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Supplementary Information for

Rhizogenic *Agrobacterium* protein RolB interacts with the TOPLESS and NINJA repressor proteins to reprogram plant jasmonate signaling

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Other supplementary materials for this manuscript include the following:

Datasets S1 to S6

MATERIALS AND METHODS

LC-MS/MS analysis. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis was carried out by the VIB proteomics Core. In brief, purified peptides were redissolved in loading solvent A (0.1% [w/v] TFA in water/acetonitrile [98:2, v/v]) and the peptide concentration was determined on a Lunatic instrument (Unchained Lab). For the LC-MS/MS analysis, 2 µg of peptides was injected on an Ultimate 3000 RSLCnano ProFLow system in-line connected to a Q Exactive HF mass spectrometer (Thermo Fisher Scientific). Trapping was done at 10 µL/min for 4 min in loading solvent A on a 20-mm trapping column (made in-house, 100 µm internal diameter, 5-µm beads, C18 Reprosil-HD; Dr. Maisch, Germany). The peptides were separated on a 200-cm µPAC™ column with C18-endcapped functionality (Pharmafluidics, Belgium) kept at a constant temperature of 50°C. Peptides were eluted by a nonlinear gradient reaching 55% MS solvent B (0.1% [w/v] TFA in water/acetonitrile [2:8, v/v]) in 80 min, and 97% MS solvent B in 90 min at a constant flow rate of 300 nL/min, followed by a 5-min wash at 97% MS solvent B and reequilibration with MS solvent A (0.1% [w/v] TFA in water). The mass spectrometer was operated in data-dependent mode, automatically switching between MS and MS/MS acquisition for the 12 most abundant ion peaks per MS spectrum. Full-scan MS spectra (375-1500 *m/z*) were acquired at a resolution of 60,000 in the Orbitrap analyzer after accumulation to a target value of 3,000,000. The 12 most intense ions above a threshold value of 13,000 were isolated for fragmentation at a normalized collision energy of 30% after filling the trap at a target value of 100,000 for maximum 80 ms. MS/MS spectra (200-2000 *m/z*) were acquired at a resolution of 15,000 in the Orbitrap analyzer.

Yeast two- and three-hybrid assays. Y2H assays were done as previously described (1). Briefly, bait and prey entry constructs were cloned into pGBT9/pGBKT7 and pGADT7 Gateway-compatible destination vectors. The *Saccharomyces cerevisiae* yeast strain PJ69-4A was cotransformed with a bait and a prey plasmid by means of the lithium acetate/single-stranded carrier DNA/polyethylene glycol (LiAc/ssDNA/PEG) method and selected on yeast synthetic defined (SD) medium lacking Leu and Trp. To verify that constructs did not autoactivate, empty pGBT9/pGBKT7 and pGADT7 vectors were used as controls. Three independent transformants were selected for replica plating in 10× and 100× dilutions and grown on SD medium lacking Leu, Trp, and His for 2 days at 30°C.

For Y3H assays, the 'bridge' construct was generated through recombination of the entry clones pEN-L4-GPD-R1, pEN-R2-NLS-3×FLAG-6×His-L3, and pEN-L1-ORF-L2 with the MultiSite

Gateway cloning with pMG426 as destination vector. Y3H was carried out as described above, except that the transformed yeast cells were selected on SD medium lacking Leu, Trpn and Ura.

Transient expression assay. Transient expression assays were carried out as previously described (2). Tobacco (*Nicotiana tabacum*) protoplasts were prepared and cotransfected with a reporter construct expressing the Firefly luciferase (fLUC) driven by a promoter containing the GAL4 upstream-activating sequences (UASs) and a construct expressing the gene of interest fused to the GAL4-DBD under the control of the constitutive CaMV35S promoter. In several experiments, effector constructs were added, cloned into the p2GW7 Gateway-compatible destination vector for overexpression. As a control, GUS was cloned into the pGAL4-DBDgate or p2GW7. For each experiment, 2 µg of each plasmid was used. Transfected protoplasts were incubated overnight and lysed. fLUC activities were determined with the Dual-Luciferase reporter assay system (Promega).

Tomato composite plant bioassay. Tomato composite plant bioassays were carried out as previously described with some adaptations (3). More specifically, the WT rhizogenic *Agrobacterium* K599 (NCPPB2659; pRi2659) strain and the K599 strain mutated in the *rolB* oncogene through base editing, were used to infect tomato seedlings (4). Both K599 strains were transformed as previously described with a plasmid expressing the mCherry fluorescent reporter under the control of the constitutive CaMV35S promoter from its T-DNA (5). In several experiments, the mCherry reporter construct was replaced by the pK7WG2D Gateway-compatible binary destination vector expressing a gene of interest under the control of the constitutive CaMV35S promoter and the GFP fluorescent marker under the control of the *pRolD* promoter from its T-DNA region.

Per strain, ~30 tomato seedlings were infected as previously described (3). Three and 4 weeks post-infection, the percentage of fluorescent transgenic roots was determined (for instance, 50% transgenic roots corresponded to 15 out of 30 tomato seedlings with fluorescent transgenic roots) under a fluorescence microscope (Leica).

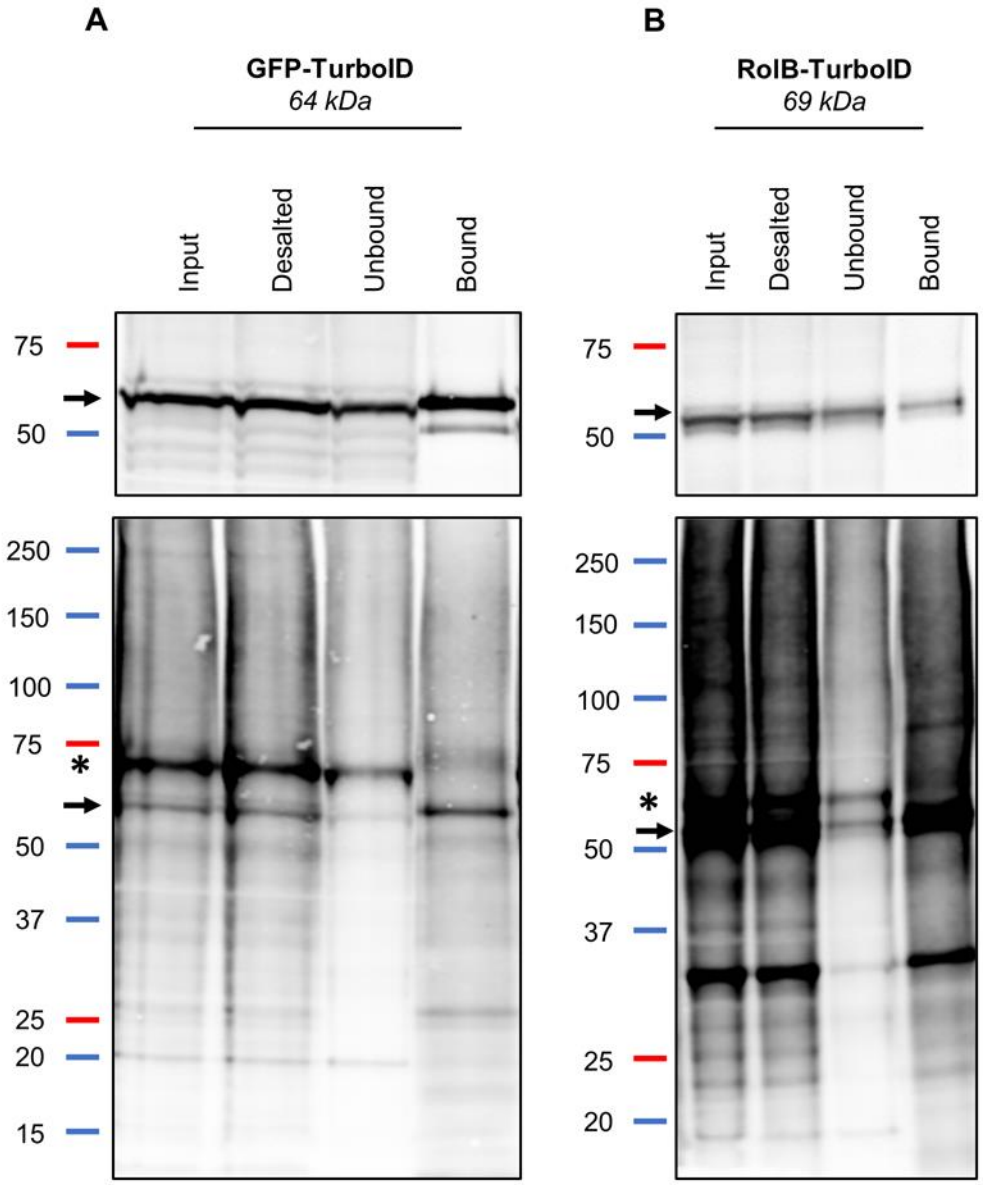

Fig. S1. Protein expression and biotinylation activity of Bait-TurboID fusion proteins. Constitutive *pCaMV35S::eGFP-TurboID* and β-estradiol-inducible *pXVE::RolB-TurboID* expression constructs used for rhizogenic *Agrobacterium*-mediated transformation of tomato. Before the protein extracts from transformed hairy roots were submitted to TurboID-MS, the quality of the TurboID-MS sample preparation was assessed by exposing the input, input desalted, unbound, and bound fractions collected at various steps of the procedure to an immunoblot analysis. (*A*) Immunoblot results of input, input desalted, unbound and bound fractions for the eGFP-TurboID fusion protein (64 kDa) and (*B*) for the RolB-TurboID fusion protein (69 kDa). A streptavidin/Alexa Fluor™ 680 conjugate was used for the detection of biotinylated proteins (bottom panels), and anti-Flag for the detection of the translational fusion of the promiscuous labeling enzyme TurboID to the bait (top panels). Arrows indicate the molecular mass of the fusion proteins. Asterisks mark TurboID-catalyzed autobiotinylation of the fusion proteins. Results are representative for the three biological TurboID replicate samples.

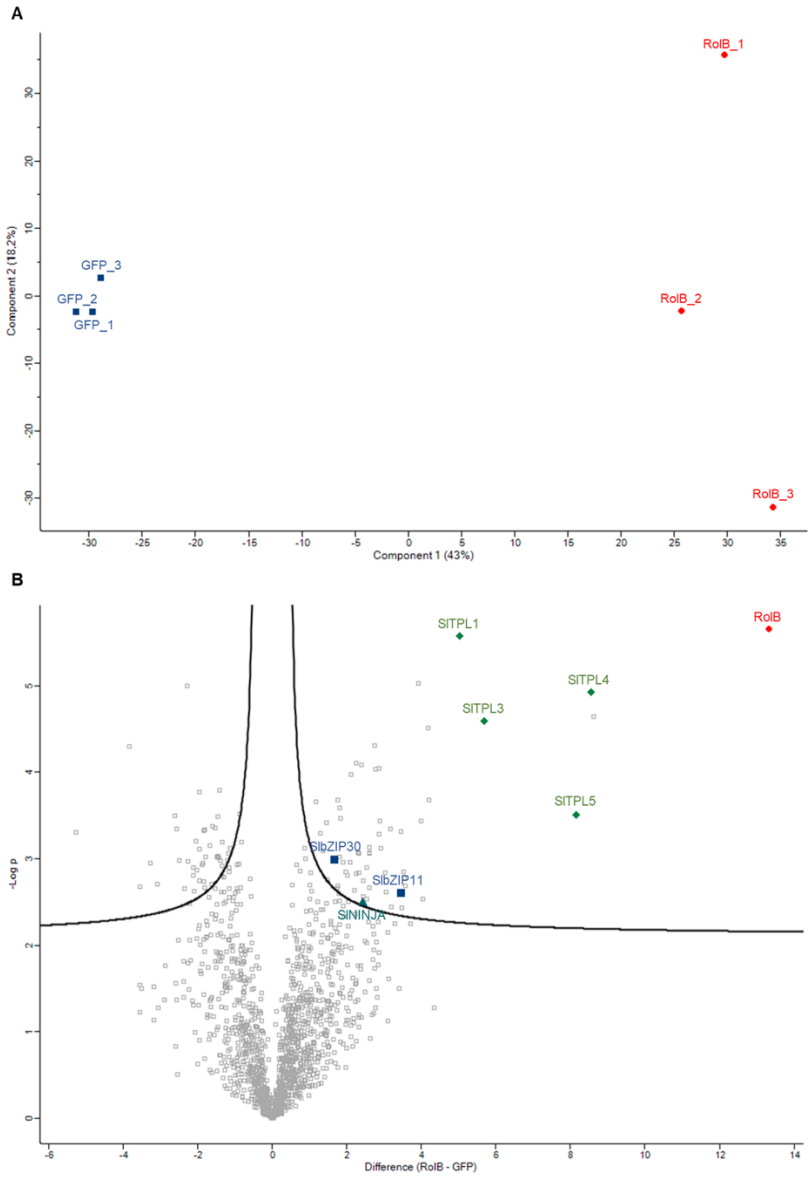

Fig. S2. Statistical analysis on RolB and eGFP TurboID samples. *35S*::*GFP-TurboID* and *XVE*::*rolB-TurboID* expression constructs were used for rhizogenic *Agrobacterium*-mediated transformation of tomato. Transformed hairy roots were treated with β-estradiol (100 µM) for 24 h and biotin (50 µM) for 2 h. Proteins were extracted from the hairy root tissue, followed by streptavidin beads enrichment, trypsin digest, and identification by mass spectrometry. The MaxQuant software was used for peptide and protein identification on the acquired raw files and the Perseus software for statistical data analysis (69, 70). (*A*) Variability of samples represented by a principal component analysis (PCA) plot. Red circles and blue squares, RolB and eGFP replicate samples, respectively. (*B*) Volcano plot of pairwise comparison between RolB and eGFP samples. A two-sample Student's *t* test was done to identify enriched proteins in the RolB samples. The full line indicates the cut-off at false discovery rate (FDR)=0.01 and S0=0.1 (i.e. artificial within groups variance; this value defines the relative importance of the *P* value and difference between means). The *t* test difference was plotted versus the *t* test –log(*P* value). Direct and indirect interactors of RolB are highlighted in the volcano plot. Red circle, RolB; light-blue triangle, SlNINJA (Solyc05g018320); dark-green diamonds, TPL family proteins (SlTPL1 [Solyc03g117360], SlTPL3 [Solyc01g100050], SlTPL4 [Solyc03g116750], and SlTPL5 [Solyc07g008040]); dark-blue squares, bZIP family proteins (SlbZIP11 [Solyc01g110480] and SlbZIP30 [Solyc04g071160]).

Fig. S3. Conservation of the EAR2 motif in the RolB protein. Alignment of the amino acid sequences of RolB homologs from five rhizogenic *Agrobacterium* strains, comprising the EAR1 and EAR2 motifs found in K599 RolB (red underlined). Residues are highlighted (yellow); when a majority or all amino acid residues are identical. The sequences used for the alignment were collected from the National Center for Biotechnology Information sequence database. Proteins and their accession numbers are: RolB_K599 (CAB65895), RolB_ATCC15834 (KEA04442), RolB_1724 (P49409), RolB_A4 (P20402), and RolB_8196 (AAA22095). Image created by Snapgene**.**

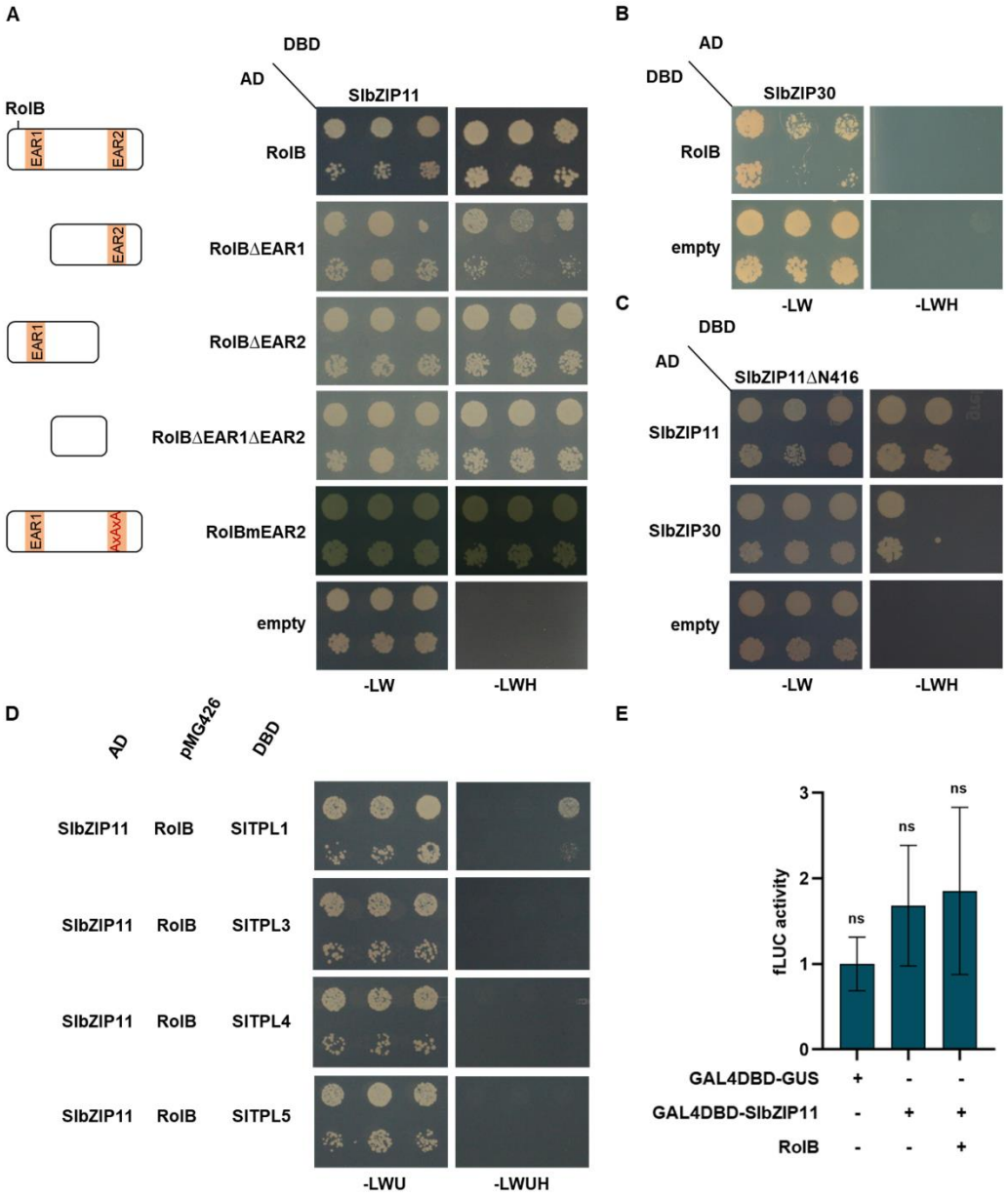

Fig. S4. Direct interaction of RolB with SlbZIP11. (*A*) Y2H assay with RolB derivatives as baits and SlbZIP11 (Solyc01g110480) as prey. Y2H analysis was done as in Fig. 1. (*B*) Y2H assay with SlbZIP30 (Solyc04g071160) as bait and RolB as prey. (*C*) Y2H assay with SlbZIP11ΔN416 as bait and SlbZIP11 and SlbZIP30 as preys. To avoid autoactivation, a truncated version of SlbZIP11, SlbZIP11ΔN416, lacking the first 416 amino acids, was generated and used as the bait. (*D*) Y3H analysis with tomato TPL homologs as baits, SlbZIP11 as prey, and RolB as 'bridge' protein. Transformed yeasts were spotted in 10-fold and 100-fold dilutions on control medium (-Leu-Trp-Ura [-LWU]) and selective medium (-Leu-Trp-Ura-His [-LWUH]). Gene constructs in the pGBT9 and pGADT7 vectors carry the GAL4- DNA-binding domain (DBD) or the transcription activation domain (AD), respectively, whereas the constructs expressed in pMG426 do not carry any additional domain. (*E*) Transactivation activity in tobacco protoplasts transfected with a *pUAS–fLUC* reporter construct, a SlbZIP11 construct fused with GAL4-DBD, and a RolB construct. Values represent the mean ± SE (n=8) relative to a control transfection with a GAL4-DBD-GUS control construct. Statistical significance was assessed using one-way ANOVA with Tukey's honest significance difference (ns, not significant).

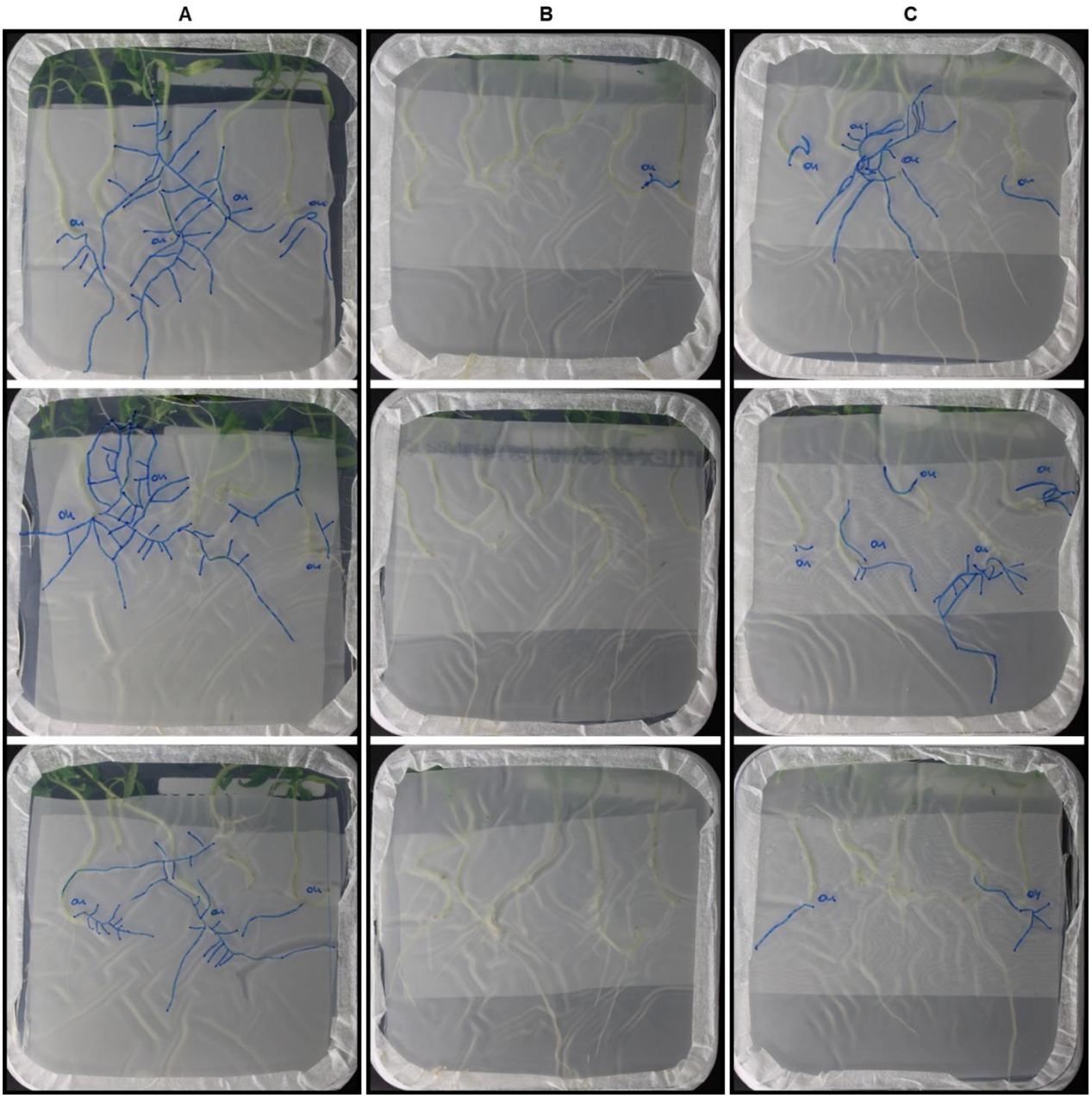

Fig. S5. Pictures of a representative outcome of a tomato infection assay with rhizogenic *Agrobacterium*. Shown are tomato seedlings 4r weeks post-infection with either (*A*) the rhizogenic *Agrobacterium* K599 wild-type strain, (*B*) the *rolB*Δ mutant strain, or (*C*) the *rolB*Δ strain transformed with a construct expressing the native *rolB* oncogene. For each strain, three square Petri dishes are shown, each containing five tomato seedlings. Tomato plants with transgenic fluorescent roots are marked with 'OK' and transgenic roots are highlighted in blue.

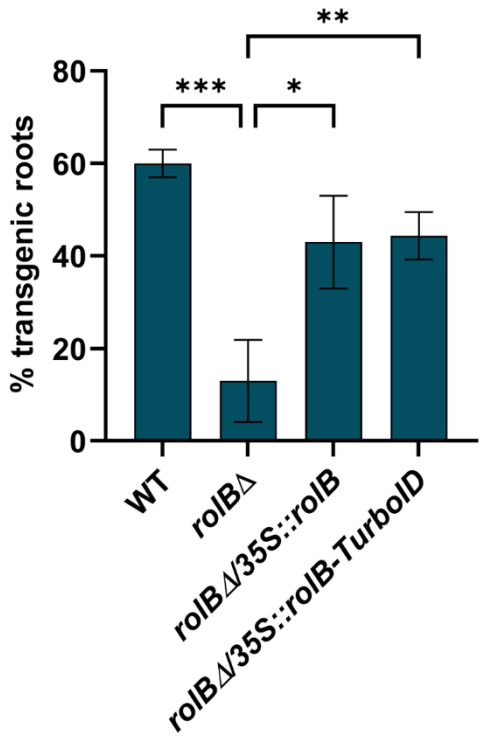

Fig. S6. Functional analysis of the RolB-TurboID fusion protein. Tomato seedlings (~30 seedlings per strain) were infected with rhizogenic *Agrobacterium* K599 strain variants and the percentage of emerging fluorescent transgenic roots was scored 4 weeks post-infection. Percentage of transgenic fluorescent tomato roots upon infection with the rhizogenic *Agrobacterium* K599 wild-type (WT) strain, the *rolB*Δ mutant strain, and the *rolB*Δ strain transformed with a construct expressing either the native *rolB* oncogene or the native *rolB* oncogene N-terminally fused to TurboID, each under the control of the constitutive *CaMV35S* promoter, along with a *GFP* fluorescent reporter gene, *rolB*Δ*/35S::rolB* and *rolB*Δ*/35S::rolB-TurboID*, respectively. The WT and *rolB*Δ strain were transformed with a construct expressing the *mCherry* fluorescent reporter gene. Values represent the mean ± SE (n=3). Statistical significance was assessed with one-way ANOVA with Tukey's honest significance difference (*** $P \leq 0.001$; ** $P \leq 0.01$; * $P \leq 0.05$).

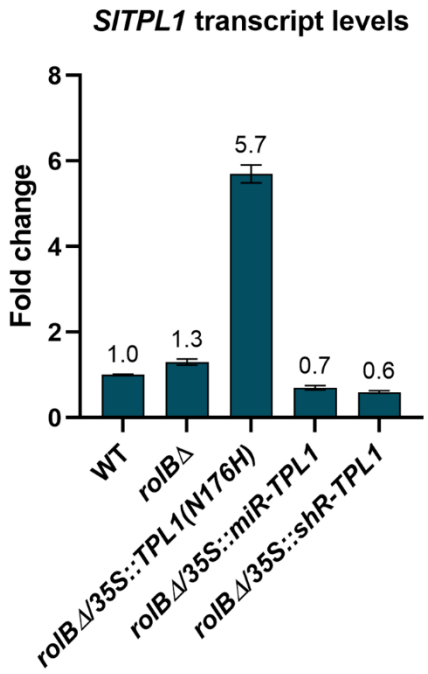

Fig. S7. Analysis of the *SlTPL1* expression in roots infected with gain- or loss-of-function constructs. qRT-PCR analysis reflects the fold-change in steady-state *SlTPL1* transcript levels in transformed tomato roots, 4 weeks post infection with the *rolB*Δ strain transformed with either the *SlTPL1* silencing constructs *microRNA(miR)-SlTPL1* and *short hairpin RNA(shR)-SlTPL1*, or a construct carrying a dominant negative mutant version of the *SlTPL1* gene, *SlTPL1(N176H)*. Fold change is relative to the transcript levels in roots upon K599 WT infection and normalized to the *SlCAC* (*Solyc08g006960*) transcript levels. Given the lack of complementation with any of the constructs, the assay was not repeated, hence *n*=1 without error bars.

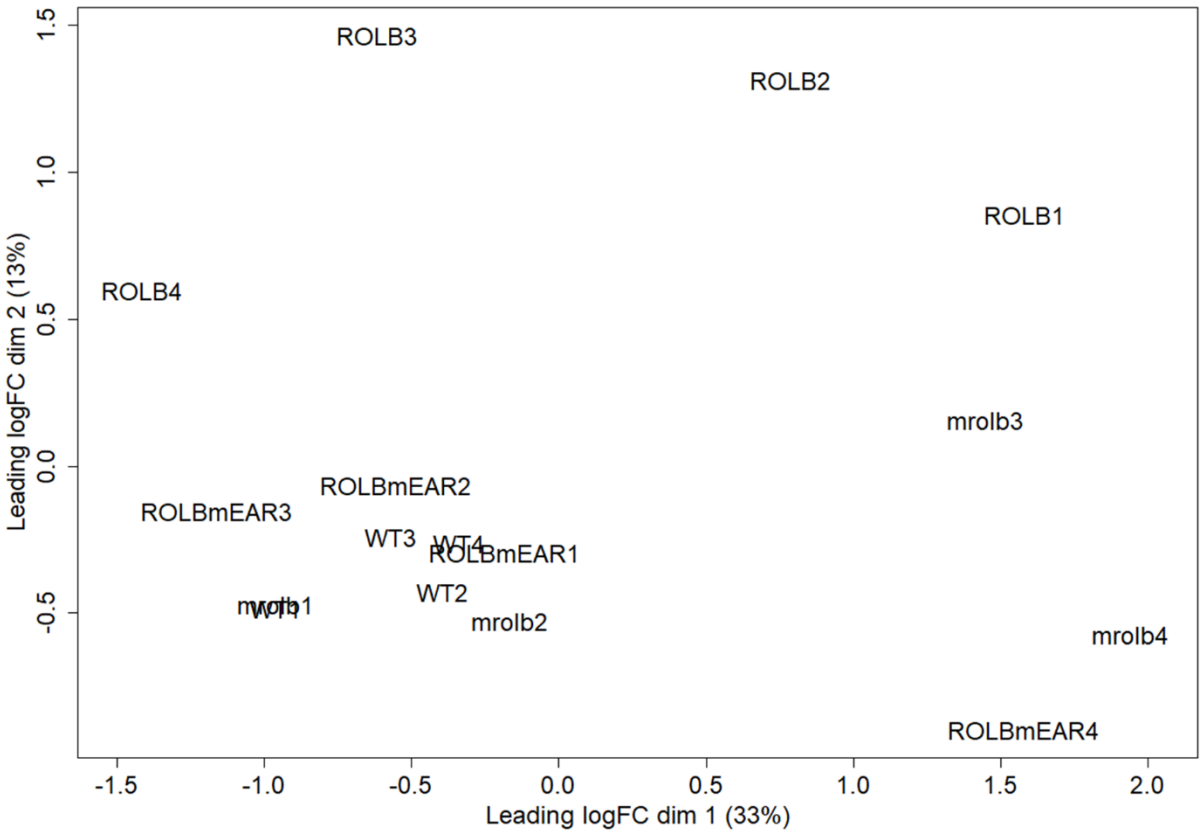

Fig. S8. RNA sequencing of tomato roots infected with the WT K599 strain, the *rolB*Δ strain, and the *rolB*Δ strain with a construct expressing either the native *rolB* oncogene or the *rolBmEAR2* mutant, each under the control of the constitutive *CaMV35S* promoter, *rolB*Δ*/35S::rolB*, and *rolB*Δ*/35S::rolBmEAR2*, respectively. Principal component analysis (PCA) plot showing the variance between all samples. WT, wild-type K599-infected tomato root samples; mrolb, *rolB*Δ-infected tomato root samples; ROLB, *rolB*Δ*/35S::rolB*-infected tomato root samples; ROLBmEAR, *rolB*Δ*/35S::rolBmEAR2*-infected tomato root samples. Each point represents a sample and each sample group consists of four biological replicates.

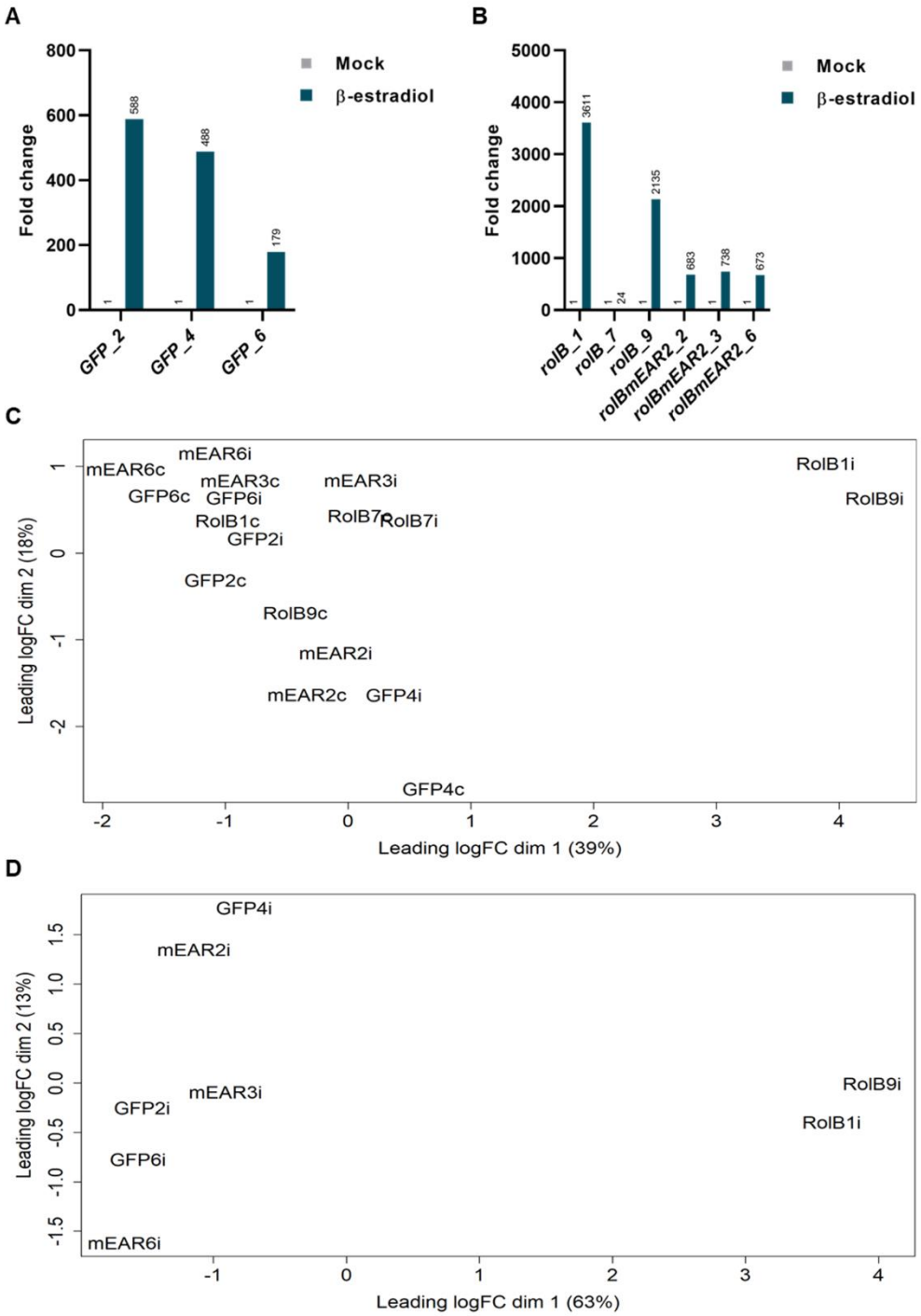

Fig. S9. RNA sequencing on *rolB*, *rolBmEAR2*, and *GFP* transgenic tomato hairy root lines after 24 h of mock and 100 µM β-estradiol treatments. (*A*) and (*B*) Preliminary assessment by qRT-PCR of *GFP* and *rolB* transcript levels in transformed hairy roots, respectively. Fold change is relative to the transcript levels in mock-treated hairy roots and normalized to the *SlCAC* (*Solyc08g006960*) transcript levels. (*C*) Principal component analysis (PCA) plot showing the variance between all samples. Each point represents a sample and each sample group consists of three biological replicates. c, mock-treated (control) samples; i, β-estradiol-induced samples. (*D*) PCA plot showing the variance between the β-estradiol-induced samples. One sample was removed from the analysis (pRPS5α_XVE::*rolB*, line 7; RolB7i), because of divergent correlation coefficients (see panel *C*).

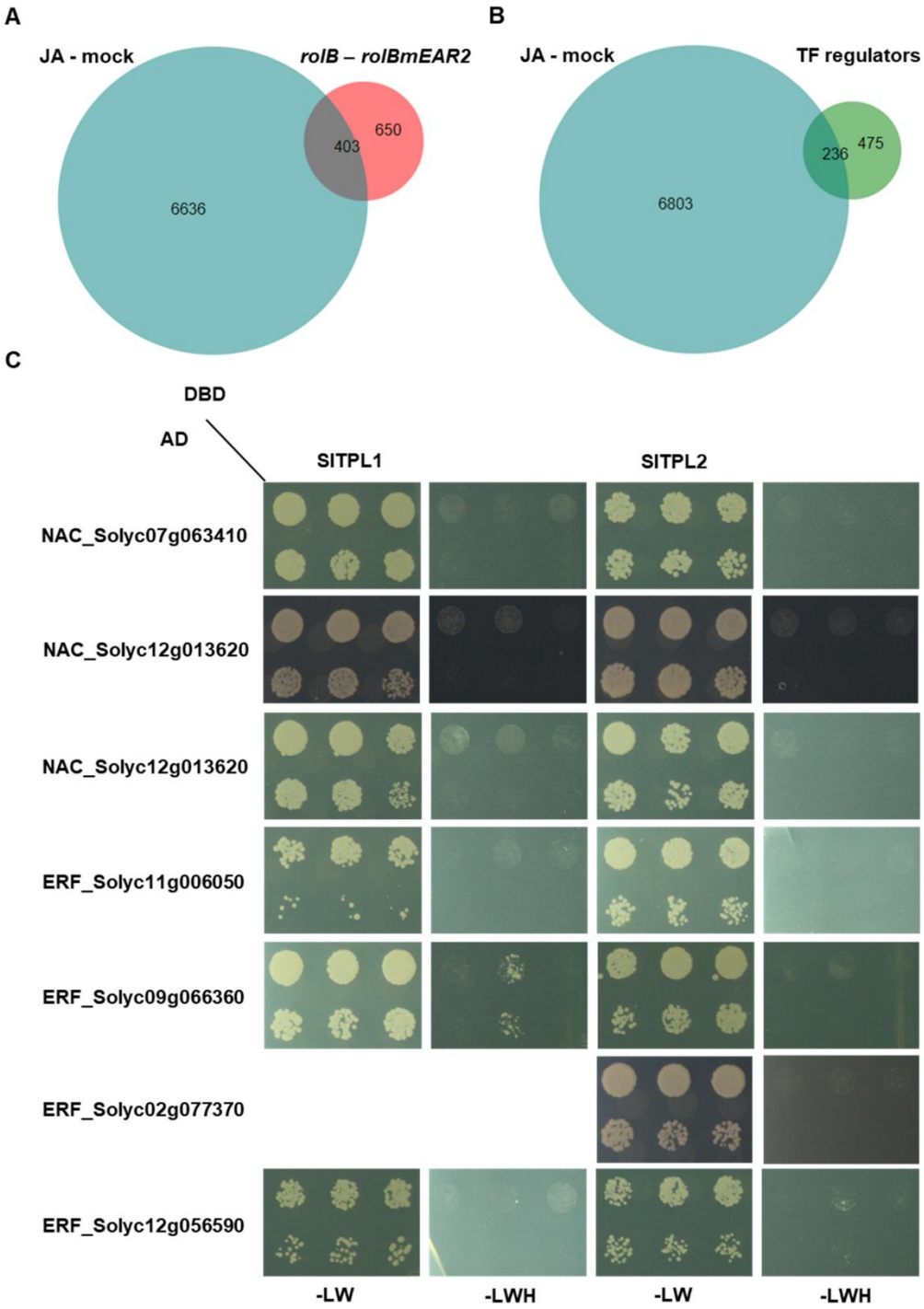

Fig. S10. *rolB*-activated genes enriched for NAC and AP/ERF TF binding targets. (*A*) Proportional Venn diagram showing overlap between DEGs upregulated in the contrast group *rolB-rolBmEAR2* and DEGs upregulated in tomato hairy roots treated with JA. (*B*) Proportional Venn diagram showing overlap between TF regulators of the DEGs upregulated in the contrast group *rolB-rolBmEAR2* and DEGs upregulated in tomato hairy roots treated with JA. (*C*) Y2H assay with SlTPL1 and SlTPL2 as baits and the NAC and AP/ERF TFs as preys. Y2H analysis was done as in Fig. 1.

Table S1. Primer sequences used in this study.

Primer name	Primer sequence	SolycID	Species
Fw_attB1_rolB	GGGGACAAGTTTGTACAAAAAAGCAGGCTTAATGGCACTGAACTTGCCG	N/A	*Agrobacterium K599*
Rv_attB2_rolB	GGGGACCACTTTGTACAAGAAAGCTGGGTATCAAGTCGCCGAGGTTTCTTTC	N/A	*Agrobacterium K599*
Fw_attB1_rolBΔEAR1	GGGGACAAGTTTGTACAAAAAAGCAGGCTTAATGTTCCACAGTTATCCCCAACC	N/A	*Agrobacterium K599*
Rv_attB2_rolBΔEAR2	GGGGACCACTTTGTACAAGAAAGCTGGGTATCAGTCTGGCTCCGGTGACGG	N/A	*Agrobacterium K599*
Fw_attB1_rolBmEAR1	GGGGACAAGTTTGTACAAAAAAGCAGGCTTAATGGCAGCTAACGCTCCGGCTTTCCACAGTTATCCCC	N/A	*Agrobacterium K599*
Fw_rolBmEAR2	TCACCGGAGCCAGACGCTACCGCTCGGGCTTCGGGACCCAAC	N/A	*Agrobacterium K599*
Rv_rolBmEAR2	GTTGGGTCCCGAAGCCCGAGCGGTAGCGTCTGGCTCCGGTGA	N/A	*Agrobacterium K599*
Fw_attB1_SlTPL1ΔN	GGGGACAAGTTTGTACAAAAAAGCAGGCTCCATGAAGTACTTTGAAGATGAAGTGCATAATGG	N/A	*Agrobacterium K599*
Rv_attB2_SlTPL1ΔN	GGGGACCACTTTGTACAAGAAAGCTGGGTTCATCTTGGTGCTTGATCGGAGC	N/A	*Agrobacterium K599*
Fw_attB1_NAC72	GGGGACAAGTTTGTACAAAAAAGCAGGCTTAATGGGTGTTCAAGAAATGGATC	Solyc07g063410	*S. lycopersicum*
Rv_attB2_NAC72	GGGGACCACTTTGTACAAGAAAGCTGGGTATTACCCAGTAAAACCCATATTTACTG	Solyc07g063410	*S. lycopersicum*
Fw_attB1_JA2	GGGGACAAGTTTGTACAAAAAAGCAGGCTTAATGGGTGTTCAAGAAAAAGATCC	Solyc12g013620	*S. lycopersicum*
Rv_attB2_JA2	GGGGACCACTTTGTACAAGAAAGCTGGGTACTACTGCTTGAACCCGAGATTTAAC	Solyc12g013620	*S. lycopersicum*
Fw_attB1_ERF2	GGGGACAAGTTTGTACAAAAAAGCAGGCTTAATGGATTCTTCATCTTCCTCATCATC	Solyc11g006050	*S. lycopersicum*
Rv_attB2_ERF2	GGGGACCACTTTGTACAAGAAAGCTGGGTACTAGGAATTGCTTTCAGACAACATC	Solyc11g006050	*S. lycopersicum*
Fw_attB1_ERF_C3	GGGGACAAGTTTGTACAAAAAAGCAGGCTTAATGGATTATTCATCTCGGGATGATC	Solyc09g066360	*S. lycopersicum*
Rv_attB2_ERF_C3	GGGGACCACTTTGTACAAGAAAGCTGGGTATCACCAATTTGTGATACTTTCTGAAG	Solyc09g066360	*S. lycopersicum*
Fw_attB1_ERF_C5	GGGGACAAGTTTGTACAAAAAAGCAGGCTTAATGGTTCCAACTCCTCAAAGTG	Solyc02g077370	*S. lycopersicum*
Rv_attB2_ERF_C5	GGGGACCACTTTGTACAAGAAAGCTGGGTATTAACATCTTGATTCAAATACATCATTAGTTCC	Solyc02g077370	*S. lycopersicum*
Fw_attB1_ERF_D2	GGGGACAAGTTTGTACAAAAAAGCAGGCTTAATGTCGGAGATGGTGACGG	Solyc12g056590	*S. lycopersicum*
Rv_attB2_ERF_D2	GGGGACCACTTTGTACAAGAAAGCTGGGTATTAACTAGAAGATGGAGGATATTGGCTAG	Solyc12g056590	*S. lycopersicum*
Fw_attB1_SlTPL1	GGGGACAAGTTTGTACAAAAAAGCAGGCTCCATGTCATCTCTCAGTAGAGAGCTTG	Solyc03g117360	*S. lycopersicum*
Rv_attB2_SlTPL1	GGGGACCACTTTGTACAAGAAAGCTGGGTTCATCATCTTGGTGCTTGATCGGAG	Solyc03g117360	*S. lycopersicum*
Fw_attB1_SlTPL2	GGGGACAAGTTTGTACAAAAAAGCAGGCTCCATGTCTTCCTTGAGTAGGGAACTGG	Solyc08g076030	*S. lycopersicum*
Rv_attB2_SlTPL2	GGGGACCACTTTGTACAAGAAAGCTGGGTCCTTGAAGGTGTTTCTGATGGCTG	Solyc08g076030	*S. lycopersicum*

Fw_attB1_SlTPL3	GGGGACAAGTTTGTACAAAAAAGCAGGCTCCATGTCTTCTCTTAGCAGAGAATTGGTG	Solyc01g100050	*S. lycopersicum*
Rv_attB2_SlTPL3	GGGGACCACTTTGTACAAGAAAGCTGGGTTCTTTGAACTTGGTCAGCAGCATG	Solyc01g100050	*S. lycopersicum*
Fw_attB1_SlTPL4	GGGGACAAGTTTGTACAAAAAAGCAGGCTCCATGACTTCTTTAAGCAGAGAGCTGG	Solyc03g116750	*S. lycopersicum*
Rv_attB2_SlTPL4	GGGGACCACTTTGTACAAGAAAGCTGGGTTCACTACCTTGATGCTTGATCAAGACC	Solyc03g116750	*S. lycopersicum*
Fw_attB1_SlTPL5	GGGGACAAGTTTGTACAAAAAAGCAGGCTCCATGAGGCATTTTGATGAAATGGTGG	Solyc07g008040	*S. lycopersicum*
Rv_attB2_SlTPL5	GGGGACCACTTTGTACAAGAAAGCTGGGTTCACTACCTTTGAGGTTGATCTGAATTTGC	Solyc07g008040	*S. lycopersicum*
Fw_attB1_SlTPL6	GGGGACAAGTTTGTACAAAAAAGCAGGCTCCATGTCTCTTAGTAAGGACCTTATATTCTTG	Solyc08g029050	*S. lycopersicum*
Rv_attB2_SlTPL6	GGGGACCACTTTGTACAAGAAAGCTGGGTTATTGGTTGCTCATTGGTAAGAGTC	Solyc08g029050	*S. lycopersicum*
Fw_attB1_SlNINJA	GGGGACAAGTTTGTACAAAAAAGCAGGCTCGCCCACATCCCACCTACTG	Solyc05g018320	*S. lycopersicum*
Rv_attB2_SlNINJA	GGAAGGCCTATTGACAAGGAACCCAGCTTTCTTGTACAAAGTGGTCCCC	Solyc05g018320	*S. lycopersicum*
Fw_attB1_SlJAZ6	GGGGACAAGTTTGTACAAAAAAGCAGGCTTAATGGAGAGGGACTTTATGGG	Solyc01g005440	*S. lycopersicum*
Fw_attB1_SlJAZ6	GGGGACCACTTTGTACAAGAAAGCTGGGTACTAGGTCTCCTTACCGGCTAAC	Solyc01g005440	*S. lycopersicum*
Fw_attB1_SlbZIP11	GGGGACAAGTTTGTACAAAAAAGCAGGCTTAATGGCTGGTGACAATGACGAAG	Solyc01g110480	*S. lycopersicum*
Rv_attB2_SlbZIP11	GGGGACCACTTTGTACAAGAAAGCTGGGTACTACTTTGATTCATGCTTTG	Solyc01g110480	*S. lycopersicum*
Fw_attB1_SlbZIP30	ggggacaagtttgtacaaaaaagcaggctccATGGACCCGAAGTTCACC	Solyc04g071160	*S. lycopersicum*
Rv_attB2_SlbZIP30	ggggaccactttgtacaagaaagctgggtcTCAACCCCTGTTATTGAAGTTCA	Solyc04g071160	*S. lycopersicum*
Fw_attB1_SlbZIP30ΔN179	GGGGACAAGTTTGTACAAAAAAGCAGGCTTAATGTTGATTGATCCCAAGAGAGC	Solyc04g071160	*S. lycopersicum*
Fw_attB1_SlbZIP11ΔN416	GGGGACAAGTTTGTACAAAAAAGCAGGCTTAATGTTAGCAGATCCAAAACGAG	Solyc01g110480	*S. lycopersicum*
Fw_SlCAC (qRT-PCR)	CCTCCGTTGTGATGTAACTGG	Solyc08g006960	S. lycopersicum
Rv_SlCAC (qRT-PCR)	ATTGGTGGAAAGTAACATCATCG	Solyc08g006960	S. lycopersicum
Fw_RolC (qRT-PCR)	CAGGCATCAGGATTCTTTGG	N/A	*Agrobacterium K599*
Rv_RolC (qRT-PCR)	TGGCATAAAGGTCGAAGGTC	N/A	*Agrobacterium K599*
Fw_RolA (qRT-PCR)	GGAACTAGCCGGAATAAACG	N/A	*Agrobacterium K599*
Rv_RolA (qRT-PCR)	ACACACGTTTGGCCTTCTTC	N/A	*Agrobacterium K599*
Fw_RolB (qRT-PCR)	GACGGTTTTCCTCCAAATCA	N/A	*Agrobacterium K599*
Rv_RolB (qRT-PCR)	CCAACCTCACATCACAATGC	N/A	*Agrobacterium K599*

LEGENDS TO DATASETS

Dataset S1. Significantly enriched protein interactors of RolB identified by TurboID-mediated proximity labeling in tomato hairy roots**.** *eGFP* and *rolB* transgenic hairy roots were generated and processed for TurboID-mediated PL as previously described (Fig. S2). The Perseus software was used for the statistical data analysis (6, 7). The three biological TurboID replicate samples were grouped and a two-sample Student's *t* test (with settings S0 = 0.1, i.e., artificial within groups variance; this value defines the relative importance of the *P* value and difference between means); FDR= 0.01 or FDR= 0.001; and *P* value <0.05) was performed in Perseus to identify significantly enriched proteins in the RolB samples compared to the eGFP samples. Log_2(difference): the difference between the means of the imputed and log_2 transformed label-free quantification (LFQ) intensity values from each sample group (RolB and eGFP sample group). -log(*P* value): significance of the observed difference.

Dataset S2. Differentially expressed genes in the contrast groups after RNA-seq analysis**.** RNA sequencing was performed on tomato roots infected with the WT K599 strain, the *rolB*Δ strain, and the *rolB*Δ strain with a construct expressing either the native *rolB* oncogene or the *rolBmEAR2* mutant, each under the control of the constitutive *CaMV35S* promoter. WT, wild-type K599-infected tomato root samples; mrolb, *rolB*Δ-infected tomato root samples; ROLB, *rolBΔ/35S::rolB*-infected tomato root samples; ROLBmEAR, *rolBΔ/35S::rolBmEAR2*-infected tomato root samples. Each sample group consists of four biological replicates. The data were filtered for low expressed genes that did not meet the following criteria: minimum read count in at least two samples >5 and count per million reads (CPM) >1.5. RNA-seq read counts were normalized with the Trimmed Mean of M values method (TMM) (8). Models of expression contrasts were fitted with a generalized linear regression model. Genes were significantly differentially expressed in the contrast groups, when the *P* value <0.05, the false discovery rate (FDR) <0.05, and the log(FC) >2 (upregulated) or <-2 (downregulated) differentially expressed genes (DEGs). (A) Normalized RNA-seq read count per sample. (B) Number of significantly up- and downregulated DEGs in each contrast group. Significantly upregulated (C) and downregulated (D) DEGs in tomato root samples infected with ROLB *vs* WT. FC, fold-change; F, variance of the group means/mean of the within group variances; *P* value, significance of the observed difference. (E) GO categories significantly enriched in upregulated DEGs in tomato root samples infected with ROLB *vs* WT. GO term enrichment for biological process (BP), cellular component (CC), and molecular function (MF) was analyzed with PLAZA 4.5 Dicots.

Dataset S3. Differentially expressed genes in the contrast groups after RNA-seq analysis**.** RNA sequencing was performed on *rolB, rolBmEAR2,* and *GFP* transgenic tomato hairy root lines after 24 h of mock and β-estradiol (100 µM) treatment. C, mock-treated (**c**ontrol) samples; I, β-estradiol-induced samples. Each sample group consists of three biological replicates. One sample was removed from further analysis (pRPS5α_XVE::*rolB*, line 7) due to divergent correlation coefficients. The data was filtered for low expressed genes that did not meet the following criteria: minimum read count in at least two samples >25 and count per million reads (CPM) >1.5. RNA-seq read count were normalized with the Trimmed Mean of M values method (TMM) (8). Models of expression contrasts were fitted with a generalized linear regression model. Genes were significantly differentially expressed in the contrast groups; when the *P* value <0.05, false discovery rate (FDR) <0.05, and log(FC) >2 (upregulated DEGs) or <-2 (downregulated DEGs). (A) Normalized RNA-seq read count per sample. (B) Number of significantly up- and downregulated DEGs in each contrast group.

(C) Significantly upregulated DEGs in *rolB* compared to *GFP* transgenic hairy root lines. (D) Significantly downregulated DEGs in *rolB* compared to *GFP* transgenic hairy root lines. (E) Significantly upregulated DEGs in *rolB* compared to *rolBmEAR2* transgenic hairy root lines. (F) Significantly downregulated DEGs in *rolB* compared to *rolBmEAR2* transgenic hairy root lines. FC, fold-change; F, variance of the group means/mean of the within group variances; *P* value, significance of the observed difference.

Dataset S4. GO categories enriched in differentially expressed genes (DEGs) of each contrast group. GO term enrichment for biological process (BP), cellular component (CC), and molecular function (MF) was analyzed with PLAZA 4.5 Dicots. GO categories significantly enriched in (A) upregulated and (B) downregulated DEGs in *rolB* compared to *GFP* transgenic hairy root lines. GO categories significantly enriched in (C) upregulated and (D) downregulated DEGs in *rolB* compared to *rolBmEAR2* transgenic hairy root lines. The cut-off *P* value and FDR were <0.05. (E) Upregulated DEGs in *rolB* compared to *rolBmEAR2* transgenic hairy root lines categorized in the GO category 'response to ethylene'. (F) Upregulated DEGs in *rolB* compared to *rolBmEAR2* transgenic hairy root lines categorized in the GO category 'response to jasmonic acid'.

Dataset S5. TF-binding sites enriched in differentially expressed genes (DEGs) between *rolB* and *rolBmEAR2*. Binding sites significantly enriched in (A) upregulated and (B) downregulated DEGs in *rolB* compared to *rolBmEAR2* transgenic hairy root lines. Each row contains the enrichment statistics of the corresponding binding site (original binding site IDs form CisBP) (9) and JASPAR (10) along with information of the associated TFs, Arabidopsis orthologs, and expression values in *rolB* and *rolBmEAR2*. Set ID, identifier for the set of input genes for which we want to predict potential regulators; binding site ID, identifier of the binding site predicted to be enriched in the promoters of the genes in the set; *P* value, significance of the enrichment of the binding site in the promoters of the genes in the set; *Q* value, *P* value after correction for multiple testing; enrichment fold, how higher is the enrichment of the binding site in the promoters of the genes in the set than expected by chance; number of genes of interest, how many genes there are on the input gene set; number of genes targeted by binding site, how many genes are targeted by the binding site in the whole genome; percentage of genes targeted by binding site, percentage of genes targeted by the binding site divided by all the genes in the genome; number of hits, how many of the total genes targeted by the binding site are present in the input gene set; gene ID, gene ID of the transcription factor (TF) associated to the binding site predicted as enriched (can be more than one per binding site); TF description, description of the TF by Sol Genomics (https://solgenomics.net/); binding site family, TF family to which the enriched binding site is characteristic; Arabidopsis ortholog gene ID, gene ID of the corresponding tomato TF in Arabidopsis; Arabidopsis ortholog gene symbol, gene symbol of the corresponding Arabidopsis ortholog; logFC of the TF, log fold-change of the TF predicted as regulator of the gene set, if this one is among the differentially expressed genes (up- or downregulated); up- or downregulated, column specifying whether the corresponding TF is up- or downregulated; target genes, gene ID of the genes targeted by the enriched binding site and present in the input gene set.

Dataset S6. Upregulated differentially expressed genes in tomato hairy roots upon JA treatment. RNA sequencing was done on control tomato hairy root lines 24 h after mock and 50 µM JA treatments. Each sample group consists of three biological replicates. RNA-seq read counts were normalized with Trimmed Mean of M-values method (TMM) (8). Models of expression contrasts were fitted with a generalized linear

regression model. Genes were significantly differentially expressed in the contrast group, when the *P* value and FDR <0.05. FC, fold-change; CPM, count per million reads; F, variance of the group means/mean of the within group variances; *P* value, significance of the observed difference; FDR, false discovery rate.

SI REFERENCES

1. A. Pérez Cuéllar, L. Pauwels, R. De Clercq, A. Goossens, Yeast two-hybrid analysis of jasmonate signaling proteins. *Methods Mol. Biol.* **1011**, 173-185 (2013).
2. V. De Sutter *et al.*, Exploration of jasmonate signalling via automated and standardized transient expression assays in tobacco cells. *Plant J.* **44**, 1065-1076 (2005).
3. T. Ho-Plágaro, R. Huertas, M. I. Tamayo-Navarrete, J. A. Ocampo, J. M. García-Garrido, An improved method for *Agrobacterium rhizogenes*-mediated transformation of tomato suitable for the study of arbuscular mycorrhizal symbiosis. *Plant Methods* **14**, 34 (2018).
4. S. D. Rodrigues *et al.*, Efficient CRISPR-mediated base editing in *Agrobacterium* spp. *Proc. Natl. Acad. Sci. USA* **118**, e2013338118 (2021).
5. S. Wen-jun, B. G. Forde, Efficient transformation of *Agrobacterium* spp. by high voltage electroporation. *Nucleic Acids Res.* **17**, 8385-8385 (1989).
6. J. Cox, M. Mann, MaxQuant enables high peptide identification rates, individualized p.p.b.-range mass accuracies and proteome-wide protein quantification. *Nat. Biotechnol.* **26**, 1367-1372 (2008).
7. S. Tyanova, T. Temu, J. Cox, The MaxQuant computational platform for mass spectrometry-based shotgun proteomics. *Nat. Protoc.* **11**, 2301-2319 (2016).
8. M. D. Robinson, A. Oshlack, A scaling normalization method for differential expression analysis of RNA-seq data. *Genome Biol.* **11**, R25 (2010).
9. M. T. Weirauch *et al.*, Determination and inference of eukaryotic transcription factor sequence specificity. *Cell* **158**, 1431-1443 (2014).
10. O. Fornes *et al.*, JASPAR 2020: update of the open-access database of transcription factor binding profiles. *Nucleic Acids Res.* **48**, D87-D92 (2020).