

Agrochemical control of plant water use using engineered abscisic acid receptors

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Rising temperatures and lessening fresh water supplies are threatening agricultural productivity and have motivated efforts to improve plant water use and drought tolerance. During water deficit, plants produce elevated levels of abscisic acid (ABA), which improves water consumption and stress tolerance by controlling guard cell aperture and other protective responses^{1,2}. One attractive strategy for controlling water use is to develop compounds that activate ABA receptors, but agonists approved for use have yet to be developed. In principle, an engineered ABA receptor that can be activated by an existing agrochemical could achieve this goal. Here we describe a variant of the ABA receptor PYRABACTIN RESISTANCE 1 (PYR1) that possesses nanomolar sensitivity to the agrochemical mandipropamid and demonstrate its efficacy for controlling ABA responses and drought tolerance in transgenic plants. Furthermore, crystallographic studies provide a mechanistic basis for its activity and demonstrate the relative ease with which the PYR1 ligand-binding pocket can be altered to accommodate new ligands. Thus, we have successfully repurposed an agrochemical for a new application using receptor engineering. We anticipate that this strategy will be applied to other plant receptors and represents a new avenue for crop improvement.

The phytohormone ABA (Fig. 1a) has an essential role in regulating plant water use and drought tolerance. A land-plant-specific signalling network composed of receptors, phosphatases and kinases mediates ABA responses¹. ABA receptors control the activity of a subfamily of three SNF1-related protein kinases (SnRK2 kinases) in response to environmental stress. These SnRK2 kinases autoactivate by *cis*- and *trans*-autophosphorylation on their activation loops^{3,4}, but are continuously inactivated by type 2C protein phosphatases (clade A PP2Cs), which results in low basal kinase activity. When ABA levels rise during stress, the phytohormone binds to soluble ABA receptors and stabilizes their activated conformations, enabling them to bind to and inhibit PP2Cs^{5–11}. This in turn allows accumulation of activated SnRK2 kinases, whose direct targets include SLOW ANION CHANNEL 1, an anion channel that controls guard cell aperture, and ABA RESPONSE-ELEMENT-BINDING FACTORS, b-ZIP transcription factors that mediate ABA-regulated gene expression¹². Thus, ABA controls water use and stress physiology by receptor-mediated inhibition of PP2C activity and resultant SnRK2 kinase activation.

Plants regulate their transpiration rates by modifying stomatal aperture, and consequently ABA receptors have emerged as attractive targets for water use optimization; however, ABA agonists approved for this use have yet to be developed. We reasoned that agrochemical control of plant water use could be accomplished in transgenic plants that express an engineered ABA receptor that responds to an existing agrochemical, a strategy based on orthogonal ligand–receptor systems, which have enabled selective chemical control of diverse targets^{13,14}. To identify ligands suitable for our strategy, we constructed a collection of PYR1 mutants with saturating mutations in ligand-contacting residues and screened it to identify activating agrochemical ligands. Once identified, the sensitivities

of specific ligand–receptor pairs were improved through targeted mutagenesis and functional selection. This scheme was facilitated by a previously constructed set of PYR1 mutants that contains all possible 475 single amino acid substitutions in the 25 residues that line the ABA binding pocket¹⁵. Since overexpression of wild-type receptors has negative yield consequences¹⁶, we inactivated the intrinsic ABA responsiveness of each of the 475 mutants by introducing an arginine at position K59, a highly conserved residue that forms a salt bridge with the carboxylate of ABA in wild-type receptors^{7–11}. Each member of the mutant collection was individually tested for responsiveness to a panel of 15 commonly used non-herbicidal agrochemicals at high concentrations (100 μ M) using a yeast two-hybrid-based assay that measures agonist-induced binding of receptor to PP2C^{6,17}. This screening effort, which involved 7,125 mutant receptor–ligand response assays, identified receptors weakly responsive to 4 of the 15 compounds tested (Extended Data Fig. 1). This high hit rate is probably a consequence, in part, of the intrinsically low basal activity of PYR1, which facilitated the identification of weak responders.

We next attempted to optimize response sensitivities using targeted mutagenesis and functional selections. This worked most successfully

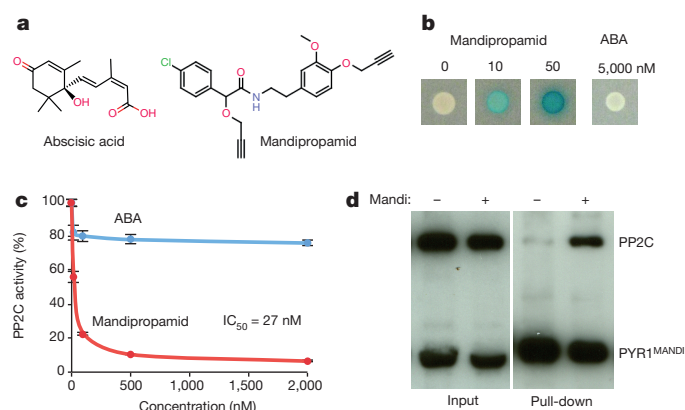


Figure 1 | PYR1^{MANDI} possesses nanomolar sensitivity to mandipropamid and functions *in vitro* and *in vivo*. **a**, Structures of ABA and mandipropamid. **b**, PYR1^{MANDI} binds to the PP2C HAB1 in response to mandipropamid, but not ABA, as measured using a yeast two-hybrid assay. **c**, PYR1^{MANDI} inhibits PP2C phosphatase activity in response to mandipropamid (red line), but not ABA (blue line). Shown are data using ABI1; ABI2 and HAB1 were also tested (IC_{50} = 76 and 32 nM respectively). **d**, 6 $\times$ His–GFP–PYR1^{MANDI} and GFP–HAB1 were co-expressed in *N. benthamiana* leaves using *Agrobacterium tumefaciens*. Plants were treated with mock (–) or 50 μ M mandipropamid (Mandi; +) solutions. Twenty hours later PYR1^{MANDI} was affinity purified from treated leaves. Input extracts and affinity-purified proteins were separated by SDS–polyacrylamide gel electrophoresis (SDS–PAGE) and detected using an anti-GFP antibody.

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with receptors responsive to mandipropamid (Fig. 1a), a mandelamide compound used to control oomycete (blight) pathogens that is sold under the trade name Revus. Mutations in four separate residues (in combination with K59R) were initially observed to confer mandipropamid responsiveness (Extended Data Figs 1 and 2). We constructed combinations of a subset of these mutations and identified a triple mutant (K59R, S122G, F108A) with low micromolar responsiveness in yeast-based assays (Extended Data Fig. 2). This mutant was next subjected to saturation mutagenesis at 22 pocket-lining residues and separately mutagenized by DNA shuffling, which together identified mutations in five residues that individually enhance sensitivity of the triple mutant (Extended Data Fig. 2). These enhancing mutations were assembled combinatorially using multiple site-directed mutagenesis and the mutagenized receptors were characterized directly. These efforts yielded a hexuple mutant, PYR1^{MANDI} (PYR1(Y58H/K59R/V81I/F108A/S122G/F159L)) that possesses nanomolar mandipropamid sensitivity in *in vitro* PP2C inhibition assays (half-maximum inhibitory concentration (IC₅₀) = 27 nM; Fig. 1b, c). To rule out potential artefacts caused by our reliance on yeast-based receptor activation assays and selections, we examined PYR1^{MANDI} activity in *Nicotiana benthamiana*. PYR1^{MANDI}, but not PYR1, binds to the PP2C HYPERSENSITIVE TO ABA1 (HAB1) in response to mandipropamid when both proteins are co-expressed in *N. benthamiana*, demonstrating that PYR1^{MANDI} functions in a plant cell environment (Fig. 1d). PYR1^{MANDI} is not activated by ABA *in vitro*, nor does mandipropamid substantially activate wild-type PYR1 or ten other *Arabidopsis* ABA receptors tested (Extended Data Table 1). Thus, PYR1^{MANDI} is selectively activated by mandipropamid.

To understand the molecular basis for the engineered agrochemical sensitivity we solved the X-ray crystal structure of a quadruple mutant receptor, PYR1(K59R/V81I/F108A/F159L), in complex with mandipropamid and HAB1 at 2.25 Å resolution (data collection structural statistics are shown in Extended Data Table 2). This quadruple mutant contains four of the six mutations present in PYR1^{MANDI} and possesses nanomolar sensitivity to mandipropamid (IC₅₀ = 50 nM; Extended Data Fig. 3A); it was used because it formed higher-quality crystals than could be obtained with the hexuple mutant PYR1^{MANDI}. The mutant

receptor binds the (S)-stereoisomer of mandipropamid, which adopts a U-shaped orientation (Extended Data Fig. 3B, C) reminiscent of sulfonamide ABA receptor agonists^{17–21} and induces a closed-gate receptor conformation (Extended Data Fig. 3D) that is nearly indistinguishable from previously determined PYR1-agonist-PP2C complexes (C α root mean squared deviation (r.m.s.d.) of ~0.45 Å).

The structure obtained reveals how the ABA-binding pocket of PYR1 was transformed to bind mandipropamid. The most conspicuous change in the mutant receptor is an increase in the volume of the ligand-binding pocket created by two mutations, F108A and F159L, that enable mandipropamid's lengthy propargyl substituents to fit in the binding pocket (Fig. 2). F108A and F159L also enable hydrophobic contacts between mandipropamid and S109 and between mandipropamid and the G392 of HAB1, neither of which makes ABA contacts in wild-type structures (Extended Data Fig. 4). In addition, the K59R mutation enables a hydrogen bond between the N ϵ of R59 and the amide carbonyl of mandipropamid, mimicking the direct contact between K59 and ABA's carboxylate in wild-type PYR1 (Extended Data Fig. 4). A second direct hydrogen bond occurs between E94 and mandipropamid's amide carbonyl oxygen. Binding is also stabilized by extensive hydrophobic contacts to residues that would normally contact ABA in wild-type PYR1 and two separate water-mediated hydrogen-bond networks that interact with mandipropamid's amide carbonyl or its 3- and 4-alkoxy phenethyl substituents and the Trp lock (Extended Data Fig. 4). The contributions that the Y58H, V81I and S122G mutations present in PYR1^{MANDI} make to ligand binding are less obvious, as they occur in residues too distant to make direct mandipropamid contacts. Collectively, these structural observations show that a relatively small number of mutations are sufficient to reshape PYR1's ligand-binding pocket so that it can be activated by an unnatural ligand.

An unusually large family of receptors binds ABA in land plants. The restricted activation of PYR1 and closely related receptors by the sulfonamide agonist quinabactin is sufficient to induce a full ABA response, indicating that simultaneous activation of all 14 ABA receptors is not necessary for synthetic pathway activation^{20,21}. It is likely, although untested, that activating PYR1 should be sufficient to elicit global pathway

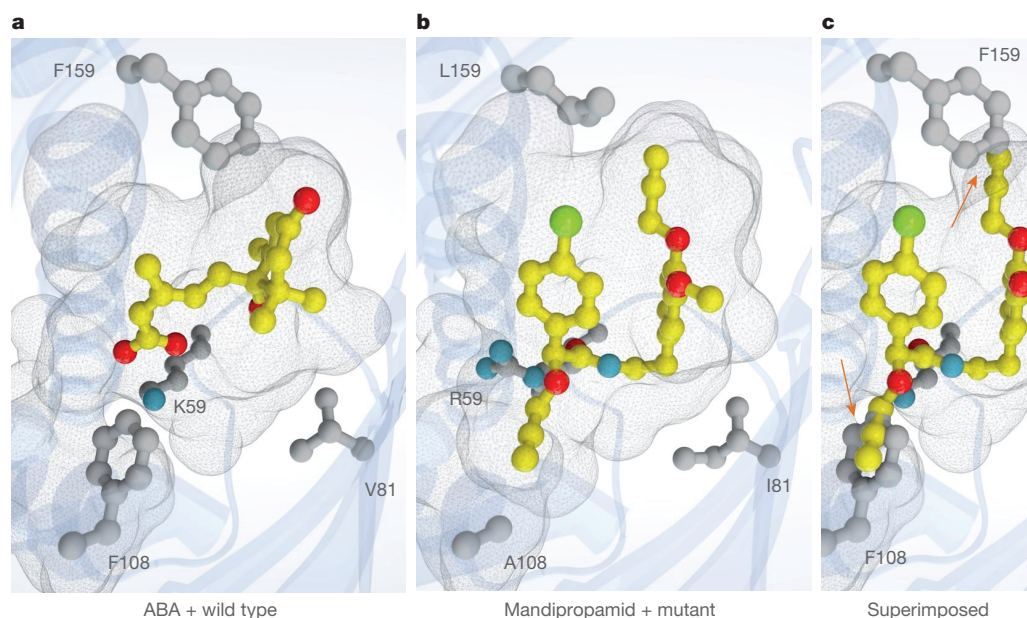


Figure 2 | Crystal structure of a mandipropamid responsive receptor—F108A and F159L prevent steric clash. **a**, Previously published coordinates (Protein Data Bank accession 3QN1) were used to represent the inner surface of wild-type PYR1, which is shown as a mesh enclosing ABA (shown in yellow); the side chains altered in the mandipropamid-responsive mutant are shown in grey. **b**, X-ray coordinates for a PYR1(K59R/V81I/F108A/F159L)–mandipropamid–HAB1 complex were obtained experimentally and used to

represent the ligand-binding pocket. Mandipropamid (yellow) is sold as mixed stereoisomers, but the mutant receptor selectively binds the (S)-isomer. **c**, Superimposition of mandipropamid (yellow) onto the wild-type receptor shows that the wild-type receptor disfavours binding due to steric clash with F108 and F159 (red arrows). Structures were rendered in Cinema4D using ePMV²³. The inner surface meshes shown were exported from PyMol; the latch loop has been omitted for clarity.

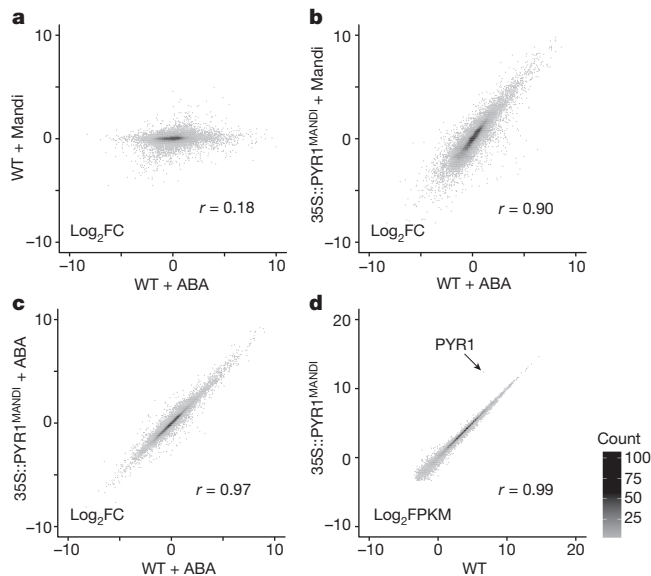


Figure 3 | Mandipropamid induces an ABA-like transcriptional response selectively in the PYR1^{MANDI} genotype. **a–d**, The wild-type (WT) and transgenic PYR1^{MANDI} genotypes were treated with 50 μ M ABA, 2 μ M mandipropamid (Mandi) or mock solutions for 8 h in biological triplicate. RNA was then isolated and used for RNA-seq experiments. **a**, Mandipropamid does not induce an ABA-like transcriptional response in the wild-type genotype. **b**, Mandipropamid induces an ABA-like effect in the PYR1^{MANDI} genotype. **c**, The PYR1^{MANDI} genotype responds normally to ABA. **d**, The PYR1^{MANDI} transgene does not substantially alter basal transcript levels in the absence of mandipropamid treatment. **a–c**, Hexbin plots of $\log_2$ -transformed fold change (FC) (chemical/mock) values for $\sim 21,000$ transcripts with fragments per kilobase of exon per million fragments mapped (FPKM) abundance values above 0.1 in all experimental samples; **d**, Log₂ FPKM values are plotted.

activation in adult plants. To test this, we made and characterized transgenic *Arabidopsis* plants that express PYR1^{MANDI} under the control of the constitutive viral 35S promoter. Seed germination is classically used to assess ABA effects, owing to ABA's role in inhibiting germination under environmentally unfavourable conditions. Mandipropamid inhibits seed germination in two independent 35S::PYR1^{MANDI} transgenic, but not wild-type or 35S::GFP–PYR1, strains. As expected, the mandipropamid sensitivity of the transgenic strains constructed correlates with PYR1^{MANDI} protein abundance (Extended Data Fig. 5A–C). Mandipropamid also inhibits primary root growth in the 35S::PYR1^{MANDI} strains, but not wild-type or 35S::GFP–PYR1 genotypes (Extended Data Fig. 5D). These data show that activating PYR1^{MANDI} is sufficient to control seed and root ABA responses.

To characterize the ABA response of 35S::PYR1^{MANDI} transgenic lines more closely, we used RNA sequencing (RNA-seq) experiments to compare the transcriptional responses induced by mandipropamid and ABA treatments in both the wild-type and 35S::PYR1^{MANDI} strains. As shown in Fig. 3a, mandipropamid does not induce a substantial ABA response in wild-type non-transgenic plants (Pearson's correlation coefficient $r = 0.17$); however, it does induce a global ABA-like response in the 35S::PYR1^{MANDI} line ($r = 0.90$; Fig. 3b). Additionally, the transcriptional responses of the wild-type and 35S::PYR1^{MANDI} genotypes to ABA treatments are highly correlated ($r = 0.97$), indicating that the 35S::PYR1^{MANDI} transgene does not interfere substantially with the endogenous ABA response mediated by wild-type receptors (Fig. 3c). The basal transcript levels of untreated wild-type and 35S::PYR1^{MANDI} genotypes are also highly correlated ($r = 0.99$; Fig. 3d), which indicates that the transgene has minimal effects on transcript abundances in the absence of mandipropamid treatment. Consistent with this, we observe negligible differences between the fresh weights or flowering times of the wild-type and two 35S::PYR1^{MANDI} genotypes grown in the absence

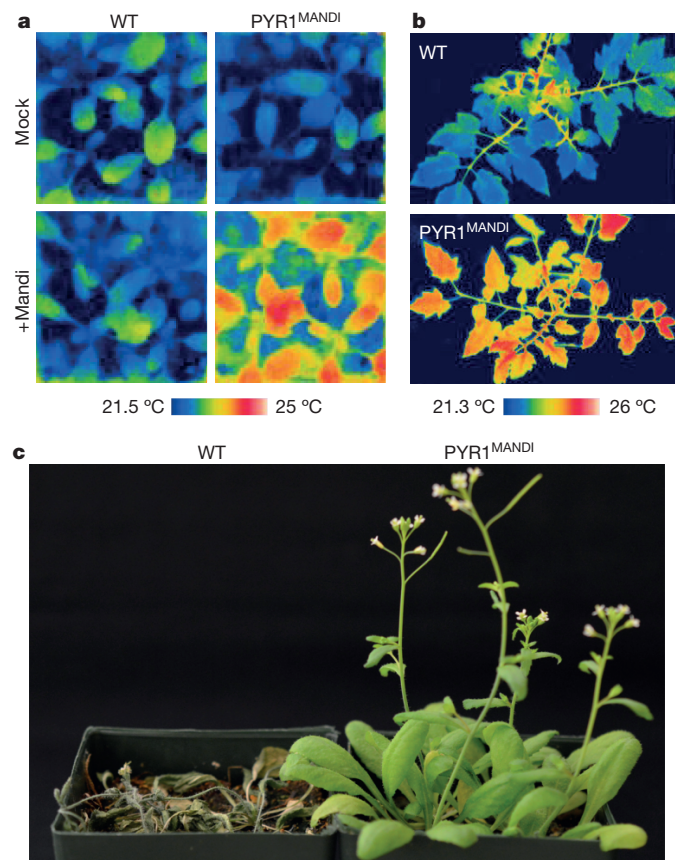


Figure 4 | Agrochemical control of transpiration and drought tolerance in the PYR1^{MANDI} genotype. **a–c**, A ~ 2 °C increase in leaf temperature is selectively observed in response to mandipropamid in the PYR1^{MANDI} genotype in *Arabidopsis* and tomato. WT, wild type. **a**, Three-week-old *Arabidopsis* seedlings were treated with 1 μ M mandipropamid (Mandi) or a mock solution and imaged by thermography 24 h after application. Leaf warming is a consequence of reduced transpiration. **b**, Transgenic 35S::PYR1^{MANDI} tomato plants were grown alongside wild-type controls and treated with 25 μ M mandipropamid and thermographed 24 h after application. **c**, Induction of drought tolerance by mandipropamid treatments of the PYR1^{MANDI} genotype. Three-week-old wild-type or PYR1^{MANDI} genotype plants were treated with mandipropamid twice over the course of an 11-day water deprivation period. Photographs were taken 24 h after re-watering. Drought survival experiments were conducted on three separate occasions with each experiment conducted using a minimum of three biological replicates. The data shown for each figure panel are subsets of larger experiments shown completely in Extended Data Figs 6–8.

of mandipropamid treatment (Extended Data Fig. 5E, F). Thus, mandipropamid induces a genome-wide ABA-like transcriptional response selectively in transgenic *Arabidopsis* plants expressing PYR1^{MANDI} and the expression of PYR1^{MANDI} is not associated with substantial changes in basal transcript levels or background ABA responsiveness.

A critical physiological role of ABA is to control guard cell aperture and transpiration rates. This can be measured indirectly through leaf temperature, which increases when guard cells close owing to decreased evaporative cooling. After treatment with mandipropamid, transgenic *Arabidopsis* plants expressing PYR1^{MANDI} show elevated leaf temperatures (Fig. 4a and Extended Data Fig. 6), indicating that PYR1^{MANDI} can function in guard cells. The effects of mandipropamid persist ~ 2 days longer than those of ABA for wild-type plants (Extended Data Fig. 6), which could be due to multiple reasons, including differences in metabolism between ABA and mandipropamid. To establish whether PYR1^{MANDI} is active in other species, we constructed transgenic 35S::PYR1^{MANDI} tomato plants and observed that they too show a similar increase in leaf temperature in response to mandipropamid treatments (Fig. 4b and

Extended Data Fig. 7), indicating that PYR1^{MANDI} can control transpiration rates in two divergent eudicotyledonous species. Furthermore, like the effects of ABA on wild-type plants, the action of mandipropamid on the 35S::PYR1^{MANDI} genotypes is sufficient to improve *Arabidopsis* survival after water deprivation (one measure of drought tolerance). When we subjected the wild-type, 35S::GFP-PYR1 and 35S::PYR1^{MANDI} genotypes to a water deprivation regime, mandipropamid treatments selectively improved survival in two independent 35S::PYR1^{MANDI} transgenic lines in three separate experiments (Fig. 4c and Extended Data Fig. 8), as expected based on the broad activation of ABA responses we have demonstrated with the PYR1^{MANDI}/mandipropamid system.

Our data demonstrate selective agrochemical control of ABA signaling in *Arabidopsis* using an engineered receptor and illustrate the power of synthetic biological approaches for manipulating plant physiology. PYR1^{MANDI} is a new tool that can be used to control water use and to probe the ABA response pathway. Given the relatively simple structural basis underlying PYR1^{MANDI} function, we anticipate that it should be possible to modify other ABA receptors so that individual family members can be selectively activated, which will facilitate functional analyses at the whole-plant and cell-type-specific levels. Our work also has biotechnological implications. Although the genetic manipulation of ABA responses has been validated in the field as a strategy for improving drought tolerance in canola²², the broad use of the ABA pathway for manipulating drought tolerance is a relatively new idea that requires further validation. Moreover, the specific orthogonal control strategy outlined here will require testing in crops before it is suitable for use in the field. Nonetheless, our work demonstrates that it is possible to repurpose an existing agrochemical using receptor engineering. This strategy can be broadly applied to other plant receptors and agrochemicals and therefore opens new avenues for crop improvement.

Online Content Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

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Author Contributions S.-Y.P. and A.M. conducted protein mutagenesis experiments. S.-Y.P. conducted and J.Y. analysed the RNA-seq experiments. F.C.P. conducted the protein crystallography experiments. S.-Y.P. constructed and analysed transgenic plants. B.F.V. and S.R.C. designed and supervised experiments collaboratively with all co-authors. S.R.C. conceived the project and wrote the manuscript with input from all co-authors.

Author Information The X-ray crystallographic coordinates and structure factor files for the engineered PYR1 mandipropamid receptor in complex with mandipropamid and HAB1 have been deposited in the Protein Data Bank under accession number 4WVO. Reprints and permissions information is available at www.nature.com/reprints. The authors declare competing financial interests: details are available in the online version of the paper. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to S.R.C. (sean.cutler@ucr.edu).

METHODS

Construction of the K59R site saturation mutagenized 'pocket' library. Site-saturation mutagenesis involves directly constructing all 19 possible single amino acid substitution mutations at target residues of interest and enables systematic coverage of substitutions at sites of interest²⁴. We constructed a library of site-saturated mutants at 25 pocket-lining residues in the ABA non-responsive PYR1(K59R) backbone. In preliminary experiments screening error-prone PCR-mutagenized wild-type PYR1 templates for mutants that would respond to agrochemicals, we isolated the K59R mutation in screens against structurally dissimilar agrochemicals. It is possible that, in addition to eliminating PYR1's sensitivity to ABA, that the K59R mutation may sensitize PYR1 to non-specific chemical activation.

A set of site-saturated mutations was previously constructed in a wild-type PYR1 backbone as part of a larger effort focused on engineering constitutively active ABA variants¹⁵. The PYR1 template mutagenized in those experiments was a pBD-PYR1 plasmid that encodes a GAL4-DNA binding domain (BD) fusion to PYR1; this plasmid can be directly used for assaying receptor-PP2C interactions in an appropriate yeast strain co-transformed with pACT-PP2C, which express a GAL4 activation domain fusion (ACT) to a PP2C of interest⁶. We used pACT-HAB1 in the experiments shown.

We incorporated the K59R mutation into each of the original PYR1 wild-type backbone mutants using PCR-based mutagenesis, which yielded a collection of 475 PYR1(K59R) mutants in the following sites: P55, F61, I62, V81, V83, L87, P88, A89, S92, E94, E141, F108, I110, H115, R116, L117, Y120, S122, M158, F159, A160, T162, V163, V164 and N167. This was accomplished in two ways. Plasmids containing mutations in twenty-two of the sites targeted (all except P55, F61 and I62) were mutagenized using inverse PCR with two mutagenic primers oriented in opposite directions and directly flanking K59. After phosphorylating with polynucleotide kinase, these primers were used for PCR amplification of each of 418 pBD-PYR1 mutant templates. Three ligand-contacting residues (P55, F61 and I62) are too close to K59 to utilize this method. To introduce K59R into mutants at these sites, individual K59R mutagenic primers were designed complementary to each of the 57 remaining mutant templates. These primers were then used for inverse PCR mutagenesis, as described earlier. The linear PCR products generated using either method were ligated using T4 DNA ligase, digested with the restriction enzyme Dpn1 (to remove original template DNA) and transformed into competent *Escherichia coli* cells. Transformed colonies were screened by PCR using K59R allele-specific primers to identify plasmids that had successfully incorporated the K59R mutation. K59R mutant plasmids were isolated and sequenced to verify that they contained both the introduced K59R mutation and the original ligand-site mutation. This mutagenesis effort created a set of 475 PYR1(K59R) variants containing all possible single amino acid substitutions at 25 ligand-contacting residues. Sanger sequencing was used to validate all clones constructed.

Assays of the pocket library for agrochemical responsiveness. The set of 475 mutant plasmids were individually transformed into the Y190 yeast two-hybrid reporter strain co-transformed with pACT-HAB1, as previously described⁶. The yeast strains generated were arrayed into 96-well plates yielding what we refer to as the 'pocket library'. The pocket library strains were spotted onto duplicate agar plates containing selective synthetic dextrose (minus Leu and Trp) medium that was supplemented with a single test compound at 100 μ M. The pocket library strains were separately tested for responsiveness to the following compounds: benzothiadiazole, mandipropamid, fludioxonil, benoxacor, mesotrione, thiamethoxam, cyprodinil, azoxystrobin, primicarb, lufenuron, tefluthrin, fimasafen, cloquintocet, fenclorim and cloquintocet-mexyl. All agrochemicals used were purchased from Sigma-Aldrich. After incubating test plates at 30 °C for 2 days, colonies were chloroform lysed and stained to reveal β -galactosidase expression levels, using previously described methods⁶. Mutants displaying responsiveness to the test compound, if present, were identified by virtue of X-gal staining and then subjected to subsequent optimization efforts.

First round optimization by combinatorial mutagenesis. To identify potential additive or synergistic interactions between the mutations that improve receptor function, we constructed combinations of the best variants identified in the first round of screening for mandipropamid, benzothiadiazole, benoxacor and fludioxonil sensitivity. The mutations selected for combinatorial mutagenesis are marked with asterisks in Extended Data Fig. 1. The mutant combinations were constructed using the QuickChange Lightning Multi Site-Directed PCR Mutagenesis kit (Agilent) using pBD-PYR1(K59R) template DNA and mutagenic primers, essentially as previously described¹⁵. The mutant combinations were sequence validated, introduced into the pACT-HAB1 Y190 reporter strain and then tested for responsiveness to a range of compound concentrations (100, 50, 25, 10, 1, 0.2, 0.1 or 0 μ M each test compound). These efforts yielded double-mutant receptor variants with improved sensitivity for the four compound-receptor pairs examined; however, only mandipropamid and benzothiadiazole receptors displayed responses at concentrations as low as 1 μ M. Efforts to improve the benzothiadiazole receptor further were abandoned because we could not produce active recombinant protein for biochemical

characterization. The most sensitive response to mandipropamid was obtained with the triple mutant PYR1(K59R/F108A/S122G) (Extended Data Fig. 2).

Site-saturation mutagenesis. We screened for additional pocket-located mutations that would improve the mandipropamid responsiveness of the PYR1(K59R/F108A/S122G) receptor. Extended Data Figure 2A outlines the engineering scheme for developing the receptor and Extended Data Fig. 2B the specific mutants tested for activity. We first conducted site-saturation mutagenesis of 23 pocket-lining residues using NNK primers, which enable all amino acids at a targeted site to be encoded while only encoding one of the three possible stop codons. Each NNK primer was used to generate a pool of PYR1(K59R/F108A/S122G) receptors which were then combined and transformed into a pACT-HAB1 MAV99 reporter strain, which allows URA3-based negative selection against constitutively active receptors and positive selection of activated receptors¹⁷. The pooled yeast cells were first plated onto synthetic dextrose (without Trp and Leu) media containing 0.1% FOA to select against constitutively active receptors. After 2 days, the surviving yeast cells were plated onto SD (without Ura, Trp and Leu) plates containing 1 μ M mandipropamid, a concentration too low to allow growth of a control MAV99 pACT-HAB1 reporter strain expressing pBD-PYR1(K59R/F108A/S122G). Colonies displaying growth on the selective medium were identified and subsequently re-tested on selective media with and without mandipropamid. These efforts yielded ten mutations in five residues (V81C, V81I, V81T, V83L, L87A, F159L, F159M, F159V, A160V, V164I) that enhance the mandipropamid sensitivity of PYR1(K59R/F108A/S122G) (see Extended Data Fig. 2B).

DNA shuffling. We also used recombination-based mutagenesis to identify mutant combinations that enhance PYR1(K59R/F108A/S122G) sensitivity using nucleotide excision and exchange technology (NExT)²⁵. An equal amount of PYR1(K59R/F108A/S122G) template was recombined with an equal amount of template DNA that was made by pooling plasmid DNAs from the PYR1(K59R/F108A/S122G) NNK plasmid libraries described earlier. A ~200,000 member library of mutagenized clones was generated and was transformed into the MAV99 pACT-HAB1 reporter strain. Selections were conducted on plates containing 1 μ M mandipropamid and characterized for ligand-dependent interactions, as described earlier. These efforts identified Y58H as an additional mutation that improves the mandipropamid sensitivity of the PYR1(K59R/F108A/S122G) receptor. Y58 was not targeted in the PYR1(K59R/F108A/S122G) NNK library and was therefore a spontaneous mutant that arose during the mutagenesis process. The side chain of Y58 projects into PYR1's ligand-binding pocket, but is not within the 5 Å cut-off that we initially employed for targeting pocket residues for site-saturation mutagenesis.

Final optimization using combinatorial mutagenesis. Mutagenic primers for all of the strongest enhancing mutations identified in the NNK-mutagenesis (V81I, V83L, F159L, A160V and V164I) were designed and used simultaneously with the QuickChange Lightning Multi Site-Directed Mutagenesis kit (Agilent) using PYR1(Y58H/K59R/F108A/S122G) template DNA. Individual clones were sequenced to identify combination mutants, which were transformed into the Y190 pACT-HAB1 yeast strain and assayed for mandipropamid sensitivity on varying concentrations of mandipropamid. This led to the identification of PYR1(Y58H/K59R/V81I/S122G/F108A/F159L), PYR1^{MANDI}, which responds to mandipropamid concentrations as low as 10 nM in the yeast assay (Fig. 1b and Extended Data Fig. 2B).

Receptor- and ligand-mediated PP2C inhibition. PYR1^{MANDI} and 9 of the 11 wild-type receptors characterized (previously cloned²⁰) were expressed as 6 $\times$ His-tagged fusion proteins in pET28; PYL9 and PYL11 were expressed as maltose-binding fusion proteins using the pMAL-c expression vector²⁰. Recombinant 6 $\times$ His-tagged receptors²⁰ and GST-PP2Cs⁶ were expressed and purified as previously described^{6,20}. PP2C assays were conducted in 96-well polystyrene flat-bottom microtitre plates (Greiner). Assays for all receptors except PYL9 were conducted using the following assay conditions: 100 nM 6 $\times$ His-receptor, 50 nM GST-PP2C, 100 mM Tris-HCl (pH 7.9), 100 mM NaCl, 1 mM MnCl₂, 1% β -mercaptoethanol and 0.3% BSA. Reactions were mixed with probe molecules (or mock carrier solvent-only controls), equilibrated for 30 min, after which 4-methylumbelliferyl phosphate was added (1 mM final concentration). The plates were read using a Victor 2 plate reader (PerkinElmer) (355 nm excitation, 460 nm emission). PYL9 was assayed at 300 nM, under otherwise identical conditions. GST-HAB1, GST-ABI1 and GST-ABI2 were all tested with identical reactions, which were run in triplicate. The ratio of receptor to PP2C used in our assays was selected based on titration experiments, which showed that maximal inhibition of HAB1 PP2C activity (at saturating ABA concentrations, 10 μ M) required a twofold excess of each receptor to PP2C; the total PP2C concentration used (50 nM; established empirically) probably overestimates active PP2C concentrations since we observed some IC₅₀ values below 50 nM. PP2C activity values reported are expressed as per cent control values, which were calculated by including the carrier solvent (1% dimethylsulphoxide (DMSO)) and the specific receptor assayed, but no probe molecule.

Receptor-PP2C pull-down assays. PYR1^{MANDI} was cloned as a 6 $\times$ His-GFP fusion protein in pEGAD²⁶. HAB1 was cloned as a GFP fusion protein in the vector pEGAD

(without a 6×His tag). Both constructs were transformed into *Agrobacterium tumefaciens* (GV3101) and these strains and a strain expressing the silencing suppressor p19 were mixed together in ratios corresponding to 0.1 receptor, 1.0 HAB1 and 0.5 p19 final OD_{600 nm} units, respectively; this yielded roughly equimolar amounts of PP2C and receptor. The mixture was infiltrated into two separate *N. benthamiana* leaves and 2 days later the leaves were treated with either 50 µM mandipropamid or mock solutions made in water containing 0.02% Silwet L-77 (obtained from Lehle Seeds). After 20 h, the leaves were homogenized in liquid nitrogen, resuspended in an extraction buffer composed of 1× TBS, 0.1% NP-40, 1 mM dithiothreitol (DTT), 10% glycerol and 1× plant protease inhibitor cocktail (USB) and clarified by centrifugation. Twenty-five milligrams of PrepEase Ni-TED beads (USB) was added to 2 ml of each extract to isolate 6×His-tagged PYR1^{MANDI} receptors and associated proteins. The resin was washed three times; bound proteins were eluted in SDS-PAGE loading buffer, separated by SDS-PAGE and then electroblotted onto nitrocellulose membranes. Both PYR1^{MANDI} and HAB1 were expressed as GFP fusion proteins and detected using an anti-GFP monoclonal primary antibody (Clontech) and an anti-mouse IgG-HRP sheep secondary antibody (GE Healthcare) using ECL (Perkin Elmer) development.

Protein crystallization. PYR1(K59R/V81I/F108A/F159L) and ΔNHAB1 (residues 1–178 deleted) were expressed in *E. coli* and purified as described previously^{6,9}. Purified PYR1(K59R/V81I/F108A/F159L) was mixed with an equimolar amount of ΔNHAB1 and a fivefold molar excess of mandipropamid and incubated at room temperature for 10 min. The mixture was then exchanged into 20 mM Tris (pH 7.6), 50 mM NaCl solution and concentrated to 15 mg ml⁻¹. Crystallization was conducted at 19 °C by sitting-drop vapour diffusion by mixing equal volumes of the protein with well solution containing 100 mM Bis-Tris propane (pH 7.0), 22.5% PEG 2000 monomethyl ether, and 150 mM sodium malonate. The resulting crystals were flash frozen after passing through a cryoprotection solution of the well solution plus an additional 20% glycerol. All diffraction data were collected at 100 K using an R-Axis IV⁺⁺ detector equipped with a MicroMax007 generator and an Osmic mirror set. Diffraction data were processed with HKL2000.

X-ray crystal structure determination. Molecular replacement was used to evaluate the initial phases using the PYR1-ABA-HAB1 complex (Protein Data Bank accession 3QN1) as the search model. Phenix.AutoMR solved the initial phases and automatically built in the majority of the residues for the ternary complex. Models were completed through iterative rounds of manual model building in Coot and refinement with Phenix.refine using translational libration screw-motion (TLS) and individual atomic displacement parameters. Mandipropamid was modelled using the ProgDrg server and placed into the complex after several rounds of manual refinement to limit model bias. The geometry of the final structure was validated using Molprobity and Procheck. Ramachandran statistics for the ternary complex were 98.3 and 1.7% for the favoured and additionally allowed regions of the Ramachandran plot, respectively. Data collection and refinement statistics for the final model are listed in Extended Data Table 2.

Production of transgenic *Arabidopsis*. The PYR1^{MANDI} coding sequence was PCR amplified from the pBD-PYR1^{MANDI} template and cloned into the plant transformation vector pEGAD under control of the 35S promoter. This construct was introduced into *A. tumefaciens* GV3101 and then used to transform *Arabidopsis* using the floral dip method²⁷ and the resultant seed was germinated in soil and treated with glufosinate to identify transformed plants. Seed from approximately 15–16 transgenic plants were harvested individually and used to identify single insert lines. Three independent homozygous single insert 35S::PYR1^{MANDI} insertion lines (referred to as lines 1, 2, 3 in Extended Data Figs 5 and 8) were used in this work. The RNA-seq experiments used line 1, which had the highest-level expression of PYR1^{MANDI} protein, and the drought experiments used both lines 1 and 2. Protein blots comparing protein expression levels in the lines are presented in Extended Data Fig. 4. Western blots characterizing PYR1 protein expression levels used a previously described and validated polyclonal PYR1 antibody²⁸.

Root growth and seed germination assays. The wild-type, PYR1^{OX} and PYR1^{MANDI} genotypes were surface sterilized in bleach and plated on to 0.7% agar Petri plates containing one-half MS salts and one-half 0.5% sucrose. After 4 days of stratification at 4 °C, the plates were transferred to a growth chamber in darkness and allowed to germinate for 24 h and then transferred to Petri plates (0.7% agar containing one-half MS salts and one-half 0.5% sucrose) supplemented with differing concentrations of mandipropamid. These plates were then grown vertically under darkness. The amount of new root growth after transfer was measured 72 h after transfer. Seeds for germination assays were prepared similarly except that the plates contained differing concentrations of mandipropamid.

RNA-seq experiments. We examined the effects of ABA and mandipropamid on gene expression in wild type and the PYR1^{MANDI} (line 1) transgenic lines. Seed of the wild type or transgenic lines were surface sterilized, stratified for 4 days at 4 °C and then grown for 10 days at room temperature under continuous illumination in a liquid culture consisting of one-half MS salts and one-half 0.5% sucrose and

grown with continuous shaking to provide aeration. After 10 days, the culture solutions were adjusted to contain 50 µM ABA, 2 µM mandipropamid, or a mock treatment. Each compound/mock exposure was conducted in biological triplicate. The (+)-stereoisomer of ABA (Biosynth, AG) was used in this study and mandipropamid (mixed stereoisomer) was purchased from Sigma-Aldrich. After 8 h exposure to the test compounds, RNA was isolated using RNeasy Plant RNA isolation kit (Qiagen) and treated with DNase. The total RNA was prepared for RNA-seq using the NEBNext platform, which consists of a Poly(A) mRNA Magnetic Isolation Module, NEBNext Multiplex Oligos for Illumina, and NEBNext Ultra RNA Library Prep Kit for Illumina, New England BioLabs. poly(A) mRNA was isolated using NEBNext oligo d(T)₂₅ magnetic beads and 5 µg total RNA input, as described by the manufacturer. mRNA was eluted using the kit's first-strand synthesis reaction buffer and hybridized to a random primer mix by incubating the sample at 94 °C for 15 min followed by cooling. First-strand cDNA was synthesized using ProtoScript II reverse transcriptase and subsequently second-strand synthesis reactions were conducted using the kit's components. The double-stranded cDNA produced was purified using Agencourt AMPure XP beads and NEBNext adaptors were ligated to the purified cDNAs. The adaptor-ligated DNA was then size-selected using Agencourt AMPure XP beads. Quantities of the size-selected cDNA were increased by PCR enriched (using the manufacturer's protocol) and purified using AMPure XP beads. Library quality was assessed using a Bioanalyzer. The libraries were sequenced using a HiSeq instrument. Each lane was used to analyse six barcoded libraries. Data from 51 cycles of single-end reads were collected. This ultimately yielded approximately 20 million mapped reads per barcoded sample.

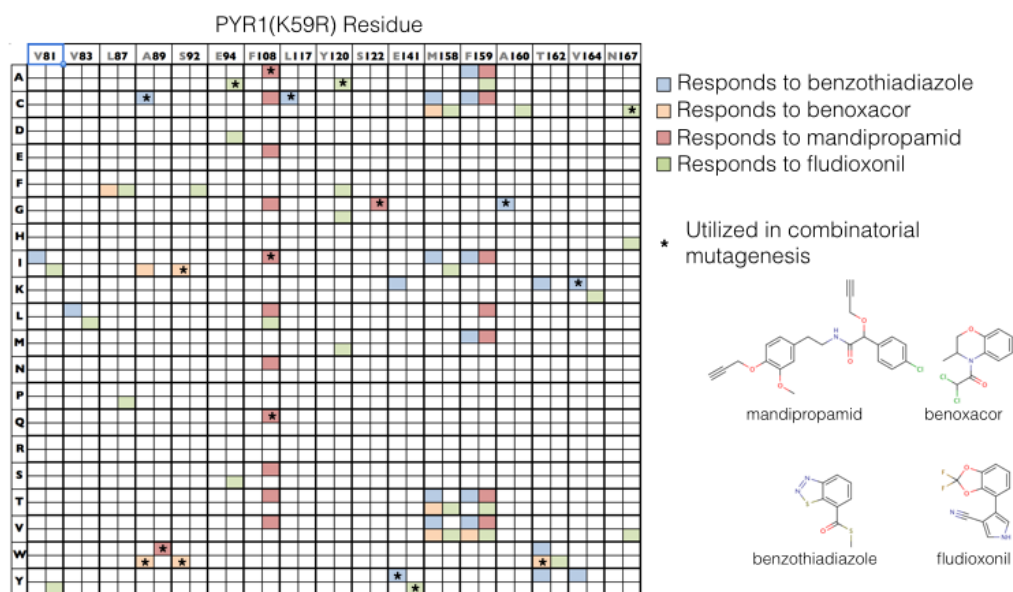
The raw HiSeq data was mapped to the *Arabidopsis* genome using TOPHat, which matches Illumina reads to the *Arabidopsis* reference genome (TAIR 10) sequence and calculates the abundance of each gene using the FPKM (fragments per kilobase of exon per million fragments mapped) metric. The Cufflinks²⁹ package was used to compare samples and the cuffdiff results (showing mean transcript FPKM values and significant differences for all pairwise comparisons) is available (see Source Data associated with Fig. 3). Twenty-two thousand three-hundred and twenty-six genes displayed non-zero mean FPKM values in all samples and, of these, 20,534 genes had mean FPKM values greater than 0.1 across all samples and these were used to make the comparisons of global gene expression patterns shown in Fig. 3.

Drought stress assays. The wild-type Columbia, 35S::PYR1-GFP (PYR1^{OX}) and PYR1^{MANDI} genotypes were used for drought stress tolerance assays. Each experiment was conducted independently of the other at different times over the course of 8 months. Lines 1 and 2 are two independent homozygous single-insertion transfer-DNA lines (their construction is described earlier); the PYR1^{OX} line, which complements the *pyr1-1* mutant phenotype, has previously been described⁶. Each experiment was initiated with five pots of each genotype (four plants per pot) and entire pots were discarded if any of the seedlings died after transplantation; however, each experimental replicate contained a minimum of three pots (raw data shown in Extended Data Fig. 8). After 2 weeks (experiment 1) or 3 weeks (experiments 2 and 3) growth under standard water regimes, watering was ceased and the plants were treated with a mock or 1 µM mandipropamid solution made in water containing 0.02% Silwet L-77; the plants were treated a second time 4 days (experiment 1) or 3 days (experiments 2 and 3) after the initial treatment. Leaf turgor of the wild-type controls was monitored in each of the three experimental sets to identify endpoints based on wilting (between 10 to 12 days, which varies between the three experiments because of differences in ambient humidity and other growth parameters), at which point all plants in the experimental series were re-watered and then survival rates were assessed by restoration of leaf turgor 24 h after re-watering.

Generation and characterization of transgenic tomato expressing PYR1^{MANDI}. The 35S::PYR1^{MANDI} construct used for construction of *Arabidopsis* transgenics was modified to contain a kanamycin selection marker. Tomato was transformed by *Agrobacterium*-mediated transformation as described previously³⁰, with minor modifications. Surface-sterilized tomato seeds (strain UC82B) were germinated on sterilized wet filter paper. Cotyledons from 7-day-old seedlings were excised and dipped into a suspension of *Agrobacterium* in MS medium containing 100 µM acetosyringone and 10 µM 2-mercaptoethanol for 10 min. Explants were then placed on co-cultivation medium containing MS salts, 3% sucrose, 0.3% Gelite and 1.5 mg l⁻¹ zeatin. After 3 days of co-culture in darkness, the explants were transferred onto callus induction medium containing MS salts, 3% sucrose, 0.3% Gelite, 1.5 mg l⁻¹ zeatin, 100 mg l⁻¹ kanamycin and 125 mg l⁻¹ carbenicillin. Explants were transferred to freshly prepared medium every 2 weeks. Calli-displaying shoot buds were transferred to the medium containing 1 mg l⁻¹ zeatin to simulate shoot elongation. Transgenic shoots 1 cm in length were cut and transferred onto rooting medium containing MS salts, 1.5% sucrose, 0.3% Gelite, 1 mg l⁻¹ isobutyric acid, 50 mg l⁻¹ kanamycin and 125 mg l⁻¹ carbenicillin. After 2–3 weeks young plants displaying well-developed roots were transferred to the soil. The experiments shown in Extended Data Fig. 7D were conducted using a primary transgenic line and those shown in

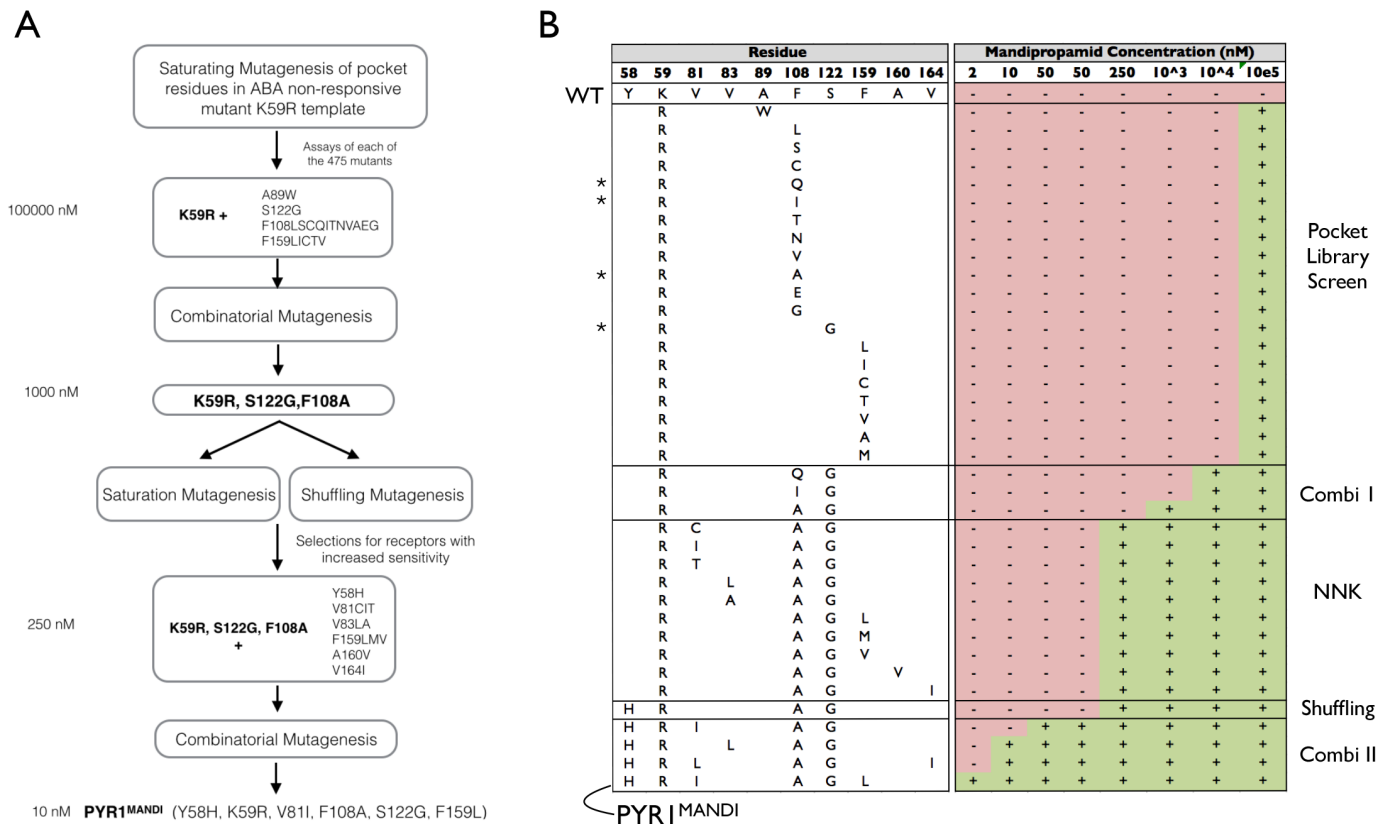
Fig. 4 and Extended Data Fig. 7A, C used T2 progeny from an independent single-insert transgenic line. The T2 seed was germinated on filter paper and transferred to soil. Proteins from leaf tissue were probed with an anti-PYR1 antibody to infer protein expression levels. Plants lacking expression were confirmed by PCR to be non-transgene-containing segregants (that is, null segregants). We propagated the transgenic plants, null segregants and wild-type controls by making cuttings from plants of the same age. To make these clones, ~5-cm-long shoots were excised and planted in soil after treatment with a commercial rooting powder (Bonide). Plants were grown in a growth chamber on a 16 h light cycle at 25 °C. About 3 weeks after clone establishment, thermal images were collected and the plants were then treated with a solution containing 25 µM mandipropamid, 0.1% DMSO and 0.05% Silwet-77. Thermal images were then collected 24 h after treatment. The experiment shown in Extended Data Fig. 7D used clones derived from an independent primary transgenic line and treated with a lower mandipropamid concentration (10 µM). For this experiment, ~3 weeks after clones were established, the transgenic and wild-type controls were treated with a mock solution (0.1% DMSO and 0.05% Silwet-77) and then thermal images were collected after 24 h. Three days later the plants were treated with a solution containing 10 µM mandipropamid, 0.1% DMSO and 0.05% Silwet-77. Thermal images were then taken again 24 h after treatment.

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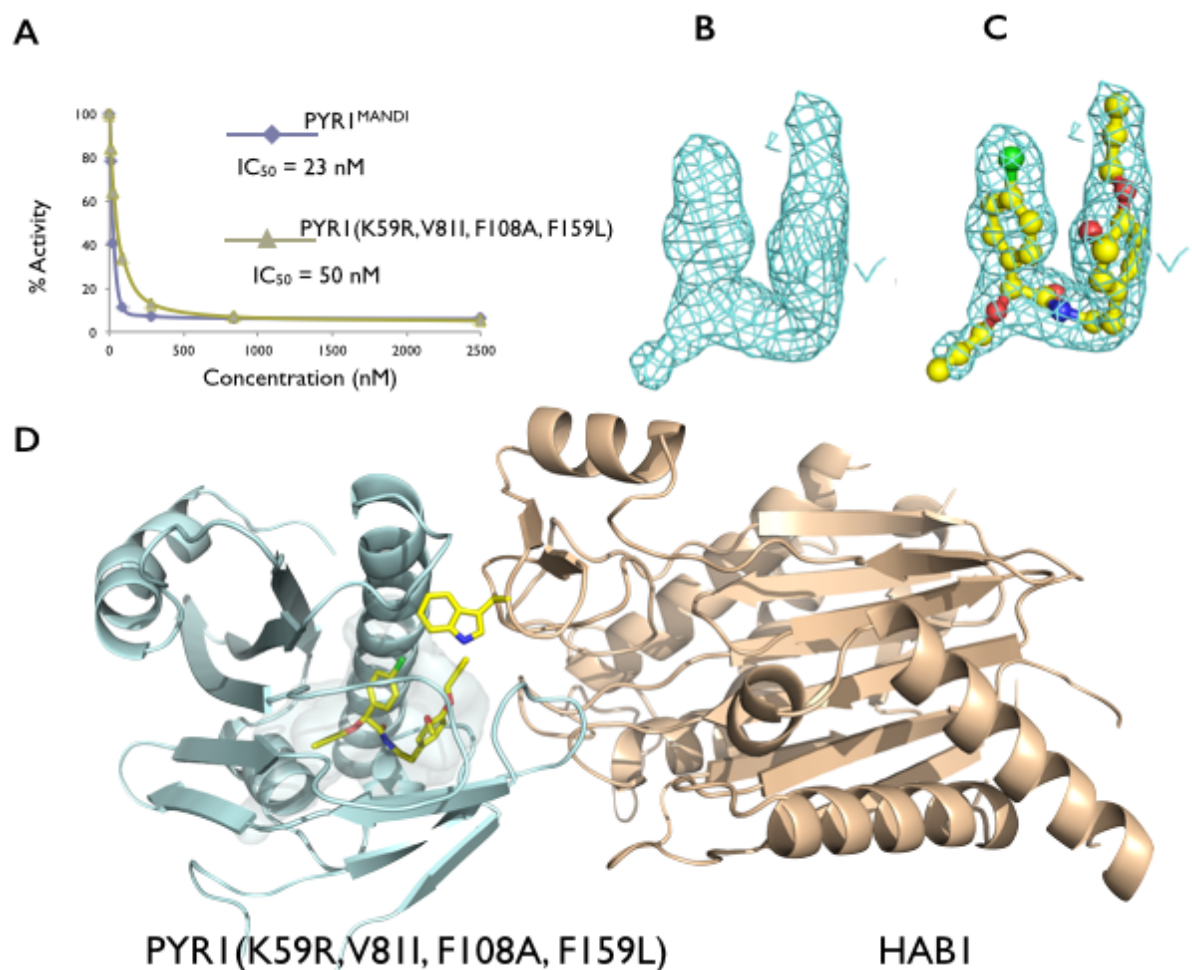
Extended Data Figure 1 | The PYR1 pocket library enables identification of new receptors. To create a screening platform for identifying orthogonal ligand–PYR1 receptor pairs, we inactivated the intrinsic ABA responsiveness of PYR1 by introducing the K59R mutation and constructed all possible substitution mutations in 25 ligand-proximal residues. Each mutant receptor in this ‘pocket library’ was tested for responsiveness to 15 agrochemicals (at 100 μ M) using a yeast two-hybrid assay that reports agonist-induced binding of receptor to HAB1 (ref. 6). The 15 compounds screened are listed in the

Methods. The table on the left-hand side summarizes the results of the 7,125 ligand–receptor interactions tested. Orthogonal receptors were identified for 4 of the 15 compounds tested, shown on the right. Coloured boxes in the table indicate the specific mutations that confer responsiveness to one or more of the compounds screened. An asterisk indicates a specific mutation that was used in subsequent combinatorial mutagenesis experiments. Eight of the 25 residues mutagenized did not confer ligand responsiveness and are not shown in the table.



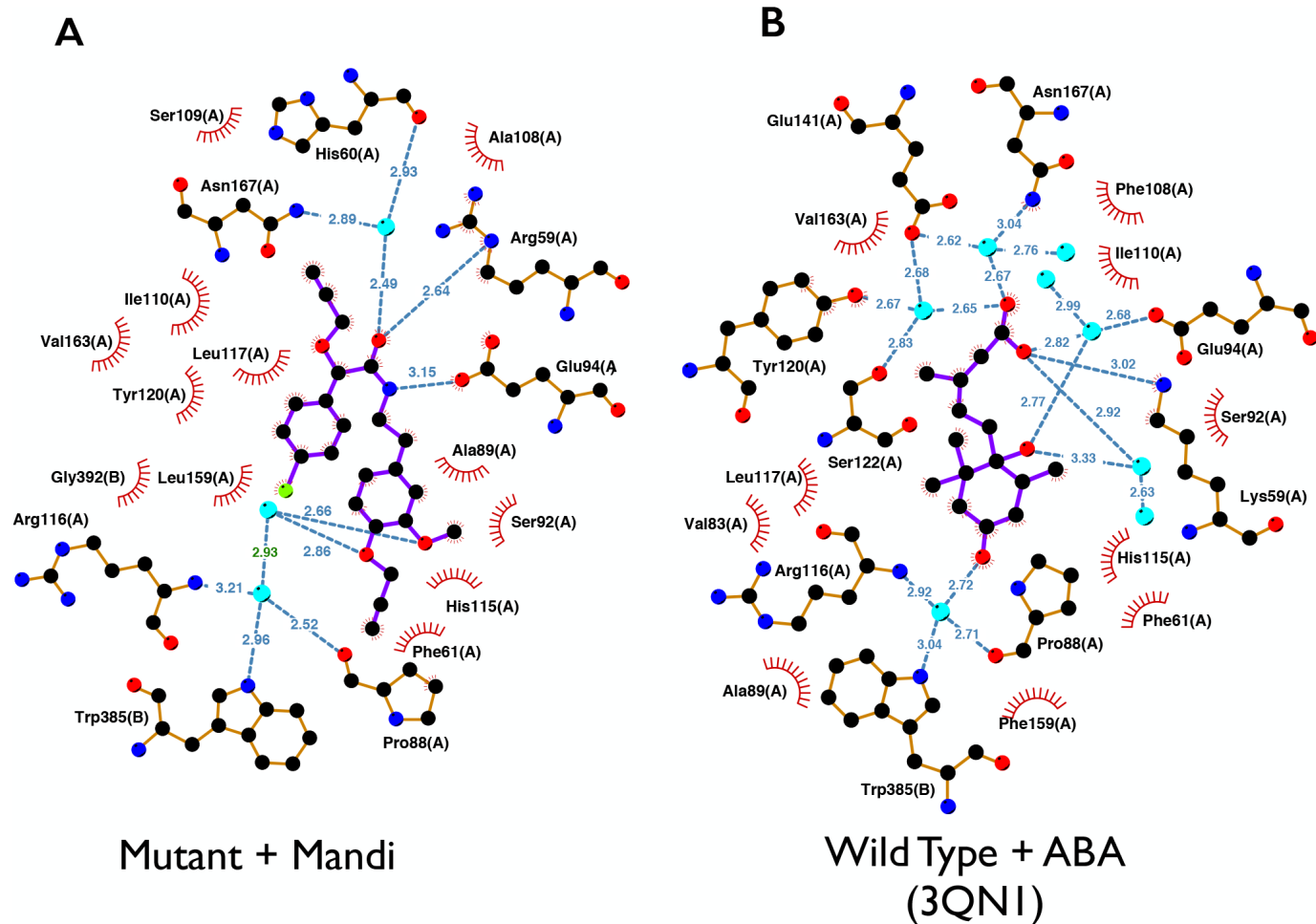
Extended Data Figure 2 | Mandipropamid receptor optimization process.

A, Flow chart for the receptor optimization process. The mutations identified at each step are shown as insets in each box and the minimum concentration of mandipropamid required to elicit a detectable response in yeast two-hybrid assays for the mutants is shown on the left. **B**, Summary of the mutations tested using the yeast two-hybrid assay at different stages of the optimization process.



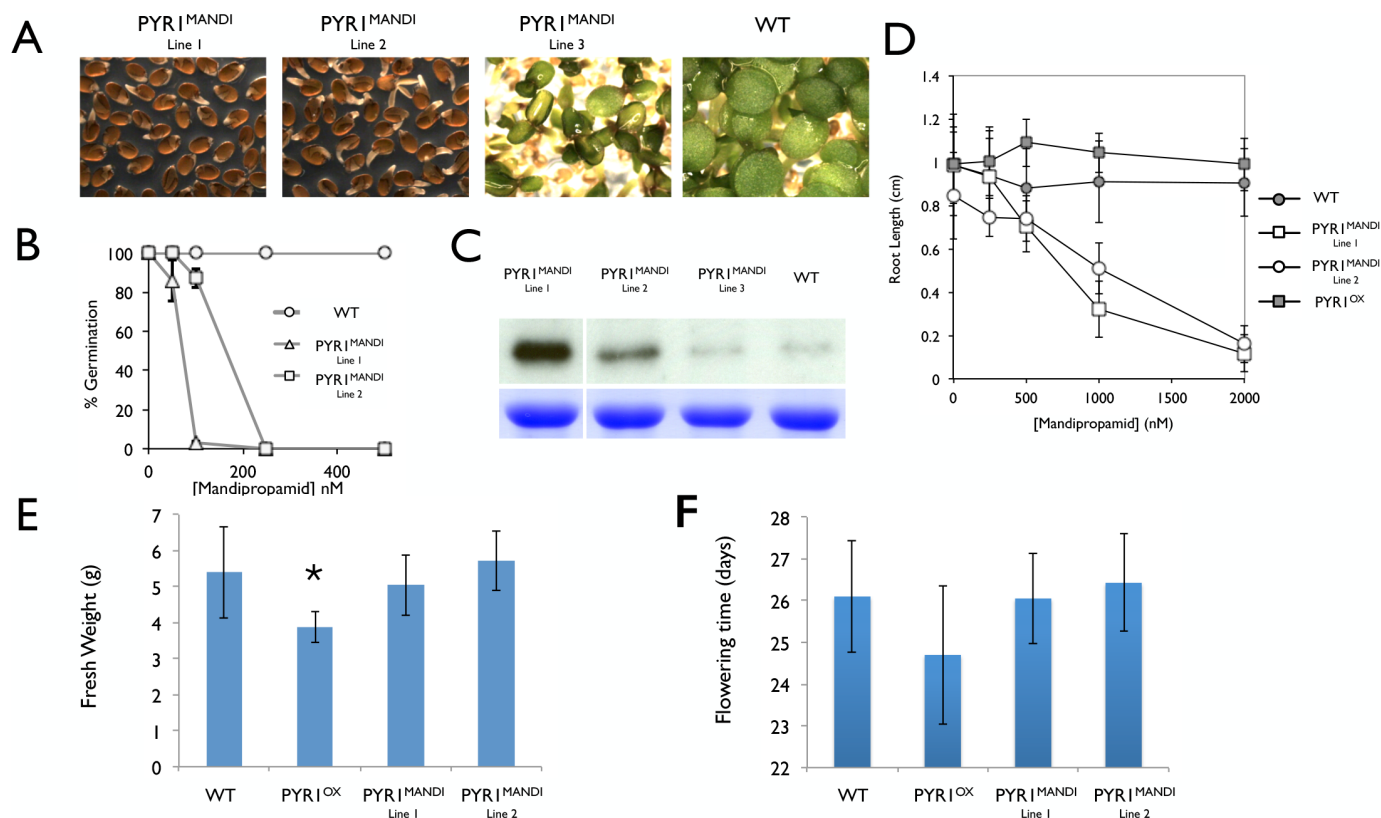
Extended Data Figure 3 | Structure of the PYR1(K59R/V81I/F108A/F159L) receptor. **A**, PYR1(K59R/V81I/F108A/F159L) is a sensitive mandipropamid receptor. Crystallization experiments with PYR1^{MANDI} were unsuccessful; however, reverting S122G and Y58H present in PYR1^{MANDI} to wild-type residues yielded the quadruple mutant receptor, which crystallized successfully. Recombinant PYR1^{MANDI} and PYR1(K59R/V81I/F108A/F159L) proteins were tested side by side for activity in HAB1 PP2C activity assays. The recombinant PYR1(K59R/V81I/F108A/F159L) mutant protein was the same material used in crystallization experiments (which had its 6×His tag

proteolytically removed) whereas the PYR1^{MANDI} protein used did not have its tag cleaved. **B**, The $F_o - F_c$ density present in the binding pocket of the PYR1(K59R,V81I,F108A,F159L)–mandipropamid–HAB1 structural model after several rounds of refinement in the absence of ligand; $F_o - F_c$ is shown using a sigma level of 3. **C**, The profile of the unbiased electron density shown in **B** allowed us to unambiguously place mandipropamid into the structural model. **D**, PYR1(K59R/V81I/F108A/F159L) adopts a closed-gate conformation in the presence of mandipropamid and HAB1.



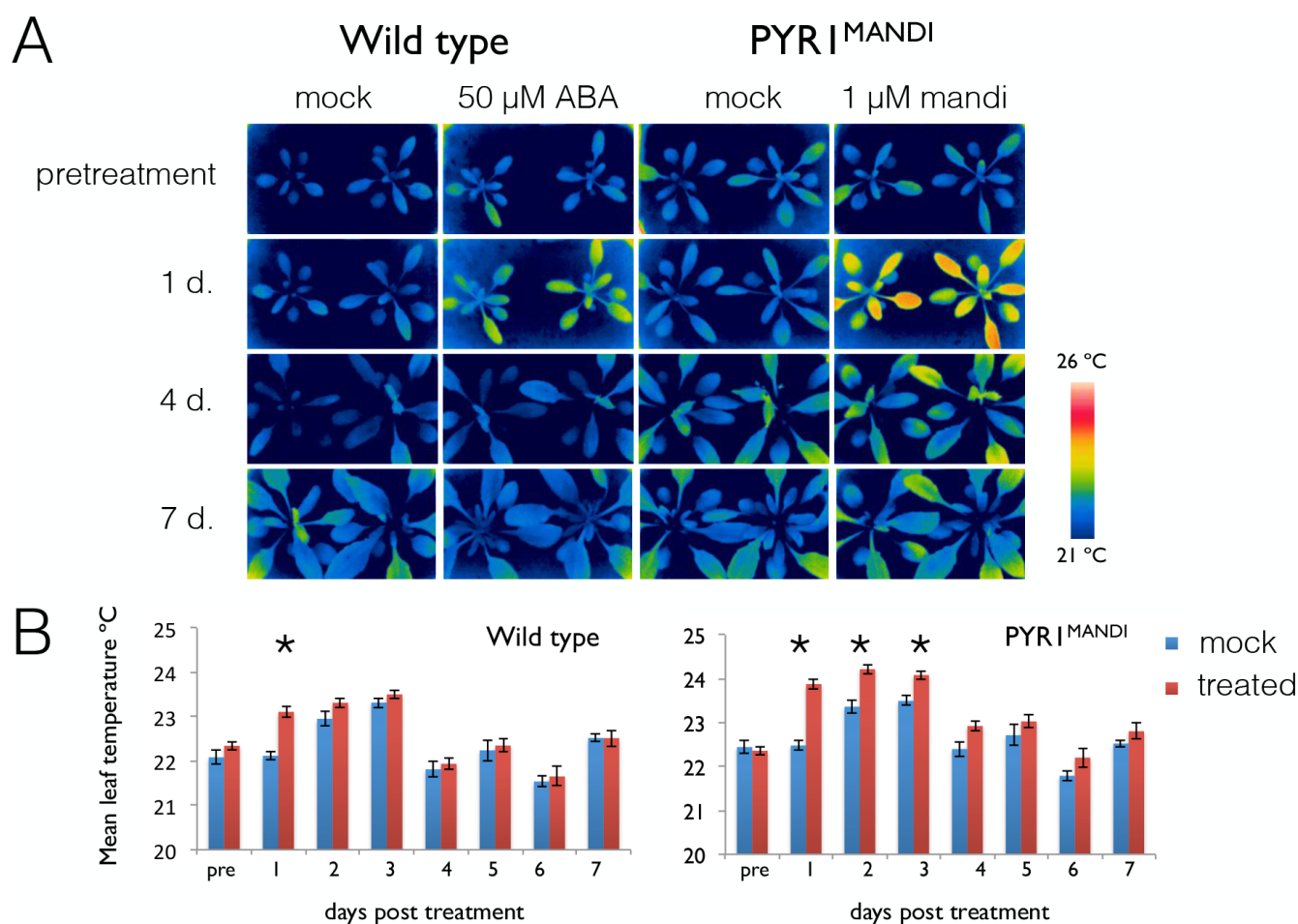
Extended Data Figure 4 | Mandipropamid-PYR1(K59R, V81I, F108A, F159L) interactions. **A**, Ligplot of mandipropamid interactions with PYR1(K59R/V81I/F108A/F159L) and HAB1 in the ternary complex. **B**, Ligplot of ABA interactions with PYR1 and HAB1 in the ternary complex of previously

published coordinates (Protein Data Bank accession 3QN1), shown for comparison. Ligplots were made using LigPlot+³¹. In both plots the residues from PYR1 are denoted by chain A and HAB1 by chain B.



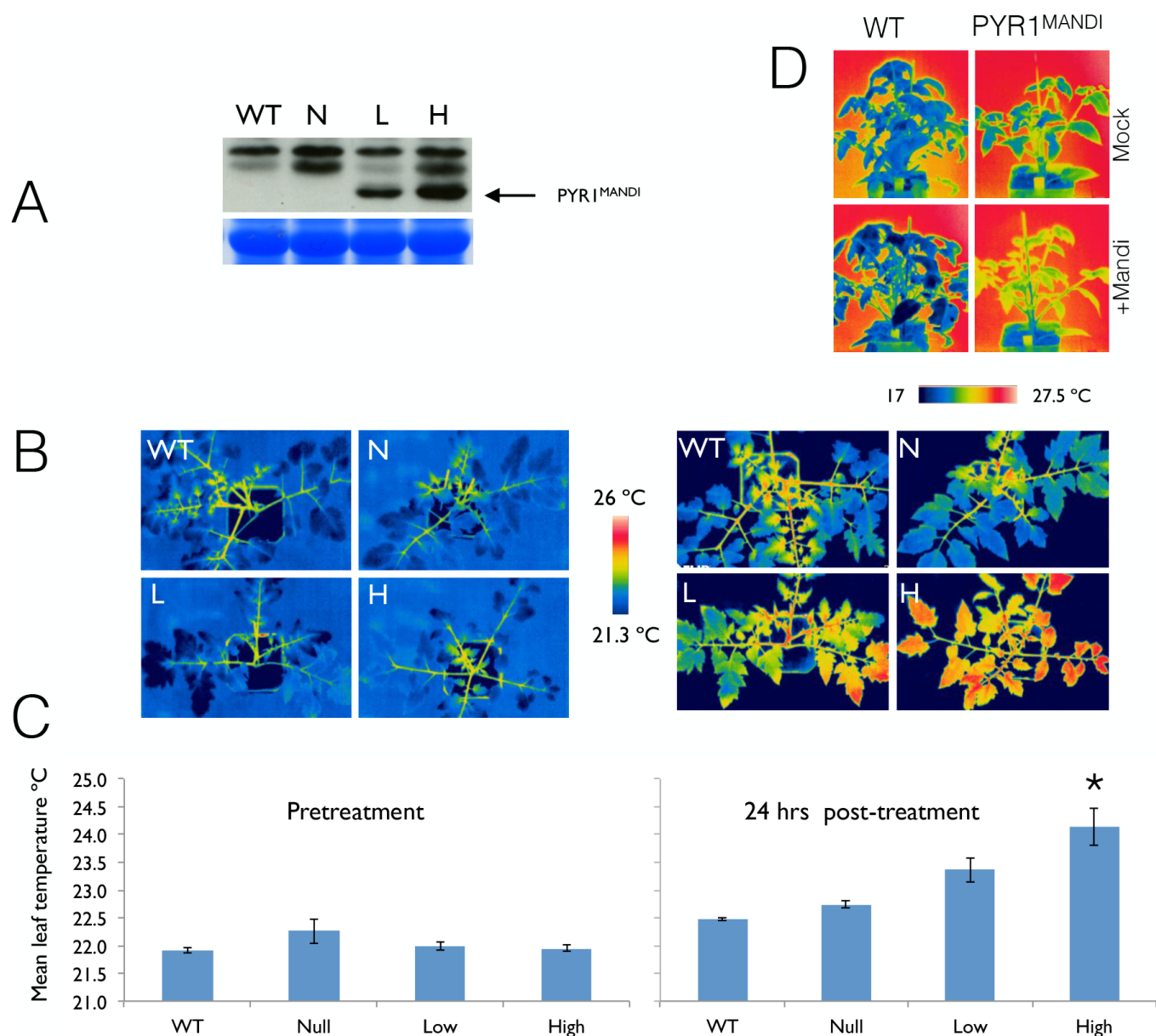
Extended Data Figure 5 | PYR1^{MANDI} is functional in *Arabidopsis* using seed germination and root growth inhibition assays. **A**, Germination of wild-type and three independent PYR1^{MANDI} transgenic lines (lines 1, 2 and 3) on Petri plates containing 250 nM mandipropamid. Images taken 3 days after stratification. **B**, Dose–response curves for germination sensitivity to mandipropamid in the two independent PYR1^{MANDI} transgenic lines and wild-type lines. The PYR1^{MANDI} transgenic line 3 shown in **A** was excluded from this experiment because its germination is not inhibited by mandipropamid at the concentrations tested. **C**, PYR1 protein levels in the wild-type and three PYR1^{MANDI} transgenic lines (detected using an anti-PYR1

antibody). The bottom panel is a loading control showing the rubisco large subunit. **D**, Selective inhibition of root primary growth by mandipropamid in PYR1^{MANDI} genotypes. **E**, Fresh weights of 7-week-old wild-type and transgenic plants ($n = 20$). **F**, Flowering times of the wild-type and transgenic genotypes used. Neither of the two PYR1^{MANDI} lines characterized displayed statistically significant differences in their fresh weight or flowering time in comparison to wild-type controls; however, PYR1 overexpression decreased fresh weight in comparison to the wild type ($P < 0.05$, two-sided t -test). Error bars show standard deviation (s.d.).



Extended Data Figure 6 | Mandipropamid induces a persistent ABA response in the $\text{PYR1}^{\text{MANDI}}$ genotype. **A, B,** To compare the ABA and mandipropamid responses of wild-type and $\text{PYR1}^{\text{MANDI}}$ *Arabidopsis* genotypes respectively, we treated 3-week-old plants with mock solutions (0.02% Silwet) or either 50 μM ABA (wild type Columbia) or 1 μM mandipropamid ($\text{PYR1}^{\text{MANDI}}$). Leaf surface temperatures were monitored immediately before treatment and at 24 h intervals for 7 days after treatment. **A, B,** Representative images from the experiment (**A**) and quantification of

mean leaf temperatures (**B**). These data show that the response induced by mandipropamid in *Arabidopsis* is more persistent than that induced by ABA, which we speculate may be because mandipropamid evades metabolism by the CYP707A enzymes that mediate ABA catabolism. Asterisks indicate a significantly warmer mean leaf temperature (at a $P < 0.01$ cutoff) relative to the mock-treated control analysed at the same time point, as determined using a two-sided t -test ($n = 8$ or $n = 7$ for wild-type and $\text{PYR1}^{\text{MANDI}}$ samples, respectively); error bars show s.d.



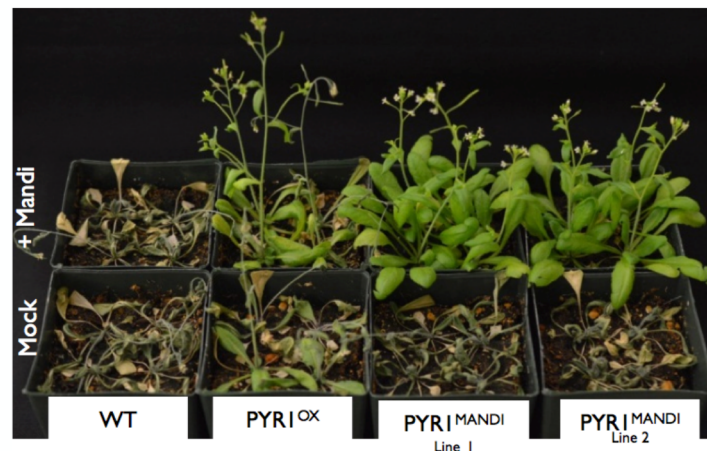
Extended Data Figure 7 | PYR1^{MANDI} is functional in tomato.

A–D, Transgenic tomato plants expressing the 35S::PYR1^{MANDI} transgene were made as described in the Methods. A single-insert line was obtained that segregated plants with high (H) and low (L) PYR1^{MANDI} protein levels as well as non-transgenic (N, Null) segregants. **A**, Western blot analyses of SDS–PAGE separated proteins from T2 segregants using an anti-PYR1 antibody. The bottom panel shows the large subunit of Rubisco as a loading control.

B, Thermography of representative T2 transgenic plants and wild-type control before and 24 h after treatment with a solution containing 25 μ M mandipropamid. The wild-type and H plant images shown in **B** are the same

images shown in Fig. 4. **C**, Quantification of mean leaf temperatures (measured from the three oldest leaves) before and after mandipropamid treatment. The asterisk indicates a statistically significant ($P < 0.05$) difference between the high expressing PYR1^{MANDI} line in comparison to the null segregant control, as determined using a two-sided t -test; $n = 2$ for each of the four sample groups. Error bars show s.d. **D**, Analysis of an independent primary transgenic tomato PYR1^{MANDI} line. The line was grown alongside a wild-type control and treated with a mock solution and then thermographed 24 h later. Three days after this, the plants were treated with a solution containing 10 μ M mandipropamid and thermographed 24 h after application.

A



B

	WT	PYR1 ^{OX}	PYR1 ^{MANDI} Line 1	PYR1 ^{MANDI} Line 2
Mock	Survivors Total Exp. 1 0 / 16 Exp. 2 0 / 12 Exp. 3 2 / 20	0 / 12 5 / 12 8 / 20	0 / 16 2 / 12 3 / 12	1 / 16 1 / 12 2 / 16
+ Mandi	0 / 16 0 / 12 0 / 16	0 / 16 4 / 12 10 / 16	12 / 12 12 / 12 20 / 20	16 / 20 11 / 12 12 / 12

Extended Data Figure 8 | Mandipropamid confers drought tolerance to the PYR1^{MANDI} genotype in *Arabidopsis*. A, B, The genotypes shown were treated with 1 μ M mandipropamid or mock solution two times over the course of a water deprivation experiment. Photographs were taken 24 h after re-watering. Drought experiments were conducted on three separate occasions over an 8-month period; each experiment was conducted using a minimum of three replicate pots each containing four plants of either the wild type, a 35S::GFP-PYR1 wild-type overexpressor line (PYR1^{OX}), or one of two independent 35S::PYR1^{MANDI} lines. A, Shown are representative images 24 h after recovery for one set of replicates. B, Summary of survival data for the three

independent experiments conducted. The inset survival values have been separated for each experiment. The images shown in A are from the second experiment conducted and those in B are taken from the third experiment conducted. The numerator of each value is the number of plants that survived 24 h after re-watering and the denominator is the total number of plants tested. Experiment 1 initiated the water deprivation at 2 weeks after germination while experiments 2 and 3 initiated at 3 weeks; further experimental details are described in Methods. The mock-treated wild-type control and mandipropamid-treated PYR1^{MANDI} (line 1) pots were photographed separated for Fig. 4.

Extended Data Table 1 | Activity of ABA and mandipropamid on *Arabidopsis* ABA receptors and PYR1^{MANDI}

Receptor	mandipropamid	abscisic acid
PYR1	94.2	6.4
PYL1	93.8	5.5
PYL2	97.0	5.7
PYL3	97.8	5.3
PYL4	96.4	12.4
PYL5	99.3	5.1
PYL6	95.4	12.1
PYL8	96.4	7.8
PYL9	95.9	7.7
PYL10	97.9	13.2
PYL11	92.7	7.1
PYR1 ^{MANDI}	5.4	103.3

Assays were conducted using recombinant proteins as described in the Methods. Each reaction contained recombinant HAB1, the specified receptor and either 10 μ M ABA or 10 μ M mandipropamid. Shown are the per cent PP2C activity levels relative to control (activity level in the absence of ligand). The data indicate that mandipropamid is not an ABA agonist for the receptors tested and that the PYR1^{MANDI} receptor is not activated by ABA.

Extended Data Table 2 | Structure statistics

PYR1^{K59R,V81I,F108A,F159V} (1-181):Mandipropamid:HAB1 (refinement)

Data collection

Space group	P2 ₁ 2 ₁ 2 ₁
Cell dimensions	
<i>a</i> , <i>b</i> , <i>c</i> (Å)	45.5, 67.0, 172.0
α , β , γ (°)	90, 90, 90
Resolution (Å)	50-2.25(2.29-2.25)
<i>R</i> _{merge}	0.087(0.763)
<i>I</i> / σ <i>I</i>	23.6(2.1)
Completeness (%)	98.5(96.4)
Redundancy	6.6(6.0)

Refinement

Resolution (Å)	36.2-2.25(2.31-2.25)
No. reflections	23,833
<i>R</i> _{work} / <i>R</i> _{free}	0.181/0.229
No. atoms	3,924
Protein	3,700
Ligand/ion	32
Water	192
<i>B</i> -factors	
Protein	50.2
Ligand/ion	48.8
Water	49.3

R.m.s. deviations

Bond lengths (Å)	0.012
Bond angles (°)	1.146

Values in parentheses are for highest-resolution shell. A single crystal was used for each structure determination.