM1), which is an essential
 33 regulator of gemma cup development in *M. polymorpha*. Although gemma cups are a
 34 characteristic gametophyte organ for vegetative reproduction in a taxonomically restricted
 35 group of liverwort species, phylogenetic and interspecific complementation analyses
 36 supported the orthologous relationship of GCAM1 to regulatory factors for axillary
 37 meristem formation, e.g. Arabidopsis RAXs and tomato Blind, in angiosperm sporophytes.
 38 The present findings in *M. polymorpha* suggest an ancient acquisition of a regulatory
 39 mechanism for production of secondary meristems, and the use of the mechanism for
 40 diverse developmental programs during land plant evolution.

41

42

43 **Introduction**

44 The plastic nature of plant architecture can be traced back to the activity of meristems,
 45 which are pools of pluripotent cells located at the tips of growing plant bodies. In seed
 46 plants, the primary shoot apical meristem (SAM) is established during embryonic
 47 development. Following germination, secondary meristems are continuously produced in
 48 the axils of leaves, and form the basis of branching in flowering plants. Many plant species
 49 also develop ectopic meristems at different positions of the plant body^{1,2}.

50 Vegetative reproduction, a form of asexual reproduction in plants, is a
 51 developmental process by which clonal progeny arise directly from parental tissues. This
 52 process is based on the remarkable potential of plants to proliferate meristems, which
 53 develop whole plantlets from differentiated tissues^{1,3,4}. Diverse plants have the capability
 54 for vegetative reproduction, which occurs naturally from a variety of vegetative tissues, e.g.
 55 stems (rhizomes: asparagus, iris, ginger, bulbs: onion, tubers: potato, corms: crocus, and
 56 runners: strawberry, wild mint), roots (sweet potato), and leaves (*Kalanchoe*), or can be
 57 induced artificially by means of various techniques, including cuttings, grafting, and tissue
 58 culture. Vegetative reproduction is considered to be an important strategy for efficient plant

propagation and survival in the natural environment⁵, as well as for agriculture and horticulture⁶.

The liverwort, *Marchantia polymorpha* L., as well as certain related species in the class Marchantiopsida, develops specialized organs for vegetative reproduction, termed the gemma cup or cupule⁷. Clonal propagules, called gemmae, develop from single epidermal cells at the base, or floor, of gemma cups. Thus, the floor cells have the capability to produce clonal plantlets. Gemma cups are formed periodically along the dorsal midrib of a thallus. The restricted occurrence of gemma cups along the midrib suggests that they originate from the dorsal merophyte above the apical cell⁷. Over 100 gemmae can develop in a single gemma cup. The development of gemma cups has been well described on the basis of a series of histological observations on *M. polymorpha*⁸. According to the detailed observations by Barnes and Land⁸, the precursors of the floor epidermis in a gemma cup are recognizable as close as the third cell back from the apical cell on the dorsal surface. The floor epidermis is a region where periclinal cell divisions to generate protodermal and subprotodermal cell layers of the air chamber are suppressed, and consecutive elongate epidermal cells are instead observed. The single-layered epidermal cells comprise the gemminiferous region, an area that will become the basal epidermis of a gemma cup. These cells undergo repeated anticlinal divisions to enlarge the area of the gemma cup floor, allowing for increase proliferation of gemma production. Some gemma cup floor cells begin to develop gemmae from an early stage of gemma cup development, and growth of the gemma cup occurs concomitantly with gemma development and maturation (Supplementary Figure 1). The frequency of gemma cup formation is influenced by a variety of environmental factors, such as light and nutrients^{9,10,11}. However, the molecular mechanisms underlying gemma and gemma cup development are largely unknown.

Marchantia polymorpha is a member of an early diverging lineage among land plants. The species offers a number of advantages for genetic studies: low genetic redundancy¹², a haploid-dominant life cycle, and practicable genetic tools¹³, such as high-efficiency transformation^{14,15} and gene modification techniques^{16,17,18,19}. Recent molecular genetic studies on bryophytes, including *M. polymorpha*, have revealed several key developmental regulators for the gametophyte generation that have orthologous counterparts in angiosperms shown to control analogous, but not homologous, developmental processes in the sporophyte generation^{20,21,22,23,24,25}. These observations

suggest that a considerable number of developmental regulatory modules were acquired in the common ancestor of land plants before the divergence of bryophytes and vascular plants^{26,27}.

In the present study, we undertook a comprehensive transcriptome analysis and isolated a R2R3-MYB transcription factor gene, *GCAM1*, which was predominantly upregulated in developing *M. polymorpha* gemma cups. Molecular and genetic characterization revealed a critical role for GCAM1 in gemma cup development. Cell proliferation without tissue differentiation was observed in plants ectopically expressing *GCAM1*. Although the gemma cup is a characteristic gametophytic organ for vegetative reproduction in certain species of Marchantiopsida, phylogenetic and interspecific complementation analyses supported the orthologous relationship of *GCAM1* with angiosperm genes of the R2R3-MYB subfamily 14²⁸, which are involved in axillary meristem formation. Potential mechanisms shared between vegetative reproduction in the gametophyte of liverworts and axillary meristem formation in the sporophyte of angiosperms are discussed.

Results

An R2R3-MYB transcription factor is upregulated in the gemma cup

To screen for potential key regulators involved in vegetative reproduction in *M. polymorpha*, we performed RNA-seq analyses comparing the transcriptome of gemma cups with that of the entire young thallus yet to form gemma cups and that of the midrib region (Fig. 1a). We obtained more than 14×10^6 reads from each of the samples (triplicates per tissue; Supplementary Table 1). More than 90% of the reads per library were mapped to the *M. polymorpha* genome sequence version 3.1¹². Genes for which the reads per kilobase per million (RPKM) value was > 1 were considered to be ‘expressed’. Among 19,287 annotated genes, 11,699 genes (61%) were expressed in the gemma cup. We identified 1391 and 1483 genes with greater than 2-fold changes in the gemma cup compared with their expression in the young thallus and midrib tissue, respectively. A total of 664 genes were upregulated in both comparisons (Fig. 1b and Supplemental Data Sets 1–3). Of the 664 genes, 10 were annotated as transcription factors (Supplementary Table 2).

Among the transcription factors, we focused on Mapoly0034s0034, a gene encoding an R2R3-MYB transcription factor. The RPKM value of this gene in the gemma

cup was 82.1, which represented 100-fold and 13-fold upregulation compared with expression in the young thallus and the midrib, respectively (Supplementary Table 2). Quantitative reverse-transcription PCR (qRT-PCR) analysis confirmed that Mapoly0034s0034 transcripts accumulated at a significantly higher level in gemma cups than in the young thallus and midrib tissue (Fig. 1c). We designated the *Mapoly0034s0034* gene as *GEMMA CUP-ASSOCIATED MYB 1* (*GCAM1*). Genetic nomenclature is as outlined in Bowman *et al.*²⁹.

Next, we generated transgenic *M. polymorpha* plants expressing a β -glucuronidase (GUS) reporter gene under the control of the *GCAM1* promoter (*GCAM1pro:GUS*). We generated a construct in which the GUS reporter gene was translationally fused to a *GCAM1* genomic fragment, containing 5.2-kb upstream of the ATG start codon. In *GCAM1pro:GUS* transgenic lines, histological GUS staining was detected in the apical notches of the 10-day-old thallus (Fig. 2a). In mature thalli with gemma cups, significant promoter activity was detected in the apical notches, the floor of gemma-cups, and developing gemmae (Fig. 2b–d).

To analyze the localization of protein accumulation, we inserted the yellow fluorescent protein (Citrine) sequence into the 3' end of the *GCAM1* coding sequence (CDS) in the *M. polymorpha* genome (Supplementary Figure 2a, b). The *GCAM1-Citrine* knock-in plants exhibited normal organ development, including gemma cups during vegetative growth (Supplementary Figure 2c). Consistent with the promoter–reporter analysis, we detected Citrine signals in the nucleus of cells in the developing gemma and gemma-cup floor cells (Fig. 2e–m).

GCAM1 is essential for gemma cup formation

To investigate the function of *GCAM1*, we disrupted *GCAM1* using homologous recombination-mediated gene targeting technology¹⁶. Two independent *GCAM1* knock-out lines (*gcam1^{ko}*) were isolated from 522 candidates (Supplementary Figure 2d, e). In the wild-type 3-week-old thallus developed from a thallus explant containing an apical notch, over ten gemma cups filled with gemmae were observed on the midrib of the dorsal surface (Fig. 3a, c, e). In contrast, no gemma cup development was observed on the dorsal surface of the *gcam1^{ko}* midrib (Fig. 3b and d), resulting in no gemma generation. No distinct impairment of thallus growth or development of other vegetative organs, i.e. air chamber,

rhizoid, and ventral scales, was observed in *gcaml^{ko}* thalli compared with those of wild-type thalli (Fig. 3c–f). We also generated *GCAMI* mutants using CRISPR/Cas9-mediated targeted genome editing³⁰. Two independent *GCAMI* mutants for each of two independent target sequences of *GCAMI* exhibited absence of gemma cup development, but no other obvious aberrant phenotype was observed during vegetative growth (Supplementary Figure 3), and thus the phenotypes were essentially identical to those of *gcaml^{ko}* plants. Altogether, these results demonstrated that *GCAMI* performs a critical role in gemma cup formation.

Generation of gemma cups is correlated with bifurcation (i.e. branching of the apical meristem) of thalli⁷. To clarify whether *GCAMI* may also be involved in branching, we investigated the number of gemma cups and apical notches in the *gcaml^{ko}* line in comparison with those of the wild type (Fig. 3g, h). About 20 gemma cups were observed on 3-week-old wild-type thalli, whereas no gemma cups developed in the *gcaml^{ko}* mutants. In contrast to the conspicuous gemma cup phenotype, no significant decrease in the number of bifurcations was observed in the *gcaml^{ko}* lines compared with that of the wild type, which suggested that *GCAMI* plays no role in bifurcation of the apical notch.

171

172 **Overexpression of *GCAMI* promotes proliferation of undifferentiated cells**

To further investigate the developmental function of *GCAMI*, we generated transgenic plants that constitutively expressed *GCAMI* under the control of the *MpELONGATION FACTOR 1α* regulatory sequence in *M. polymorpha* (*MpEFpro:GCAMI*). We obtained a significantly lower number of transformants using the *MpEFpro:GCAMI* construct than usual, and the majority of the obtained *MpEFpro:GCAMI* lines formed a mass of tiny flat thallus-like structures (Supplementary Figure 4). Thus, we utilized an inducible system (Fig. 4a), in which protein nuclear localization is modulated by a glucocorticoid receptor (GR) domain^{31,32}. In these transgenic plants, *GCAMI* function was expected to be induced by treatment with dexamethasone (DEX). In the wild type, treatment with DEX caused no morphological effect (Supplementary Figure 5). In the absence of DEX, *MpEFpro:GCAMI-GR* plants exhibited the wild-type phenotype and formed normal dorsal/ventral organs, i.e. air chambers, gemma cups, scales, and rhizoids (Fig. 4b, d, f). Treatment of *MpEFpro:GCAMI-GR* plants with DEX severely suppressed thallus growth, and cell clumps with no dorsal/ventral organs and few rhizoids were observed (Fig. 4c, e,

187 and g). After suspension of the DEX treatment, a number of small thallus branches were
188 generated in random positions (Fig. 4h, i and Supplementary Figure 6). These results
189 implied that GCAM1 functions in the maintenance and proliferation of pluripotent cells
190 that possess the capability to develop into individual thalli.

191

Orthologous relationship of GCAM1 to R2R3-MYBs in subfamily 14

193 Gemmae and gemma cups are specialized and derived gametophytic organs for vegetative
194 reproduction in certain Marchantiopsida species, a group of complex thalloid liverworts
195 including *M. polymorpha*. However, orthologs of *GCAM1* have been identified across
196 diverse branches of land plants. The inferred amino acid sequence of GCAM1 contained an
197 R2R3-MYB DNA-binding domain towards the N-terminus, and showed the highest
198 similarity (3e-59) to REGULATOR OF AXILLARY MERISTEM 3 (RAX3) in a BLASTP
199 search against the *Arabidopsis thaliana* protein database. Phylogenetic analysis suggested
200 that GCAM1 belonged to a clade, the R2R3-MYB subfamily 14, which includes the
201 *Arabidopsis* *RAX* genes and tomato *Blind* gene (Fig. 5a), with highly conserved exon–
202 intron structures (Fig. 5b). Some angiosperm proteins in the R2R3-MYB subfamily 14 are
203 regulatory factors for axillary meristem formation, e.g. *Blind* and *RAX* genes^{33,34,35,36}.
204 Relative to other R2R3-MYB proteins, those of the R2R3-MYB subfamily 14 are
205 characterized by an additional amino acid insertion between the first and second conserved
206 Trp residues of the R2 repeat²⁸, and GCAM1 conforms to this rule (Fig. 5c). These findings
207 support the orthologous relationship of GCAM1 to angiosperm proteins in the R2R3-MYB
208 subfamily 14.

209 To examine whether *M. polymorpha* *GCAM1* is functionally equivalent to the
210 angiosperm orthologs and is able to function in control of axillary meristem formation in
211 angiosperms, we introduced *GCAM1* into the *Arabidopsis rax1 rax2 rax3* triple mutant,
212 which exhibits significant inhibition of axillary bud formation compared with the wild
213 type³⁴. Interestingly, expression of *GCAM1* under the control of the *Arabidopsis RAX1*
214 promoter (*RAX1pro:GCAM1*) further inhibited axillary meristem formation in the *rax1*
215 *rax2 rax3* triple mutant background (Fig. 5 and Supplementary Figure 7). Given that a
216 non-conserved N-terminal domain is present upstream of (28 amino acids from) the
217 R2R3-MYB domain of *GCAM1*, which does not exist in angiosperm R2R3-MYB
218 subfamily 14 members (Fig. 5b, c), we also tested complementation with a shorter open

reading frame of *GCAM1* (*GCAM1-s*), which starts from the second start codon in the original *GCAM1* ORF. The *RAXIpro:GCAM1-s* transgenic plants showed notable recovery of axillary meristem formation compared with that of the *rax* triple mutant (Fig. 5 and Supplementary Figure 7). These results demonstrated the capability of *M. polymorpha* *GCAM1* to interact with the genetic machinery of axillary meristem formation in the angiosperm *A. thaliana*.

Discussion

Identification of an essential regulator for vegetative reproduction in *M. polymorpha*

The basis of vegetative reproduction in seed plants is the *de novo* meristem formation, which allows development of clonal plants. At present, the molecular mechanism of natural vegetative reproduction is poorly known. In the current study, we identified a key regulator for vegetative reproduction in the basal land plant *M. polymorpha*. Using comprehensive transcriptome analysis and molecular genetic approaches, we identified an R2R3-MYB gene, *GCAM1*, which is an essential factor for gemma cup development in *M. polymorpha*. Loss-of-function mutants of *GCAM1* showed no sign of gemma cup development, but no obvious abnormality was observed in the development of other thallus tissues (Fig. 3). This observation suggested that *GCAM1* plays a specific role in gemma cup development in the vegetative growth phase of *M. polymorpha*. Our observations on the accumulation of *GCAM1* transcripts in developing gemma cups and gemmae (Fig. 2e–m) corroborated the suggestion of a specific function for *GCAM1* in gemma cup and gemma formation. However, continuous expression of *GCAM1* did not confer excessive production of gemma cups (Fig. 4) and instead suppressed organ differentiation and promoted generation of undifferentiated cell clumps (Fig. 4 and Supplementary Figure 4). After *GCAM1* induction was halted at 1 week or 2 weeks after DEX treatment, numerous thalli developed directly from the cell clumps (Fig. 4), suggesting that the cell clumps consist of cells with the potential to differentiate into meristematic apical cells of the thallus. These results suggest that *GCAM1* might function to suppress tissue differentiation and maintain the undifferentiated status of cells, which may be essential for the floor cells of the gemma cup to form gemma initials. The role of *GCAM1* would be to proliferate floor cells of gemma cups, and to maintain the somewhat undifferentiated status of these cells, which may be a prerequisite for gemmae and gemma cup development.

251

252 **A common developmental mechanism between gemma cups and boundary zone for**
 253 **axillary meristems**

254 The present phylogenetic analysis indicated that *GCAM1* is orthologous to angiosperm
 255 genes in the R2R3-MYB subfamily 14²⁸, which includes regulatory factors for axillary
 256 meristem formation, e.g. tomato *Blind* and Arabidopsis *RAX* genes^{33,34,35,36} (Fig. 5a).
 257 Interspecific complementation analyses demonstrated that *M. polymorpha* *GCAM1* can
 258 interact with the genetic machinery of axillary meristem formation in Arabidopsis (Fig. 5d).
 259 These results suggested there is a common regulatory mechanism between vegetative
 260 reproduction in the gametophyte body of the liverwort *M. polymorpha* and axillary
 261 meristem formation in the sporophyte body of angiosperms. In Arabidopsis, *RAX1* and
 262 *RAX3* are specifically expressed in the boundary region, axils, between the shoot apical
 263 meristem (SAM) and leaf primordia, and regulate axillary meristem formation during early
 264 vegetative development^{34,35}. In angiosperms, boundary zones are characterized by a lower
 265 rate of cell divisions and lower concentrations of auxin and brassinosteroids than those of
 266 leaf primordia, resulting in a low degree of differentiation of boundary cells that exhibit
 267 higher competence for meristem formation^{2,37}. In Arabidopsis, *CUP-SHAPED*
 268 *COTYLEDON* genes (*CUCs*) are also known to be expressed in boundary zones and
 269 required for boundary formation². The tomato gene *Goblet*, which is the tomato orthologue
 270 of *CUCs*, promotes ectopic shoot formation at the leaflet boundary, suggesting that the
 271 specification of the boundary zone is the common basis of axillary bud formation and
 272 vegetative reproduction in angiosperms³⁸. The cells of the gemma cup floor in the liverwort
 273 *M. polymorpha*, in which the ability to regenerate clonal plantlets is maintained, could be
 274 considered to show similar characteristics to cells in the boundary zones of angiosperms.
 275 An orthologous relationship and functional equivalence between *GCAM1* in *M.*
 276 *polymorpha* and *RAX/Blind* genes in angiosperms further implies acquisition of a
 277 regulatory mechanism for establishment of the boundary zone, which retains the
 278 competence to generate meristematic cells, in the common ancestor of land plants, and such
 279 a regulatory mechanism was co-opted for axillary bud formation in the course of land-plant
 280 evolution.

281 In many plant species capable of vegetative reproduction, clonal propagules are
 282 often produced as modified forms of axillary buds, e.g. rhizomes in asparagus (*Asparagus*

283 *officinalis* L.) and iris (*Iris germanica* L.), tubers in potato (*Solanum tuberosum* L.), and
 284 stolons (runners) in strawberry (*Fragaria × ananassa*) and wild mint (*Mentha arvensis* L.).
 285 In some *Dioscorea* species, including the air potato *Dioscorea bulbifera*, aerial clonal
 286 propagules (bulbils) are produced in the leaf axils and detach from the parental plant. In
 287 addition, aerial tubers are often formed in the leaf axils of stressed potato plant³⁹. Ectopic
 288 expression of the tomato cytokinin-activating enzyme gene LONELY GUY (LOG1)
 289 transforms axillary meristems into aerial tubers⁴⁰. Thus, there is a strong link between
 290 vegetative reproduction and axillary bud formation in angiosperms that is convergent to the
 291 clonal reproduction observed in complex thalloid liverworts. The present findings on the
 292 liverwort *M. polymorpha* suggests a homology in the mechanisms controlling vegetative
 293 reproduction and axillary bud formation in land plants. Additional molecular studies on the
 294 process of vegetative reproduction in diverse lineages, such as basal tracheophytes, other
 295 bryophytes, or charophycean algae, should shed light on the validity of an evolutionarily
 296 conserved gene regulatory network for vegetative reproduction or axillary bud formation in
 297 land plants.

298

299 **Materials and Methods**

300 **Plant material and growth conditions**

301 Male and female accessions of *M. polymorpha*, Takaragaike-1 (Tak-1) and Takaragaike-2
 302 (Tak-2), respectively¹⁴, were maintained asexually. F₁ spores generated by crossing Tak-2
 303 and Tak-1 were used for generation of *gcam1*^{ko} and *GCAM1-Citrine* knock-in plants.
 304 Formation of sexual organs was induced by far-red irradiation as described previously⁴¹.
 305 Mature sporangia were collected 3–4 weeks after crossing, air-dried for 7–10 days, and
 306 stored at –80°C until use.

307 *Marchantia polymorpha* plants were cultured using half-strength Gamborg's B5
 308 medium⁴² containing 1% agar under 50–60 μmol photons m⁻² s⁻¹ continuous white light
 309 with a cold cathode fluorescent lamp (OPT-40C-N-L; Optrom, Miyagi, Japan) or
 310 light-emitting diode (VGL-1200W; SYNERGYTEC, Tokushima, Japan) at 22°C unless
 311 otherwise defined.

312

313 **RNA extraction and Illumina sequencing**

314 Developing gemma cups located within 3 mm from an apical notch of 3-week-old thalli

315 were manually dissected and immediately immersed in RNAlater (Thermo Fisher Scientific,
316 Waltham, MA, USA). For the control, the midrib region between gemma cups of
317 3-week-old thalli and 1-week-old thalli, which had no visible gemma cups, were also
318 sampled. The collected samples were divided into three pools for extraction of total RNA.

319 Total RNA was extracted with the RNeasy® Plant Mini Kit (QIAGEN) in
320 accordance with the manufacturer's protocol. The quality and quantity of resultant total
321 RNA were evaluated using a NanoDrop™ 1000 spectrophotometer (Thermo Fisher
322 Scientific), Qubit® 2.0 Fluorometer (Thermo Fisher Scientific), and a Bioanalyzer
323 RNA6000 Nano Kit (Agilent Technologies, Santa Clara, CA, USA). The sequence libraries
324 were constructed with a TruSeq™ RNA Sample Prep Kit v2 (Illumina, San Diego, CA,
325 USA) in accordance with the manufacturer's protocol. The quality and quantity of each
326 library were determined using a Bioanalyzer with High Sensitivity DNA kit (Agilent
327 Technologies), and KAPA Library Quantification Kit for Illumina (Illumina). Equal
328 amounts of each library were mixed to make the 2 nM pooled library. Illumina sequencing
329 was performed using a HiSeq 2000 platform (Illumina) to produce 101 single-end data. All
330 reads obtained have been deposited in the DDBJ and are available through the Sequence
331 Read Archive (SRA) under the accession number DRA005912.

332

333 **RNA-seq data analysis**

334 The resultant reads were mapped to the draft genome of *M. polymorpha* version 3.1 by
335 TopHat ver 2.1.1⁴³ with the default parameters. To identify differentially expressed genes
336 (DEGs), the mapped reads from different samples were compared by Cuffdiff 2.2.1⁴⁴ using
337 the default parameters and transcript annotation generated on the Marchantia genome
338 assembly ver. 3.1¹². Among DEGs, genes with a log2-fold change >1 were considered as
339 preferentially expressed in the gemma cup. The supercomputers of the Academic Center for
340 Computing and Media Studies at Kyoto University and of the Computing Center at the
341 Faculty of Biology-Oriented Science and Technology, Kindai University were used for
342 computation.

343

344 **Quantitative RT-PCR analysis**

345 One microgram of total RNA was reverse-transcribed in a 20 µl reaction mixture using
346 ReverTra Ace® qPCR RT Master Mix with gDNA remover (TOYOBO). After the reaction,

the cDNA samples were diluted with 220 µl distilled water and 2 µl aliquots were amplified with the LightCycler® Nano Real-time PCR Detection System (Roche Applied Science, Tokyo, Japan) using the KOD SYBR® qRT-PCR Mix (TOYOBO). The PCR cycling program was performed in accordance with the manufacturer's protocol. The primers used in these experiments are listed in Supplementary Table 3. MpEF1α was used as an internal control.

Generation of transformants for promoter-reporter analysis

To construct *GCAM1pro:GUS*, the *GCAM1* genomic region, including a 5593-bp fragment upstream of the 83rd Arg codon in the second exon, was amplified from Tak-1 genomic DNA by PCR with the primers GCAM1pro_L1 and GCAM1pro_R2 and cloned into the pENTR™/D-TOPO® cloning vector (Life Technologies, Rockville, MD, USA). This entry vector was used in the Gateway® LR reaction (Life Technologies) with the Gateway binary vector pMpGWB104⁴⁵ to generate the *GCAM1pro:GUS* binary construct, in which the *GUS* reporter gene was translationally fused at the middle of the second exon of *GCAM1*. The *GCAM1pro:GUS* vectors were introduced into regenerating thalli of Tak-1 by *Agrobacterium*-mediated transformation as previously described¹⁵. Transformants were selected with 10 µg/ml hygromycin B and 100 µg/ml cefotaxime.

Generation of *gcaml*^{ko} and *GCAM1-Citrine* knock-in plants

For knock-in and knock-out experiments, Tak-1 genomic sequences were amplified by PCR with KOD FX Neo DNA polymerase (TOYOBO) and the primer pair listed in Supplementary Table 3. The amplified products were inserted into the *PacI* and *AscI* sites of pJHY-TMp¹⁶ and pJHY-TMp1-Cit⁴⁶ to generate *gcaml*^{ko} and *GCAM1-Citrine* transformants, respectively. The vectors were introduced into germinating spores via *Agrobacterium tumefaciens* GV2260 as described previously¹⁶. Transformants were selected with 10 µg/ml hygromycin B and 100 µg/ml cefotaxime. The transformants harboring the targeted insertions were selected by PCR with KOD FX Neo DNA polymerase and the primer pairs listed in Supplementary Table 3 and Supplementary Figure 2.

CRISPR/Cas9-based genome editing of *GCAM1*

379 Loss-of-function mutants of *GCAM1* were generated with the CRISPR/Cas9 system as
 380 described previously^{30,47}. We selected two target sequences, one located in the first exon,
 381 and the other located in the second exon of *GCAM1* (Supplementary Figure 4). An
 382 off-target search was performed using casOT⁴⁸. Synthetic oligo DNAs for respective target
 383 sites shown in Supplementary Table 3 were annealed, inserted into the entry vector
 384 pMpGE_En01, and then introduced into the destination vector pMpGE011^{30,47}. The vectors
 385 were introduced into regenerating thalli of Tak-1 via *A. tumefaciens* GV2260¹⁵, and
 386 transformants were selected with 0.5 μ M chlorsulfuron. Genomic DNA was isolated from
 387 transformants and amplified from the target region by PCR. The PCR product was used for
 388 sequencing of the respective target sites with an ABI 3100 genetic analyzer (Applied
 389 Biosystems, Foster City, CA, USA).

390

391 **Visualization of GCAM1-Citrine**

392 Transverse sections of *GCAM1-Citrine* knock-in thalli with apical notches were generated
 393 with a pair of razors, and observed under a confocal laser scanning microscope (Olympus
 394 FLUOVIEW FV1000, Tokyo, Japan). Excitation and emission wavelengths for Citrine
 395 fluorescence were 515 nm (Ar laser) and 520–570 nm, respectively.

396

397 **Histology and light microscopy**

398 For histochemical GUS staining, *GCAM1pro:GUS* plants were grown on half-strength B5
 399 medium for respective periods under continuous white light. GUS staining was performed
 400 as described previously and at least five independent lines were observed for GUS staining
 401 patterns using S8APO (Leica Microsystems) and DMS1000 (Leica) stereoscopic
 402 microscopes, or an upright light microscope Axio Scope.A1 (Zeiss) equipped with an
 403 AxioCam ERc5c camera (Zeiss).

404 For plastic-embedded sectioning, 3-week-old thalli developed from gemmae were
 405 dissected into small pieces and transferred to fixative solution (2% glutaraldehyde in 0.05
 406 M phosphate buffer, pH 7.0), evacuated with a water aspirator until the specimens sank,
 407 and fixed for 2 days at room temperature. The samples were dehydrated in a graded ethanol
 408 series and embedded in Technovit 7100 plastic resin. Semi-thin sections (5 μ m thickness)
 409 for light microscopy were obtained with a microtome (HM 335E, Leica Microsystems,
 410 Heerbrugg, Switzerland) and stained with toluidine blue O.

For scanning electron microscopy, plant samples were frozen in liquid nitrogen and directly observed on a VHX-D500 microscope (KEYENCE, Osaka, Japan).

Generation of *GCAM1*-overexpressing plants

To generate *MpEFpro:GCAM1*, the *GCAM1* coding sequence was amplified by PCR using KOD Plus Neo DNA polymerase (TOYOBO) with the primer set GCAM1-cds-L1/GCAM1-cds-sR and cloned into the pENTR/D-TOPO cloning vector. The entry vector was used in the Gateway LR reaction with the Gateway binary vector pMpGWB103⁴⁵. The *MpEFpro:GCAM1* vector was introduced into regenerating thalli of Tak-1 as previously described¹⁵. Transformants were selected with 10 µg/ml hygromycin B and 100 µg/ml cefotaxime.

To construct *MpEFpro:GCAM1-GR*, the *GCAM1* CDS was amplified by PCR using KOD Plus Neo DNA polymerase (TOYOBO) with the primer set GCAM1-cds-L1/GCAM1-cds-nsR and cloned into the pENTR/D-TOPO cloning vector. The entry vector was used in the Gateway LR reaction with the Gateway binary vector pMpGWB313⁴⁵. The *MpEFpro:GCAM1-GR* vector was introduced into regenerating thalli of Tak-1 as previously described¹⁵. Transformants were selected with 0.5 µM chlorsulfuron and 100 µg/ml cefotaxime.

The transformants and mutants were named consistent with the nomenclatural rules for *M. polymorpha*²⁹.

Phenotypic analysis of *MpEFpro:GCAM1-GR* plants

In the vegetative phase, DEX treatment of *MpEFpro:GCAM1-GR* plants was performed by culture in half-strength Gamborg's B5 medium containing 10 µM DEX or applying 200 µL of 10 µM DEX solution to plants.

Phylogenetic analysis of *GCAM1*

For phylogenetic analysis of *GCAM1* and homologous R2R3-MYBs, sequence information was obtained from the PlantTFDB⁴⁹ and Phytozome databases. A multiple alignment of amino acid sequences of *GCAM1* and homologous R2R3-MYBs was first constructed using COBALT⁵⁰ and then using the MUSCLE program⁵¹ implemented in SeaView version 4.5.4⁵² with the default parameters. The alignment is available as

Supplemental Data Set 4. The conserved region covering the R2R3-MYB domain was extracted before phylogenetic tree construction, which was performed using the maximum likelihood method with PhyML⁵³ with the LG substitution model. A bootstrap analysis with 1000 replicates was performed in each analysis to assess statistical support for the topology. The phylogenetic tree was visualized using FigTree (<http://tree.bio.ed.ac.uk/software/figtree/>).

Introduction of *GCAM1* into an *Arabidopsis rax* triple mutant

To generate a RAX1 promoter construct, the upstream region of *Arabidopsis RAX1* including the promoter and 5' untranslated region (2939 bp) were amplified by PCR using the primers Myb37-pro-SbfIF and Myb37-pro-AscIR. The resulting PCR fragment was inserted into the *SbfI* and *AscI* sites of the pGPTV-BAR-*AscI* vector⁵⁴ in front of the *GUS* gene (Vector pFY124). A GFP fragment was amplified using the primers GFPAscIF and GFPpolylinkerR. This PCR fragment was introduced into the *AscI* and *SacI* sites of pFY124 introducing a new *AvrII* site for the following constructions (Vector pQW154).

The *RAX1* open reading frame was amplified by PCR using the primers RAX1AscIF and RAX1SacIR. The PCR fragment was inserted into the *AscI* and *SacI* sites of pQW154. The *GCMA1* open reading frame was amplified using the primers GCAM1AscIF and GCAM1AvrIIR. The PCR fragment was inserted into the *AscI* and *AvrII* sites of pQW154. The short open reading frame of the *GCAM1* coding sequence (*GCAM1-s*; from +85 to the stop codon) was amplified using the primers GCAM1NewAscIF and GCAM1AvrIIR. The PCR fragment was inserted into the *AscI* and *AvrII* sites of pQW154.

The RAX1pro:RAX1, RAX1pro:GCAM1, and RAX1pro:GCAM1-s constructs were introduced into the *Arabidopsis rax1-3 rax2-1 rax3-1* mutant³⁴ using the floral dip method, and T₃ homozygous transgenic lines were used for further analysis.

For cultivation under short-day conditions, *Arabidopsis* plants were grown in a controlled environment room under a 8 h/16 h (light/dark) photoperiod, 20°C/18°C (day/night) temperatures, and 50% relative humidity. Flowering was induced after 4 weeks by transferring the plants to a 16 h/8 h (light/dark) photoperiod. Cultivation under long-day conditions was done in a controlled greenhouse with additional artificial light when needed.

475 Axillary bud formation was analyzed after the onset of flowering using a
476 stereomicroscope as previously described⁵⁵. Buds that had produced one or two leaf
477 primordia as well as elongating lateral shoots were scored as leaf axils that had established
478 axillary meristems.

479

480 **Data Availability**

481 All reads obtained have been deposited in the DDBJ and are available through the
482 Sequence Read Archive (SRA) under the accession number DRA005912. The authors
483 declare that all data supporting the findings of this study are available within the
484 manuscript and its supplementary files or are from the corresponding author upon request.

485

486 **Supplemental Data**

487 The following materials are available in the online version of this article.

488 **Supplementary Figure 1.** Histological description for the origin and development of
489 gemma cup in *Marchantia polymorpha*.

490 **Supplementary Figure 2.** Generation of *GCAM1-Citrine* knock-in and *gcam1^{ko}* plants,
491 related to Figs. 2 and 3.

492 **Supplementary Figure 3.** Targeted disruption of *GCAM1* using CRISPR/Cas9-mediated
493 genome editing, related to Fig. 3, related to Fig. 3.

494 **Supplementary Figure 4.** Overexpression of *GCAM1*, related to Fig. 4.

495 **Supplementary Figure 5.** No morphological phenotype is conferred by DEX treatment in
496 wild-type thalli, related to Fig. 4.

497 **Supplementary Figure 6.** Induction of GCAM1 function suppresses growth and organ
498 development, related to Fig. 4.

499 **Supplementary Figure 7.** Interaction of GCAM1 with Arabidopsis mechanism for axillary
500 meristem formation, related to Fig. 5.

501

502 **Supplementary Table 1.** Summary of the RNA-sequencing analysis.

503 **Supplementary Table 2.** Transcription factor genes upregulated in the gemma cup.

504 **Supplementary Table 3.** Primers used in this study.

505

506 **Supplemental Data Set 1.** List of 1391 genes upregulated by >2 times in the gemma cup

507 in comparison with the young thallus (GC vs TH in Fig. 1b)

508 **Supplemental Data Set 2.** List of 1483 genes upregulated by >2 times in the gemma cup
509 in comparison with the midrib (GC vs MR in Fig. 1b)

510 **Supplemental Data Set 3.** List of 664 genes upregulated by >2 times in the gemma cup in
511 comparison with the young thallus and midrib (overlap of GC vs TH and GC vs MR in Fig.
512 1b)

513 **Supplemental Data Set 4.** Multiple alignment of GCAM1 and related R2R3-MYBs used
514 for phylogenetic analysis in Fig. 5a.

515

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530

531 **AUTHOR CONTRIBUTIONS**

532 K.I., S.T., and Y.Y. designed the research; S.T., Y.Y., T.S., R.N., Q.W., H.K., K.T., and K.I.
533 performed research; Y.Y., S.T., R.N., Q.W., K.T.Y., H.K., K.T., H.F., T.M., T.K., and K.I.
534 analyzed the data; K.I. and Y.Y. wrote the paper.

535

536 **Competing Interests**

537 The authors declare no competing interests.

538

539 REFERENCES

- 540 1. Steeves TA, Sussex IM. *Patterns in plant development*, 2 edn. Cambridge
541 University Press (1989).
542
- 543 2. Wang Q, Hasson A, Rossmann S, Theres K. Divide et impera: boundaries shape the
544 plant body and initiate new meristems. *New Phytol*, (2015).
545
- 546 3. Steward FC, Mapes MO, Kent AE, Holsten RD. Growth and development of
547 cultured plant cells. *Science* **143**, 20-27 (1964).
548
- 549 4. Steward FC, Mapes MO, Mears K. Growth and organized development of cultured
550 cells. II. Organization in cultures grown from freely suspended cells. *Am J Bot* **45**,
551 705-708 (1958).
552
- 553 5. Klimesčová J, Klimeš L. Bud banks and their role in vegetative regeneration – A
554 literature review and proposal for simple classification and assessment. *Perspect*
555 *Plant Ecol Evol Syst* **8**, 115-129 (2007).
556
- 557 6. Davis FT, Geneve, R.L., Wilson, S.E., Hartmann, H.T., Kester, D.E. *Hartmann &*
558 *Kester's Plant Propagation: Principles and Practices*. Pearson (2017).
559
- 560 7. Shimamura M. *Marchantia polymorpha*: Taxonomy, phylogeny and morphology of
561 a model system. *Plant Cell Physiol* **57**, 230-256 (2016).
562
- 563 8. Barnes CR, Land WJG. Bryological papers. II. The origin of the cupule of
564 *Marchantia*. *Bot Gaz* **46**, 401-409 (1908).
565
- 566 9. Voth PD, Hamner KC. Responses of *Marchantia polymorpha* to nutrient dupply
567 and p dhotoperiod. *Bot Gaz* **102**, 169-205 (1940).
568
- 569 10. Voth PD. Gemmae-cup production in *Marchantia polymorpha* and its response to
570 calcium deficiency and supply of other nutrients. *Bot Gaz* **103**, 310-325 (1941).
571
- 572 11. Benson-Evans K. Physiology of the reproduction of bryophytes. *The Bryologist* **67**,
573 431-445 (1964).
574
- 575 12. Bowman JL, *et al.* Insights into land plant evolution garnered from the *Marchantia*
576 *polymorpha* genome. *Cell* **171**, 287-304 e215 (2017).
577
- 578 13. Ishizaki K, Nishihama R, Yamato KT, Kohchi T. Molecular genetic tools and
579 techniques for *Marchantia polymorpha* research. *Plant Cell Physiol* **57**, 262-270
580 (2016).
581

- 582 14. Ishizaki K, Chiyoda S, Yamato KT, Kohchi T. *Agrobacterium*-mediated
583 transformation of the haploid liverwort *Marchantia polymorpha* L., an emerging
584 model for plant biology. *Plant Cell Physiol* **49**, 1084-1091 (2008).
585
- 586 15. Kubota A, Ishizaki K, Hosaka M, Kohchi T. Efficient *Agrobacterium*-mediated
587 transformation of the liverwort *Marchantia polymorpha* using regenerating thalli.
588 *Biosci Biotechnol Biochem* **77**, 167-172 (2013).
589
- 590 16. Ishizaki K, Johzuka-Hisatomi Y, Ishida S, Iida S, Kohchi T. Homologous
591 recombination-mediated gene targeting in the liverwort *Marchantia polymorpha* L.
592 *Sci Rep* **3**, 1532 (2013).
593
- 594 17. Flores-Sandoval E, Dierschke T, Fisher TJ, Bowman JL. Efficient and inducible use
595 of artificial microRNAs in *Marchantia polymorpha*. *Plant Cell Physiol* **57**, 281-290
596 (2016).
597
- 598 18. Nishihama R, Ishida S, Urawa H, Kamei Y, Kohchi T. Conditional gene
599 expression/deletion systems for *Marchantia polymorpha* using its own heat-shock
600 promoter and Cre/loxP-mediated site-specific recombination. *Plant Cell Physiol* **57**,
601 271-280 (2016).
602
- 603 19. Sugano SS, *et al.* CRISPR/Cas9-mediated targeted mutagenesis in the liverwort
604 *Marchantia polymorpha* L. *Plant Cell Physiol* **55**, 475-481 (2014).
605
- 606 20. Menand B, *et al.* An ancient mechanism controls the development of cells with a
607 rooting function in land plants. *Science* **316**, 1477-1480 (2007).
608
- 609 21. Kubota A, Kita S, Ishizaki K, Nishihama R, Yamato KT, Kohchi T. Co-option of a
610 photoperiodic growth-phase transition system during land plant evolution. *Nat*
611 *Commun* **5**, 3668 (2014).
612
- 613 22. Xu B, *et al.* Contribution of NAC transcription factors to plant adaptation to land.
614 *Science* **343**, 1505-1508 (2014).
615
- 616 23. Kato H, *et al.* Auxin-mediated transcriptional system with a minimal set of
617 components is critical for morphogenesis through the life cycle in *Marchantia*
618 *polymorpha*. *PLoS Genet* **11**, e1005084 (2015).
619
- 620 24. Flores-Sandoval E, Eklund DM, Bowman JL. A simple auxin transcriptional
621 response system regulates multiple morphogenetic processes in the liverwort
622 *Marchantia polymorpha*. *PLoS Genet* **11**, e1005207 (2015).
623
- 624 25. Proust H, *et al.* RSL class I genes controlled the development of epidermal
625 structures in the common ancestor of land plants. *Curr Biol* **26**, 93-99 (2016).

- 626
- 627 26. Ishizaki K. Evolution of land plants: insights from molecular studies on basal
628 lineages. *Biosci Biotechnol Biochem* **81**, 73-80 (2017).
- 629
- 630 27. Pires ND, Dolan L. Morphological evolution in land plants: new designs with old
631 genes. *Philos Trans R Soc Lond B Biol Sci* **367**, 508-518 (2012).
- 632
- 633 28. Stracke R, Werber M, Weisshaar B. The R2R3-MYB gene family in *Arabidopsis*
634 *thaliana*. *Curr Opin Plant Biol* **4**, 447-456 (2001).
- 635
- 636 29. Bowman JL, *et al.* The Naming of Names: Guidelines for Gene Nomenclature in
637 Marchantia. *Plant Cell Physiol* **57**, 257-261 (2016).
- 638
- 639 30. Sugano SS, *et al.* Efficient CRISPR/Cas9-based genome editing and its application
640 to conditional genetic analysis in Marchantia polymorpha. *PLoS One* **13**, e0205117
641 (2018).
- 642
- 643 31. Lloyd AM, Schena M, Walbot V, Davis RW. Epidermal cell fate determination in
644 Arabidopsis: patterns defined by a steroid-inducible regulator. *Science* **266**, 436-439
645 (1994).
- 646
- 647 32. Schena M, Lloyd AM, Davis RW. A steroid-inducible gene expression system for
648 plant cells. *Proc Natl Acad Sci U S A* **88**, 10421-10425 (1991).
- 649
- 650 33. Schmitz G, Tillmann E, Carriero F, Fiore C, Cellini F, Theres K. The tomato Blind
651 gene encodes a MYB transcription factor that controls the formation of lateral
652 meristems. *Proc Natl Acad Sci U S A* **99**, 1064-1069 (2002).
- 653
- 654 34. Müller D, Schmitz G, Theres K. Blind homologous R2R3 Myb genes control the
655 pattern of lateral meristem initiation in Arabidopsis. *Plant Cell* **18**, 586-597 (2006).
- 656
- 657 35. Keller T, Abbott J, Moritz T, Doerner P. Arabidopsis REGULATOR OF
658 AXILLARY MERISTEMS1 controls a leaf axil stem cell niche and modulates
659 vegetative development. *Plant Cell* **18**, 598-611 (2006).
- 660
- 661 36. Jeifetz D, David-Schwartz R, Borovsky Y, Paran I. CaBLIND regulates axillary
662 meristem initiation and transition to flowering in pepper. *Planta* **234**, 1227-1236
663 (2011).
- 664
- 665 37. Wang Q, Kohlen W, Rossmann S, Vernoux T, Theres K. Auxin depletion from the
666 leaf axil conditions competence for axillary meristem formation in Arabidopsis and
667 tomato. *Plant Cell*, (2014).
- 668
- 669 38. Rossmann S, Kohlen W, Hasson A, Theres K. Lateral suppressor and Goblet act in

670 hierarchical order to regulate ectopic meristem formation at the base of tomato
671 leaflets. *Plant J* **81**, 837-848 (2015).
672

673 39. Ewing EE, Struik PC. Tuber Formation in potato: induction, initiation, and growth.
674 In: *Horticultural Reviews* (eds Janick J) John Wiley & Sons, Inc. (1992).
675

676 40. Eviatar-Ribak T, Shalit-Kaneh A, Chappell-Maor L, Amsellem Z, Eshed Y,
677 Lifschitz E. A cytokinin-activating enzyme promotes tuber formation in tomato.
678 *Curr Biol* **23**, 1057-1064 (2013).
679

680 41. Chiyoda S, Ishizaki K, Kataoka H, Yamato KT, Kohchi T. Direct transformation of
681 the liverwort *Marchantia polymorpha* L. by particle bombardment using immature
682 thalli developing from spores. *Plant Cell Rep* **27**, 1467-1473 (2008).
683

684 42. Gamborg OL, Miller RA, Ojima K. Nutrient requirements of suspension cultures of
685 soybean root cells. *Exp Cell Res* **50**, 151-158 (1968).
686

687 43. Kim D, Pertea G, Trapnell C, Pimentel H, Kelley R, Salzberg SL. TopHat2:
688 accurate alignment of transcriptomes in the presence of insertions, deletions and
689 gene fusions. *Genome Biol* **14**, R36 (2013).
690

691 44. Trapnell C, *et al.* Differential gene and transcript expression analysis of RNA-seq
692 experiments with TopHat and Cufflinks. *Nat Protoc* **7**, 562-578 (2012).
693

694 45. Ishizaki K, *et al.* Development of Gateway binary vector series with four different
695 selection markers for the liverwort *Marchantia polymorpha*. *PLoS One* **10**,
696 e0138876 (2015).
697

698 46. Yamaoka S, *et al.* Generative cell specification requires transcription factors
699 evolutionarily conserved in land plants. *Curr Biol* **28**, 479-486 e475 (2018).
700

701 47. Sugano SS, Nishihama R, CRISPR/Cas9-based genome editing of transcription
702 factor genes in *Marchantia polymorpha*. *Methods Mol Biol* **1830**: 109-126 (2018).
703

704 48. Xiao A, *et al.* CasOT: a genome-wide Cas9/gRNA off-target searching tool.
705 *Bioinformatics* **30**, 1180-1182 (2014).
706

707 49. Jin J, *et al.* PlantTFDB 4.0: toward a central hub for transcription factors and
708 regulatory interactions in plants. *Nucleic Acids Res* **45**, D1040-D1045 (2017).
709

710 50. Papadopoulos JS, Agarwala R. COBALT: constraint-based alignment tool for
711 multiple protein sequences. *Bioinformatics* **23**, 1073-1079 (2007).
712

713 51. Edgar RC. MUSCLE: multiple sequence alignment with high accuracy and high

714 throughput. *Nucleic Acids Res* **32**, 1792-1797 (2004).
715
716 52. Gouy M, Guindon S, Gascuel O. SeaView version 4: A multiplatform graphical
717 user interface for sequence alignment and phylogenetic tree building. *Mol Biol Evol*
718 **27**, 221-224 (2010).
719
720 53. Guindon S, Dufayard JF, Lefort V, Anisimova M, Hordijk W, Gascuel O. New
721 algorithms and methods to estimate maximum-likelihood phylogenies: assessing
722 the performance of PhyML 3.0. *Syst Biol* **59**, 307-321 (2010).
723
724 54. Überlack B, Werr W. Vectors with rare-cutter restriction enzyme sites for
725 expression of open reading frames in transgenic plants. *Mol Breed* **2**, 293-295
726 (1996).
727
728 55. Raman S, Greb T, Peaucelle A, Blein T, Laufs P, Theres K. Interplay of miR164,
729 CUP-SHAPED COTYLEDON genes and LATERAL SUPPRESSOR controls
730 axillary meristem formation in *Arabidopsis thaliana*. *Plant J* **55**, 65-76 (2008).
731
732
733

Fig. 1. Identification of genes preferentially expressed in the gemma cup of *Marchantia polymorpha*.

(a) Tissues used for mRNA-seq analysis. GC, gemma cup; TH, 1-week-old thallus; MR, midrib. (b) Venn diagram of genes upregulated by more than two-fold in the gemma cup. (c) qRT-PCR analysis of Mapoly0034s0034/GCAM1 in vegetative tissues. The elongation factor 1a (MpEF1a) gene was used as a reference (Welch's *t*-test, $p < 0.01$, $n = 3$).

Fig. 2 *GCAM1* expression pattern in the vegetative growth stage of *Marchantia polymorpha*.

(a–d) Histochemical GUS assay in a representative *GCAM1**pro::GUS* line. (a) Ten-day-old thallus. (b) Three-week-old thallus with gemma cups. (c and d) Transverse sections of developing and mature gemma cups on a 3-week-old thallus. Developing gemma cups are those closely located near the apical notch (c) and mature gemma cups are those located in the basal region of the thallus (d). (e–m) Accumulation of GCAM1 in the basal floor of the gemma cup. Bright-field (e–g), single confocal (h–j), and merged (k–m) images of the basal floor of gemma cups in 17- to 18-day-old thalli in *GCAM1-Citrine* #1 knock-in plants (e, f, h, i, k, and l) and the wild type (g, j, and m). Dotted lines indicate basal floor cells. Bars, 100 μm (e–m).

Fig. 3. Phenotype of the *gcam1* mutant of *Marchantia polymorpha*.

(a and b) Three-week-old thalli grown from tip of thalli of the wild type (a) and *gcam1*^{ko} knock-out mutant (b). The thalli were grown from the tip of parental thalli, because gemmae were absent in *gcam1*^{ko} lines. (c and d) Close-up of the tip of thalli of the previous individual, showing gemma cups in the wild type (c), whereas gemma cups were absent in the *gcam1*^{ko} lines (d). Red arrowheads indicate rhizoids. (e and f) Transverse sections (5 μm thick) from 3-week-old thalli of the wild type (e) and *gcam1*^{ko} (f). Red and blue arrows indicate air chambers and ventral scales, respectively. (g) Number of gemma cups in the wild type (WT) and *gcam1*^{ko} lines. (h) Number of apical notches in the wild type and *gcam1*^{ko} lines. Values are means ± s.d (n = 10).

Fig. 4. Induction of GCAM1 function suppresses growth and organ development in thallus of *Marchantia polymorpha*.

(a) Schematic representation of the *MpEFpro:GCAM1-GR* construct. (b, d, f) Two-week-old *MpEFpro:GCAM1-GR* transgenic plant treated with mock. A number of air pores and air chambers are shown in high-magnification views of the thallus surface (d and f). (c, e, and g) Two-week-old *MpEFpro:GCAM1-GR* transgenic plant treated with 10 μM DEX. There is no indication of air chamber development (e and g). Much fewer rhizoids developed from the ventral surface (e, inset). (h) Two-week-old *MpEFpro:GCAM1-GR* transgenic plant treated with DEX for the first 7 days and mock for the latter 7 days, and a high-magnification view of the thallus surface (inset). Red arrowhead indicates a developing air chamber. (i) Three-week-old *MpEFpro:GCAM1-GR* transgenic plant treated with DEX for the first 7 days and mock for the latter 14 days, and a high-magnification view of the thallus (inset). Red arrows indicate a set of bifurcated apical notches of thalli.

Fig. 5. Relationships of GCAM1 and angiosperm R2R3-MYB subfamily 14 orthologs.

(a) Phylogenetic analysis of *GCAM1* and its homologs in plants. Unrooted maximum-likelihood tree generated from the amino acid sequences for the R2R3-MYB DNA-binding domain of *GCAM1* and related R2R3-MYB proteins across diverse plant lineages. The numbers at the nodes are bootstrap values calculated from 1000 replicates. The scale bar is evolutionary distance as the rate of amino acid substitutions. *SIBlind* and *CaBlind* are R2R3-MYB genes reported to function in axillary bud formation in *Solanum lycopersicum* and *Capsicum annuum*, respectively. From *A. thaliana*, not only R2R3-MYBs in subfamily 14, but also other R2R3-MYBs in closely related subfamilies were sampled for the phylogenetic analysis. For *Physcomitrella patens* and *Selaginella moellendorffii*, R2R3-MYBs that showed eight highest and two highest similarities to *GCAM1*, respectively, were sampled. **(b)** Exon–intron structure of *GCAM1*, *AtRAX1*, *AtRAX2*, *AtRAX3*, and *SIBlind*. Box, exon; line, intron; white, 5'- or 3'- untranslated region. Red and blue boxes indicate R2R3-MYB domains and the non-conserved N-terminal stretch of 28 a.a. in *GCAM1*, respectively. **(c)** Multiple alignment of R2R3-MYB domains of *GCAM1*, *AtRAX1*, *AtRAX2*, *AtRAX3*, and *SIBlind*. Black asterisks indicate conserved Trp residues in plant R2R3-MYBs, and the red asterisk indicates a Phe residue conserved in R2R3-MYB subfamily 14²⁸. R2R3-MYBs in subfamily 14 are characterized by an additional amino acid (20 a.a.) compared with other R2R3-MYB subfamilies (19 a.a.) between the first and the second Trp residues of the R2 repeat. **(d)** Genetic complementation of Arabidopsis *rax1-3 rax2-1 rax3-1* mutant with Arabidopsis *RAX1* and Marchantia *GCAM1* (see Supplementary Figure 7 for details). Lateral shoot formation in rosette leaf axils of the *rax1-3 rax2-1 rax3-1* mutant (*triple rax*, *n* = 30), two independent transformants containing *RAX1*-promoter-driven *RAX1* open reading frame (ORF1) in *rax* triple mutant background (*RAX1pro:RAX1 triple rax*, *n* = 15), two independent transformants containing *RAX1*-promoter-driven *GCAM1* ORF in *rax* triple mutant background (*RAX1pro:GCAM1 triple rax*, *n* = 15), and three independent transgenics containing *RAX1*-promoter-driven shorter version of *GCAM1* ORF in *rax* triple mutant background (*RAX1pro:GCAM1-s triple rax*, *n* = 15). Values represent means $\pm$ SE. Asterisks indicate significant differences relative to triple *rax* mutant (* *P* < 0.05, ** *P* < 0.02, *** *P* < 0.0001).