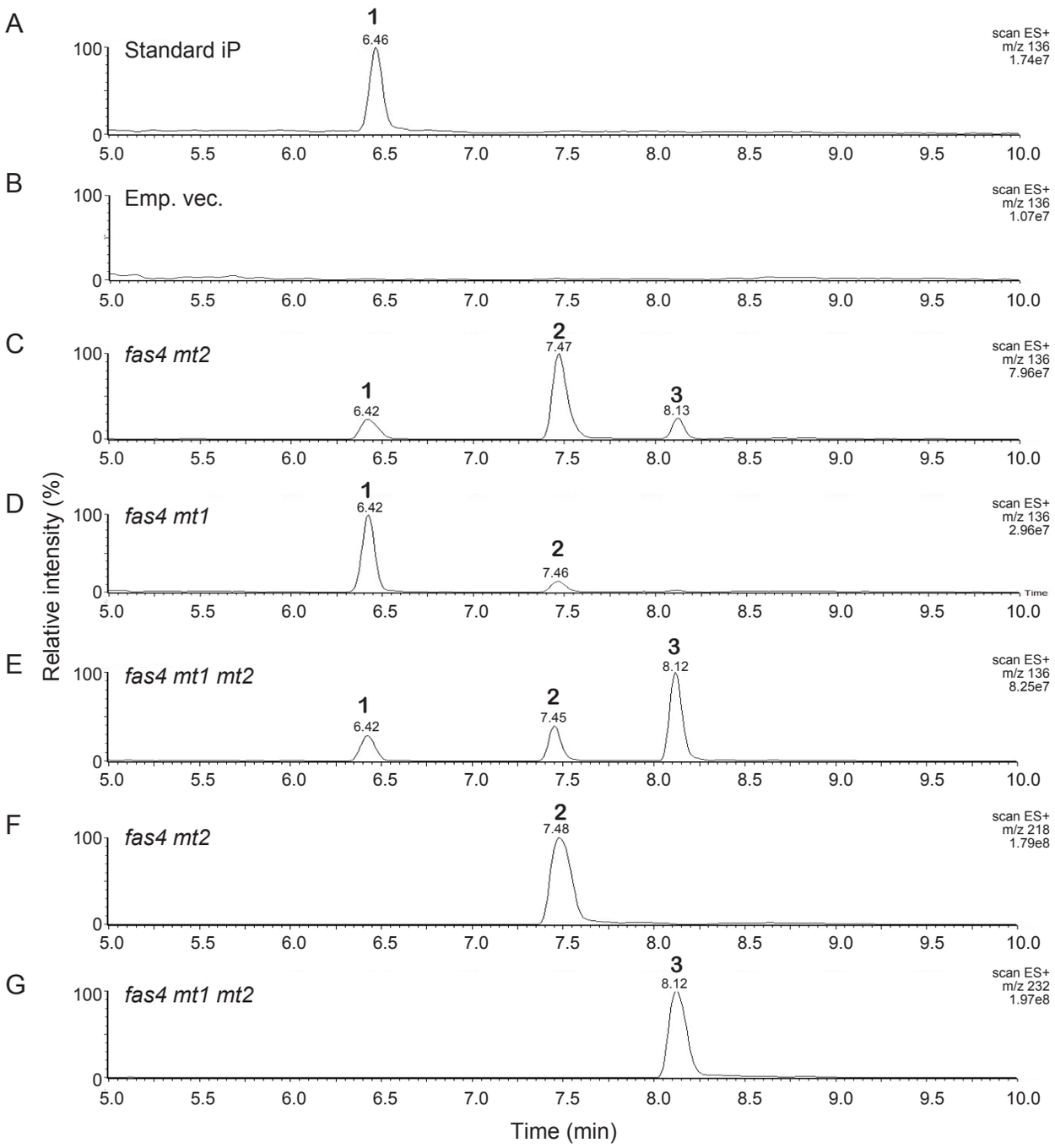
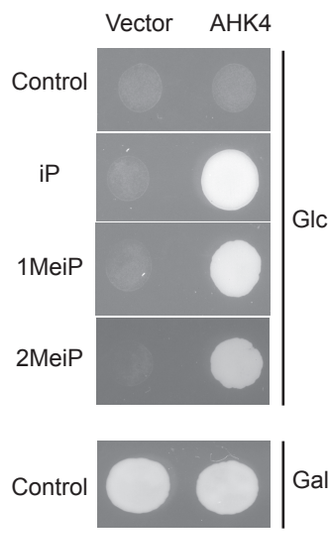


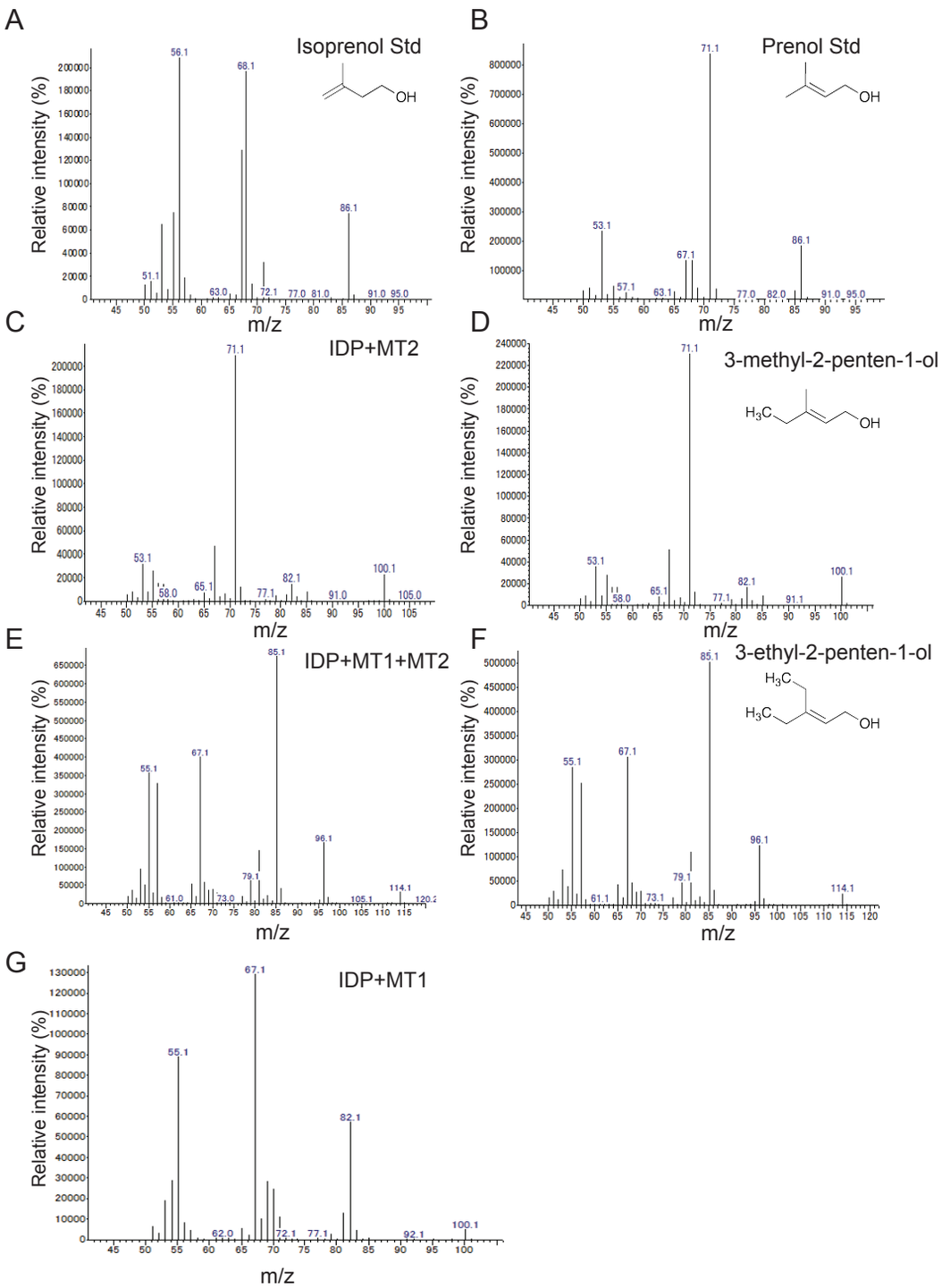
Supplemental Fig. S1. Detection of CK-like compounds in liquid culture filtrates of *E. coli* co-expressing *fas4* and *mt*s. The CK-containing fractions were prepared by solid phase extraction (1) and analyzed by liquid chromatography-quadrupole tandem mass spectrometry (LC-MS/MS). A – E, Monitoring at m/z 136 in the positive ion mode, which corresponds to the adenine moiety; F, Monitoring at m/z 218; and G, Monitoring at m/z 232. A, Standard iP; B, Control filtrate of *E. coli* harboring the empty vector (Emp. vec.); C and F, Filtrate of *E. coli* harboring *fas4mt2*; D, Filtrate of *E. coli* harboring *fas4mt1*; and E and G, Filtrate of *E. coli* harboring *fas4mt1mt2*. Peak numbers correspond to **1**, iP (m/z = 204); **2**, 1MeiP (m/z = 218); **3**, 2MeiP (m/z = 232).

(1) Kojima M, et al. (2009) Highly sensitive and high-throughput analysis of plant hormones using MS-probe modification and liquid chromatography-tandem mass spectrometry: An application for hormone profiling in *Oryza sativa*. *Plant Cell Physiol* 50(7):1201–1214.

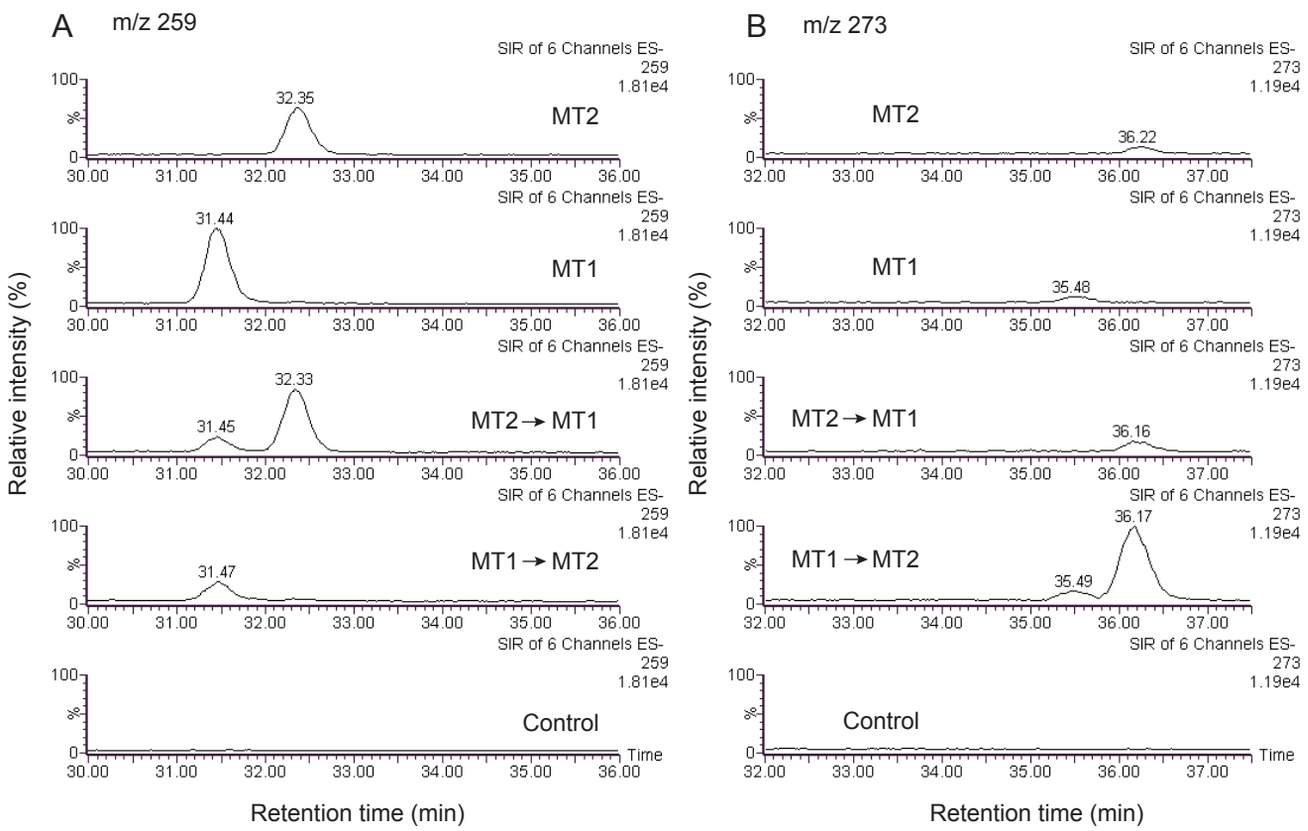


Supplemental Fig. S2. CK-dependent growth of yeast *sln1Δ* expressing *Arabidopsis* AHK4.
sln1Δ strains (TM182) (1) were transformed with the p415CYC empty vector (Vector) or p415CYC-AHK4 (AHK4). Cultures of the transformants were spotted on synthetic complete (SC) agar media containing 2% glucose (Glc) supplemented with 1 μM iP, 1MeiP, or 2MeiP. The control (Control) contains no CKs. Growth of the transformants on SC agar medium containing 2% galactose (Gal) is shown in the bottom panel. Agar plates were incubated at 30˚C for 3 days.

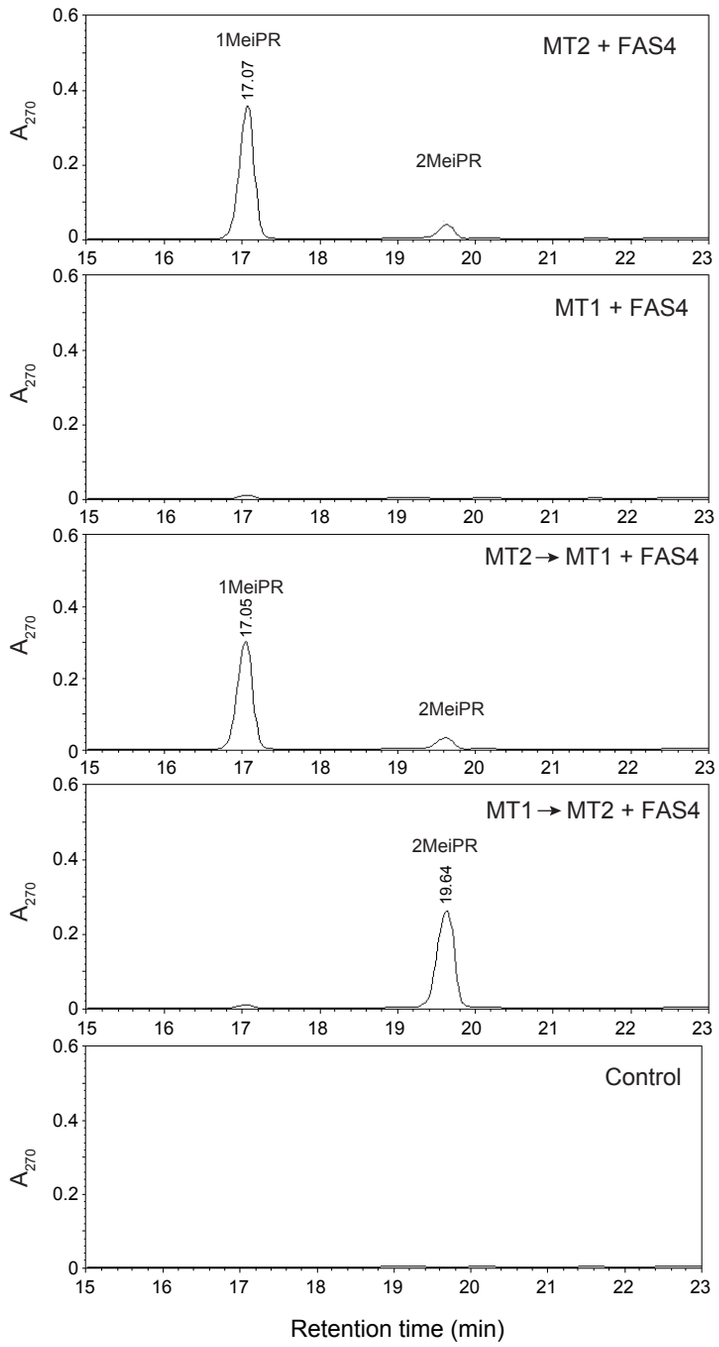
(1) Maeda T, Wurgler-Murphy SM, Saito H (1994) A two-component system that regulates an osmosensing MAP kinase cascade in yeast. *Nature* 369:242–245.



Supplemental Fig. S3. Mass spectra of methylated prenyl compounds in GC-MS with electronic ionization. A, 3-Methylbut-3-en-1-ol standard (Isoprenol Std); B, 3-Methyl-2-buten-1-ol standard (Prenol Std); C, MT2 mono-methylated product; D, Synthetic 3-methyl-2-penten-1-ol; E, Di-methylated product of the MT1+MT2 reaction; F, Synthetic 3-ethyl-2-penten-1-ol; and G, MT1 mono-methylated product.

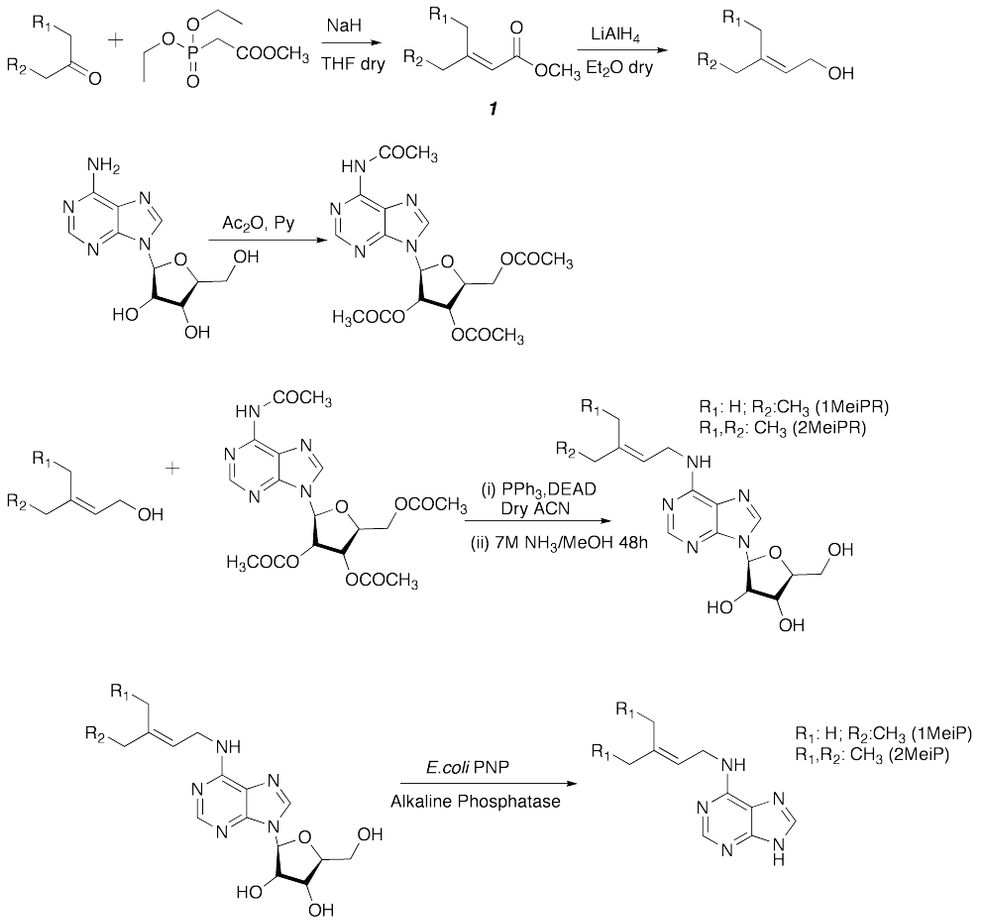


Supplemental Fig. S4. Sequential reaction assay. LC-MS chromatograms of *in vitro* MT assay reaction products. The reaction mixtures containing SAM, IDP and either MT1 or MT2 enzymes in MT buffer were incubated at 30˚C. After 20 h, the other MT enzyme was added and the reaction mixtures were further incubated for 20 h. MT1, incubation with MT1 only; MT2, incubation with MT2 only; MT2 → MT1, sequential incubation with MT2 and MT1; MT1 → MT2, sequential incubation with MT1 and MT2; Control, no enzymes. The reaction products were analyzed by LC-MS with selected ion modes: A, the mono-methylated prenyl diphosphate product (m/z = 259); B, the di-methylated prenyl diphosphate product (m/z = 273).

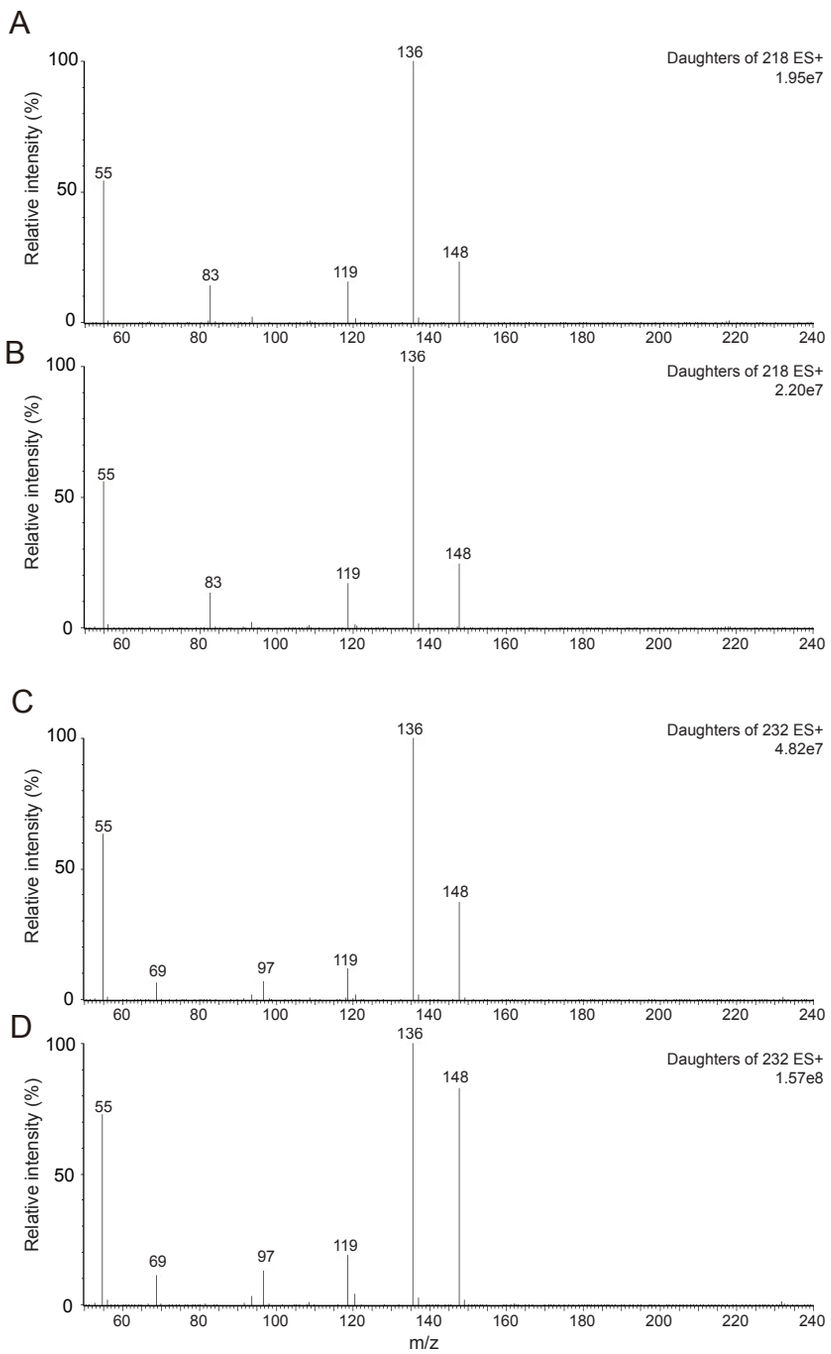


Supplemental Fig. S5. Reaction products generated by the *R. fascians* FAS4 recombinant protein using the MT products as substrates. Recombinant FAS4 protein was added to the reaction mixtures shown in Fig. S4, and the samples were incubated for 2 h at 25˚C. The generated products were detected by HPLC (Alliance 2695/PDA detector 2996, Waters) as described previously (1).

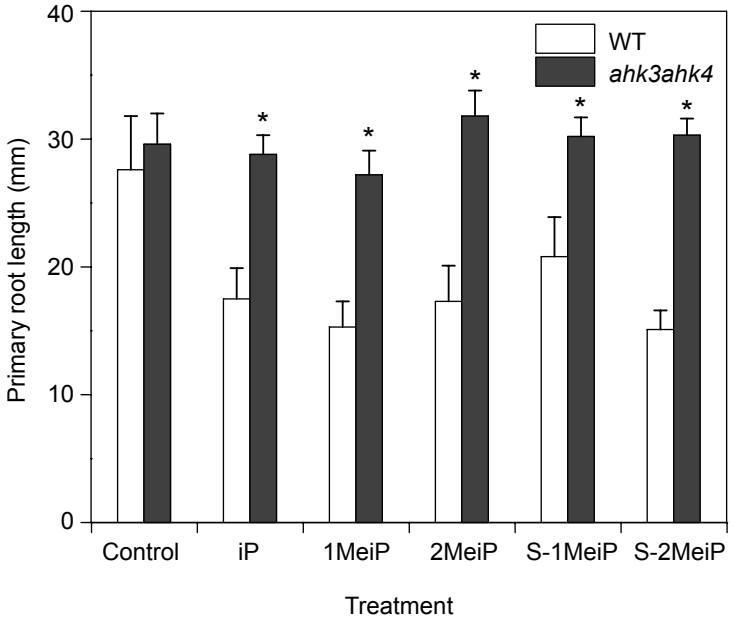
(1) Sugawara H, *et al.* (2008) Structural insight into the reaction mechanism and evolution of cytokinin biosynthesis. *Proc Natl Acad Sci USA* 105:2734-2739.



Supplemental Fig. S6. Chemical synthesis of 1MeiP and 2MeiP. THF, tetrahydrofuran; Et_2O, diethyl ether; Ac_2O, acetic anhydride; Py, pyridine; DEAD, diethyl azodicarboxylate; ACN, acetonitrile; PNP, purine-nucleoside phosphorylase.



Supplemental Fig. S7. Fragmentation patterns of purified and synthesized MeCKs. Mass spectra of daughter ions of m/z 218 (A and B) and m/z 232 (C and D) were analyzed with a LC-MS/MS in the positive ion mode. A, 1MeiP isolated from a filtrate; B, 1MeiP chemically synthesized; C, 2MeiP isolated from a filtrate; and D, 2MeiP chemically synthesized.



Supplemental Fig. S8. Effect of chemically synthesized MeCKs on primary root growth in *Arabidopsis*. Primary root length of wild type (WT) and *ahk3ahk4* double mutant *A. thaliana* plants grown on Murashige-Skoog plates containing 100 nM of the corresponding CK for 7 days. S denotes chemically synthesized MeCKs. Asterisks denote statistical significance in root length between WT and the mutant plant (one-way ANOVA, $P<0.05$). Error bars represent the SD of ten biological replicates.

Supplemental Table S1. CK concentrations in tobacco plants after infection with *R. fascians.*

Infected

dpi	iP	1MeiP	2MeiP	cZ	tZ
			pmol g^{-1} fresh weight		
1	0.13 ± 0.11	BQ	BQ	0.16 ± 0.27	2.37 ± 0.68
2	0.70 ± 0.22	0.02 ± 0.01	0.24 ± 0.02	0.09 ± 0.15	0.77 ± 0.21
3	1.57 ± 0.71	0.16 ± 0.03	1.13 ± 0.25	0.21 ± 0.19	0.78 ± 0.27
5	1.04 ± 0.43	0.09 ± 0.03	0.53 ± 0.10	0.27 ± 0.08	0.98 ± 0.30
6	0.87 ± 0.24	0.06 ± 0.00	0.59 ± 0.05	0.21 ± 0.03	0.85 ± 0.10
8	0.64 ± 0.50	0.03 ± 0.01	0.28 ± 0.04	0.19 ± 0.04	0.81 ± 0.55

Uninfected

dpi	iP	1MeiP	2MeiP	cZ	tZ
			pmol g^{-1} fresh weight		
1	BQ	BQ	BQ	BQ	4.31 ± 0.27
2	BQ	BQ	BQ	BQ	2.82 ± 0.09
3	BQ	BQ	BQ	BQ	1.32 ± 0.11
5	BQ	BQ	BQ	0.24 ± 0.07	0.91 ± 0.11
6	BQ	BQ	BQ	0.11 ± 0.06	0.85 ± 0.33
8	BQ	BQ	BQ	0.27 ± 0.08	0.79 ± 0.03

Means ± SD (*n* = 3); dpi, days post infection; BQ, below the detection limit.

Supplemental Table S2 Primers used in this study.

Sequence (5' to 3')	Purpose for use	Notes
GCTGTTGATGATAGTATGGTTG AGATATTGTAAAGCTCTTGTCG	qPCR analysis for *ARR5*	*ARR5* (At3g48100)
GAAGTTATGCTACCGAGGAAG TACGATCAACGTGACTGTCGT	qPCR analysis for *ARR6*	*ARR6* (At5g62920)
AACATTGTGCTCAGTGGTGG GTGGTGCCACGACCTTAATC	qPCR analysis for *ACT8*	*ACT8* (At1g49240)
CCGCATATGAAGGAATCAACCATGGCAC CGCGGTACCTCATCTGGCGGTCACACCTG	Construction of pCOLD-IV-fas4	Template: *R. fascians* DNA Cloning site: *Nde*I/*Kpn*I site of pCOLD-IV
AGAGGTAATACCATATGATCTCGGCAGATCAATATGCGCG ATTCGGATCCCTCGAGTCAGATGCGTTCGGCCGCAATC	Construction of pCOLD-IV-mt1	Template: *R. fascians* DNA Cloning site: *Nde*I/*Xho*I site of pCOLD-IV
CCGCATATGCCGAACCTCGACGTGGCAGTCCTC CGCCTCGAGCTACAGACGCTCGCACGCGATAACC	Construction of pCOLD-IV-mt2	Template: *R. fascians* DNA Cloning site: *Nde*I/*Xho*I site of pCOLD-IV
CGAGGGATCCGAATTCACGCCATATCGCCGAAAGGCAC AGATTACCTATCTAGAACTAGTTCAGATGCGTTCGGCCG	Construction of pCOLD-IV-fas4mt1	Template: pCOLD-IV-mt1; Cloning into *Eco*RI/*Xba*I site of pCOLD-IV-fas4
CGAGGGATCCGAATTCACGCCATATCGCCGAAAGGCAC AGATTACCTATCTAGAACTAGTCTACAGACGCTCGCACG	Construction of pCOLD-IV-fas4mt2	Template: pCOLD-IV-mt2; Cloning into *Eco*RI/*Xba*I site of pCOLD-IV-fas4
GCGTCTGTAGCTCGAGACGCCATATCGCCGAAAGGCAC CGACAAGCTTGAATTCTCAGATGCGTTCGGCCGCAATC	Construction of pCOLD-IV-mt1mt2	Template: pCOLD-IV-mt1; Cloning into *Xho*I/*Eco*RI site of pCOLD-IV-mt2
CGCCAGATGAAAGCTTACGCCATATCGCCGAAAGGCAC AGATTACCTATCTAGAACTAGTTCAGATGCGTTCGGCCG	Construction of pCOLD-IV-fas4mt1mt2	Template: pCOLD-IV-mt1mt2; Cloning into *Hin*dIII/*Xba*I site of pCOLD-IV-fas4
CCGGGTACCATGAAGGAATCAACCATGGCAC CGCAAGCTTTCATCTGGCGGTCACACCTG	Construction of pCOLD-I-fas4	Template: *R. fascians* DNA Cloning site: *Kpn*I/*Hin*dIII site of pCOLD-I
TCGAAGGTAGGCATATGATCTCGGCAGATCAATATGCGCG ATTCGGATCCCTCGAGTCAGATGCGTTCGGCCGCAATC	Construction of pCOLD-I-mt1	Template: *R. fascians* DNA Cloning site: *Nde*I/*Xho*I site of pCOLD-I
CCGCATATGCCGAACCTCGACGTGGCAGTCCTC CGCCTCGAGCTACAGACGCTCGCACGCGATAACC	Construction of pCOLD-I-mt2	Template: *R. fascians* DNA Cloning site: *Nde*I/*Xho*I site of pCOLD-I

Restriction sites in primers are underlined.