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Supplementary Information for

Robustness of plant quantitative disease resistance is provided by a decentralized immune network

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This PDF file includes:

Supplementary text
Figures S1 to S13
Tables S1 to S4
Legends for Dataset S1 and S2
SI References

Other supplementary materials for this manuscript include the following:

Dataset S1
Dataset S2

Supplementary Information Text

Material and methods.

Plant growth and bacterial inoculation.

Arabidopsis thaliana Col-0 accession was grown on Jiffy pots in a growth chamber at 22 °C with a 9-h photoperiod at 192 µmol m^{-2} s^{-1}. 4 week-old plants were used for experiments. Arabidopsis T-DNA insertion mutant lines were obtained from the Nottingham Arabidopsis Stock Centre. Homozygous lines were identified by PCR genotyping to precise the T-DNA insertion site (Table S3). The inoculation tests were done with the strain LMG568/ATCC33913 (*Xcc568*) (1) carrying the LUX operon of *Photorhabdus luminescens* (2) and *Xcc568*∆XopAC (kindly provided by L. Noel). Bacterial cultures of *Xcc568* were done at 28°C on Kado medium supplemented with 50 mg/mL rifampicin and 25 mg/mL kanamycin and Kado medium supplemented with 50 mg/mL rifampicin for *Xcc568∆XopAC*.

Constructs and plant transformation

The *RKS1-OE* construct was performed by amplification of the *RKS1* coding sequence (AT3G57710) using [attB1F-710 + attB2R-710] as primers (Table S4). PCR products were cloned in pDONR207 and transferred into the T-DNA binary vector pBIN19 using the Gateway technology (Invitrogen) for *Agrobacterium*-mediated transformation of Arabidopsis Col-0 using the floral dip transformation (3). Harvested seeds were spread on MS medium containing 50 µM of Kanamycin for selection of transgenic plants. C-terminal fusion of RKS1 with GFP was accomplished using a multisite Gateway cloning strategy (Invitrogen) described previously (4). RKS1 was amplified from cDNA using [attB1_RKS1 + attB4_RKS1] (Table S4) and recombined into the multisite Gateway entry vector pBSDONR P1-P4. YFP was cloned into the entry vector pBS-DONR P4-P2. To fuse RKS1 with eGFP both vectors were mixed with either the 35S plant expression vector pEarleyGate100 (5) and recombined with LR clonase II (Invitrogen). The catalytic RKS1 mutant was generated using RKS1_D191A_fw and RKS1_D191A_rev as primers (Table S4). For transient expression assays, *Agrobacterium* strain GV3101 or C58C1 carrying the corresponding construct

was used. Arabidopsis seedlings were transformed according to Marion *et al.* (6). Overnight cultures of *Agrobacterium tumefaciens* were resuspended in 2 ml of 5% sucrose supplemented with acetosyringone (200 µM). Then one week-old seedlings were vacuum-infiltrated. with the *Agrobacterium* solution.

Fluorescence microscopy

Fluorescence images were acquired using a Leica SP8 confocal microscope equipped with a water immersion objective lens (× 25, numerical aperture 1.20; PL APO). GFP and YFP fluorescence was excited with the 488 nm ray line of the argon laser or the 561 nm ray line of the He-Ne laser respectively. The emission recording bands were set in the 505 to 530 nm range for GFP detection and 520 and 580 nm range for YFP detection. CFP fluorescence was excited with the 458 nm ray line of the argon laser and recorded in the 465–520 nm emission range**.** Image acquisition was done in the sequential mode using Leica LCS software and analyzed using the ImageJ software. Representative confocal images are shown after histogram normalization. Two fluorescent protein fusion constructs were used that mark different subcellular compartments have been used: MIEL1:CFP as a subcellular marker of the cytoplasm and nucleus of Arabidopsis cells (7) and SYMREM as a subcellular marker of the plasma membrane (8).

Yeast Two-Hybrid screening

To identify proteins interacting with RKS1, $RKS1_{D191A}$ were amplified using [attB1_RKS1 + attB2R-710-stop] cloned into pGBKT7 using Gateway technology (Invitrogen). $RKS1_{D191A}$ was used to increase our chances to identify RKS1 interactors, as a mutation of the phosphate transfer site in active kinases is considered to be a substrate trap and has been shown to stabilize interactions with their substrates. A hybrid screen was performed (2 rounds) in the yeast *Saccharomyces cerevisiae* (strain AH109), from a cDNA library made in the vector pGADT7 from *Arabidopsis thaliana* leaves infected with the strain *Xcc147* (9). The screens were performed following the protocol described previously by Gietz and Schiestl (10). The transformed yeasts were selected on SD / -Leu / -Trp / -His solid medium (1_{st} round) and on the same medium with 25 mM 3AT (3-amino-1, 2,4-triazole) (2_{nd} round). Then the protein interactions were demonstrated using a more stringent medium, SD / -Leu / -Trp / -His / -Ade.

Plant phenotyping

After one night under high humidity conditions (9h light/15h dark and 90% relative humidity, 4 week-old plants were inoculated by piercing with a *Xcc568* bacterial suspension of 2.10_8 colony forming units (CFU).mL_{-1}. We performed a scoring of the symptoms as already described (11). Each line was tested in at least three separate experiments where Col-0 and the *rks1-1* mutant were inoculated as controls.

In planta bacterial growth analysis (colony forming unit (CFU)/cm_2 expressed in a log10 scale) was performed as described by Froidure et al. (9). Because *Xcc* is a vascular bacterium, bacterial growth was measured 0 and 7 days after inoculation by piercing with *Xcc* strain 568, at distance from the inoculation zone (at the tip of the inoculated leaves). Data were collected from three independent experiments, each time point corresponds to 6 independent measurements, each on 3–5 individual plants (four leaves/plant). At the inoculation site (basis of the inoculated leaves), bacterial growth was measured and found similar among the different lines.

RNA extraction and Quantitative Real-Time PCR analysis

Total RNA was isolated using the NucleoSpinRNA Plus kit from Macherey-Nagel following the manufacturer's instructions. Purified RNA was quantified with Nanodrop and quality control was done using Agilent. Quantitative RT-PCR analysis was performed as described (9). The housekeeping gene *MON1* (AT2G28390), *PR1* (AT2G14610), *RKS1* and *MPK3* (AT3G45640) (Table S4) genes were used to control the reproducibility between the 3 transcriptomic experiments. cDNA synthesis was performed using 1.5 µg of total RNA and the Roche Transcriptor Reverse Transcriptase (Roche diagnostics GmbH (Mannheim, Germany) according to the manufacturer's instructions. Results were analyzed using the LC480 on-board software, release version 1.5.0.39. The real-time PCR was conducted with at least five experimental replicates for each biological sample. Statistical analyses for Q-PCR were performed with the Wilcoxon test. Groups were defined for different lines as compared to Col-0.

Protein extraction and western blotting

Total protein was extracted using the Laemmli buffer from 4-week leaves and separated on SDS-PAGE (12). For detection of tagged proteins, blots were incubated with rabbit anti-GFP antibody (AMS Biotechnology, [1:5,000]) and goat anti-rabbit IgG HRP coupled secondary antibody (Millipore, [1:20,000]). Proteins were visualized using the Clarity Western ECL substrate kit (Bio-Rad).

Transcriptomic analyses

Arabidopsis plants mis-expressing *RKS1* (*AT3G57710)* (*rks1-1*, *RKS1-si24 and si15* lines (13) *RKS1-OE1* and *OE2* lines were used. Three independent experiments were performed. Each replicate included 96 plants: 6 plants per line, 4 lines (*rks1-1*, *RKS1-OE1*, *RKS1-si24* and Col-0) and 4 time points (0, 1.5, 3 and 6 hours post-inoculation). For inoculation, 5 leaves per plant were infiltrated with a blunt-ended syringe containing the bacterial suspension of *Xcc568* at 2.10_8 colony forming units (CFU).mL_{-1}.

Samples were sequenced by Fasteris on an Illumina HiSeq 2500 instrument using a base calling pipeline integrating HiSeq Control Software 2.0.5, RTA 1.17.20.0 and CASAVA-1.8.2. A single

HiSeq 2500 Flow cell (v3) was used with the kit TruSeq SBS Kit v3 in order to generate stranded single reads of 100nt. The three replicates were sequenced on three different lanes. The total number of raw reads ranged from 3,471,313 to 45,251,051 (Dataset S1). Reads were mapped on Col-0 genome downloaded from TAIR (https://www.arabidopsis.org/) using the glint software (http://lipm-bioinfo.toulouse.inrae.fr/download/glint/ release glint-1.0.rc6) with parameters set as follows: matches ≥ 50 nucleotides, with ≤ 3 mis-matches, no gap allowed, only best-scoring hits taken into account (--lmin 50 --mmis 3 --best-score --no-gap -C 0). Ambiguous matches (same best score) were removed. Bedtools (2010) were used to compute counts at the gene level (gene models version: TAIR10_GFF3_genes.gff) taking into account the strand (intersectBed –f 0.8 -s). The number of unambiguously mapped reads that span gene models ranged from 3,293,054 to 41,653,761 (Dataset S1). Raw and normalized RNAseq data have been deposited in the SRA database (accession number SRP233656).

Statistical analyses

We used the software R version 3.4.2 (2017-09-28) and gene counts were normalized using edgeR_3.16.5 limma_3.30.0 package. A Principal Component Analysis (PCA) on the 33,602 genes was performed using the ade4 1.7-6 version package in the R environment. To identify the main drivers of global change of expression across the genome, we ran the following model under the SAS environment with inference performed using REML estimation (PROC MIXED procedure in SAS9.3, SAS Institute Inc.) for each of three first Principal Components (PCs):

$$Y_{ij} = \mu_{trait} + line_i + time_j + line_i*time_j + \varepsilon_{ij}$$

where '*Y*' corresponds to the coordinates of all the samples on one of the three PCs, 'μ' is the overall mean; 'line' accounts for differences among *A. thaliana* lines; 'time' accounts for differences among the four time points; 'ε' is the residual term. The variance explained by each of the three model terms was estimated based on variance components estimated by REML (PROC VARCOMP procedure in SAS 9.3, SAS Institute Inc.), For each time point, the 'line' effect on global change of expression was tested with the following model: $Y_i = \mu_{trait} + line_i + \varepsilon_i$

Genes deregulated in their expression after *Xcc568* inoculation were identified using a hypergeometric test with a significance threshold of 0.05 after a Benjamini and Hochberg FDR correction. GO annotation analysis on gene and classification were done using using BINGO module form Cytoscape.

Comparison of kinetics of disease scores. We fitted the temporal relation between the disease index of Col-0 and each tested mutant. We fitted the temporal relation of disease index between the tested mutant and Col-0 by a second order polynomial. Kinetics of disease index were considered similar if the coefficient of the second order terms was not significantly different from 0 and if the slope was not significantly different from 1. P-value numbers represent kinetic modeling

deference with Col-0, 0 = p-value>0.05 and 1= p-value<= 0.05. The fit and statistical testes were implemented in R (https://www.R-project.org/) and based on the lm library.

Network reconstruction

Interactors of the 268 co-regulated genes and the 41 potential interactors of RKS1$_{D191A}$ identified by Yeast Two Hybrid screens were recovered from Arabidospis BioGRID protein interaction datasets version 3.5.179 (14). Each interaction was manually verified and curated with UniProtKB databases (Swiss-Prot or TrEMBLversion 01-2019). To increase the robustness of the network, a protein-protein localization-based filter was performed. 1886 protein-protein interactions were conserved corresponding to proteins described in the same or associated sub-cellular compartment. Protein-Protein Interactions were plotted with Cytoscape software v3.7.2. GO annotation analysis on gene and classification were done using BINGO module from Cytoscape. Using BINGO classification, each protein was attributed to a functional group. Expression classes were recovered for the 268 co-regulated genes and plotted in the network. Network connectivity was calculated using Cytoscape.

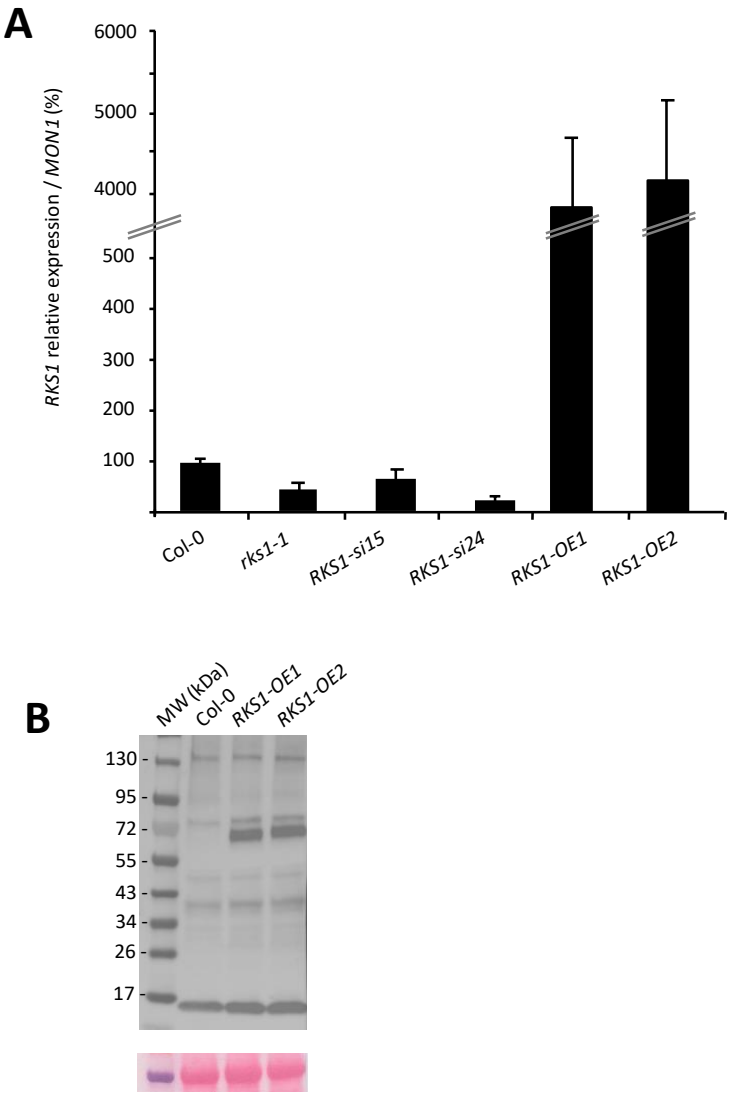


Fig. S1. Characterization of the *RKS1* overexpressing lines used in this study. Expression analysis of *RKS1* gene (A) by quantitative RT-PCR in the *RKS1-OE1* and *RKS1-OE2* lines, as compared to the *RKS1-si15* and -*si24* lines, the *rks1-1* mutant and the wild-type Col-0; (B) by Western blot analysis using YFP antibodies in the *RKS1-OE1* and *RKS1-OE2* lines, as compared to the wild type Col-0. On the 1st line, the protein ladder (Thermofisher) with the molecular weight (MW) of the proteins is indicated. Ponceau S staining of total protein transferred to the nitrocellulose membrane (bottom) demonstrates sample loading.

A

Line	Disease index					Standard deviation					nb_exp	nb_plants	Diff	Intercept	iLCI	iUCI	Slope	sLCI	sUCI
	3 dpi	5 dpi	6 dpi	7 dpi	10 dpi	3 dpi	5 dpi	6 dpi	7 dpi	10 dpi									
Col	0	0.16	0.40	0.63	0.85	0	0	0	0	0	3	12							
rks1-1	0.04	1.19	1.52	1.94	2.60	0	0.1	0.1	0.1	0	3	12	1	0	0	0	2.77	1.76	3.79
RKS1-*si15*	0.01	0.69	0.99	1.26	2.15	0	0	0	0	0	3	18	1	0	0	0	2.33	1.87	2.79
RKS1-*si24*	0.07	0.46	1.33	1.65	2.14	0	0	0.1	0.1	0.1	3	18	1	0	0	0	2.34	1.58	3.09
RKS1-*OE1*	0	0	0	0	0.17	0	0	0	0	0	3	18	1	0.55	0.06	1.04	-0.30	-0.69	0.10
RKS1-*OE2*	0	0	0	0.01	0.07	0	0	0	0	0	3	18	1	0.20	0.11	0.30	-0.10	-0.17	-0.02

B

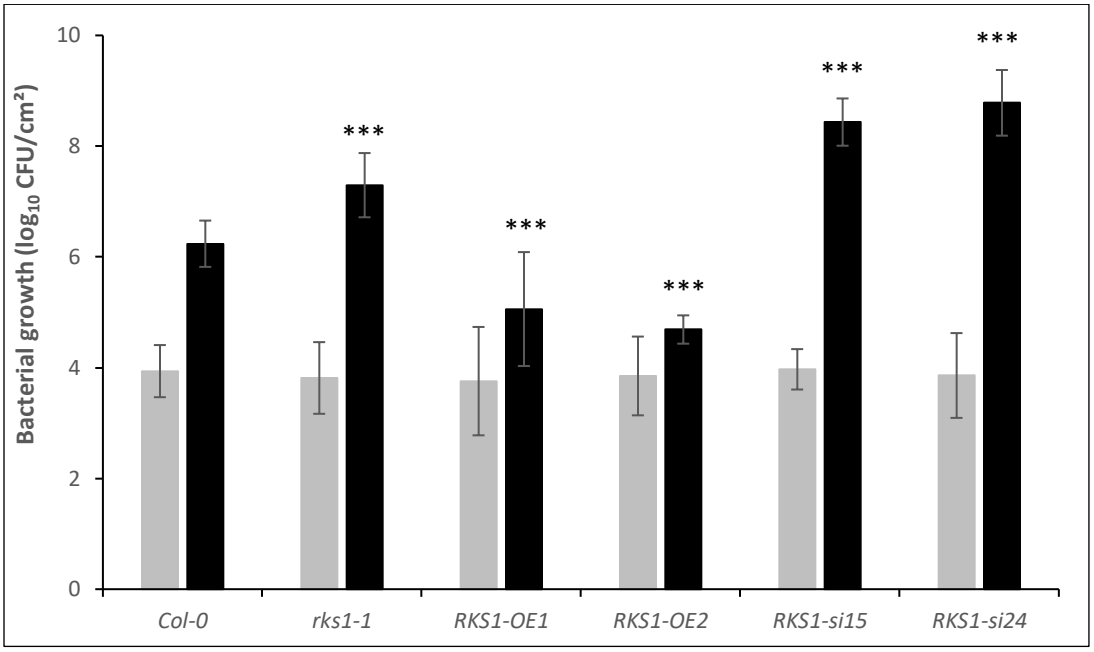


Fig. S2. Analysis of RKS1-deregulated transgenic lines in response to *Xcc568*. (A) Disease index at 3, 5, 7 and 10 days post-inoculation and statistical data. (B) Bacterial growth measurement (colony forming unit (CFU)/cm2 expressed in a log10 scale) in leaves of the wild type accession Col-0 and the different *RKS1* transgenic or mutant lines. Bacterial growth has been measured 0 (grey bars) and 7 (black bars) days after inoculation with *Xcc568* at distance from the inoculation zone (at the tip of the inoculated leaves) with a bacterial suspension adjusted to 10_9 CFU/mL. Data were collected from three independent experiments, each timepoint corresponds to measurements on 3–5 individual plants (four leaves/plant). Statistical analysis was performed using the non-parametric T-test with Welch correction and bacterial growth of Col-0 at day 0 or day 7 as reference.

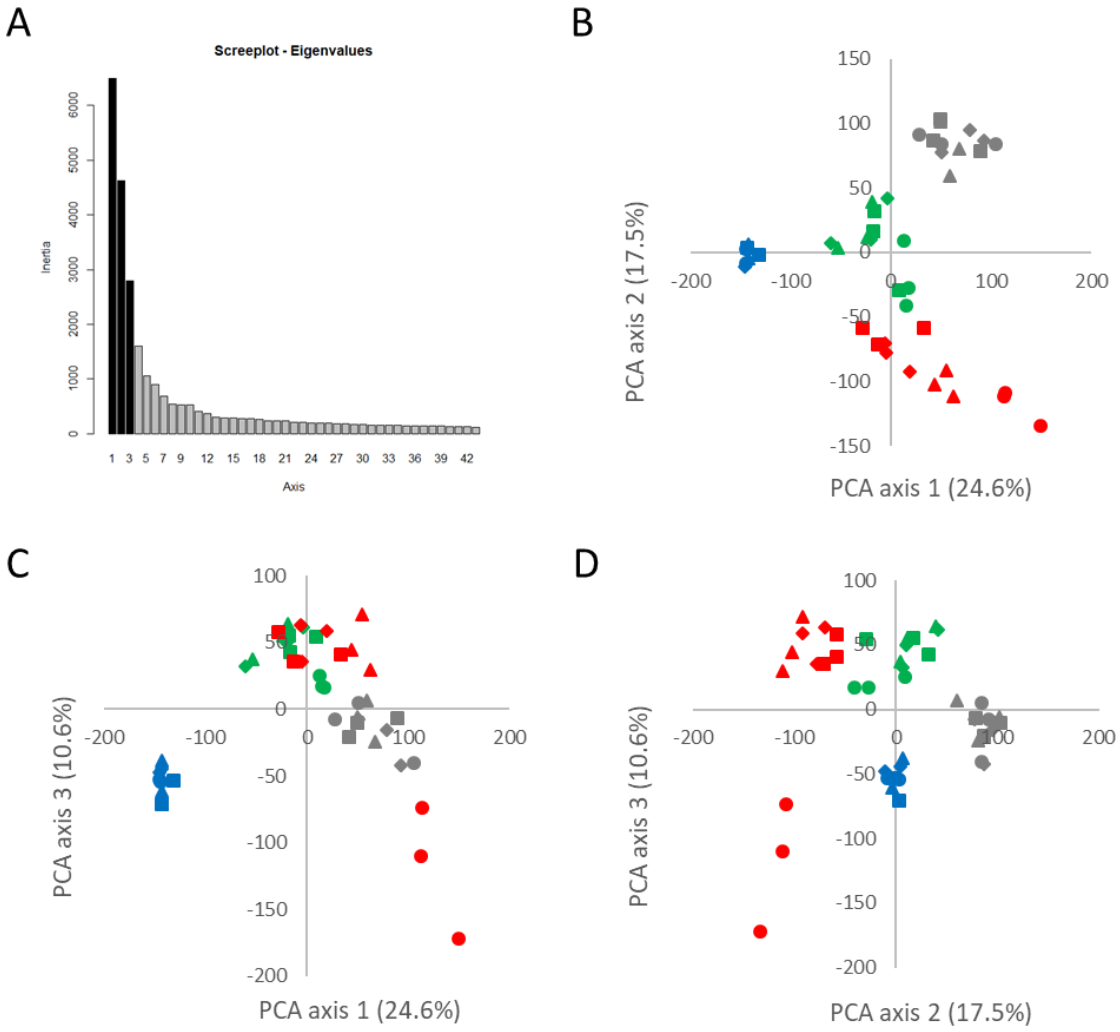


Fig. S3. Principal Component Analysis performed on the RNA-seq dataset of 33602 genes. (A) Eigenvalues distribution showing the relative importance of the Principal Components (PCs). (B) Plot of the PCA axis 1 *vs* the PCA axis 2. Each symbol indicates one genotype (Δ correspond to Col-0 line, ◊ to *rks1-1* mutant, □ to *RKS1-si24* line and ○ is RKS1-OE1) and each timepoint is illustrated by a different color (blue for T0, grey for T1.5, green for T3 and red for T6). (C) Plot of the PCA axis 1 *vs* the PCA axis 3. (D) Plot of the PCA axis 2 *vs* the PCA axis 3. Values in brackets correspond to variance explained by each of the three PCs.

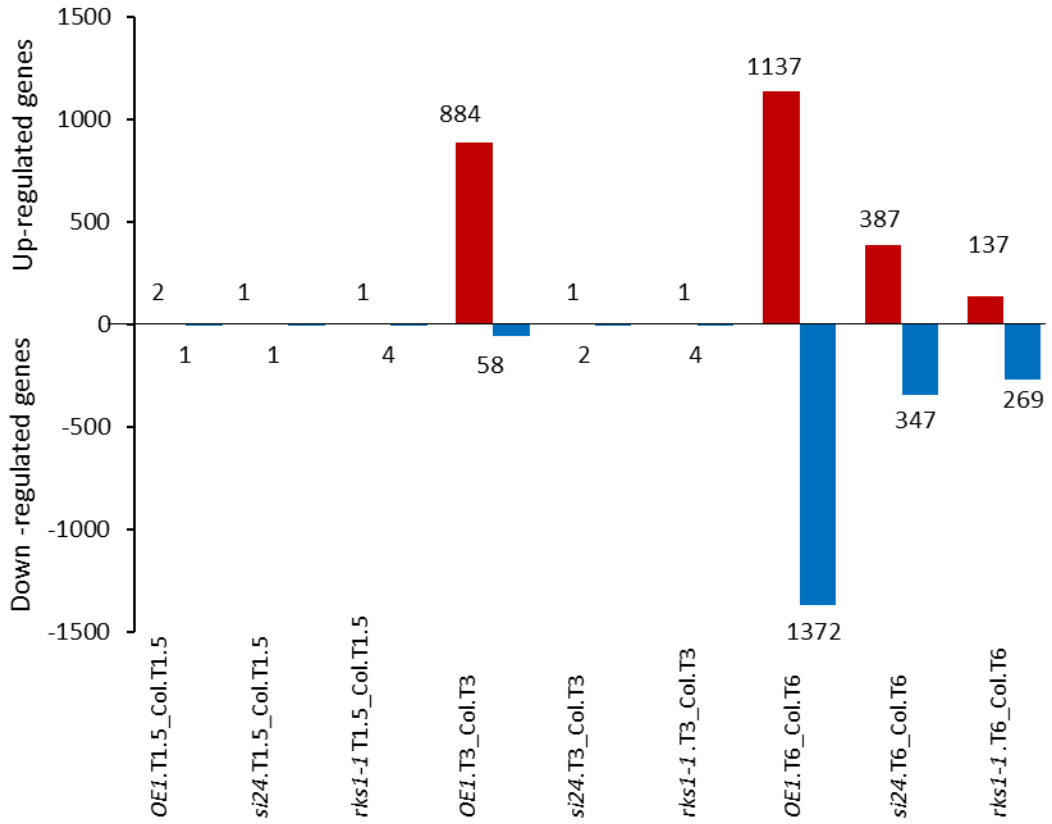


Fig. S4. Genes differentially expressed at different hours post-inoculation with *Xcc568* compared with those expressed in the wild type at the same time points. Red bars indicate up-regulated genes, blue bars indicate down-regulated genes.

A

Class	Gene number per class	Number of genes tested by RT-qPCR	Number of genes validated *	
			RNA-seq material	RNA-seq material + independent experiment + additional lines
UDD	55	8	8	6 - 7
ØDD	117	10	9	5
DUU	26	9	6	5 - 7
ØUU	70	7	4	3

* Genes were considered as validated if expression profiles (determined by using a Wilcoxon statistical test performed on qPCR gene expression data) obtained by Quantitative RT-PCR analyses using RNA extracts from the samples from transcriptomic experiments (left column) or using an independent experiment and additional lines (right column) belonged to the same regulation class. Gene numbers indicated in the last column correspond to genes validated in the 6 *RKS1* lines (first number), or in 5/6 *RKS1* lines (second number).

B

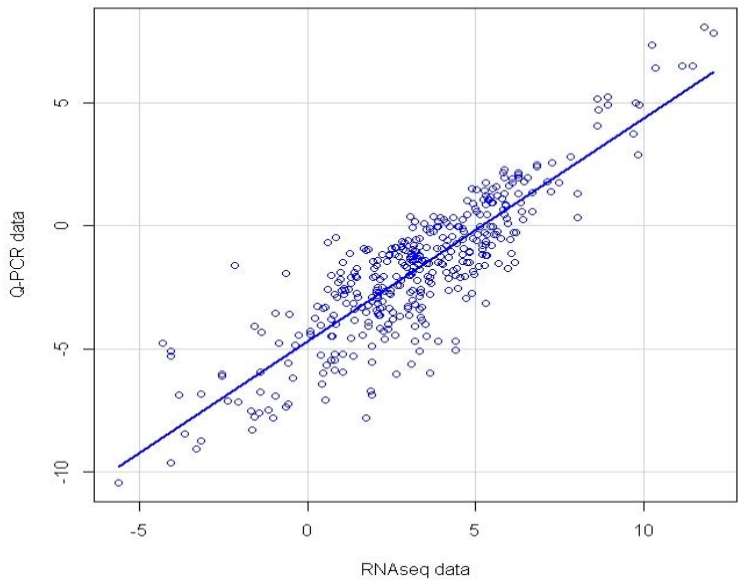


Fig. S5. Quantitative RT-PCR analysis of the expression profiles of genes belonging to the different regulation classes: UDD (Up-regulated in *RKS1-OE1* line, down regulated in *RKS1-si24* and in *rks1-1* lines), ØDD (not affected in *RKS1-OE1* line, down regulated in *RKS1-si24* and in *rks1-1* lines), DUU (down-regulated in *RKS1-OE1* line, up-regulated in *RKS1-si24* and in *rks1-1* lines) and ØUU (not affected in *RKS1-OE1* line, up-regulated in *RKS1-si24* and in *rks1-1* lines). (A) Number of genes validated as compared to the RNA-seq data (as described in *). (B) Statistical relationship between RNA-seq data and RT-qPCR data obtained on 33 genes (3 values per line and 4 lines (Col-0, *RKS1-OE1*, *rks1-1* and *RKS1-si24*)) estimated by Pearson and Spearman correlation tests from R commander (p-value< 0.05). Coefficient of correlation of Pearson = 0.862987; coefficient of correlation of Spearman= 0.8231565.

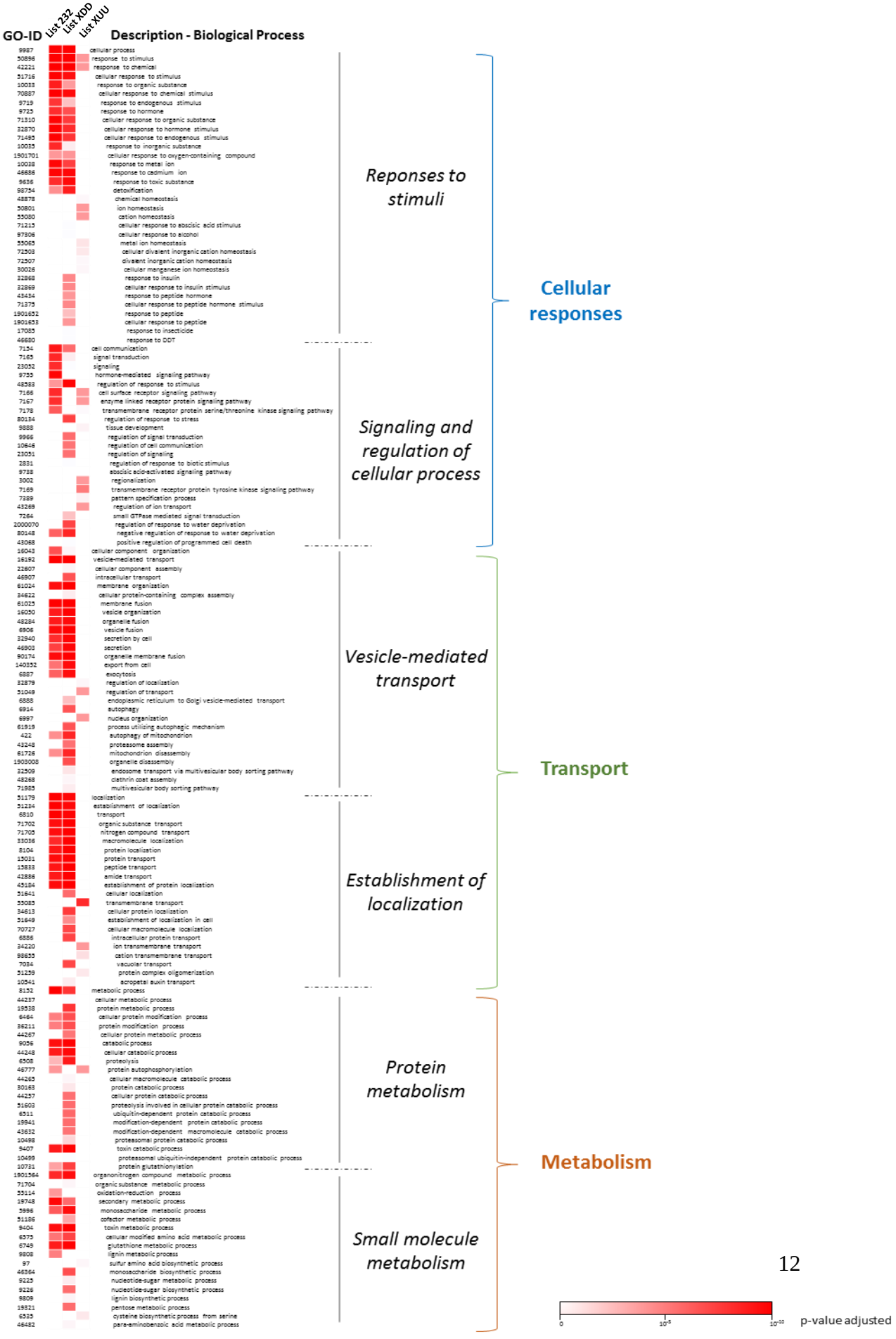
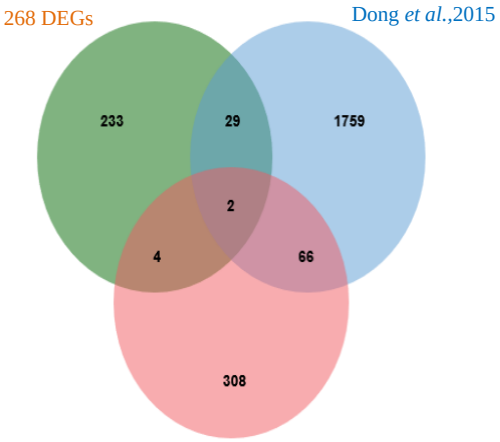

GO-ID
List 232
List XDD
List XUU
Description - Biological Process
9987 cellular process
50896 response to stimulus
42221 response to chemical
51716 cellular response to stimulus
10033 response to organic substance
70887 cellular response to chemical stimulus
9719 response to endogenous stimulus
9725 response to hormone
71310 cellular response to organic substance
32870 cellular response to hormone stimulus
71495 cellular response to endogenous stimulus
10035 response to inorganic substance
1901701 cellular response to oxygen-containing compound
10038 response to metal ion
46686 response to cadmium ion
9636 response to toxic substance
98754 detoxification
48878 chemical homeostasis
50801 ion homeostasis
55080 cation homeostasis
71215 cellular response to abscisic acid stimulus
97306 cellular response to alcohol
55065 metal ion homeostasis
72503 cellular divalent inorganic cation homeostasis
72507 divalent inorganic cation homeostasis
30026 cellular manganese ion homeostasis
32868 response to insulin
32869 cellular response to insulin stimulus
43434 response to peptide hormone
71375 cellular response to peptide hormone stimulus
1901652 response to peptide
1901653 cellular response to peptide
17085 response to insecticide
46680 response to DDT
7154 cell communication
7165 signal transduction
23052 signaling
9755 hormone-mediated signaling pathway
48583 regulation of response to stimulus
7166 cell surface receptor signaling pathway
7167 enzyme linked receptor protein signaling pathway
7178 transmembrane receptor protein serine/threonine kinase signaling pathway
80134 regulation of response to stress
9888 tissue development
9966 regulation of signal transduction
10646 regulation of cell communication
23051 regulation of signaling
2831 regulation of response to biotic stimulus
9738 abscisic acid-activated signaling pathway
3002 regionalization
7169 transmembrane receptor protein tyrosine kinase signaling pathway
7389 pattern specification process
43269 regulation of ion transport
7264 small GTPase mediated signal transduction
2000070 regulation of response to water deprivation
80148 negative regulation of response to water deprivation
43068 positive regulation of programmed cell death
16043 cellular component organization
16192 vesicle-mediated transport
22607 cellular component assembly
46907 intracellular transport
61024 membrane organization
34622 cellular protein-containing complex assembly
61025 membrane fusion
16050 vesicle organization
48284 organelle fusion
6906 vesicle fusion
32940 secretion by cell
46903 secretion
90174 organelle membrane fusion
140352 export from cell
6887 exocytosis
32879 regulation of localization
51049 regulation of transport
6888 endoplasmic reticulum to Golgi vesicle-mediated transport
6914 autophagy
6997 nucleus organization
61919 process utilizing autophagic mechanism
422 autophagy of mitochondrion
43248 proteasome assembly
61726 mitochondrion disassembly
1903008 organelle disassembly
32509 endosome transport via multivesicular body sorting pathway
48268 clathrin coat assembly
71985 multivesicular body sorting pathway
51179 localization
51234 establishment of localization
6810 transport
71702 organic substance transport
71705 nitrogen compound transport
33036 macromolecule localization
8104 protein localization
15031 protein transport
15833 peptide transport
42886 amide transport
45184 establishment of protein localization
51641 cellular localization
55085 transmembrane transport
34613 cellular protein localization
51649 establishment of localization in cell
70727 cellular macromolecule localization
6886 intracellular protein transport
34220 ion transmembrane transport
98655 cation transmembrane transport
7034 vacuolar transport
51259 protein complex oligomerization
10541 acropetal auxin transport
8152 metabolic process
44237 cellular metabolic process
19538 protein metabolic process
6464 cellular protein modification process
36211 protein modification process
44267 cellular protein metabolic process
9056 catabolic process
44248 cellular catabolic process
6508 proteolysis
46777 protein autophosphorylation
44265 cellular macromolecule catabolic process
30163 protein catabolic process
44257 cellular protein catabolic process
51603 proteolysis involved in cellular protein catabolic process
6511 ubiquitin-dependent protein catabolic process
19941 modification-dependent protein catabolic process
43632 modification-dependent macromolecule catabolic process
10498 proteasomal protein catabolic process
9407 toxin catabolic process
10499 proteasomal ubiquitin-independent protein catabolic process
10731 protein glutathionylation
1901564 organonitrogen compound metabolic process
71704 organic substance metabolic process
55114 oxidation-reduction process
19748 secondary metabolic process
5996 monosaccharide metabolic process
51186 cofactor metabolic process
9404 toxin metabolic process
6575 cellular modified amino acid metabolic process
6749 glutathione metabolic process
9808 lignin metabolic process
97 sulfur amino acid biosynthetic process
46364 monosaccharide biosynthetic process
9225 nucleotide-sugar metabolic process
9226 nucleotide-sugar biosynthetic process
9809 lignin biosynthetic process
19321 pentose metabolic process
6535 cysteine biosynthetic process from serine
46482 para-aminobenzoic acid metabolic process
Reponses to stimuli
Signaling and regulation of cellular process
Cellular responses
Vesicle-mediated transport
Establishment of localization
Transport
Protein metabolism
Small molecule metabolism
Metabolism
0
10-5
10-10
p-value adjusted

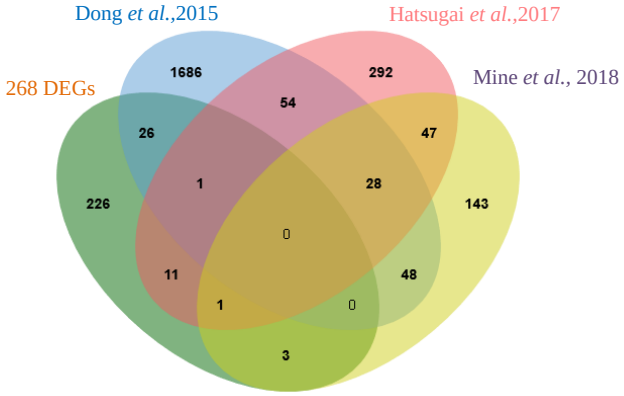
Fig. S6. Gene ontology analyses on the 268 co-regulated genes reveal multiple gene functional modules.

The analysis was conducted by using BINGO module from Cytoscape software. GO process annotation were recover for 232 genes and for genes down-regulated in *RKS1si24* and in *rks1-1* lines (XDD) and genes up-regulated in *RKS1-si24* and in *rks1-1* lines (XUU). The heatmap shows the overrepresentation significance (p-value<0.05) of GO biological process terms across the different classes. The lines highlight GO terms participating in same process.

A



B



C

23 Common genes between the 268 DEGs and PTI genes	
Accession number	Gene description
AT5G44572	Transmembrane protein
AT1G74940	DUF581
AT2G30740	Protein kinase
AT2G44210	DUF239
AT5G61520	Major facilitator protein
AT2G45920	U-box protein
AT3G03320	RNA-binding protein
AT2G47000	ABCB4
AT4G00710	BSK3
AT2G38170	CAX1
AT1G75270	DHAR2
AT5G54650	Fh5
AT3G52930	FBA8
AT4G14630	GLP9
AT1G33240	GTL1
AT4G32980	ATH1
AT4G27730	OPT6
AT1G65390	PP2-A5
AT3G45780	PHOT1
AT3G11330	PIRL9
AT5G47200	RAB1A
AT5G07250	RBL3
AT3G04670	WRKY39

30 Common genes between the 268 DEGs and ETI genes	
Accession number	Gene description
AT5G01380	Homeodomain-like protein
AT4G26470	EF-hand protein
AT1G24350	Acid phosphatase
AT4G37030	Membrane protein
AT5G64850	sorbin/SH3 protein
AT2G29670	TPR-like protein
AT5G07910	LRR protein
AT2G18193	Hydrolase protein
AT5G62890	Xanthine permease
AT1G70490	ARFA1D
AT5G63880	VPS20.1
AT1G66600	WRKY63
AT5G20910	AIP2
AT2G22470	AGP2
AT5G11520	ASP3
AT4G21980	G8A
AT2G32210	HCYSTM6
AT2G30770	CYP71A13
AT2G34500	CYP710A1
AT3G12620	PP2C.D3
AT2G30550	DALL3
AT2G31570	GPX2
AT1G78380	GSTU19
AT2G29460	GSTU4
AT5G02780	GSTL1
AT3G17420	GPK1
AT4G32190	PII1
AT4G18205	PUP21
AT5G14420	RGLG2
AT5G11390	WIT1

12 Common genes between the 268 DEGs, and PTI and ETI gene lists	
Accession number	Gene description
AT2G29320	NAD(P)-binding protein
AT2G44380	Cys/His-rich protein
AT3G57090	BIGYIN
AT2G45770	CPFTSY
AT3G48740	SWEET11
AT4G22710	CYP706A2
AT2G47730	GSTF8
AT4G39050	KIN7.4
AT1G65610	KOR2
AT2G38360	PRA1.B4
AT4G25230	RIN2
AT2G15480	UGT73B5

Fig. S7. Identification within the 268 DEGs of genes previously associated with gene networks of PTI or ETI immune responses (Dong *et al.,* 2015; Hatsugai *et al*., 2017; Mine *et al.,* 2018). Venn diagrams established with the 268 DEGs identified in this study and (A) the PTI genes and (B) the

ETI genes previously identified. (C) Accession numbers and description of genes found in common between the 268 DEGs identified in this study and the PTI (left), ETI (center) and PTI and ETI (right) genes.

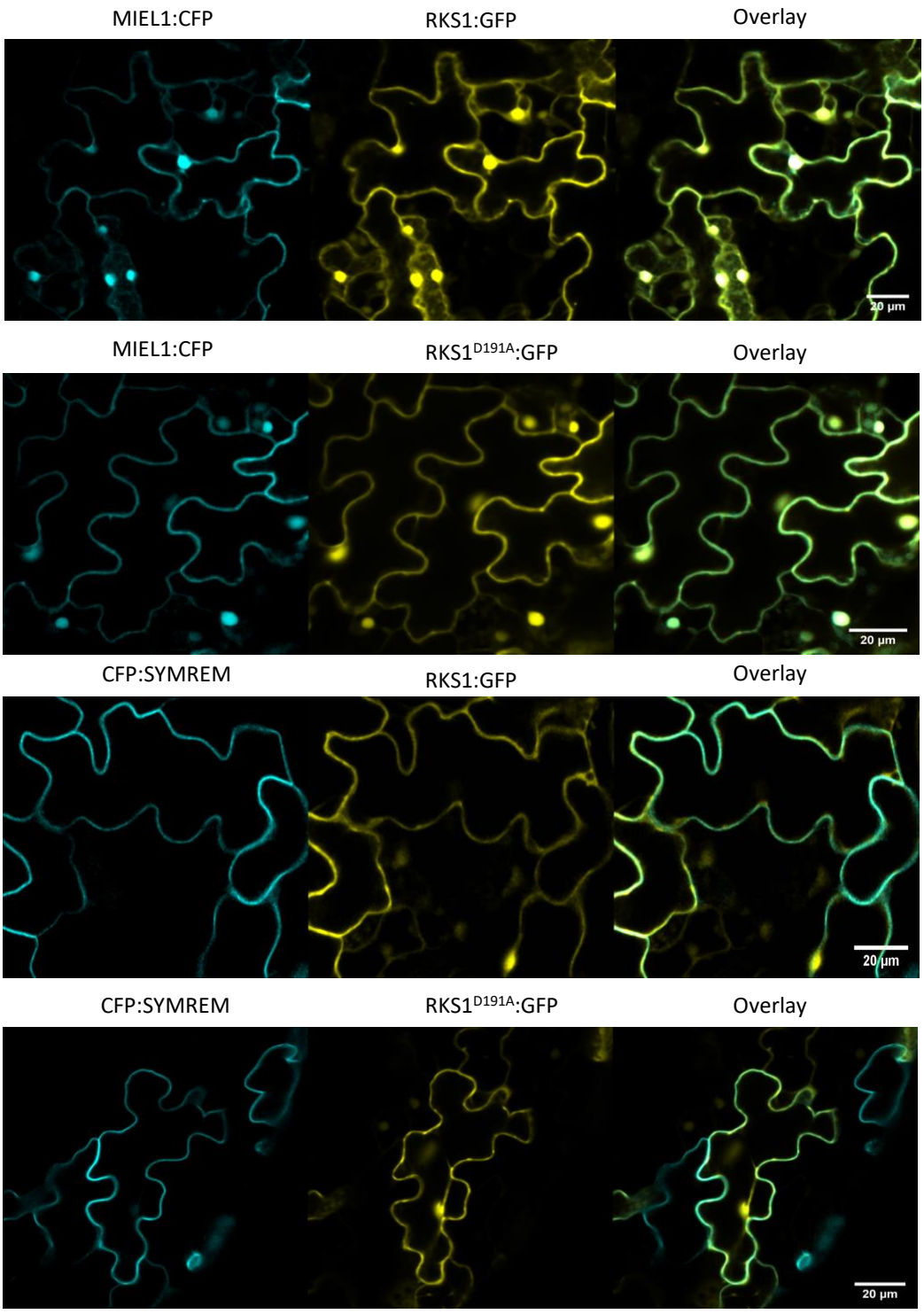
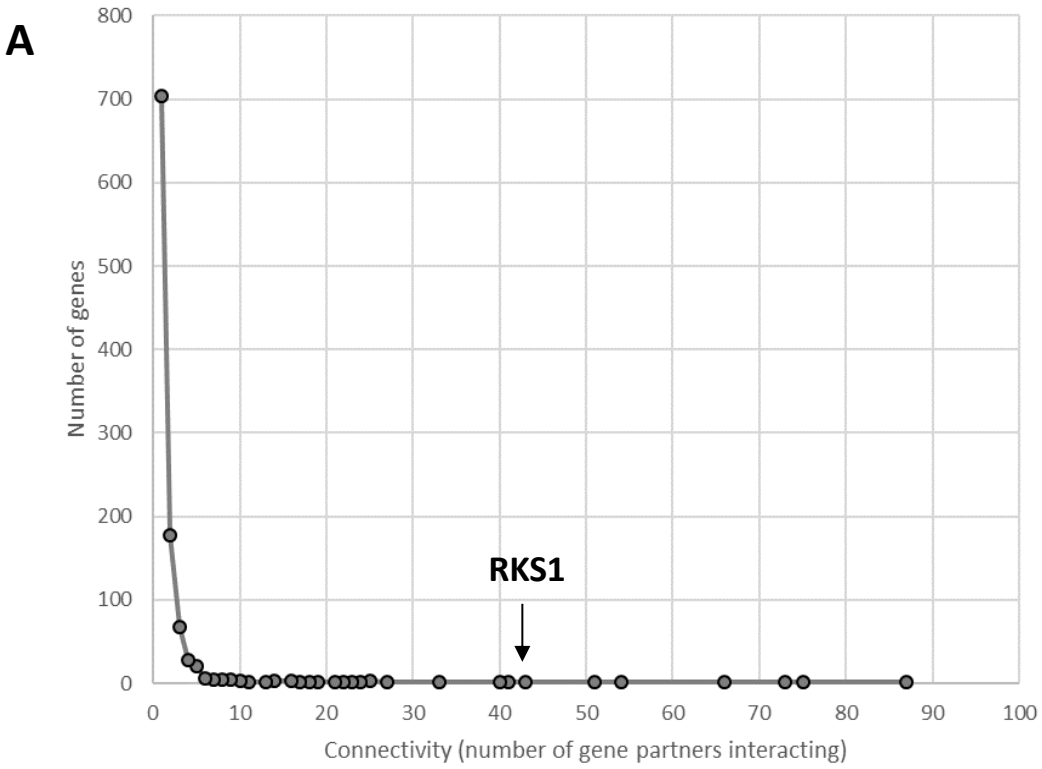


Fig. S8. RKS1 localizes in the nucleus, the cytoplasm and the plasma membrane. Confocal images of epidermal cells of Arabidopsis seedlings 72 h after Agrobacterium mediated transient expression of the indicated constructs. We used RKS1 and a mutated version of RKS1 (RKS1^{D191A}) fused to the GFP. MIEL1:CFP was used as a subcellular marker of the cytoplasm and nucleus of Arabidopsis cells (6), CFP:MtSYMREM as a subcellular marker of the plasma membrane (7). Co-localization of RKS1 or the mutated version of RKS1 with the two subcellular markers is shown in the merge panel (right). Scale bars= 20µM.



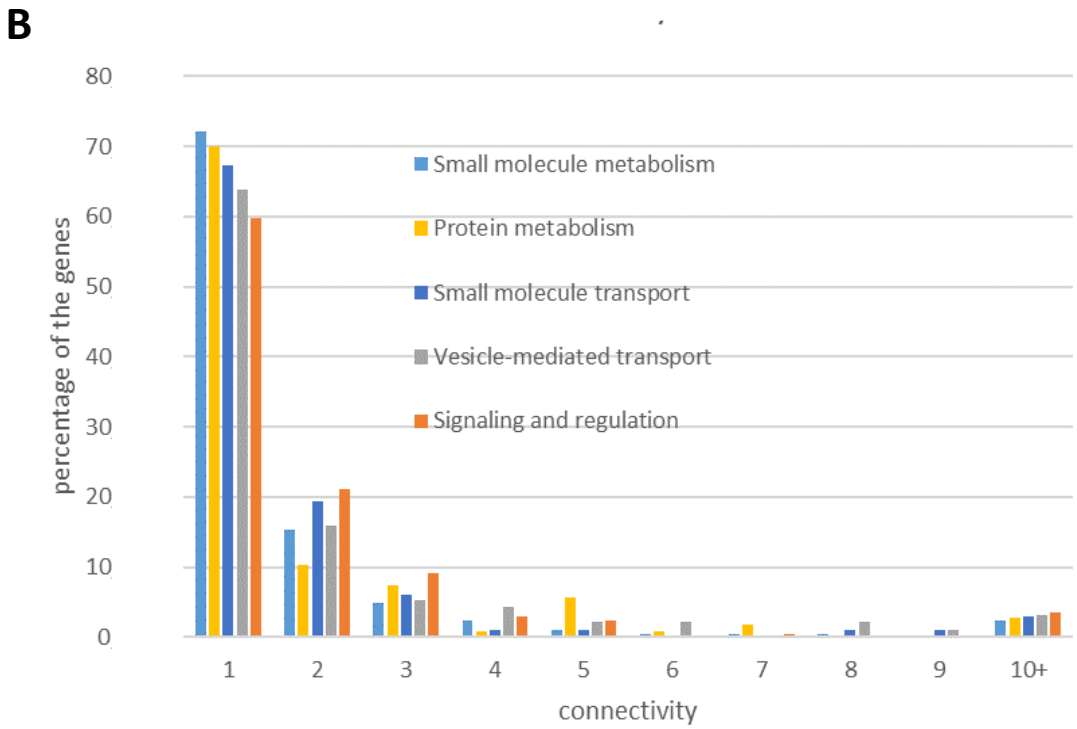
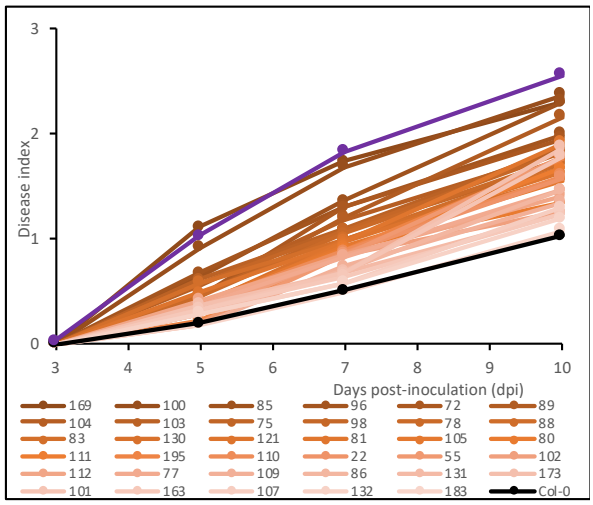


Fig. S9. Analysis of the network connectivity. (A) Number of genes by connectivity. RKS1 is the 7th top hub in the network with 44 connections. (B) Percentage of the genes by connectivity distributed in the 5 functional groups.

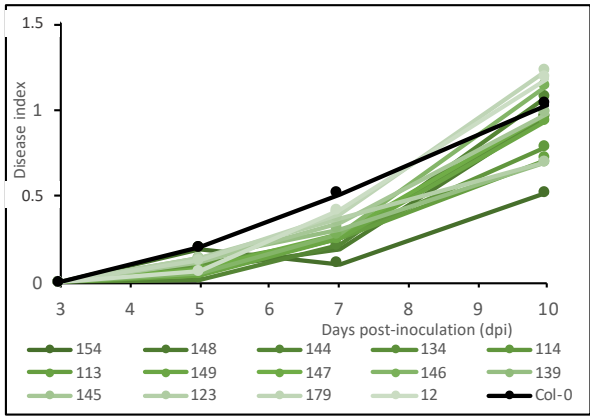
A

Gene	mutants lines	Disease index 3dpi	Disease index 5dpi	Disease index 7dpi	Disease index 10dpi	Phenotype Class	T-DNA insertion localisation	T-DNAgenome insertion site	Gene Expression Estimation
AT1G05785	170	0	0.19	0.42	1.00	WT	3'UTR	Chr1_1732442	+*
AT1G52540	134	0	0.08	0.25	0.96	R	5'UTR	Chr1_19571727	+*
AT1G53850	85	0	0.64	1.36	2.31	S	5'UTR	Chr1_20105934	+*
	128	0	0.20	0.57	0.92	WT	ORF	Chr1_20104861	+
	129	0	0.03	0.57	0.89	WT	upstream 5'UTR	Chr1_20106174	+
AT1G67070	26	0	0	0.58	1.71	WT	ORF	Chr1_24997945	-*
	111	0	0.29	0.90	1.67	S	5'UTR	Chr1_24995969	-*
AT1G69790	130	0	0.38	0.98	1.57	S	5'UTR	Chr1_26266784	-*
	131	0	0.28	0.69	1.23	S	ORF	Chr1_26266204	-*
AT1G78380	98	0	0.57	1.08	1.82	S	3' UTR	Chr1_29464359	-[a]
AT2G24850	tat3-1	0	0.37	0.54	1.35	WT	ORF	Chr2_10583243	+*[a]
AT2G26410	191	0	0.28	0.44	1.00	WT	ORF	Chr2_11235597	(-)
AT2G32390	88	0	0.34	1.00	1.71	S	3' UTR	Chr2_13444417	-*
	121	0	0.49	0.98	1.63	S	5' UTR	Chr2_13448374	-*
	77	0	0.36	0.83	1.46	S	ORF	Chr2_13445725	-*
AT2G36020	109	0	0.18	0.73	1.35	S	ORF	Ch2_14833120	+*
AT2G43490	169	0	1.11	1.74	2.30	S	ORF	Chr2_18056144	-
	168	0	0.48	0.83	1.35	WT	ORF	Chr2_18057293	+
AT3G01650	75	0	0.47	1.08	1.73	S	ORF	Chr3_240740	-*
	76	0	0.04	0.57	1.07	WT	5' UTR	Chr3_232307	-*
AT3G05710	163	0	0.33	0.65	1.25	S	ORF	Chr3_1686286	=
AT3G07370	103	0	0.61	1.09	1.87	S	ORF	Chr3_2373436	-*
	78	0	0.46	1.05	1.76	S	ORF	Chr3_2373693	-*
	54	0	0.25	0.40	1.15	WT	5' UTR	Chr3_2375359	=
AT3G12620	144	0	0.02	0.21	1.08	R	ORF	Chr3_4009571	-*
	145	0	0.12	0.35	0.98	R	ORF	Chr3_4010068	-*
AT3G13235	183	0	0.19	0.50	1.08	S/WT	ORF	Chr3_4272731	=
AT3G15980	173	0	0.38	0.69	1.88	S	ORF	Chr3_5415270	-*
AT3G17420	141	0	0.25	0.40	0.96	WT	ORF	Chr3_5960762	-*
AT3G19230	139	0	0.14	0.31	0.69	R	ORF	Chr3_6661716	-*
AT3G26340	100	0	0.92	1.69	2.37	S	ORF	Chr3_9615183	-*
	105	0	0.39	0.93	1.56	S	5'UTR	Chr3_9615855	+*
AT3G54300	154	0	0.19	0.11	0.52	R	upstream 5'UTR	Chr3_20110528	-*
AT3G57090	101	0	0.32	0.68	1.79	S	3'UTR	Chr3_20740664	=
	115	0	0.09	0.51	1.19	WT	ORF	Chr3_20740462	-*
AT3G59110	132	0	0.29	0.58	1.19	S	ORF	Chr3_21856602	-*
	133	0	0.19	0.33	0.92	WT	ORF	Chr3_21857573	=
AT4G00710	195	0	0.23	0.88	1.77	S	5'UTR	Chr4_320461	-*
	194	0	0.05	0.43	0.95	WT	5'UTR	Chr4_290458	-*
AT4G01960	104	0	0.56	1.19	1.83	S	5'UTR	Chr4_869623	=
	102	0	0.31	0.84	1.25	S	5'UTR	Chr4_853159	=
AT4G12120	55	0	0.20	0.85	1.79	S	3' UTR	Chr4_7436195	-*[a]
	61	0	0.05	0.46	1.15	WT	ORF	Chr4_7439099	=
AT4G15780	151	0	0.35	0.57	1.24	WT	5'UTR	Chr4_8981597	+
	152	0	0.23	0.62	1.07	WT	ORF	Chr4_8979601	+
AT4G19006	187	0	0.38	0.54	1.46	WT	ORF	Chr4_10410105	=
AT4G19040	72	0	0.45	1.30	1.95	S	ORF	Chr4_10695490	+
	86	0	0.29	0.69	1.46	S	ORF	Chr4_10435374	+
	80	0	0.38	0.92	1.34	S	ORF	Chr4_10435951	+
	114	0	0.11	0.25	0.79	R	5'UTR	Chr4_10437913	-
AT4G19170	107	0	0.29	0.58	1.30	S	ORF	Chr4_10483036	-*[a]
AT4G30260	164	0	0.40	0.52	1.31	WT	5'UTR	Chr4_14818697	=
	165	0	0.19	0.33	1.19	WT	ORF	Chr4_14816938	=
AT5G02320	12	0	0.06	0.42	1.19	R	ORF	Chr5_469574	-
AT5G07180	146	0	0.06	0.28	1.14	R	ORF	Chr5_2230477	-*
	147	0	0.08	0.28	0.98	R	ORF	Chr5_2231242	-*
	148	0	0.08	0.19	0.96	R	ORF	Chr5_2228526	-*
AT5G11610	110	0	0.39	0.88	1.60	S	3' UTR	Chr5_3747502	-*[a]
	112	0	0.41	0.84	1.42	S	3' UTR	Chr5_3747577	=
AT5G14420	96	0	0.68	1.31	2.00	S	ORF	Chr5_4657392	-
	122	0	0.31	0.46	0.98	WT	ORF	Chr5_4650498	-*
	123	0	0.14	0.38	0.70	R	ORF	Chr5_4648653	-
AT5G20480	149	0	0.04	0.25	0.94	R	ORF	Chr5_6924648	-*
AT5G23540	179	0	0.13	0.40	1.23	R	3' UTR	Chr5_7937377	+[a]
AT5G28020	89	0	0.45	1.20	2.17	S	ORF	Chr5_10053642	-
	83	0	0.58	1.00	1.68	S	ORF	Chr5_10053872	-*
	22	0	0.29	0.87	1.58	S	ORF	Chr5_10107148	-*
AT5G44190	190	0	0.38	0.52	1.13	WT	ORF	Chr5_17800212	-
AT5G47200	156	0	0.33	0.64	1.19	WT	ORF	Chr5_19166902	-
AT5G67600	81	0	0.30	0.96	1.91	S	ORF	Chr5_26716016	-*
	113	0	0.06	0.25	0.71	R	upstream 5'UTR	Chr5_26717084	+
NA	Col-0	0	0.20	0.51	1.03				
AT3G57710	rks1-1	0	1.02	1.83	2.56				

B



C



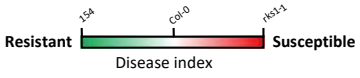


Fig. S10. Molecular and phenotypic analysis of insertional mutants corresponding to genes belonging to functional modules of the *RKS1* dependent network.

(A) Mutant lines, corresponding gene accessions and mutant phenotypes after inoculation with a bacterial suspension of *Xcc568* adjusted to 2.10^8 cfu/mL. A heatmap highlights time course evaluation of mutant disease index as compared to the wild type (Col-0) disease index. Disease symptoms were observed on leaves of mutant and wild-type plants at 3, 5, 7 and 10 days post-inoculation (dpi). Means were calculated from 4–24 plants. Green represents disease index significantly reduced as compared to Col-0 (more resistant), Red represents disease index

significantly increased as compared to Col-0 (more susceptible) and white, not significantly different from Col-0. P-value numbers represent kinetic modeling deference with Col-0, 0 = p-value>0.05 and 1= p-value<= 0.05. The phenotype is indicated as R (resistant), S (susceptible) or WT (not significantly different from Col-0). For each mutant, location of the T-DNA insertion was determined by sequencing and indicated (5'UTR, ORF or 3'UTR regions, T-DNA genome insertion site). Gene expression was evaluated by RT-qPCR in the mutant plants, and indicated as + (increased in the mutant as compared to Col-0), - (decreased in the mutant as compared to Col-0) and by = (not affected in the mutant as compared to Col-0) in the last column. Statistical analysis was performed by comparing the kinetic of the average disease index of each mutant to the kinematic of the average disease index of Col-0 (see Material and Methods section) and * indicates that gene expression is significantly different in the mutant as compared to Col-0 (p-value $\leq$ 0.05). RT-qPCR was performed with primers downstream the T-DNA insertion except for mutants mentioned by [a]. (B and C) Time course evaluation of mutant phenotype in response to inoculation with *Xcc*, according to their "phenotype class" susceptible (B) or resistant (C). *rks1-1* phenotype is represented in purple.

A

	Disease index				Standard deviations												
Line	**3 dpi**	**5 dpi**	**7 dpi**	**10 dpi**	**3 dpi**	**5 dpi**	**7 dpi**	**10 dpi**	**nb_exp**	**nb_plants**	**Diff**	**Intercept**	**iLCI**	**iUCI**	**Slope**	**sLCI**	**sUCI**
dde2-2	0	0	0	0.45	0	0	0	0.1	2	10	1	3.30	2.69	3.91	-0.34	-0.61	-0.08
ein2-1	0	0	0.07	0.21	0	0	0	0.1	3	18	1	0	0	0	0.50	0.39	0.61
pad4-1	0	0.03	0.12	0.24	0	0	0	0.1	3	19	1	-1.82	-2.46	-1.18	1.34	1.05	1.62
sid2-2	0	0.01	0.12	0.43	0	0	0	0.1	3	19	0	0	0	0	1.03	0.94	1.12
dde2-2/ein2-1	0	0	0.05	0.10	0	0	0	0.0	2	10	1	0	0	0	0.24	0.11	0.36
dde2-2/sid2-2	0	0.05	0.41	0.82	0	0	0.1	0.1	2	11	0	0	0	0	1.92	0.96	2.88
dde2-2/pad4-1	0	0	0.05	0.22	0	0	0	0.1	3	15	1	0	0	0	0.52	0.46	0.58
ein2-1/pad4-1	0	0.02	0.06	0.24	0	0	0	0.1	3	18	1	0	0	0	0.57	0.54	0.60
dde2-2/ein2-1/sid2-2	0	0	0.03	0.10	0	0	0	0.0	2	10	1	0	0	0	0.24	0.21	0.27
dde2-2/ein2-1/pad4-1	0	0	0	0.13	0	0	0	0.0	2	10	1	0.92	0.75	1.09	-0.10	-0.17	-0.02
dde2-2/pad4-1/sid2-2	0	0	0	0.15	0	0	0	0.0	2	10	1	1.10	0.90	1.30	-0.11	-0.20	-0.03
ein2-1/pad4-1/sid2-2	0	0	0	0.08	0	0	0	0.0	2	10	1	0.55	0.45	0.65	-0.06	-0.10	-0.01
quadruple	0	0	0	0.05	0	0	0	0.0	2	11	1	0.33	0.27	0.39	-0.03	-0.06	-0.01
Col-0	0	0.03	0.10	0.42	0	0	0	0.1	3	29							
rks1-1	0	0.26	0.69	1.73	0	0.1	0.2	0.2	3	32	1	-8.20	-14.66	-1.74	7.50	4.66	10.34
Kas-1	0	0.65	1.60	2.87	0	0.1	0.2	0.2	3	15	1	-27.47	-45.60	-9.33	18.28	10.30	26.26

B

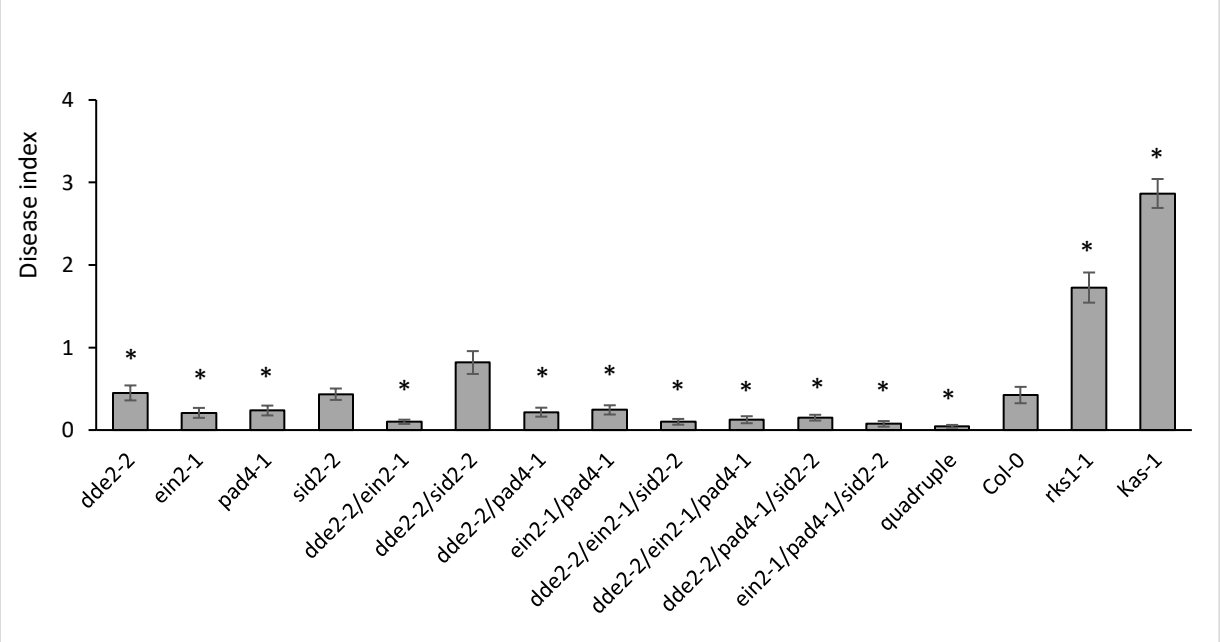


Fig. S11. Analysis of mutant lines described to impair PTI/ETI networks in response to *Xcc568*. (A) Disease index at 3, 5, 7 and 10 days post-inoculation and statistical data. (B) Evaluation of disease index 10 dpi after inoculation with a bacterial suspension adjusted to 2.10^8 cfu/mL. Means and standard errors were calculated from 10-32 plants in two or three independent experiments. * represent kinematic modeling deference with Col-0 time course. 0 = p-value> 0.05 and 1 = p-value= 0.05.

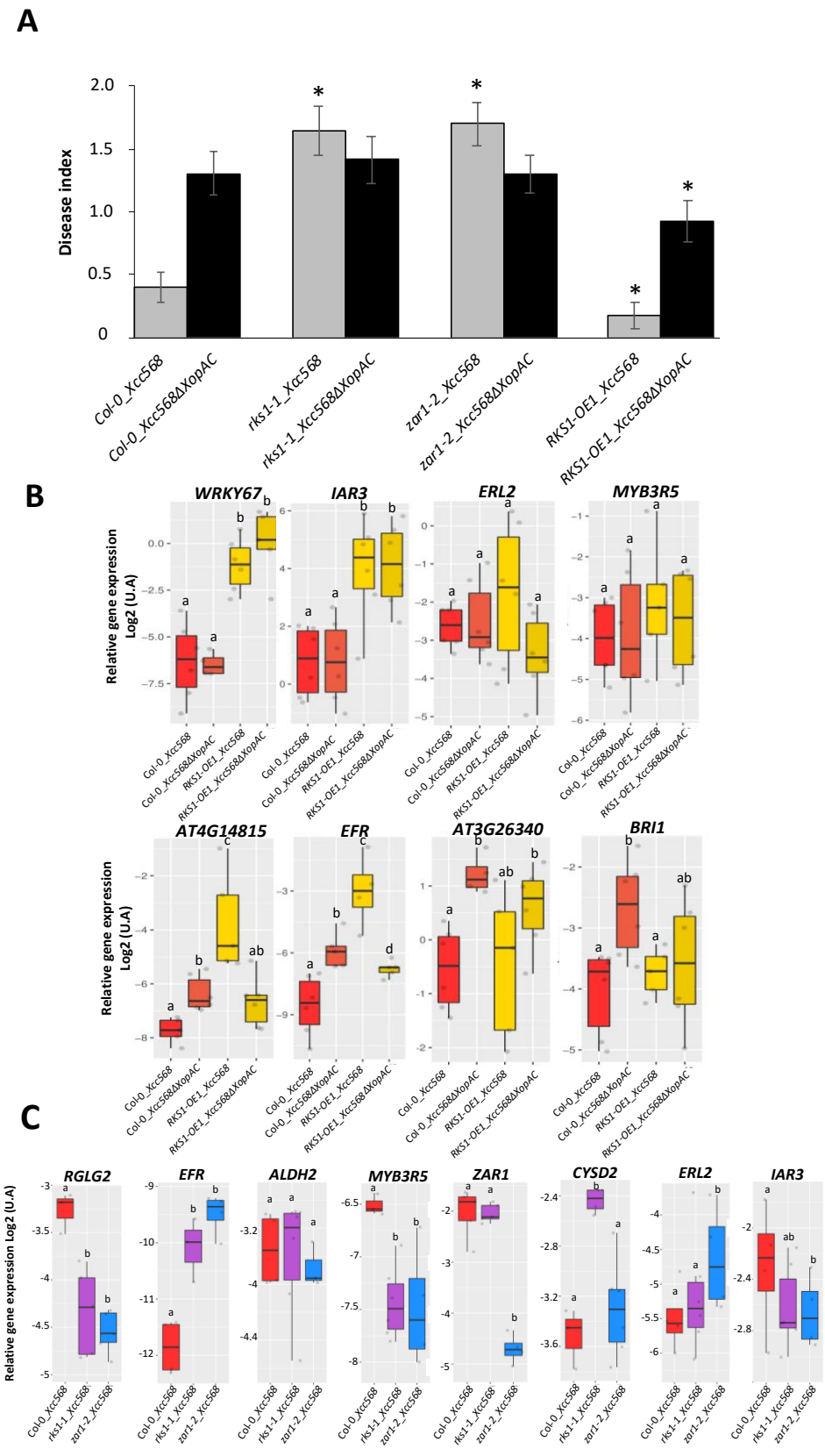

A
Disease index
2.0
1.5
1.0
0.5
0
Col-0_Xcc568
Col-0_Xcc568ΔXopAC
rks1-1_Xcc568
rks1-1_Xcc568ΔXopAC
zar1-2_Xcc568
zar1-2_Xcc568ΔXopAC
RKS1-OE1_Xcc568
RKS1-OE1_Xcc568ΔXopAC
B
WRKY67
IAR3
ERL2
MYB3R5
AT4G14815
EFR
AT3G26340
BRI1
Relative gene expression Log2 (U.A)
C
RGLG2
EFR
ALDH2
MYB3R5
ZAR1
CYSD2
ERL2
IAR3
Relative gene expression Log2 (U.A)

Fig. S12. Analysis of resistance phenotypes of *RKS1* mutant or transgenic lines and of DEG expression profiles in response to *Xcc568* and *Xcc568ΔXopAC*. (A) Disease index at 7 dpi after inoculation of *rks1-1, zar1-2, RKS1-OE1* and the wild-type accession Col-0 with a bacterial suspension of *Xcc568* or *Xcc568ΔXopAC* strains adjusted to 2.10^8 cfu/mL. Means were calculated from 8–10 plants on 2 independent experiments. * represents kinetic modeling deference with the corresponding Col-0 (Col-0_*Xcc568* or Col-0_*Xcc568ΔXopAC)* time course, * = p-value≤0.05. (B) Analysis by quantitative RT-PCR of the expression profile for 8 specific genes, 6 hours after inoculation in *RKS1-OE1* and the WT leaves inoculated with *Xcc568* or *Xcc568ΔXopAC* (2.10^8 cfu/mL) . Each gene corresponds to a DEG expression class or RKS1 network component: *WRKY67 and IAR3* (class UDD), *ERL2* (ØUU class), *MYB3R5 (*ØDD class), *AT4G14815*, *AT3G26340* and *BRI1* as components of the RKS1 dependent network, EFR as Yeast Two Hybrid candidate. The 4 genes *WRKY67, IAR3, ERL2* and *MYB3R5* present a XopAC expression profile independent of the presence of XopAC, while *AT4G14815*, *EFR*, *AT3G26340* and *BRI1* exhibit a XopAC-dependent expression profile. Statistical groups were generated with the Wilcoxon test, based on 6 plants/strain/line. (C) Analysis by quantitative RT-PCR of the expression profile for 8 specific genes, 6 hours after inoculation in Col-0, *rks1-1* and *zar1-2* inoculated with *Xcc568* (2.10^8 cfu/mL). Each gene corresponds to a DEG expression class or RKS1 network component: *IAR3* (class UDD), *CYSD2* and *ERL2* (ØUU class), *RGLG2* and *MYB3R5* (ØDD class), *ALDH2* and *ZAR1* as components of the *RKS1* dependent network, *EFR* as a Yeast Two Hybrid candidate. Statistical groups were generated with the Wilcoxon test, based on 6 plants/strain/line.

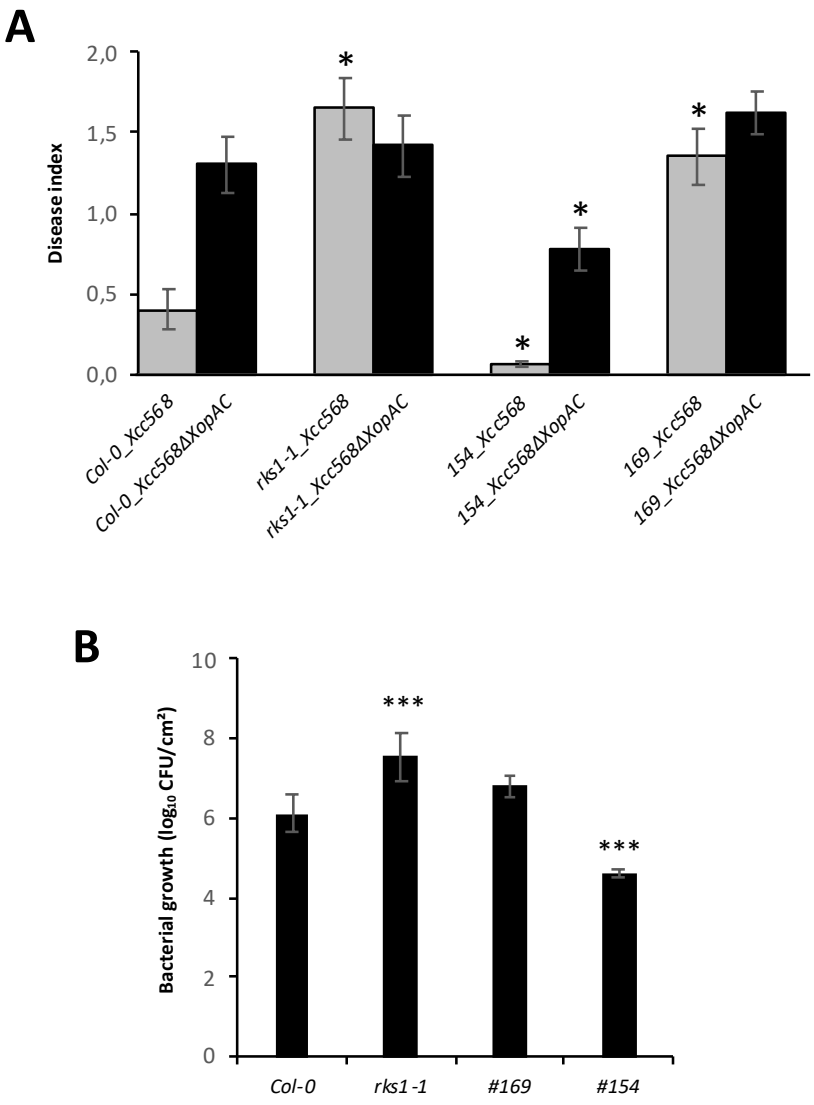


Fig. S13. Analysis of resistance phenotypes of two mutants corresponding to genes of the RKS1-dependent network in response to *Xcc568* and *Xcc568ΔXopAC*. (A) Disease index of *rks1-1, 154, 169* mutants and the WT at 7 dpi with a bacterial suspension of *Xcc568* or *Xcc568ΔXopAC* (2.10^8 cfu/mL). Data were collected from three independent experiments. Means were calculated from 8–10 plants on 2 independent experiments. * represents kinetic modeling deference with the corresponding Col-0 (Col-0_*Xcc568* or Col-0_*Xcc568ΔXopAC)* time course, * = p-value≤ 0.05. (B) Bacterial growth measurement in leaves of the WT and the mutants *rks1-1, 154 and 169*. Bacterial growth has been measured 7 dpi with *Xcc568* at distance from the inoculation zone (at the tip of the inoculated leaves) with a bacterial suspension adjusted to 2.10^8 CFU/mL. Data were collected from three independent experiments, each timepoint corresponds to measurements on 3–5 individual plants (four leaves/plant). Statistical analysis was performed using the parametric test ANOVA and bacterial growth of Col-0 at day 0 as reference.

Table S1. Effects of genetic line and time on global change of expression across the genome. (A) Complete model. Var Expl : variance explained by each model term (expressed in percent). (B) Reduced model for each time point.

A	**PCA axis 1 (24.6%)**			**PCA axis 2 (17.5%)**			**PCA axis 3 (10.6%)**		
Terms	**F**	***P***	**Var Expl (%)**	**F**	***P***	**Var Expl (%)**	**F**	***P***	**Var Expl (%)**
Line	7.8	6.1E-04	0.9	3.9	1.9E-02	1.0	18.1	1.0E-06	6.9
Time	185.0	1.5E-18	86.5	237.4	5.2E-20	92.5	42.7	1.4E-10	36.1
Line * Time	6.2	8.2E-05	8.3	2.0	7.9E-02	1.8	12.5	1.1E-07	45.8

B	**PCA axis 1 (24.6%)**		**PCA axis 2 (17.5%)**		**PCA axis 3 (10.6%)**	
Time	**F**	***P***	**F**	***P***	**F**	***P***
T0	1.23	0.4075	0.15	0.9262	0.91	0.5114
T1.5	0.22	0.8772	0.24	0.8628	0.36	0.7836
T3	3.87	0.0559	1.67	0.2501	5.95	**0.0195**
T6	24.14	**0.0002**	14.79	**0.0013**	25.59	**0.0002**

Table S2. A list of candidate genes identified by Yeast Two-Hybrid screening (2 rounds) using RKS1^{D191A} as bait and cDNAs generated from mRNA isolated from Arabidopsis leaves infected with *Xcc* (strain 147) as prey, including their accession number or gene description (TAIR10), the known symbol and hit number.

Accession number	Primary Gene Symbol / Gene description	Hit number
AT1G09070	SOYBEAN GENE REGULATED BY COLD-2 (SRC2)	2
AT1G12900	GLYCERALDEHYDE 3-PHOSPHATE DEHYDROGENASE A SUBUNIT 2 (GAPA-2)	1
AT1G17100	HAEM-BINDING PROTEIN 1 (HBP1)	1
AT1G58180	BETA CARBONIC ANHYDRASE 6 (BCA6)	2
AT1G66160	"CYS, MET, PRO, AND GLY PROTEIN 1" (CMPG1)	1
AT1G66200	GLUTAMINE SYNTHASE CLONE F11 (GSR2)	2
AT1G68140	zinc finger/BTB domain protein, putative (DUF1644)	2
AT1G72430	SMALL AUXIN UPREGULATED RNA 78 (SAUR78)	1
AT2G20560	DNAJ PROTEIN (DNAJ)	3
AT2G24850	TYROSINE AMINOTRANSFERASE 3 (TAT3)	6
AT2G29450	GLUTATHIONE S-TRANSFERASE TAU 5 (GSTU5)	1
AT2G47450	CHAOS (CAO)	1
AT2G47710	Adenine nucleotide alpha hydrolases-like superfamily protein	3
AT3G10350	GUIDED ENTRY OF TAIL-ANCHORED PROTEINS 3B (GET3B)	1
AT3G26450	Polyketide cyclase/dehydrase and lipid transport superfamily protein	2
AT3G26650	glyceraldehyde-3-phosphate dehydrogenase A subunit	1
AT3G29160	SNF1 KINASE HOMOLOG 11 (KIN11)	1
AT3G44110	Putative dnaj-like protein (J3)	4
AT3G45030	Ribosomal protein S10p/S20e family protein	1
AT3G48000	ALDEHYDE DEHYDROGENASE 2B4 (ALDH2B4)	11
AT3G55270	FRUCTOSE-BISPHOSPHATE ALDOLASE 8 (FBA8)	1
AT3G55605	Mitochondrial glycoprotein family protein	2
AT3G57520	SEED IMBIBITION 2 (SIP2)	1
AT4G04770	ATP-BINDING CASSETTE I8 (ABCI8)	4
AT4G13430	ISOPROPYL MALATE ISOMERASE LARGE SUBUNIT 1 (IIL1)	1
AT4G23570	(SGT1A)	1
AT4G24020	NIN LIKE PROTEIN 7 (NLP7)	6
AT4G26910	Dihydrolipoamide succinyltransferase	4
AT4G31850	PROTON GRADIENT REGULATION 3 (PGR3)	1
AT4G33670	L-galactose dehydrogenase	1
AT4G35090	CATALASE 2 (CAT2)	1
AT4G35830	ACONITASE 1 (ACO1)	3
AT5G08280	HYDROXYMETHYLBILANE SYNTHASE (HEMC)	1
AT5G09590	MITOCHONDRIAL HSO70 2 (MTHSC70-2)	1
AT5G11040	(TRS120)	1
AT5G12020	17.6 KDA CLASS II HEAT SHOCK PROTEIN (HSP17.6II)	10
AT5G20480	EF-TU RECEPTOR (EFR)	2
AT5G24460	RING-H2 zinc finger protein	1
AT5G50210	QUINOLINATE SYNTHASE (QS)	3
AT5G58590	RAN BINDING PROTEIN 1 (RANBP1)	1

Table S3. List of primers and oligonucleotide sequences used for mutant genotyping.

Mutant code in this study #	Gene Accession	Mutant Accession no	Forward primer	Reverse primer
12	AT5G02320	N531972	GTTCATTTTGTGCTCAATCCC	CCATTTGTTTCCGTACATTCG
22	AT5G28020	N552788	TGATTGGTAACACACCAATGG	CCACCTGCCAAATGAATTATC
26	AT1G67070	N570922	GCCCTTTAGTTGAGTGGTGTG	CGTGAACTGTCGGAGAAAGAG
54	AT3G07370	N612098	CACAAGGCTGAGAGCAAAATC	CTCGATTGGTCCAATAAGCAG
55	AT4G12120	N620492	TGGCTTCTTTGTATTTGTGGG	TTGAAATTTCGGGTGTGTCTC
61	AT4G12120	N635470	AGGCAACCTATGACAGCAATG	GAATGAGAGGTGCGATCTGAG
72	AT4G19040	N654195	CTGTTCTTCCGCTGTATTTGC	ATAGCAATGATTTTTGGTGCG
75	AT3G01650	N655271	TTTTGATTTGGGTCCCTTACC	AGCAATGAATGGACAGGTTTG
76	AT3G01650	N655733	ACAAACCTGTCCATTCATTGC	TTCCCTTTGACCTGTTGAGTG
77	AT2G32390	N656359	TGGCCAGGGGAAGTAATAAAG	TGTCGACATGTCCACAGCTAG
78	AT3G07370	N657213	TACCTTATGATGGCCCATGTC	GAAGATGCTCGAATCCAACAG
80	AT4G19040	N657975	AGGCTTGAAAACACATCATGG	TAACCATTTTGGCACTGAAGG
81	AT5G67600	N658196	TATGGACTAACATGTGGGTGG	CCAATTCTTCTCGAGCAAAAG
83	AT5G28020	N659687	TTCAATAGCCTCTTCACCTGC	ACAGACCAACATGGAAGATCG
85	AT1G53850	N661066	TTTGTGCGTTGAACTTGAGTG	AGCTTCAATGGCATATTCCAC
86	AT4G19040	N661471	TACATTGGAAACCAGTCGAGC	GGCAGAGGATGAAGAGGACTC
87	AT2G41440	N661599	CAGAGCAAGAAGTGGGAAATG	TTTTCCAGATATGGATGCAGC
89	AT5G28020	N663434	CCTCCACTAGGAAAAACCACC	TGAGATGATGGAGCCTTGTTC
96	AT5G14420	N670818	AAAGAGAGAGAAGGGTCGTCG	TGACGAGGACAACTTGATTCC
98	AT1G78380	N672766	TTTTTGTTGTGCGTGAACAAG	TTCGTCTCTGAGCTCAGGAAG
100	AT3G26340	N673661	ACATAGTACAATCCAGGGCCC	ACAACTATGCCTGTGATTGGC
101	AT3G57090	N673702	AAGTCATTGCTCCAATACCCC	GCTGATTGGAGACAAGCTTTG
102	AT4G01960	N674268	GTGAAAGGCGAAGGAAGATTC	TCCAATTCAAAGAACGAATGC
103	AT3G07370	N674983	CAACCACAATGGTGGGTTTAC	CTTGGAACTGTGCTTTTGTGG
104	AT4G01960	N675193	GTGAAAGGCGAAGGAAGATTC	TCCAATTCAAAGAACGAATGC
105	AT3G26340	N675921	ATGCACGTTCGCATATAGGAC	GTTGTACCCTTTGCAGGCTTC
107	AT4G19170	N680044	TTCTCCAATCACAAACCCAAG	TTAGCGTCCATCACCAGAAAC
109	AT2G36020	N682206	GAACGTGTATGAACCAATGGC	TTGGCGCTAATTTCATCATTC
110	AT5G11610	N682800	TCTCTTTTCGCTTTCCCTTTC	ATTTTGTACGGTTGTGTTCCG
111	AT1G67070	N682894	TCTGTCATTTCAGACGAGGAAG	ATTCAACATGACTCGGTCCAG
112	AT5G11610	N684240	GCCTTCACAAAAGTTTTGCAG	GAGCTTGCACGGTTACGTTAG
113	AT5G67600	N685290	ATGCATCCAAGAGACAGCAAC	ATTGGGTTTTTAGGTTGCGTC
114	AT4G19040	N685788	GAAACTGCTGGAGCAATATGG	TCCACTTCGACGAAAAACAAG
115	AT3G57090	N686450	AAGATCCTCCTTGACCTCGAC	GGGAATTACTCAAGGAGCAGG
121	AT2G32390	N859735	GTCAGCTTCTCCTACATTGCG	CTGAAGATTGTGGACCAATGG

122	AT5G14420	N450195	TTATTCGTACCTGCCCATCTG	ACGTGACGTGATTGAATCTCC
123	AT5G14420	N548485	GACGAATTGGTACCGTCATTC	GGAAACAGAGTTTGCCCTTTC
128	AT1G53850	N651939	GATGGCGATTAATTAGGAGCC	GAAGGAGTTGTGCTTGCTGTC
129	AT1G53850	N645344	AATGTGTGATATTTGGGGTGG	TAATTGATCTGCGAATCGGAG
130	AT1G69790	N527863	AAAGGGCTTTTGAGCTGCTAG	GAGTCGAGATGACTGATTCGG
131	AT1G69790	N597486	TGCCAACCCAAAATCAGATAG	CTTCTCTTCTCGAATTTCGCC
132	AT3G59110	N683911	TCATTTTAGGCCGTTTCTGTG	TGTGCATCATCATGAGAGAGC
133	AT3G59110	N684083	AGTGGCGTGGATGACAAGTAC	TTTGGTGTCCTGCTGCTAGAG
134	AT1G52540	N685325	TGATTCAGCCAAACGGTAAAC	ATACACACTGCCAAATCTGCC
139	AT3G19230	N680649	TTTGATCAGATCATTGGAGGC	TTTGGTTGCAAAAAGGCATAC
141	AT3G17420	N668207	GAGTTAGCGTATTCAGGTGCG	AACCCCTTGTTGGACTTCTTC
144	AT3G12620	N656211	TCTTATCCCAAACGTATCGTTG	AAGCCCATCCTTAGAGCAGAG
145	AT3G12620	N662121	CGCGACAAATGTGTATTGATG	TTGCTGCCTCTCTTAGAGCTG
146	AT5G07180	N681024	TATGGCAAAGGTGATACCTGG	TATCTCCATGGCAACAAGCTC
147	AT5G07180	N800028	CAAGCTCAGCAGGTATTTTGC	ATATGTGTCAGCTGACGGGTC
148	AT5G07180	N684732	ACTCGTGAAGATGTCCATTGG	AGCTGGTGATTCTTCACATGG
149	AT5G20480	N654241	TTTCAAACAAGGTTTCTCCAATC	CGCTTCTCTTCAACCAATTTG
151	AT4G15780	N670510	TCCGTGGAACGATAAATTCAG	TATCGCCAAGTAATGCGAATC
152	AT4G15780	N874999	TGTGATAGGGTTGTTTTCCCC	GAAATCAGCTTTCACACGCTC
154	AT3G54300	N527783	ACCAAAGCCATTGTCAACAAG	AACTGGAGTTGGAGGAACCTC
156	AT5G47200	N653446	CTGACAAGGAAAAACGCAAAG	TTGCCTATCTTTGCAGGTCAC
163	AT3G05710	N677254	CATCTAGACGCCGAGATCTTG	TCTTGCTCATTTGATGCTCAC
164	AT4G30260	N653255	TGGAACCCTCAAAGAATCATG	TCAATGTATTTGGCGGAGATC
165	AT4G30260	N660179	TTGGAAGTTGGAACCTTTGTG	GCGGCTGGAGAATTCTCTATC
168	AT2G43490	N668005	TAACCAGTTGCAGGATCAACC	CAAGCGTCTTTTGATAGCGAC
169	AT2G43490	N682830	GGTTATTTCGACTTTCCCTCG	AAACGACACAAGGGACATGTG
170	AT1G05785	N672831	TTAAGTCAACGCCAATCCTTG	CTAACTTTGCAGCGGATTTTG
173	AT3G15980	N678521	ACTCTTGAGTACCTCGCCTCC	TGTATTCTGGCATGGGAAAAC
179	AT5G23540	N681956	TGGATGTAAGTAGGATTGGCG	TTTTGCTTGTGTTGTGTTTGC
183	AT3G13235	N674812	TGCTCGAGGTTGAGGTAAGAG	TGTCGACTGTGCACCACTATC
187	AT4G19006	N682762	GGCTTAAGCCTTAAAGGCAAC	TGTGGACTCACTCTCGGAATC
190	AT5G44190	N661106	GATTGGTAATCTCTATCGCATGG	GCATCAGCAACCACTCTATCC
191	AT2G26410	N661133	CATTTGCAACACCAGTTGTTG	TGAAATTTCATAGGCGAGACG
194	AT4G00710	N666828	GGTAAAGAGTACGGCCTTTGC	ACGTTCATGTCGATTCCTTTG
195	AT4G00710	N661796	CTCACTCCGTAGCTGACCAAC	TATATAAAAAGCCCATGGGCC
tat3-1	AT2G24850	N403572	TGGGGAAGTTTGCATCAATAG	AATGTGGACTTGTGGCATAGG
LB1_SAIL		GCCTTTTCAGAAATGGATAAATAGCCTTGCTTCC		
LBb1.3_SALK		ATTTTGCCGATTTCGGAAC		
GABi_o8409		ATATTGACCATCATACTCATTGC		

Table S4. List of primers and oligonucleotide sequences used for RT-qPCR and RKS1 constructs.

Gene or Mutant line in this study #	Primer (5' - 3')	Primer (5' - 3')
AT2G28390	AACTCTATGCAGCATTTGATCCACT	TGATTGCATATCTTTATCGCCATC
AT3G57710	GAAATTGTTGGTGGCTTTCAA	TCCGTTATCCAAGAACCACCTC
AT1G66560	AGACGAATCCCCGACTCC	GCATAGGTACACCGATAGTAACACC
AT1G51760	AGGGCTGATATGGATGCACT	GTGCATCTTCCCTGGAACC
AT4G01895	CAGAGAACAAAAGGATCAACGAG	TTCGTTTCTTAGCATCCTGGTAG
AT3G01650	CGAGCTTTCGATAACTTCCAGT	GGGATCTCCATGAGAGCAGA
AT4G31860	CAGCAGGTTCTTAGTGATGAGG	TGCATCATCTCATCCATTCTG
AT5G14420	CCTCAGTATGGTGCAGAAAGC	CAGGAGGTTGGGCATAAGAA
AT5G67600	AGGATAATTATATACGTTGCCTCA	GAAAGAGAGAGATAATTATGGTCGG
AT5G19080	ATGCCTTATTCGATGGCAGT	GGGACGATTGTGCACTTTG
AT3G26340	TGCGTTTATCTTTAAGGAAGGTG	TCTTCACAGATTGCGACGAG
AT2G23600	TGAAGCACCGTCTCTACCAA	GGCCTCTTTAGAAGCAATCCA
AT5G11610	AAACATCATCTCAAGCTCTTACCTG	GCTCAAAATACTGAAGGCCTGA
AT5G44572	CTTAGCCAATCGGCTCCTT	TGCTATTATTCCCCCATTCG
AT4G32980	TCTGAAATCTTCCCAAAGGTTC	TTGTTGTTGTCCATTGGGTTT
AT5G28030	CGGTTACTGGAACAGGGAAG	TCAAATGTGGACCTGGTTTTC
AT1G20823	ATTCTGATCTCGTCGTCATCC	TTAAGCCGAGAACGCAAATC
AT5G28020	TTCCTCAAGGAGCAGAACAAA	TCAAATGTGGACCTGGTTGA
AT2G14610	GGAGCTACGCAGAACAACTAAGA	CCCACGAGGATCATAGTTGCAACTGA
AT3G45640	TGACCCCAACAGAAGAATCAC	AAAAGAGAATGGCTTTTGACAGA
AT3G50950	CAAAACAACAACTAGTACATGGATGG	TTCCGTTTCTCCACATGACA
AT3G48000	CCATGTTTGCAAGATTGTTCC	GCTGGAATTGTTAGTCCATGAAT
12	TGGGAAACAATGTCGAGAAA	CGATGAGAATTCATGAGAGCTG
22 / 83 / 89	CAAGCTTCTTGCCCTCAAAG	AGTTTCCCCGCGTTTTCT
26 / 111	CCAATTCTCCAAAGTGTTAGGAA	AAATGACAACTCCTTGAATTTGC
54 / 78 / 103	CGTTCCAAATCTGGCTATCAA	TGTAAGCCCAGACGTGTTTTT
55	AGTTTTGGCCTCACTAAAGGAA	GAATCAACTCTCGGTAAGTTGTCA
61	GAGCAGGACCTTGTTTTTGG	AGCTTGCTTTCGTGGCTTAT
72 / 80 / 86 / 114	CACAACATATCCATCTTCAAAAGG	GCCTGTACAAGGAAAGCCATT
75 / 76	ACCGGTGCAGAGTGGATCATCA	GTAAAGCTTGATTCTGGTCT
77 / 88 / 121	CGAGGAGGCTGGTTCTTCTA	TCTCTGCTTCTCTCTTATCAACAACT
81 / 113	AGGATAATTATATACGTTGCCTCA	GAAAGAGAGAGATAATTATGGTCGG
85 / 128 / 129	GAATGACATGGGAGCCAAAAG	GGGTAATTTACCTATCAGCTTGTG
96 / 122 / 123	GACATGGCCTTTGGTTGTG	GCAAATCGGACACATTTGAA
98	CCTTCTGATCCTTACCTGAGAGC	CCTCTGAGCATCATACAGCTTC
100 / 105	GGGACTTTCTGTTGGCACA	TCGTTGTCAACATAGTACAATCCA
101 / 115	TGTGTATACAATGGCAGTTTACTTTTT	TGAAGTAAGCAGTGGAGAACACA
102 / 104	TGAGTTTGCTCCTCGTGAGA	TGTCTGCACCTGAGACTCTCTTA
107	TTCGCTCCTGTCCTCGAC	AAGAGTGCCGTGGATGATTT
109	AGCTGCGACGTTCTAACTCC	CGTATATGTACGTTTGCGTTACTATG
110 / 112	TCGATCATAAATCATACATCCGTTA	CTTCCTTAGATTCGGGATCTCTT
130 / 131	AAGGAAGCCTGGAGAACCAT	AAGCTACTTTCATCCTAGTCTTCCAC
132 / 133	TGACACAAGCAAAGGGCCTG	ACGAGAAAACTCATTTGCCATC
134	CGCAGCAACAAACAGTTTTAAT	CCTTCAATCTCTTGACTGCAATTT
139	TGCAAAGCTCCCTAGTCTCC	TCAGGAGACGCCTGGATAGT
141	ATTATGCGCGGCCTAAAGA	TCCACCACTTCCTCAAACTGT
144 / 145	TAGAGTCAAGTAGAAGGTCCAGCTAA	CCGACCTCTCCTCTCCATCT
146 / 147 / 148	TCGGTATTGTCCTTCTTGAGC	CATCCGCCTTTGATAGAATCAT
149	TCATGTTAGTGACTTTGGTTTGG	GCCTCCCATTCCATACTCTG
151	TTGTCGAAGATGGCTACGC	CTCCATATCTCTTCTTGAAATCAGC
152	GACCGAGAATCTACGCTCTCA	TCCCAAGAACCACCAGTTTT
154	CCAGATTCCTGAGAATTTGAGC	GCGAGAGAGGAATGAGTACCTT
156	TCTTGATTCAATTAGTCACCACTCC	ATGGGTTTCAAACTTCAACG
163	GGATCCTGGGAATTCGAGA	TGAAATCTCCTCAGATACATCAACC
164 / 165	TCAGTTTGGGAAGTCATTTGG	TCCACAGGGGAGTTTAACAAA
168 / 169	AGCTCTCAGGATGTGGGAGA	GCAATCATTCTCCAAAGTTTCAGT
170	AGAGGTGCTCGTTGAAAAAGA	AAAGAAAGGCTTTGATGTCGTT
173	ACGCTGGGTAGATTGGAAGA	TCAGAGCAGCTTCTGGTATCC
179	ACAGACCGGAAGACCTGAGA	CTCGCTGATTCAAAGCTTCA
183	ATGCTGCGTAAGCATCAGTG	GGGACGGGATGTCTTTCTCT

187	GCCTTATTGAGATCATTTTCAGC	AGTACGCTCGGCAATGACA
190	CGGAGTTACAACGTCAAGGAG	TTCGACAAATTTTGGAGATCTTT
191	TGTCTGTTTCGGTTTGGTGT	CATTAACGCGAAATAAATGGTTT
194 / 195	TGAAACACTAGCCAAACACCTTT	CGTAGCCTCATAGTCCATTTCA
tat3-1	CCCTGGAGTTTTCAAGGCTA	CGTCGGAAGTTCTCCGTTTA
attB1F-710	GGGGACAAGTTTGTACAAAAAAGCAGGCTTAATGAAGAAGCAGTATCTGAAA	
attB2R-710	GGGGACCACTTTGTACAAGAAAGCTGGGTCGCTAGAATTTTTCAATGATGC	
attB1_RKS1	GGGGACAAGTTTGTACAAAAAAGCAGGCTTAATGAAGAAGCAGTATCTGAAATCTGG	
attB2R-710-stop	GGGGACCACTTTGTACAAGAAAGCTGGGTCCTAGCTAGAATTTTTCAATGATGC	
attB4_RKS1	GGGGACAACTTTGTATAGAAAAGTTGGGTGGCTAGAATTTTTCAATGATGCTTC	
RKS1_D191A_fw	CCTAAGATCATCATACATAGAGCTGTTAAACCGATGCATGTTTTC	
RKS1_D191A_rev	GAAAACATGCATCGGTTTAACAGCTCTATGTATGATGATCTTAGG	

Dataset S1 (separate file). Annotation, logFC and categorization in expression classes of 268 expressed genes. Genes significantly found up-regulated are represented in red, those significantly down-regulated in green.

Dataset 2 (separate file). Protein-protein interactions used for the generation of the RKS1 PPI network.

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