Supporting Information

General synthetic and analytical methods

5-(and-6)-Carboxyfluorescein succinimidyl ester and 5-carboxyfluorescein succinimidyl ester were purchased from Invitrogen. All other chemicals were purchased from Sigma-Aldrich and used as received unless otherwise noted. Anhydrous solvents and reagents (DMF) were obtained as SureSeal bottles from Sigma-Aldrich. Thin-layer chromatography and flash chromatography were performed using EMD pre-coated silica gel 60 F-254 plates and silica gel 60 (230-400 mesh). UV absorbance and fluorescence spectra were recorded on a Cary 3E (Varian) and Fluorolog 2 (Spex) fluorimeter, respectively. Analytical and preparative HPLCs were performed on Agilent HPLCs, with Luna C18(2) columns (Phenomenex) using water (solvent A) and acetonitrile (solvent B) with 0.05% TFA as an additive. Low resolution ESI mass spectrometry was performed on an Agilent LC/MSD Trap XCT coupled to an Agilent HPLC. High-resolution mass spectra were acquired on a ThermoFisher Orbitrap XL hybrid mass spectrometer. ^{1}H- and ^{13}C-NMR spectra were collected in d_6-DMSO (Cambridge Isotope Laboratories, Cambridge, MA) at 25 ºC using a Varian Unity Inova spectrometer at 500 MHz at the Department of Chemistry and Biochemistry NMR Facility at the University of California, San Diego. All chemical shifts are reported in the standard δ notation of parts per million using the peak of residual proton signals of d_6-DMSO as an internal reference.

Synthetic chemistry methods

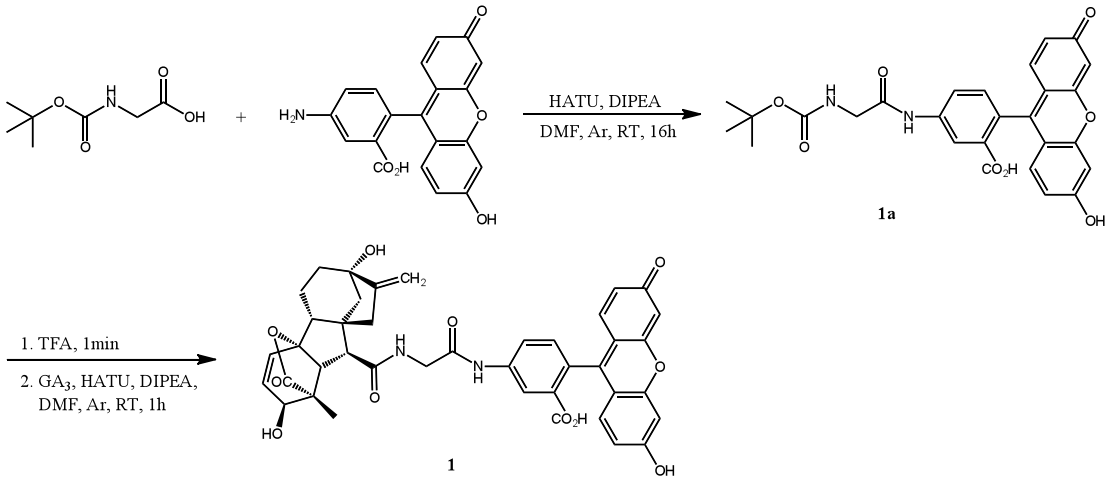

Fig. S1. Synthetic scheme of compound **1**.

Compound **1a**

Boc-glycine (50 mg, 286 µmol, 2.05 eq) was dissolved in 1 mL dry DMF and DIPEA (50 µL, 286 µmol, 2.05 eq) and HATU (109 mg, 286 µmol, 2.05 eq) were added. The mixture was stirred under argon atmosphere for 1 minute then 5-aminofluorescein (48 mg, 139 µmol, 1 eq) and DIPEA (50 µL, 286 µmol, 2.05 eq) were added and the reaction mixture was stirred for 16 hours at room temperature. Then, solvent was removed under reduced pressure. The residue was dissolved in 2 mL MeOH:water 3:1, LiOH monohydrate (11.5 mg, 278 µmol, 2 eq) was added and the mixture stirred for 2 hours at room temperature. The reaction mixture was diluted with 20 mL water, acidified with 1 M HCl (pH ~2-3) and extracted 3 times with EtOAc. The combined organic layers were dried on $MgSO_4$, filtered and the solvents removed under reduced pressure. The residue was dissolved in 2 mL DMSO:ACN:water (2:1:1), filtered with Costar® Spin-X® centrifuge filter tube (9,000 rpm for 5 minutes) and the desired product was purified using preparative HPLC (see general HPLC purification conditions below) (retention time: 14.59 minutes). 25 mg of compound **1** (50 µmol, yield 36%) as a yellow solid were obtained.

^{1}H-NMR (DMSO-d_6, 500 MHz): δ = 1.40 (9H, s), 3.76 (2H, d, J = 6.3 Hz), 6.53 (2H, dd, J_1 = 8.3 Hz, J_2 = 2.9 Hz), 6.58 (2H, d, J = 9.2 Hz), 6.66 (2H, d, J = 2.0 Hz), 7.18 (1H, t, J = 6.1 Hz), 7.21 (1H, d, J = 8.3 Hz), 7.82 (1H, dd, J_1 = 8.3, J_2 = 2.0 Hz), 8.31 (1H, d, J = 2.0 Hz), 10.14 (1H, s), 10.44 (1H, s). ^{13}C-NMR (DMSO-d_6, 125 MHz): δ = 169.1, 168.6, 159.4, 156.1, 152.0, 146.7, 140.6, 129.2, 129.1, 127.0, 126.3, 124.5, 113.4, 112.2, 109.4, 102.0, 78.1, 43.9, 28.2. ESI-MS (positive mode) calculated ($C_{27}H_{24}N_2O_8$) 504.1, found m/z $[M+H]^+$ 505.3.

Compound **1**

Compound **1a** (25 mg, 50 µmol, 1 eq) was dissolved in 1 mL TFA and stirred for 1 minute. TFA was immediately removed under reduced pressure. The residue was dissolved in dry DMF under argon atmosphere and DIPEA (22 µL, 125 µmol, 2.5 eq) was added. In a separate 1.5 mL eppendorf, Gibberellin A_3 (29 mg, 75 µmol, 1.5 eq) was dissolved in dry DMF, then DIPEA (13 µL, 75 µmol, 1.5 eq) and HATU (29 mg, µL, 75

µmol, 1.5 eq) were added. The mixture was vortexed for 1 minute and then added to the TFA-treated compound **1a**. The reaction was stirred at room temperature for 1 hour and the solvent removed under reduced pressure. The residue was dissolved in 2 mL DMSO:ACN:water (2:1:1), filtered with Costar® Spin-X® centrifuge filter tube and the desired product was purified using preparative HPLC (see general HPLC purification conditions below) (retention time: 12.77 minutes). 22 mg of compound **1** (30 µmol, yield 60%) as a yellow solid were obtained.

^{1}H-NMR (DMSO-d_6, 500 MHz): d = 1.11 (3H, s), 1.53-1.76 (5H, m), 1.77-1.93 (2H, m), 2.06 (1H, d, J = 16.8 Hz), 2.22 (1H d, J = 16.8 Hz), 2.70 (1H, d, J = 10.3 Hz), 3.12 (1H, d, J = 10.7 Hz), 3.85 (1H, d, J = 3.5 Hz), 3.95 (2H, d, J = 5.4 Hz), 4.79 (1H, s), 5.08 (1H, s), 5.78 (1H, dd, J_1 = 9.8 Hz, J_2 = 3.4 Hz), 6.33 (1H, d, J = 8.8 Hz), 6.53 (2H, dd, J_1 = 8.8 Hz, J_2 = 2.5 Hz), 6.59 (2H, d, J = 8.8 Hz), 6.66 (2H, d, J = 2.5 Hz), 7.22 (1H, d, J = 8.3 Hz), 7.79 (1H, dd, J_1 = 8.3 Hz, J_2 = 1.5 Hz), 8.30 (1H, d, J = 2.0 Hz), 10.13 (1H, s), 10.51 (1H, s). ^{13}C-NMR (DMSO-d_6, 125 MHz): d = 179.2, 168.6, 168.4, 159.5, 158.2, 152.0, 146.7, 140.5, 131.6, 129.2, 127.1, 126.2, 113.3, 112.6, 109.6, 104.5, 102.0, 90.9, 76.8, 68.6, 52.6, 51.0, 50.5, 44.8, 43.4, 43.0, 40.4, 16.7, 14.5. ESI-MS (positive mode) calculated ($C_{41}H_{36}N_2O_{11}$) 732.2, found m/z $[M+H]^+$ 733.4.

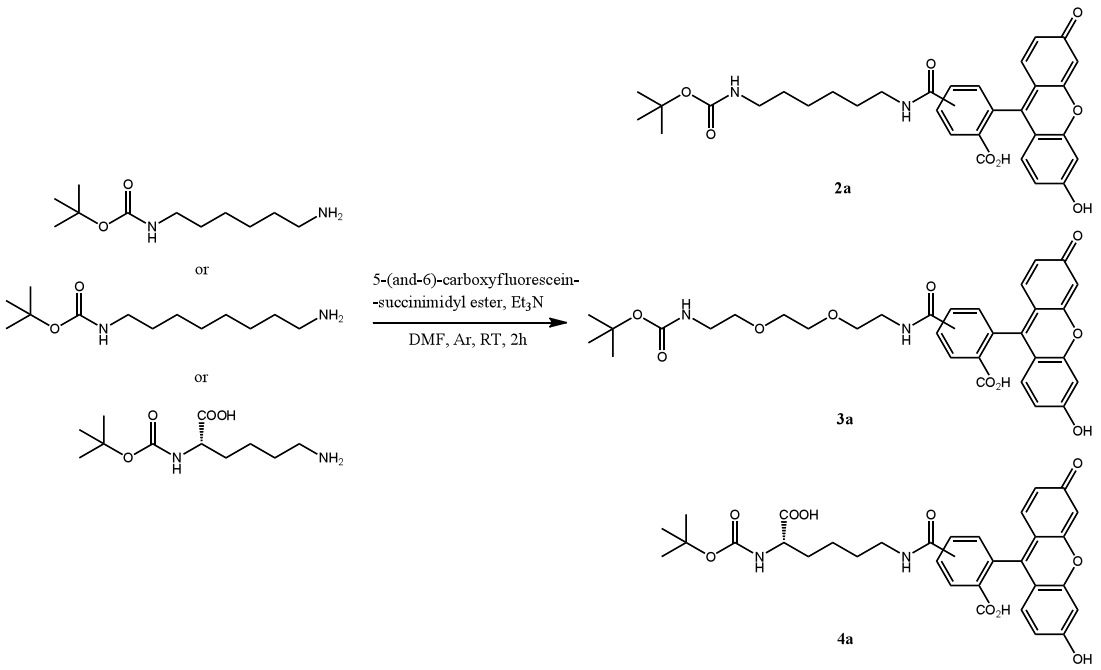

Fig. S2. Synthetic scheme of compounds **2a**, **3a** and **4a**.

General procedure for synthesis of **2a**, **3a**, and **4a**.

5-(and-6)-Carboxyfluorescein succinimidyl ester (mixed isomers) (20 mg, 42 µmol, 1 eq) was dissolved in 750 µL dry DMF under argon atmosphere. The appropriate amine (N-Boc-1,6-hexanediamine, *N*-Boc- 2,2′-(ethylenedioxy)diethylamine or N_α-Boc-lysine) was added (1.1 eq), followed by Et_3N (6.5 µL, 46 µmol, 1.1 eq). The reaction mixture was stirred for 2 hours at room temperature then the solvent was evaporated under reduced pressure. The residue was dissolved in 2 mL DMSO:ACN:water 2:1:1, filtered with Costar® Spin-X® centrifuge filter tube and the desired product was purified using preparative HPLC (see general HPLC purification conditions below). Compounds **2a**, **3a** and **4a** were isolated as a mixture of the 5-(and-6)-carboxy-fluorescein isomers (^{1}H-NMR signals are labeled ^{5} for the 5-carboxyfluorescein isomer and ^{6} for the 6-carboxyfluorescein isomer whenever signals differ, and ^{5,6} when signals overlap. Signals integration is calibrated for the 5-carboxyfluorescein isomer. ^{13}C-NMR signals are reported for the mixture).

Compound **2a**: Prepared from N-Boc-1,6-hexanediamine. Preparative HPLC retention time: 14.26 min. Obtained 20 mg yellow solid (35 µmol, yield 83%).

^{1}H-NMR (DMSO-d_6, 500 MHz): d = 1.14-1.33 (8H, m), 1.34 (3.6H, s)^6, 1.36 (9H, s)^5, 1.41-1.47 (0.8H, m)^6, 1.48-1.60 (2H, m)^5, 2.85 (0.8H, dd, J_1 = 6.8 Hz, J_2 = 5.9 Hz)^6, 2.89 (2H, dt, J_1 = 6.8 Hz, J_2 = 6.4 Hz)^5, 3.17 (0.8H, dt, J_1 = 6.8 Hz, J_2 = 6.3 Hz)^6, 3.29 (2H, dt, J_1 = 6.3 Hz, J_2 = 6.3 Hz)^5, 6.52-6.62 (5.6H, m)^{5,6}, 6.66-6.71 (2.8H, m)^{5,6}, 6.75 (0.4H, t, J = 5.3 Hz)^6, 6.80 (1H, t, J = 5.3 Hz)^5, 7.36 (1H, d, J = 7.8 Hz)^5, 7.66 (0.4H, s)^6, 8.06 (0.4H, d, J = 8.3 Hz)^6, 8.16 (0.4H, dd, J_1 = 8.3 Hz, J_2 = 1.0 Hz)^6, 8.23 (1H, dd, J_1 = 8.3 Hz, J_2 = 1.5 Hz)^5, 8.25 (1H, s)^5, 8.67 (0.4H, t, J = 5.8 Hz)^6, 8.81 (1H, t, J = 5.4 Hz)^5, 10.17 (1.4H, s)^{5,6}. ^{13}C-NMR (DMSO-d_6, 125 MHz): d = 168.2, 164.4, 159.6, 155.6, 154.6, 151.8, 136.4, 129.2, 126.5, 123.2, 112.6, 109.2, 102.3, 77.4, 77.2, 31.0, 30.8, 29.5, 29.4, 29.0, 28.9, 28.3, 26.2, 26.1. ESI-MS (positive mode) calculated ($C_{32}H_{34}N_2O_8$) 574.2, found *m*/*z* $[M+H]^+$ 575.4.

Compound **3a**: Prepared from *N*-Boc- 2,2′-(ethylenedioxy)diethylamine. Preparative HPLC retention time: 14.46 min. Obtained 21 mg yellow solid (34 µmol, yield 81%).

^{1}H-NMR (DMSO-d_6, 500 MHz): δ = 1.34 (3.6H, s)[6], 1.36 (9H, s)[5], 2.99 (0.8H, q, *J* = 6.4 Hz)[6], 3.03 (2H, q, *J* = 5.9 Hz)[5], 3.30 (0.8H, t, *J* = 5.9 Hz)[6], 3.37 (2H, t, *J* = 5.9 Hz)[5], 3.42-3.55 (11.2H, m)[5,6], 6.51-6.56 (2.8H, m)[5,6], 6.59-6.70 (2.8H, m)[5,6], 6.73 (0.4H, t, *J* = 5.4 Hz)[6], 6.77 (1H, t, *J* = 5.9 Hz)[5], 7.37 (1H, d, *J* = 8.8 Hz)[5], 7.68 (0.4H, s)[6], 8.06 (0.4H, d, *J* = 7.8 Hz)[6], 8.16 (0.4H, dd, J_1 = 7.8 Hz, J_2 = 1.5 Hz)[6], 8.24 (1H, dd, J_1 = 7.8 Hz, J_2 = 1.5 Hz)[5], 8.46 (1H, s)[5], 8.77 (0.4H, t, *J* = 5.4 Hz)[6], 8.90 (1H, t, *J* = 5.4 Hz)[5], 10.17 (1.4H, s)[5,6]. ^{13}C-NMR (DMSO-d_6, 125 MHz): δ = 168.2, 164.7, 159.6, 155.6, 154.7, 151.8, 140.6, 136.1, 134.8, 129.2, 128.2, 126.5, 124.3, 123.3, 122.3, 112.7, 112.6, 109.2, 109.1, 102.3, 102.2, 77.6, 69.6, 69.5, 69.4, 69.3, 69.2, 68.8, 68.7, 68.6, 34.3, 34.0, 31.0, 30.7, 28.2, 28.1, 24.8, 22.1, 14.0. ESI-MS (positive mode) calculated ($C_{32}H_{34}N_2O_{10}$) 606.2, found *m*/*z* $[M+H]^+$ 607.3.

Compound **4a**: Prepared from N_a-Boc-lysine. Preparative HPLC retention time: 14.07 minutes. Obtained 21 mg yellow solid (34 μmol, yield 81%).

^{1}H-NMR (DMSO-d_6, 500 MHz): δ = 1.16-1.44 (5.2H, m)[5,6], 1.33 (2.7H, s, partially overlaps with 1.16-1.44 signal)[6], 1.36 (9H, s, partially overlaps with 1.16-1.44 signal)[5], 1.45-1.72 (2.6H, m)[5,6], 3.15-3.41 (2.6H, m)[5,6], 3.76-3.81 (0.3H, m)[6], 3.82-3.88 (1H, m)[5], 6.52-6.61 (5.2H, m)[5,6], 6.67-6.71 (2.6H, m)[5,6], 7.02 (0.3H, d, *J* = 8.3 Hz)[6], 7.07 (1H, d, *J* = 7.8 Hz)[5], 7.36 (1H, d, *J* = 7.4 Hz)[5], 7.66 (0.3H, s)[6], 8.06 (0.3H, d, *J* = 8.3 Hz)[6], 8.16 (0.3H, dd, J_1 = 8.3 Hz, J_2 = 1.5 Hz)[6], 8.23 (1H, dd, J_1 = 7.8 Hz, J_2 = 1.5 Hz)[5], 8.45 (1H, s)[5], 8.69 (0.3H, t, *J* = 5.4 Hz)[6], 8.30 (1H, t, *J* = 5.4 Hz)[5], 10.17 (1.3H, s)[5,6]. ^{13}C-NMR (DMSO-d_6, 125 MHz): δ = 174.3, 168.2, 164.4, 159.6, 155.6, 154.7, 151.2, 140.81, 136.6, 134.8, 126.5, 124.9, 124.2, 112.8, 109.1, 102.2, 82.3, 78.0, 53.4, 34.2, 34.0, 31.0, 30.1, 30.5, 28.6, 28.2, 24.8, 23.2, 22.1, 18.7, 14.0, 11.3. ESI-MS (positive mode) calculated ($C_{32}H_{32}N_2O_{10}$) 604.2, found *m*/*z* $[M+H]^+$ 605.3.

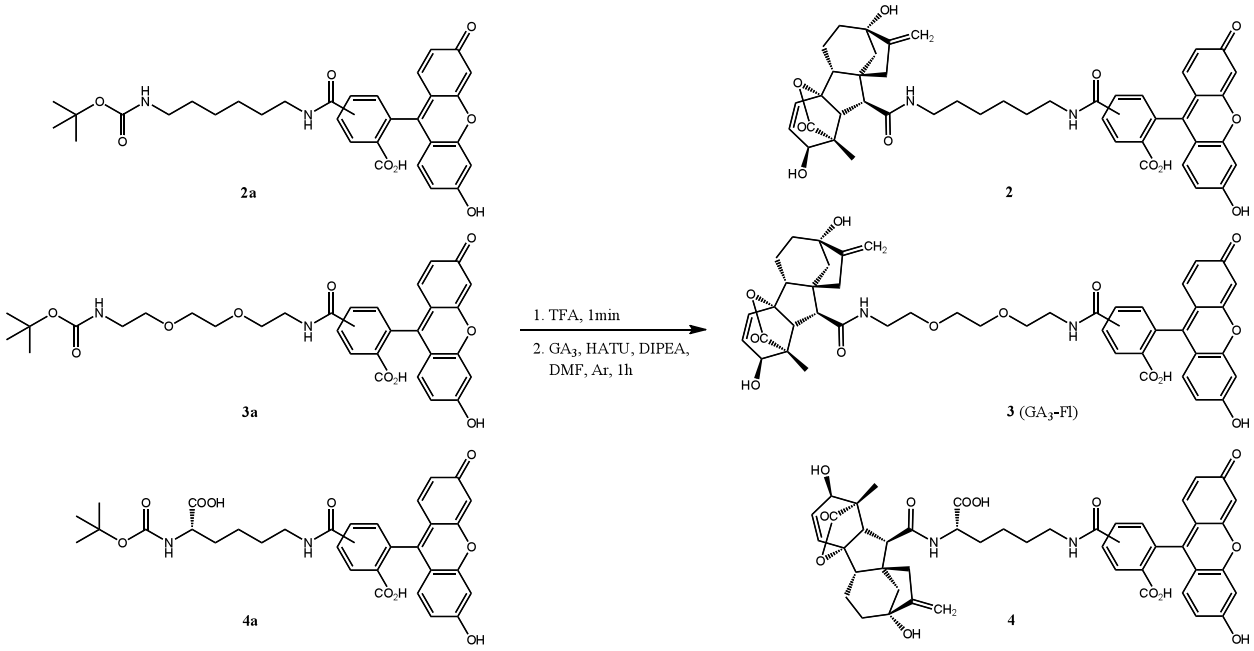

Fig. S3. Synthetic scheme of compounds **2**, **3** (GA_3-Fl) and **4**.

General procedure for synthesis of **2**, **3** (GA_3-Fl) and **4**.

The appropriate compound **2a**, **3a**, or **4a** (30 µmol, 1 eq) was dissolved in 1 mL TFA and stirred for 1 minute. TFA was immediately removed under reduced pressure. The residue was dissolved in dry DMF under argon atmosphere and DIPEA (5.7 µL, 33 µmol, 1.1 eq) was added. In a separate 1.5 mL eppendorf, Gibberellin A_3 (11.5 mg, 33 µmol, 1.1 eq) was dissolved in dry DMF, then DIPEA (5.7 µL, 33 µmol, 1.1 eq) and HATU (12.5 mg, 33 µmol, 1.1 eq) were added. The mixture was vortexed for 1 minute and then added to the TFA-treated compound **2a**, **3a**, or **4a**. The reaction was stirred at room temperature for 1 hour and the solvent removed under reduced pressure. The residue was dissolved in 2 mL DMSO:ACN:water (2:1:1), filtered with Costar® Spin-X® centrifuge filter tube and the desired product was purified using preparative HPLC (see general HPLC purification conditions below).

Compound **2**: Starting from **2a** (17 mg). Preparative HPLC retention time: 13.57 minutes. Obtained 18 mg yellow solid (22.5 µmol, yield 75%).

^{1}H-NMR (DMSO-d_6, 500 MHz): δ = 1.04 (1.2H, s)[6], 1.06 (3H, s)[5], 1.17-1.74 (7H, m)[5,6], 1.75-1.91 (2.8H, m)[5,6], 1.97-2.12 (2.8H, m)[5,6], 2.53 (0.4H, s)[6], 2.54 (1H, s)[5], 2.93-3.07

(2.8H, m)[5,6], 3.10 (0.4H, d, J = 10.8 Hz)[6], 3.11 (1H, d, J = 10.7 Hz)[5], 3.17 (0.8H, dt, J_1 = 6.8 Hz, J_2 = 5.8 Hz)[6], 3.29 (2H, dt, J_1 = 7.3 Hz, J_2 = 5.8 Hz)[5], 3.81-3.85 (2.8H, m)[5,6], 4.76 (0.4H, s)[6], 4.78 (1H, s)[5], 5.03 (0.4H, s)[6], 5.05 (1H, s)[5], 5.75-5.78 (1.4H, m)[5,6], 6.32 (0.4H, d, J = 9.2 Hz)[6], 6.33 (1H, d, J = 9.2 Hz)[5], 6.52-6.57 (2.8H, m)[5,6], 6.58-6.61 (2.8H, m)[5,6], 6.67-6.71 (2.8H, m)[5,6], 7.36 (1H, d, J = 8.3 Hz)[5], 7.66 (0.4H, s)[6], 7.99 (0.4H, t, J = 5.4 Hz)[6], 8.04 (1H, t, J = 5.4 Hz)[5], 8.06 (0.4H, d, J = 8.3 Hz)[6], 8.16 (0.4H, dd, J_1 = 8.3 Hz, J_2 = 1.5 Hz)[6], 8.23 (1H, dd, J_1 = 8.3 Hz, J_2 = 1.5 Hz)[5], 8.45 (1H, s)[5], 8.67 (0.4H, t, J = 5.8 Hz)[6], 8.82 (1H, t, J = 5.8 Hz)[5], 10.17 (1.4H, s). ^{13}C-NMR (DMSO-d_6, 125 MHz): δ = 206.6, 179.2, 170.5, 170.4, 168.2, 168.1, 164.5, 164.3, 159.9, 158.0, 154.6, 151.7, 140.8, 136.4, 134.7, 133.2, 131.6, 129.2, 128.2, 126.5, 124.3, 123.3, 112.8, 108.9, 105.4, 102.9, 91.1, 76.7, 68.7, 53.2, 52.4, 51.5, 50.5, 49.7, 44.8, 43.4, 40.4, 38.5, 34.2, 33.9, 31.0, 30.7, 29.1, 29.0, 28.9, 26.2, 26.1, 26.0, 24.8, 22.1, 20.6, 16.6, 14.3, 14.0, 11.3. ESI-MS (positive mode) calculated ($C_{46}H_{46}N_2O_{11}$) 802.3, found m/z $[M+H]^+$ 803.5.

Compound **3** (GA_3-Fl): Starting from **3a** (18 mg) preparative HPLC retention time: 13.05 minutes. Obtained 18 mg yellow solid (21 μmol, yield 71%).
^{1}H-NMR (DMSO-d_6, 500 MHz): δ = 1.04 (1.2H, s)[6], 1.06 (3H, s)[5], 1.53-1.76 (7H, m)[5,6], 1.77-1.93 (2.8H, m)[5,6], 2.02-2.14 (2.8H, m)[5,6], 2.53 (0.4H, s, partially obscured by DMSO signal)[6], 2.54 (1H, s, partially obscured by DMSO signal)[5], 3.10 (0.4H, d, J = 10.8 Hz)[6], 3.12 (1H, d, J = 10.8 Hz)[5], 3.13-3.60 (16.8H, m)[5,6], 3.82 (1.4H, s)[5,6], 4.76 (0.4H, s)[6], 4.78 (1H, s)[5], 5.03 (0.4H, s)[6], 5.05 (1H, s)[5], 5.75-5.78 (1.4H, m)[5,6], 6.32 (0.4H, d, J = 9.2 Hz)[6], 6.33 (1H, d, J = 9.2 Hz)[5], 6.52-6.57 (2.8H, m)[5,6], 6.58-6.61 (2.8H, m)[5,6], 6.67-6.71 (2.8H, m)[5,6], 7.36 (1H, d, J = 8.3 Hz)[5], 7.66 (0.4H, s)[6], 7.99 (0.4H, t, J = 5.4 Hz)[6], 8.04 (1H, t, J = 5.4 Hz)[5], 8.06 (0.4H, d, J = 8.2 Hz)[6], 8.16 (0.4H, dd, J_1 = 8.2 Hz, J_2 = 1.5 Hz)[6], 8.23 (1H, dd, J_1 = 8.3 Hz, J_2 = 1.5 Hz)[5], 8.45 (1H, s)[5], 8.67 (0.4H, t, J = 5.8 Hz)[6], 8.82 (1H, t, J = 5.8 Hz)[5], 10.17 (1.4H, s). ^{13}C-NMR (DMSO-d_6, 125 MHz): δ = 179.2, 170.9, 168.2, 164.7, 159.6, 158.1, 154.7, 151.8, 146.0, 144.5, 140.5, 136.2, 134.6, 131.6, 129.2, 126.4, 124.4, 123.3, 112.7, 109.1, 105.5, 102.3, 91.0, 76.8, 69.5, 69.1, 68.8, 68.6, 53.2, 52.4, 51.3, 50.4, 49.7, 44.9, 43.2, 38.6, 16.7, 14.3. ESI-MS (positive mode) calculated ($C_{46}H_{46}N_2O_{13}$) 834.3, found m/z $[M+H]^+$ 835.4.

Compound **4**: Starting from **4a** (18 mg) preparative HPLC retention time: 12.77 minutes. Obtained 14 mg yellow solid (17 µmol, yield 57%).

^{1}H-NMR (DMSO-d_6, 500 MHz): d = 1.04 (0.9H, s)[6], 1.06 (3H, s)[5], 1.16-1.44 (5.2H, m)[5,6], 1.44-1.76 (6.5H, m)[5,6], 1.77-1.93 (2.6H, m)[5,6], 2.02-2.14 (2.6H, m)[5,6], 2.53 (0.3H, s, partially obscured by DMSO signal)[6], 2.54 (1H, s, partially obscured by DMSO signal)[5], 3.11 (0.3H, d, J = 10.8 Hz)[6], 3.12 (1H, d, J = 10.8 Hz)[5], 3.15-3.41 (2.6H, m)[5,6], 3.82 (1.4H, s)[5,6], 4.08-4.14 (0.3H, m)[6], 4.15-4.22 (1H, m)[5], 4.74 (0.3H, s)[6], 4.76 (1H, s)[5], 5.03 (0.3H, s)[6], 5.05 (1H, s)[5], 5.74-5.77 (1.3H, m)[5,6], 6.31 (0.3H, d, J = 9.7 Hz)[6], 6.32 (1H, d, J = 9.7 Hz)[5], 6.50-6.65 (5.2H, m)[5,6], 6.66-6.78 (2.6H, m)[5,6], 7.36 (1H, d, J = 7.4 Hz)[5], 7.66 (0.3H, s)[6], 8.06 (0.3H, d, J = 8.3 Hz)[6], 8.16 (0.3H, dd, J_1 = 8.3 Hz, J_2 = 1.5 Hz)[6], 8.23 (1H, dd, J_1 = 7.8 Hz, J_2 = 1.5 Hz)[5], 8.45 (1H, s)[5], 8.69 (0.3H, t, J = 5.4 Hz)[6], 8.30 (1H, t, J = 5.4 Hz)[5], 10.17 (1.3H, s)[5,6]. ^{13}C-NMR (DMSO-d_6, 125 MHz): d = 179.3, 173.8, 171.0, 168.4, 164.4, 159.7, 158.3, 154.7, 151.9, 136.3, 134.8, 133.4, 131.7, 129.4, 126.6, 124.2, 123.4, 112.7, 109.2, 105.3, 102.4, 91.2, 77.0, 68.7, 53.3, 52.6, 51.8, 50.9, 50.5, 50.2, 44.9, 43.2, 35.9, 31.1, 30.6, 29.7, 28.6, 23.1, 22.2, 16.8, 14.4. ESI-MS (positive mode) calculated ($C_{46}H_{44}N_2O_{13}$) 832.3, found m/z $[M+H]^+$ 833.4.

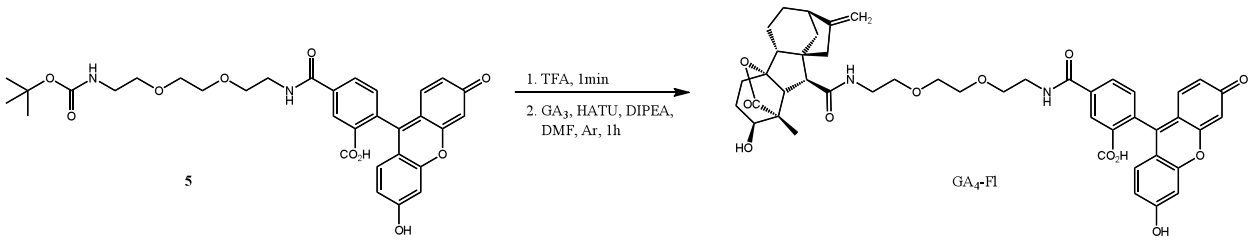

Fig. S4. Synthetic scheme of compound GA_4-Fl.

Compound **5**

Compound **5** for synthesized according to published procedure (1) (general HPLC purification conditions below). Compound **5** eluted at retention time of 12.88 minutes. After solvents removal under reduced pressure, 8 mg of yellow solid were obtained (13 µmol, yield 63%).

Compound GA_4-Fl

Synthesized according to the general procedure for synthesis of **2**, **3** and **4** (above). Starting from **5** (8 mg, 13 µmol). Preparative HPLC retention time: 14.98 minutes. Obtained 7.5 mg yellow solid (9 µmol, yield 70%).

^{1}H-NMR (DMSO-d_6, 500 MHz): d = 0.97 (3H, s), 1.21-1.30 (2H, m), 1.42-1.57 (5H, m), 1.63-1.73 (3H, m), 1.79-1.88 (2H, m), 1.93-2.03 (2H, m), 2.45 (1H, d, *J* = 10.2 Hz), 3.06 (1H, d, *J* = 10.2 Hz), 3.09-3.18 (2H, m), 3.19-3.28 (2H, m), 3.38 (2H, t, *J* = 6.4 Hz), 3.41-3.57 (10H, m), 4.77 (1H, s), 4.88 (1H, s), 6.51-6.60 (4H, m), 6.66-6.69 (2H, m), 7.37 (1H, d, *J* = 8.3 Hz), 8.04 (1H, t, *J* = 5.9 Hz), 8.24 (1H, dd, J_1 = 8.3 Hz, J_2 = 1.5 Hz), 8.46 (1H, s), 8.91 (1H, t, *J* = 5.9 Hz), 10.17 (1H, s). ^{13}C-NMR (DMSO-d_6, 125 MHz): d = 178.6, 171.2, 168.2, 164.7, 159.7, 157.3, 151.8, 136.2, 134.7, 129.2, 126.4, 123.3, 112.7, 109.0, 106.5, 102.2, 93.8, 69.5, 69.1, 68.8, 68.7, 54.3, 52.9, 52.1, 50.7, 50.5, 44.4, 40.4, 36.8, 31.1, 30.7, 28.0, 26.8, 22.1, 15.7, 14.5. ESI-MS (positive mode) calculated ($C_{46}H_{48}N_2O_{12}$) 820.3, found *m*/*z* $[M+H]^+$ 821.5.

Preparative HPLC purification conditions. All preparative separations were performed on Agilent HPLC system (1200 series) with Luna 10 µm PREP C18(2) column (250.0 X 21.2 mm, 100 Å), using a water-acetonitrile gradient of 10% to 100% solvent B in 20 minutes at flow rate of 15 mL/min (solvent A = water, solvent B = acetonitrile, both with 0.05% TFA as an additive).

Quantum yield measurements. The quantum yields of GA_3-Fl and GA_4-Fl were determined using the comparative method with Fluorescein in 0.1 N NaOH as standard (Φ_{yield} = 0.91). GA_3-Fl or GA_4-Fl (0.5 µM) in 50 mM MOPS buffer pH 7.2. Recordings were performed at room temperature.

Supporting Methods

Plant material, growth conditions and chemicals application

Arabidopsis thaliana seeds were stratified for 2-days at 4 °C and plated on medium containing 1/2× Murashige-Skoog medium, 1% sucrose, and 1% agar (both w/v) on vertical plates at 22 °C, under short-day fluorescent light (8 h light/16 h dark). All *Arabidopsis* lines are *Col-0*. Tomato plants (*Solanum lycopersicum* cv M82, sp) were grown in growth chambers at 16-h-day and 8-h-night conditions at 25˚C. The following *Arabidopsis* transgenic plants and mutants were previously described: *ga1-1* (2-3) *casp1-1 casp3-1* double mutant (4), *scr-3* (5), *shr-2* (6), *ctr1-1* (7), *ein2-2* (8), *pRGA::GFP::RGA* (9). *35S:H2B-RFP* (10).

Chemicals were supplied to the agar medium at concentrations as indicated for each experiment in figure legends (starting from stock solutions of 10 mM). Seedlings were placed on agar plates in a way such that the chemical was applied uniformly to the whole root.

Imaging and analysis

Seedlings were imaged on a laser scanning confocal microscope (models Leica SP/2 and Zeiss LSM 710 inverted microscopes), with argon laser set at 488 nm for excitation, 566-617 nm filter for propidium iodide (PI) emission, and 493-543 nm filter for Fl derivatives and GFP-RGA emission. Seedlings were stained in 10 mg/L PI for 2-5 minutes, rinsed, and mounted in water. Image analysis and signal quantification were done with the measurement function of Zeiss Zen 2011 software. Signal intensity of a circle region of interest (20x20 μM) was quantified. The number of quantified biological repeats and sampling points is indicated for each graph in figure legends.

Preparation of root extracts and analysis by HPLC-HRMS

6-days-old plants were treated with either GA_3-Fl or GA_4-Fl (5 μM) or not treated (control) for the indicated time on agar plates. The roots and shoots were separated, placed in 1.5 mL eppendrofs containing metal beads, flash-frozen in liquid nitrogen and grinded by vortexing for 3 minutes. To the grinded powder was added 50 μL of 80%

MeOH (in water). The suspension was vortexed for additional 1 minute then centrifuged for 3 minutes at 14,000 rpm. The clear supernatant (extract) was separated and kept at -20^{o}C until analysis. Before analysis, 10-20 µL of extract sample were diluted with 60-70 µL of 50% ACN:water (final volume 80 µL), vortexed and centrifuged for 2 minutes at 14,000 rpm.

Extracts samples were analyzed by a Magic 2002 HPLC (Michrom Bioresources Inc., Auburn, California) using 2X150 mm C18 Phenomenex Gemini column, connected to a FP-920 fluorescence detector (Jasco Inc., Easton, Maryland) with 5 µl flow cell. The eluant then flowed to an LTQ Orbitrap XL mass spectrometer with electrospray interface (ThermoFisher Scientific, San Jose, California). Solvents system: A = double-distilled water, 4 mM ammonium bicarbonate, B = acetonitrile, no pH modifier (pH 7.0). Fluorescence detection conditions: λ_{ex} = 480 nm, λ_{em} = 520 nm, gain 100. Gradient for GA_3-Fl- and GA_4-Fl-treated root and shoot extracts: 0-4 minutes 10% B, then 10-100% B in 20 minutes. Flow rate 220 µL/min. Gradient for GA_4-Fl-treated root extract for HRMS analysis: 0-4 minutes 10% B, then 10-55% B in 55 minutes. Flow rate 220 µL/min. As reference control, we also analyzed samples of GA_3-Fl, GA_4-Fl, product of C6 amide cleavage and 5(6)-carboxyfluorescein using the same gradient and HPLC setup used for extracts (above). All solutions percentages are expressed as v/v.

Plasmid construct

RGA coding DNA sequence CDS sequence was amplified from *Arabidopsis* Col-0 cDNA by PCR using primers 5'-CACCATGAAGAGAGATCATCACCAATTCC-3' and 5'-TCAGTACGCCGCCGTCGAGAG-3' and cloned into the pENTR/D TOPO vector (Invitrogen). pTnT® vector (Promega) was modified to create a C-4xMyc tagged Gateway compatible version, designated pCCM95. For construction of pCCM95 the --4xMyc-R1- CmR-ccdB-R2-- cassette was amplified by PCR from vector pGWB18 using primers 5'-GGGACTCTAGAATGAGCGGG-3' and 5'-TATCACCACTTTGTACAAGAAAGC-3'. The resulting fragment was digested with *Xba*I, and cloned between the *Xba*I and *Sma*I sites of pTnT®. RGA sequence was cloned into pCCM95 following a LR reaction using LR Clonase® II mix (Invitrogen). The

resulting vector, pCCM97, was used as a template for in vitro transcription/translation of 4x-Myc-RGA.

Statistical analysis

Two-tailed student's *t*-test was performed whenever two groups were compared. Statistical significance was determined at $P < 0.001$.

Hormones and inhibitors stock solutions

Compounds **1-4** and GA_4-Fl (10 mM methanol stock), GA_3 (Sigma G7645, 10 mM methanol stock), GA_4 (Sigma G7276, 10 mM methanol stock), paclo (Sigma 64046, 10 mM ethanol stock), Piperonylic acid (Sigma P49805, 10 mM water stock), Antimycin A (Sigma A8674, 10 mM ethanol stock), Oligomycin A (SIGMA 75351, 10 mM ethanol stock), Myxothiazol (SIGMA T5580, 10 mM DMSO stock). ACC (Sigma A3903, 10 mM water stock).

Imaging and analysis

Seedlings were imaged on a laser scanning confocal microscope (models Leica SP/2 and Zeiss LSM 710 inverted microscopes), with argon laser set at 488 nm for excitation, 566-617 nm filter for PI emission, and 493-543 nm filter for Fl derivatives emission. Seedlings were stained in 10 mg/L PI for 2-5 min, rinsed, and mounted in water. Image analysis and signal quantification was done with the measurement function of the Zeiss Zen 2011 software. Signal intensity of a circle region of interest (20x20 µM) was quantified. The number of quantified biological repeats and sampling points is indicated for each graph in figure legends.

Pull-down assays

Recombinant GST-GID1b protein was expressed in BL21DE3(*LysS*) *E.Coli* cells transformed with pGEX-GID1b (GST-GID1b) vector (11) . 100 mL *E.Coli* culture (OD = 0.5) was induced with 1 mM IPTG at 28 ºC for 3 hours. Pelleted cells were resuspended with 10 mL of GST lysis buffer (20 mM Tris-Cl pH 8, 200 mM NaCl, 5 mM DTT, 1 mM PMSF, 1x Complete Protease Inhibitor (Roche cat. #04693159001), 1 tablet per 10 mL

buffer) and lysed by sonication. The lysate was rotated together with 500 µL of 50% Glutathione-Agarose (Sigma cat. #G4510) at 4 ºC for 1 hour. After washing once with GST lysis buffer, the glutathione-agarose bound by GST-GID1b was resuspended in 400 µL GST lysis buffer and frozen with liquid nitrogen before storage in -80 ºC. Myc-RGA was produced by TNT® SP6 Coupled Wheat Germ Extract System (Promega, cat. #L4130) with pCCM97 vector (see plasmid construct below). For each pulldown assay, 20 µL of glutathione-agarose slurry, 10 µL of reaction mix for Myc-RGA *in vitro* expression products and Fl, GA_3, GA_3-Fl (100 µM) or Fl, GA_4 and GA_4-Fl (50 µM) were mixed and rotated at room temperature for 1 hour. The pull-down was performed in 400 µL reaction containing 1x pull-down buffer (20 mM Tris-Cl pH 7.5, 100 mM NaCl, 10% Glycerol, 0.01% NP40, 1 mM DTT, 1 mM PMSF, 10 µM MG132 and 1x Complete Protease Inhibitor). The glutathione-agarose was transferred onto Micro Bio-Spin™ Chromatography Columns (Biorad, cat. #732-6204) and washed five times each with 1 mL of 1x pulldown buffer without MG132 and Complete Protease Inhibitor. Each sample was eluted with 28 µL elution buffer (50 mM Tris-Cl pH 8, 200 mM NaCl and 15 mM L-Glutathione reduced). 10 µL of each elute was loaded onto SDS-PAGE for the detection of Myc-RGA, with 2 µL (0.5%) of pull-down mixture as loading and quantification controls. The western blot was performed with anti-c-Myc-Peroxidase (Roche, cat. #11814150001).

The quantification of the western blot bands was done using ImageJ software and is presented as a ratio between the pull down samples and the input.

Yeast Two-Hybrid assay

DB-GID1a in pLexA-NLS derivative (Trp selection) and AD-RGA in pACTII derivative (Leu selection) fusion constructs (bait and pray constructs) were previously described (11). Bait and pray constructs were co-transformed into *Saccharomyces cerevisae* strain EGY48 [p8opLacZ] (Clontech) and transformants were selected on SD supplemented with –Ura/–Trp/–Lue/ drop-out solution (BD Biosciences). To test the interaction between GID1a and RGA proteins, transformed yeast colonies were plated on SD–Ura/–Trp/–Lue/ drop out, glucose, 80 µg/ml X-Gal, in the abscense and prescense of 100 µM of the indicated GAs and GA-Fl molecules. Plates were incubated for 3 days at 30°C. A

control strain, containing the DB-GID1a and an empty vector of the AD (no RGA protein) was used as a negative control. The quantification of the Y2H assay was done using ImageJ software by measuring the mean intensity yeast colonies signal, and presented after subtracting the background signal of control colonies (empty vector).

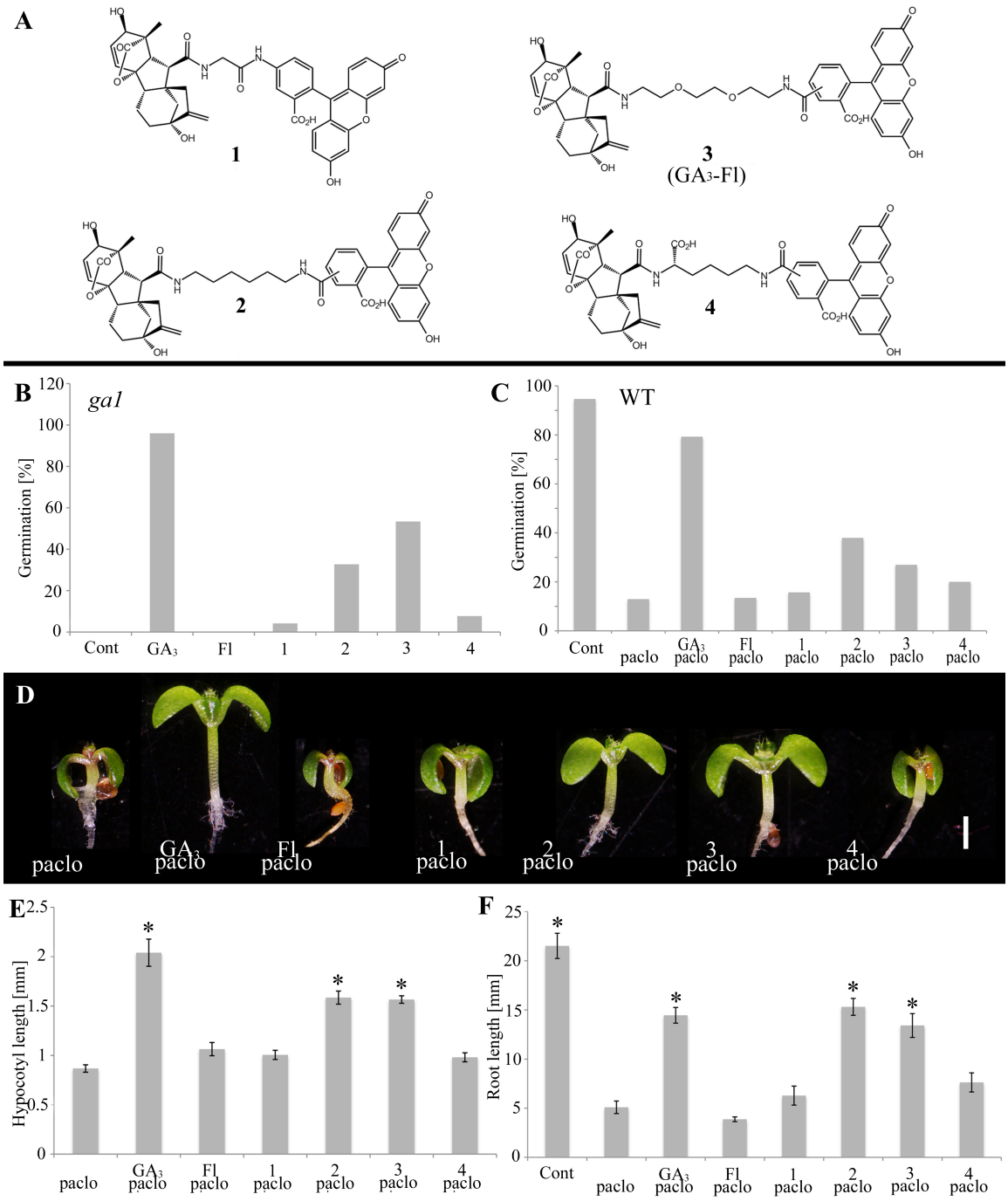

Fig. S5. Bioactivity of labeled-GAs. (*A*) Molecular structures of Fluorescein (Fl) labeled GA_3 derivatives **1**-**4**. (*B*) Germination of GA biosynthesis mutant *ga1* treated with **1**-**4**, Fl or GA_3 (100 µM). (*C*) Germination of WT seeds treated with paclobutrazol-(paclo) (2 µM) and **1**-**4**, Fl or GA_3 (10 µM), (n = 100). (*D*-*E*) Hypocotyl elongation of seedlings treated with paclobutrazol (2 µM) plus **1**-**4**, Fl or GA_3 (10 µM). Shown are averages ± SE (n = 10). Scale bar is 1 mm. (*E*) Graphical representation of the experiment in *D*. (*F*)

Root elongation of seedlings treated with paclo and **1-4**, Fl or GA_3 (10 µM). Shown are averages ± SE (n = 10) * Significantly different relative to respective paclo treatment at P ≤ 0.001 by Student's t test.

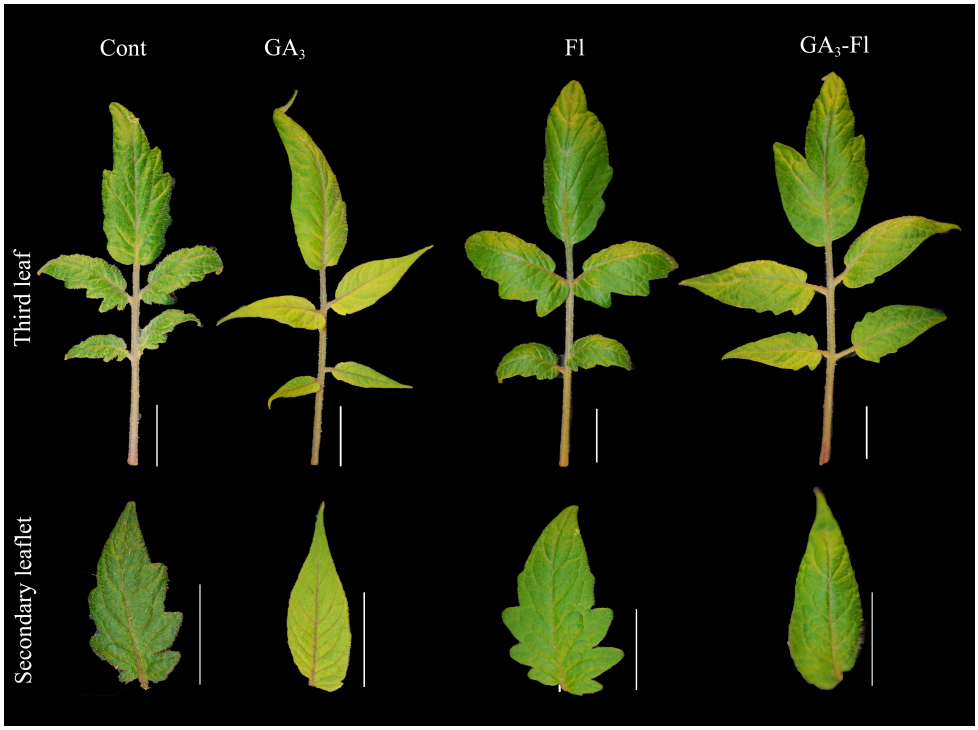

Fig. S6. GA_3-Fl is bioactive in tomato. GA_3 and GA_3-Fl application to developing tomato leaves results in the formation of simpler leaves with smooth margins compared to control and Fl treatment. Tomato WT (M82 sp) seedlings were treated (spray) with 10 µM of the indicated compound. Top row presents a mature third leaf. Bottom row present a magnification of the second leaflet. Scale bars are 1 cm.

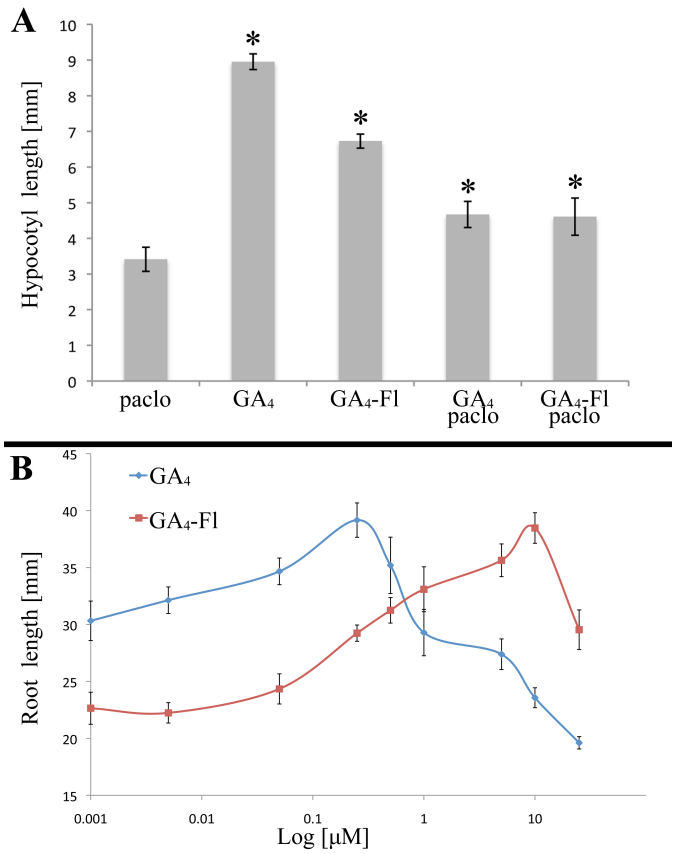

Fig. S7. GA_4-Fl bioactivity in *Arabidopsis*. (*A*) Hypocotyl elongation WT seeds treated with paclo (2 µM) + GA_4-Fl, Fl or GA_4 (10 µM). Shown are averages ±SE (n = 10). * Significantly different relative to paclo treatment at $P \leq 0.001$ by Student's t test. (*B*) Root elongation response of paclo-treated seedlings to increasing concentrations of GA_4 and GA_4-Fl. Shown are averages ±SE (n = 10).

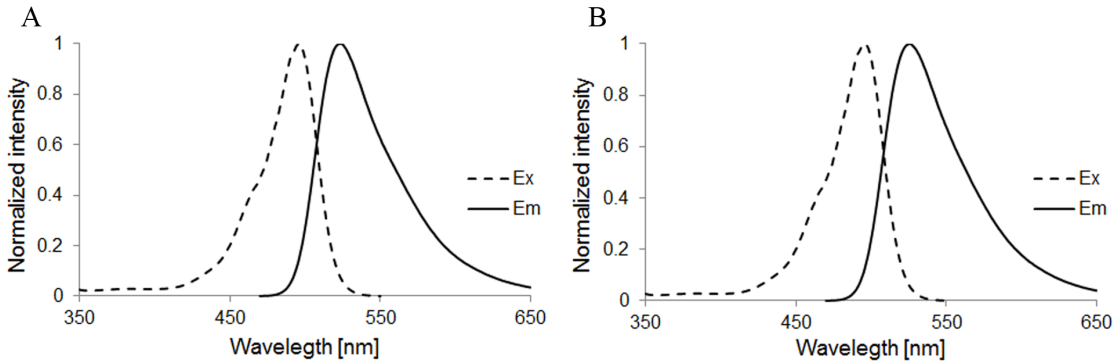

Fig. S8. Excitation and emission spectra of GA_3-Fl and GA_4-Fl. Excitation (dashed line) and emission (solid line) spectra of 0.5 μM GA_3-Fl (*A*) and GA_4-Fl (*B*) were measured in 50 mM MOPS buffer (pH 7.2) at room temperature.

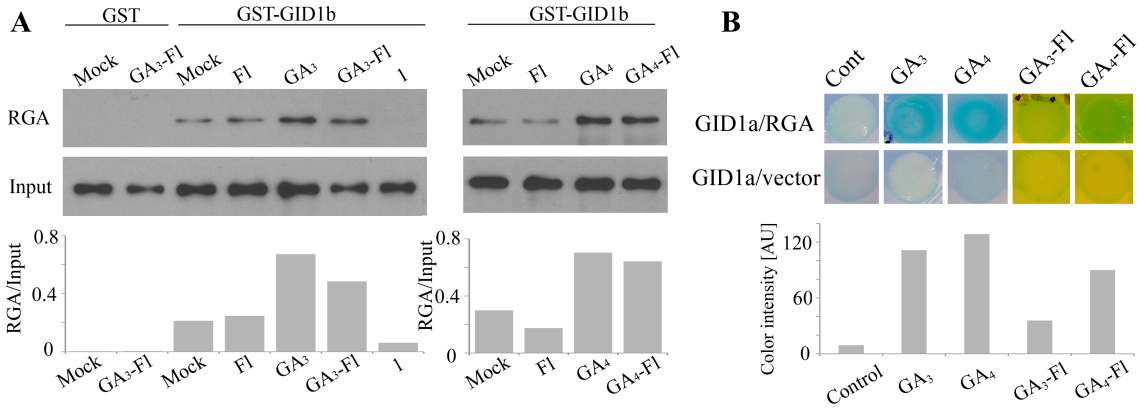

Fig. S9. GA-Fls promote interaction between GID1b and RGA. (*A*) GA-Fls promote interaction between GID1b and RGA *in vitro*. Pull-down assays with recombinant GST-GID1b and Myc-RGA in the presence of indicated molecules. Left panel: 100 μM (Fl, GA_3, GA_3-Fl and **1**. Right panel: 50 μM (Fl, GA_4 and GA_4-Fl). Lower bar graphs show average quantification of Myc-RGA pull-down and input band ratio intensity of two experiments. (*B*) Yeast two-hybrid assay testing the interaction between GID1a and RGA in the presence of indicated molecules. Bottom row is a control yeast strain with empty Activation-Domain vector (no RGA). GA_3, GA_4, GA_3-Fl, GA_4-Fl are all 100 μM. Lower bar graph show average quantification of the yeast two-hybrid. Mean intensity signal was reduced from the relative control (empty vector treatment with relative GA compounds).

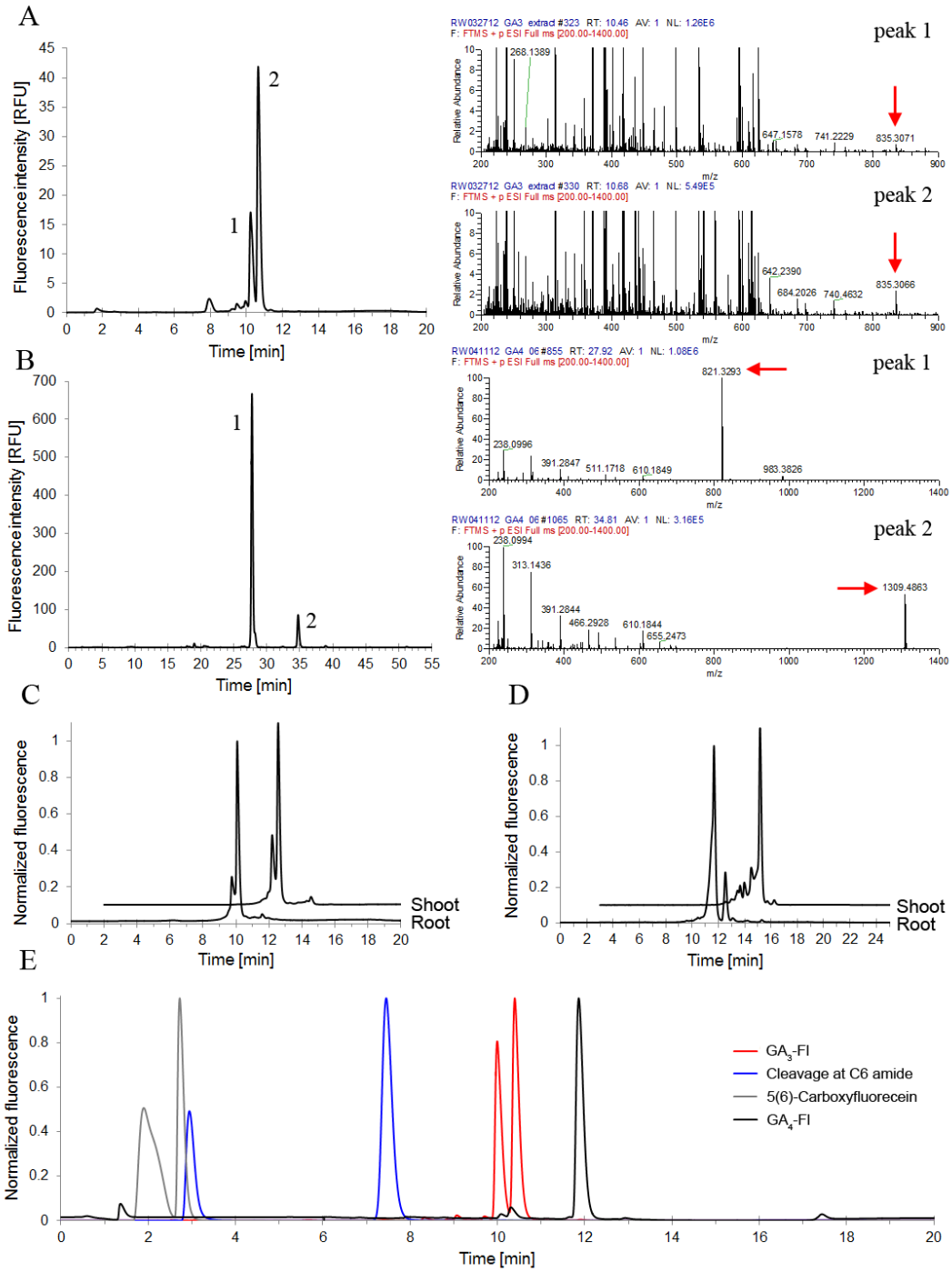

Fig. S10. HPLC and mass spectra analysis of fluorescent molecules in GA_3-Fl and GA_4-Fl treated plants. Fluorescence HPLC traces (left) and mass spectra chromatograms (right) of the major fluorescent components in GA_3-Fl- (*A*) and GA_4-Fl- (*B*) treated plants roots extracts (peaks 1 and 2). The found masses (marked by red

arrows) for peaks 1 and 2 in the GA_3-Fl-treated extracts correspond to 5- and 6-carboxyfluorescein derivatives of GA_3-Fl, respectively. Calculated for $C_{46}H_{46}N_2O_{13}$ (M) 834.3000, found ($M+H^+$) peak 1 835.3071, peak 2 835.3066. Found masses for peak 1 and 2 are < 3 ppm from the calculated $M+H^+$ (835.3073). The found mass for peak 1 in the GA_4-Fl-treated extract corresponds to GA_4-Fl. Calculated for $C_{46}H_{48}N_2O_{12}$ (M) 820.3207, found ($M+H^+$) peak 1 821.3293, peak 2 1309.4863. Found mass for peak 1 is < 3 ppm from the calculated $M+H^+$ (821.3280). (*C*) Normalized fluorescence HPLC chromatograms of root and shoot extracts from plants treated with GA_3-Fl for 3 days. (*D*) Normalized fluorescence HPLC chromatograms of root and shoot extracts from plants treated with GA_4-Fl for 2 days. (*E*) Normalized fluorescence HPLC chromatograms of GA_3-Fl, GA_4-Fl, product of C6 amide cleavage and 5(6)-carboxyfluorescein using the same gradient and HPLC setup used for root extracts analysis.

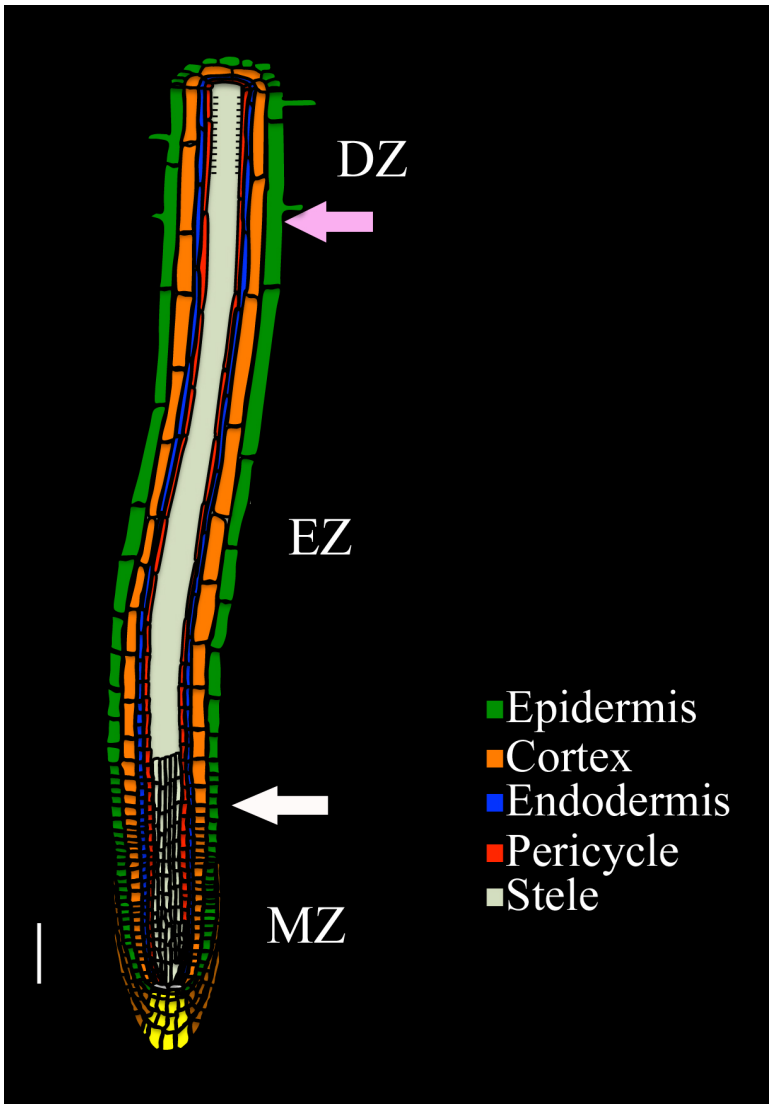

Fig. S11. Diagram of the *Arabidopsis* root. High resolution image of Fig 2*A* showing tissue organization and developmental zones of the *Arabidopsis* primary root. MZ, Meristematic Zone; EZ, Elongation Zone; DZ, Differentiation Zone. Scale bar is 50 µm.

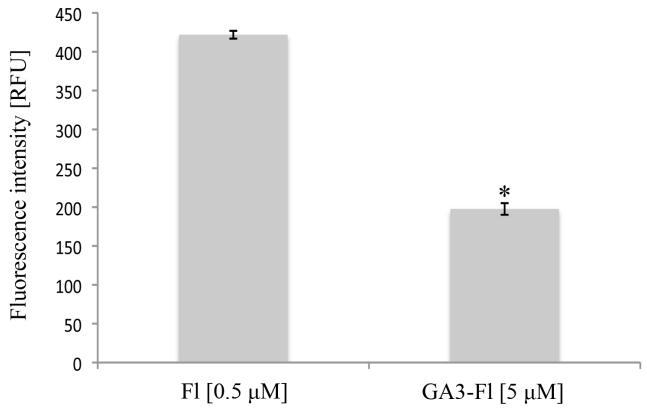

Fig. S12. Root uptake of GA_3-Fl is significantly lower compared to Fl. Quantification of fluorescence intensity as imaged in root endodermal elongating cells treated with 0.5 µM Fl and 5 µM GA_3-Fl. Shown are averages. ±SE (3 root images, 10 cells/image, 2 sampling points/cell, n = 60).

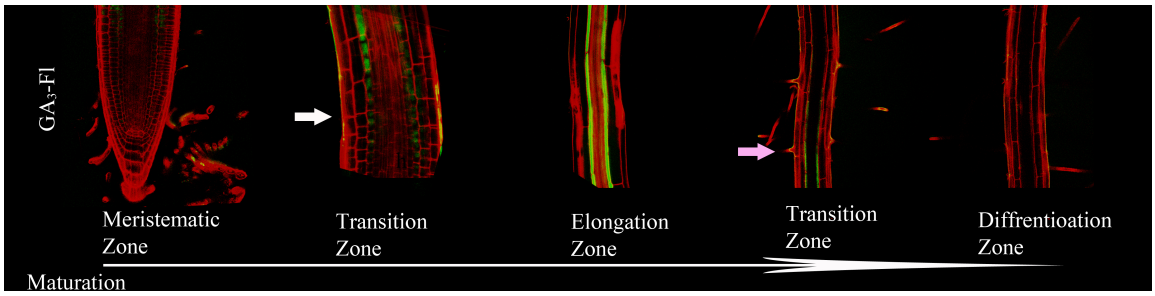

Fig. S13. GA_3-Fl localization during root maturation. GA_3-Fl accumulates at relatively high levels in the elongating endodermal cells. 6 days old plants were treated with 5 µM GA_3-Fl for 2 hours. White arrow marks transition from meristematic to elongation zone; pink arrow marks transition from elongation to differentiation zone.

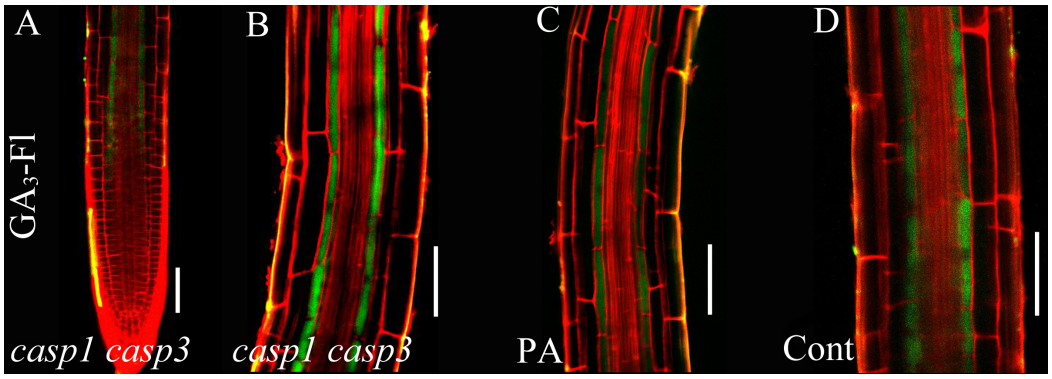

Fig. S14. Casparian strip does not affect GA_3-Fl accumulation in the elongating endodermal cells. (*A-B*) GA_3-Fl (5 µM, 2h) accumulates in the elongating endodermal cells of the double mutants *casp1-1 casp3-1*. (*A*) Root tip. (*B*) Elongation zone. (*C*) GA_3-Fl (5 µM, 2h) distribution in piperonylic acid (PA) (10 µM, 24 hours) treated WT plants. (*D*) GA_3-Fl (5 µM, 2h) distribution in WT plants (Cont). Scale bars are 50 µm.

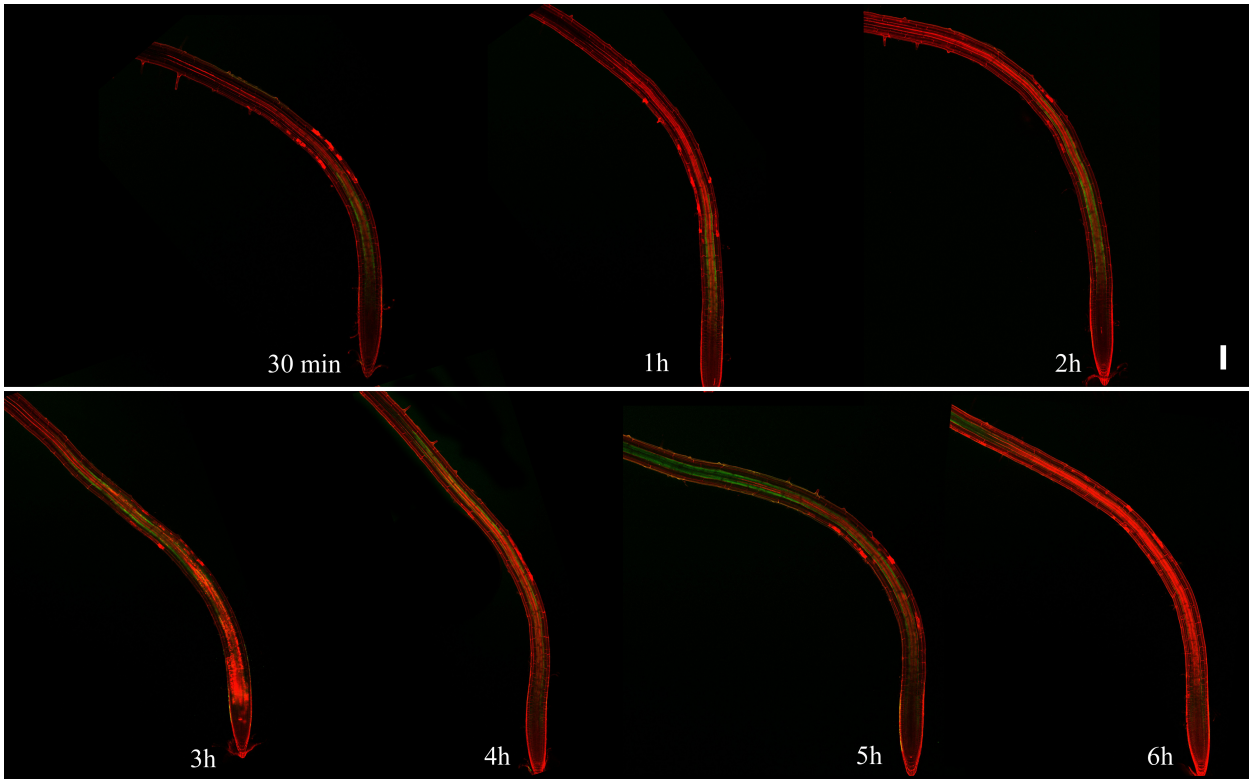

Fig. S15. Temporal dynamic distribution of GA_3-Fl in the root. Time-lapse images taken after GA_3-Fl (5 µM) application. Time is indicated for each image. Each image is of different root. A graph quantifying these experiments is presented in Fig 2*G*. Scale bar is 100 µm.

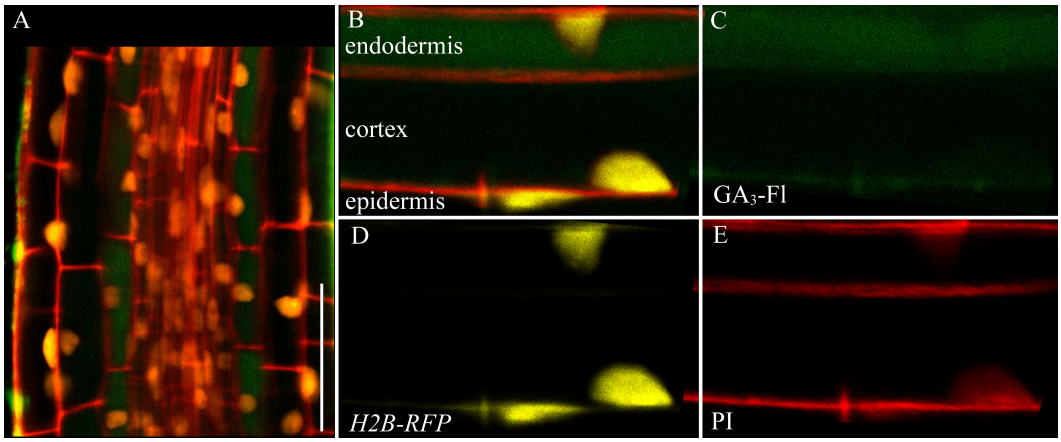

Fig. S16. GA_3-Fl accumulation in *35S:H2B-RFP* plants. Green, GA_3-Fl; Red, propidium iodide; Yellow, H2B-RFP signal marking the nucleus. (*A*) Merged elongation zone image. (*B-E*) Magnification of an endodermal and cortex cell in the elongation zone. (*B*) Merged composite of the images presented in *C-E*. Scale bar in *A* is 50 µm.

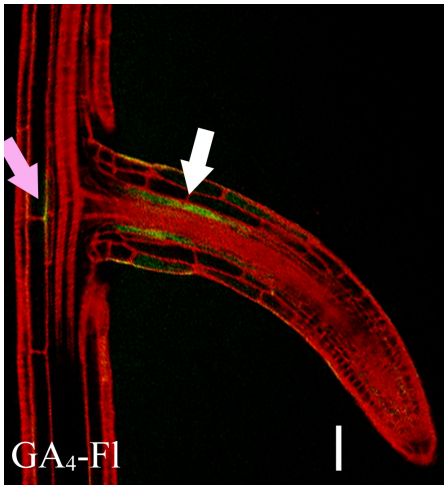

Fig. S17. Distribution of GA_4-Fl in a developing lateral root. White arrows mark the GA_4-Fl signal in elongating endodermal cells. Pink arrows mark the additional accumulation at the endodermal cell of the main root opposite to the lateral root formation site. Scale bar is 50 µm.

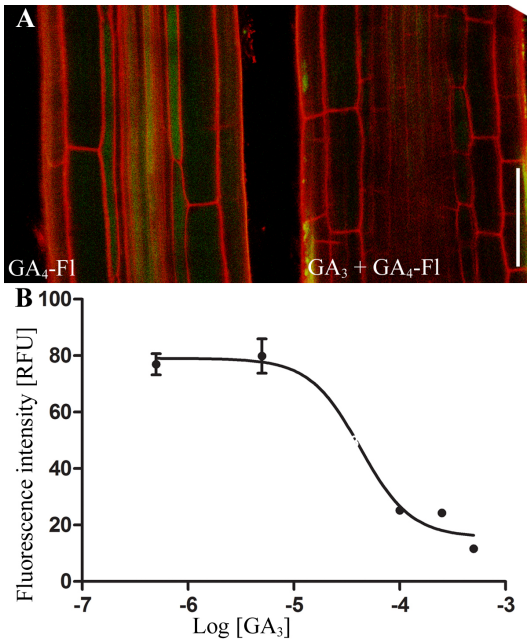

Fig. S18. GA_4-Fl and GA_3-Fl uptake in the root is competed by unlabeled GA. (*A*) *Arabidopsis* seedlings were pretreated with 200 µM GA_3 for 10 minutes and transferred to a media containing 5 µM GA_4-Fl + 200 µM GA_3. The elongation zone was imaged 3 hours later. Scale bar is 50 µm. (*B*) *Arabidopsis* seedlings were pretreated with GA_3 for 1.5 hours and transferred to a media containing 5 µM GA_3-Fl and the indicated concentration of GA_3. Fluorescence intensity was measured at the elongating endodermal cells. Shown are averages. ±SE (3 root images, 10 cells/image, 2 sampling points/cell, n=60). Data were fit with non-linear regression curve (variable slope, 4 parameters) using GraphPad Prism 5.

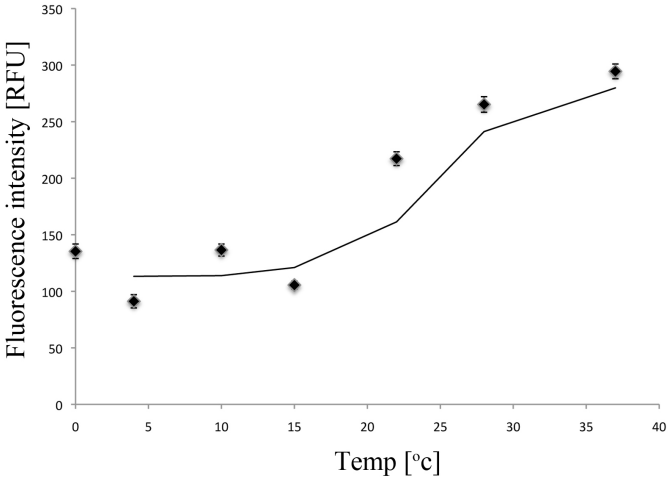

Fig. S19. Temperature dependence of Fl uptake in the root. Fluorescence intensity in endodermal cells of the elongation zone in seedlings treated with Fl (0.5 µM) for 2 hours at the indicated temperatures. Shown are averages. ±SE (n = 60). Moving-average trend line (period = 2) was fitted to the data.

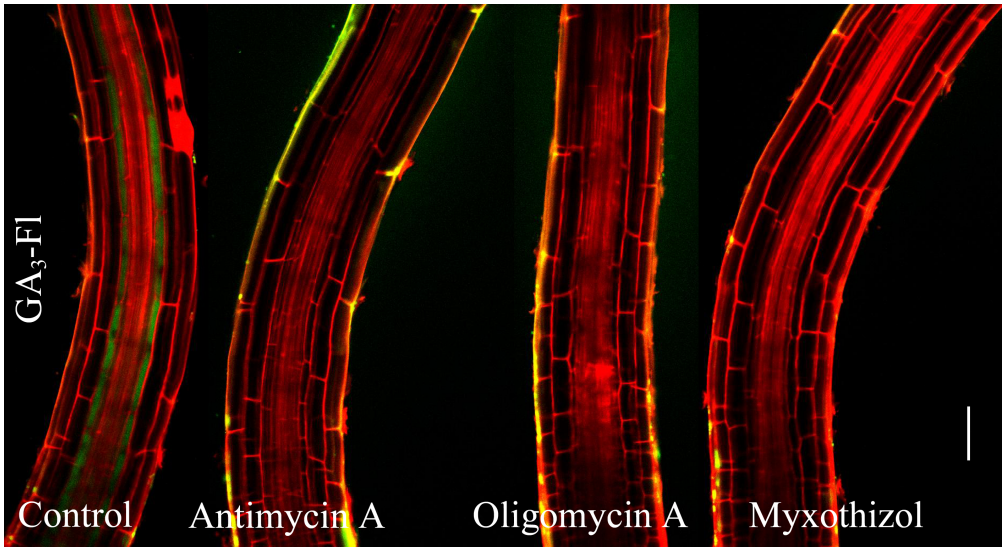

Fig. S20. GA_3-Fl uptake in the root is energy dependent. ATP synthesis inhibitors Antimycin A, Oligomycin A and Myxothiazol disrupt GA_3-Fl accumulation in the elongation zone. Seedlings were treated with 10 µM of the indicated inhibitor for 1.5h and transferred to plates with 10 µM inhibitor + GA_3-Fl or Fl (5 µM or 0.5 µM, respectively) for an additional 1.5 hours. Graph of fluorescence intensity is presented in Fig 4*H*. Scale bar is 50 µm.

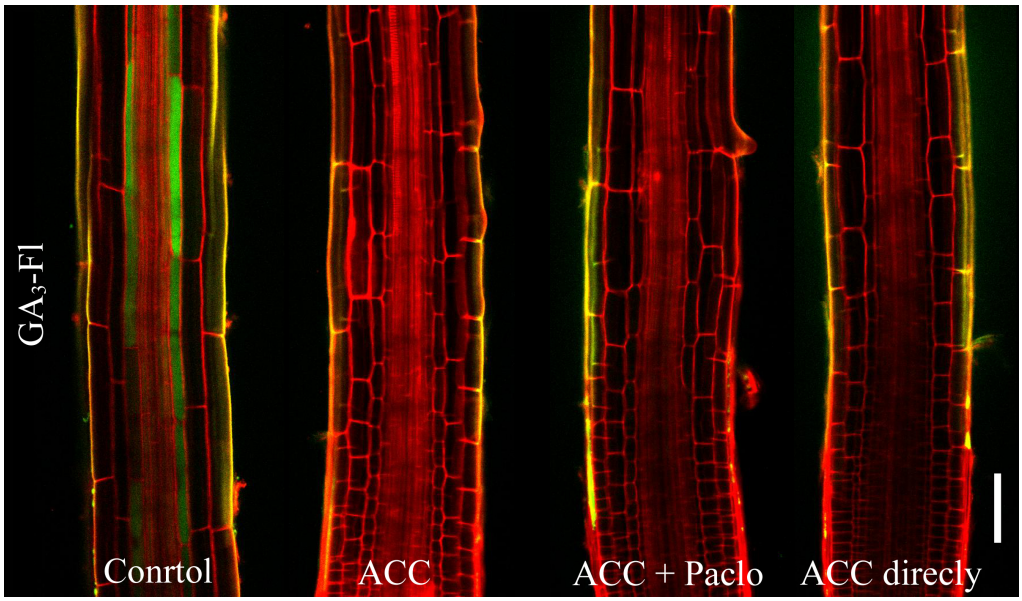

Fig. S21. ACC inhibition of GA_3-Fl uptake is independent of GA biosynthesis. Seedlings were treated with ACC or ACC + Paclo for 2 hours and transferred to plates with similar treatment + GA_3-Fl for an additional hour. The right image present seedlings that were treated directly with ACC + GA_3-Fl for 1 hour and imaged. Concentrations; ACC, 1 µM; Paclo, 2 µM; GA_3-Fl 5 µM. Scale bar is 50 µm.

Supporting references

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