TITLE

Extensive signal integration by the phytohormone protein network

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Running title: Arabidopsis phytohormone interactome map

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Plant hormones orchestrate responses to environmental cues with developmental programs[1], and are fundamental for stress resilience and agronomic yield[2]. The core signaling pathways have been elucidated by genetic screens and hypothesis-driven approaches, and extended by interactome studies for select pathways[3]. However, fundamental questions remain about how information from different pathways is integrated. Genetically most phenotypes are regulated by multiple hormones, whereas transcriptional profiling suggests that hormones trigger largely exclusive transcriptional programs[4]. We hypothesized that protein-protein interactions play an important role in phytohormone signal integration. Therefore, we generated experimentally a systems-level map of the Arabidopsis phytohormone signaling network consisting of more than 2,000 binary protein-protein interactions. In the highly interconnected network, pathway communities and hundreds of novel pathway contact points can be identified that represent potential points of crosstalk. Functional validation of candidates in seven hormone pathways demonstrate novel functions for 74% of tested proteins in 84% of candidate interactions, and indicate that a large majority of signaling proteins function pleiotropically in multiple pathways. Moreover, we identify several hundred largely small-molecule-dependent interactions of hormone receptors. Comparison with previous reports suggests that non-canonical and non-transcription mediated receptor-signaling is more common than currently appreciated.

Phytohormone network mapping and analysis

To examine phytohormone signal integration by the plant protein network we first identified 1,252 genes with likely or genetically demonstrated functions in phytohormone signaling (**Fig. 1a**, **Supplementary Table 1**). The corresponding network of literature curated binary interactions (LCI) from the IntAct database[5] (LCI_{IntA}) shows extensive intra-pathway but sparse inter-pathway connectivity (**Extended Data Fig. 1**), which could reflect an insulated organization of hormone signaling or be an artifact of inspection biases[6]. We therefore experimentally

generated a systematic (unbiased design) map of the phytohormone signaling network. After cloning open reading frames (ORFs) for 1,226 (98%) of the selected genes (PhyHormORFeome), five-fold interrogation of the pairwise matrix using a high-quality yeast-2-hybrid (Y2H)-based mapping pipeline[7] yielded the phytohormone interactome main (PhI_{MAIN}) network. To find links into the broader Arabidopsis network, PhyHormORFeome was screened against ~13,000 Arabidopsis ORFs[8] resulting in an asymmetric PhI_{EXT} dataset. Moreover, we conducted focused screens for pathway-specific repressors with transcription factors[9] (TFs), and for hormone-dependent interactions of phytohormone receptors. In the stringent final step of the common Y2H pipeline all candidate pairs were four-fold verified (**Fig. 1b**). The combined PhI network contains 2,072 interactions, of which 1,572 are novel (**Fig. 1c, Extended Data Fig. 1**, **Supplementary Table 2**). The interaction-density in the symmetrically interrogated PhI_{MAIN} (0.4‰) is higher than in the proteome-scale Arabidopsis Interactome-1 (AI-1, 0.1‰)[10], but lower than in the ABA-focused interactome (7.5‰)[3]. Likely, the increasing focus on functionally coherent proteins is underlying this trend, but also system differences[11] and screening parameters[12] affect overall sensitivity. We implemented our interactome mapping framework[6,12] to compare PhI to literature-based network maps from IntAct and BioGrid[13] (LCI_{BioG}). Sampling sensitivity of PhI_{MAIN} after five repeat screens was 86% ± 5% (**Fig. 1d**). For benchmarking, we recurated[12] a positive and a random reference set (PRS_{PhI}/RRS_{PhI}) of 92 and 95 protein pairs (**Supplementary Table 2**), respectively. Benchmarking our Y2H system yielded an unconditional assay sensitivity of 20.4% (**Fig. 1e**); excluding hormone-dependent PRS_{PhI} interactions increased this to 23%. The resulting overall completion of 16.0% ± 6.8% matches the overlap with LCI datasets (**Fig. 1g**). Thus, missed interactions explain the incomplete overlap between PhI_{MAIN} and LCI_{PhI} suggesting a low false-discovery rate. This is substantiated by the observation that no RRS_{PhI} pair scored positive (**Fig. 1e**). To further assess PhI quality, we used a pull-down assay in which protein pairs are expressed in wheat-germ lysate and, following an anti-FLAG immunoprecipitation, interactions are detected via activity of renilla luciferase-fused

second protein. Benchmarking this assay with PRS_{PhI}/RRS_{PhI} revealed an assay performance similar to previous implementations[10,11]; the slightly increased background likely results from the functionally relative coherent search space from which RRS was sampled. Subsequent testing of 285 interactions from the unconditional PhI_{MAIN}, $PhI_{EXT,}$ and PhI_{REP} subsets yielded a PhI validation rate of 22.5%, which is indistinguishable from PRS_{PhI} (23.5%, **Fig. 1f**) and similar for the individual subsets (**Extended Data Fig. 1**). These data demonstrate that PhI is a high-quality map of the Arabidopsis phytohormone signaling network on par with high-quality literature data.

For hypothesis generation and local network analyses the full PhI will be most useful. For topological and systems-level questions the symmetrically mapped PhI_{MAIN} should be employed to avoid biases[6]. PhI_{MAIN} has a scale-free degree-distribution and, in contrast to LCI_{PhI} networks, a hierarchical modularity as expected for unbiased network maps (**Fig. 1h, Extended Data Fig. 1**)[14]. We used PhI_{MAIN} to investigate the topological organization of phytohormone signaling pathways.

Important features of hierarchical networks are highly connected hubs and interconnected communities[14]. Using an edge-betweenness-based detection algorithm[15], we identified 21 network communities in PhI_{MAIN}, of which nine were significantly enriched in different phytohormone pathways (**Fig. 1i, Extended Data Fig. 2**, **Supplementary Tables 3, 4**). Thus, the topology of PhI_{MAIN} recapitulates biological knowledge and confirms that at least some pathway proteins are highly interconnected. Additionally, most communities encompass proteins from different pathways that possibly mediate crosstalk. In the JA community, e.g., the canonical JA TF MYC2 is physically linked to ABA signaling via interaction with the protein kinase CIPK14 (**Fig. 1j**), validated by *in vitro* pull-down and bimolecular fluorescence complementation (BiFC) (**Extended Data Fig. 2**). Additional pathway contacts occur between different communities (**Fig. 1j**). However, on average only 27% of pathway proteins reside within the corresponding communities indicating that phytohormone signaling may not be predominantly organized in topological communities (**Supplementary Table 3**).

We next analyzed inter-pathway connectivity. The distances between the phytohormone pathways are considerably shorter in PhI_{MAIN} than in LCI_{PhI} (**Fig. 1k, l**). This is mirrored by significantly more pathway contact points (PCPs) in PhI_{MAIN} than LCI_{PhI}, *i.e.* protein interaction-mediated contacts between different pathways. As some proteins operate in multiple pathways, we distinguished 192 Type I PCPs (PCP_I) of proteins with strictly different annotations from 248 Type II PCPs (PCP_{II}), where the interactors share annotations, but at least one has additional functions (**Fig. 1m**). Bootstrap subsampling confirmed that PhI_{MAIN} contains significantly more PCP_I (**Fig. 1n**), but not PCP_{II} (not shown), than LCI_{IntA} or LCI_{BioG}, and this is valid for essentially all pathway-pairs (**Extended Data Fig. 3**). Each PCP supports a specific crosstalk hypothesis and the abundance of PCPs suggests extensive protein-interaction mediated information exchange among pathways.

Validation of pathway contact points

We experimentally tested if PCPs reflect yet unknown functions of the interacting partners. Assays for most hormones are established in seedlings. Therefore, and for standardization, we focused on seedling-expressed PCP interaction pairs. Validated homozygous T-DNA lines for 19 pairs were evaluated in response-assays for six different phytohormones to establish whether the candidates function in the pathway of their respective partner (**Fig. 2a - f, Extended Data Figs. 4 - 7, Supplementary Table 5**).

ABA regulates seed germination and desiccation stress responses including root growth[16]. In the presence of 0.3 µM ABA, germination of *WT* seeds was ~40% decreased. In contrast, the candidate lines ddl_{Lit_ET} and $eds1_{Lit\_SA}$ displayed a similar ABA-hypersensitivity as the *rcar1* control. Root growth was significantly less affected in five candidate lines resulting in altogether six lines (66%) with a novel ABA phenotype (**Fig. 2a, b, Extended Data Fig. 4**).

Anthocyanin production is a widely used assay for CK signaling[17]. At low concentrations CK-induced anthocyanin accumulation was impaired in the candidate lines similar to the *spy* control.

At higher concentrations *myc2*$_{Lit\_JA/ABA}$ remained similar to *spy* whereas *jaz1*$_{Lit\_JA/ABA}$ over-accumulated anthocyanin indicating complexity in CK signaling (**Fig. 2c, d**).

For ET we assayed the triple response, *i.e.* formation of exaggerated apical hooks (loops) and development of shorter and thicker roots and hypocotyls in dark-grown seedlings[18]. Ten of our twelve candidates (83%) displayed an apical loop phenotype; seven of these additionally displayed a root growth phenotype, and *ttl* also had a hypocotyl growth defect following ACC treatment (**Fig. 2e, Extended Data Fig. 5**). To ensure specificity we tested six mutant lines for proteins in PhI that showed no interaction with ET annotated proteins. Of these controls only one displayed a weak root growth phenotype and none exhibited a hypocotyl or loop formation defect (**Fig. 2e, Extended Data Fig. 6**).

Salicylic acid (SA) mediates defense responses to (hemi-) biotrophic pathogens[19]. Following inoculation with *Pseudomonas syringae* pv. tomato (*Pst*), titers in the *gi*$_{Lit\_GA}$ mutant were significantly elevated indicating enhanced disease susceptibility and impaired SA signaling. Similarly, leaves of mature *rcar1*$_{Lit\_ABA}$ and *pp2ca*$_{Lit\_ABA}$ plants supported enhanced *Pst* growth (**Fig. 2f**). Assays for root growth inhibition by brassinosteroids, gibberellins, and jasmonates revealed new phenotypes for two or one candidates, respectively (**Extended Data Fig. 4**).

Altogether, interactome-guided phenotyping revealed a function in new pathways for 74% of tested proteins (20/27) involved in 84% of interactions in the validation set (**Fig. 2g; Extended Data Fig. 7**). Notably, for all PCP_I pairs a novel function was revealed for at least one partner, such that all interactions are substantiated by phenotypes in at least one common pathway (**Fig. 2g**). For three of the six PCP_{II} pairs an additional common pathway was identified, such that more than half (11/19) of all PCP pairs genetically operate in two common pathways (**Fig. 2g**). To support these functional data we demonstrate for nine pairs *in planta* interactions by BiFC (**Fig. 2h, Extended Data Fig 7**). Intriguingly, prior to our experiments a large majority of signaling proteins in the literature and in our validation set were considered pathway-specific (**Fig. 2i**). After the interactome-guided phenotyping however, 82% of proteins in the validation

set are known to function in multiple pathways, whereas only one-fifth is single-pathway specific (**Fig. 2g, i**). The new annotations are distributed across different pathways (**Extended Data Fig. 7)** and the network degree is not correlated to the number of phenotypes (not shown). As the validation set is not obviously biased, the observation of widespread pleiotropy may extrapolate to most of the phytohormone signaling network. Thus, our data point to a highly integrated central signal-processing network that channels different inputs into a balanced multifactorial output. To facilitate further studies, we provide an expression-based 'edge-score' indicating the possibility of each PhI interaction occurring in different plant tissues (**Supplementary Table 6**).

Hormone-receptor interactions

Input into the central processing unit is provided by hormone receptors, which often initiate signaling via small molecule-regulated protein-interactions[20]. To better understand initial phytohormone-signaling, we conducted interaction screens with soluble hormone receptors in the presence and absence of their cognate hormone. For ABA, GA, IAA, KAR, SA, and SL-receptors 241 interactions were identified, of which 101 are hormone-dependent. Re-identified pairs include interactions of GA-receptors with DELLA proteins, and of RCAR/PYR/PYL ABA-receptors with type 2C protein phosphatases (PP2Cs) (**Fig. 3a**, **Extended Data Fig. 8**), which display known patterns of hormone dependence[21]. Notably, several ABA-receptors interacted also with TFs and other non-PP2C proteins (**Fig. 3a**). As some of these additionally link to PP2Cs, we wondered if interactions are combinatorially modulated and investigated by yeast-3-hybrid the effect of different PP2Cs on RCAR1/PYL9 interactions with MYB-family TFs. The RCAR1-MYB73 interaction was blocked by several PP2Cs, whereas the RCAR1-MYB77 interaction was enabled by ABI1/2, together demonstrating dynamic modulation of complex formation (**Fig. 3b, c**). In addition, PP2C-independent RCAR-functions have been described for RCAR9/PYL6 via MYC2[22] and for RCAR3/PYL8 via MYB77[23]. Our data suggest that such core-pathway-independent functions may be more widespread. The independently validated interaction of DELAY-OF-GERMINATION 1 (DOG1) with PP2Cs[24] similarly points to non-

canonical PP2C-signaling mechanisms. Thus, core-pathway independent signaling and complex multimeric interaction-regulation are important mechanisms underlying the functional diversification in the ABA signaling system.

Receptors for the defense hormone SA are the NON-EXPRESSOR OF PATHOGEN RELATED PROTEIN 1 (NPR1) and its orthologues NPR3 and NPR4[25]. While NPR1 is a well-studied positive regulator of defense-gene transcription, NPR3 and NPR4 are emerging as alternative negative or complementary transcriptional regulators[25,26]. The pattern of SA-regulated NPR3 interactions (**Fig. 3d; Extended Data Fig. 9**), especially with NIMIN proteins, differs from the described NPR1 pattern[27], suggesting dynamic complexity of this signaling system. EMB1968/RFC4, a member of the replication factor C (RFC) complex, is a new interactor common to NPR1 and NPR3 possibly integrating defense with DNA repair or replication. Most novel NPR3/NPR4 interactors can be linked to immunity via mutant phenotypes or known interactions with virulence effectors and immune receptors[8] (**Fig. 3d; Extended Data Fig. 9**). These data support the biological validity of the interactions and indicate that SA-receptors also act via non-transcriptional signaling.

The karrikin (KAR) and strigolactone (SL) pathways have been discovered most recently and mediate germination (KAR) and diverse aspects of development and organismal interactions[28]. We screened the KAR-receptor KAI2 and SL-receptor D14 together with the F-box protein MAX2 in the absence and presence of a stereoisomer-mix of two synthetic strigolactones, which bind to D14 and KAI2, respectively[29]. For KAI2 we found the previously described interaction with MAX2 and 21 novel interactors of which fifteen were hormone-dependent (**Fig. 3f, g; Extended Data Fig. 9**). Recently we described that *KAI2* regulates root hair length (RHL) and density (RHD)[30]. As both phenotypes are also regulated by auxin, and the hormone-dependent KAI2-interactor PP2AA2 regulates PIN auxin exporters we wondered whether PP2AA2 mediates the KAR effect on these phenotypes. Similar to *kai2-2*, *pp2aa2-2* displayed a lower RHL and RHD than Col-0 (**Fig. 3h, i**) (**Supplementary Table 5**). Strikingly, in both *kai2-2* and *pp2aa2-2* the response to

exogenous karrikin treatment was abolished, indicating that they jointly mediate signaling by the karrikin pathway.

Transcriptional changes are common outcomes of phytohormone signaling. Investigating PhI_{REP} we found no evidence of significant hormone crosstalk at the level of transcriptional regulators from different pathways converging on TFs (not shown). Nonetheless, only a quarter of TFs interacting with regulators were previously implicated in hormone signaling (**Extended Data Fig. 10**). While most pathways converge on TCP-family TFs, which are known for their high connectivity[10] the vast majority of TFs interacts with repressors from one to three pathways suggesting more specific signal integration at this level.

Taken together, we present a systematic map of the Arabidopsis phytohormone signaling network, which reveals an unexpectedly high interconnectivity of the signaling pathways. If the observed level of functional pleiotropy extends into the larger hormone signaling network, the concept of dedicated signal transduction pathways may need to be revised in favor of network based models. The small-molecule dependent interactions of hormone-receptors point towards prominent roles for non-canonical signaling mechanisms. We expect that our findings and the PhI resource will stimulate important mechanistic and systems-level analysis in Arabidopsis with a significant outreach into crops.

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Figure legends

Fig. 1 | **Phytohormone network mapping and analysis. a**, PhyHormORFeome candidates selected by mutant phenotypes or membership in therein overrepresented families. **b**, Protein interaction mapping pipeline consists of five steps. **c**, Phytohormone interactome (PhI) network. Node colors indicate hormone annotations according to legend in a. **d**, Sampling sensitivity of PhI_{MAIN}: number of verified interactions in first three repeats of primary screen (black dots; n = 3); screen saturation model based on first three repeats (black line, grey corridor: standard error); identified interactions after five screens (blue dot). **e,** Assay sensitivity of Y2H: fraction of PRS_{PhI} (n = 92), RRS_{PhI} (n = 95) pairs scoring positive. Error-bars indicate standard error. **f**, Validation results: fraction of PRS_{PhI} (n = 69), RRS_{PhI} (n = 83) and PhI (n = 285) pairs testing positive. One-sided Fisher-exact and error bars indicate standard error of proportion. **e, f** Individual results in **Supplementary Table 2**. **g**, Expected and observed overlap of PhI_{MAIN} with LCI_{IntA} (n = 109) and LCI_{BioG} (n = 150 interactions). Error bars indicate propageted standard error. **h**, Degree and clustering coefficient distribution of PhI_{MAIN}. **i**, Number of hormone-signaling-function enriched communities in PhI_{MAIN} (red arrow) compared to n = 1,000 randomized control networks (experimental $P < 0.001$). **j**, Links within and between JA- and CK-enriched communities. Node colors according to legend in a. **k**, **l**, Distances between indicated pathway combinations in PhI_{MAIN} (**k**) and LCI_{IntA} (**l**). Color indicates average shortest distance, circle size indicates number of connections. Insets show shortest distance distributions. **m,** Count of type I (n = 192) and type II PCPs (n = 248) in PhI_{MAIN} – P from analysis in n. **n,** Proportion of PCP_I in PhI_{MAIN} and LCI networks obtained by bootstrap subsampling (n = 1,000) of 100 interactions (two-sided Welch two sample t-test). Boxes represent the interquartile range (IQR) and median; whiskers indicate highest and lowest data point within 1.5 IQR; outliers plotted individually. Pathway abbreviations throughout manuscript: ABA - abscisic acid, AUX - auxin, BR - brassinosteroids, CK - cytokinins, ET - ethylene, GA - gibberellic acid, JA - jasmonic acid, KAR - karrikin, SA - salicylic acid, SL - strigolactone.

Figure 2 | Validation of pathway contact points. a, Proportion of germinating seeds in absence (MS) or presence of 0.3 µM ABA (n ≥ 20, three repeats). **b**, Root elongation in absence (MS) or presence of 30 µM ABA. Boxes represent the interquartile range (IQR), with the bold black line representing the median; whiskers indicate highest and lowest data point within 1.5 IQR; outliers are plotted individually (n ≥ 8; two repeats). **c**, **d**, CK-dependent anthocyanin accumulation in response to indicated concentrations of 6-benzylamino purine (BA). **c**, Seedlings at 10 d after stratification following the indicated treatment. **d**, Quantified anthocyanin content per g fresh weight for lines in c (n = 15; four repeats). **e**, ET induced apical loop formation in response to 10 µM 1-aminocyclopropane-carboxylic acid (ACC) (n ≥ 10; three repeats). **f**, SA-associated phenotypes in response to inoculation with *Pseudomonas syringae* pv. tomato (*Pst*). *In planta Pst* titers (n = 9). **g**, Summary of hormone validation assays for 19 PCP. Node colors indicate known pathway annotations. Square colors indicate new phenotypes. Colors according to legend in 1a. **h**, Bimolecular fluorescent complementation assay of indicated PCP_I candidate pairs and matched negative controls. Scale-bar: 10 µm. Assay was performed in duplicate for all constructs. **i**, Literature reported specificity (single pathway annotation) and pleiotropy (multiple pathway annotations) of genes encoding 1,252 target proteins (total) and 27 proteins in validation set (above line), updated specificity and pleiotropy after hormone validation assay (below line). In **a**, **b**, **d** – **f**, Two sided t-test * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$. **a – f**, Precise P values, biological repeats, and n for each test are shown in **Extended Table 5**.

Fig. 3 | Hormone receptor interactions. **a**, ABA-dependent Y2H interactions. All identified interactors were systematically tested against all receptors in presence and absence of ABA. **b**, **c**, Y3H assays for indicated protein triplets. In all sets DB-RCAR1 is tested for interactions with AD-MYB proteins in the presense of the indicated PP2Cs and in presence and absence of ABA. **b**, One of four representative Y3H results. * indicates ABA-dependent interaction. **c**, Y3H subnetwork of data in b. **d**, SA-dependent interactors of NPR1,3,4. **e**, One representative yeast colony of four repeats in presence and absence of 100 µM SA for identified NPR interactors. **f**, Hormone-dependent and -independent interactions of KAI2, D14 and MAX2. **g**, One representative of four yeast spots for selected KAI2 interactors in presence and absence of *rac*-GR24. **h**, Representative images for analysis in i show root hair phenotypes of the indicated genotypes. Scale bar: 1 mm. **i**, Quantification of RHD (right top) and RHL (right bottom) after indicated treatment. Letters indicate statistical groups (ANOVA, post-hoc Tukey, $P \leq 0.05$). Boxes represent interquartile range (IQR) and bold line median; whiskers indicate highest and lowest data point within 1.5 IQR; outliers plotted individually. Precise n and P values for all group comparisons in **Supplementary Table 5**. **a**, **c**, **d**, **g**, Modulated interactions are represented by line shape as in legend c. Node colors represent hormone annotations as in legend g.

Material and Methods

PhyHormORFeome selection and cloning. We selected target genes with i) a known mutant phenotype in phytohormone signaling based on AHD2.0[31] annotations, ii) all members of gene families were enriched in (ii) and iii) input from colleagues. In total 1,252 genes were selected, for which 1,226 full-length Open Reading Frames (ORFs) could be obtained. To physically assemble the PhyHormORFeome, 688 ORFs were picked from our published AtORFeome collection[8], 276 ORFs were obtained from ABRC, 11 ORFs were obtained from colleagues and 277 ORFs were amplified from Col-0 cDNA-mix from different tissues. For RNA extraction, 6-10 d old *Arabidopsis thaliana* Col-0 seedlings, separated organs and plant organs from mature plants were used (flower and silique - all developmental stages, node, internode, rosette leaves, cauline leaves, root from 15 d old plants grown on solid MS agar plates in vertical orientation, imbibed seeds). From all plant organs, tissue types, and seedlings, specific total RNA was extracted using the NucleoSpin RNA kit from Macherey and Nagel, following the manufacturer's recommendations. For cDNA synthesis, Superscript III (Thermo Fisher 18080044) protocol was modified using 25 ng random primers and 250 ng oligo d(T) 16 per 1 µg total RNA. Mixture was heated to 70 °C/5 min and incubated at 21°C/10 minutes. A mixture of 2.5 µl (0.1 µM) DTT, 10 U RNase OUT (40 U/µl), 250 U SSIII (200 U/µl), 4 µl SSIII 5x buffer, 2.5 µL 2 µM dNTPs was added and incubated at 21 °C for 10 minutes followed by 42 °C for 120 min incubation. To generate cDNA longer than 5 KB an additional 250 U of SSIII (200 U/µl) were added to the mixture followed by 55 °C for 30 minutes incubation for elongation and 70 °C for 15 minutes inactivation. All generated cDNAs from different organs, tissues and seedlings were mixed in equal amounts and 2 µl non diluted cDNA mixture (~100 ng) was used to amplify the ORFs of interest. ORF amplification was conducted as nested PCR to attach attB cloning-sites for further Gateway cloning. The specific primers consist of 18 bp specific and 12 bp of a partial attB site (for attB overhang - GCAGGCTCAGGA, rev attB overhang – GAAAGCTGGGTC). All ORFs were generated with a stop codon. In the second PCR, full attB sites were added to the ORFs

(attB for – GGGACAAGTTTGTACAAAAAAGCAGGCTCAGGAATG, attB rev – GGGGACCACTTTGTACAAGAAAGCTGGGTC). Gateway cloning and yeast transformation were performed as described[7]. ORFs cloned in this project are available from stock centers.

Y2H interaction mapping pipeline. Network mapping was performed according to Altmann et al., 2018[7]. Briefly, bait ORFs were expressed as genetic fusions to the GAL4 DNA binding domain (**pDEST-DB**), prey ORFs were expressed as genetic fusions to the minimal GAL4 activation domain. Both constructs were maintained on low copy centromeric (*cen*) plasmids (**pAD-DEST**) and expressed from weak *adh2* promoters. Primary screening was done by mating individual DB plasmid-containing haploid yeast strains (Y8930, MATα) with a mini-pool of haploid Y8800 (MATa) AD-plasmid containing strains. Following 3 day selection on selective plates containing 1mM 3-Amino-1,2,4-triazole to repress background HIS3 activity, positive single colonies were picked and retested on selective media and cycloheximide control plates. Colonies showing specific selective growth were lysed, the respective ORFs amplified with generic primers that include position-specific barcodes and subsequently identified using the kiloSeq service by seqWell (Beverly, MA, US). All primary Y2H screens were performed once, except for the PhI_{MAIN} screen, which was performed with five repeats. The receptor screens and the PhI_{REP} screen were verified systematically, i.e. in the final verification all identified interaction candidates were tested against all receptors or repressors/regulators, respectively. The receptor screens were performed in the absence and presence of the respective phytohormones applied to the selective media. For the ABA receptor screen, 30 µM abscisic acid was used, for the IAA receptor screen 100 µM indol-3-acetic acid, for the GA receptor screen 100 µM GA3 and for the SA receptor screen 100 µM salicylic acid was used. The receptors of strigolactone (D14) and karrikin (KAI2) signaling pathways were both screened with 5 µM *rac*-GR24.

Y3H assay. RCAR1 was genetically fused to the GAL4 DNA binding domain using **pDEST DB**, the MYB proteins were genetically fused to the minimal GAL4 activation domain using **pAD-**

DEST. To test for modulation of these interactions, the indicated PP2Cs were expressed from the helper plasmid **pVTU-DEST** maintained via the URA3 selection marker. All combinations RCAR1 and PP2Cs were transformed into the haploid yeast strain Y8930 and mated against Y8800 transformed with the AD-MYB constructs. The Y3H assays were performed in four independent repeats in presence and absence of 30 µM ABA treatment on selective plates (Sc-W-L-U-H) containing 1 mM 3-Amino-1,2,4-triazole to repress activity of background HIS3 reporter activity. Interactions that were verified in three repeats were counted as Y3H interactions.

Protein-protein interaction reference set. Candidate interactions for the positive reference set (PRS) were compiled from protein-protein interactions from IntAct (downloaded august 2014)[5] and BioGRID (Version 3.2.115)[32]. At this time, the IntAct dataset contained 17,574 interactions and the BioGRID dataset contained 21,474 interactions among *Arabidopsis thaliana* molecules. In both datasets protein-DNA interactions, interactions derived from papers that reported more than 100 interactions, and non-binary interactions in protein complexes were removed. Subsequently, both datasets were filtered for interactions described in at least two publications or identified in at least two binary interaction detection methods. This resulted in 233 interactions from which 140 interactions described in 247 publications were randomly picked for re-curation. This recuration yielded a selection of 92 highly reliable binary protein-protein interactions, which constitute the PRS_{PhI}. 10 of these 92 interactions were phytohormone dependent interactions. To assemble the random reference set (RRS_{PhI}) we sampled randomly 95 protein pairs from proteins in our PhyHormORFeome, excluding already described protein-protein interaction pairs.

Implementation of interaction mapping framework parameters. To assess the quality of PhI map, i.e. false positive and false negative interactions, the interactome mapping framework was implemented as described[33] and the assay sensitivity, sampling sensitivity, precision and completeness were estimated.

Completeness of the PhI_{MAIN} screening space, i.e. the proportion of tested protein pairs in comparison to the theoretical number in the full search space was based on the number of available ORFs in PhyHormORFeome. The initially defined search space comprised 1,252 loci and thus 1,567,504 possible protein pairs. For the screen of PhI_{MAIN} 1,254 ORFs corresponding to 1,199 gene loci were tested, of which 1,179 were present as AD- and DB-hybrid constructs, 15 only as AD-hybrid constructs, and 5 only as DB-hybrid constructs. Together, AD- and DB-hybrid constructs for 90.2% of locus combinations were tested for interactions, corresponding to the completeness.

The assay sensitivity of our Y2H system for detection of phytohormone signaling related proteins was estimated by benchmarking the system using PRS_{PhI}/RRS_{PhI}. Of the 92 tested PRS_{PhI} pairs 19 pairs were detected, whereas no RRS_{PhI} scored positive, thus yielding an assay sensitivity of 20.7% ± 4.2%. Excluding the 9 interactions from PRS_{PhI} that are dependent on presence of a phytohormone, none of which was detected by the unconditional Y2H, resulted in an unconditional assay sensitivity of 22.8% ± 4.6%.

Sampling sensitivity was estimated as described[10]. Briefly, a modified Michaelis-Menten function was fitted to the number of identified interactions with increasing number of iterations of the experiment using the R-package drc (3.0-1). Using the first three repeats of the PhI_{MAIN} screen for developing the saturation model we estimated saturation to occur at 616 ± 38 interactions. The model was then challenged by two additional repeats of the primary screen. These resulted in a dataset of 529 interactions, which matches the model prediction of 519 ± 31 interactions after 5 repeats.

Overall sensitivity is the product of assay sensitivity and sampling sensitivity. With an assay sensitivity of 20.7% ± 4.2% and sampling sensitivity of 85.9% ± 5.3%, the overall sensitivity is 17.8% ± 6.8% including conditional interactions in PRS_{PhI}. The unconditional overall sensitivity of 19.4% ± 7.0% is the product of the unconditional assay sensitivity of 22.8% ± 4.6% and

sampling sensitivity of 85.9% ± 5.3%. Overall completion of the screen was estimated as the product of overall sensitivity and completeness of the screen; overall completion of PhI_{MAIN} is thus 16.0% ± 6.8%.

Luciferase validation assay. Protein expression: Proteins constituting PRS_{PhI}/RRS_{PhI} pairs and the interaction pairs from the different subsets were expressed in cell-free coupled transcription translation wheat-germ lysate (Promega, L3260) using SP6 promoters. Of each protein pair, one partner was expressed as an N-terminal FLAG-fusion protein, the second protein carried an N-terminal renilla luciferase fusion. Protein pairs were co-expressed according to the manufacturer's protocol, except that the amounts were proportionally adjusted to 20 µl final reaction volume. Input DNA plasmids were isolated from 1.5 ml bacterial cultures grown in Terrific Broth for 20 h on a vibration platform shaker (Union Scientific) using a Qiagen Biorobot3000 and Turbo Prep 96-well plasmid isolation kits. These yielded approximately 20-40 ng $µl^{-1}$ DNA of which 4 µl were used in a 20 µl fv. TnT reaction. Protein expression was done by incubating the reaction mixture containing both plasmids for 2 h at 30 °C. Immunoprecipitation (IP) plate preparation: anti-FLAG antibody coated plates were made in-house by incubating white 96-well Lumitrac high binding plates (Greiner) over night at 4 °C with 75 µl PBS (pH 7.4) per well containing 8 µg ml^{-1} M2 anti-Flag antibody (Sigma). 2 h before use, the antibody solution was replaced with 100 µl blocking buffer containing 10 µg $µl^{-1}$ bovine serum albumin (BSA) followed by 2 h shaking at room temperature. Following protein expression 2 µl lysate were diluted in 28 µl PBS (pH7.4) to quantify expression of the prey protein by addition of 10 µl Renilla glow luciferase substrate. The remaining expression lysate was diluted in 42 µl blocking buffer and added to the empty wells of the IP plates. The plates were incubated with gentle shaking for 2 h at 4 °C, washed 3 times with 100 µl blocking buffer. Co-IP efficiency was determined by addition of 10 µl Renilla glow luciferase substrate (Promega) diluted in 30 µl PBS (pH7.4). Interaction pairs were scored as positive when the expression level was at least 10% of the median of the respective plate (expression positive), the immunoprecipitation (IP) exceeded

the median IP of the plate (min IP signal) and the Z-test on the IP efficiency gave a score greater that 0.4 (IP ratio of sample relative to those of the plate). For determination of dataset precision a total of 446 pairs were tested from PRS_{PhI} (78), PRS_{unc} (69), RRS_{PhI} (83), PhI_{MAIN} (115), PhI_{EXT} (110), PhI_{REP} (60). Dataset differences were statistically compared using one-sided Fisher exact test.

Network topology. To determine network topology of PhI_{MAIN} the distributions of degree and clustering coefficients were calculated for the indicated networks using the igraph package. The distributions were used to determine the underlying network topology[34].

Network visualization and annotation. Networks were visualized with Cytoscape[35] (v. 3.7.2) using protein annotations from Araport11[36]. Hormone annotations were downloaded from AHD2.0, and extracted from TAIR10 GO annotations (03/08/2018). Hormone annotations were inferred from GO annotations when a gene has a GO term that contains one of these key words: "auxin", "abscisic acid", "brassinosteroid", "cytokinin", "ethylene", "gibberellin", "jasmonic acid", "salicylic acid", "strigolactone", "karrikin". GO annotations with evidence code IEP were excluded from all analyses.

Community detection. Communities in PhI_{MAIN} were determined using the edge betweenness algorithm[15] implemented in R-package igraph (v. 1.2.4)[37].

Hormone enrichment. Communities were tested for enrichment with proteins functioning in the hormone signaling pathways using the hormone annotations from AHD2.0 and TAIR10. For each community the number of proteins with a given pathway annotation was compared to the total in the full PhI_{MAIN} network using two-sided Fisher's exact test and multiple hypothesis corrected with Benjamini-Hochberg algorithm.

GO enrichment. All communities were tested for GO enrichment using R package GOstats (2.50.0)[38]. GO annotation data were derived from R package GO.db (3.7.0). Communities were

tested for overrepresentation of GO terms using a hypergeometric test function hyperGTest invoked with parameter conditional = TRUE. *P* values of each community were corrected for testing multiple GO terms using the Benjamini-Hochberg method.

Pathway distance calculation. To determine the distance between different hormone pathways, all shortest paths between proteins of the respective hormone signaling pathways were determined. Only shortest paths were considered that do not contain proteins in the same pathways as those under consideration. The mean path length was calculated from all shortest paths between the two pathways.

Pathway contact point determination and network comparison. Hormone pathway annotations from AHD2.0 and GO were used for this analysis. From the PhI_{MAIN} network we extracted interactions between two proteins annotated with distinct hormone signaling pathways (Type I) and for interactions between two proteins involved in distinct but also common pathways (Type II). To compare the number of PCPs in PhI_{MAIN} with LCI networks, we used a subsampling bootstrapping approach. From each network we conducted 1,000 iterations of sampling 100 interactions without replacement. For each sampling the total number of PCPs of type I and type II and the number of PCPs for each specific hormone combination were determined. The derived distributions for total PCPs from PhI_{MAIN} were compared to the distributions obtained from LCI networks using a two-sided Welch Two-Sample t-test. The distributions of hormone combination-specific PCPs were compared using a two-sided Wilcoxon test and multiple testing corrected by the number of hormone combinations tested (45).

Literature curated interactions. Interactions curated from literature were downloaded from IntAct[5] and BioGRID[39]. Arabidopsis protein-protein interactions were extracted from IntAct database downloaded in June 2016 and from BioGRID database version 3.4.142 (downloaded November 2016).

Phytohormone sources. 1-aminocyclopropane-carboxylic acid (ACC) from SIGMA (A-3903), 6-benzylamino purine (BA) from SIGMA (B3408), brassinolide (BL) from SIGMA (B1439), karrikin2 (KAR2) from Olchemim (025 682), karrikin2 (KAR2) from Toronto Research Chemicals (F864800) for Y2H experiments, gibberellic acid 3 (GA) from Duchefa (G0907), *rac*-GR24 from Chiralix (CX23880), indol-3-acetic acid (IAA) from SIGMA (I2886), paclobutrazol (Pac) from Duchefa (P0922), salicylic acid from SIGMA (S5922), abscisic acid (ABA) from SIGMA (A1049), and methyl-jasmonate (Me-JA) from SIGMA (392707).

Plant material and growth conditions. All *Arabidopsis thaliana* lines, i.e. WT, *ahp2, as1, bee1, bee2, bim1, bpm3, cbl9, cos1,cpk1, ddl, eds1, ga3ox1, gai, gi, hub1, ibr5, jaz1, jaz3, kai2-2, myb77, myc2, nap1;1, nia2, pks1, pp2aa2-2, pp2ca, rcar1, rcn1, rgl1, tt4, ttl, wrky54, rga, rga-28, spy,* and *ein3* are in the Col genetic background. Seeds were obtained from NASC and propagated for three generations in a greenhouse environment at 21 °C and LD light (16 h / 8 h). For genotyping, one leaf of a 12 - 14 days old plant was frozen in liquid nitrogen and genomic DNA was extracted in 1.5 ml tubes using Edwards DNA extraction buffer[40]. For expression level analysis of the mutant lines, RNA was extracted using NucleoSpin RNA kit from Macherey-Nagel and the M-MuLV Reverse Transcriptase (Biozym 350400201) according to the manufacturer's recommendations. All seeds were surface sterilized and stratified for 3 d at 4 °C in the dark on MS plates or plates containing the indicated additives. LD light conditions were 75-85 µM m^{-2} s^{-1} measured with LI-250A light sensor (LI-COR). *Nicotiana benthamiana* seeds were spread on soil and grown in a greenhouse environment with 23 °C and LD light (16 h / 8 h). For all assays, measurements were done with distinct samples (no repeat measurements on the same sample). For statistical tests of significance a normal distribution of the measured variable (e.g. root length) was assumed; hormone treatments and genotype were tested as covariates.

ET triple response measurement. Sterile seeds were placed directly on standard MS or 10 µM ACC containing plates, stratified for 3 d at 4 °C in the dark, transferred into light for 1 h to induce

germination, and then incubated for 3 d at 23 °C in the dark. Apical hook vs loop formation was scored visually, image analysis for hypocotyl and root length determination was performed using the Fiji imaging software[41] and herein the Simple Neurite Tracer[42] plugin (v 3.1.3).

Root elongation measurements. Seedlings were grown on MS plates to 5 DAG and then transferred to MS mock plates or MS containing the appropriate phytohormone additive as indicated in the figures (Pac 0.5 µM, 1.0 µM; BL 0.1 µM, 0.5 µM; 25 µM Me-JA). Transferred seedlings grew in vertical position for another 4 days at 23 °C in LD light conditions (16 h / 8 h). Root lengths were determined as described above.

Anthocyanin accumulation. Anthocyanin content in response to the indicated treatments was determined as described by Nakata et al, 2014[43] and expressed per g fresh weight.

Root hair growth. Analysis was performed according to Villaécija-Aguilar et al., 2019[44] using 1 µM KAR2. Arabidopsis seeds were stratified in the dark for 3 d at 4 °C and then transferred to a growth cabinet at 22 °C, 16 h / 8 h light/dark cycle (intensity ~100 µM m^{-2} s^{-1}). Images were taken with a Zeiss SteREO Discovery.V8 microscope (Carl Zeiss, Germany) equipped with a Zeiss Axiocam 503 color camera (Carl Zeiss, Germany). The number of root hairs was determined by counting the root hairs between 2 and 3 mm from the root tip on each root, and root hair length was measured for 10 - 12 different root hairs per root as described above. For karrikin treatments, KAR2 (Olchemim, Olomouc) was dissolved in 75% methanol for the preparation of a 10 mM stock solution. Analysis and data are based on two repeats.

Infection assay. To measure bacterial proliferation in 4 - 5 week old plants, assays were conducted as described[18] using *Pseudomonas syringae* pv. tomato DC3000. To prepare the inoculum, bacteria were grown overnight on NYGA medium (5 g/l bactopeptone, 3 g/l yeast extract and 20 m/l glycerol) and resuspended and diluted to 5 x 10^5 colony forming units ml^{-1} in 10 mM $MgCl_2$. Bacteria were inoculated by syringe infiltration of two leaves per plant, and

harvested at 4 days post inoculation as described[45]. In short, 3 leaf discs per sample were incubated for 1 hour in 10 mM $MgCl_2$ containing 0.01% Silwett. The resulting suspension was then serially diluted, 20 µl of each dilution were plated, and colonies were counted after two days.

Bimolecular fluorescent complementation assay (BiFC). For BiFC the vectors pMDC43-YFC, pMDC43-YFN[46], and pDEST-VYNE(R), pDEST-VYCE(R)[47] were used. After Gateway recombination, the ORF-containing destination clones were introduced into *Agrobacterium tumefaciens* GV3101 strain. Transformed *A. tumefaciens* cells were grown overnight and resuspended in infiltration buffer (10 mM $MgCl_2$, 10 mM MES pH 5.6, and 150 µM acetosyringone) with a final OD_{600} of 0.3 for each expression vector. The abaxial leaf surface of *N. benthamiana* plants was transiently transformed by *A. tumefaciens*, harboring the constructs and the p19 silencing inhibitor protein, by infiltration using a needleless syringe. Two days after infiltration, two leaves from two independently transformed plants were used for fluorescence detection. Reconstitution of fluorescence was observed under an epifluorescence microscope (Olympus BX61) using YFP and RFP band-pass filters for the YFC-MYC2 and YFN-CIPK14 interaction, and either a TCS SP8 (Leica) or a LSM880 laser scanning confocal microscope (Carl Zeiss) was used for the remaining BiFC assays. Laser excitation wavelength for both microscopes was 488 nm and the detection band was set to 493-545 for Venus protein. The objectives were a PL APO 40x/1.10 and a Plan-Apochromat 20x/0.8 M27 for the TCS SP8 and LSM880, respectively. Image analysis was performed using the Fiji imaging software[41]. Analyses were performed in duplicate for all constructs.

***In vitro* pull-down assays.** For *in vitro* pull down assays, Amylose Resin (New England Biolabs) coated with MBP-MYC2 was incubated for 2 hours at 4 °C with equimolar amount of purified GST-CIPK14. Wash and elution steps were performed following manufacturer's

instructions. Pull-downs were analyzed by western blot using α-GST (Amersham Biosciences) and α-MBP (New England Biolabs) antibodies.

Estimation of the protein-protein interaction likely scores We developed the Edge-score model to determine the protein-protein interaction likely score in different plant tissues and development states. The Edge-score modelling was designed to exploit transcript abundance to estimate possibility and to some extent likelihood of an interaction taking place in a given tissue and condition. It is based on using transcript abundance as a proxy for protein concentration and modeling binary complex formation by the law of mass action. Tissue specific transcriptome data were collected from Kleptikova[48]. FastQC (v0.11.7) was used for read quality control before and after trimming. Adaptor sequences and low quality reads were trimmed with Trimmomatic v0.36[49], using the ILLUMINACLIP:TruSeq3-SE.fa:2:30:10, LEADING:3, TRAILING:3, SLIDING WINDOW:4:15 and MINLEN:36 options. High quality reads were mapped to the *Arabidopsis thaliana* (TAIR10) reference genome. The estimation of gene abundance was performed with Kallisto v0.45[50]. To estimate the chance of two proteins i and j to interact in a given condition, the law of mass action was used to obtain a quantitative estimate of their interaction feasibility. The amount of protein i and j was estimated using their respective transcript levels as proxy t_i and t_j. Edge-scores were calculated using the following scheme: The score of the interaction between protein i and protein j in tissue t_k sets as $S_{ij}^{t_k}$ (Equation 1). In each tissue, let $t_i^{t_k}$ and $t_j^{t_k}$ denote the abundance of genes i and j in tissue t_k.

$$S_{ij}^{t_k} = t_i^{t_k} * t_j^{t_k} \quad (1)$$

After obtaining a score for each interaction in each tissue, the Edge-score of a specific interaction in tissue t_k was computed with Z-transformation (Equation 2).

$$es_{ij}^{t_k} = \frac{s_{ij}^{t_k} - \overline{s_{ij}^{t_k}}}{\sqrt{\frac{1}{N-1}\sum_{i=1}^{N}(s_{ij}^{t_k} - \overline{s_{ij}^{t_k}})^2}} \quad (2)$$

Finally, we normalized this score to fit the range of [0, 1] (Equation 3).

$$es_{ij}^{t_k} = \frac{es'^{t_k}_{ij} - \min(es'^{t_k}_{ij})}{\max(es'^{t_k}_{ij}) - \min(es'^{t_k}_{ij})} \quad (3)$$

A higher Edge-score indicates that an interaction in this tissue is more likely as both proteins are expressed jointly. A higher Z-score indicates that an interaction in this tissue is more likely as both proteins are expressed jointly.

Data Availability

All functional, genetic, and interaction data generated in this study are available as supplementary information. The genes selected for interactome mapping (search space) are presented in **Supplementary Information Table 1**. All protein-protein interaction data acquired in this study can be found in **Supplementary Information Table 2**. The data for genetic validation assays can be found in **Supplementary Information Table 5**. The preliminary edge-scores for all interactions identified in this study are presented in **Supplementary Information Table 6**. Additionally, all protein interactions from this work have been submitted to the IMEx (http://www.imexconsortium.org) consortium through IntAct[5] and assigned the identifier IM-27834.

Code Availability

Custom scripts used in this manuscript are available at https://github.com/INET-HMGU/PhyHormInteractome

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Author contributions

Project conception: PFB; ORF selection and cloning: MA, SA, PFB, GWB, SC, CG; Y2H screening: MA, PAR, LEV, MS, VY, RP; NMR; Hormone-dependent Y2H screens: MA, JP; PRS_{Phl}/RRS_{Phl} curation: MA, SA, NMR, AGM, PFB; network analyses: SA, PFB, MA, KK, KFXM,; Edge-score calculation: CWL, SA, PFB; pulldown experiment: JP, NMR; Systematic validation assay: BW, PAR, MS, MA, AS, VY, PFB; BiFC validations and figure panel: PAR, MA, NMR, JP; genetic validation assays: ABA: MA, EG, LG, SA, PFB; CK: MA, LEV; ET: MA, LEV; SA: ACV, MW, JS, MA; JA: NMR, MA, PAR; GA: MA; Karrikin/GR24 vignette and figure panel: JAVA, CG; Figures: MA; SA, CF, PFB; manuscript writing: PFB, CF, MA, SA, CG, ACV.

Competing interests The authors declare no competing interests

Extended Data Figure Legends

Extended Data Fig. 1 | Network analyses. a, Network map of binary protein-protein interactions among search space proteins derived from IntAct (LCI_{IntA}). Color code indicates hormone pathway annotations as indicated in legend. **b**, Degree distribution and clustering coefficient distribution on log-log scale of network in a. **c**, Network map of binary protein-protein interactions among search space proteins derived from BioGRID (LCI_{BioG}). Color code indicates hormone pathway annotations as indicated in the legend. **d**, as in b, but for network shown in c. **e**, number of proteins and interactions in the PhI interactome subsets. **f**, number of total and new interactions in PhI for all proteins belonging to each pathway and the non-redundant total for PhI. **g**, Fraction of positive scoring pairs of PRS_{PhI} (78), PRS_{unc} (hormone-independent PRS interactions) (69), RRS_{PhI} (85), combined PhI subsets (green) (285) and the individual subsets from the single Y2H screens: PhI_{EXT} (110), PhI_{MAIN} (115), and Rep-TF (60). Error bars indicate standard error of proportion. Individual results for all pairs are provided in **Supplementary Table 2**.

Extended Data Fig. 2 | Communities and validation. a, Communities with three or more nodes identified in PhI_{MAIN}. Community numbers correspond to the numbering in Supplementary Table 3. Color code indicates hormone pathway annotations as indicated in legend. Node labels are gene symbols when available, otherwise Locus IDs. **b**, Bimolecular fluorescence complementation (BiFC) for CIPK14-MYC2. *Nicotiana benthamiana* epidermal leaves transiently co-expressing cYFP-MYC2 and nYFP-CIPK14 restore YFP fluorescence, whereas co-expression of the non-interacting cYFP-JAZ1 and nYFP-CIPK14 does not. **c**, maltose-binding-protein (MBP) pull-down of MBP-MYC2 and glutathione-S-reductase (GST) tagged CIPK14 shows specific co-purification of the latter. **b**, **c** Shown are representative results of two experiments with similar results.

Extended Data Fig. 3 | Pathway contact points enrichment. a, Number of pathway contact points (PCPs) per hormone combination for type I and type II are shown. * indicates a significantly higher number of PCPs compared to LCI_{IntA} as obtained by bootstrap subsampling analysis (n = 1,000) of 100 interactions followed by two-sided Welch two sample t-test. Precise *P* Values for PCP_{I} and PCP_{II} and pathway combinations are listed in **Supplementary Table 2**.

Extended Data Fig. 4 | Hormone response assays I. a, ABA germination rate for Col-0 (*WT* background), *cbl9, gai, myc2, ibr5, rcar1 and rcn1* (positive controls), *ahp2, as1, bee1, bee2, bim1, ddl, eds1, jaz3, myb77* and *wrky54* in absence (MS) or presence of 0.3 µM ABA. **b**, Root elongation in absence (MS) or presence of 30 µM ABA for the same lines as in a. **c**, BR root length inhibition in absence (mock) or presence of indicated concentrations of BL for Col-0 (*WT* background), *bee1*, *bee2*, *bim2* (controls), and *ddl*, *rcn1*, and *ttl* (candidates) lines. **d**, GA root length inhibition in the presence of indicated concentrations of Paclobutrazol (Pac) for Col-0 (*WT* background), *as1, gai, gi, rga and rga-28* (controls) and *hub1, jaz3, nia2,* and *rcn1* (candidate) lines. **b – d**, Boxes represent IQR, bold black line represents median; whiskers indicate highest and lowest data point within 1.5 IQR; outliers are plotted individually. **a** – **d**, Two sided t-test * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$. Precise n for each repeat and precise *P* values are provided in **Supplementary Table 5.**

Extended Data Fig. 5 | Hormone response assays II. a, SA-associated phenotypes: Pst titers following 3 dpi with *Pseudomonas syringae* pv. tomato (*Pst*) by syringe infiltration. In planta *Pst* titers were elevated in mature plants of indicated genotypes relative to *WT* Col-0 plants. **b**, JA root growth in absence (MS) or presence of 25 µM Me-JA. **c – f**, ET triple response in control conditions compared to Col-0. Apical hook formation graph indicates hook or loop formation following 10 µM ACC treatment. The hypocotyl and root length values are shown with and without 10 µM 1-aminocyclopropane-carboxylic acid (ACC) treatment. **c**, Apical hook formation in absence or presence of 10 µM ACC. Representative results underlying quantitation in d. **d**, Proportion of apical loop formation in presence of ACC treatment for same lines as in c. **e**, Hypocotyl length in absence or presence of 10 µM ACC for same lines as in d. **f**, Root elongation in

absence or presence of 10 µM ACC for same lines as in d. Two sided t-test * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$. **b**, **e**, **f**, Boxes represent IQR, black line represents median; whiskers indicate highest and lowest data point within 1.5 IQR; outliers are plotted individually. **a**, **b**, **d – f**, Two sided t-test * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$. Precise n for each repeat and exact *P* values are provided in **Supplementary Table 5.**

Extended Data Fig. 6 | ET triple response assays (negative controls). ET triple response in negative control lines compared to Col-0 and *ein3*. **a**, proportion of apical loop formation in response to 10 µM ACC. **b**, Hypocotyl length in absence or presence of 10 µM ACC. **d**, Root elongation in absence or presence of 10 µM ACC. Two sided t-test * $P \leq 0.05$, ** $P \leq 0.01$. **b**, **c**, Boxes represent IQR, black line represents median; whiskers indicate highest and lowest data point within 1.5 IQR; outliers are plotted individually. Precise n for each repeat and precise *P* values are provided in **Supplementary Table 5.**

Extended Data Fig. 7 | PCP validation. a, Summary of hormone-assay results for 27 candidate genes. Light colors indicate known hormone pathway annotations. Bright colors indicate significant new phenotypes observed in validation assays. **b**, Bimolecular fluorescent complementation assay (BiFC) in *N. benthamiana* of two PCP_I pairs (AHP2-MYC2, MYB77-RCAR1) and five PCP_{II} pairs (CBL9-IBR5, PP2CA-IBR5, TT4-COS1, AS1-NIA2, EDS1-HUB1). PCP pairs are additionally tested with one or two negative controls in the BiFC assay. Each construct was tested in duplicate and in two independent assays and one representative result is shown. Scale-bar = 10 µm.

Extended Data Fig. 8 ABA Y2H interactions. a, ABA-dependent and -independent interactions of RCAR1-14 ABA receptors. All identified interactors were systematically tested against all receptors in presence and absence of 30 µM ABA. Except for PP2Cs, single RCAR-specific interactors are displayed above, interactors common to multiple RCARs are displayed below receptors. Color of nodes represent hormone annotations. Solid lines indicate ABA independent interactions, dashed lines indicate ABA-dependent interactions as indicated in legend. **b – f**, one representative set of Y2H results, out of four repeats, showing yeast growth on selective media in presence and absence of 30 µM ABA as indicated. All candidate interactors identified in primary screens were tested systematiclly against all receptors in the shown representative verification experiments. **g**, plate layout of candidate-interactors tested with the indicated RCARs in b-f.

Extended Data Fig. 9 Hormone dependent Y2H interactions. **a**, SA-dependent interactors of NPR1,3,4. in presence and absence of 100 µM SA. **b**, Evidence for NPR4 interactor functions in defense. **c**, MAX2, D14 and KAI2 interactions in presence and absence of 5 µM *rac*-GR24. **a, c** One representative set of Y2H results, out of four, showing yeast growth in presence and absence of hormone. All candidate interactors identified in primary screen were tested against all receptors in the shown representative verification experiments.

Extended Data Fig. 10 | Pathway convergence on transcription factors. a, Y2H-derived interaction map of repressor and non-DNA binding transcriptional regulators (boxed and color coded for the respective main pathway involvement) with Arabidopsis TFs. Above repressors are TFs interacting specifically with regulators from one pathway. Lower layers show the TFs intecating with regulators from multiple number of pathways. Node annotations are represented by color-code as indicated.