Supporting Information

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SI Materials and Methods

Growth Conditions and Feeding Experiments. Rice seedlings were grown hydroponically as described previously (1). Surface-sterilized rice seeds were incubated in sterile water at 28 °C in the dark for 2 d. The germinated seeds were transferred to hydroponic culture medium (2) solidified with 0.6% agar and cultured at 25 °C under fluorescence white light (150 μmol·m^{-2}·s^{-1}) with a 16-h light/8-h dark photoperiod for 5 d. The 1-wk-old seedlings were transferred to glass vials containing hydroponic culture media (13 mL) containing 1 μM [1-$^{13}CH_3$]-carlactone (CL) and 0.02% acetone. Hydroponic culture media were renewed every 2 d. Control plants were grown in the same volume of hydroponic culture media containing 0.02% acetone. All culture media contained 5 mM Mes and were adjusted to pH 5.7. For the −Pi treatment, 7-d-old seedlings were transferred to hydroponic culture media without Pi. For orobanchol analysis using the *d10-2* mutant, agar medium without Pi was used to increase the strigolactones (SLs) production. The following experimental procedures were the same as described above.

Arabidopsis seedlings were grown on agar medium. Seeds were sterilized in 1% sodium hypochlorite solution for 5 min, rinsed with sterile water, and stratified for 2 d at 4 °C. The seeds were placed on the half-strength Murashige and Skoog (MS) medium (3) containing 1% sucrose and 1% agar (pH 5.7) at 22 °C under fluorescence white light (60–70 μmol·m^{-2}·s^{-1}) with a 16-h light/8-h dark photoperiod for 14 d. Plants were then transferred to a glass pot containing 400 mL hydroponic solution (4) and grown under the same environmental condition for an additional 14 d.

Hydroponic culture media were renewed after 7 d. For the −Pi treatment, plants were grown in the +Pi hydroponic culture medium for 7 d, and then they were transferred to the −Pi medium and grown for another 7 d.

For feeding experiments using *Arabidopsis*, 3-wk-old plants (the *max4-7* single or the *max1-4 max4-7* double mutant, the first 2 wk on agar plates and another week in the hydroponic culture system) were transferred to glass vials containing hydroponic culture media (7 mL) containing 1 μM (*R*)-[1-$^{13}CH_3$]-CL and 0.02% acetone, and were grown for an additional 2 d. Control plants were grown in the same volume of hydroponic culture media containing 0.02% acetone.

LC-MS/MS Analysis of 5DS and CL. To analyze 2′-*epi*-5-deoxystrigol (5DS) and orobanchol released from roots, the hydroponic culture media were collected and extracted with ethyl acetate twice. The ethyl acetate phase was evaporated to dryness under nitrogen gas and dissolved in ethyl acetate:*n*-hexane (15:85). The extracts were loaded onto Sep-Pak Silica 1-mL cartridges (Waters), washed with ethyl acetate:*n*-hexane (15:85), and then eluted with ethyl acetate:*n*-hexane (50:50) for 2′-*epi*-5DS. For orobanchol, the column was washed with ethyl acetate:*n*-hexane (35:65) and then eluted with ethyl acetate:*n*-hexane (50:50). The eluates were evaporated to dryness and dissolved in 50% (vol/vol) acetonitrile, then subjected to LC-MS/MS analysis. For quantification of endogenous 2′-*epi*-5DS, internal standard was added before purification steps.

To analyze 2′-*epi*-5DS in root samples, the roots (0.5–1.0 g) were homogenized in 10 mL of acetone. The filtrates were evaporated to dryness under nitrogen gas, dissolved in deionized water, and extracted with ethyl acetate twice. The ethyl acetate phase was evaporated to dryness under nitrogen gas. The extracts were then dissolved in 20% (vol/vol) acetone and loaded onto Oasis HLB 1-mL cartridges (Waters) and eluted with 50% (vol/vol) acetone after washing with 20% (vol/vol) acetone. The eluates were evaporated to dryness under nitrogen gas, dissolved in ethyl acetate:*n*-hexane (15:85), and loaded onto Sep-Pak Silica 1-mL cartridges (Waters). The column was washed with ethyl acetate:*n*-hexane (15:85) and then eluted with ethyl acetate:*n*-hexane (50:50). The purified 2′-*epi*-5DS-containing fractions were evaporated to dryness and dissolved in 50% (vol/vol) acetonitrile and subjected to LC-MS/MS analysis. For quantification of endogenous 2′-*epi*-5DS, internal standard was added before purification steps.

To analyze CL in root samples, the roots (1–1.5 g) were homogenized in 10 mL of acetone containing [1-$^{13}CH_3$]-CL as an internal standard. The filtrates were evaporated to dryness under nitrogen gas, dissolved in deionized water, and extracted with ethyl acetate twice. The ethyl acetate phase was evaporated to dryness under nitrogen gas. The extracts were then dissolved in *n*-hexane and loaded onto Sep-Pak Silica 1-mL cartridges (Waters), washed with *n*-hexane, and then eluted with chloroform:*n*-hexane (10:90). For quantitative analysis, the eluates were evaporated to dryness under nitrogen gas, dissolved in 50% (vol/vol) acetonitrile, and subjected to LC-MS/MS analysis. For qualitative analysis of CL, the eluates of silica gel column purification step were dissolved in ethyl acetate:*n*-hexane (5:95), and further purified by HPLC with a normal-phase column (Capcel Pack silica, Shiseido, 5% (vol/vol) ethyl acetate/*n*-hexane). The CL-containing fractions were collected and evaporated to dryness under nitrogen gas and dissolved in 50% (vol/vol) acetonitrile and subjected to LC-MS/MS analysis.

To analyze SL-LIKE1 in *Arabidopsis* root samples, the roots were homogenized in 10 mL of acetone. The filtrates were evaporated to dryness under nitrogen gas, dissolved in deionized water, and extracted with ethyl acetate twice. The ethyl acetate phase was evaporated to dryness under nitrogen gas, dissolved in *n*-hexane, and loaded onto Sep-Pak Silica 1-mL cartridges (Waters), washed with *n*-hexane and chloroform:*n*-hexane (10:90), and then eluted with chloroform:*n*-hexane (30:70). The eluates were evaporated to dryness under nitrogen gas, dissolved in 50% (vol/vol) acetonitrile, and subjected to LC-MS/MS analysis.

LC-MS/MS analysis of 5DS, orobanchol, CL, and SL-LIKE1 was carried out using a system consisting of a quadrupole/time-of-flight tandem mass spectrometer (TripleTOF 5600; AB SCIEX) and an ultra high performance liquid chromatography (Nexera; Shimadzu) equipped with a reverse phase column [For 2′-*epi*-5DS, orobanchol, and SL-LIKE1, Acquity UPLC BEH-C18, ϕ2.1 × 50 mm, 1.7 μm (Waters); for CL, Acquity UPLC phenyl, ϕ2.1 × 50 mm, 1.7 μm (Waters)]. For chiral LC-MS/MS analysis, chiral column (CHIRALCEL OD-RH, ϕ4.6 × 150 mm, 5 μm; DAICEL Chemical) was used for separation.

For reverse phase chromatography of 2′-*epi*-5DS and orobanchol, the elution of the samples was carried out with 0.05% acetic acid (solvent A2) and acetonitrile with 0.05% acetic acid (solvent B2), and the mobile phase was changed from 30% (vol/vol) B2–40% (vol/vol) and 70% (vol/vol) at 1 and 6 min after the injection, respectively, at a flow rate of 0.4 mL·min^{-1}. For chiral column analysis of 5DS isomers, the elution of the samples was carried out with 60% (vol/vol) B2 at a flow rate of 0.6 mL·min^{-1}. The column temperature was 40 °C. MS/MS analysis conditions were as follows: Declustering potential, 40; collision energy, 17 V; and parent ion (*m*/*z*), 331.2 for unlabeled 5DS and 332.2 for labeled 5DS, 347.2 for unlabeled orobanchol, and 348.2 for [^{13}C]-orobanchol. Quantification was carried out by using the

fragment ion, 234.13 and 235.13, for 2′-*epi*-5DS and [6′-d_1]- 2′-*epi*-5DS, respectively.

For reverse phase chromatography of CL, the elution of the samples was carried out with 0.05% acetic acid (solvent A2) and acetonitrile with 0.05% acetic acid (solvent B2), and the mobile phase was changed from 30% (vol/vol) B2–65% (vol/vol) in 4 min after the injection at a flow rate of 0.4 mL·min^{-1}. For chiral column analysis of CL, the elution of the samples was carried out with 80% (vol/vol) B2, at a flow rate of 0.6 mL·min^{-1}. The column temperature was 40 °C. MS/MS analysis conditions were as follows: Declustering potential, 70; collision energy, 15 V; parent ion (*m/z*), 303.2 for unlabeled CL and 304.2 for labeled CL. Quantification was carried out by using a fragment ion, 97.03, both for CL and [1-$^{13}CH_3$]-CL.

For reverse phase chromatography of SL-LIKE1, the elution of the samples was carried out with 0.05% acetic acid (solvent A2) and acetonitrile with 0.05% acetic acid (solvent B2), and the mobile phase was changed from 30% (vol/vol) B2–65% (vol/vol) in 4 min after the injection at a flow rate of 0.4 mL·min^{-1}, then kept at 65% (vol/vol) B2 for 2 min at the same flow rate. The column temperature was 40 °C. MS/MS analysis conditions were as follows: Declustering potential, 70; collision energy, 15 V; parent ion (*m/z*), 347.2 for unlabeled SL-LIKE1 and 348.2 for [^{13}C]-SL-LIKE1.

***Striga hermonthica* and *Orobanche minor* Germination Assays.** *Arabidopsis atd14-2* mutant plants were grown hydroponically as described above. The roots of 3-wk-old plants were homogenized in 10 mL of acetone. The filtrates were evaporated to dryness under nitrogen gas, dissolved in deionized water, and extracted with ethyl acetate twice. The ethyl acetate phase was evaporated to dryness under nitrogen gas and dissolved in ethyl acetate:*n*-hexane (15:85). The extracts were loaded onto Sep-Pak Silica 1-mL cartridges (Waters) and then eluted with ethyl acetate:*n*-hexane (50:50). The flow-through and eluted fractions were combined and evaporated to dryness, dissolved in 5% ethyl acetate:*n*-hexane (5:95), and then subjected to HPLC separation using a normal-phase column (SG120, ϕ4.6 × 200 mm, 5 μm; Shiseido). The samples were eluted with a linear gradient system from 5 to 60% (vol/vol) ethyl acetate/*n*-hexane in 30 min. The samples were fractionated every min and the presence of SL-LIKE1 in each fraction was checked by LC-MS/MS. The fraction containing SL-LIKE1 (fraction no. 16) was evaporated to dryness using nitrogen gas, and the germination tests were carried out using *S. hermonthica* and *O. minor* seeds. The germination assays were performed as described previously (5).

Synthesis of [1-$^{13}CH_3$]-CL. ***General experimental procedure.*** Mass spectra were recorded on a JEOL JMS-700 instrument. ^{1}H- and ^{13}C-NMR spectra were obtained with a JEOL JNM-AL400 NMR spectrometer. Chemical shifts were referenced to tetramethylsilane as an internal standard. Optical rotations were recorded on a JASCO P-2100 polarimeter. CD spectra were measured with a JASCO J-820 spectropolarimeter. Column chromatography was performed with Wakogel C-200 (Wako Pure Chemical), Kieselgel 60 (Merck), Inertsil ODS-3 (ϕ10 × 250 mm, 5 μm; GL Sciences), and Chiralpak AD-H (ϕ 10 × 250 mm; Daicel Chemical).

[2-$^{13}CH_3$]-2,2,6-trimethylcyclohexanone. A solution of 2,6-dimethylcyclohexanone (2.96 g, 23.5 mmol) in tetrahydrofuran (6 ml) was added dropwise to a solution of lithium diisopropylamine (0.5 M in tetrahydrofuran and hexane, 48.7 ml, 24.4 mmol) at −78 °C under argon. The mixture was stirred for 1.5 h and then [^{13}C]-methyl iodide (5.00 g, 35.0 mmol) was added. After stirring for 1 h at −78 °C, the mixture was warmed to room temperature and stirred for 21 h. The reaction mixture was quenched by adding a mixture of saturated aqueous NH_4Cl (60 mL), water (8 mL), and ether (30 mL), and extracted with ether. The organic phase was dried over anhydrous Na_2SO_4, filtered, and evaporated to give [2-$^{13}CH_3$]-2,2,6-trimethylcyclohexanone as a colorless oil (3.29 g, 23.3 mmol, 99%). The spectral data are as follows: ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 0.99 (3H, d, $^3J_{HH}$ = 6.3 Hz, 6-CH_3), 1.04 (3H, appeared as two doublets, $^1J_{CH}$ = 126 Hz, $^3J_{CH}$ = 4.9 Hz, 2-$^{13}CH_3$), 1.18 (3H, appeared as two doublets, $^1J_{CH}$ = 127 Hz, $^3J_{CH}$ = 4.9 Hz, 2-$^{13}CH_3$), 1.26–2.09 (6H, m, H-3, -4 and -5), 2.63–2.69 (1H, m, H-6); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 15.0, 21.6, 25.3 (2-$^{13}CH_3$), 25.7 (2-$^{13}CH_3$), 36.8, 40.8, 41.8, 45.5 (appeared as two doublets, $^1J_{CC}$ = 33.2 Hz coupled to 25.3 ppm 2-$^{13}CH_3$, $^1J_{CC}$ = 38.2 Hz coupled to 25.7 ppm 2-$^{13}CH_3$), 217.5; EIMS *m/z*: 141 $[M]^+$, 126, 83; HREIMS *m/z*: 141.1249 $[M]^+$ (calcd. for $C_8{}^{13}CH_{16}O$, *m/z* 141.1235).

[6-$^{13}CH_3$]-2,6,6-trimethylcyclohex-1-enyl trifluoromethanesulfonate. To a solution of lithium diisopropylamide (0.67 M in 1,2-dimethoxyethane and hexane, 35.6 mL, 23.3 mmol) was added [2-$^{13}CH_3$]-2,2,6-trimethylcyclohexanone (1.10 g, 7.80 mmol) in 1,2-dimethoxyethane (11 mL) dropwise at −78 °C under argon. The mixture was stirred for 2 h and then *N*-phenylbis (trifluoromethanesulfonimide) (8.41 g, 23.5 mmol) in 1,2-dimethoxyethane (33 mL) was added. The mixture was warmed to 0 °C and stirred for 15 h. The reaction mixture was poured into 10% (wt/vol) HCl, and extracted with *n*-hexane. The organic phase was successively washed with 10% (wt/vol) HCl, 10% (wt/vol) NaOH, and water, dried over anhydrous Na_2SO_4, and concentrated in vacuo. Purification by silica gel column chromatography eluting with *n*-hexane gave the triflate as a colorless oil (2.00 g, 7.32 mmol, 94%). The spectral data are as follows: ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 1.15 (6H, appeared as two doublets, $^1J_{CH}$ = 127 Hz, $^3J_{CH}$ = 4.9 Hz, 6-CH_3 and 6-$^{13}CH_3$), 1.58–1.68 (4H, m, H-4 and -5), 1.75 (3H, s, 2-CH_3), 2.15 (2H, t, $^3J_{HH}$ = 5.9 Hz, H-3); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 17.6, 18.6 (d, $^3J_{CC}$ = 1.7 Hz), 26.4 (6-$^{13}CH_3$), 32.5, 35.6 (d, $^1J_{CC}$ = 35.7 Hz), 40.5, 118.8 (q, $^1J_{CF}$ = 317 Hz), 126.1, 150.2 (d, $^2J_{CC}$ = 2.5 Hz); EIMS *m/z*: 273 $[M]^+$, 258; HREIMS *m/z*: 273.0702 $[M]^+$ (calcd. for $C_9{}^{13}CH_{15}F_3O_3S$, *m/z* 273.0728).

[6′-$^{13}CH_3$]-(E)-4-(2,6,6-Trimethylcyclohex-1-enyl)but-3-en-2-one. A mixture of [6-$^{13}CH_3$]-2,6,6-trimethylcyclohex-1-enyl trifluoromethanesulfonate (5.58 g, 20.5 mmol), triethylamine (8.29 g, 81.9 mmol), methyl vinyl ketone (2.87 g, 41.0 mmol), and bis(triphenylphosphine)palladium(II) dichloride (273 mg, 0.390 mmol) in *N,N*-dimethylformamide (54 mL) was stirred at 75 °C for 22 h under argon. The reaction mixture was ice-cooled, quenched by adding water, and extracted with *n*-hexane. The organic phase was washed with water, dried over anhydrous Na_2SO_4, and concentrated in vacuo. Purification by silica gel column chromatography using 2% (vol/vol) stepwise elution with ether and *n*-hexane gave the ionone as a colorless oil (3.59 g, 18.6 mmol, 91%). The spectral data are as follows: ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 1.07 (6H, appeared as two doublets, $^1J_{CH}$ = 126 Hz, $^3J_{CH}$ = 4.9 Hz, 6′-CH_3 and 6′-$^{13}CH_3$), 1.47–1.50 (2H, m, H-5′), 1.60–1.66 (2H, m, H-4′), 1.77 (3H, s, 2′-CH_3), 2.07 (2H, t, $^3J_{HH}$ = 5.9 Hz, H-3′), 2.30 (3H, s, H-1), 6.12 (1H, d, $^3J_{HH}$ = 16.1 Hz, H-3), 7.28 (1H, d, $^3J_{HH}$ = 16.1 Hz, H-4); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 18.9, 21.8, 27.2, 28.8 (6′-$^{13}CH_3$), 33.6, 34.1 (d, $^1J_{CC}$ = 35.7 Hz), 39.7, 131.6, 135.9, 136.2, 143.2, 198.9; EIMS *m/z*: 193 $[M]^+$, 178; HREIMS *m/z*: 193.1543 $[M]^+$ (calcd. for $C_{12}{}^{13}CH_{20}O$, *m/z* 193.1548).

[6′-$^{13}CH_3$]-(E)-2-methyl-4-(2,6,6-trimethylcyclohex-1-enyl) but-3-enal. To a mixture of [6′-$^{13}CH_3$]-(*E*)-4-(2,6,6-trimethylcyclohex-1-enyl) but-3-en-2-one (1.35 g, 7.00 mmol), ethyl chloroacetate (1.16 g, 9.51 mmol), pyridine (687 mg, 8.68 mmol), and phenothiazine (3.5 mg) was slowly added sodium methoxide (567 mg, 10.5 mmol) at −15 °C under argon. The mixture was stirred at −5 °C for 30 min, warmed to room temperature, and was again cooled to −5 °C. After stirring for 3 h, 15% (wt/vol) sodium hydroxide in methanol (2.84 mL) was slowly added at −5 °C. The mixture was stirred at 10 °C for 1 h and cooled to 0 °C. The reaction mixture was quenched by adding acetic acid (4.2 mL) at 0 °C, diluted with water, extracted with ether. The organic phase was

washed with water, dried over anhydrous Na_2SO_4, and concentrated in vacuo. Purification by silica gel column chromatography eluting with 2% (vol/vol) ether in *n*-hexane gave the C_{14}-aldehyde as an orange oil (583 mg, 2.82 mmol, 40%). The spectral data are as follows: ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 0.98 (6H, appeared as two doublets, $^1J_{CH}$ = 125 Hz, $^3J_{CH}$ = 4.6 Hz, 6′-CH_3 and 6′-$^{13}CH_3$), 1.24 (3H, d, $^3J_{HH}$ = 6.8 Hz, 2-CH_3), 1.42–1.63 (4H, m, H-4′ and -5′), 1.67 (3H, s, 2′-CH_3), 1.96–1.99 (2H, m, H-3′), 3.12–3.19 (1H, m, H-2), 5.31 (1H, dd, $^3J_{HH}$ = 16.1 Hz, 7.8 Hz, H-3), 6.04 (1H, d, $^3J_{HH}$ = 16.1 Hz, H-4), 9.62 (1H, d, $^3J_{HH}$ = 1.5 Hz, H-1); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 13.6, 19.2, 21.4, 28.6 (6′-$^{13}CH_3$), 32.6, 33.8 (d, $^1J_{CC}$ = 35.7 Hz), 39.2, 50.8, 129.1, 129.4, 131.9, 137.0, 201.8; EIMS *m/z*: 207 $[M]^+$, 178; HREIMS *m/z*: 207.1689 $[M]^+$ (calcd. for $C_{13}{}^{13}CH_{22}O$, *m/z* 207.1704).

[6″-$^{13}CH_3$]-3-methyl-5-((1Z,3E)-2-methyl-4-(2,6,6-trimethylcyclohex-1-enyl) buta-1,3-dienyloxy)furan-2(5H)-one ([1-$^{13}CH_3$]-CL) and [6″-$^{13}CH_3$]-3-methyl-5-((1E,3E)-2-methyl-4-(2,6,6-trimethylcyclohex-1-enyl) buta-1,3-dienyloxy)furan-2(5H)-one ([1-$^{13}CH_3$]-9E-CL). To a mixture of [6′-$^{13}CH_3$]-(*E*)-2-methyl-4-(2,6,6-trimethylcyclohex-1-enyl)but-3-enal (600 mg, 2.90 mmol) and phenothiazine (14.3 mg) in dimethyl sulfoxide (5.8 mL) was added a solution of dimsyl sodium (1.2 M in dimethyl sulfoxide, 5.84 mL, 7.01 mmol) at room temperature under argon. After stirring for 1 min, (±)-4-bromo-2-methyl-2-buten-4-olide (616 mg, 3.48 mmol) was added, and the mixture was stirred at room temperature for 2.5 h. The reaction mixture was poured into 0.1 N HCl, extracted with ether. The organic phase was washed with water, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The residue was subjected to silica gel column chromatography eluted with 3, 10, and 15% (vol/vol) ethyl acetate in hexane. The 10% (vol/vol) ethyl acetate eluate containing CLs was purified by a semipreparative Inertsil ODS-3 HPLC (ϕ10 × 250 mm, 5 μm; GL Sciences), using isocratic elution with 80% (vol/vol) acetonitrile in water at a flow rate of 4.0 mL·min^{-1} and monitoring at 280 nm to give ^{13}C-labeled CL [3.7 mg, 0.012 mmol, 0.4%, retention time (*Rt*) 18.7 min] and 9*E*-CL (38.2 mg, 0.126 mmol, 4.3%, *Rt* 20.0 min) as a pale yellow oil. The spectral data of [1-$^{13}CH_3$]-CL are as follows: ^{1}H -NMR (CD_2Cl_2, 400 MHz) δ: 0.99 (6H, appeared as two doublets, $^1J_{CH}$ = 125 Hz, $^3J_{CH}$ = 4.6 Hz, $^{13}CH_3$-16,17), 1.43–1.47 (2H, m, H-2), 1.58–1.63 (2H, m, H-3), 1.68 (3H, s, CH_3-18), 1.73 (3H, d, $^4J_{HH}$ = 1.5 Hz, CH_3-19), 1.94 (3H, t, $^{4,5}J_{HH}$ = 1.6 Hz, CH_3-15), 1.99 (2H, br t, $^3J_{HH}$ = 5.9 Hz, H-4), 5.97 (1H, br p, $^{3,5}J_{HH}$ = 1.4 Hz, H-11), 6.04 (1H, br d, $^3J_{HH}$ = 16.5 Hz, H-7), 6.20 (1H, s, H-10), 6.46 (1H, d, $^3J_{HH}$ = 16.5 Hz, H-8), 6.90 (1H, br p, $^{3,4}J_{HH}$ = 1.6 Hz, H-12); ^{13}C-NMR (CD_2Cl_2, 100 MHz) δ: 10.7, 14.4, 19.6 (d, $^3J_{CC}$ = 1.7 Hz), 21.8, 29.0 ($^{13}CH_3$-16,17), 33.2, 34.4 (d, $^1J_{CC}$ = 35.7 Hz), 39.8, 100.7, 117.5, 127.4, 127.5, 129.3 (d, $^3J_{CC}$ = 2.5 Hz), 135.0, 138.2, 138.3, 142.6, 171.5; EI-MS *m/z* (rel. int): 303 $[M]^+$ (17), 206 (41), 178 (96), 121 (90), 107 (51), 97 (35), 95 (63), 70 (100); HREIMS *m/z*: 303.1899 $[M]^+$ (calcd. for $C_{18}{}^{13}CH_{26}O_3$, *m/z* 303.1915). The spectral data of [1-$^{13}CH_3$]-9*E*-CL are as follows: ^{1}H-NMR (CD_2Cl_2, 400 MHz) δ: 0.986 (3H, appeared as two doublets, $^1J_{CH}$ = 125 Hz, $^3J_{CH}$ = 4.9 Hz, $^{13}CH_3$-16 or 17), 0.990 (3H, appeared as two doublets, $^1J_{CH}$ = 125 Hz, $^3J_{CH}$ = 4.6 Hz, $^{13}CH_3$-17 or 16), 1.43–1.47 (2H, m, H-2), 1.57–1.63 (2H, m, H-3), 1.67 (3H, s, CH_3-18), 1.77 (3H, d, $^4J_{HH}$ = 1.2 Hz, CH_3-19), 1.95 (3H, t, $^{4,5}J_{HH}$ = 1.6 Hz, CH_3-15), 1.99 (2H, br t, $^3J_{HH}$ = 6.3 Hz, H-4), 5.88 (1H, d, $^3J_{HH}$ = 15.9 Hz, H-8), 6.01 (1H, d, $^3J_{HH}$ = 15.9 Hz, H-7), 6.01 (1H, br p, $^{3,5}J_{HH}$ = 1.4 Hz, H-11), 6.42 (1H, s, H-10), 6.92 (1H, br p, $^{3,4}J_{HH}$ = 1.6 Hz, H-12); ^{13}C-NMR (CD_2Cl_2, 100 MHz) δ: 9.7, 10.7, 19.6 (d, $^3J_{CC}$ = 1.6 Hz), 21.7, 28.9 ($^{13}CH_3$-16,17), 33.2, 34.4 (d, $^1J_{CC}$ = 34.9 Hz), 39.8, 100.7, 119.5, 125.2, 128.8 (d, $^3J_{CC}$ = 1.7 Hz), 132.0, 135.1, 138.1, 141.7, 142.6, 171.5; EIMS *m/z* (rel. int) 303 $[M]^+$ (31), 206 (47), 178 (100), 121 (76), 107 (43), 97 (39), 95 (51), 70 (90).

Optical Resolution of [1-$^{13}CH_3$]-CL. (±)-[1-$^{13}CH_3$]-CL was chromatographed on a semipreparative Chiralpak AD-H HPLC column (ϕ10 × 250 mm; Daicel Chemical) using isocratic elution with 0.5% isopropanol-*n*-hexane at a flow rate of 2.4 mL·min^{-1} and monitoring at 260 nm. Two single peaks at 15.8 and 19.1 min were collected. The enantiomeric purities of two enantiomers were estimated to be >99% e.e., respectively, by analytical HPLC using a Chiralpak AD-H column (ϕ 4.6 × 250 mm, 5 μm; Daicel Chemical) using isocratic elution with 0.5% isopropanol-*n*-hexane at a flow rate of 0.5 mL·min^{-1} monitored at 260 nm. The specific rotation and CD data of fast-moving [1-$^{13}CH_3$]-CL enantiomer are as follows: $[\alpha]_D{}^{25}$ +68° (*c* 0.059, acetonitrile); CD (acetonitrile) λ_{max} (Δε) nm: 271 (3.43), 244 (1.33), 215 (3.46), 194 (–5.26). The specific rotation and CD data of slow-moving [1-$^{13}CH_3$]-CL enantiomer are as follows: $[\alpha]_D{}^{25}$ −58° (*c* 0.092, acetonitrile); CD (acetonitrile) λ_{max} (Δε) nm: 269 (−3.50), 247 (−1.57), 217 (−3.25), 195 (3.99).

Synthesis of CL Diels-Alder Adduct Enantiomers. ***2-Methyl-(5S)-((1Z,3E)-2-methyl-4-(2,6,6-trimethylcyclohex-1-enyl) buta-1,3-dien-1-yloxy)-4-oxa-endo tricyclo[5.2.1.0^{2,6}]dec-8-en-3-one and 2-methyl-(5S)-((1E,3E)-2-methyl-4-(2,6,6-trimethylcyclohex-1-enyl) buta-1,3-dien-1-yloxy)-4-oxa-endo tricyclo[5.2.1.0^{2,6}]dec-8-en-3-one.*** To a mixture of the C_{14}-aldehyde (268 mg, 1.30 mmol) and phenothiazine (6.5 mg) in dimethyl sulfoxide (2.63 mL) was slowly added a solution of dimsyl sodium (1.2 M in dimethyl sulfoxide, 2.61 mL, 3.13 mmol) at room temperature under argon. (5*R*)-Chloro-(2*R*)-methyl-4-oxa-*endo* tricyclo[5.2.1.0^{2,6}]dec-8-en-3-one (310 mg, 1.56 mmol) (6) was added, and the mixture was stirred at room temperature for 3 h. The reaction mixture was poured into 0.1 N HCl, extracted with ether. The organic phase was washed with water, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The residue was subjected to silica gel column chromatography eluted stepwise with *n*-hexane and ethyl acetate. The 3 and 6% (vol/vol) ethyl acetate eluates were purified by a semipreparative Inertsil ODS-3 HPLC column (ϕ10 × 250 mm, 5 μm; GL Sciences), using isocratic elution with 90% (vol/vol) acetonitrile in water at a flow rate of 4.0 mL·min^{-1} and monitoring at 260 nm to give (11*S*)-CL Diels-Alder adduct (4.1 mg, 0.011 mmol, 0.9%, *Rt* 13.7 min) and (11*S*)-9*E*-CL Diels-Alder adduct (31.0 mg, 0.084 mmol, 6.5%, *Rt* 14.9 min). The spectral data of (11*S*)-CL Diels-Alder adduct are as follows: ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 1.01 (3H, s, 6′′-CH_3), 1.03 (3H, s, 6′′-CH_3), 1.45–1.65 (4H, m, H-4′′ and -5′′), 1.58 (3H, s, 2-CH_3), 1.68 (2H, t, *J* = 1.5 Hz, H-10), 1.70 (3H, d, *J* = 1.2 Hz, 2′-CH_3), 1.71 (3H, s, 2′′-CH_3), 2.01 (2H, t, *J* = 6.0 Hz, H-3′′), 2.66 (1H, d, *J* = 4.2 Hz, H-6), 2.85 (1H, s, H-7), 3.15 (1H, br s, H-1), 5.02 (1H, s, H-5), 6.01 (1H, d, *J* = 16.3 Hz, H-4′), 6.06 (1H, s, H-1′), 6.17 (1H, dd, *J* = 5.7, 2.9 Hz, H-8 or -9), 6.27 (1H, dd, *J* = 5.7, 3.1 Hz, H-8 or -9), 6.47 (1H, d, *J* = 16.3 Hz, H-3′); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 14.4, 19.3, 21.7, 22.6, 28.9, 29.0, 33.0, 34.1, 39.6, 45.2, 49.8, 51.9, 53.7, 54.4, 103.0, 116.6, 126.5, 127.2, 129.0, 133.7, 137.6, 137.9, 138.3, 179.8; EIMS *m/z* (rel. Int): 368 $[M]^+$(23), 206 (20), 177 (33), 163 (88), 97 (100); HREIMS *m/z*: 368.2348 $[M]^+$ (calcd. for $C_{24}H_{32}O_3$, *m/z* 368.2351); $[\alpha]_D{}^{25}$–40° (*c* 0.20, acetonitrile). The spectral data of (11*S*)-9*E*-CL Diels-Alder adduct are as follows: ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 0.98 (3H, s, 6′′-CH_3), 0.99 (3H, s, 6′′-CH_3), 1.43–1.61 (4H, m, H-4′′ and -5′′), 1.57 (3H, s, 2-CH_3), 1.66 (3H, d, *J* = 1.0 Hz, 2′′-CH_3), 1.69 (2H, t, *J* = 1.6 Hz, H-10), 1.76 (3H, d, *J* = 1.2 Hz, 2′-CH_3), 1.98 (2H, t, *J* = 6.3 Hz, H-3′′), 2.68 (1H, dd, *J* = 4.1, 1.0 Hz, H-6), 2.86 (1H, m, H-7), 3.17 (1H, br s, H-1), 5.05 (1H, d, *J* = 1.0 Hz, H-5), 5.84 (1H, d, *J* = 15.9 Hz, H-3′), 5.96 (1H, d, *J* = 15.9 Hz, H-4′), 6.18 (1H, dd, *J* = 5.9, 2.9 Hz, H-8 or -9), 6.28 (1H, m, H-8 or -9), 6.28 (1H, s, H-1′); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 9.7, 19.3, 21.6, 22.6, 28.8, 28.9, 32.8, 34.1, 39.5, 45.2, 49.8, 51.9, 53.7, 54.4, 103.1, 118.6, 124.3, 128.3, 131.9, 133.7, 137.6, 137.8, 141.7, 179.7; EIMS *m/z* (rel. Int): 368 $[M]^+$(9), 204(16), 193(18), 177(25), 163(86), 97(100); HREIMS *m/z*: 368.2362 $[M]^+$ (calcd. for $C_{24}H_{32}O_3$, *m/z* 368.2351); $[\alpha]_D{}^{25}$ +90.0° (*c* 1.72, acetonitrile).

2-Methyl-(5R)-((1Z,3E)-2-methyl-4-(2,6,6-trimethylcyclohex-1-enyl) buta-1,3-dien-1-yloxy)-4-oxa-endo tricyclo[5.2.1.0^{2,6}]dec-8-en-3-one and 2-methyl-(5R)-((1E,3E)-2-methyl-4-(2,6,6-trimethylcyclohex-1-enyl) buta-1,3-dien-1-yloxy)-4-oxa-endo tricyclo[5.2.1.0^{2,6}]dec-8-en-3-one. To a mixture of the C_{14}-aldehyde (361 mg, 1.75 mmol) and phenothiazine

(8.7 mg) in dimethyl sulfoxide (3.54 mL) was slowly added a solution of dimsyl sodium (1.2 M in dimethyl sulfoxide, 3.52 mL, 4.22 mmol) at room temperature under argon. (5*S*)-Chloro-(2*S*)-methyl-4-oxa-*endo* tricyclo[5.2.1.$0^{2,6}$]dec-8-en-3-one (417 mg, 2.10 mmol) (6) was added, and the mixture was stirred at room temperature for 3 h. The reaction mixture was poured into 0.1 N HCl, extracted with ether. The organic phase was washed with water, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The residue was subjected to silica gel column chromatography eluted stepwise with *n*-hexane and ethyl acetate. The 3 and 6% (vol/vol) ethyl acetate eluates were purified by a semipreparative Inertsil ODS-3 HPLC column (ϕ10 × 250 mm, 5 μm; GL Sciences), using isocratic elution with 90% (vol/vol) acetonitrile in water at a flow rate of 4.0 mL·min^{-1} and monitoring at 260 nm to give (11*R*)-CL Diels-Alder adduct (4.7 mg, 0.013 mmol, 0.7%, *Rt* 13.7 min) and (11*R*)-9*E*-CL Diels-Alder adduct (32.1 mg, 0.087 mmol, 5%, *Rt* 14.8 min). The spectral data of (11*R*)-CL Diels-Alder adduct are as follows: $[\alpha]_D^{25}$ +40° (*c* 0.30, acetonitrile); ^{1}H- and ^{13}C-NMR and mass data were the same as for (11*S*)-CL Diels-Alder adduct. The spectral data of (11*R*)-9*E*-CL Diels-Alder adduct are as follows: $[\alpha]_D^{25}$ −85° (*c* 1.59, acetonitrile); ^{1}H- and ^{13}C-NMR and mass data were the same as for (11*S*)-9*E*-CL Diels-Alder adduct.

Determination of the Absolute Configuration at C-11 of CL Enantiomers. To a solution of bis(trifluoromethanesulfonyl)methane (Tf_2CH_2) (14 mg, 0.05 mmol) in dichloromethane (1.0 mL) was added trimethylaluminum (Me_3Al) (1.4 M in *n*-hexane, 72 μL, 0.10 mmol), and stirred for 1 h at room temperature under nitrogen (7). The Tf_2CH_2-Me_3Al solution (13 μL, 0.66 μmol Tf_2CH_2) was added to a solution of (+)- or (−)-CL (100 μg, 0.33 μmol) in cyclopentadine (100 μL, 79 mg, 1.2 mmol). The mixture was stirred at room temperature for 3 h under nitrogen, and was subjected to silica gel chromatography. After washing the column with 10 bed volumes of *n*-hexane, the reaction product was eluted stepwise with *n*-hexane and ethyl acetate. The 4% (vol/vol) ethyl acetate eluate containing the Diels-Alder adduct was concentrated in vacuo, and dissolved in acetonitrile. The fraction was subjected to LC/electrospray ionization-MS analysis using a system consisting of a single quadrupole mass spectrometer (LCMS-2020; Shimadzu) and an ultra-fast liquid chromatograph (Prominence $UFLC_{XR}$; Shimadzu) equipped with a chiral HPLC column (Chiralcel OD-RH, ϕ 4.6 × 150 mm, 5 μm; Daicel Chemical) using isocratic elution with 85% (vol/vol) methanol-water at a flow rate of 0.6 mL·min^{-1}. The column temperature was 35 °C. The Diels-Alder adducts were detected by SIM (*m*/*z* 391.2 $[M+Na]^+$) and identified by comparing the retention times with those of authentic standards [(11*R*)-CL Diels-Alder adduct, *Rt* 14.1 min; (11*S*)-CL Diels-Alder adduct, *Rt* 16.4 min].

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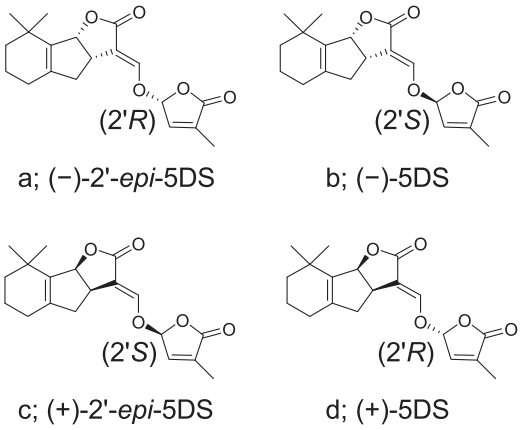

Fig. S1. Chemical structures of four stereoisomers of 5DS.

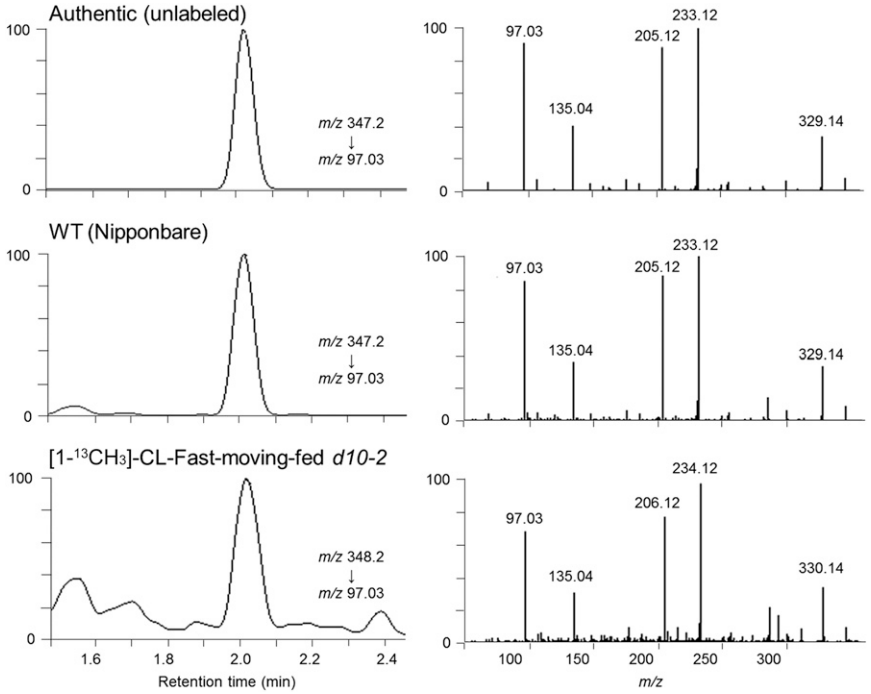

Fig. S2. LC-MS/MS analysis of orobanchol from *d10-2* root exudates after feeding [1-$^{13}CH_3$]-CL-fast-moving. Selected reaction monitoring (*Left*) and the full-scan spectrum of fragment ions (*Right*) are shown.

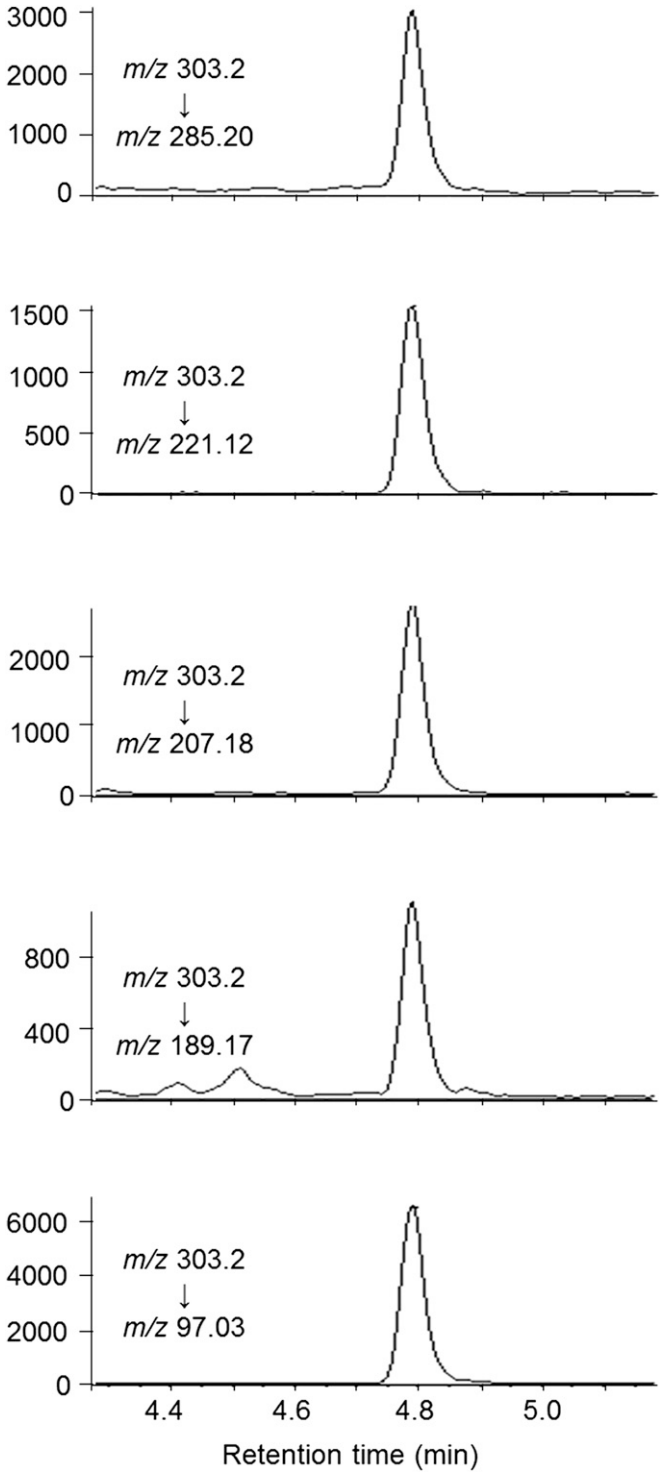

Fig. S3. LC-MS/MS analysis of endogenous CL from root extracts of rice *d14-1*. Selected reaction monitoring using major fragment ions are shown.

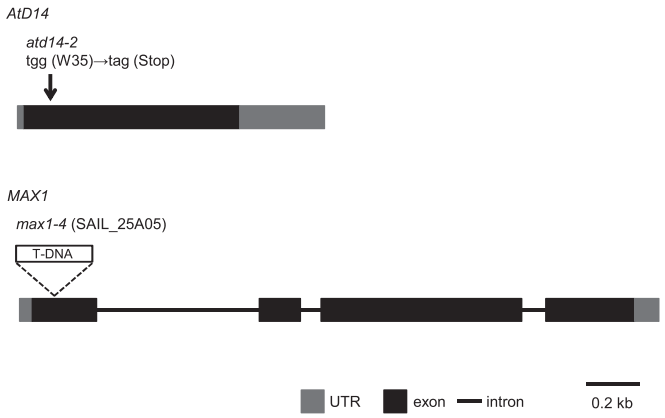

Fig. S4. Schematic diagram showing the *atd14-2* and *max1-4* mutations in the *AtD14* (At3g03990) and *MAX1* (At2g26170) genes, respectively.

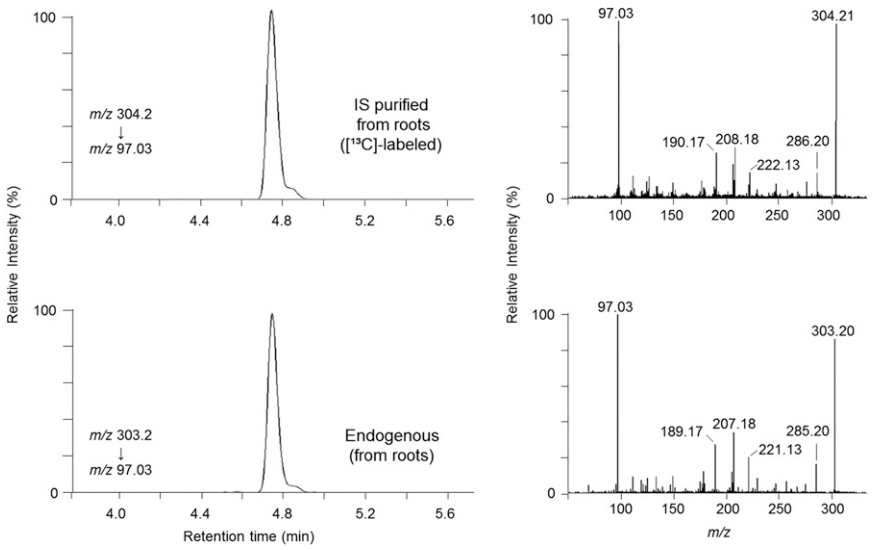

Fig. S5. LC-MS/MS analysis of CL from root extracts of *Arabidopsis atd14-2*. Selected reaction monitoring (*Left*) and full-scan spectra of fragment ions (*Right*) of [1-$^{13}CH_3$]-CL purified from root extracts and endogenous CL.

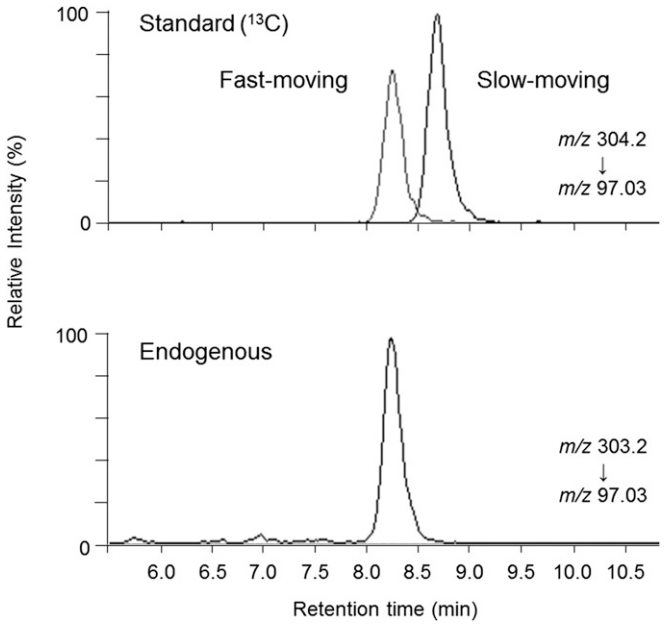

Fig. S6. Chiral LC-MS/MS analysis of CL from root extracts of *Arabidopsis atd14-2*. Selected reaction monitoring of authentic standards of two enantiomers of [1-$^{13}CH_3$]-CL and endogenous CL.

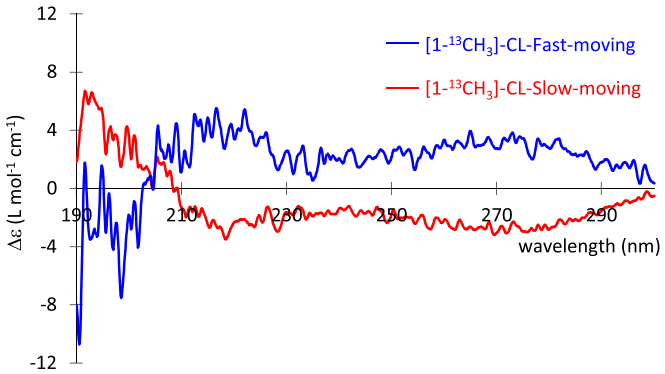

Fig. S7. Circular dichroism (CD) spectra of two stereoisomers of synthetic [1-$^{13}CH_3$]-CL.

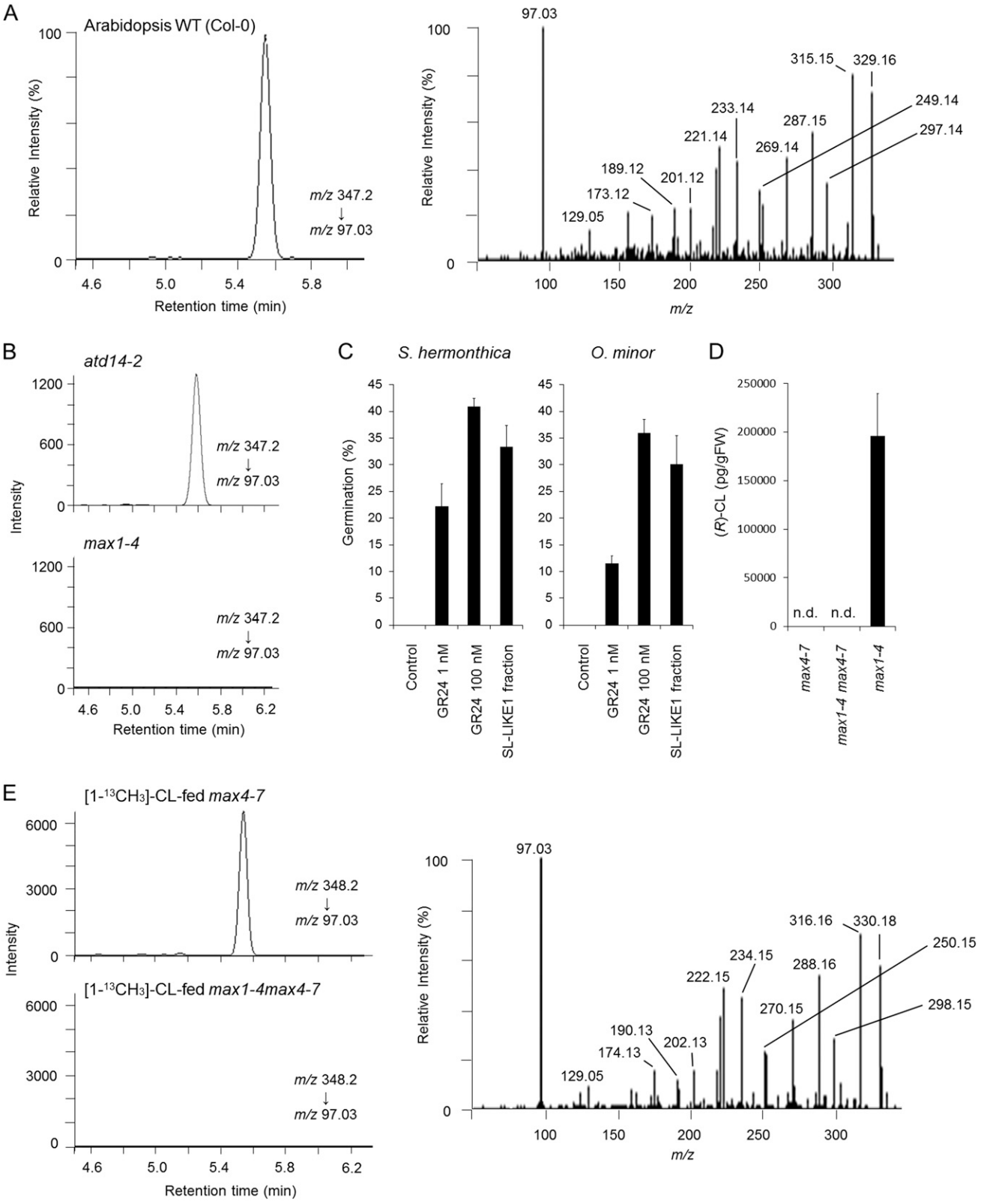

Fig. S8. Contribution of MAX1 to the conversion of CL to SL-LIKE1 in *Arabidopsis*. (*A*) LC-MS/MS analysis of SL-like molecule (SL-LIKE1) from *Arabidopsis* WT root extracts. Selected reaction monitoring (*Left*) and the full-scan spectrum of fragment ions (*Right*) are shown. (*B*) LC-MS/MS analysis of SL-LIKE1 from the root extracts of *Arabidopsis atd14* and *max1-4* mutants. (*C*) Germination assay of the SL-LIKE1 containing fraction using *S. hermonthica* and *O. minor* seeds. Control is distilled water. Data are the means ± SD ($n = 3$). (*D*) Quantitative analysis of CL in *Arabidopsis max4-7*, *max1-4 max4-7*, and *max1-4*. Data are the means ± SD ($n = 3$). N.d., not detected due to low abundance. (*E*) LC-MS/MS analysis of SL-LIKE1 after feeding (*R*)-[1-$^{13}CH_3$]-CL using *max4-7* and *max1-4 max4-7*. Selected reaction monitoring (*Left*) and the full-scan spectrum of fragment ions of SL-LIKE1 from (*R*)-[1-$^{13}CH_3$]-CL fed *max4-7* (*Right*) are shown.