

Supplemental Data. Ramšak et al. ().

## Supplemental Data

Supplemental Figure 1: Comparison of predicted connections for three selected network inference algorithms.

Supplemental Figure 2: Shortest path search from EIN3 to NPR1 in *Solanum tuberosum* integrated network (StIN).

Supplemental Figure 3: Results of the replicated experiment for validation of direct transcriptional regulation of NPR1 by the ethylene (ET) signalling module in potato leaves.

Supplemental Table 1: Contribution of four knowledge layers to the built *Arabidopsis thaliana* comprehensive knowledge network (AtCKN).

Supplemental Table 2: List of selected plant hormone pathway receptors and transmitters.

Supplemental Table 3: Primers and probes used for functional validation in *Arabidopsis* and potato and their properties according to MIQE guidelines.

Supplemental Data Set 1: Plant immune signalling model, version 2 (PIS-v2).

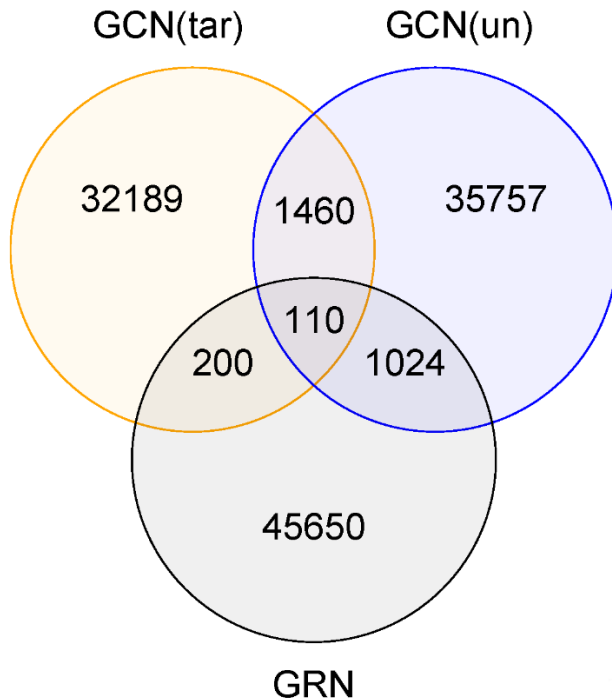
Supplemental Data Set 2: Comparison of contributions in the PIS model and AtCKN subnetwork.

Supplemental Data Set 3: Gene connections for network analysis results between EIN3 and NPR1.

Supplemental Data Set 4: Results of in silico regulatory element search for AtNPR1 and StNPR1.

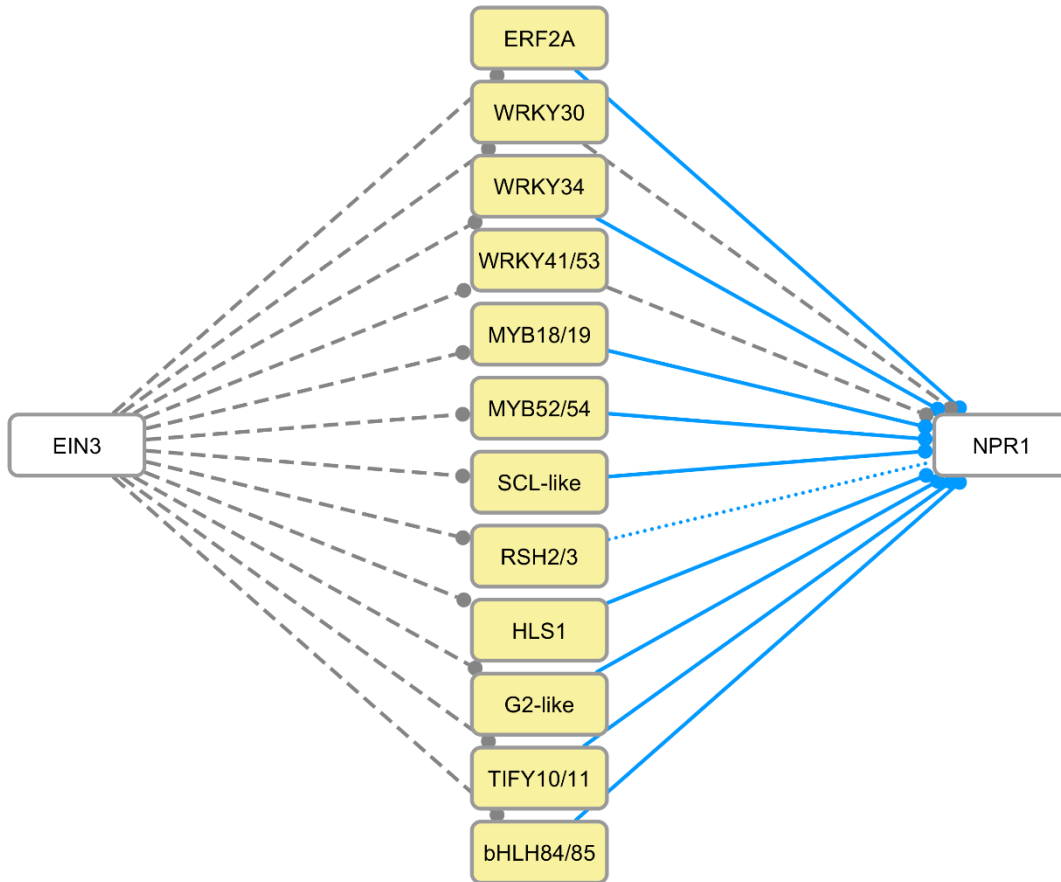
**Supplemental Figure 1: Comparison of predicted connections for three selected network inference algorithms.**

A Venn diagram of common and unique predicted connections depending on the network inference algorithm: targeted gene co-expression (GCN(tar); CoExpNetViz), untargeted gene co-expression (GCN(un); BioLayout) and gene regulatory network inference (GRN; Genie3).



**Supplemental Figure 2: Shortest path search from EIN3 to NPR1 in *Solanum tuberosum* integrated network (StIN).**

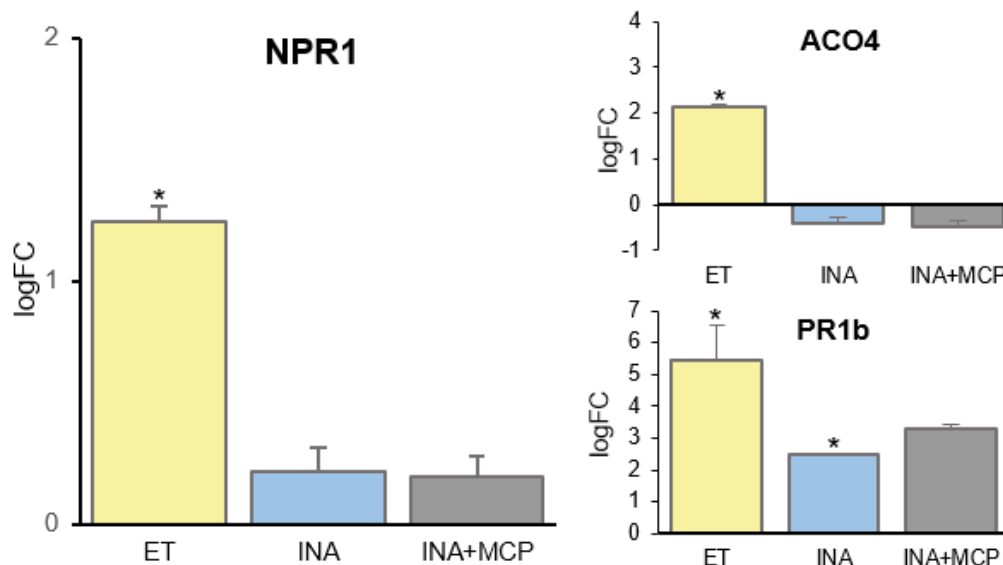
Results of the shortest path search from EIN3 to NPR1 in a reduced StIN, where only transcriptional layer connections were kept (TF, gene co-expression and gene regulatory type connections).



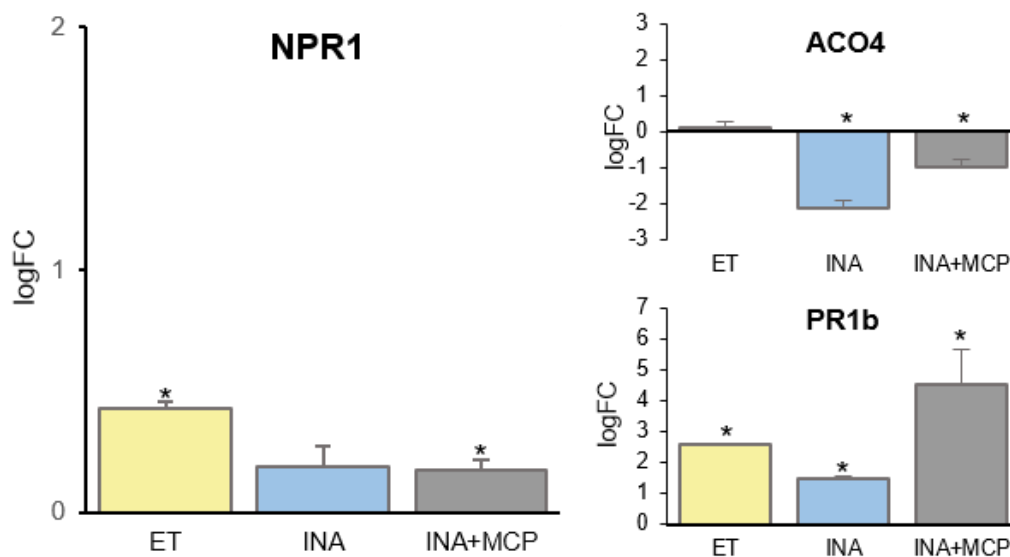
**Supplemental Figure 3: Results of the replicated experiment for validation of direct transcriptional regulation of NPR1 by the ethylene (ET) signalling module in potato leaves.**

Results of the replicated experiment for validation of direct transcriptional regulation of NPR1 by the ethylene (ET) signalling module in potato leaves. Potato plants cv. Rywal (top) and its transgenic line NahG-Rywal (bottom) were treated with either ET (orange), INA (SA analogue; green) or a combination of INA with 1-MCP (ET inhibitor; INA+MCP). Log2 fold change in gene expression of treated versus control plants is shown (\*-  $p < 0.05$ ;  $n = 5$ ) for ACO4 (ET signalling marker), PR1b (SA signalling marker) and NPR1 (SA signal transmitter). Error bars denote standard error of biological replicates. Note different y-axis scales for different genes.

**Potato cv. Rywal**



**Potato NahG-Rywal**



**Supplemental Table 1: Contribution of four knowledge layers to the built *Arabidopsis thaliana* comprehensive knowledge network (AtCKN).**

Each knowledge layer (PIS-v2 – plant immune signalling model, PPI – protein-protein interactions, TR – transcriptional regulation, miRNA – regulation through microRNA) was assessed on the subset credibility, forming ranks: rank1 (R1) denotes highly reliable data obtained from manually curated sources; rank2 (R2) high-throughput studies data and rank3 (R3) *in silico* computationally predicted connections. For each contribution, the source and number of connections per source is given. In the last column, the final unique kept connection count in AtCKN is given.

Knowledge layer	Rank (reliability)	Source	Connections per Source	Connections kept in AtLKN
PPI	R1	AtPIN (Brandão et al., 2009)	6637	34683
		STRING-v10 (Szklarczyk et al., 2015)	9140	
		PPI individual	31	
PPI	R2	Arabidopsis interactome (Arabidopsis Interactome Mapping Consortium, 2011)	11351	
		membrane interactome (Jones et al., 2014)	12102	
		viral component interactions (Elena et al., 2011)	175	
		immune component interactions (Mukhtar et al., 2011)	2795	
TF	R1	atRegNet (Palaniswamy et al., 2006)	4540	31907
		ATRM (Jin et al., 2015)	1440	
		TF individual	34	
TF	R2	atRegNet (Palaniswamy et al., 2006)	12334	
		ChIP-seq (EIN3) (Chang et al., 2013)	1314	
		ChIP-seq (WRKY33) (Liu et al., 2015)	214	
TF	R3	Arabidopsis TF targets (Srivastava et al., 2010)	12333	
miRNA	R1	miRTarBase (Chou et al., 2015)	68	2904
		miRNA individual	36	
miRNA	R2	PMRD (Zhang et al., 2010)	1999	
miRNA	R3	PNRD (Yi et al., 2014)	1617	
PIS-v2	R1	activation	204	180
		binding	98	76
		inhibition	132	132
		synthesis	209	209
			78803	70091

**Supplemental Table 2: List of selected plant hormone pathway receptors and transmitters.**

The list of Arabidopsis gene short names and corresponding gene identifiers for receptors and transmitters of seven major plant hormonal pathways. All combinations were generated, and for each pair the corresponding shortest path and walk was searched for in Pajek (Batagelj and Mrvar, 1998).

Plant Hormone	Receptor	Transmitter
Ethylene	ETR1 (AT1G66340)	EIN3 (AT3G20770)
		ERF1A (AT4G17500)
Salicylic acid	NPR1 (AT1G64280)	TGA2 (AT5G06950)
Jasmonic acid	COI1 (AT2G39940)	MYC2 (AT1G32640)
Abscisic acid	PYR1 (AT4G17870)	SNRK (AT5G66880)
Auxins	TIR1 (AT3G62980)	ARF2 (AT5G62000)
Cytokinins	AHK2 (AT5G35750)	/
	AHK3 (AT1G27320)	
	AHK4 (AT2G01830)	
Brassinosteroids	BRI1 (AT4G39400)	BES1 (AT1G19350)

**Supplemental Table 3: Primers and probes used for functional validation in Arabidopsis and potato and their properties according to MIQE guidelines.**

Gene name, organism, primer and probe sequence, their source, target genes (according to the StNIB-v1 paralogue grouping) their description and assay amplification efficiency for the five target and three reference genes are shown.

	Gene Name	Primer and probe sequences (5' --> 3')	Source	Target gene(s)	Description	Amplicon efficiency
Potato	Target	ACO AAGGGACTCCGCGCTCATA CAAGTTGGTCACCAAGGTTAACC FAM-TCGATGTTCTCCCATGCGCC-MGB	(Chersicola et al., 2017)	Sotub07g018820.1.1	1-aminocyclopropane-1-carboxylate oxidase	98%
		PR1b GTATGAATAATTCCACGTACCATATGTTC GTGGAAACAAGAAGATGCAATACTTAGT FAM-TGGTATAGTGGCTTACGTTGA-Zen	This work	Sotub09g006090.1.1 Sotub09g006100.1.1 Sotub09g006110.1.1	pathogenesis related protein 1b	92%
		NPR1 TCCCTCAAACGTCTATCAGAAACAC CGTCAGCGAAGAAGTCGAAAT FAM-AGTCTATCTTCGATGCGTCTTCGCCG-TAMRA	(Petek et al., 2014)	Sotub07g011600.1.1	non-expressor of PR1	99%
	Reference	COX CGTCGCATTCCAGATTATCCA CAACTACGGATATATAAGRRCCRAACTG FAM-TGCTTACGCTGGATGGAATGCCCT-TAMRA	(Weller et al., 2000)	Sotub04g015050.1.1	cytochrome oxidase	96%
		EF-1 GGAAGCTGCTGAGATGAACAAGA CTCACGTTACGCTTAAGTTTGTC FAM-TCATTCAAGTATGCCTGGGTGCT-TAMRA	(Baebler et al., 2009)	Sotub06g010680.1.1	elongation factor 1 $\alpha$	96%
		Target	NPR1 GATCGCAAAACAAGCCACTATGG GCCTTTGAGAGAATGCTTGCA FAM-TTGCTCCGGGATATTAT-MGB	This work	AT1G64280	non-expressor of PR1
Reference	COX TCAGGTATGCCACGTCGTATTC CGGATATATAAGAGCCAAAACACTGGAA FAM-TGCTTACGCTGGATGGAATGCCCT-TAMRA		(Gašparič et al., 2013)	ATMG01360	cytochrome oxidase	99%

## Supplemental References

- Arabidopsis Interactome Mapping Consortium** (2011). Evidence for Network Evolution in an Arabidopsis Interactome Map. *Science* (80-. ). **333**: 601–607.
- Baebler, Š., Krečič-Stres, H., Rotter, A., Kogovšek, P., Cankar, K., Kok, E.J., Gruden, K., Kovač, M., Žel, J., Pompe-Novak, M., and Ravnikar, M.** (2009). PVYNTN elicits a diverse gene expression response in different potato genotypes in the first 12 h after inoculation. *Mol. Plant Pathol.* **10**: 263–275.
- Batagelj, V. and Mrvar, A.** (1998). Pajek – Program for Large Network Analysis. *Connections* **21**: 47–57.
- Brandão, M.M., Dantas, L.L., and Silva-Filho, M.C.** (2009). AtPIN: Arabidopsis thaliana protein interaction network. *BMC Bioinformatics* **10**: 454.
- Chang, K.N. et al.** (2013). Temporal transcriptional response to ethylene gas drives growth hormone cross-regulation in Arabidopsis. *Elife* **2013**: 1–20.
- Chersicola, M., Kladnik, A., Tušek-Žnidarič, M., Mrak, T., Gruden, K., and Dermastia, M.** (2017). 1-Aminocyclopropane-1-Carboxylate Oxidase Induction in Tomato Flower Pedicel Phloem and Abscission Related Processes Are Differentially Sensitive to Ethylene. *Front. Plant Sci.* **8**.
- Chou, C.-H. et al.** (2015). miRTarBase 2016: updates to the experimentally validated miRNA-target interactions database. *Nucleic Acids Res.* **44**: gkv1258-.
- Elena, S.F., Carrera, J., and Rodrigo, G.** (2011). A systems biology approach to the evolution of plant-virus interactions. *Curr. Opin. Plant Biol.* **14**: 372–377.
- Gašparič, M.B., Lenassi, M., Gostinčar, C., Rotter, A., Plemenitaš, A., Gunde-Cimerman, N., Gruden, K., and Žel, J.** (2013). Insertion of a Specific Fungal 3'-phosphoadenosine-5'-phosphatase Motif into a Plant Homologue Improves Halotolerance and Drought Tolerance of Plants. *PLoS One* **8**: e81872.
- Jin, J., He, K., Tang, X., Li, Z., Lv, L., Zhao, Y., Luo, J., and Gao, G.** (2015). An arabidopsis transcriptional regulatory map reveals distinct functional and evolutionary features of novel transcription factors. *Mol. Biol. Evol.* **32**: 1767–1773.
- Jones, A.M. et al.** (2014). Border control—a membrane-linked interactome of Arabidopsis. *Science* (80-. ). **344**: 711–716.
- Liu, S., Kracher, B., Ziegler, J., Birkenbihl, R.P., and Somssich, I.E.** (2015). Negative regulation of ABA Signaling By WRKY33 is critical for Arabidopsis immunity towards Botrytis cinerea 2100. *Elife* **4**: 1–27.
- Mukhtar, M.S. et al.** (2011). Independently evolved virulence effectors converge onto hubs in a plant immune system network. *Science* (80-. ). **333**: 596–601.
- Palaniswamy, S.K., James, S., Sun, H., Lamb, R.S., Davuluri, R. V., and Grotewold, E.** (2006). AGRIS and AtRegNet. A Platform to Link cis-Regulatory Elements and Transcription Factors into Regulatory Networks. *Plant Physiol.* **140**: 818–829.
- Petek, M., Rotter, A., Kogovšek, P., Baebler, Š., Mithöfer, A., and Gruden, K.** (2014). Potato virus Y infection hinders potato defence response and renders plants more vulnerable to Colorado potato beetle attack. *Mol. Ecol.* **23**: 5378–5391.
- Srivastava, G.P., Li, P., Liu, J., and Xu, D.** (2010). Identification of transcription factor's targets using tissue-specific transcriptomic data in Arabidopsis thaliana. *BMC Syst. Biol.* **4 Suppl 2**: S2.
- Szklarczyk, D. et al.** (2015). STRING v10: Protein-protein interaction networks, integrated over the tree of life. *Nucleic Acids Res.* **43**: D447–D452.
- Weller, S.A., Elphinstone, J.G., Smith, N.C., Boonham, N., and Stead, D.E.** (2000). Detection of *Ralstonia solanacearum* Strains with a Quantitative, Multiplex, Real-Time, Fluorogenic PCR (TaqMan) Assay. *Appl. Environ. Microbiol.* **66**: 2853–2858.
- Yi, X., Zhang, Z., Ling, Y., Xu, W., and Su, Z.** (2014). PNRD: a plant non-coding RNA database. *Nucleic Acids Res.*: 1–8.
- Zhang, Z., Yu, J., Li, D., Zhang, Z., Liu, F., Zhou, X., Wang, T., Ling, Y., and Su, Z.** (2010). PMRD: plant microRNA database. *Nucleic Acids Res.* **38**: 806–813.