Supplementary Information

Stabilization of dimeric PYR/PYL/RCAR family members relieves abscisic acid-induced inhibition of seed germination

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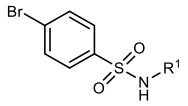
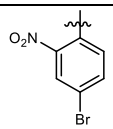
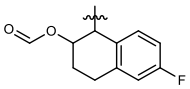
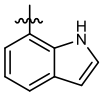
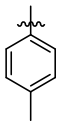
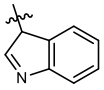
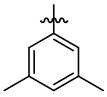
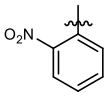
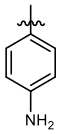
Supplementary Table 1. The binding free energy (kcal/mol) of compounds generated using fragment growing approach.



No.	Substituents	$\triangle E_{ele}$	$\triangle E_{vdw}$	$\triangle E_{MM}$	$\triangle G_{sol}$	$\triangle E_{bind}$	$-T\triangle S$	$\triangle G_{cal}$
1		-20.65	-49.85	-70.50	38.21	-32.29	20.06	-12.23
2		-20.66	-46.00	-66.66	36.07	-30.59	18.58	-12.01
3		-23.58	-53.19	-76.58	44.66	-32.92	21.56	-11.36
4		-23.86	-47.37	-71.23	38.32	-32.81	21.54	-11.27
5		-19.37	-43.62	-62.99	33.68	-29.31	18.31	-11.00
6		-18.32	-50.99	-69.31	39.67	-29.64	18.65	-10.99
7		-16.89	-45.08	-61.97	33.72	-28.25	17.35	-10.90
8		-17.90	-47.08	-64.98	34.79	-30.19	18.35	-10.84
9		-22.40	-47.51	-69.91	38.61	-31.30	20.75	-10.55
10		-18.86	-40.48	-59.34	33.53	-25.71	16.33	-9.38

Supplementary Table 2. Data collection, phasing, and refinement statistics of PYL1-DBSA (PDB code: 9J6I).

	PYL1-DBSA (PDB ID: 9J6I)
Data collection	
Space group	$P6_5$
Cell dimensions	
a, *b*, *c* (Å)	126.99, 126.99, 60.195
α, β, γ (°)	90, 90, 120
Resolution (Å)	41.67- 2.29 (2.37- 2.29)
Rmerge	0.027 (0.435)
I/σ (*I*)	13.35 (1.82)
Completeness (%)	100.00 (98)
Redundancy	2.0 (2.0)
Refinement	
Resolution (Å)	2.29
No. reflections	50745 (4891)
Rwork/Rfree (%)	20.67/25.23
No. atoms	
Protein	2722
Ligand/ion	24
Water	38
B-factors	
Protein	71.2
Ligand/ion	76.8
Water	61.2
R.m.s. deviations	
Bond lengths (Å)	0.009
Bond angles (°)	1.035

Values in parentheses are for the highest resolution shell. $R_{merge}=\Sigma_h\Sigma_i|I_{h,i}-I_h|/\Sigma_h\Sigma_i I_{h,i}$, where I_h is the mean intensity of the *i* observations of symmetry related reflections of *h*. $R=\Sigma|F_{obs}-F_{calc}|/\Sigma F_{obs}$, where F_{calc} is the calculated protein structure factor from the atomic model (R_{free} was calculated with 5% of the reflections selected).

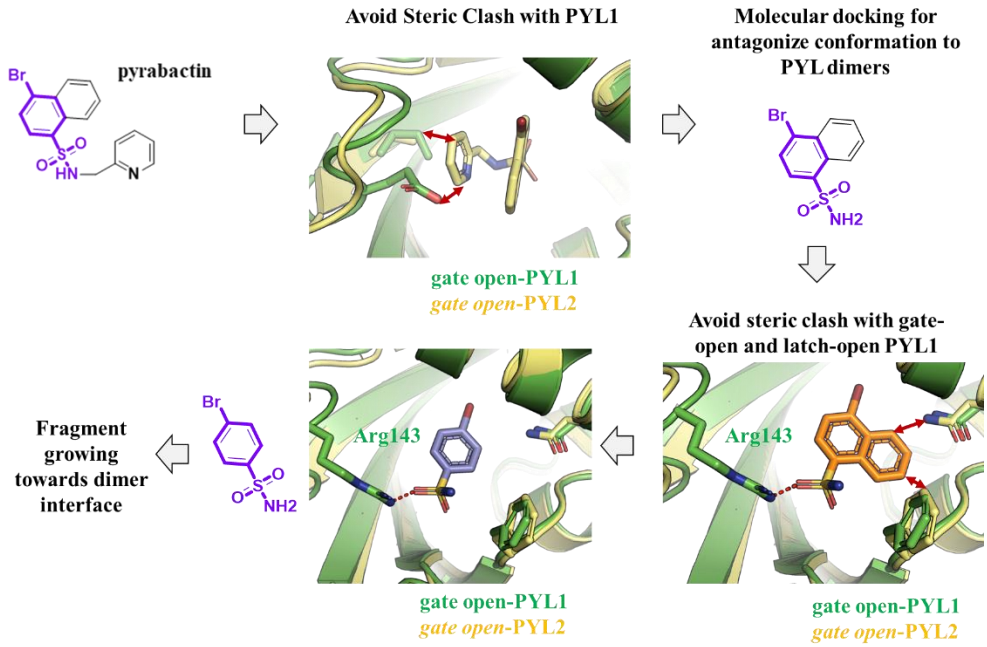
Supplementary Table 3. The binding free energy between two monomers of PYL dimers under the existence of ABA or BDSA (kcal/mol).

	ABA-PYR1	***apo*-PYL1**	**DBSA-PYL1**
ΔE_{ele}	-96.42	-113.69	-138.42
ΔE_{vdw}	-55.10	-104.97	-129.37
ΔE_{GB}	102.24	160.33	187.85
ΔE_{SA}	-12.91	-12.86	-11.25
ΔG_{cal}	-62.19	-71.19	-91.18

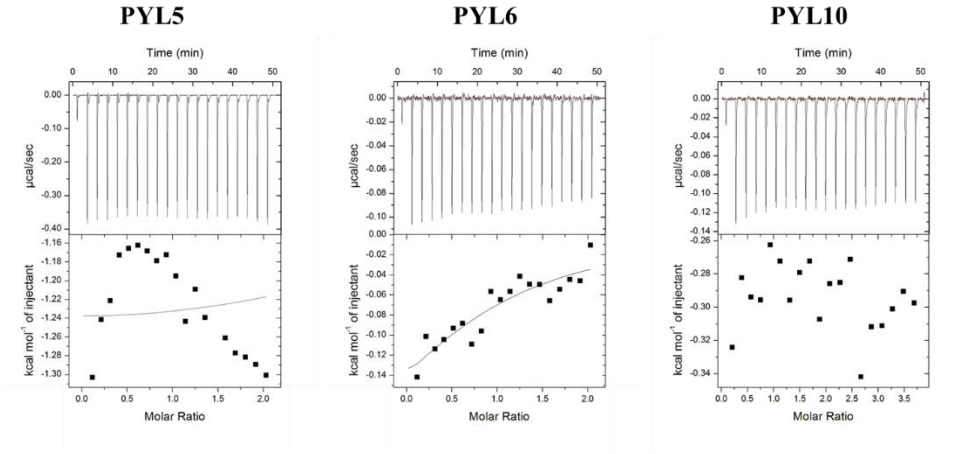
Supplementary Table 4. The hydrogen bond analysis of PYR1-ABA, *apo*-PYL1 and PYL1-DBSA during MD simulations.

System	Acceptor	Donor	Frac[a]	AvgDist[b]	AvgAng[c]
PYR1-ABA	B_ Lys_63@NZ	A_Asp_155@OD1	0.6264	2.9	138.9
	B_ Lys_63@NZ	A_Asp_155@OD2	0.5880	3.0	156.2
	A_Ser_85@OG	B_Asp_155@O	0.5636	2.9	172
apo-PYL1	B_ Lys_69@NZ	A_Asp_164@OD1	0.8288	3.3	130.9
	A_ Lys_69@NZ	B_Asp_164@OD1	0.7664	3.0	167.2
	B_Pro_94@ND2	B_Arg_122@ND2	0.6636	2.9	159.7
	B_Pro_94@ND2	B_Arg_122@ND2	0.6616	3.2	132.7
DBSA-PYL1	DBSA@O^-	A_SER_112@OG	0.9044	2.9	140.4
	A_Ser_112@O	B_Asn_160@ND2	0.7380	2.9	140.5
	A_Asn_88@ND2	A_ Lys_69@NZ	0.6672	2.8	144.1
	A_ Lys_69@NZ	B_Asp_164@OD1	0.9244	2.9	155.0
	A_ Lys_69@NZ	B_Asp_164@OD2	0.9632	2.9	135.1
	B_ Lys_69@NZ	A_Asp_164@OD1	0.8912	2.8	161.5
	B_ Lys_69@NZ	A_Asp_164@OD2	0.8536	2.9	138.2
	A_Asn_160@ND2	B_Ala_95@ND2	0.5632	3.0	153.3
	B_Pro_94@ND2	B_Arg_122@ND1	0.6896	2.7	140.9
	B_Pro_94@ND2	B_Arg_122@ND2	0.7132	2.8	144.8

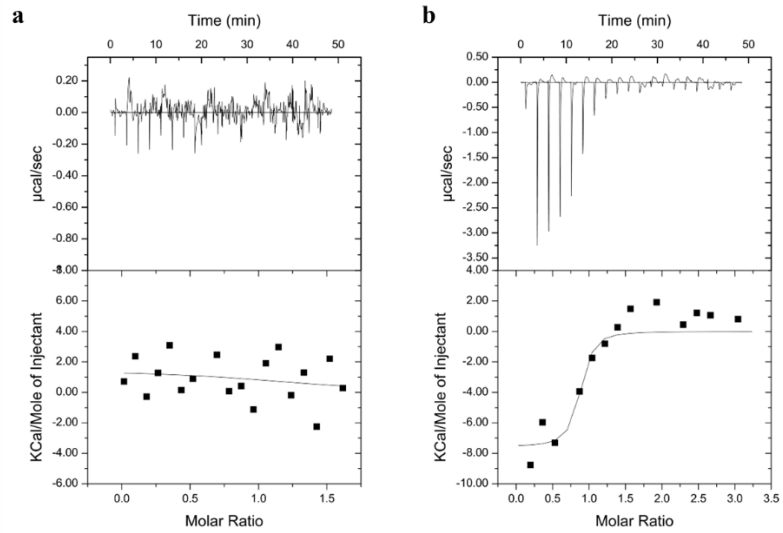
[a] Hydrogen bond occupancy. [b] Average hydrogen bond distance. [c] Average hydrogen bond angle.



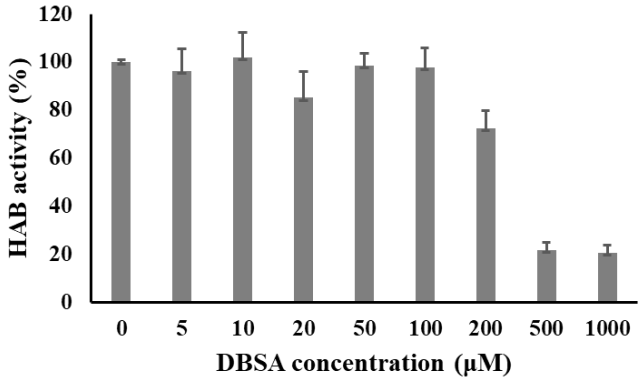
Supplementary Fig. 1. The detailed optimization of pyrabactin for fragment growing. Pyrabactin could act as ABA receptor agonist (PYR1 and PYL1) or antagonist (PYL2). Binding mode of pyrabactin as antagonist to gate open PYL2 (PDB code 3NR4) was analyzed, and steric clash was occurred to gate open PYL1 (PDB code 3KAY). To avoid steric clash and find a proper conformation for dimer stabilizer design, the pyridine group should be removed, and molecular docking was performed on the rest of molecules. It could be noticed that conformation towards dimer interface was easy to cause steric clash with gate open and latch open PYL1. And molecule was finally optimized into 4-bromobenzenesulfonamide group, which was binding tightly to gate-open and latch-open PYL1 by forming hydrogen bonds with Arg143.



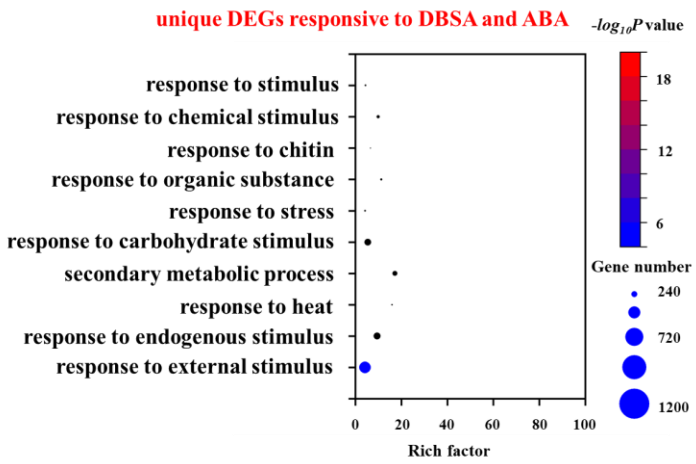
Supplementary Fig. 2. The ITC results of DBSA to PYL5, PYL6 and PYL10.



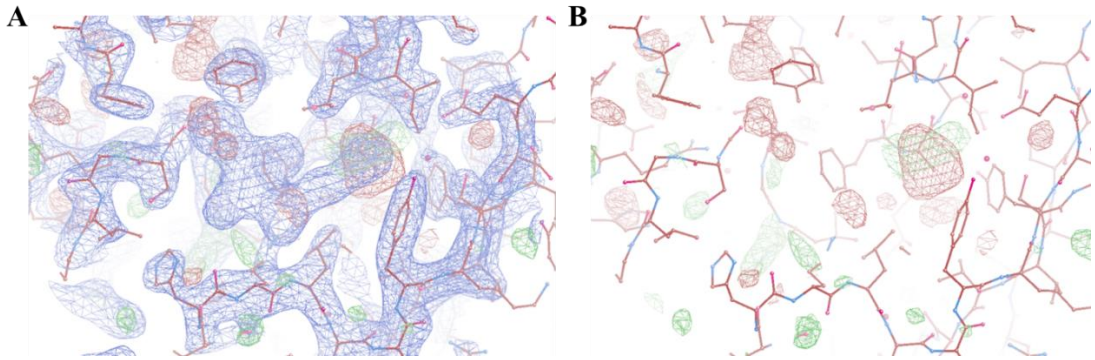
Supplementary Fig. 3. (a) ITC experiment results of dropping ABA into a cell containing PYR1 and DBSA. (b) ITC experiment results of dropping DBSA into a cell containing PYR1 and ABA.



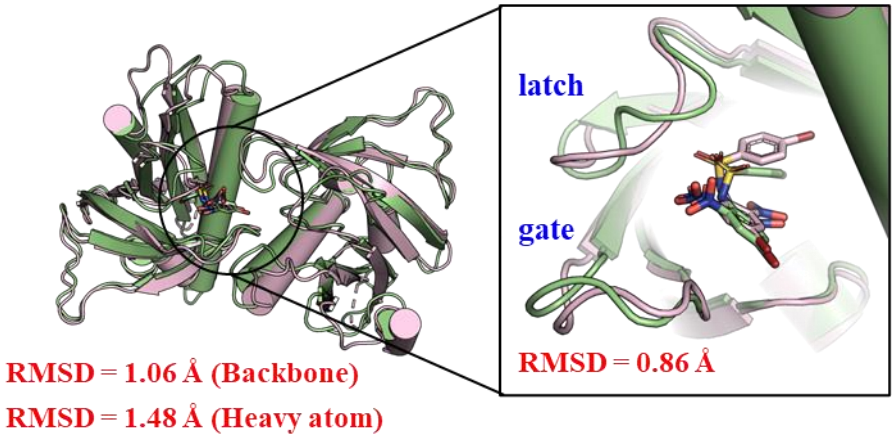
Supplementary Fig. 4. The HAB1 activity of treatment with different concentration of DBSA but without PYLs. (n = 3 biologically replicates). The data are presented as the mean ± SD, the line within the box marks the median and the whiskers represent the minimum and maximum values.



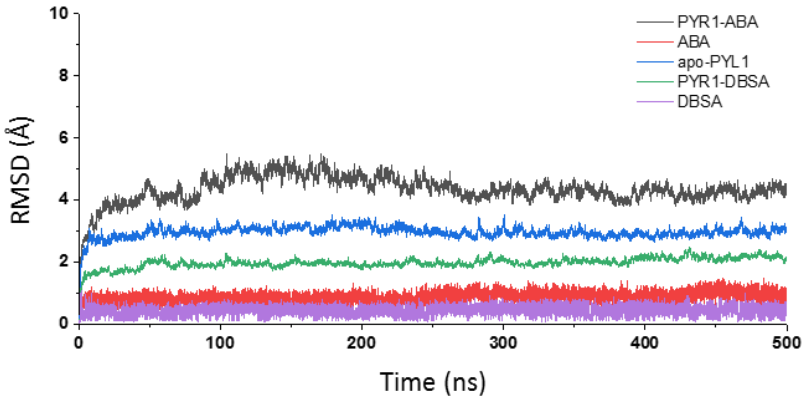
Supplementary Fig. 5. The representative response unique DEGs and pathways enriched for both ABA and DBSA based on functional enrichment analysis ($p < 0.01$).



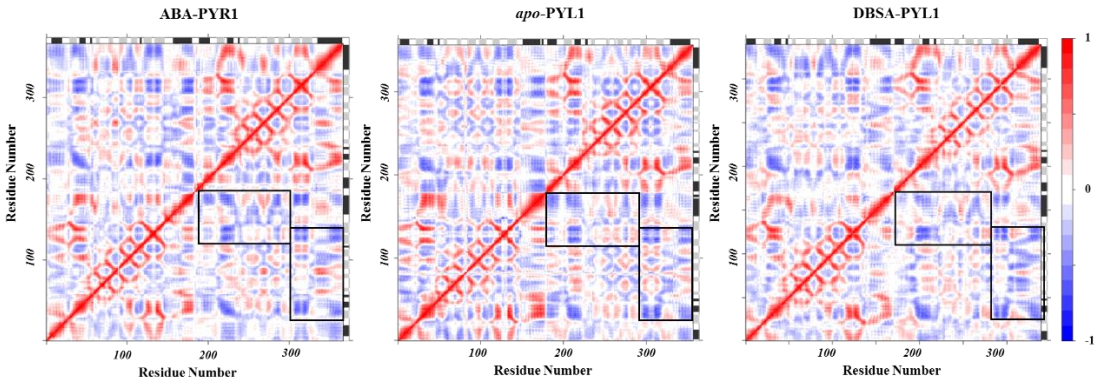
Supplementary Fig. 6. The final 2*F*o-*F*C map (A) and an unbiased *F*o-*F*c map (B) obtained after omitting the ligand from the X-ray crystal structure.



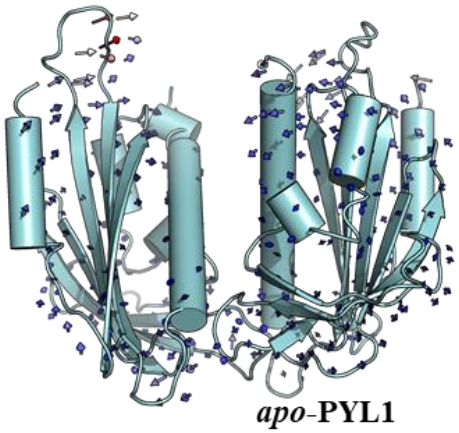
Supplementary Fig. 7. The comparison between predicted model and x-ray crystal structure of PYL1-DBSA complex (PDB code 9J6I). The crystal structure shared a similar structure with our predicted model. The prediction model was shown in pink stick model, and X-ray crystal structure was shown in green stick model.



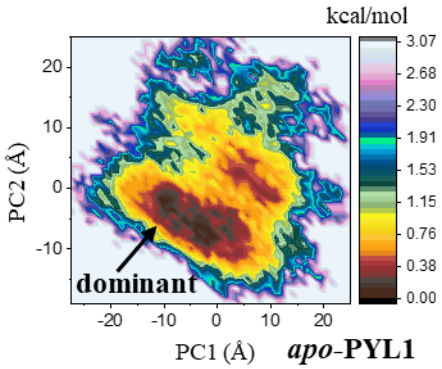
Supplementary Fig. 8. The RMSD plot during MD simulations of PYRR1-ABA, apo-PYL1 and PYL1-DBSA.



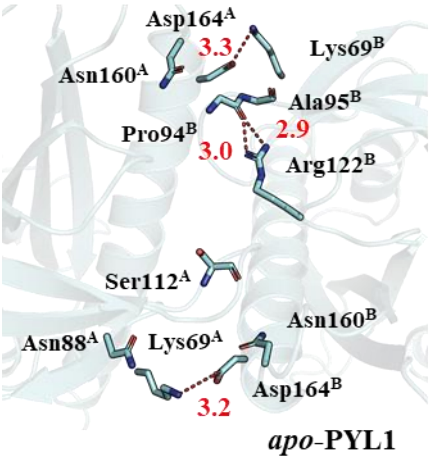
Supplementary Fig. 9. The function motion of ABA-PYR1, *apo*-PYL1 and DBSA-PYL1.



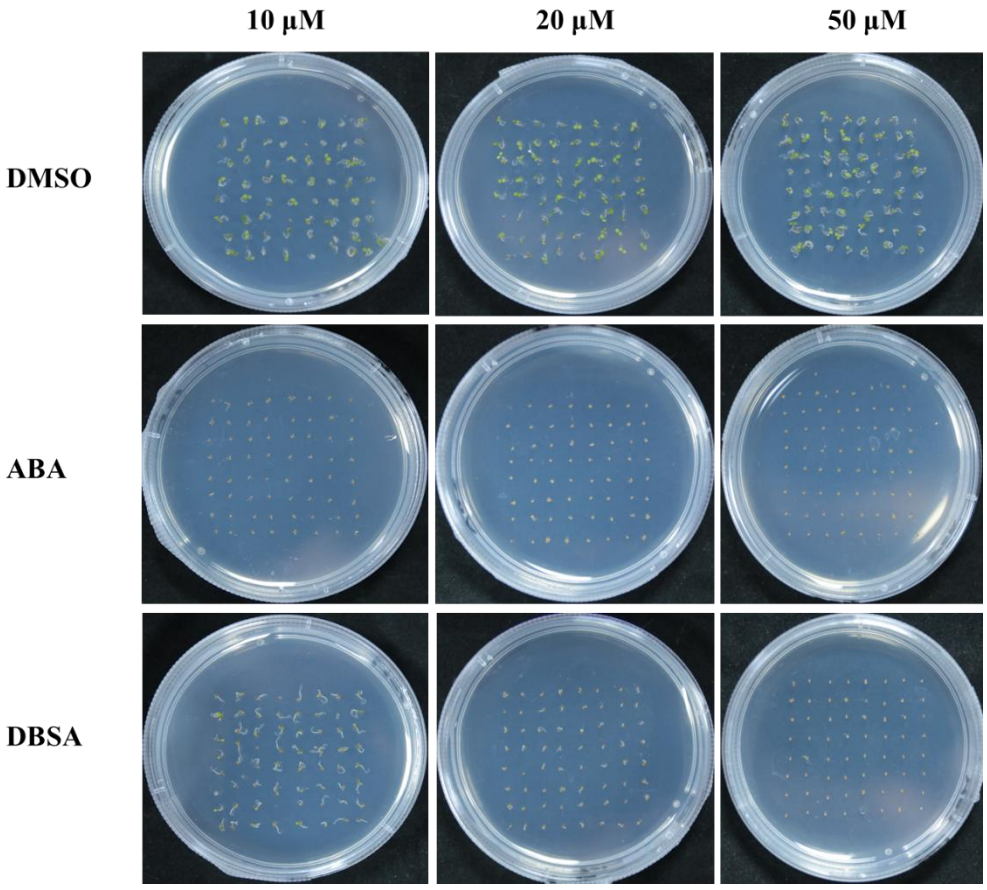
Supplementary Fig. 10. The motion trends of apo-PYL1.



Supplementary Fig. 11. The dominant conformations were determined using FEL analysis.

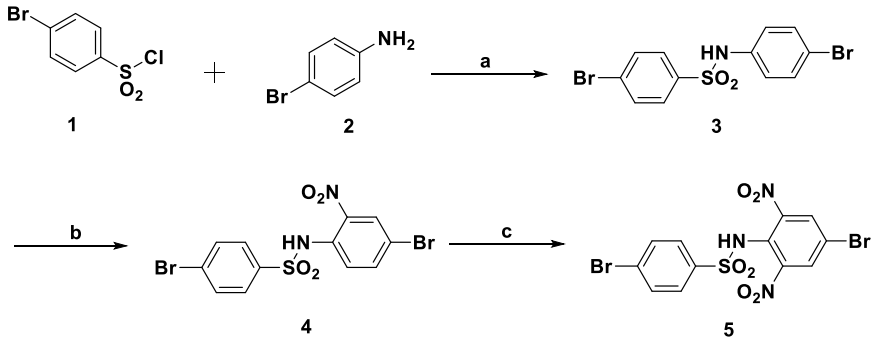


Supplementary Fig. 12. The hydrogen networks mediated by apo-PYL1 in the dimer interfaces.



Supplementary Fig. 13. Seed germination experiment of different concentration of ABA and DBSA on WT *Arabidopsis thaliana* seeds.

Supplementary Note 1. The synthesis of NBSA and DBSA



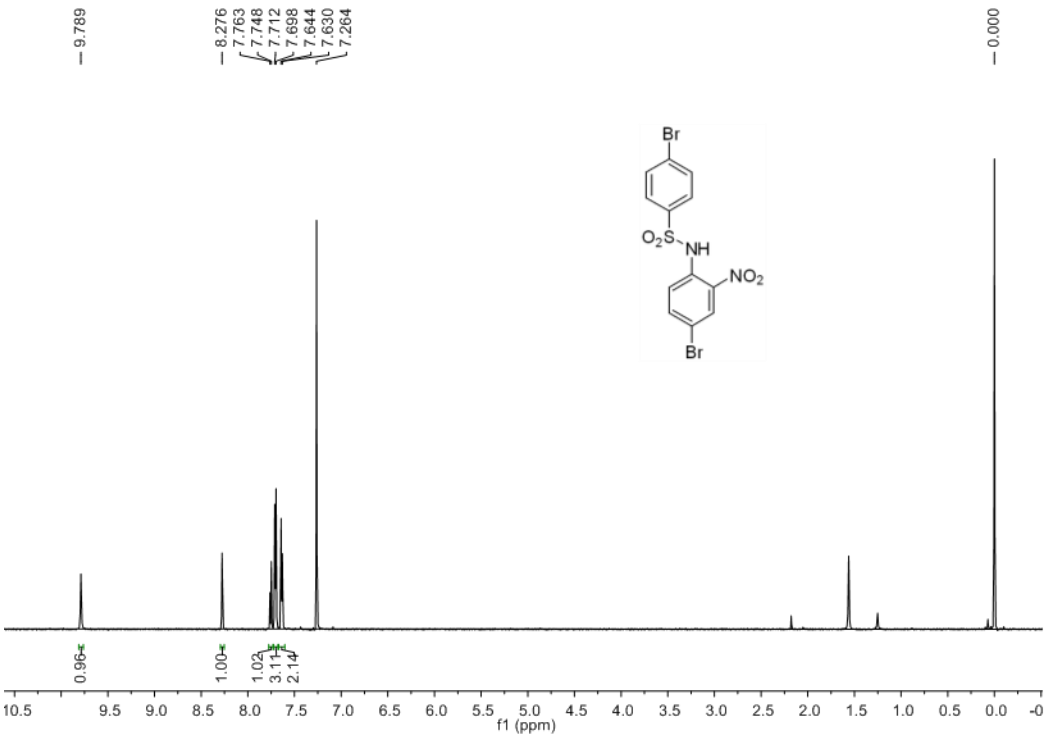
Reagents and conditions: a). Pyridine, CH_2Cl_2, 0℃- reflux; b). nitric acid, CH_3COOH, r.t.-80℃; c). nitric acid, CH_3COOH, 90℃.

Synthesis of 4-bromo-N-(4-bromophenyl)benzenesulfonamide (3):

A solution of 4-bromobenzenesulfonyl chloride(2.2 mmol, 561 mg) in CH_2Cl_2(4 mL) was added dropwise to a solution of 4-bromoaniline(2 mmol, 344 mg) and pyridine(6 mmol, 474 mg) in ice-cold CH_2Cl_2(4 mL). Then, the resulting mixture was heated at reflux for 2 h, cooled, acidified with hydrochloric acid, and extracted with CH_2Cl_2(3×15 mL). The CH_2Cl_2 was removed in vacuo to give the crude mixture which was purified by column chromatography on silica gel to the desired product as a white solid (**3**).

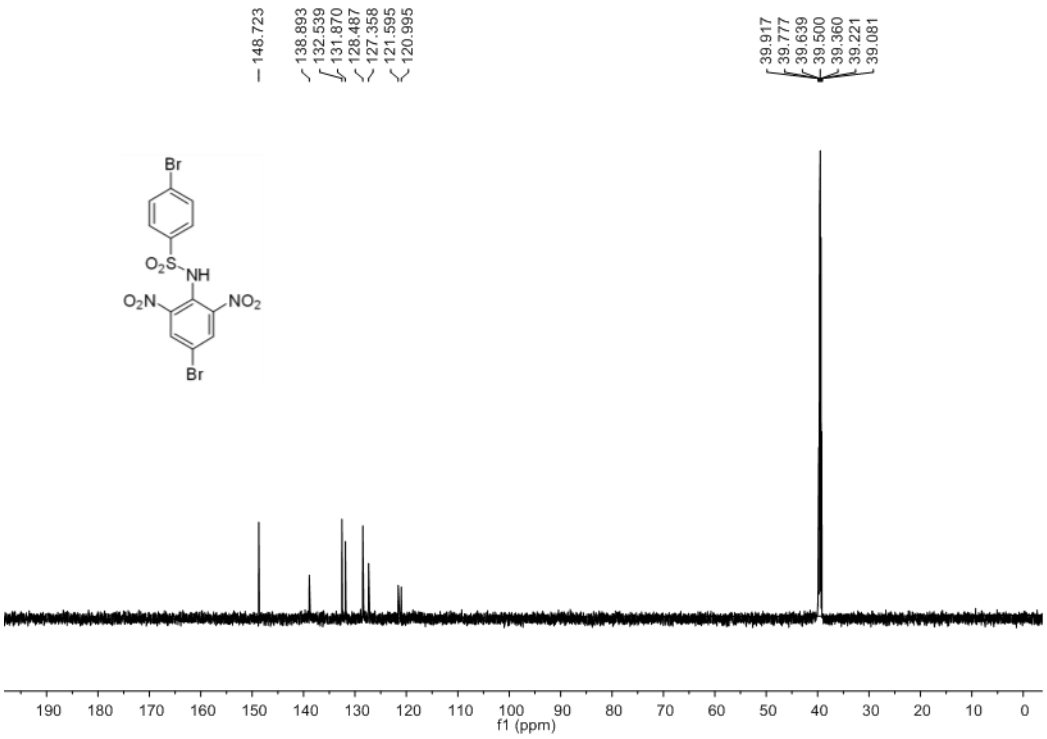
Synthesis of 4-bromo-N-(4-bromo-2-nitrophenyl)benzenesulfonamide (4, NBSA):

Intermediate **3**(1 mmol, 391 mg) was dissolved in acetic acid (6 mL) in a 25 mL single neck flask, and nitric acid (0.4 mL) was added by dropwise to the solution at room temperature. The reaction mixture was allowed to 80°C and stirred for a further 0.5 h before the residue was cooled with ice water (10 mL), and extracted with ethyl acetate (3×10 mL). Then the ethyl acetate solution was concentrated to give the crude mixture which was purified by column chromatography on silica gel to the desired product as a yellow solid (**4**). Yield 72 %; m.p. 127.3-128.1 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 9.79 (s, 1H), 8.28 (s, 1H), 7.76 (d, J = 9.0 Hz, 1H), 7.71 (d, J = 9.0 Hz, 3H), 7.64 (d, J = 8.4 Hz, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 138.79, 137.26, 137.22, 132.79, 132.43, 129.20, 128.80, 128.60, 122.25, 116.53. HRMS (ESI): m/z $[M+Na]^+$ calcd for, $C_{12}H_8Br_2N_2NaO_4S$: 456.8469 ; found: 456.8464.



4-bromo-N-(4-bromo-2,6-dinitrophenyl)benzenesulfonamide (5, DBSA):

Additional nitric acid (0.4 mL) was added to the reaction after the mononitration reaction is completed. The resulting mixture was then heated at 90°C for 1.5 h, cooled, added ice water (10 mL), and extracted with ethyl acetate (3×10 mL). The ethyl acetate solution was concentrated, and the crude mixture was purified by column chromatography on silica gel to the desired product as a yellow solid (**5**). Yield 55 %; m.p. 198.1-198.9 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.33 (s, 1H), 8.30 (s, 2H), 7.64 (d, J = 8.4 Hz, 2H), 7.50 (d, J = 8.4 Hz, 2H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 148.72, 138.89, 132.54, 131.87, 128.49, 127.36, 121.60, 121.00. HRMS (ESI): m/z $[M+Na]^+$ calcd for, $C_{12}H_7Br_2N_3NaO_6S$: 501.8320 ; found: 501.8332.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132.539
131.870
128.487
127.358
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120.995
39.917
39.777
39.639
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39.360
39.221
39.081
Br
O2S
NH
O2N
NO2
Br
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)