Supporting Information

Structure-based chemical design to abscisic acid antagonists that block PYL-PP2C receptor interactions

Jun Takeuchi[1,a]*, Naoki Mimura[2,a], Masanori Okamoto[3,4,a], Shunsuke Yajima[5], Masayuki Sue[6], Tomonori Akiyama[6], Keina Monda[7], Koh Iba[7], Toshiyuki Ohnishi[1,8] &Yasushi Todoroki[1,8]*

[1]*Faculty of Agriculture, Shizuoka University, Shizuoka 422-8529, Japan.*

[2]*Graduate School of Science and Technology, Shizuoka University, Shizuoka 422-8529, Japan.*

[3] *Center for Bioscience Research and Education, Utsunomiya University, Utsunomiya 321-8505, Japan.*

[4]*PRESTO, Japan Science and Technology Agency, Kawaguchi 332-0012, Japan.*

[5]*Department of Bioscience, Tokyo University of Agriculture, Tokyo 243-0034, Japan.*

[6]*Department of Applied Biology and Chemistry, Tokyo University of Agriculture, Tokyo 243-0034, Japan.*

[7]*Department of Biology, Faculty of Science, Kyushu University, Fukuoka 819-0395, Japan,*

[8]*Research Institute of Green Science and Technology, Shizuoka University, Shizuoka 422-8529, Japan.*

[a]*These authors contributed equally to this work.*

*E-mail: takeuchi.jun@shizuoka.ac.jp; todoroki.yasushi@shizuoka.ac.jp

Supplementary material

Methods

Synthesis of PANs

General procedures. (+)-ABA was a gift from Dr. Y. Kamuro and Toray Industries Inc. ^{1}H NMR spectra were recorded with tetramethylsilane as the internal standard using JEOLJNM-EX270 (270 MHz) and JNM-LA500 (500 MHz) NMR spectrometers (JEOL Ltd.). ^{13}C NMR and 2D-correlation NMR experiments were recorded using a JNM-LA500 (500 MHz) NMR spectrometer (JEOL Ltd.). All peak assignments refer to the numbering in structure **PANH**. High resolution mass spectra were obtained with a JEOL JMS-T100LC AccuTOF mass spectrometer (ESI-TOF, positive mode; JEOL Ltd.). Column chromatography was performed using silica gel (Wakosil C-200, Wako Pure Chemical Industries, Ltd.).

(2*Z*,4*E*)-5-((1*S*,4*S*)-1,4-Dihydroxy-2,6,6-trimethylcyclohex-2-en-1-yl)-3-methylpenta-2,4-dienoic acid (6)

With stirring at 0 °C, cerium (III) chloride heptahydrate (42.8 g, 115 mmol) and $NaBH_4$ (14.3 g 79.9 mmol) were added to an ABA solution (10.0 g, 37.8 mmol) in methanol (MeOH, 400 mL) and the mixture stirred for 15 min at the same temperature. After quenching with sat. NH_4Cl (200 mL), it was concentrated *in vacuo* to remove MeOH and then acidified pH 2 by the addition of 2 M HCl. The resulting mixture was extracted with ethyl acetate (EtOAc, 500 mL × 3), washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. The residual solid was purified and separated into two isomers (6: 4 ratios of 1′,4′-*trans*-diol-ABA and 1′,4′-*cis*-diol-ABA, respectively) by silica gel chromatography (0–60% EtOAc/ hexane containing 0.1 % acetic acid (AcOH, v/v)) to obtain 1′,4′-*trans*-diol-ABA (6; 5.48 g, 54%) as a white solid. The absolute configuration at C4′ of the two isomers was determined by comparing the NMR data with those of the corresponding esters[1]. 1′,4′-*trans*-diol-ABA. ^{1}H

NMR (CD_3OD with 0.05% v/v TMS, 270 MHz): δ 0.91 (3H, s, H_3-8′ or H_3-9′), 1.01 (3H, s, H_3-8′ or H_3-9′), 1.60 (1H, dd, *J*=12.9 and 9.6 Hz, H-5′), 1.64 (3H, br s, H_3-7′), 1.72 (1H, ddd, *J*=12.9, 6.6 and 1.3 Hz, H-5′), 2.02 (3H, d, *J*=0.9Hz, H_3-6), 4.18 (1H, m, H-4′), 5.52 (1H, br s, H-3′), 5.70 (1H, br s, H-2), 6.20 (1H, d, *J*=16.2 Hz, H-5), 7.67 (1H, d, *J*=16.2 Hz, H-4). The data were consistent with the previous data[2].

3-Phenylprop-2-yn-1-ol (8a)

With stirring at room temperature, triethylamine (2.7 mL), copper(I) iodide (CuI, 132 mg, 0.692 mmol) and bis(triphenylphosphine)palladium(II) dichloride (139 mg, 0.198 mmol) were added to a solution of iodobenzene (2.01 g, 9.87 mmol) in tetrahydrofuran (THF, 14 mL) under an atmosphere of Ar. After being stirred for 30 min at room temperature, a solution of propargyl alcohol (554 mg, 9.89 mmol) in THF (4 mL) was added to the stirred mixture. The reaction mixture was stirred for 15 min at room temperature, and then it was filtered through silica gel (Et_2O). The filtrate was successively washed with water and brine, and then dried over Na_2SO_4 and concentrated *in vacuo*. The residual solid was purified by silica gel chromatography (0–20% EtOAc/ hexane) to obtain **8a** (1.18 g, 91%). ^{1}H-NMR ($CDCl_3$ with 0.05% v/v TMS, 270 MHz): δ 4.50 (2H, d, *J*=6.3 Hz, -*CH*$_2$OH), 7.30-7.35 (3H, m, Ar-*H*), 7.44 (2H, m, Ar-*H*).

3-(*p*-Tolyl) prop-2-yn-1-ol (8b)

With stirring at room temperature, propargyl alcohol (5.01 g, 89.4 mmol) and bis(triphenylphosphine)palladium(II) dichloride (1.05 g, 1.50 mmol) were added to a solution of *p*-iodotoluene (16.2 g, 74.3 mmol) in diethylamine (90 mL). After being stirred for 20 min at room temperature, copper(I) iodide (CuI, 142 mg, 0.475 mmol) was added to the stirring

mixture, and it was stirred for 2 h at 70 °C. After cooling down to room temperature, it was quenched with 1 M HCl (300 mL) and extracted with EtOAc (150 mL × 3). The organic layer was washed, dried and concentrated, as described above. The residual oil was purified by silica gel chromatography (10% EtOAc/ hexane) to obtain **8b** (9.32 g, 86%) as a brown oil. ^{1}H NMR ($CDCl_3$ with 0.05% v/v TMS, 270 MHz): δ 1.74 (1H, t, *J*=5.7 Hz, -OH), 2.34 (3H, s, -CH_3), 4.49 (2H, d, *J*=5.7 Hz, -*CH_2*OH), 7.10-7.13 (2H, m, Ar-*H*), 7.31-7.34 (2H, m, Ar-*H*).

1-(4-(3-Hydroxyprop-1-yn-1-yl) phenyl) ethan-1-one (8c)

With stirring at room temperature, triethylamine (5 mL), CuI (66.8 mg, 0.352 mmol) and bis(triphenylphosphine)palladium(II) dichloride (57.3 mg, 0.0817 mmol) were added to a solution of 4′-iodoacetophenone (1.02 g, 4.14 mmol) in THF (5 mL). After being stirred for 30 min at room temperature, a solution of propargyl alcohol (355 mg, 6.32 mmol) in THF (2.0 mL) was added to the stirred mixture. The reaction mixture was stirred for 30 min at room temperature, and then it was filtered through silica gel (EtOAc). The filtrate was successively washed with water and brine, and then dried and concentrated as above. The residual solid was purified by silica gel chromatography (0–30% EtOAc/ hexane) to obtain **8c** (715.3 mg, 99%) as a yellowish white solid. ^{1}H NMR ($CDCl_3$ with 0.05% v/v TMS, 270 MHz): δ 1.83 (1H, t, *J*=6.2 Hz, -OH), 2.60 (3H, s, *CH_3*CO-), 4.52 (2H, d, *J*=6.2 Hz, -*CH_2*OH), 7.49-7.53 (2H, m, Ar-*H*), 7.88-7.92 (2H, m, Ar-*H*).

3-Phenylprop-2-yn-1-yl 4-methylbenzenesulfonate (9a)

With stirring at −10 °C, triethylamine (1.70 g, 16.8 mmol) and a solution of *p*-toluenesulfonyl chloride (1.61 g, 8.42 mmol) in THF (3 mL) were added to a solution of **8a** (556 mg, 4.21 mmol) in THF (1 mL) under an atmosphere of Ar. The reaction mixture stirred for 1 h at 0 °C.

After removing THF under reduced pressure, it was added EtOAc (20 mL) and successively washed with sat. NH_4Cl, water and brine. The organic layer was dried and concentrated as above. The residual solid was purified by silica gel chromatography (0–10% EtOAc/ hexane) to obtain **9a** (190 mg, 16%) as a pale-yellow oil. ^{1}H NMR ($CDCl_3$ with 0.05% v/v TMS, 270 MHz): δ 2.39 (3H, s, Ph*CH*$_3$), 4.95 (2H, s, -*CH*$_2$OSO$_2$-), 7.24-7.37 (7H, m, Ar-*H)*, 7.85 (2H, m, Ar-*H*).

3-(*p*-Tolyl)prop-2-yn-1-yl 4-methylbenzenesulfonate (9b)[3]

A mortar was charged with **8b** (5.00 g, 34.4 mmol), potassium carbonate (K_2CO_3, 17.7 g, 128 mmol) and *p*-toluenesulfonyl chloride (9.62 g, 50.5 mmol), and grinded vigorously for 30 min. After the completion of tosylation, remaining tosyl chloride was removed by addition of potassium hydroxide (KOH, 10.3 g, 184 mmol) and *tert*-butyl alcohol (1 mL), and then vigorously grinded for 30 min. The product was extracted by addition of EtOAc (150 mL × 3) and water (100 mL). The organic layer was washed with brine, dried, and concentrated as above to obtain **9b** (8.25 g, 80%) as a brown oil. ^{1}H NMR ($CDCl_3$ with 0.05% v/v TMS, 270 MHz): δ 2.34 (3H, s, Ph*CH*$_3$), 2.40 (3H, s, Ph*CH*$_3$), 4.94 (2H, s, -*CH*$_2$OSO$_2$-), 7.10-7.16 (4H, m, Ar-*H*), 7.30-7.33 (2H, m, Ar-*H*), 7.83-7.88 (2H, m, Ar-*H*); HRMS (*m/z*): $[M+Na]^+$ calc'd for $C_{17}H_{16}O_3SNa$, 323.0724; found, 323.0718.

3-(4-Acetylphenyl) prop-2-yn-1-yl 4-methylbenzenesulfonate (9c)

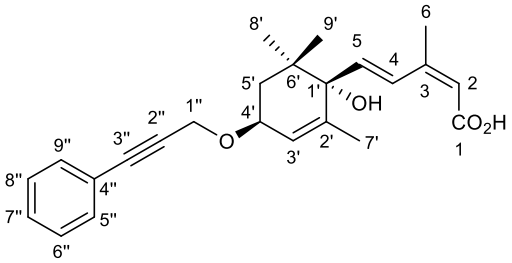
With stirring at −10 °C, triethylamine (1.62 g, 16.0 mmol) and a solution of *p*-toluenesulfonyl chloride (1.48 g, 7.76 mmol) in THF (4 mL) were added to a solution of **8c** (715.3 mg, 4.11 mmol) in THF (4 mL) and the mixture stirred for 5.5 h at 0 °C. After quenching with 1 M HCl (4 mL) at room temperature, the resulting mixture was extracted with EtOAc (30 mL). The

organic layer was successively washed with 1 M HCl and brine, and then dried, and concentrated as above. The residual oil was purified by silica gel chromatography (0–30% EtOAc/ hexane) to obtain **9c** (644.4 mg, 47%) as a colorless oil. ^{1}H NMR ($CDCl_3$ with 0.05% v/v TMS, 270 MHz): δ 2.41 (3H, s, Ph*CH*$_3$), 2.59 (3H, s, *CH*$_3$CO-), 4.96 (2H, s, -*CH*$_2$OSO$_2$-), 7.24-7.36 (4H, m, Ar-*H*), 7.84-7.88 (4H, m, Ar-*H*); HRMS (*m/z*): $[M+Na]^+$ calc'd for $C_{18}H_{16}O_4SNa$, 351.0667; found, 351.0671.

(2Z,4E)-5-((1S,4S)-1-Hydroxy-2,6,6-trimethyl-4-((3-phenylprop-2-yn-1-yl)oxy)cyclohex-2-en-1-yl)-3-methylpenta-2,4-dienoic acid, PANH (1)

With stirring at 0 °C, sodium hydride (NaH, 60% in oil, 27.8 mg, 0.696 mmol) was added to a solution of **6** (60.0 mg, 0.225 mmol) in THF (3 mL) under an atmosphere of Ar. After being stirred for 30 min at room temperature, a solution of **9a** (33.4 mg, 0.113 mmol) was added to the stirred mixture. The reaction mixture was stirred for 44 h at room temperature. After quenching with 1 M HCl (1.5 mL), it was diluted with water (20 mL) and extracted with EtOAc (15 mL × 3). The organic layer was washed with brine, dried, and concentrated as above. The residual solid was purified by silica gel chromatography (0–30% EtOAc/ hexane containing 0.1% AcOH) to obtain **1** (24.7 mg, 56%) as a yellow oil, which was further purified for bioassays by HPLC (YMC-Pack SIL-06, 150 × 20.0 mm i.d.; solvent, 20% EtOAc in hexane containing 0.1% AcOH; flow rate, 10 ml min^{-1}; detection, 254 nm) to obtain a colorless oil. ^{1}H NMR ($CDCl_3$ with 0.05% v/v TMS, 270 MHz): δ 0.93 (3H, s, H$_3$-8′ or H$_3$-9′), 1.07 (3H, s, H$_3$-8′ or H$_3$-9′), 1.68 (3H, d, *J*=1.6 H, H$_3$-7′), 1.72 (1H, dd, *J*=12.8 and 9.9 Hz, H-5′), 1.89 (1H, ddd, *J*=12.8, 5.9 and 1.0 Hz, H-5′), 2.02 (3H, d, *J*=1.3 Hz, H$_3$-6), 4.27 (1H, m, H-4′), 4.41 (1H, d, *J*=15.8 Hz, H-1″), 4.48 (1H, d, *J*= 15.8 Hz, H-1″), 5.69 (1H, m, H-3′), 5.71 (1H, br s, H-2), 6.22 (1H, d, *J*=16.2 Hz, H-5), 7.28–7.36 (3H, m, H-6″, 7″ and 8″),

7.41-7.48 (2H, m, H-5″ and 9″), 7.74 (1H, d, *J*=16.2 Hz, H-4); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 17.7 (C7′), 21.5 (C6), 22.7 (C8′), 25.3 (C9′), 39.7 (C6′), 40.7 (C5′), 56.1 (C1″), 72.3 (C4′), 79.3 (C1′), 85.5 (C2″), 86.0 (C3″), 117.0 (C2), 122.7 (C4″), 124.6 (C3′), 126.5 (C4), 128.3 (C6″ and 8″), 128.4 (C7″), 131.7 (C5″ and 9″), 139.1 (C2′), 140.3 (C5), 152.2 (C3), 170.6 (C1); UV λ_{max} (MeOH) nm (ε): 242.4 (27,600), 250.4 (29,000); HRMS (*m/z*): $[M+Na]^+$ calc'd. for $C_{24}H_{28}O_4Na$, 403.1885; found, 403.1880.



(2*Z*,4*E*)-5-((1*S*,4*S*)-1-Hydroxy-2,6,6-trimethyl-4-((3-(p-tolyl)prop-2-yn-1-yl)oxy)cyclohex-2-en-1-yl)-3-methylpenta-2,4-dienoic acid, PANMe (2)

With stirring at 0 °C, a solution of **6** (5.37 g, 20.2 mmol) in THF (150 mL) was added to a suspension of NaH (1.66 g, 69.0 mmol) in THF (90 mL). After being stirred for 15 min at the same temperature, **9b** (6.06 g, 20.2 mmol) in THF (60 mL) was added dropwise to the mixture. The mixture was stirred for 2 d at room temperature. After quenching with 1 M HCl (200 mL), it was extracted with EtOAc (500 mL × 3). The organic layer was washed, dried, and concentrated as above. The residual solid was purified by silica gel chromatography (20% EtOAc/ hexane containing 0.1% AcOH) to obtain **2** (2.45 g, 31%) as a yellow oil. ^{1}H NMR ($CDCl_3$ with 0.05% v/v TMS, 500 MHz): δ 0.81 (3H, s, H_3-8′ or H_3-9′), 1.05 (3H, s, H_3-8′ or H_3-9′), 1.66 (3H, s, H_3-7′), 1.71 (1H, dd, *J*=13.1 and 9.8 Hz, H-5′), 1.89 (1H, m, H-5′), 2.00 (3H, s, H_3-6), 2.34 (3H, s, Ph*C*H_3), 4.26 (1H, m, H-4′), 4.41 (1H, d, *J*=15.6 Hz, H-1″), 4.45 (1H, d, *J*= 15.6 Hz, H-1″), 5.67 (1H, s, H-3′), 5.71 (1H, s, H-2), 6.19 (1H, d, *J*=15.9 Hz, H-5),

7.10–7.12 (2H, m, H-6″ and 8″), 7.32-7.34 (2H, m, H-5″ and 9″), 7.74 (1H, d, *J*=15.9 Hz, H-4); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 17.7 (C7′), 21.5 (C6 and Ph*CH*$_3$), 22.7 (C8′), 25.3 (C9′), 39.6 (C6′), 40.8 (C5′), 56.2 (C1″), 72.2 (C4′), 79.3 (C1′), 84.8 (C2″), 86.1 (C3″), 117.3 (C2), 119.6 (C4″), 124.6 (C3′), 126.5 (C4), 129.0 (C6″ and C8″), 131.6 (C5″ and C9″), 138.5 (C7″), 139.1 (C2′), 151.7 (C3), 170.7 (C1); UV λ_{max} (MeOH) nm (ε): 245.8 (27,000), 254.6 (28,000); HRMS (*m/z*): $[M+Na]^+$ calc'd for $C_{25}H_{30}O_4Na$, 417.2042; found, 417.2033.

(2*Z*,4*E*)-5-((1*S*,4*S*)-4-((3-(4-Acetylphenyl)prop-2-yn-1-yl)oxy)-1-hydroxy-2,6,6-trimethylcyclohex-2-en-1-yl)-3-methylpenta-2,4-dienoic acid, PANAc (4)

With stirring at 0 °C, potassium *tert*-butoxide (513 mg, 5.47 mmol) and a solution of **9c** (644 mg, 1.96 mmol) were added to a solution of **6** (379 mg, 1.42 mmol) in THF (15 mL) and the mixture stirred for 5.5 h at room temperature. After quenching with 1 M HCl (15 mL), it was extracted with EtOAc (30 mL × 3). The organic layer was washed, dried, and concentrated as above. The residual solid was purified by silica gel chromatography (0–40% EtOAc/ hexane containing 0.1% AcOH). A portion of the 40% EtOAc elute containing **4** was further purified by HPLC (YMC Hydrosphere C18, 150 × 20.0 mm i.d.; solvent, 70% MeOH; flow rate, 5 ml min^{-1}; detection, 254 nm) to obtain **4** (8.6 mg, 1.4%) as a colorless oil. ^{1}H NMR ($CDCl_3$ with 0.05% v/v TMS, 500 MHz): δ 0.94 (3H, s, H_3-9′), 1.04 (3H, s, H_3-8′), 1.68 (3H, s, H_3-7′), 1.68 (1H, dd, *J*=13.1 and 10.1 Hz, H-5′), 1.84 (1H, ddd, *J*=13.1, 6.3 and 1.5 Hz, H-5′), 2.00 (3H, s, H_3-6), 2.56 (3H, s, *CH*$_3$CO-), 4.28 (1H, m, H-4′), 4.45 (1H, d, *J*=16.2 Hz, H-1"), 4.49 (1H, d, *J*=16.2 Hz, H-1″), 5.66 (1H, s, H-3′), 5.71 (1H, s, H-2), 6.17 (1H, d, *J*=16.1 Hz, H-5), 7.53–7.54 (2H, m, H-5″ and 9″), 7.67 (1H, d, *J*=16.1 Hz, H-4), 7.96–7.97 (2H, m, H-6″ and 8″); ^{13}C NMR ($CDCl_3$, 125 MHz); δ 18.4 (C7′), 21.3 (C6), 23.3 (C8′), 25.7 (C9′), 26.7 (*CH*$_3$CO-), 40.9 (C6′), 41.6 (C5′), 56.6 (C1″), 74.2 (C4′), 80.2 (C1′), 85.9 (C3″), 90.4 (C2″),

119.7 (C2), 125.4 (C3′), 128.3 (C4), 129.0 (C4″), 129.5 (C6″ and 8″), 132.8 (C5″ and 9″), 137.8 (C7″), 140.7 (C5), 141.4 (C2′), 150.6 (C3), 170.3 (C1), 199.6 (C10″); UV λ_{max} (MeOH) nm (ε): 255.8 (40,000); HRMS (*m/z*): $[M+Na]^+$ calc'd. for $C_{26}H_{30}O_5Na$, 445.1989; found, 445.1988.

Prop-2-yn-1-yl 4-methylbenzenesulfonate (10)

With stirring at 0 °C, propargyl alcohol (1.54 g, 27.4 mmol), dimethylamine hydrochloride (0.447 g, 5.48 mmol) and triethylamine (4.01 g, 39.4 mmol) were added to a solution of *p*-toluenesulfonyl chloride (7.64 g, 40.1 mmol) in dichloromethane (CH_2Cl_2, 30 mL) under an atmosphere of Ar, and then the mixture stirred for 1.5 h at the same temperature. After quenching with water (30 mL), the resulting mixture was extracted with EtOAc (200 mL × 3). The organic layer was washed, dried, and concentrated as above. The residual oil was purified by silica gel chromatography (0–20% EtOAc/ hexane) to obtain **10** (4.40 g, 76%) as a colorless oil. ^{1}H NMR ($CDCl_3$ with 0.05% v/v TMS, 270 MHz): δ 2.44-2.51 (4H, m, *HC*≡CCH$_2$- and -C$_6$H$_4$*CH$_3$*), 4.70 (2H, d, *J*=2.6 Hz, HC≡C*CH$_2$*-), 7.33-7.37 (2H, m, Ar-*H*), 7.79-7.84 (2H, m, Ar-*H*).

(2*Z*,4*E*)-5-((1*S*,4*S*)-1-Hydroxy-2,6,6-trimethyl-4-(prop-2-yn-1-yloxy)cyclohex-2-en-1-yl)-3-methylpenta-2,4-dienoic acid (11)

With stirring at 0 °C, NaH (69.2 mg, 3.27 mmol) and a solution of **10** (555 mg, 2.64 mmol) in THF (6.5 mL) were added to a solution of **6** (234 mg, 0.880 mmol) in THF (5 mL) and the mixture stirred for 6 h at the same temperature. After quenching with 1 M HCl (2 mL), it was diluted with water (10 mL) and extracted with EtOAc (50 mL × 3). The organic layer was washed, dried, and concentrated as above. The residual oil was purified by silica gel

chromatography (0–40% EtOAc/ hexane containing 0.1% AcOH) to obtain **11** (45.9 mg, 17%) as a colorless oil. ^{1}H NMR ($CDCl_3$ with 0.05% v/v TMS, 270 MHz): δ 0.92 (3H, s, H_3-8′ or H_3-9′), 1.05 (3H, s, H_3-8′ or H_3-9′), 1.63-1.71 (4H, m, H-5' and H_3-7′), 1.85 (1H, ddd, *J*=13.2, 6.6 and 1.3 Hz, H-5′), 2.02 (3H, d, *J*=1.0 Hz, H_3-6), 2.43 (1H, t, *J*=2.3 Hz, H-3′′), 4.16–4.23 (3H, m, H-4′ and H_2-1′′), 5.63–5.64 (1H, m, H-2 or H-3′), 5.72 (1H, s, H-2 or H-3′), 6.19 (1H, d, *J*=16.2 Hz, H-5), 7.35 (1H, d, *J*=16.2 Hz, H-4); HRMS (*m/z*): $[M+Na]^+$ calc'd for $C_{18}H_{24}O_4Na$, 327.1572; found, 327.1569.

(2*Z*,4*E*)-5-((1*S*,4*S*)-1-Hydroxy-4-((3-(4-methoxyphenyl)prop-2-yn-1-yl)oxy)-2,6,6-trimethylcyclohex-2-en-1-yl)-3-methylpenta-2,4-dienoic acid, PANOMe (3)

With stirring at room temperature, CuI (10.8 mg, 0.057 mmol) and bis(triphenylphosphine)palladium(II) dichloride (9.8 mg, 0.014 mmol) were added to a solution of 4-iodoanisole (84.7 mg, 0.36 mmol) in triethylamine (2 mL) under an atmosphere of Ar. After being stirred for 30 min at the same temperature, a solution of **11** (48.6 mg, 0.16 mmol) in THF (2.0 mL) was added to the stirred mixture. The reaction mixture was stirred for 15 min at room temperature, and then it was filtered through silica gel (EtOAc). The filtrate was successively washed with 1 M HCl and brine, and then dried and concentrated as above. The residual solid was purified by silica gel chromatography (0–30% EtOAc/ hexane containing 0.1% AcOH). A portion of the 30% EtOAc elute containing **3** was further purified by HPLC (YMC Hydrosphere C18, 150 × 20.0 mm i.d.; solvent, 75% MeOH containing 0.1 % AcOH; flow rate, 5.5 ml min^{-1}; detection, 254 nm) to obtain **3** (14.3 mg, 22%) as a colorless oil. ^{1}H NMR (CD_3OD with 0.05% v/v TMS, 500 MHz): 0.93 (3H, s, H_3-9′), 1.04 (3H, s, H_3-8′), 1.68 (3H, m, H_3-7′), 1.68 (1H, m, H-5′), 1.84 (1H, ddd, *J*=13.3, 6.5 and 1.5 Hz, H-5′), 2.01 (3H, d, *J*=1.2 Hz, H_3-6), 3.80 (3H, s, -O*C*H_3), 4.28 (1H, m, H-4′), 4.39 (2H, d,

J=16.0 Hz, H_2-1″), 4.43 (2H, d, *J*=16.0 Hz, H_2-1″), 5.65 (1H, m, H-3′), 5.70 (1H, s, H-2), 6.19 (1H, d, *J*=16.2 Hz, H-5), 6.87–6.89 (2H, m, H-6″ and 8″), 7.33–7.36 (2H, m, H-5″ and 9″), 7.69 (1H, d, *J*=16.2 Hz, H-4); ^{13}C NMR (CD_3OD, 125 MHz); δ 18.3 (C7′), 21.3 (C6), 23.3 (C9′), 25.7 (C8′), 40.8 (C6′), 41.7 (C5′), 55.8 (-O*C*H_3), 56.8 (C1″), 73.9 (C4″), 80.2 (C1′), 85.1 (C2″), 86.8 (C3″), 115.1 (C5″ and 9″), 116.1 (C4″), 119.9 (C2), 125.5 (C3′), 128.2 (C4), 134.1 (C6″ and 8″), 140.5 (C5), 141.2 (C2'), 150.1 (C3), 161.4 (C7″), 169.8 (C1); UV λ_{max} (MeOH) nm (ε): 255.8 (40,000); HRMS (*m/z*): $[M+Na]^+$ calc'd. for $C_{25}H_{30}O_5Na$, 433.1991; found, 433.1994.

(2*Z*,4*E*)-5-((1*S*,4*S*)-1-hydroxy-2,6,6-trimethyl-4-((3-(4-(trifluoromethyl)phenyl)prop-2-yn-1-yl)oxy)cyclohex-2-en-1-yl)-3-methylpenta-2,4-dienoic acid, PANCF3 (5)

With stirring at room temperature, CuI (4.0 mg, 0.021 mmol) and bis(triphenylphosphine)palladium(II) dichloride (2.0 mg, 2.8 μmol) were added to a solution of 4-iodobenzotrifluoride (26 mg, 95 μmol) in triethylamine (0.5 mL) under an atmosphere of Ar. After being stirred for 30 min at the same temperature, a solution of **11** (29 mg, 95 μmol) in triethylamine (0.5 mL) was added to the stirred mixture. The reaction mixture was stirred for 6 h at room temperature, and then it was filtered through silica gel (EtOAc). The filtrate was successively washed with 1 M HCl and brine, and then dried and concentrated as above. The residual solid was purified by silica gel chromatography (0–50% EtOAc/ hexane containing 0.1% AcOH). A portion of the 30% EtOAc elute containing **5** was further purified by HPLC (YMC Hydrosphere C18, 150 × 20.0 mm i.d.; solvent, 80% MeOH containing 0.1 % AcOH; flow rate, 8.5 ml min^{-1}; detection, 254 nm) to obtain **5** (4.3 mg, 10%) as a colorless oil. ^{1}H NMR ($CDCl_3$ with 0.05% v/v TMS, 270 MHz): δ 0.93 (3H, s, H_3-8′ or H_3-9′), 1.07 (3H, s, H_3-8′ or H_3-9′), 1.68 (3H, m, H_3-7′), 1.72 (1H, dd, *J*=13.1 and 9.7 Hz, H-5′), 1.90

(1H, ddd, *J*=13.1, 6.4 and 1.4 Hz, H-5′), 2.02 (3H, s, H_3-6), 4.25 (1H, m, H-4′), 4.42 (1H, d, *J*=15.9 Hz, H_2-1″), 4.49 (1H, d, *J*=15.9 Hz, H_2-1″), 5.68 (1H, m, H-3′), 5.71 (1H, br s, H-2), 6.21 (1H, d, *J*=16.2 Hz, H-5), 7.52-7.59 (4H, m, H-5″, H-6″, H-8″ and H-9″), 7.74 (1H, d, *J*=16.2 Hz, H-4); ^{13}C NMR ($CDCl_3$, 68 MHz); 17.7 (C7′), 21.5 (C6), 22.6 (C8′), 25.2 (C9′), 39.7 (C6′), 40.6 (C5′), 55.9 (C1″), 72.6 (C4′), 79.2 (C1′), 84.6 (C2″), 88.1 (C3″), 116.9 (C2), 123.8 (Ph*C*F_3, q, J_{CF}=272.0 Hz), 124.4 (C3′), 125.2 (C6″ and 8″, q, $^3J_{CF}$=3.9 Hz), 126.5 (C4″), 126.6 (C4), 130.2 (C7″, q, $^2J_{CF}$=32.3 Hz), 131.9 (C5″ and 9″), 139.4 (C2′), 140.3 (C5), 152.4 (C3), 170.9 (C1); UV λ_{max} (MeOH) nm (ε): 254.6 (33,000); HRMS (*m/z*): $[M+Na]^+$ calc'd for $C_{25}H_{27}F_3O_4Na$, 471.1759; found, 471.1751.

Seed germination assays

The classic definition of radical emergence was used for seed germination assays. All assays were performed at least three times. For *Arabidopsis*, 60 to 80 seeds (Columbia accession) were sterilized by soaking in 70% aqueous ethanol (EtOH, v/v) for 30 min and reagent-grade EtOH for 1 min. Seeds were then soaked in distilled water and incubated in the dark at 4°C for 3 days. The stratified seeds were then soaked in 1 mL of a test medium liquid agar containing 1/2 Murashige and Skoog (MS) in 24-well plates and allowed to germinate under continuous illumination at 22°C.

For lettuce, 25 seeds (*Lactuca sativa* L. cv. Cisco) were placed in a dish on two sheets of filter paper soaked in 2 mL of a test solution and allowed to germinate and grow under continuous illumination at 22°C.

For leaf celery, 25 seeds (*Apium graveolens* var. *secalinum*) were sterilized by soaking in 70% EtOH for 5 min and reagent-grade EtOH for 1 min. Seeds were then soaked in distilled water and incubated in the dark at 4°C for 3 days. The stratified seeds were then soaked in 2 mL

of a test medium liquid agar containing 1/2 MS in 6-well plates and allowed to germinate under continuous illumination at 22°C.

For mitsuba, 25 seeds (*Cryptotaenia canadensis* subsp. *japonica*) were sterilized by soaking in 70% EtOH for 5 min and reagent-grade EtOH for 1 min. Seeds were placed in a dish on two sheets of filter paper soaked in 2 mL of a test solution and allowed to germinate under continuous illumination at 22°C.

For perilla herba, 25 seeds (*Perilla frutescens* var. *Frutescens*) were placed in a dish on two sheets of filter paper soaked in 2 mL of a test solution and allowed to germinate under continuous illumination at 22°C.

For komatsuna, 25 seeds (*Brassica rapa* var. *perviridis*) were placed in a dish on two sheets of filter paper soaked in 2 mL of a test solution and allowed to germinate under continuous illumination at 22°C.

For rice, 25 seeds (*Oryza sativa* L. cv. Nipponbare) were placed in a dish on two sheets of filter paper soaked in 2 mL of a test solution and allowed to germinate under continuous illumination at 30°C.

For carrot, 25 seeds (*Daucus carota* subsp. *sativus*) were placed in a dish on two sheets of filter paper soaked in 2 mL of a test solution and allowed to germinate under continuous illumination at 22°C.

For proso millet, 25 seeds (*Panicum miliaceum*) were placed in a dish on two sheets of filter paper soaked in 2 mL of a test solution and allowed to germinate under continuous illumination at 30°C.

Arabidopsis seedling growth assay

Arabidopsis seeds were sowed on solid 1/2 MS medium. After vernalization at 4°C for 3 days, the plants were grown for 10 days at 22°C under continuous illumination and then transplanted into pots containing 40 g of a 1:1 mixture of vermiculite:organic potting soil. Plants were sprayed with 1 mL of 5 μM ABA and 50 μM PYL antagonists (AS6 or PANMe) dissolved in a solution containing 0.1% DMSO and 0.1% agrochemical spreader, Approach BI (Kao Co., Ltd.) once a day for 3 weeks under continuous illumination at 22°C.

Rice seedling elongation assay

Seven seeds (*Oryza sativa* L. cv. Nipponbare) were sterilized with reagent-grade EtOH for 5 min and washed with running tap water. They were then soaked in distilled water and incubated under continuous illumination at 25°C for 3 days to germinate. The germinated seeds were then soaked in 2 mL of a test medium in a glass tube and grown under continuous illumination at 30°C. When the seedlings were 7 days old, the length of the second leaf sheath was measured. All assays were performed at least three times.

Thermal imaging

The stock solutions of ABA and the PYL antagonists (AS6 and PANMe) were adjusted 100 mM by DMSO. *Arabidopsis* plants (23 days old) were sprayed with 50 μM ABA and/or 100 μM PYL antagonists (AS6 or PANMe) dissolved in a solution containing 30 mM KCl, 5 mM MES-KOH, pH 6.15, 1 mM $CaCl_2$, and 0.012% Silwet L-77 (*Bio Medical Science*). Control plants were sprayed with the same solution with DMSO only. The final concentration of DMSO was 0.15% (v/v) in all test solution conditions. After overnight incubation under the conditions of constant white light at 80 μmol m^{-2} s^{-1}, 22°C, and 60% relative humidity (RH), each plant

was transferred to a custom-made growth cabinet equipped with an automatic CO_2 control unit (TMC-LW1208A/K, TM Systems Ltd.) and incubated under the conditions of constant white light at 100 μmol m^{-2} s^{-1} at 22°C, 40% RH, and 200 μL L^{-1} [CO_2] for 3 h. Thermal images were captured using a thermography camera (InfReC Thermography R300, NEC Avio Infrared Technologies Co. Ltd.) and an InfRec Analyzer NS9500 Standard (NEC Avio Infrared Technologies Co. Ltd.).

Stomatal aperture response analysis

Stomatal aperture measurements in chemical-treated leaves were performed as described previously[4], with minor modifications. Plants (23 days old) were sprayed with 50 μM ABA and/or 50 μM or 100 μM PYL antagonists (AS6 or PANMe) using the same method as that used for thermal imaging measurement. After overnight incubation in a growth chamber, each plant was transferred to a custom-made growth cabinet and incubated under the conditions of constant white light at 100 μmol m^{-2} s^{-1} at 22°C, 40% RH, and 200 μL L^{-1} [CO_2] for 3 h. The abaxial epidermis of rosette leaves was peeled using Scotch tape and photographed using a microscope (IX71, Olympus). Stomatal images were later analyzed to determine aperture using ImageJ software (https://imagej.nih.gov/ij/; NIH).

qRT-PCR analysis

Total RNA was isolated using Plant RNA Reagent (Thermo Fisher Scientific Inc.), according to the manufacturer's protocol. cDNA was synthesized using the QuantiTec reverse transcription kit (Qiagen GmbH). Real-time PCR using SYBR® Premix Ex Taq™ (Takara Bio. Inc.) was performed with the StepOnePlus™ Real-Time PCR system (Thermo Fisher Scientific Inc.). The relative amount of target mRNA was based on a standard curve and normalized to the

relative amount of internal control mRNA. Biological quadruplicate experiments were performed, and primer sets were used as previously described[5].

GUS staining assay.

For ABA-responsive reporter gene analyses, transgenic *Arabidopsis* expressing β-glucuronidase (GUS) under the control of the *AtMAPKKK18* promoter were used, as previously described[5]. Seedlings were grown on agar plates containing 1/2 MS and 0.5% sucrose for 6 days at 22°C and an 18/6-h light/dark cycle. Transgenic seedlings were transferred to incubation solution containing 1/2 MS and 0.5% sucrose and acclimated in incubation solution overnight before chemical treatment. For ABA agonist and antagonist analyses, a single chemical or a mixture was added to the incubation solution with seedlings and then incubated for 6 h under the 22°C light condition. For osmotic stress analysis, seedlings were transferred to 400 mM mannitol solution containing 1/2 MS, 0.5% sucrose, and chemicals, and incubated for 6 h under the 22°C light condition. GUS detection was performed in reaction buffer containing 50 mM sodium phosphate (pH 7.0), 0.05% Tween-20, 2.5 mM potassium ferrocyanide, 2.5 mM potassium ferricyanide, and 1 mM 5-bromo-4-chloro-3-indolyl-β-D-glucuronide (X-gluc) at 37°C. The reaction was stopped by adding ethanol (EtOH), and the chlorophyll pigment of the seedlings was bleached with 70% EtOH at 65°C. GUS staining was observed using stereo and optical microscopes.

PP2C enzyme assay.

The protocol of the PP2C enzyme assay was described elsewhere[6]. Briefly, PYLs (AtPYLs and OsPYL2) and PP2Cs (HAB1 and OsPP2C06) were expressed in *E. coli* and purified by affinity column chromatography, as previously described (16, 34). Purified proteins were preincubated

in 80 µL of a buffer containing 12.5 mM $MnCl_2$, 0.125% 2-mercaptoethanol, and test compound at 22°C for 30 min. After adding 20 µL of substrate buffer (165 mM Tris-acetate, pH 7.9, 330 mM potassium acetate, 0.1% BSA, and 250 mM *p*NPP), reactions were immediately monitored for hydrolysis of *p*NPP at 405 nm. For AtPYL, reactions contained 600 nM HAB1 and 600 nM (PYR1, PYL1–6, and PYL10) or 1200 nM (PYL8 and PYL9) AtPYL proteins. For OsPYL, reactions contained 3000 nM OsPP2C06 and 600 nM OsPYL2.

Pull-down assay

Purified GST-HAB1 and 6xHis-tagged PYLs were used 100 µg and 20 µg, respectively, and were incubated in 300 µL of Tris-buffered saline (TBS) containing 100 µg BAS, 0.025% 2-mercaptoethanol, 10 mM $MnCl_2$ and 10 mg PrepEase His-tagged protein purification resin (Affymetrix, Inc.) in the presence or absence of test compounds with gentle shaking at 4 °C for 60 min. The resin was then washed five times with TBS containing 0.025% 2-mercaptoethanol and 10 mM $MnCl_2$ at 4 °C. The bound proteins were eluted in 60 µL of SDS-sample buffer with 250 mM imidazole and denatured at 95 °C for 5 min. Then, 5 µL of eluate was loaded on a 12% SDS-PAGE gel, and proteins were detected after development by Coomassie brilliant blue staining.

Isothermal titration calorimetry (ITC)

The ITC experiments were performed with an iTC_{200} calorimeter (Microcal, GE Healthcare Bio-Sciences AB) as described previously[6]. Briefly, His_6-tagged PYL5 was assayed at a concentration of 50 µM with PANH and PANMe stock solutions in the injection syringe at a concentration of 500 µM. All titrations were carried out via a series of 25 injections of 1.25 µL each. The data were corrected by subtracting the mixing enthalpies for the PANH or PANMe

solutions into protein-free solutions and fitted by Origin for ITC (GE Healthcare Bio-Sciences AB) with a 1/1 binding model.

For measurement of the heat capacity change, ITC experiments were performed at different temperature ranges (25–35°C) using 50 μM His_6-tagged PYL5 and 500 μM ABA or PANMe. The ΔC_p values were determined from the slopes of the fitted line of Figure 3 using the standard thermodynamic relationship $\Delta C_p = d\Delta H/dT$.

X-Ray diffraction analysis of the PYR1-PANMe complex

PYR1 was expressed in *E. coli* and purified as described previously[6]. Briefly, protein was expressed in *E. coli* strain BL21-CodonPlus (DE3)-RIPL (Agilent Technologies), and purified using a Ni-Sepharose resin (GE Healthcare Bio-Sciences AB) and eluted with binding buffer (150 mM phosphate, pH 8.0, and 300 mM NaCl) supplemented with 250 mM imidazole. The protein was further purified using a Resource Q column (GE Healthcare Bio-Sciences AB) and eluted with binding buffer (10 mM Tris-HCl, pH 8.0) supplemented with 150 mM NaCl. Peak fractions were concentrated using an Amicon Ultrafilter (30,000 MWCO, Millipore Corp.).

For crystallization, 7 mg/mL of the purified PYR1 (1.5 μL) in 50 mM Tris-HCl (pH 8.0) containing 1 mM PANMe were mixed with an equal volume of reservoir solution consisting of 0.1 M MES, pH 6.5, and 16% (w/v) PEG3000. Crystals were prepared by the hanging drop vapor diffusion method at 20°C.

Diffraction data of PYR1 cocrystallized with PANMe were collected on beamline NW12A (λ = 1.000 Å) at the Photon Factory, KEK. The crystal was flash-frozen in a cold stream of nitrogen gas at 100 K after instant soaking in the reservoir solution containing 10% PEG1000 as a cryoprotectant. The datasets were processed with the program HKL2000[7]. The initial structure of PYR1 was solved by molecular replacement using the program MOLREP[8] in the

CCP4 suite[9] with the coordinates of PYR1 (PDB code: 3WG8) as a target model. Initial refinement was performed with the program Phenix[10] to run simulated annealing; further refinements were carried out with the program REFMAC5[11] in the CCP4 suite. The restraint file for the PANMe molecule was obtained from the PRODRG server[12]. The manual model building was performed with the program Coot[13]. The structure was refined at 2.5 Å to R/R_{free}-factors of 25.6/29.4%. A Ramachandran plot by the program Rampage reported that 98.5% of total residues are in most favored and 1.2% in additional allowed region and 0.3% in outlier. Residues 112–114 and 149–154 in chain A, and 149–154 in chain B were not modeled because of a lack of electron density. The statistics for data collection and refinement are provided in Table S1, Supporting Information.

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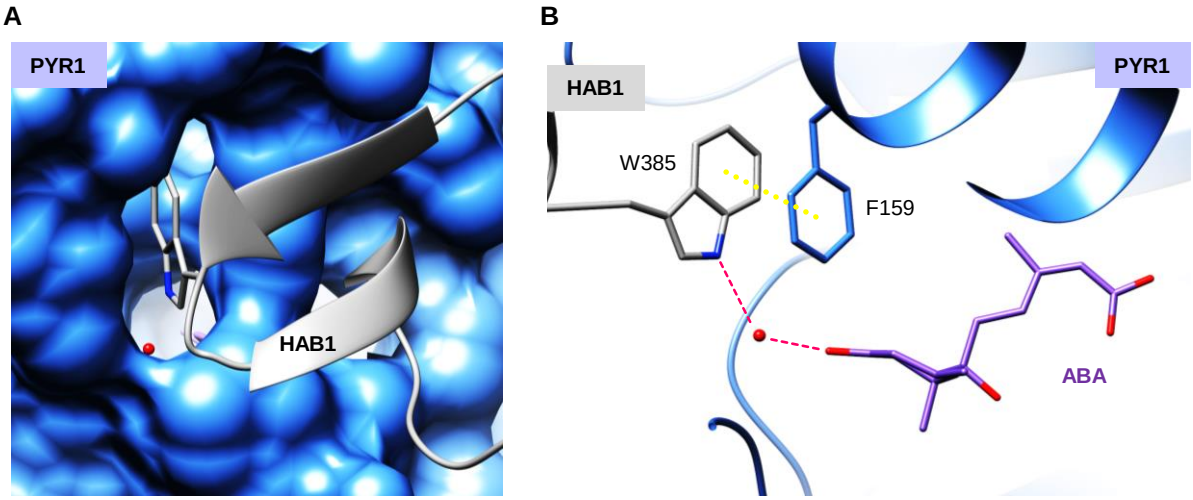

Figure S1. A conserved tryptophan in PP2Cs is inserted into the 4′-tunnel, forming a π-π interaction with Phe of PYLs and a water-mediated hydrogen bond with the carbonyl oxygen of ABA. (A) Close-up view of the 4′-tunnel of PYR1 in the PYR1-ABA-HAB1 (PDB code 3QN1) complex. The solvent-excluded surface (probe radius: 1.4 Å) as determined by Chimera software[14]. (B) Intermolecular interaction between PYR1 and HAB1 around Trp 385 of HAB1. Dotted yellow line, π-π interaction; dashed magenta lines, hydrogen bonds; red sphere, water molecule.

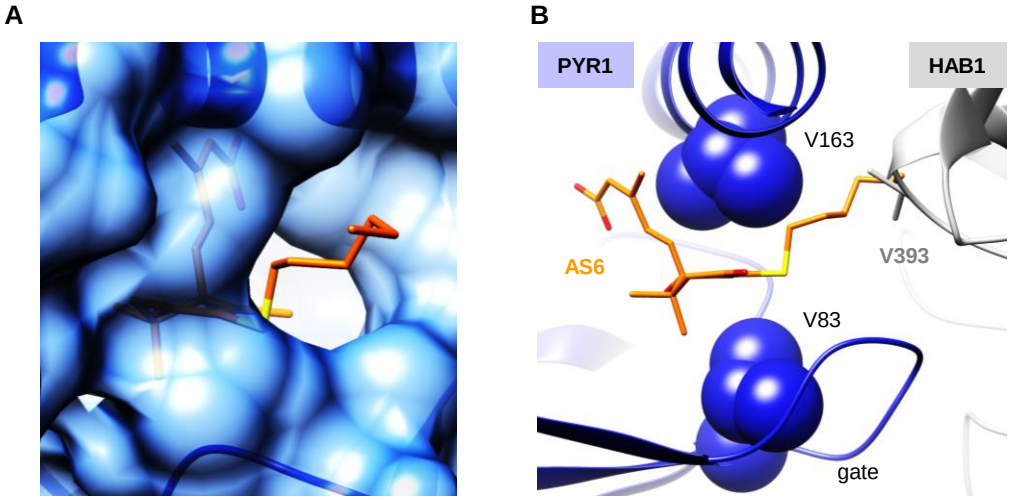

Figure S2. The 3′-S-hexyl chain of AS6 is accommodated by the 3′-tunnel and protrudes out onto the PP2C-interaction surface of PYL. (A) Crystal structure of the PYR1-AS6 complex (PDB code 3WG8). (B) Superposition of the PYR1-AS6 complex and the PYR1-ABA-HAB1 (gray, PDB code 3QN1) complex.

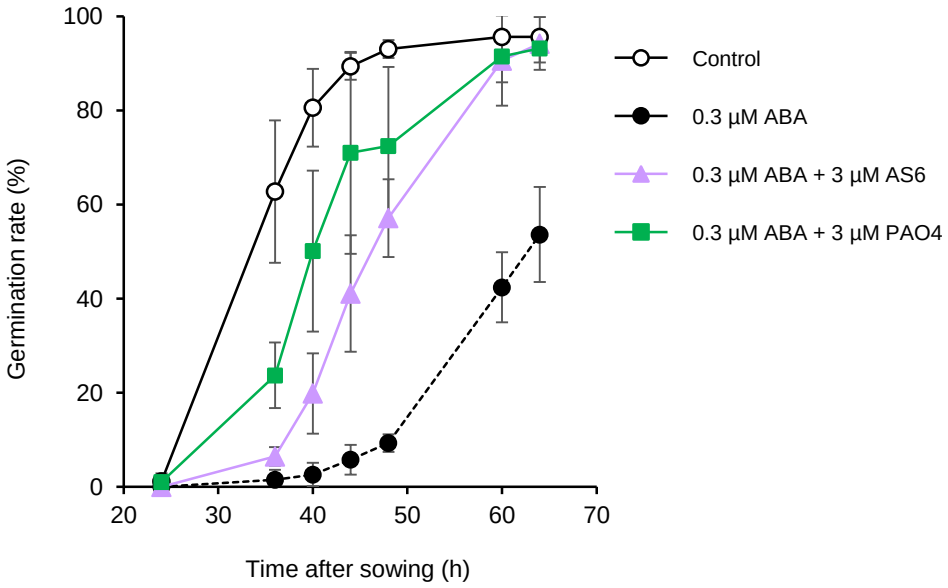

Figure S3. Antagonistic effects of AS6 and PAO4 on *Arabidopsis* seed germination. Seed germination rate in the presence of 0.3 µM ABA and 3 µM AS6 or PAO4. Seed germination rate in the presence of 0.3 µM ABA is also shown (*n* = 3; error bars, sd).

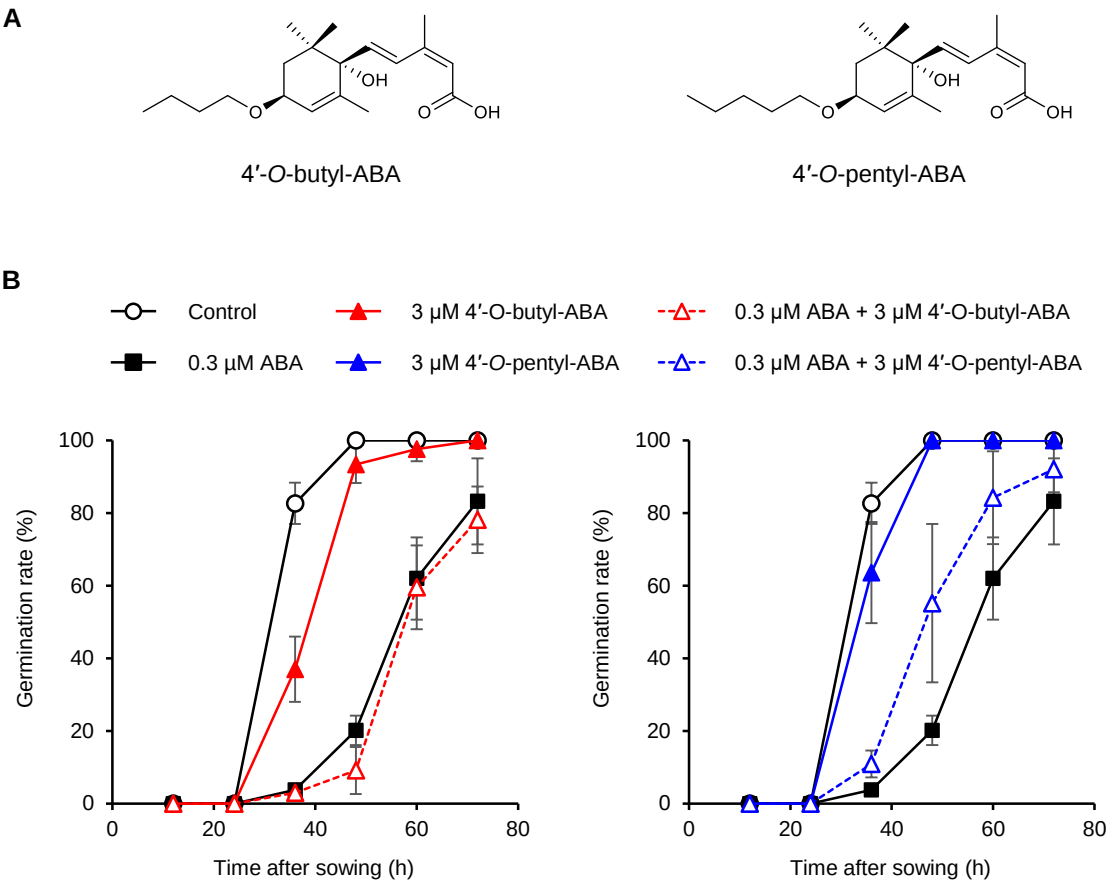

Figure S4. Characterization of 4′-O-alkyl-ABAs. (A) Structure of 4′-*O*-butyl-ABA and 4′-*O*-pentyl-ABA. (B) Seed germination rate in the presence of 3 μM 4′-*O*-butyl-ABA (left) or 4′-*O*-pentyl-ABA (right) with or without 0.3 μM ABA. Seed germination rate in the presence of 0.3 μM ABA is also shown (*n* = 3; error bars, sd).

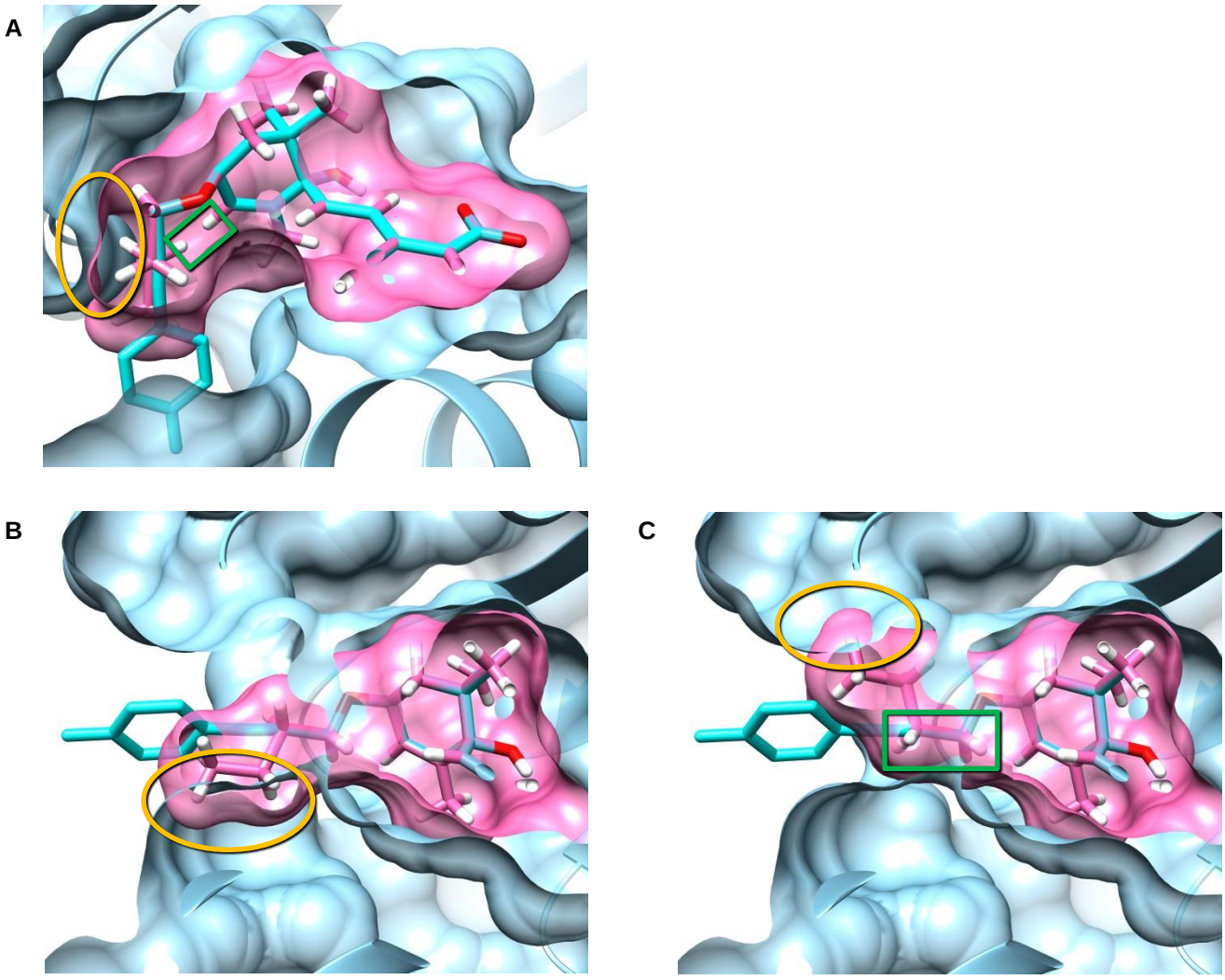

Figure S5. 4′-*O*-butyl-ABA superimposed in PYR1-PANMe complex A (A) and B (B and C: two conformations of 4′-*O*-butyl chain). Orange open circles show steric hindrances between 3′- or 4′-tunnel and 4′-*O*-butyl chain. These steric hindrances should cause the low affinity of 4′-*O*-butyl-ABA for PYR1. Green open squares show unstable conformations of 4′-*O*-butyl-ABA. These also should cause low affinity. Solvent-excluded surface area is represented in light blue (PYR1-PANMe) and pink (4′-*O*-butyl-ABA). PANMe and 4′-*O*-butyl-ABA are represented in cyan and pink, respectively, with stick-bond model.

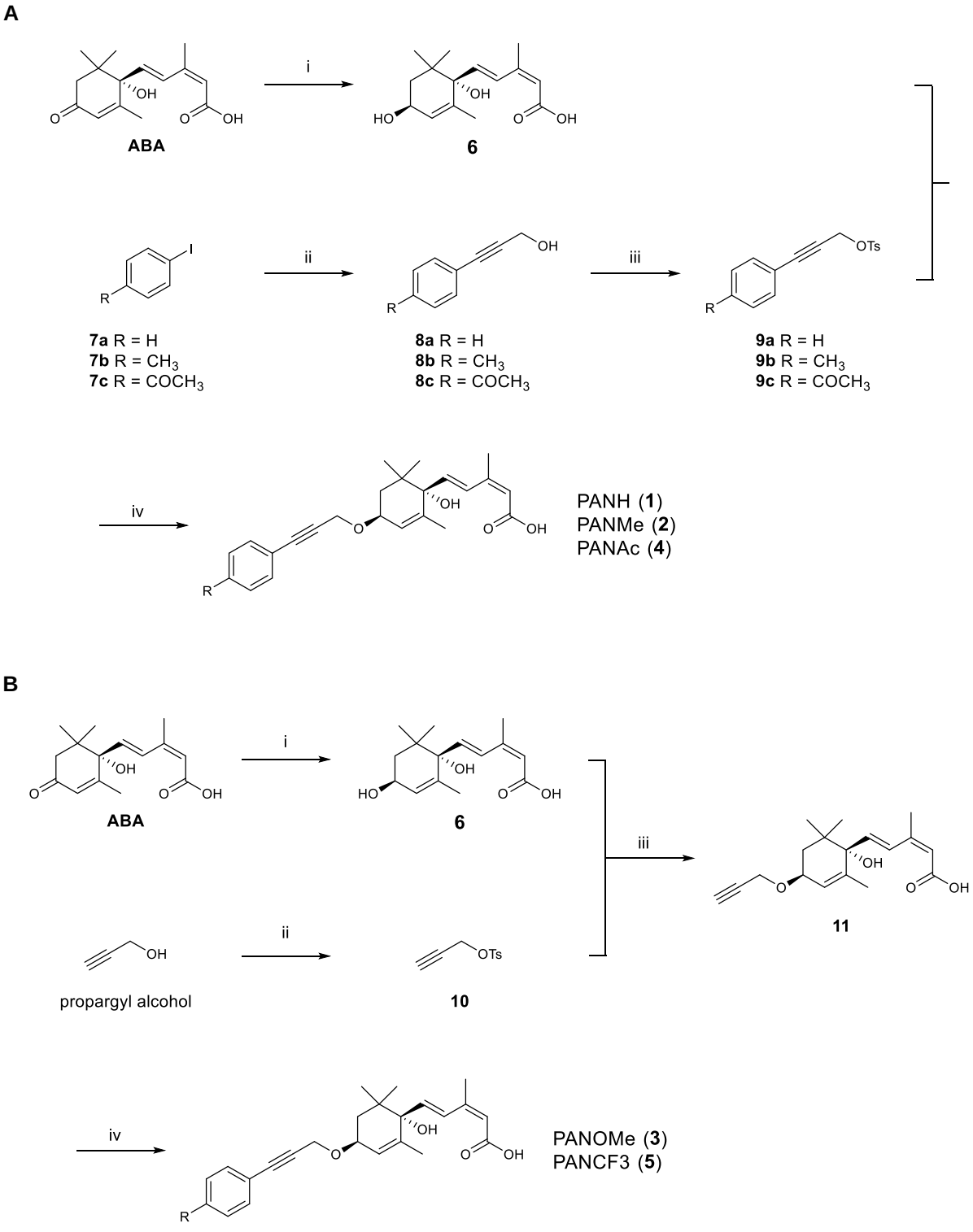

Figure S6. Synthesis of PAN compounds. (A) Synthesis of PANH, PANMe and PANAc. Reagents: (i) $NaBH_4$, $CeCl_3 \cdot 7H_2O$, MeOH; (ii) propargyl alcohol, CuI, $Pd(PPh_3)_2Cl_2$, THF; (iii) *p*-TsCl,Et_3N, THF; (iv) NaH, THF. (B) Synthesis of PANOMe and PANCF3. Reagents: (i) $NaBH_4$, $CeCl_3 \cdot 7H_2O$, MeOH; (ii) *p*-TsCl, Et_3N, THF; (iii) NaH, THF; (iv) CuI, $Pd(PPh_3)_2Cl_2$, benzyl iodide, THF

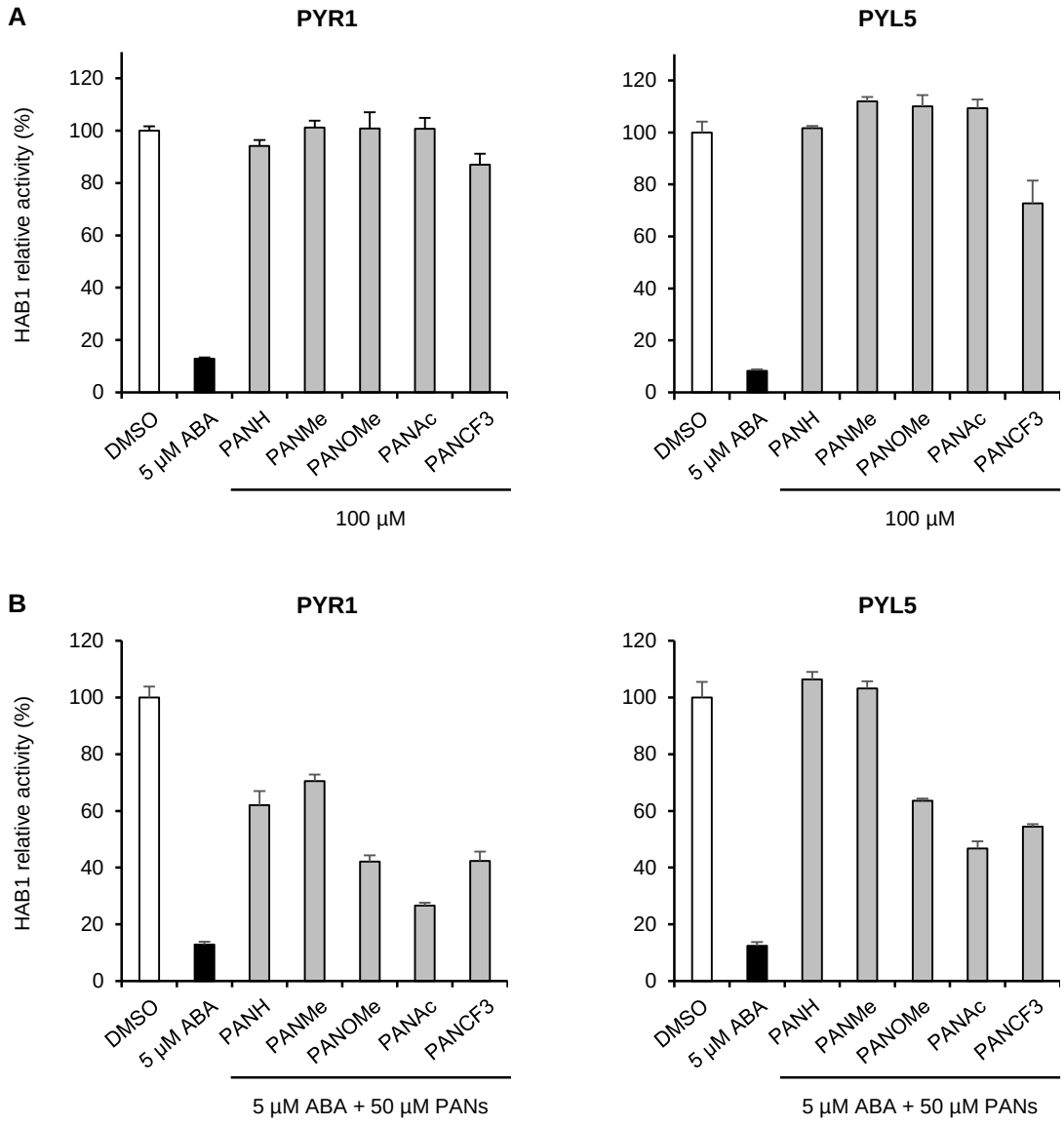

Figure S7. Effects of PAN compounds on HAB1 inhibition by ABA receptors. (A) Chemical inhibition of HAB1 by PYR1 and PYL5 in the presence of 100 µM PANs. (B) Antagonistic effect of each test compound on PYR1 and PYL5. The HAB1 phosphatase activity of each reaction was normalized to a control (DMSO-treated) value of 100% and expressed as relative activity. PYL and HAB1 proteins were used at the same molar ratio of 60 pmol (*n* = 3, error bars represent sd).

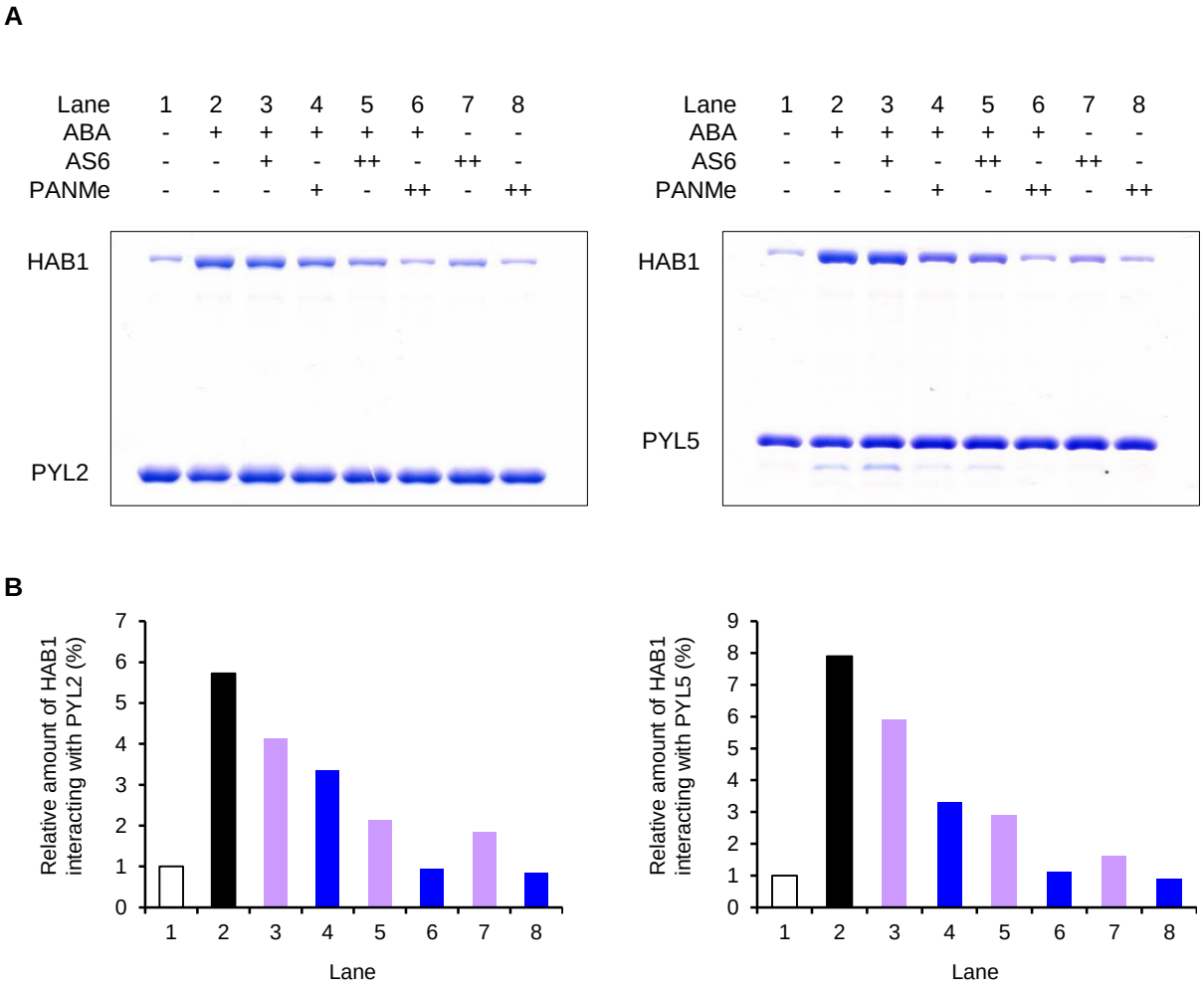

Figure S8. Antagonistic effect of AS6 and PANMe on ABA-mediated PYL-HAB1 interaction. (A) Pull-down assay performed using purified glutathione S-transferase (GST)-HAB1 and 6xHis-tagged PYLs (100 and 20 µg, respectively). Signals = (-) for 0 µM; (+) for 25 µM and (++) for 250 µM. (B) Relative amount of GST-HAB1 interacting with 6xHis-tagged PYLs. The amount of GST-HAB1 and 6xHis-tagged PYLs on the gel (A) was measured using Image J 1.48v software, and the relative pull-down GST-HAB1 was calculated by normalizing 6xHis-tagged PYLs.

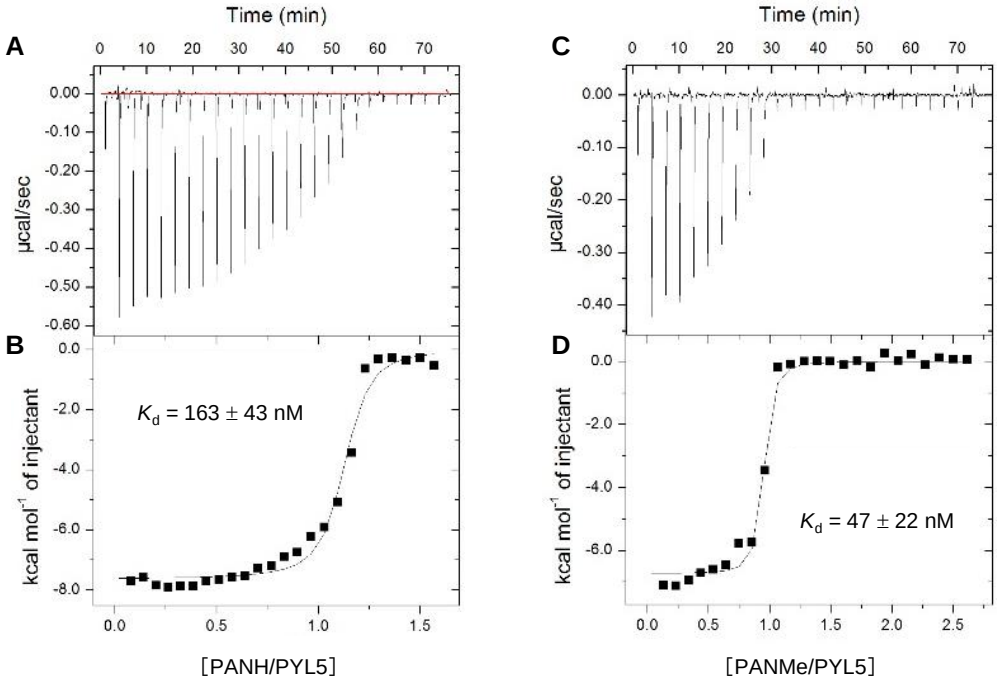

Figure S9. Isothermal titration calorimetry profiles and thermodynamic data for PANH/PANMe-PYL5 binding experiments. (A, C) Raw data for 25 sequential injections of 1.25 µL of 0.5 mM PANH (A) or PANMe (C) stock solution into cells containing 50 µM 6×His-tagged PYL5 in 0.1 M phosphate buffer, pH 8.0. Injections were performed over a period of 5 sec. with 3 min intervals between injections. (B, D) Plot of heat evolved (kcal) per mole of PANH (B) or PANMe (D) dilution, against the molar ratio of PANH or PANMe to PYL5. Data were fitted using software 'one set of sites' and the solid line represents best fit.

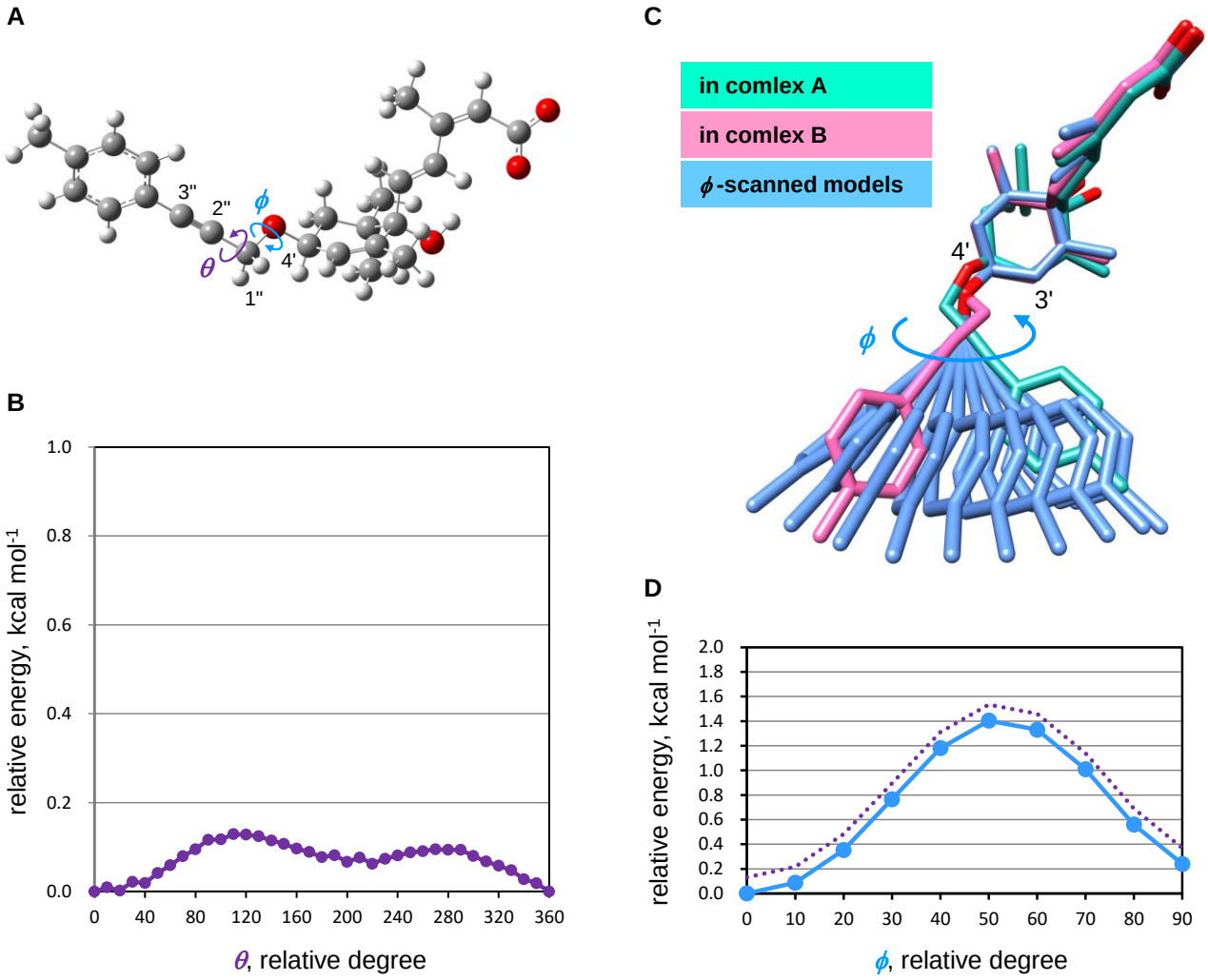

Figure S10. Flexibility of the 4'-chain of PANMe. (A) The ball and bond type model of PANMe optimized at the B3LYP/6-31G(d) level of theory. The dihedral angles θ (4'O-C1"-C2"-C3") and ϕ (C4'-4'O-C1"-C2") were scanned. The single point energy at each angle was calculated with no geometry optimization (θ) and with partial geometry optimization (only the 4'-chain) (ϕ) at the B3LYP/6-31G(d) level of theory. (B) The relative energy diagram for the dihedral angle θ. The maximum energy difference is 0.13 kcal mol^{-1}. (C) Superposition of the ϕ-scanned structures (light blue) and those (light green and pink) in the PYR1-PANMe crystal. (D) The relative energy diagram for the dihedral angle ϕ. The dotted line (purple) represents the relative energies in the unfavored θ in each model.

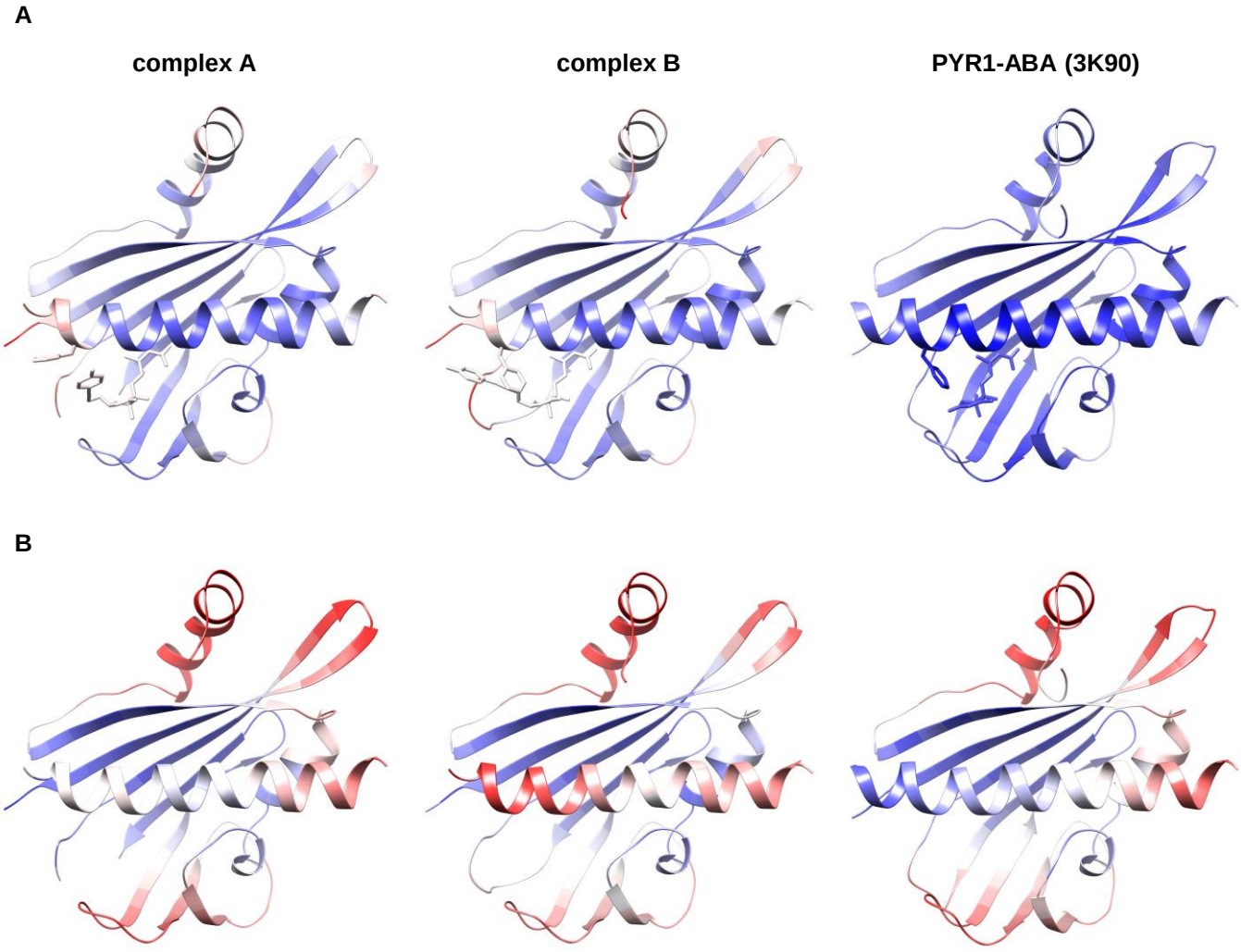

Figure S11. The 4′-O-chain of PANMe should increase the flexibility of the α-helix in the PYR1-PANMe complex. (A) The average B-factor per residue of the PYR1-PANMe complexes A (left) and B (middle) and the PYR1-ABA complex (right, PDB code 3K90) is color-coded on a blue-to-red spectrum. (B) The PYR1-PANMe complexes A (left) and B (middle) and the PYR1-ABA complex (right, PDB code 3K90) are color-coded by the size of motions driven by the lowest frequency (slowest) two GNM modes (blue: almost rigid; and red: highly mobile).

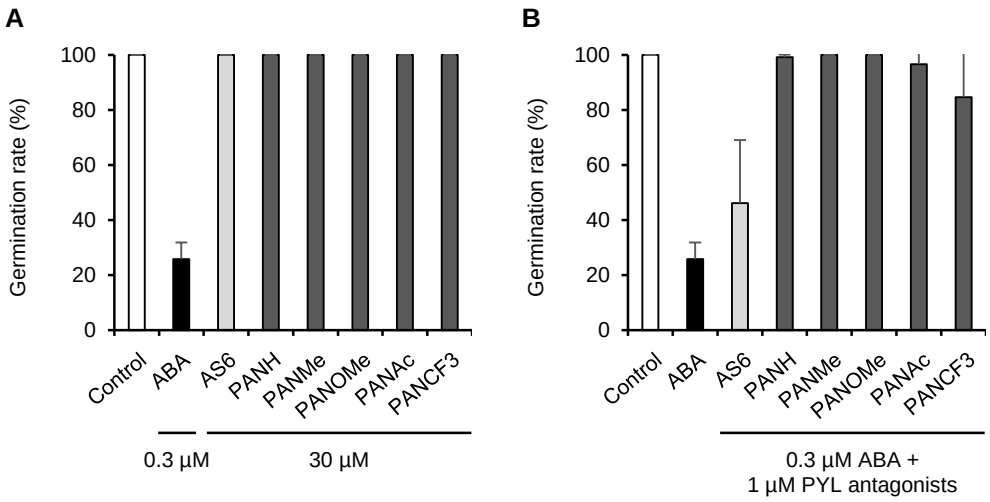

Figure S12. Effects of PAN compounds on *Arabidopsis* seed germination. (A) Seed germination rate in the presence of PANs at 48 h after stratification (*n* = 3; error bars represent sd). (B) Seed germination rate in response to 0.3 µM ABA and 1 µM AS6 or PANs at 48 h after stratification (*n* = 3; error bars represent sd).

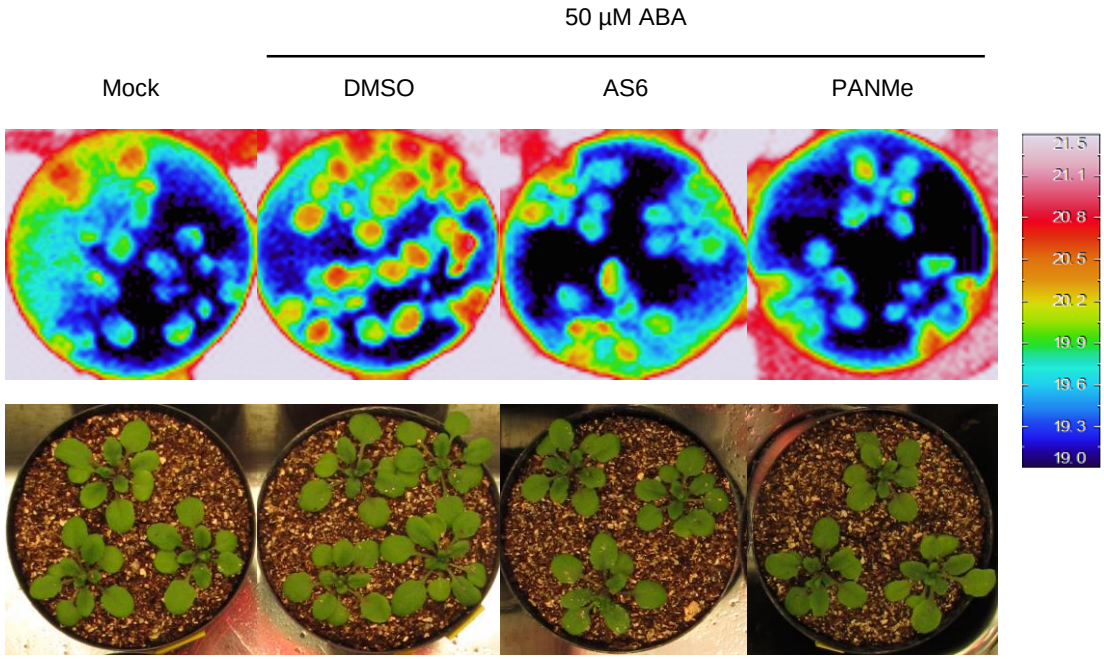

Figure S13. Thermal imaging showing the inhibitory effects of AS6 and PANMe on ABA-induced stomatal closure in *Arabidopsis*. All plants were pretreated overnight with a test solution containing 50 µM ABA and 100 µM PYL antagonists or mock (DMSO only).

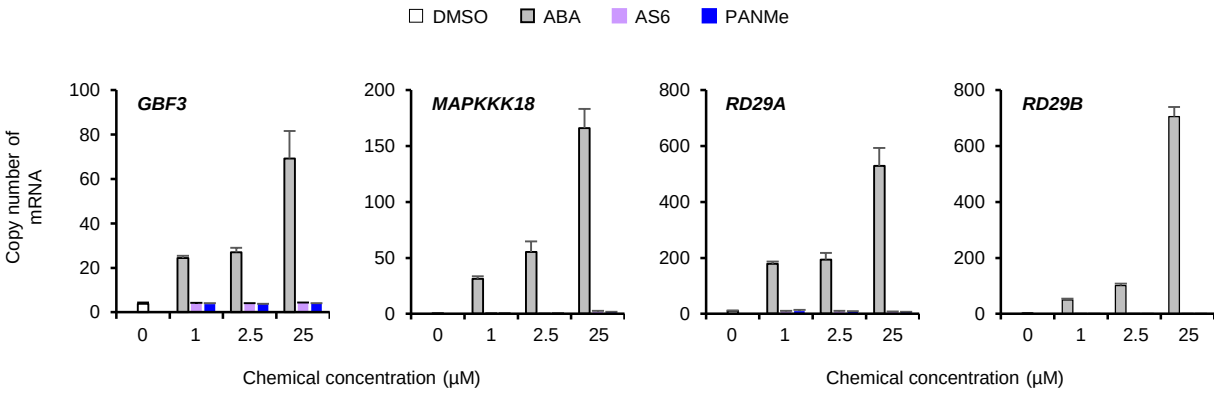

Figure S14. Effects of PANMe and AS6 on the expression of *Arabidopsis* ABA-responsive genes. Expression of ABA-responsive genes after ABA, AS6, or PANMe treatments. Chemical concentrations used were 0, 1, 2.5, and 25 μM. Six-day-old *Arabidopsis* wild-type (Columbia accession) seedlings were incubated in a solution containing chemicals in 0.5× MS and 0.5% sucrose for 6 h (*n* = 4, error bars represent sd).

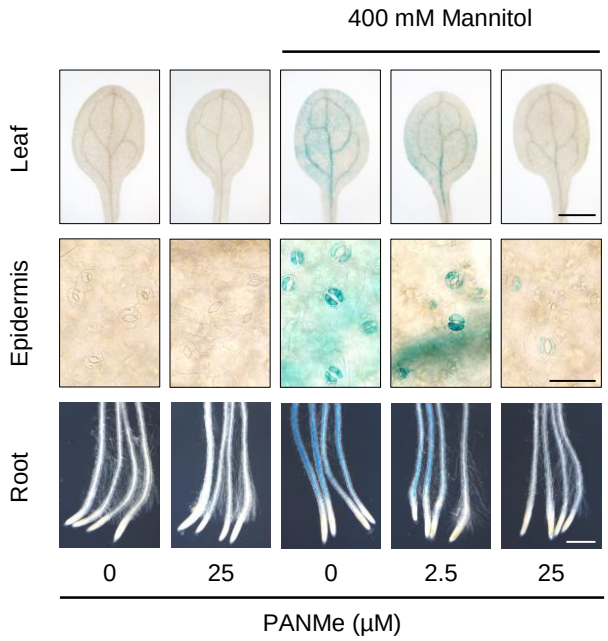

Figure S15. PANMe antagonized endogenous ABA activity. Spatial expression pattern of *MAPKKK18* after osmotic stress treatment with PANMe. Six-day-old seedlings of promoter *MAPKKK18:GUS* transgenic *Arabidopsis* were incubated in 400 mM mannitol solution containing PANMe for 6 h under the 22° C light condition. Scale bars represent 1 mm (leaf); 50 μm (epidermis); or 0.5 mm (root).

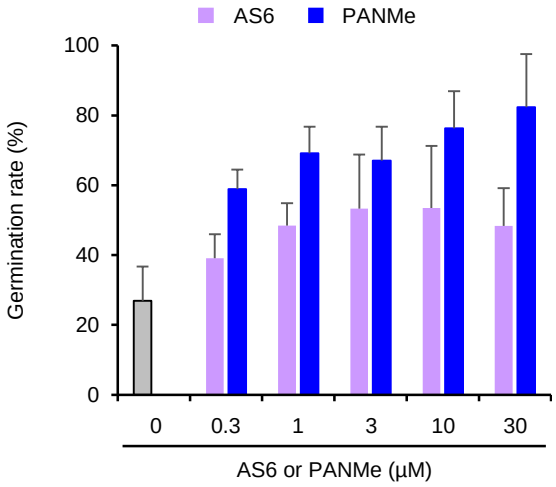

Figure S16. Effect of PANMe on thermoinhibition of *Arabidopsis* seed germination. *Arabidopsis* seeds were treated with AS6 or PANMe at 33° C, and the germination rate was determined at 72 h after stratification (*n* = 3, error bars represent sd).

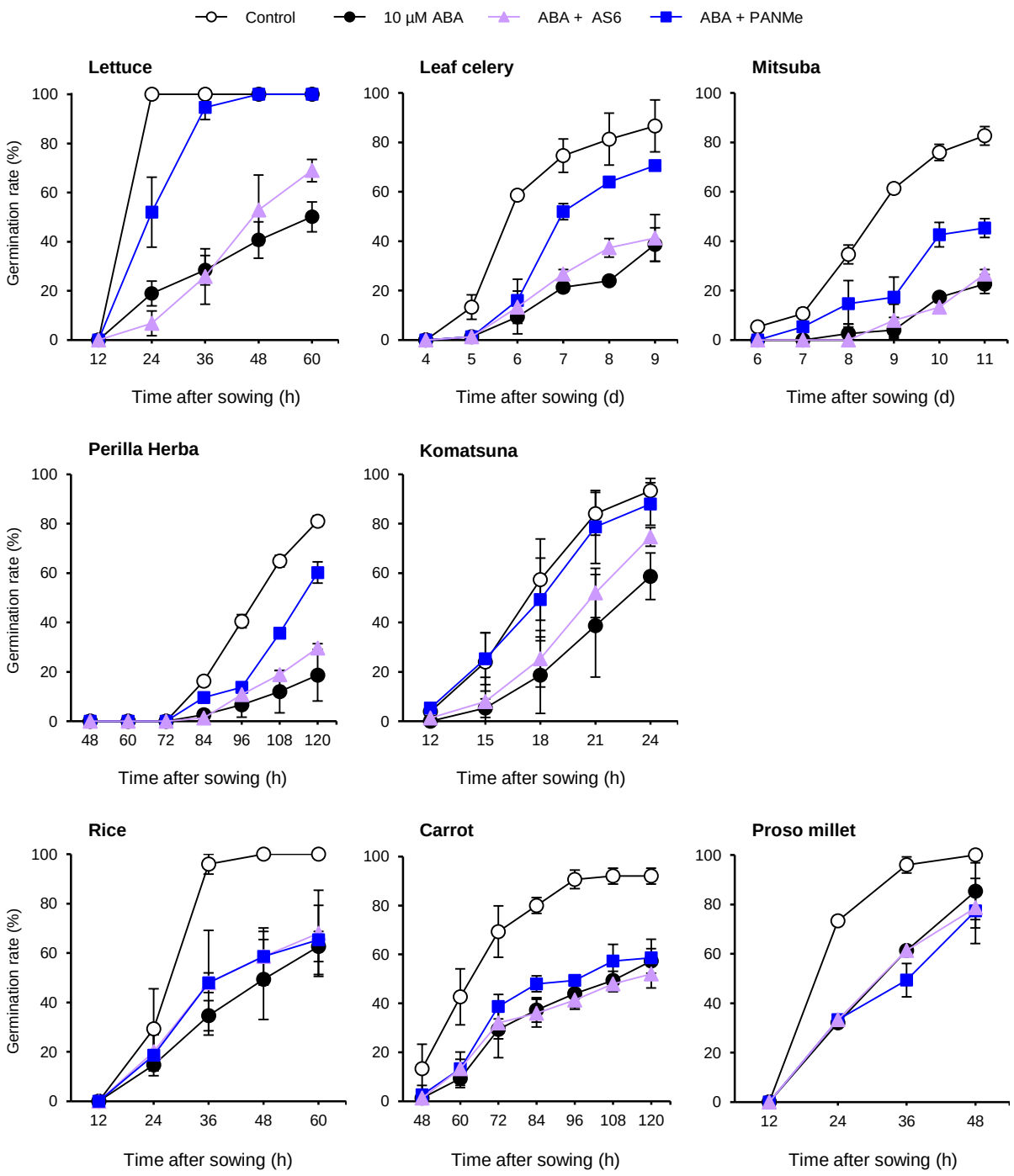

Figure S17. Effect of PANMe on seed germination of crop plants. Seed germination rate in the presence of ABA and AS6 or PANMe (*n* = 3, error bars represent sd). ABA was administered at 1 µM (mitsuba), 3 µM (carrot), or 10 µM (lettuce, leaf celery, Perilla Herba, komatsuna, rice, and proso millet). AS6 and PANMe were administered at 10 µM (mitsuba), 100 µM (lettuce, Perilla Herba, rice and carrot), or 300 µM (leaf celery, komatsuna, and proso millet).

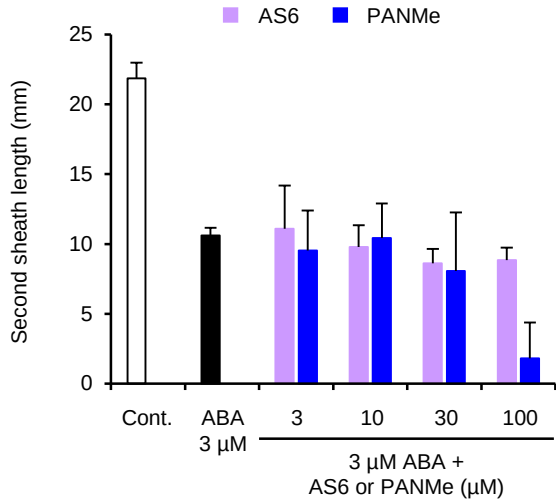

Figure S18. Effect of PANMe on rice seedling growth. Seedlings were grown on test media containing 3 µ M ABA and the indicated concentrations of AS6 or PANMe for 7 days (*n* =3, error bars represent sd).

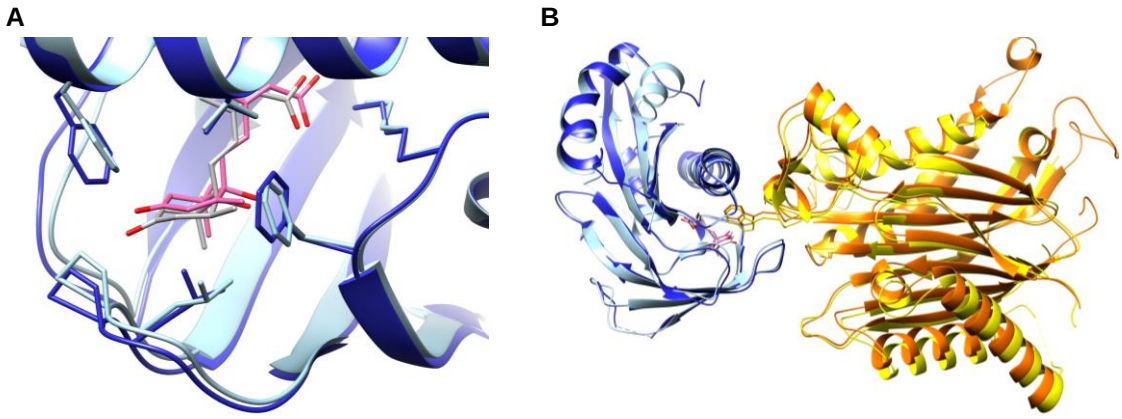

Figure S19. Structural comparisons of OsPYL2 and AtPYR1 in the PYL-ABA-PP2C complexes. (A) Close-up view of the ABA-bound OsPYL2 ligand binding pocket (cyan) overlaid with the AtPYR1 structure (blue) in the AtPYR1-ABA-HAB1 complex. ABA in OsPYL2 and AtPYR1 is shown as pink sticks and gray sticks, respectively. (B) Superposition of the OsPYL2-ABA-OsPP2C06 (orange, PDB code 4OIC) complex and the AtPYR1-ABA-HAB1 (yellow, PDB code 3QN1) complex. Trp 339 of OsPYL2 and Trp 385 of HAB1 are highlighted in sticks.

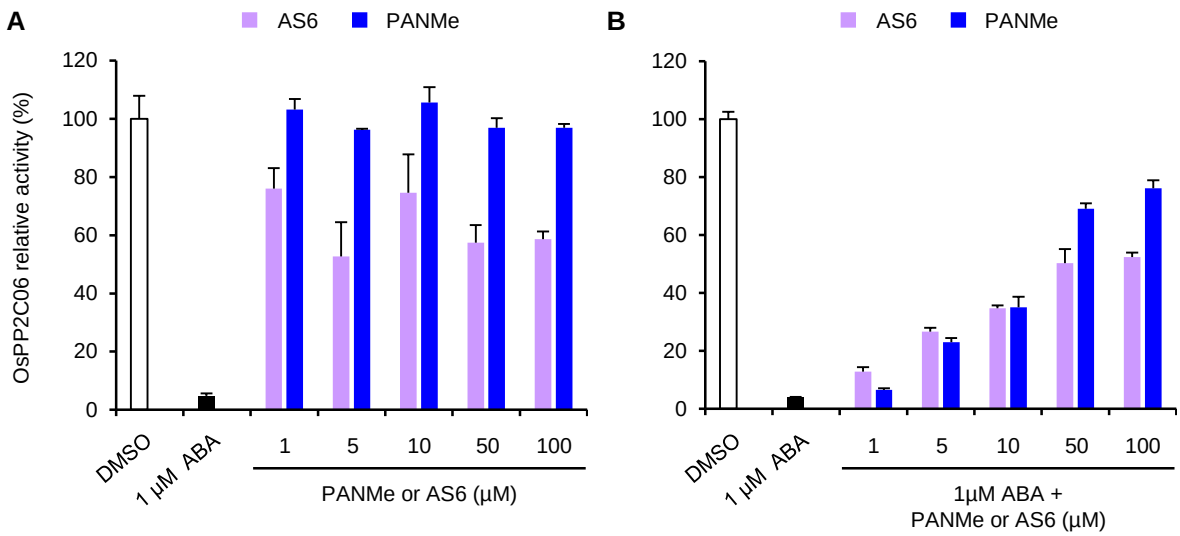

Figure S20. Effects of PANMe and AS6 on the OsPYL2-OsPP06 interaction. (A) Chemical inhibition of OsPP2C06 by OsPYL2 in the presence of various concentrations (0, 1, 5, 10, 50, and 100 μM) of PANMe or AS6. (B) Antagonistic effect of PANMe and AS6 on OsPYL2. Assays were performed in the presence of 1 μM ABA and various concentrations (0, 1, 5, 10, 50, and 100 μM) of PANMe or AS6. The OsPP2C06 activity of each reaction was normalized to a control (DMSO-treated) value of 100% and expressed as relative activity. OsPYL2 and OsPP2C06 proteins were used at 300 pmol and 60 pmol, respectively (n = 3, error bars represent sd).

Table S1. Data collection and refinement statistics (molecular replacement)

Crystal	PYR1-PANMe
Data collection	
Beamline	PF NW12A
Wavelength (Å)	1.000
Space group	$P4_1$
Unit cell dimensions	38.20, 38.20, 263.35
a, *b*, *c* (Å), a, b, g (°)	90, 90, 90
Resolution range (Å)	50.0 – 2.5 (2.54 – 2.5)
R_{merge}	0.114 (0.536)
I/sI	20.7 (2.6)
Completeness (%)	98.9 (93.7)
Number of unique reflections	12809 (638)
Redundancy	3.3 (2.8)
Refinement	
Resolution range (Å)	35.0 – 2.5 (2.57 – 2.5)
Number of reflections	12089 (895)
R_{work}/R_{free}	25.6/29.4 (32.6/32.2)
RMSD from ideal	
Bond angle (°)	1.326
Bond length (Å)	0.008
Number of atoms	
Protein	2743
Water	18
Ligand	58
Average b-factor (Å^2)	
Protein	47.0
Water	32.2
Ligand	58.3
Ramachandran plot (%)	
Favored region	98.5
Additional allowed region	3.6
Outlier region	0.3

R_{free} was calculated by randomly omitting 5% of the observed reflections from the refinement. $R_{merge} = \Sigma_h\Sigma_j|I_{hj} - I_h|/\Sigma_h\Sigma_j|I_{hj}|$, where *h* represents a unique reflection and *j* represents symmetry-equivalent indices. *I* is the observed intensity and $<I>$ is the mean value of *I*. Values in parentheses are those in the highest resolution shells.

Table S2. Thermodynamic characterization of ABA/PANMe-PYL5 binding over a range of temperature

Compounds	Temperature (°C)	K_d [a](nM)	ΔH (kcal/mol)	$-T\Delta S$[b] (kcal/mol)	ΔS (kcal/mol)	ΔG[c] (kcal/mol)
ABA	20	772 ± 35	−6.2 ± 0.1	−2.0 ± 0.1	7.0 ± 0.3	−8.2 ± 0.0
	25	883 ± 187	−8.8 ± 0.3	0.6 ± 0.4	−2.1 ± 1.4	−8.1 ± 0.1
	30	620 ± 151	−11.8 ± 0.6	3.4 ± 0.5	−11.3 ± 1.8	−8.3 ± 0.1
	35	820 ± 262	−14.5 ± 0.4	6.3 ± 0.2	−20.4 ± 0.6	−8.2 ± 0.2
PANMe	20	81 ± 40	−6.8 ± 0.1	−2.8 ± 0.1	9.6 ± 2.1	−9.6 ± 0.3
	25	47 ± 13	−8.9 ± 0.4	−1.0 ± 0.4	3.3 ± 1.5	−9.8 ± 0.2
	30	97 ± 49	−9.8 ± 0.3	0.3 ± 0.2	−1.1 ± 0.8	−9.5 ± 0.4
	35	104 ± 56	−12.8 ± 0.6	3.4 ± 0.3	−11.0 ± 1.1	−9.4 ± 0.3

[a] K_d, ΔH obtained from single-set-of-sites fit to date.

[b] $T\Delta S = \Delta H - \Delta G$

[c] $\Delta G = -RTln(1/K_d)$. Uncertinties for K_d, ΔH, and ΔG calculated by curve fitting program of MicroCal Origin 7.0.