Supplemental informations

Endogenous diterpenes derived from *ent*-kaurene, a common gibberellin precursor, regulate protonema differentiation of the moss *Physcomitrella patens*

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Supplemental Figure S1-S7

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Supplemental Figure S1

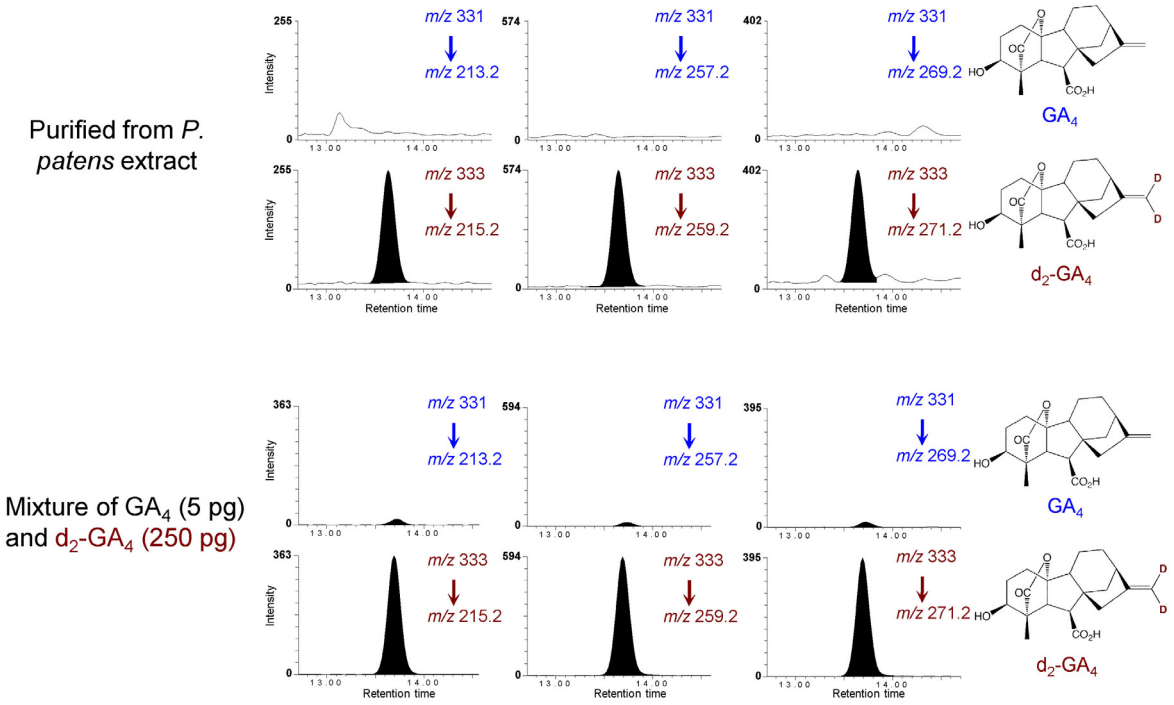

Figure S1.LC-MS/MS chromatograms of GA_4 and d_2-GA_4 in a purified fraction from 3.3 g of *P. patens* tissues (upper panel). Five hundred pg of d_2-GA_4 were added to the crude extract as an internal standard. Three fragment ions, *m/z* 213.2, 257.2 and 269.2, derived from the molecular ion (*m/z* 331) were monitored for GA_4. For d_2-GA_4, three fragment ions, *m/z* 215.2, 259.2 and 271.2, derived from the molecular ion (*m/z* 333) were detected. The d_2-GA_4 peaks were clearly detected in the *P. patens* sample, and their signal intensities were equivalent to those of 250 pg of standard d_2-GA_4 (comparing the *y*-axis scale in the upper and lower panels). In contrast, no fragment ions for the endogenous GA_4 were detected (upper panel). We generated a standard curve by co-injecting 250 pg of d_2-GA_4 and varying concentrations of cold GA_4. Five pg of GA_4 were still detectable (lower panel), suggesting that the amount of GA_4 in our *P. patens* sample, if any, was less than 5 pg (less than 1.5 pg/ g fresh weight). We analyzed $GA_{1,8,9,12,15,19,20,24,34,44,51,53}$ by the same method using the corresponding d_2-labeled internal standards, and none of them were detected.

Supplemental Figure S2

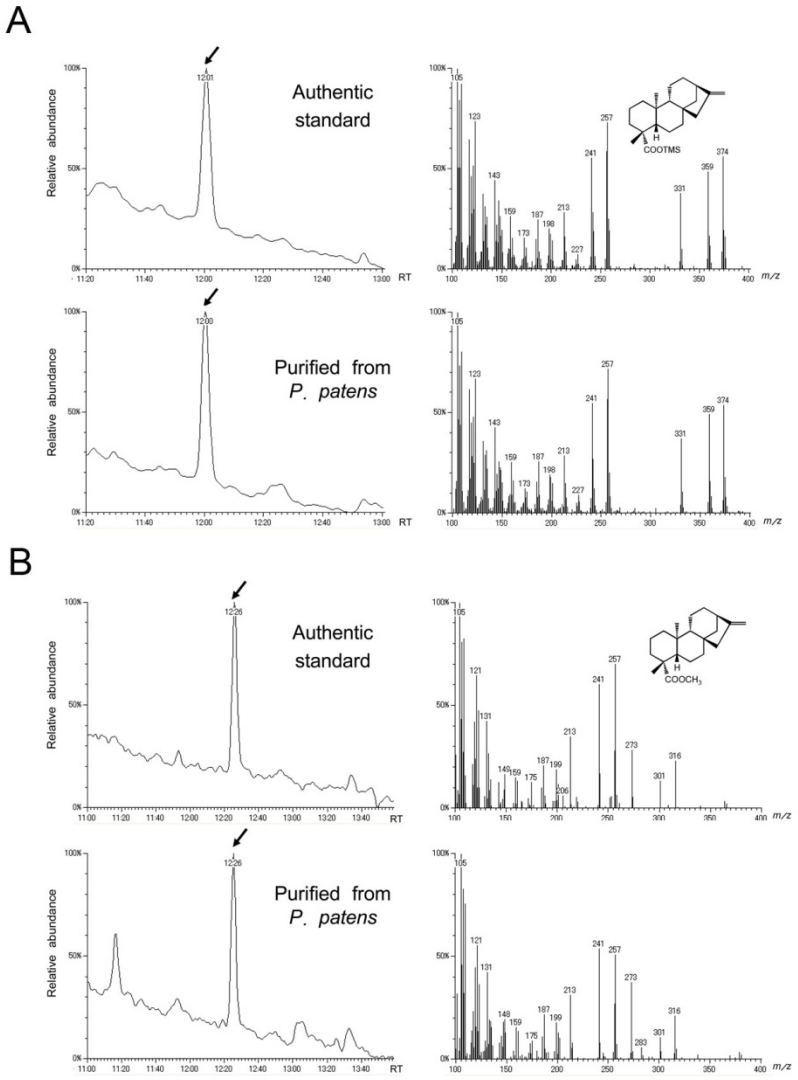

Figure S2. Identification of *ent*-kaurenoic acid (*ent*-KA) from *P. patens* as a trimethylsilylester (A) or methylester (B) derivative by GC-MS analysis. Total ion chromatograms (left) and full-scan mass spectra (right) are shown. Arrows indicate the peak for the *ent*-kaurenoic acid derivatives

Supplemental Figure S3

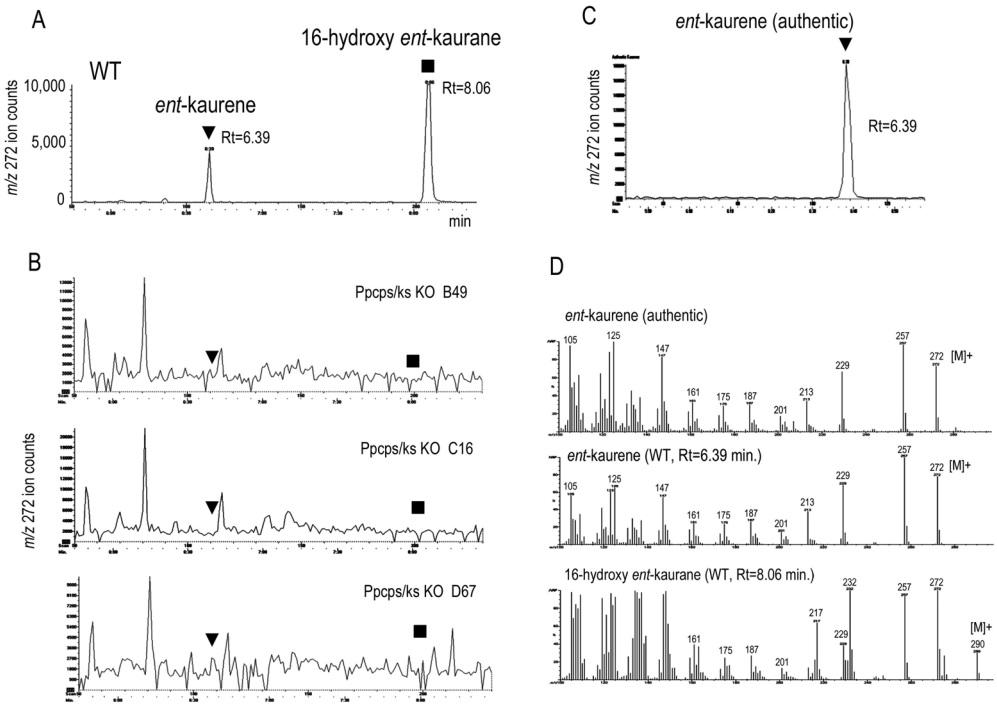

Figure S3. GC-MS chromatogram and MS spectra of *ent*-kaurene and 16-hydroxy *ent*-kaurane from wild-type and Ppcps/ks KO *P. patens* protonemata. GC-MS analysis of wild-type and *Ppcps/ks* KO lines. The selected ion (*m/z* 272) in an MS chromatogram of a wild-type extract (A) and Ppcps/ks KO B49, C16, and D67 extracts (B) and authentic sample of *ent*-kaurene (C). The triangular and square symbols indicate the peaks of *ent*-kaurene and 16-hydroxy *ent*-kaurane, respectively. D, MS spectra of *ent*-kaurene and 16-hydroxy *ent*-kaurane.

Supplemental Figure S4

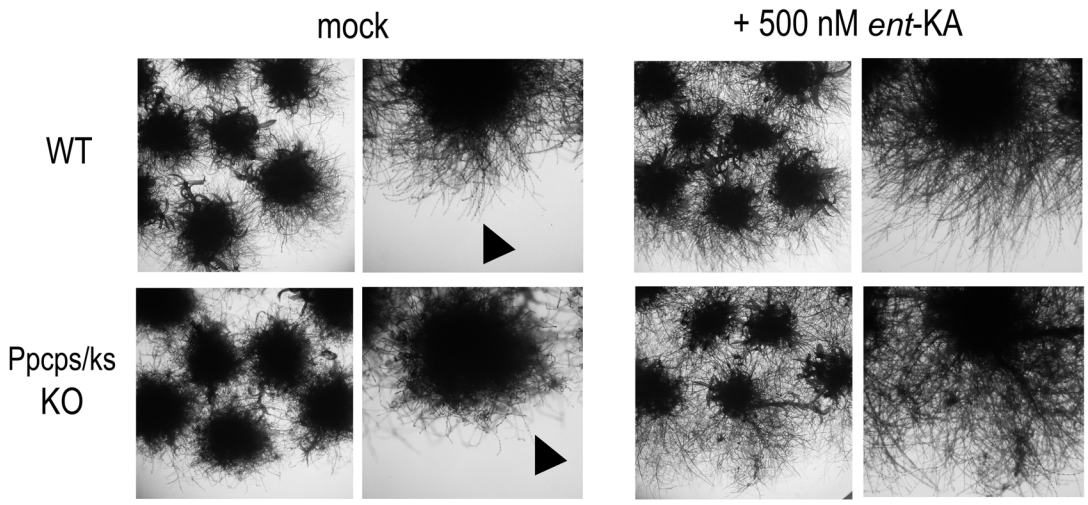

Figure S4. Effects of *ent*-kaurenoic acid on the differentiation to caulonemata. The wild-type and *Ppcps/ks* KO A74 line were grown on BCD-ATG medium with or without *ent*-kaurenoic acid for 18 days under red light. Arrow heads represent protruded caulonemata.

Supplemental Figure S5

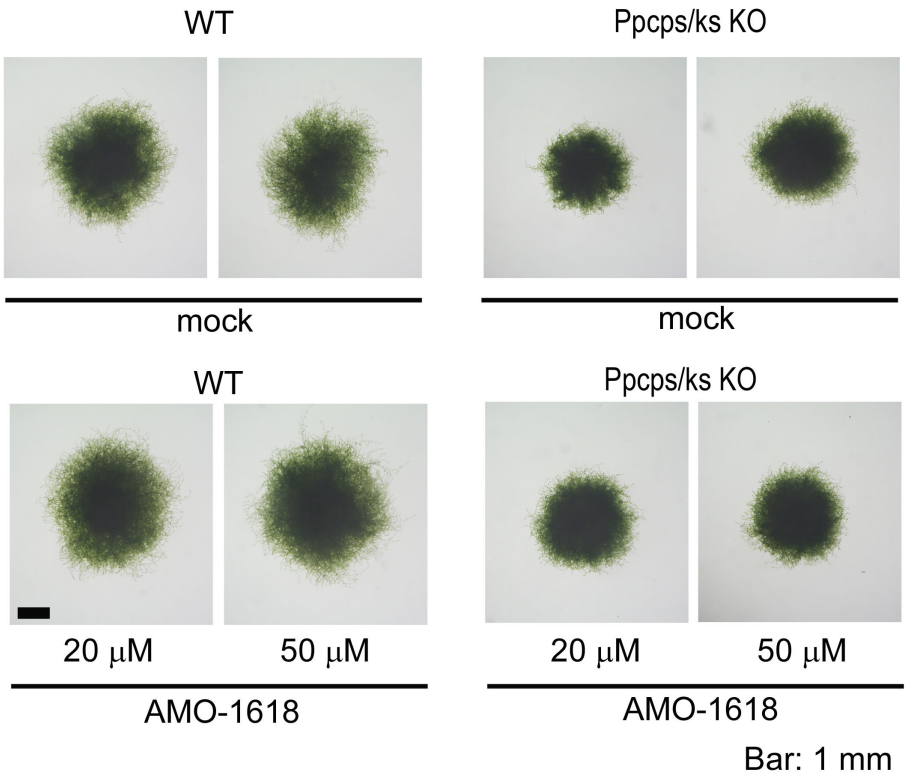

Figure S5. Effects of AMO-1618, an inhibitor of the angiosperm enzyme coparyl diphosphate synthase on *P. patens* protonemata. The wild-type (WT) and the Ppcps/ks KO A74 mutant line of *P. patens* were grown on BCD-ATG medium with or without AMO-1618 for 8 days under red light.

Supplemental Figure S6

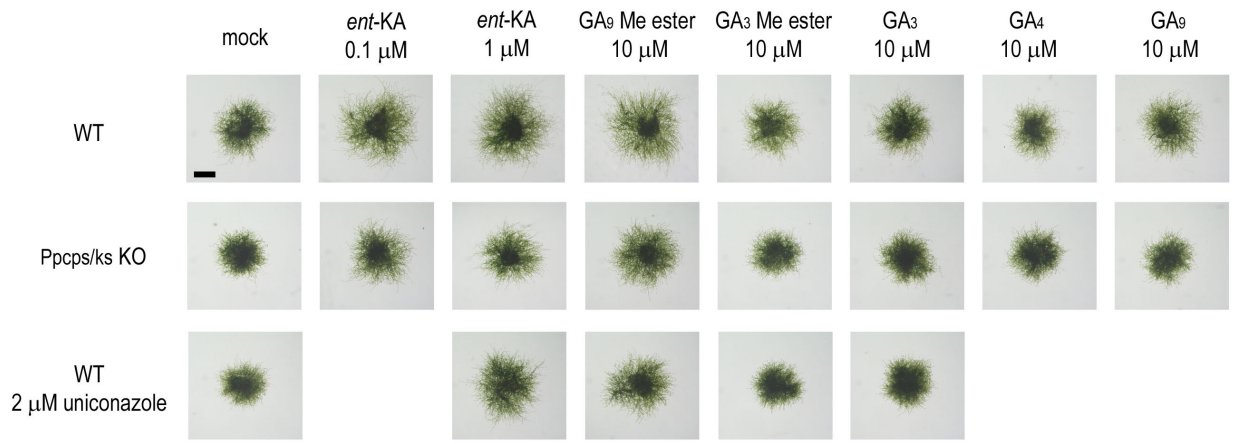

Figure S6. Effects of *ent*-kaurenoic acid, GA_9 methyl ester, GA_3 methyl ester, GA_3, GA_4, and GA_9 on the differentiation to caulonemata. Protonema of the wild-type and Ppcps/ks KO A74 line were inoculated on BCD-ATG medium supplemented with *ent*-kaurenoic acid (*ent*-KA), GA_9 methyl ester, GA_3 methyl ester, GA_3, GA_4, and GA_9 and cultivated under red light for 7 days in the presence or absence of 2 μM uniconazole. Scale bar: 1 mm.

Supplemental Figure S7

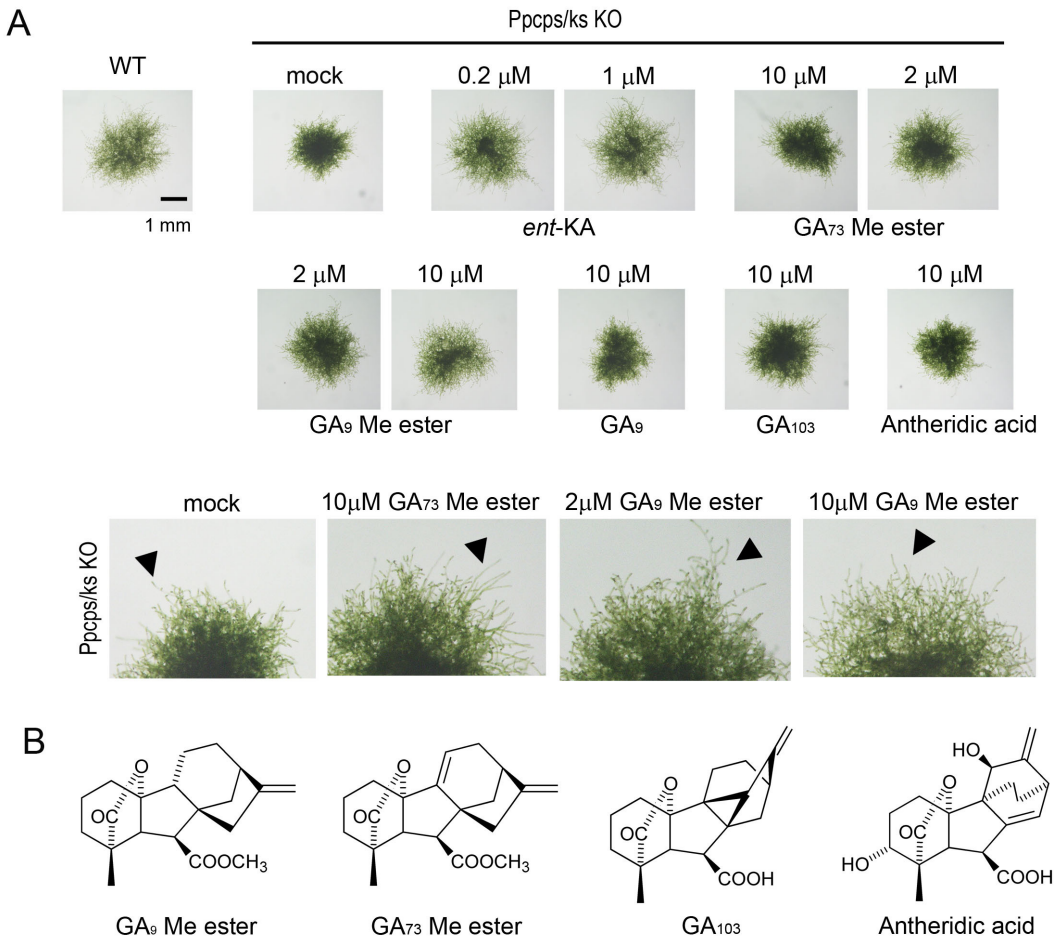

Figure S7. Effects of gibberellin-related compounds on the differentiation of chloronemata to caulonemata. A, Effects of GA_{73} methyl (Me) ester, GA_{103} and antridic acid on the differentiation to caulonemata. The WT and KO A74 mutant line were cultured on BCD-ATG medium supplemented with *ent*-kaurenoic acid, GA_{73} methyl ester, GA_9 methyl ester, GA_9, GA_{103}, and antheridic acid under red light for 7 days. Representative images of protonema colonies after cultivation were shown. The arrows represent the caulonemata. Scale bar: 1 mm. GA_{103} and antheridic acid did not restore the defects in caulonemal formation of the KO mutant. On the other hand, GA_{73} methyl ester partially recovered the caulonemal formation of the KO mutant, but only weakly. B, Structures of GA_9 methyl ester, GA_{73} methyl ester, GA_{103}, and antheridic acid, the GA-type antheridiogens of the fern.