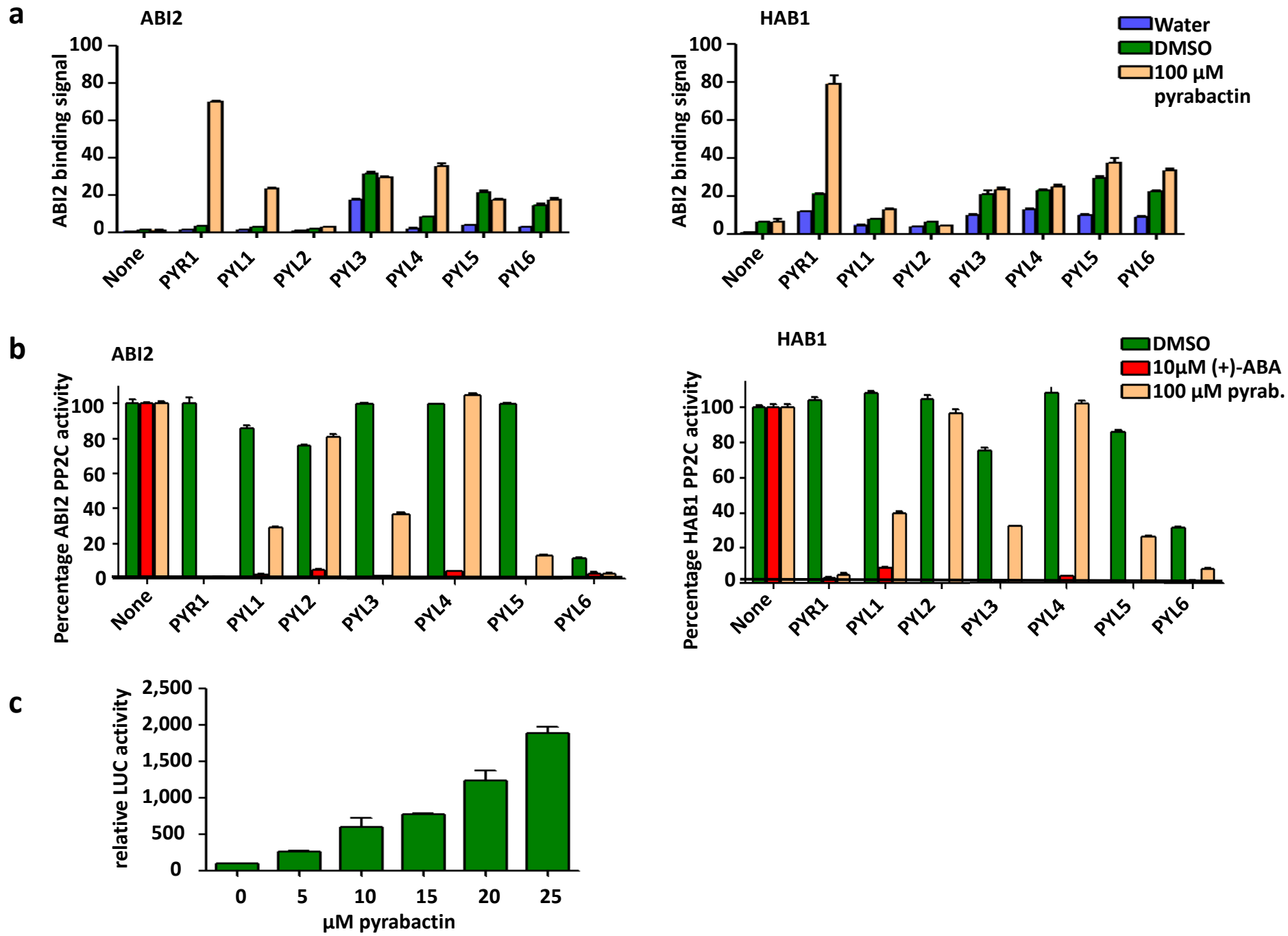


# Identification and Mechanism of ABA Receptor Antagonism

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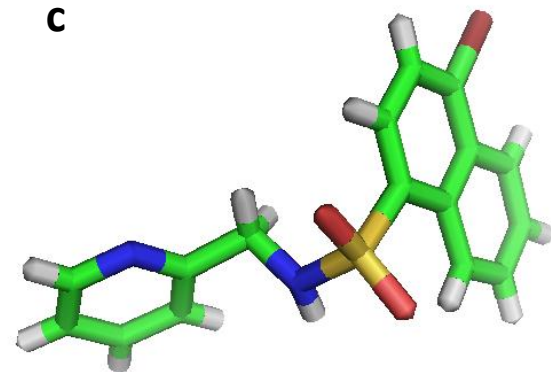
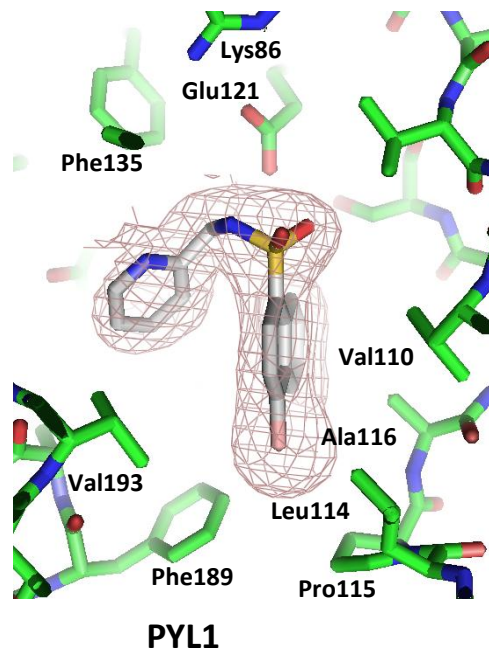
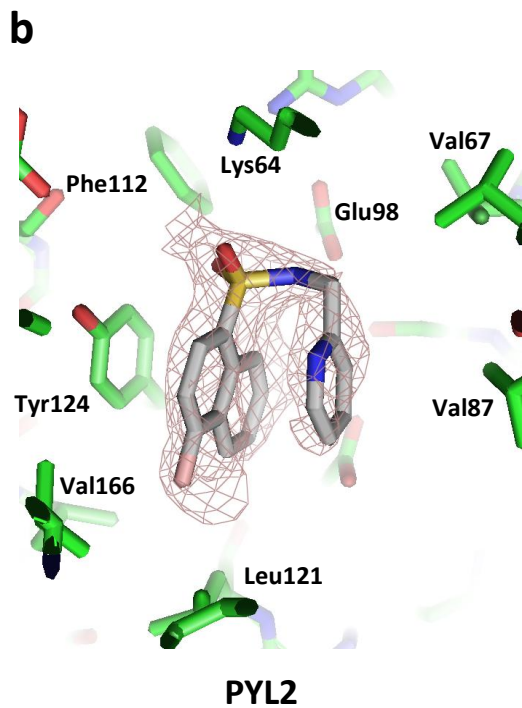
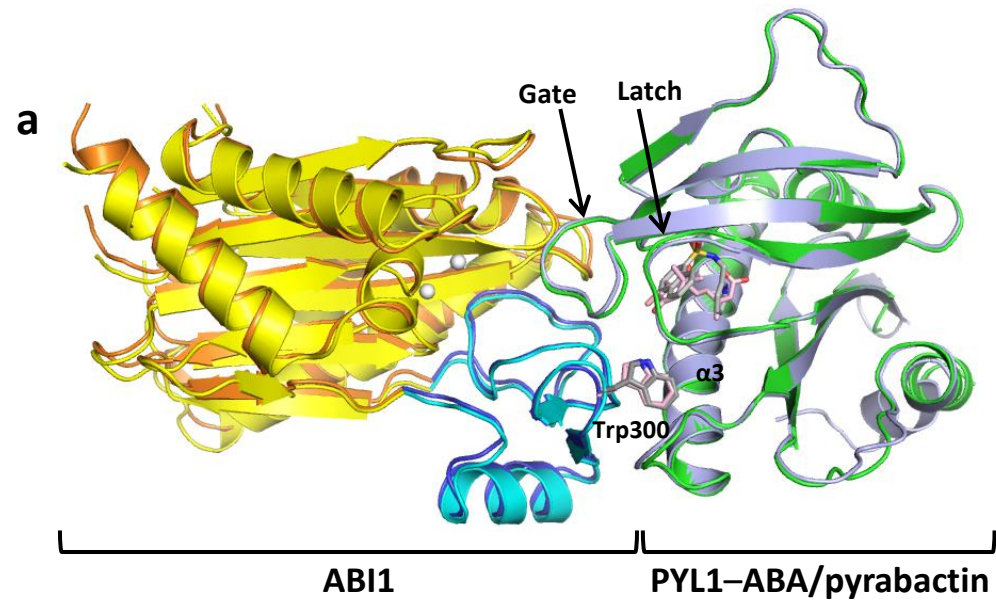
## Supplementary Information

# Supplementary Figure 1:



**Supplementary Figure 1** Pyrabactin functions both as a selective ABA receptor agonist and antagonist. **(a)** Stimulation of the interactions between PYR1–PYL6 and the PP2Cs ABI2 and HAB1 by pyrabactin. Binding was determined by AlphaScreen assays (n=3, error bars=s.d.). **(b)** Pyrabactin-dependent inhibition of ABI2 and HAB1 phosphatase activities by PYL proteins (n=3, error bars=s.d.). **(c)** Pyrabactin is a PYR1 agonist in an *Arabidopsis* protoplast reporter gene assay. Expression plasmids for PYR1, ABI1, SnRK2.6, and the transcription factor ABF2 were cotransfected into protoplasts together with a luciferase reporter plasmid driven from the ABA-responsive *RD29B* gene (n=3, error bars=s.d.).

## Supplementary Figure 2:



**Supplementary Figure 2** Structures of pyrabactin complexes and apo pyrabactin. **(a)** Overlay of the PYL1–pyrabactin–ABI1 and the PYL1–ABA–ABI1 structure. The PYL1–pyrabactin–ABI1 complex is shown in green (PYL1), grey (pyrabactin and ABI1 Trp300), and yellow (ABI1), the PYL1–ABA–ABI1 complex is shown in pale blue (PYL1), pink (ABA and ABI1 Trp300), and brown (ABI1). **(b)** Pyrabactin omit maps. Simulated  $F_c - F_c$  annealing omit maps of pyrabactin in the ligand binding pockets of PYL1 and PYL2 at  $2\sigma$ . Ligand binding residues are indicated for orientation. **(c)** Crystal structure of free pyrabactin at 0.7 Å resolution.

# Supplementary Figure 3:

PYL2 EQKTLLEPVIKTYHQFEPDPTTCTSLITQRIHAPASVVWPLIRRFDNPERYKHEVVKRCRL-ISGDG-----DVGVSREVTIVISGLPASTSTERL gate  
 PYR1 ERSELKNSIAEFHTYQLDPGSCSSLHAQRIHAPPELVWSIVRRFDKPQTYKHEIKSCSV----EQNF-EMRVGCTRDVIVISGLPANTSTERL  
 PYL1 EFTQLSQSIAEFHTYQLGNRCSSLLAQRIHAPPETVWSVVRREDRQIYKHEIKSCNV----SEDF-EMRVGCTRDVNVISGLPANTSRERL

PYL2 V114  
 PYL1 I137

↓

latch

PYL2 V166  
 PYL1 A190

↓

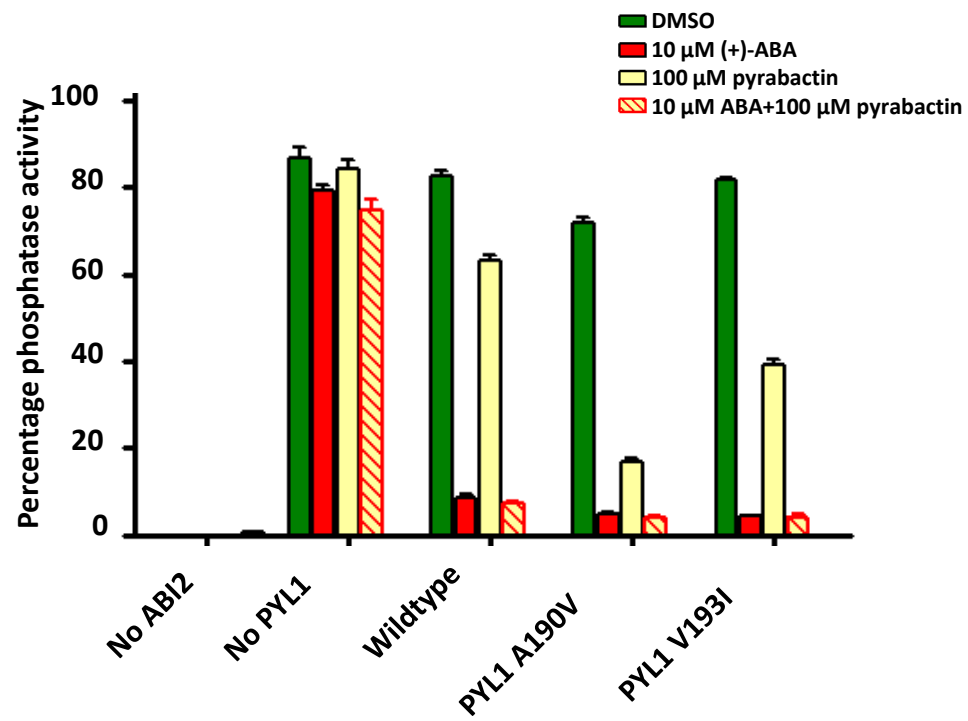
PYL2 V170  
 PYL1 I194

↓

PYL2 EFVDDHRLVLSFRVVGGEHRLKNYKSVTSVNEFL---NQDSGKVYTVVLESYTVDIPEGNTEEDTKMFVDTVVKINLQKLGVAATSAPMHD-  
 PYR1 DILDDERRVTGFSIIIGGEHRLTNYKSVTTVHRFE--KEN--RIWTVVLESYVVDMPGNSSEDDTRMFADTVVKINLQKLATVAEAMARNS-  
 PYL1 DLLDDRRVTGFSIIIGGEHRLRNYKSVTTVHRFE--KEEEEERIWTVVLESYVVDVPEGNSEEDTRLFADTVIRLNQKLASITEAMNRNN-

**Supplementary Figure 3** Alignment of PYR1, PYL1, and PYL2. Residues conserved within the PYR/PYL family are highlighted and ligand binding residues boxed. Red arrows indicate the three ligand binding residues that differ between PYL1 and PYL2.

## Supplementary Figure 4:

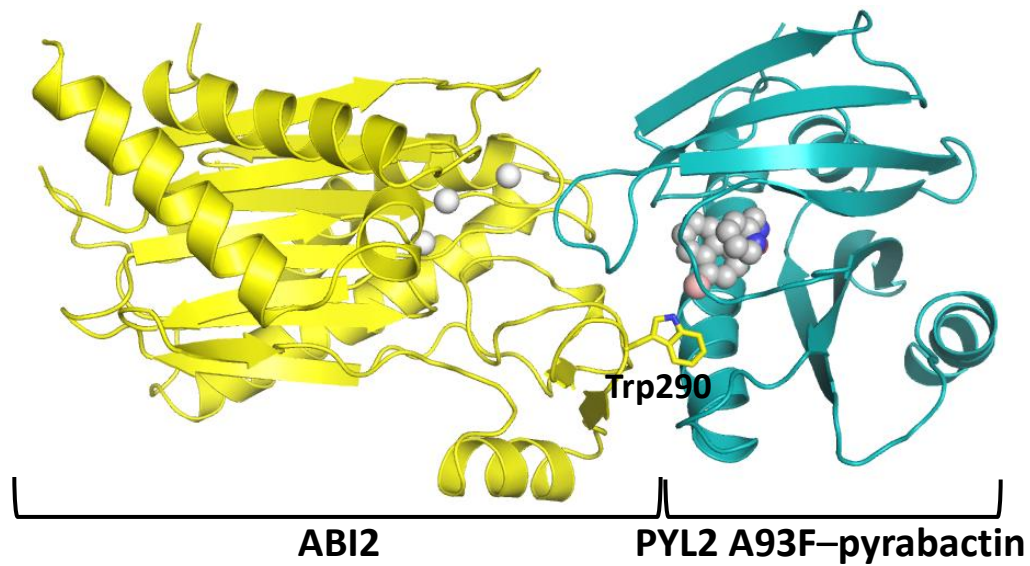




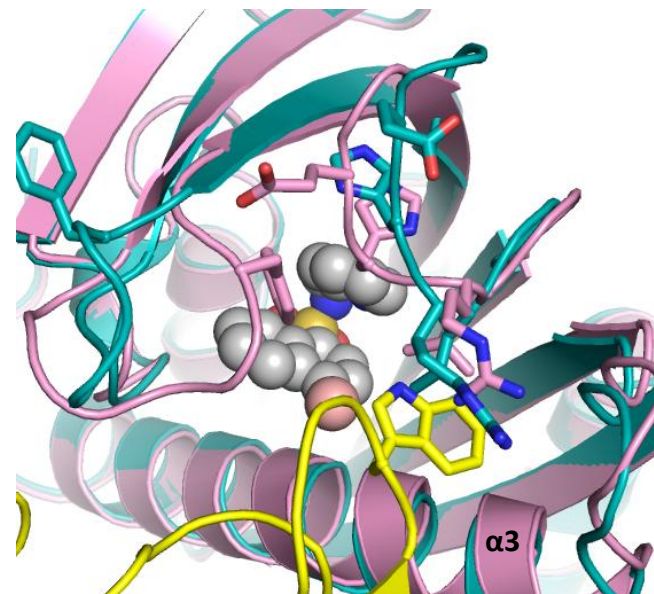
**Supplementary Figure 4** A190V and V193I enhance PYL1-mediated inhibition of ABI2 phosphatase activity by pyrabactin (n=3, error bars=s.d.).

# Supplementary Figure 5:

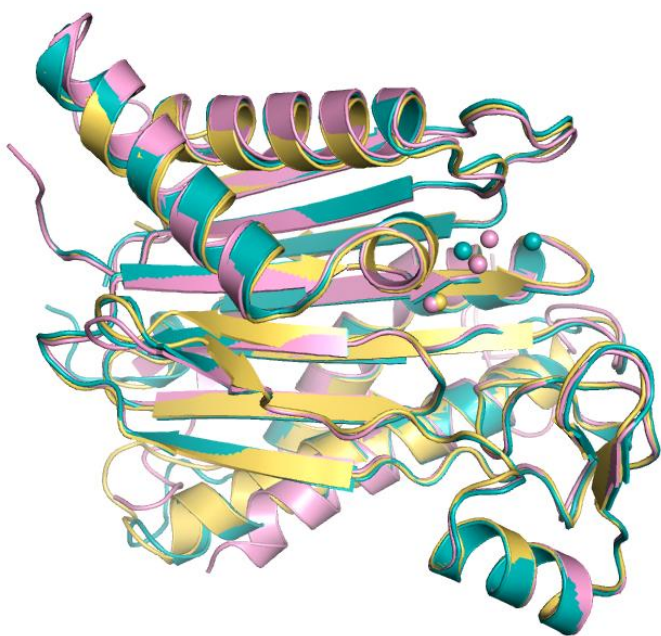
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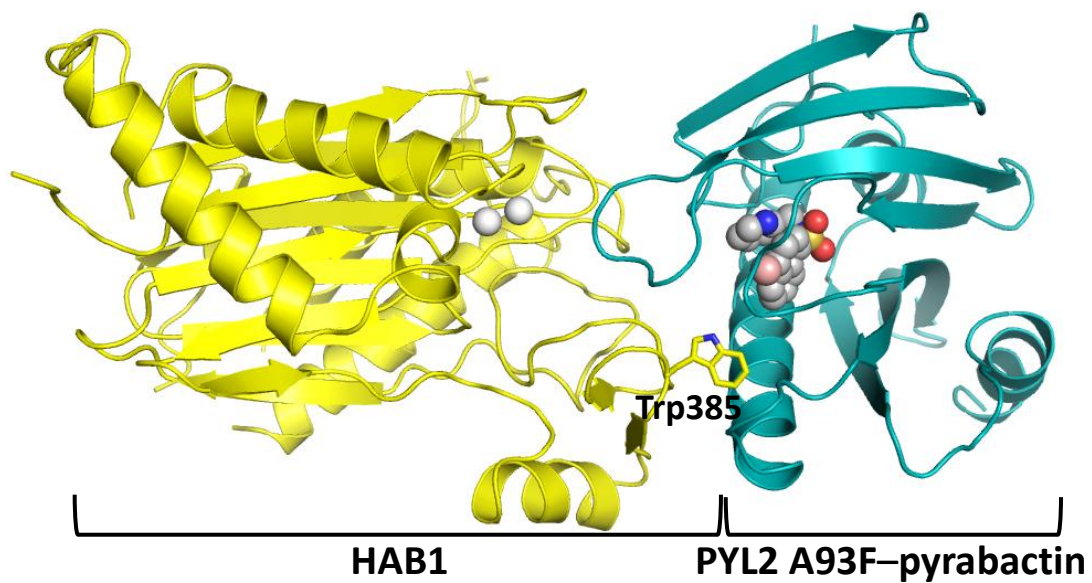
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b

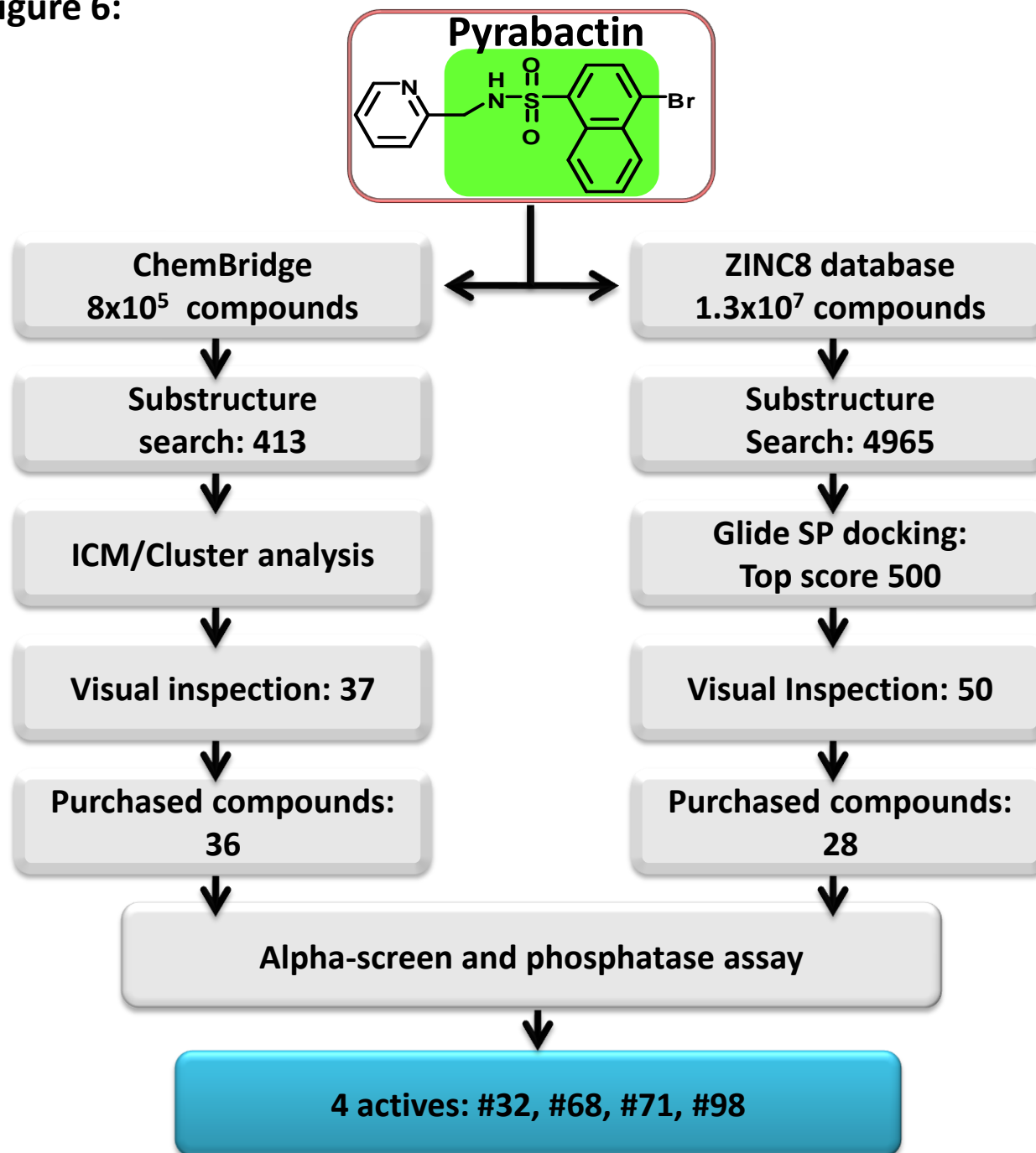


d



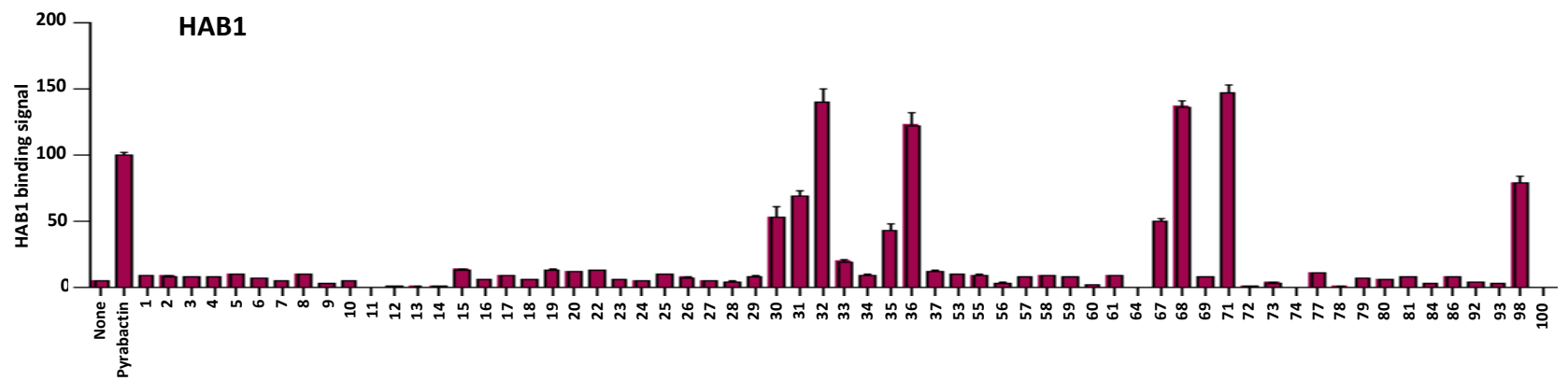
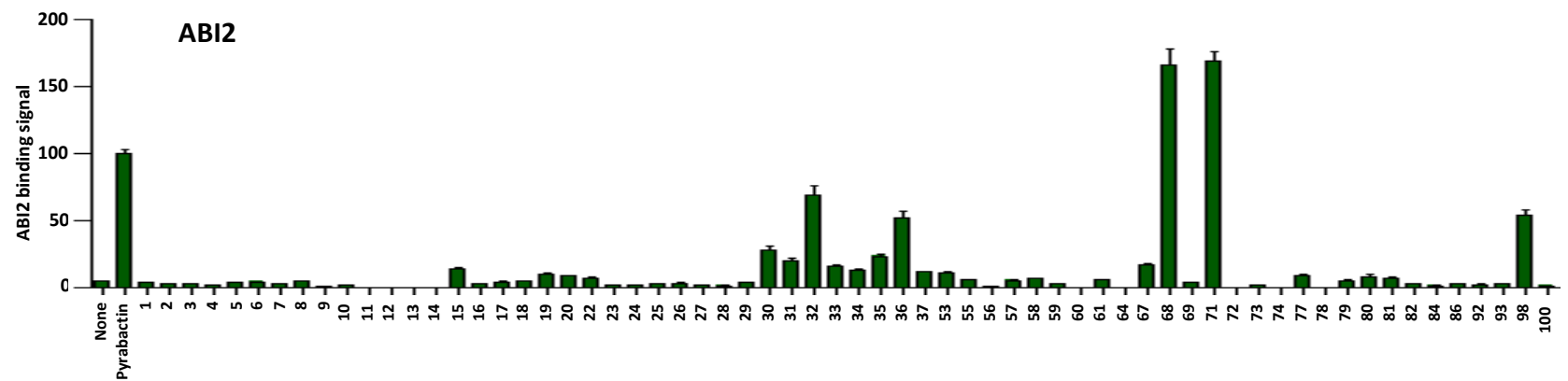
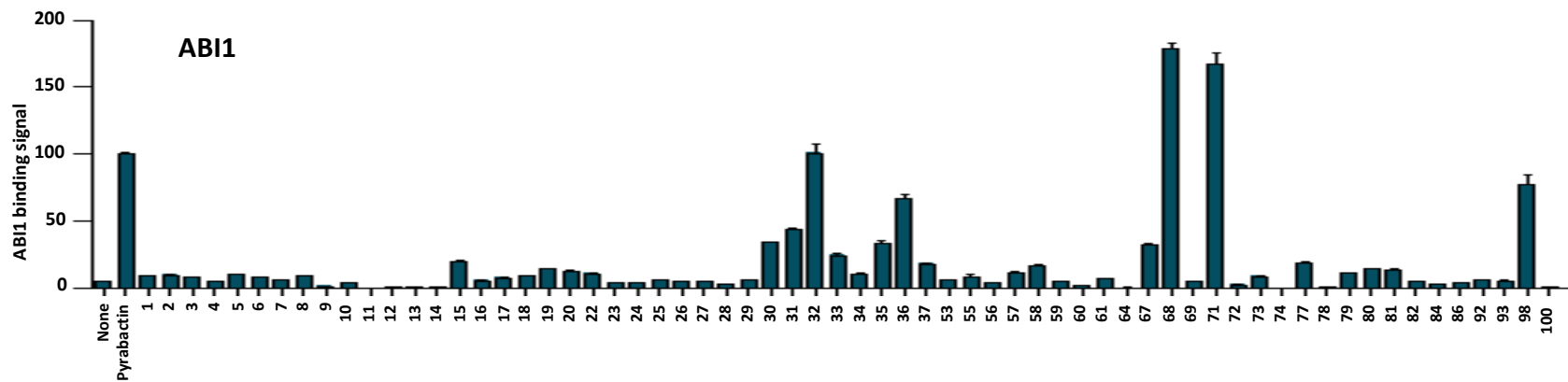
**Supplementary Figure 5** PYL2 A93F–pyrabactin and PYL2 A93F–pyrabactin–PP2C structures. **(a)** Overview of the PYL2 A93F–pyrabactin–ABI2 complex. ABI2 is shown in yellow, its three Mg<sup>2+</sup> ions in its catalytic center as grey spheres, and the locking residue Trp290 in a stick representation. PYL2 A93F is shown in cyan with pyrabactin as ball model. **(b)** Overlay of ABI2 (cyan) from the PYL2 A93F–pyrabactin–ABI2 structure with ABI1 (yellow) from the PYL1–pyrabactin–ABI1 structure and HAB1 (pink) from the PYL2–ABA–HAB1 structure. **(c)** Overlay of pyrabactin (grey balls) in the PYL2 A93F–pyrabactin–ABI2 (PYL2 pink, ABI2 yellow) structure with PYL2 A93F in the PYL2 A93F–pyrabactin structure (cyan). **(d)** Overview of the PYL2 A93F–pyrabactin–HAB1 complex. HAB1 is shown in yellow, its Mg<sup>2+</sup> ions in its catalytic center as grey spheres, and the locking residue Trp385 in a stick representation. PYL2 A93F is shown in cyan with pyrabactin as ball model.

Supplementary Figure 6:



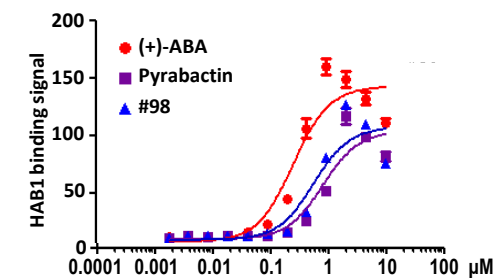
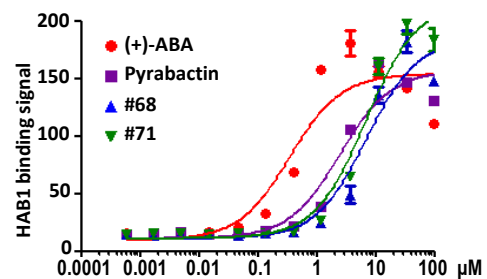
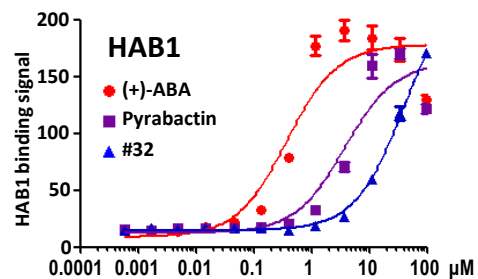
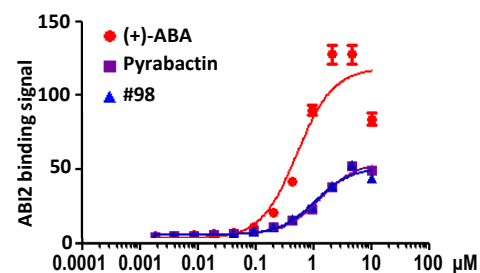
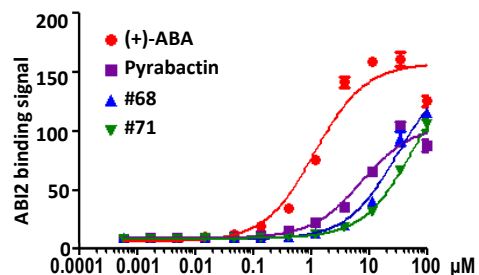
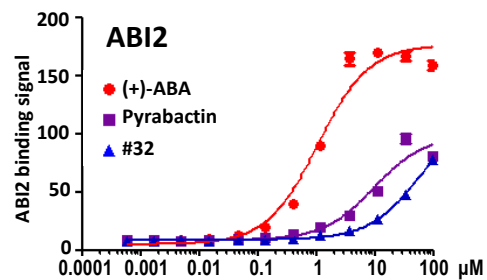
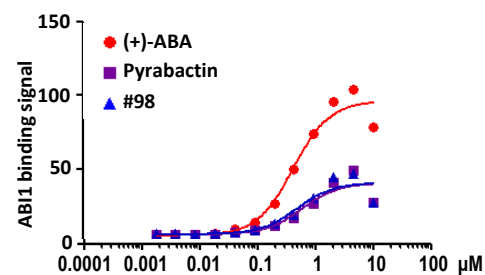
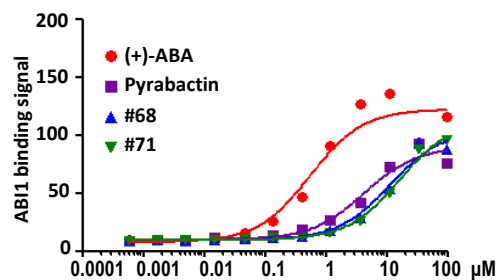
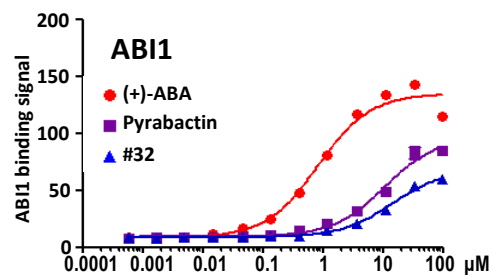
**Supplementary Figure 6** Strategy for the identification of new ABA receptor agonists. See Result and Methods sections for details.

# Supplementary Figure 7:



**Supplementary Figure 7** Screening of ABA receptor agonist candidates. AlphaScreen interaction in the presence of absence of 100  $\mu$ M compound (n=3, error bars=s.d.). Note that #36 is identical to pyrabactin B, a compound identified as an ABA agonist in the same chemical genetic screen that identified pyrabactin<sup>1</sup>.

## Supplementary Figure 8:





**Supplementary Figure 8** Agonist dose response curves. Concentration-dependent stimulation of the interaction between PYR1 and PP2Cs by (+)-ABA, pyrabactin, and compounds #32, #68, #71, and #98 as determined by AlphaScreen assays (n=3, error bars=s.d.).



**Supplementary Figure 9** ABI1, ABI2, HAB1, and PYL6 share similar expression profiles. **(a–d)** Expression profiles of ABI1, ABI2, HAB1, and PYL6. Expression profiles in different plant tissues depicted as tissue heat maps. **(e)** ABI1, ABI2, HAB1, and PYL6 are selectively expressed in guard cells. Pictograms represent surface views of leaves with heat maps. Expression data were taken from and heat maps generated using eFP browser (<http://bar.utoronto.ca>).

**Supplementary Reference:**

1. Zhao, Y. et al. Chemical genetic interrogation of natural variation uncovers a molecule that is glycoactivated. *Nat Chem Biol* 3, 716-21 (2007).