Structural basis for selective activation of ABA receptors.

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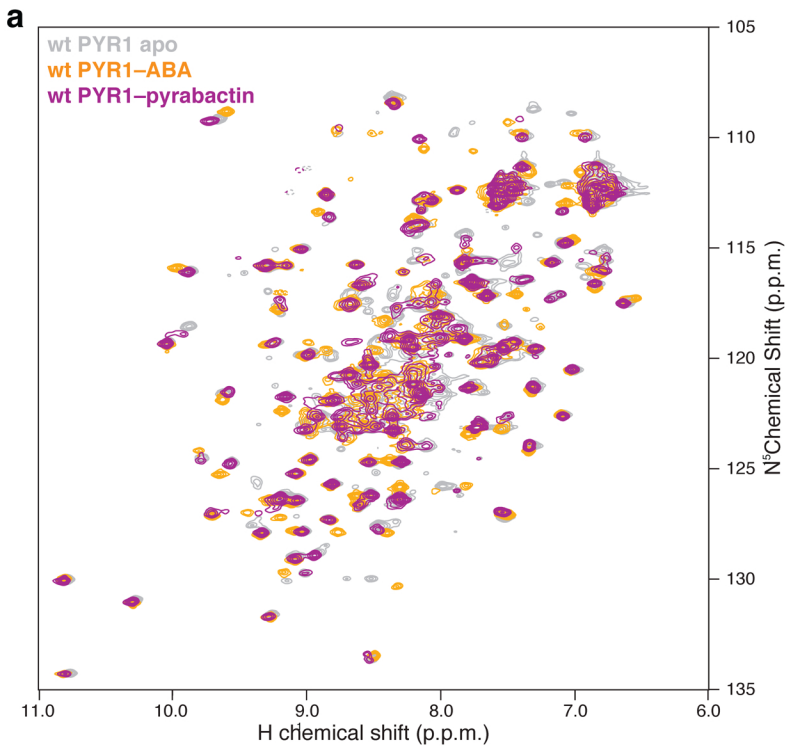
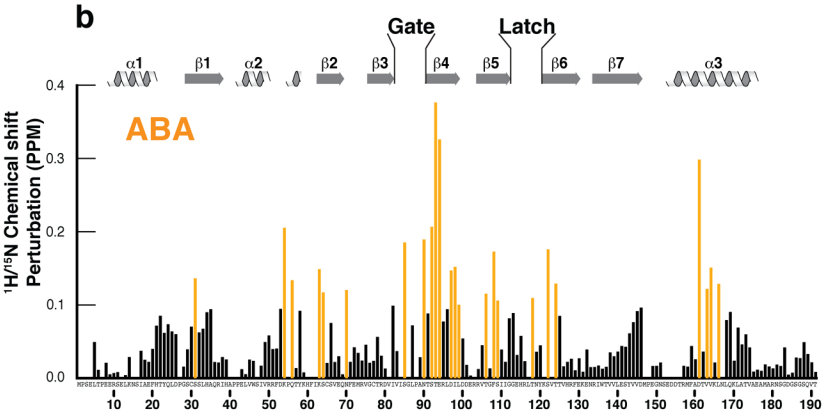
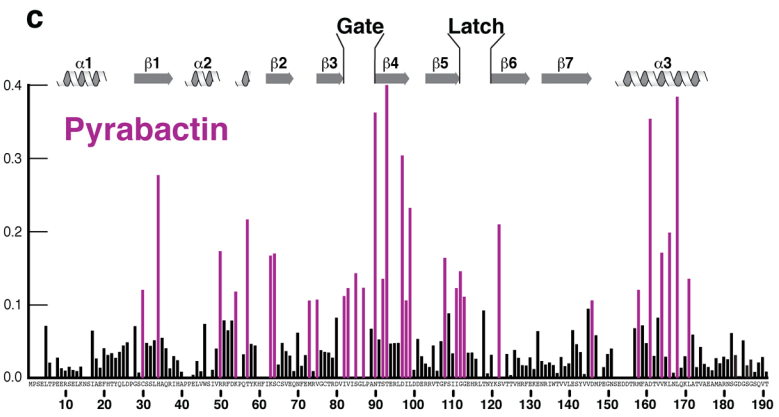
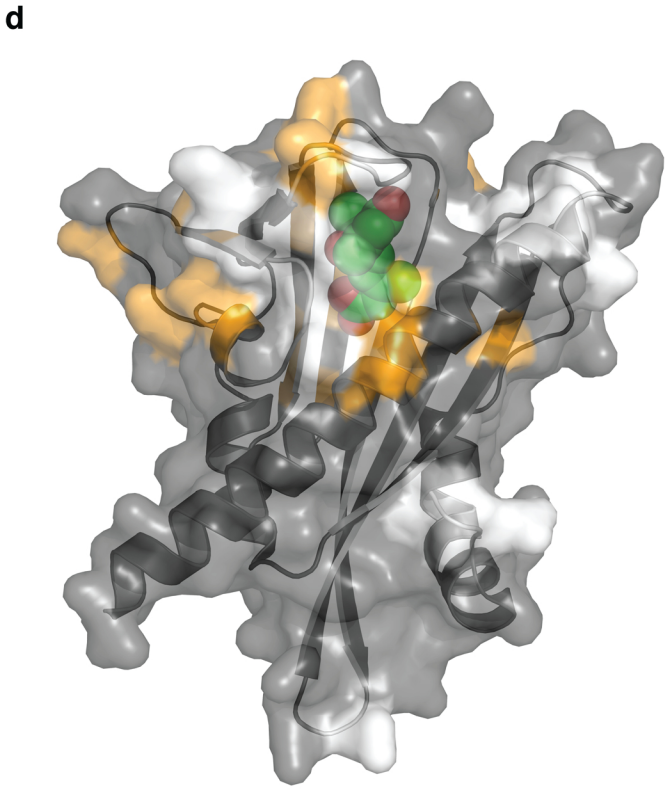
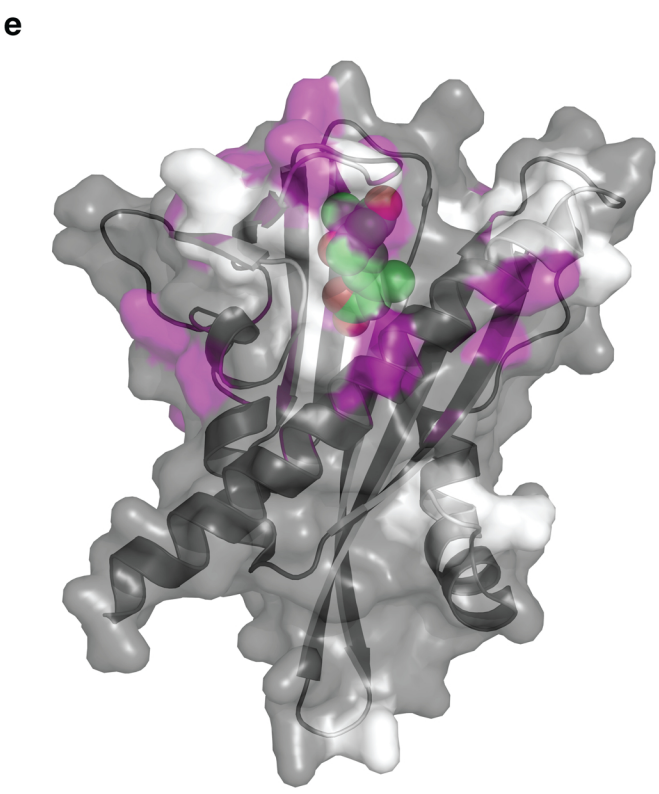


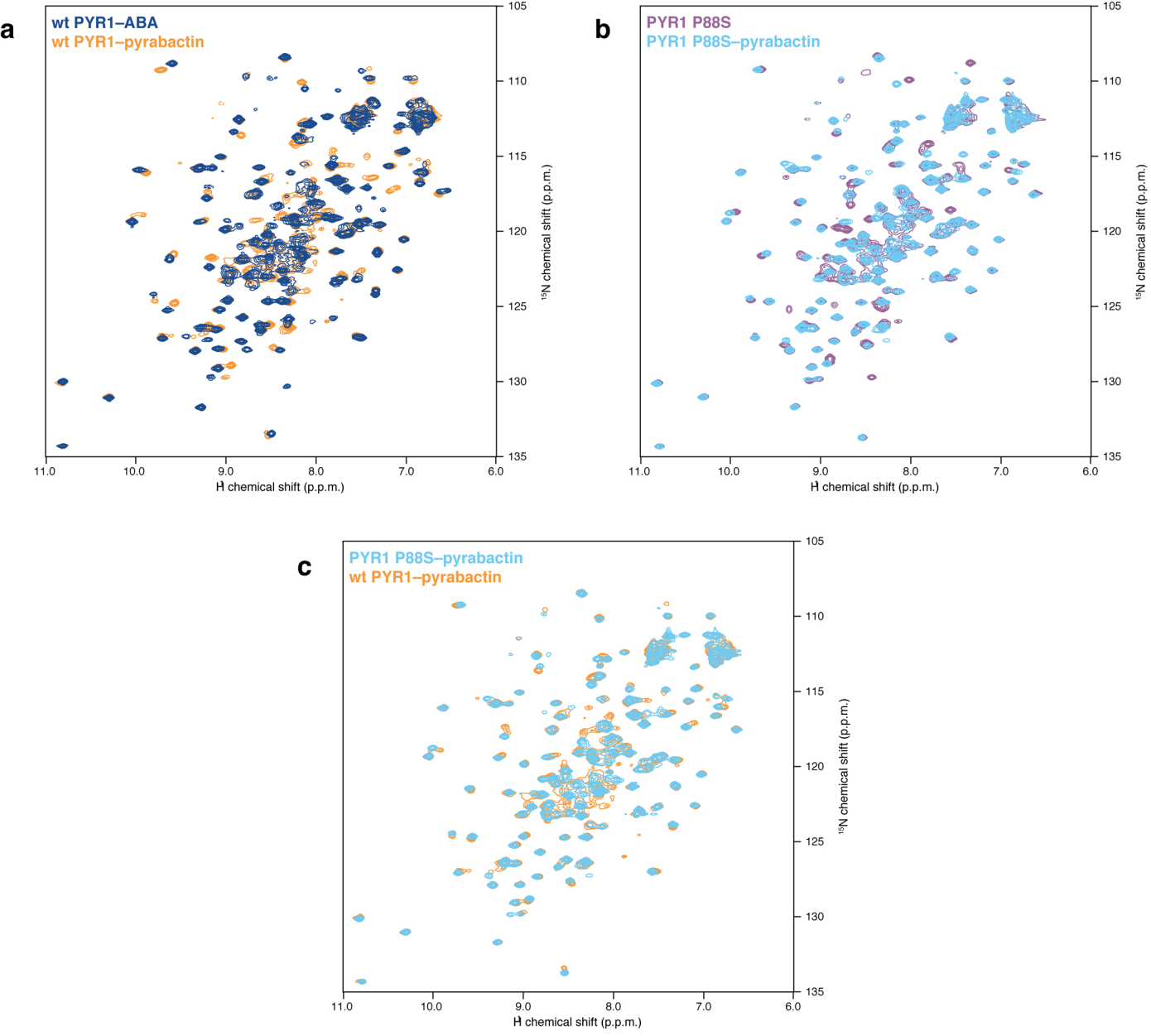
Supplementary Fig. 1 Pyrabactin and ABA bind the same site in PYR1. (**a**) ^{1}H-^{15}N HSQC spectra of wild-type PYR1 shown in the unliganded (gray), abscisic acid-bound (orange), and pyrabactin-bound (purple) states. (**b**) and (**c**) Combined ^{1}H/^{15}N chemical shift change graphed against residue number in monomeric PYR1 L166R. Colored bars denote significant changes ($\Delta\delta > 0.10$ PPM combined chemical shift) between apo and abscisic acid-bound (orange) or pyrabactin-bound states (purple). Note similarly large shifts in the residues following the region marked “Gate” and moderate shifts around “Latch.” (**d**) and (**e**) Significant chemical shift changes from (**b**) and (**c**) were then highlighted onto the crystal structure of abscisic-acid bound PYR1 (PDB ID: 3K3K). Gray and white signify unperturbed and unassigned r esidues, respectively.



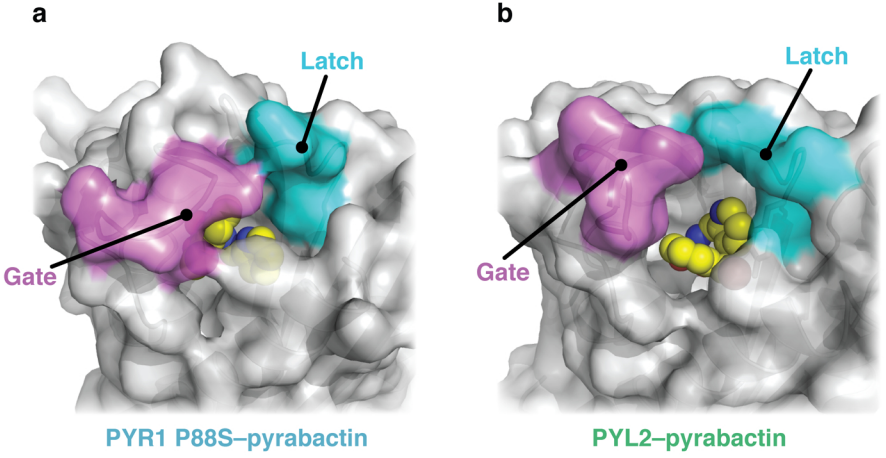








Supplementary Fig. 2 PYR1 P88S and wild-type PYR1 respond similarly to pyrabactin. (**a**) ^{1}H-^{15}N HSQC overlay of wild-type PYR1 saturated with ABA (blue) or pyrabactin (orange). (**b**) Differences in the HSQC spectra of PYR1 P88S in the absence (purple) or presence (cyana) of pyrabactin show that pyrabactin binds the mutant protein. (**c**) HSQC spectra of wild-type PYR1–pyrabactin (orange) and PYR1 P88S–pyrabactin (cyana) are highly similar, suggesting that pyrabactin induced equivalent conformational changes in both proteins.



Supplementary Fig. 3 Surface representations of PYR1 P88S and PYL2 bound to pyrabactin. Binding of pyrabactin elicits closure of the gate loop (magenta surface) in (**a**) PYR1 P88S but not (**b**) PYL2. The orientation of the latch loop (cyan surface) is similar in both PYR1 P88S and PYL2. The closed gate loop conformation sequesters pyrabactin in the binding pocket, reflects receptor activation and promotes PP2C binding.

Supplementary Table 1. Pyrabactin response mutants.

Seq #	Mutation(s)			
1	I48T	F61L	T91A	
2	V45A	F61L	W136R	
3	***I110V***	T125A		
4	**I110S**			
5	V138A	K170E		
6	I111T			
7	I111T	K170E	T173A	
8	E19G	L44H	F61L	
9	F61L	**I110T**	E132G	
10	F61L	M73V	E149G	
11	***I62V***	K170E		
12	F61L	**I110T**	I111T	
13	H60R	K170E		
14	K170E			
15	**I110S**			
16	I111T	K170E	T173A	
17	F61L	N90D	**I110T**	
18	**I110S**	E141G		
19	F61L	N90D	**I110T**	
20	***I62V***	K170E		
22	F61L	I111M		
24	Y23C	F61L	V75I	**I110T**
26	F61L	I82T	**I110T**	E132G
27	**I110S**			
28	I111T	K170E	T173A	
29	K14R	K170E		
30	**I110S**			
31	P41R	F71L	N133D	K170E
32	***I62V***	K170E		
33	N119K	K131I		
34	N119K	K131I	K170E	
35	F61L	V67A		
36	H127R	N133S	K170E	
37	V45A	F61L	W136R	
38	N119K	K131I	K170E	
40	M73V	K121N	K170E	
41	N119K	K131I	K170E	
42	N119K	K131I	K170E	
43	T57A	E141Q		
44	V45A	F61L	W136R	
45	N119K	K131I	K170E	
46	H60R	**I62T**	T104A	E153G
47	I111T	K170E	T173A	
48	I135T			
49	N133S	K170E		

Shown are the mutations present in 45 receptor clones isolated. These mutant receptors prevent pyrabactin response but allow ABA response. These mutants were isolated to define residues that are selectively needed for pyrabactin response. Shown in bold are the mutations isolated in either Ile110 or Ile62, italics denote the naturally occurring Ile->Val substitutions that were isolated.