

Supporting information- Experimental Procedures

Sustained NIK-mediated antiviral signaling confers broad-spectrum tolerance to begomoviruses in cultivated plants

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Yeast strain and two-hybrid assays. The NSP ORF was amplified from ToYSV DNA-B using PCR with the appropriate linkers and introduced through recombination into the entry vector pDONR201 to generate pDON-NSP-ToYSV, also designated as pUFV1323; the NSP ORF was subsequently transferred to the pDEST22 vector. The resulting clone pAD-NSP-ToYSV, also designated pUFV1370 or AD-NSPTY (Figure 1B), contained the GAL4 DNA-activating domain fused to the NSP-ToYSV sequence. Sequences encoding the mutant (T474D) kinase domain of NIK were transferred from pDON-T474D (Santos et al., 2009) to pDEST32 to yield pBD-T474D or BD-T474D (Figure 1B). The clones pAD-NSP-CaLCuV (AD-NSPCL, in Figure 1B) and pBD-KDNIK (BD-NIK in Figure 1B) have been previously described (Fontes et al., 2004).

The yeast two-hybrid assays were performed as previously described (Florentino et al., 2006). The yeast reporter strain AH109 (MATa, Trp1-901, leu2-3, 112, ura3-52, his3-200, gal4 Δ , LYS2::GAL1_{UAS}-GAL1_{TATA}-HIS3, MEL1 GAL2_{UAS}.GAL_{TATA}::MEL_{UAS}-MEL1_{TATA}.lacZ,) was cotransformed with AD-NSP fusions and pBD-NIK1 or pBD-T474D. The interactions were monitored by the ability of the reporter strain to grow on media lacking leucine, tryptophan, uracil and histidine, but supplemented with 25 mM 3-aminotriazole for 7 days at 30 °C. The interactions were further confirmed by measuring β -galactosidase activity in yeast extracts using o-nitrophenyl β -D-galactopyranoside substrate, as described previously (Uhrig et al., 1999).

Phosphorylation assay. To create plasmids for *E. coli* expression, the mutant NIK1 C-terminal kinase domain or intact NIK (KD, encoding amino acids 298–638) were amplified from the appropriate mutated clone, introduced by recombination into the entry vector pDONR201 and then transferred to the bacterial expression vector pDEST15, resulting in GST fused to mutant kinase domains, designated pGST-KDNIK1T474A or pGST-KDNIK1. The *E. coli*-expressed and purified KDNIK1T474D and KDNIK1 recombinant proteins were incubated alone or with NSP from CaLCuV, ToYSV or ToSRV at 25°C for 45 min in 30 μ L of kinase buffer containing 18 mM HEPES pH 7.4, 10 mM MgCl₂, 10 mM MnSO₄, 1 mM DTT, 10 μ M ATP and 5 μ Ci [γ -³²P]ATP. Phosphoproteins were resolved by SDS-PAGE.

The gel was stained with Coomassie brilliant blue to verify protein loading, dried, and subjected to autoradiography. Incorporated radioactivity in protein bands was quantified by phosphoimaging and protein loading by densitometry using the Multi Gauge V3.0 software (Fujifilm).

Bimolecular fluorescence complementation (BiFC) analysis. For biochemical complementation of fluorescent fragments of yellow fluorescent protein (YFP), NSP and NIK were fused to the N-terminus (N) or C-terminus (C) of the reporter gene. Then, constructs expressing NSP-YFPC, NSP-YFPN, NIK-YFPC and NIK-YFPN in different combinations were co-agroinfiltrated in tobacco leaves in the presence of the suppressor of silencing HC-Pro according to a previously described protocol (Carvalho et al., 2008c). YFP fluorescence was observed by confocal microscopy.

Tomato transformation. The clone pK7F-NIK1T474D has been previously described (Santos et al., 2009). It harbors a GFP gene fused in-frame after the last codon of the mutant cDNA T474D under the control of the CaMV 35S promoter. In the mutant cDNA T474D, the threonine residue at position 474 within the activation loop of NIK1 was mutated to an aspartate residue. Leaf discs from *in vitro*-grown tomato plants (*Solanum lycopersicum*, cultivar Moneymaker) were transformed with pK7F-NIK1T474D via *Agrobacterium*-mediated plant transformation (strain LBA4404). The transformed shoots were selected on MS medium supplemented with 6-benzylaminopurine (500 mg.L^{-1}), cefotaxime (300 mg.L^{-1}), and kanamycin sulfate (50 mg.L^{-1}). The regenerated shoots were rooted, transferred into soil and grown under standardized greenhouse conditions to generate seeds. The transgenic lines were confirmed using PCR. The analysis of transgene expression was performed by RT-PCR and real-time RT-PCR using transgene-specific primers, and actin was used as an endogenous control to normalize all values. The transgenic lines 35S:NIK1-4 and 35S:NIK1-6 have been previously described (Carvalho et al., 2008c). They harbor the Arabidopsis NIK cDNA fused to a GFP cDNA under the control of the CaMV 35S promoter.

RT-PCR and real-time RT-PCR analyses. Total RNA was extracted from tomato leaves using TRIzol (Invitrogen). The reverse transcription (RT)-PCR assays were performed with 2 µg of total RNA, 0.5 mM of poly-dT and 1 U of M-MLV reverse transcriptase (Invitrogen Life Technologies, Inc.) as previously described (Delu-Filho et al., 2000). The PCR reaction was performed using virus-specific primers. The PCR comprised 30 cycles of 45 s at 94 °C, 30 s at 55 °C and 2 min at 72 °C. The real-time RT-PCR reactions were performed on an ABI7500 instrument (Applied Biosystems, Foster City, CA) using the SYBR Green PCR Master Mix (Applied Biosystems) as previously described (Costa et al., 2008). The amplification reactions were performed as follows: 2 min at 50°C, 10 min at 95°C and 40 cycles of 94°C for 15 sec and 60°C for 1 min. To confirm the quality and primer specificity, we verified the size of amplification products using 1.5% agarose gel electrophoresis and analyzed the T_m (melting temperature) of the amplification products in a dissociation curve performed on the ABI7500 instrument. Actin was used as an endogenous control to normalize all values in the real-time RT-PCR assays. Gene expression was quantified using the 2^{ΔC_T} method. The fold variation of gene expression was quantified using the comparative Ct method: $2^{-(\Delta C_{T\text{Treatment}} - \Delta C_{T\text{Control}})}$.

Immunoblot analysis. Total protein was extracted from the leaves of wild-type and T474D-overexpressing tomato transgenic seedlings as previously described (Cascardo et al., 2000). Following SDS-PAGE, the proteins were transferred from 10% SDS-polyacrylamide gels to nitrocellulose membranes by electroblotting. The membranes were blocked in NaCl/Tris containing 0.05% v/v Tween-20 and 5% w/v non-fat dry milk, and they were subsequently incubated with rabbit anti-GFP at a 1:10,000 dilution for 2 h at room temperature. The bound antibody was detected using alkaline-phosphatase-conjugated goat anti-rabbit IgG serum in conjunction with nitroblue tetrazolium/5-bromo-4-chloro-3-indolyl phosphate detection reagents (Bio-Rad).

RNA sequencing method and data analysis. The transgenic and wild-type lines were infected at the six-leaf stage with ToYSV-[MG-Bi2], as described below in infectivity assay. After 10 days post-inoculation,

total RNA from systemically infected leaves, as diagnosed by PCR, and mock-inoculated leaves from wild-type, 35S::NIK1-4 and 35S::T474D lines was isolated using TRIzol (Invitrogen). For the RNA sequencing experiments we used two biological replicates of a pool of 10 plants at 10 days after inoculation when we detected high levels of viral DNA in systemic leaves but symptoms were not visible as yet. The experimental design was 6 treatments as follow: mock-inoculated (mock WT, mock NIK1-OX, mock T474D) lines and infected (inf WT, inf NIK1-OX, inf T474D) lines with 2 repetitions. The RNA sequencing Illumina data was obtained using a Genome Analyzer in the Fasteris facilities. The GEX-NIaIII protocol was used with the following quality filter parameters: maximum of 1 base below a quality of 5 in the first 30 bases, a minimum average quality of 10, no “N” calls allowed and not more than 35 identical bases (low information reads). The data were stored in a comma-separated values (csv) spreadsheet file.

A systematic comparison in our tomato data set of five representative normalization methods with and without the correction factors for CG-content and gene length was performed using the Bioconductor packages edgeR (Robinson et al., 2010), DESeq (Yang et al., 2013), and EDASeq (Risso et al., 2011). We have also adopted as a comparative experimental design a pairwise comparison and a false discover rate (FDR) p-value adjustment with the cutoff < 0.05 . Four of those normalization methods were implemented on the edgeR, such as total count (TC), upper quartile (UQ) (Bullard et al., 2010), relative log expression (RLE) (Anders and Huber, 2010) and trimmed mean of M values (TMM) (Robinson and Oshlack, 2010); the fifth was the normalization method implemented direct in DESeq package. The GC-content and gene length correction factors were performed by the Bioconductor package EDASeq (Risso et al., 2011). For differential gene expression (DGE) analysis, we employed the normalized data provided by the counting table to the most common negative binomial methods present in R/Bioconductor software, such as edgeR (Robinson et al., 2010), DESeq (Anders and Huber, 2010) and baySeq (Hardcastle et al., 2010). The parameter of dispersion was estimated by the tagwise program. Differential expression was determined using the FDR adjusted cutoff p-value of 0.05. The read mapping process was executed using the Bowtie program (Langmead et al., 2009) with the cDNA data set retrieved from the International Tomato

Annotation Group (ITAG - http://solgenomics.net/organism/Solanum_lycopersicum/genome), second release. Gene ontology classification was performed using the R/Bioconductor packages GSEABase and GOstats. The entire annotation data set from ITAG/Phytozome (<http://www.phytozome.net>) was stored in the relational database PostgreSQL 9.3 (<http://www.postgresql.org>). To detect gene set enrichment from our RNA-seq DE data, we used the GSEA method provided by the R/Bioconductor GSEABase package based on the Gene Ontology (GO) database (Ashburner et al., 2000). Clustering analysis was performed using the R package pvcust (Hierarchical Clustering with P-Values via Multiscale Bootstrap Resampling) using Ward's method (Ward, 1963), and heatmaps were generated using gplots. The results were stored in a relational database created in PostgreSQL, and a web interface was created using PHP to allow the database to be accessed and navigated (<http://tomatodb.inctipp.ufl.edu>). The RNA-seq data of T474D lines were submitted to NCBI-GEO, <http://www.ncbi.nlm.nih.gov/geo/info/linking.html>, accession number GSM932558.

***In vivo* labeling of leaf proteins.** Tomato seedlings (300 mg) were incubated with 1 mL of nutrient solution containing 50 µg/ml chloramphenicol and 20 µCi of [³⁵S]methionine (EasyTag Protein Labeling Mix, [³⁵S]-, 2mCi (74MBq), Perkin Elmer) for 3 h at room temperature. To quantitate incorporation of [³⁵S]methionine into protein, aliquots of protein extracts were placed in 10% (w/v) TCA and incubated on ice for 30 min. The samples were filtered onto glass microfiber filters and the filters were washed three times with 5 ml of cold 5% (w/v) TCA and two times with 5 ml of 95% ethanol. After drying, the filters were counted with a scintillation counter.

Polysome fractionation. Polysomes were fractionated over sucrose gradients as described (Wang et al., 2003). Briefly, 500 mg of 15-day-old tomato seedlings were ground in liquid nitrogen and 1 mL of extraction buffer (0.2 M Tris-HCl, pH 8.0, 50 mM KCl, 25 mM MgCl₂, 1% Triton X-100, 400 units/mL of RNasin and 50 mg/mL of cycloheximide). After centrifuging for 10 min, the supernatant was loaded onto a 10-mL 15% to 50% sucrose gradient and spun in a Beckman SW41Ti rotor at 135,000 g for 3.5 h.

Fractions were collected manually from the bottom, and total RNA was extracted with phenol/chloroform/isoamyl alcohol, precipitated with isopropanol, and treated with DNase I. The specific transcripts were amplified from RNA of T474D, NIK1 and wilt-type infected lines using qRT-PCR.

Infectivity assays. For the infectivity assays, we used T2 transgenic plants harboring the T474D mutant gene construct, which were derived from four independently regenerated kanamycin-resistant plants (35S::T474D-2, 35S::T474D-5, 35S::T474D-6 and 35S::T474D-9). We also used the previously described transgenic lines expressing AtNIK1 under the control of the CaMV 35S promoter, 35S::NIK1-4 and 35S::NIK1-6 (Carvalho et al., 2008c). The transgenic and wild-type lines were infected at the six-leaf stage with either ToYSV-[MG-Bi2] or ToSRV by biolistic delivery using tandemly repeated viral DNA-A and DNA-B and a microprojectile bombardment model PDS-1000/He accelerator (BIORAD) at 900 psi. In each experiment, 20 plants of each line were inoculated with 2 µg of tandemly repeated DNA-A plus DNA-B per plant and grown in a greenhouse under natural conditions of light, 70% relative humidity and approximately equal day and night lengths. Total nucleic acid was extracted from the systemically infected leaves (young leaves), and viral DNA was detected by PCR using DNA-A and DNA-B begomovirus-specific primers (PBL1v 2040, GCCTCTGCAGCARTGRTCKATCTTCATACA, and PCRC1, CTAGCTGCAGCATATTTACRARWATGCCA, or PAL1v1978, GCATCTGCAGGCCACATYGTCTTYCCNGT, and PAR1c496, AATACTGCAGGGCTTYCTRTRACATRGG) at 10 days post-infection.

Quantitation of viral DNA in infected plants. Viral DNA accumulation was measured by quantitative PCR (qPCR). The reactions were prepared in a final volume of 10 µl using the Fast SYBR Green Master Mix (Applied Biosystems) according to the manufacturer's instructions and analyzed on a 7500 Real Time PCR System (Applied Biosystems). Virus-specific primers were designed using Primer Express 3.0 (Applied Biosystems) and tested by conventional PCR using plasmids containing the complete DNA-A of each virus (10^6 copies per reaction). The following primer sequences were used: ToSRVFwd,

CACGTGCCCACATCGTCTT, and ToSRVRev, GGCCGGAACGACCTATTA-3', or ToYSVFwd, CCACGATTTTAAAGCTGCATTCT, and ToYSVRev, CAATCCTGGTGAGGGAGTCAGT. For viral DNA quantitation, standard curves were prepared using serial dilutions of these clones (10^0 to 10^6 copies of viral genome per reaction). The genomic unit refers to one copy of the DNA-A of ToYSV or ToSRV. Standard curves were obtained by regression analysis of the Ct values of each of the three replicates of a given dilution in relation to the log of the amount of DNA in each dilution. For the absolute quantitation of the number of viral DNA molecules in the different treatments, 100 ng of total DNA from the infected plants was used in the qPCR reactions containing virus-specific primers. Each sample was analyzed in triplicate from at least two biological replicates.

Physiological measurements of tomato transgenic lines. Photosynthetic CO₂ assimilation (*A*), transpiration rate (*E*), and stomatal conductance (*g_s*) measurements were performed with a portable open-flow gas exchange system (LICOR 6400, Li-COR, Lincoln, Nebraska, USA) under ambient CO₂ concentrations ($370 \pm 10 \mu\text{mol mol}^{-1}$) and temperature conditions under artificial, saturating PAR ($1,000 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ at the leaf level).

Estimation of total carotenoids, lycopene, and β -carotene. Total carotenoids from fully ripened tomato fruits were extracted with cold acetone and petroleum ether, as described by Rodriguez-Amaya et al. (1976). The extract (30 μL) was separated by HPLC using reversed phase C18 column (Phenomenex Gemini, 250 x 4,6 mm, 5 μm) and a C18 guard column (Phenomenex ODS, 4 mm x 3 mm) on a Shimadzu, SCL 10AT VP HPLC system coupled to a DAD detector (Shimadzu, SPD-M10A) and operating at a flow rate of 1.7 mL/min. The mobile phase buffer used was methanol:ethyl acetate:acetonitrile (70:20:10, v/v/v). The chromatograms were obtained at 450 nm and integrated using the software Multi System Class Vp 6.12. Lycopene and β -carotene were quantified from HPLC profile

by using a purified lycopene standard (Sigma Chemical Co.) and a β -carotene standard purified from carrots.

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Supporting information - Figures S1-S6

Sustained NIK-mediated antiviral signaling confers broad-spectrum tolerance to begomoviruses in cultivated plants

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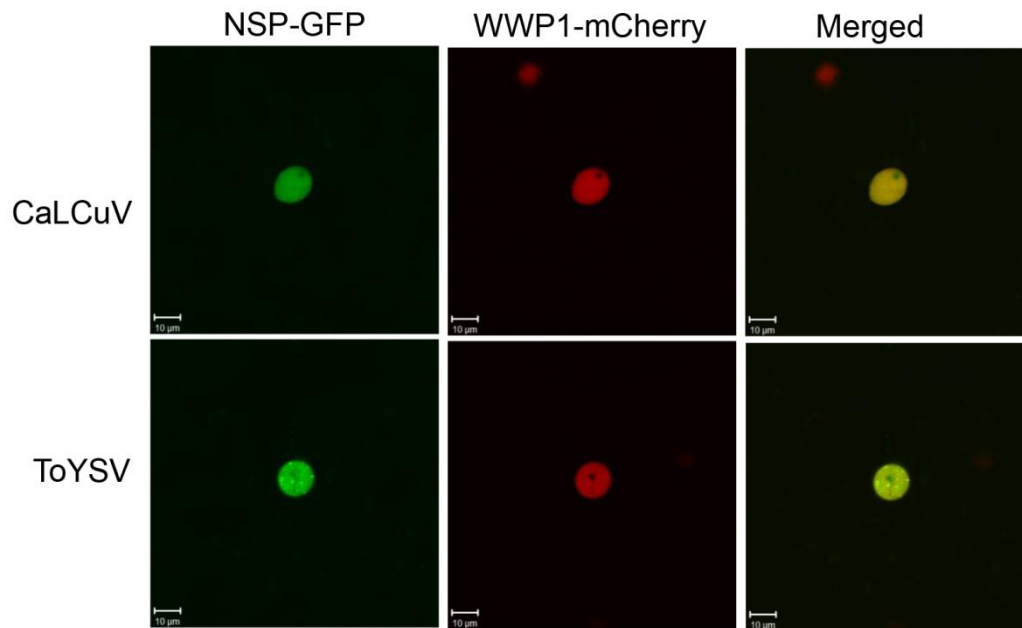


Figure S1. NSP from CaLCuV and ToYSV concentrates in the nucleus when ectopically expressed in *N. benthamiana* leaves. *N. benthamiana* leaves were co-infiltrated with *A. tumefaciens* carrying a 35S::NSP-GFP construct from CaLCuV or ToYSV together with a 35S::AtWWP1-mCherry construct. After 48 h post-infiltration, the subcellular localizations of the fluorescent fusion proteins were examined by confocal microscopy.

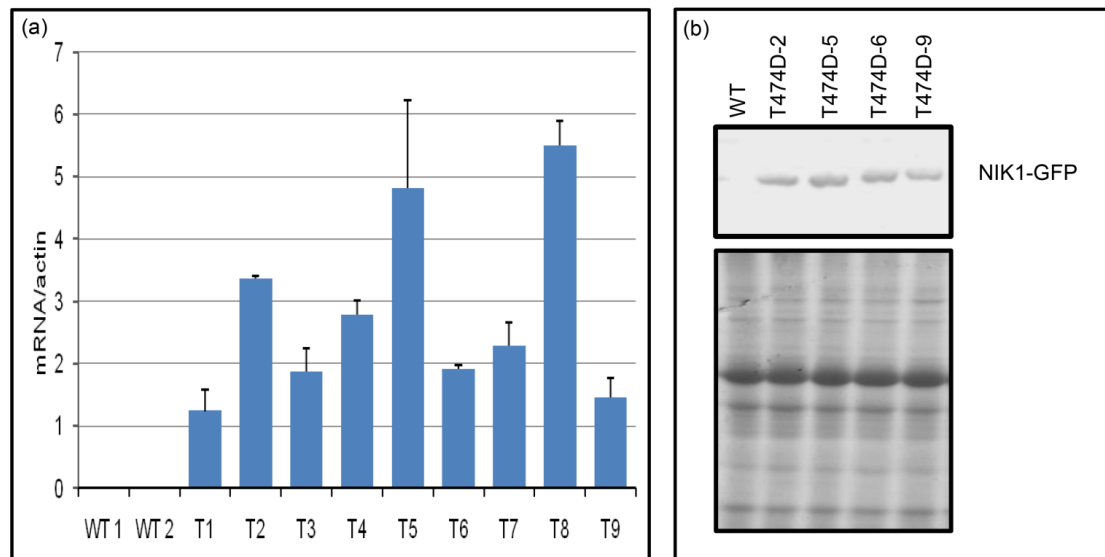


Figure S2. Overexpression of T474D mutant receptor in tomato leaves.

(a) T474D transcript accumulation in primary transformants. The expression of T474D in the leaves of several independent transgenic lines was monitored by quantitative RT-PCR. Values of expression were calculated using the $2^{-\Delta Ct}$ method with actin as an endogenous control. Values represent the mean \pm SD of three replicates. **(b)** Accumulation of T474D-GFP in transgenic lines. Total protein was extracted from the leaves of independent transgenic lines (as indicated), fractionated by SDS-PAGE (bottom) and immunoblotted with an anti-GFP antiserum.

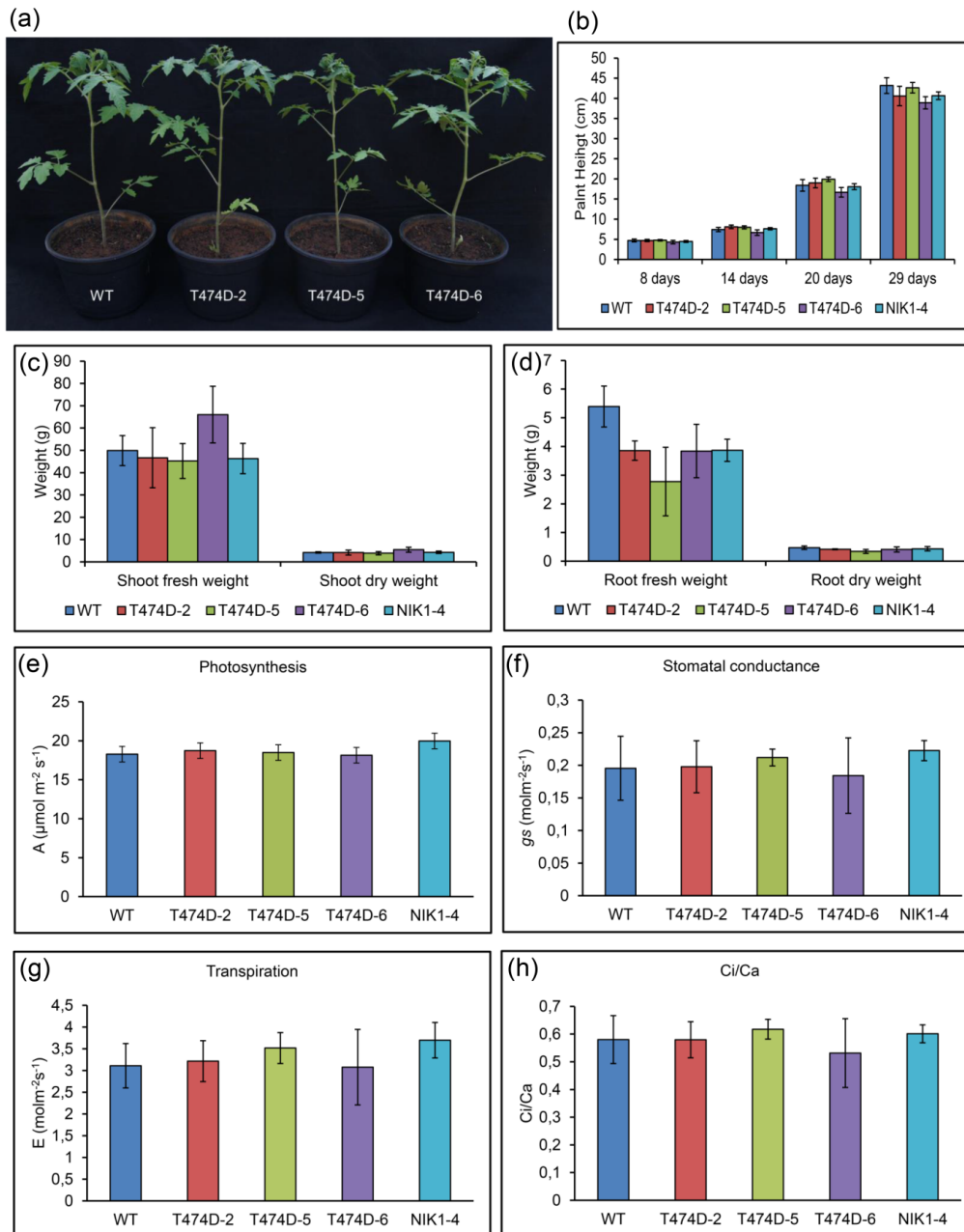


Figure S3. Characterization of the T474D-overexpressing lines during the vegetative phase.

(a), (b), (c) and (d) Developmental phenotypes associated with overexpression of the T474D gain-of-function mutant in the R3 generation of tomato transgenic lines. (a) The images are plants of T474D-2, T474D-5, T474D-6 and wild-type lines grown for 30 days under normal greenhouse conditions. The transgenic lines are visibly undistinguishable from the wild-type plant. The indicated day correspond to the period of time after transferring germinated seedlings to the soil. (b) Plant height of wild-type line and T474D-2, T474D-5, T474D-6 and NIK1-4 transgenic lines grown for 8, 14, 20 or 29 days in the greenhouse. The indicated days correspond to the period of time after transferring germinated seedlings to the soil. Values represent the mean \pm IC ($\alpha=0,05$) of 11 biological replicates and did not differ between wild-type and transgenic lines in each period of the measurement. (c) Shoot fresh and dry weight and (d) root fresh and dry weight of wild-type and transgenic lines (as indicated) grown for 30 days in greenhouse. Values represent the mean \pm IC ($\alpha=0,05$) of three biological replicates. (e-h) Physiological measurements of transgenic lines. The net CO_2 assimilation rate (A), transpiration rate (E), stomatal conductance to water vapor (g_s) and internal-to-ambient CO_2 concentration ratio (C_i/C_a) of fully expanded leaves of wild-type, T474D-2, T474D-5 and T474D-6 transgenic lines were measured by the LI-6400 infrared (IR) gas analyzer at growth irradiance. The error bars represent the confidence interval ($\alpha = 0.05$) of measurements from five individual plants.

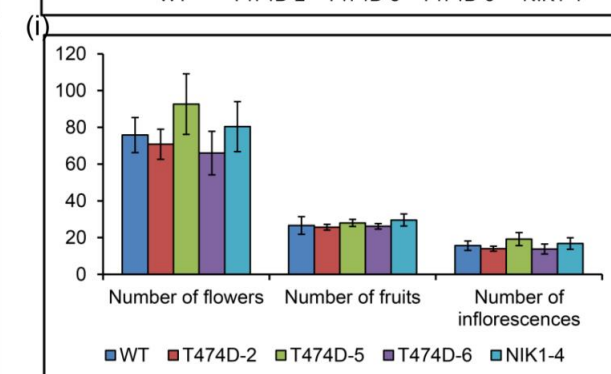
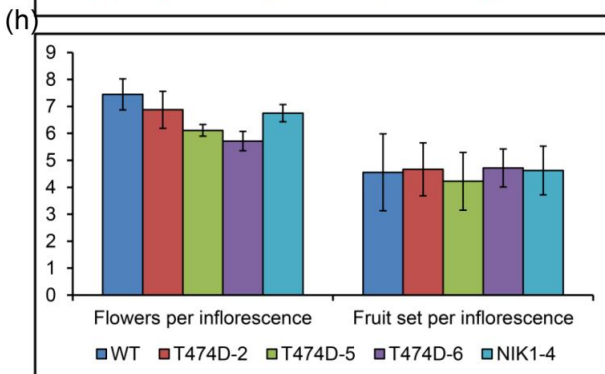
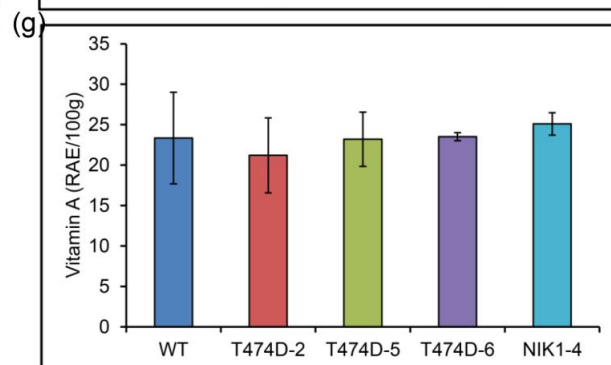
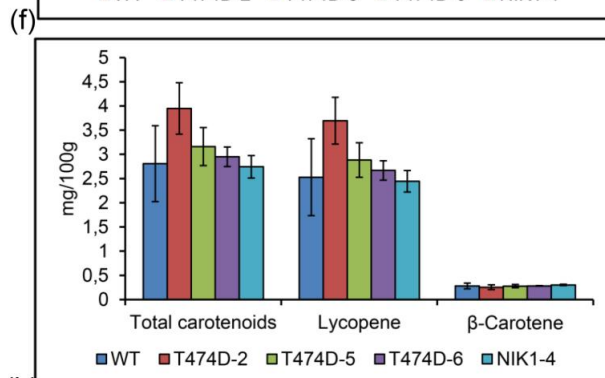
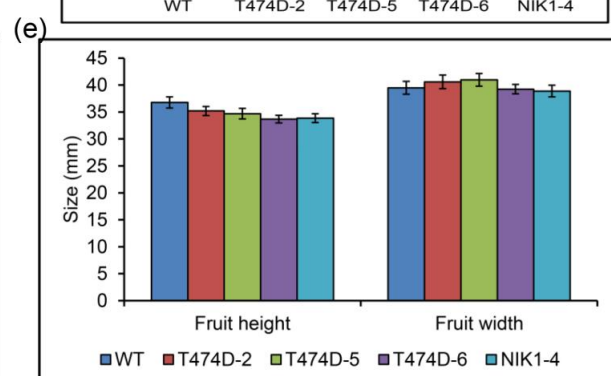
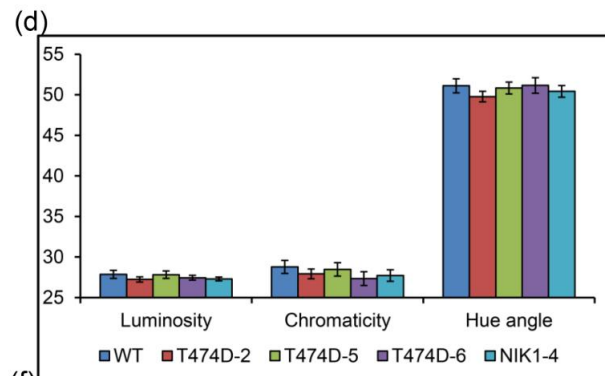
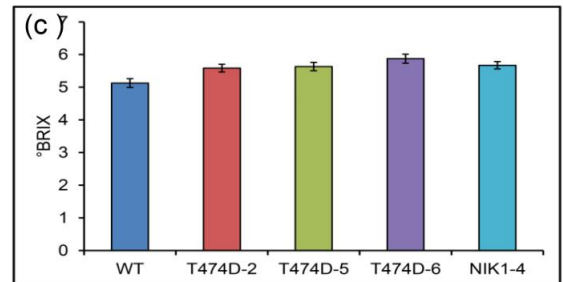
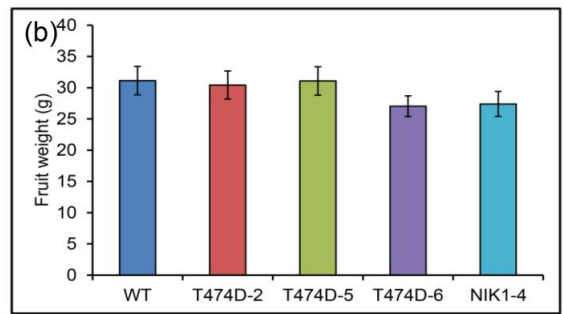
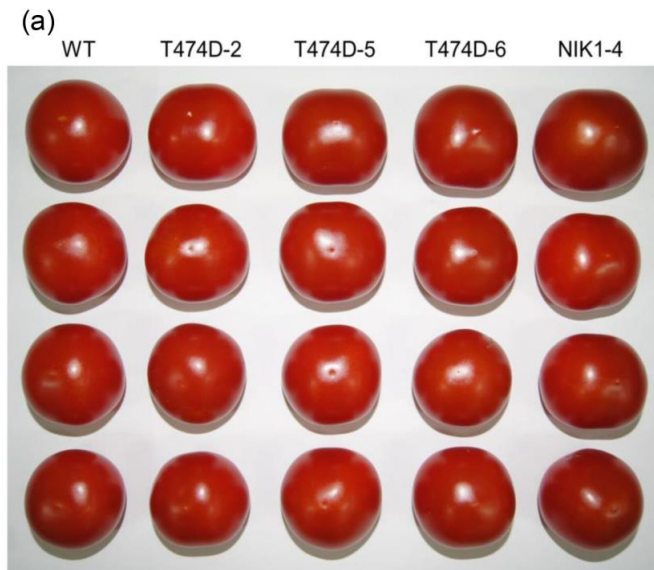


Figure S4. Fruit quality and yield of the T474D-overexpressing lines (a) Morphology and color of fresh ripe tomato fruits from T474D-overexpressing lines. The ripe fruit of the transgenic lines were bright red and were classified as small round, varying in size as indicated in the figure. (b) Fruit weight. Error bars, 95% confidence intervals (n=5) based on bootstrap resampling replicates from 160 fruits. (c) Content of soluble solids of tomato fruits from T474D-, NIK1-overexpressing lines and wild-type. Fully ripened tomato fruits were also analyzed for total soluble solids (TSS), which were not different between the transgenic lines and wild-type. Total soluble solids were determined using a manual refractometer model ATC103 (BIOBRIX). The tomato sample was squashed manually and about three or four drops were transferred to the refractometer. Results were expressed as degrees of Brix. Error bars, 95% confidence intervals (n=3) based on bootstrap resampling replicates from 85 fruits. (d) Skin color of tomato fruits. Skin coloration was analyzed according to luminosity (*L*), chromaticity (*C*) and hue angle (*H*) parameters (two readings per fruit), by means of reflectometry in a colorimeter brand KONICA MINOLTA. Values represent the mean \pm IC ($\alpha=0,05$) of 53 fruits. (e) Fruit Size. Fully ripened tomato fruits were also analyzed for size. Values represent the mean \pm IC ($\alpha=0,05$) of 160 fruits. (f) Content of carotenoids. Total carotenoids, lycopene and β -carotene were determined by HPLC. Values represent the mean \pm IC ($\alpha=0,05$, n=3) from three biological replicates. (g) Vitamin A content. The vitamin A content is expressed as recommended by the Institute of Medicine (2001), in which 1 retinol activity equivalent (RAE) corresponds to 1 μg of retinol, 12 μg of β -carotene and 24 μg of other pro-vitamin carotenoids. Values represent the mean \pm IC ($\alpha=0,05$, n=3) from three biological replicates. (h) and (i). Developmental and yield performance of wild type and transgenic lines. The number of days to flowering (30-31 days) did not differ among transgenic lines and wild-type control. The number and size of inflorescence as well as fruit yield were measured. Values represent the mean \pm IC ($\alpha=0,05$) of seven biological replicates.

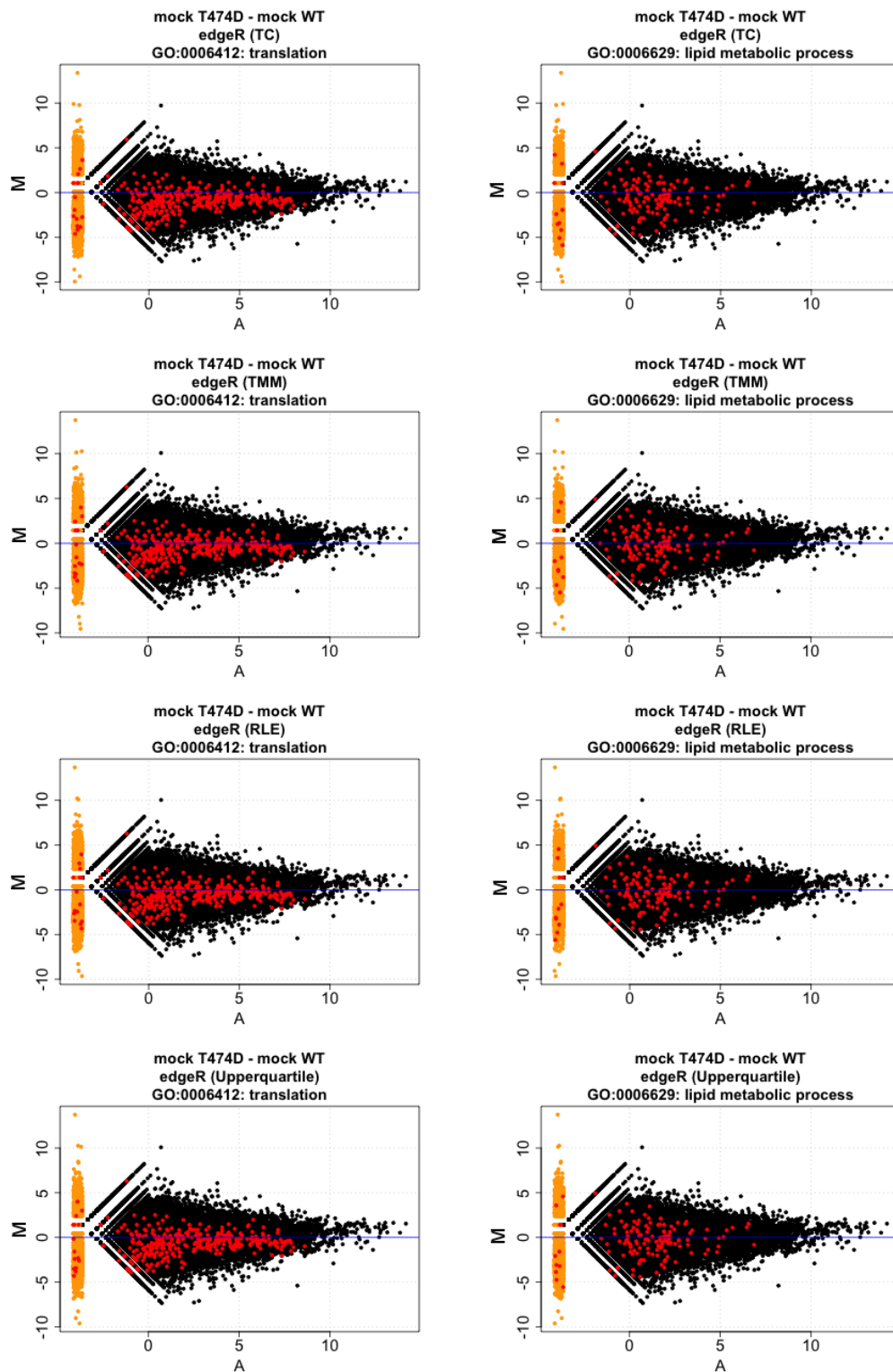


Figure S5. Representation of the translational machinery-related genes in the down-regulated changes. The 'MA' plots show the log of the ratio of expression levels against log-concentration, and each dot represents a gene. These plots are from the contrast mock T474D-mock WT and the normalization method used is shown on the top of the plots. The smear of points on the left side indicates genes that were observed in only one group of replicated samples, and the red points denote the translation related genes (GO:0006412) or lipid metabolic process-related genes (GO:0006629). For the translation related genes, the density of the red dots is concentrated below zero, whereas for the lipid metabolic process-related genes, the red dots are spread to the overall dispersion.

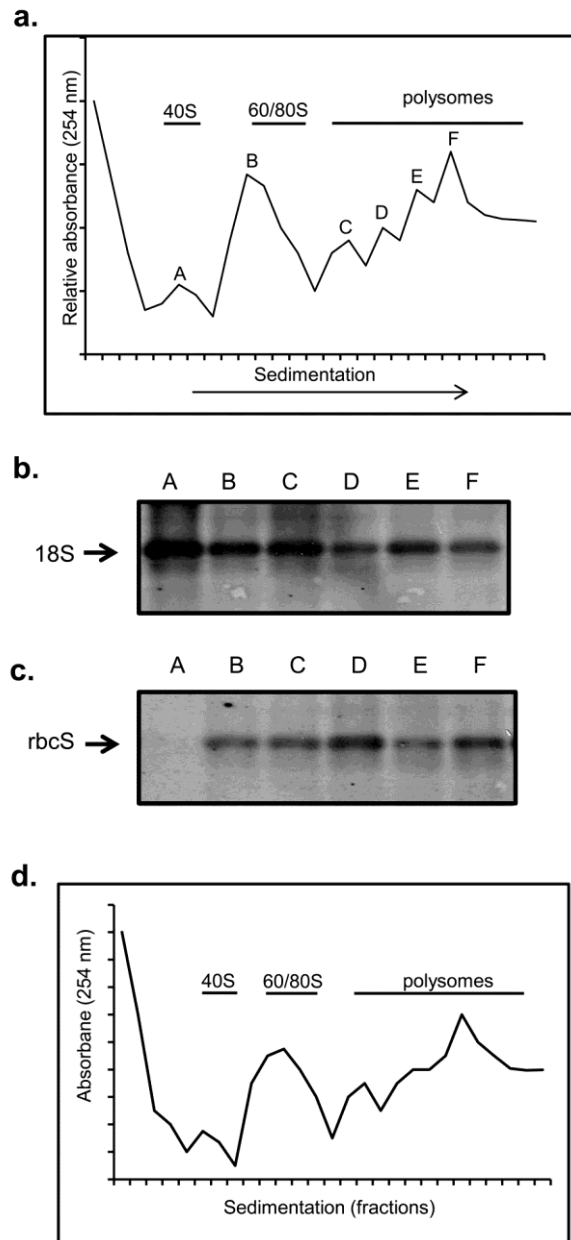


Figure S6. Isolation of polysomal fractions from tomato seedlings.

(a) UV absorbance profiles of the sucrose gradient used for RNA fractionation from infected T474D-overexpressing lines. Polysomes from infected T474D-overexpressing lines at 10 DPI were fractionated on a sucrose gradient, and the fractions were manually collected. (b) Distribution of 18S rRNA on the sucrose gradient. Total RNA from the fractions, as indicated in A by the letters, was extracted with phenol/chloroform/isoamyl alcohol, precipitated with isopropanol, blotted and probed with 18S rDNA. The result indicates the distribution of the 40S small subunit. The higher concentration of 18S in fraction A indicates a 40S-enriched fraction. The heavier fractions are monosomes (RNPs) and polysomes. (c) Levels of *rbcS* mRNA per fraction. The levels of the small subunit of rubisco (*rbcS*) mRNA were examined by northern blotting. This control was used to ensure the quality and distribution of a specific mRNA. (d) UV absorbance profiles of the sucrose gradient used for RNA fractionation of the infected wild type leaves at 10 DPI. Polysomes from wild type leaves at 10 DPI were isolated as in (a). The pooled fractions were used for RNA extraction and for the amplification of specific resistance-like gene transcripts. The bars indicate actin and *rbcS* transcripts.

Table S1. Enriched biological process categories from the GO database using the GSEA* method

Infected WT – mock WT (UP-regulated)			
Enriched Categories	DGE** methods: number of genes / number of genes in the GO group (p-value)	AN***	Average p-value
GO:0006073: cellular glucan metabolic process	edgeR/RLE: 4/93 (0.007615) edgeR/upper: 4/93 (0.008759) baySeq: 7/93 (0.006532)	5	0.007635
GO:0009765: photosynthesis, light harvesting	edgeR/RLE: 3/33 (0.002581) edgeR/upper: 3/33 (0.002895) baySeq: 4/33 (0.007371)	3	0.004282
Infected WT – mock WT (DOWN-regulated)			
Enriched Categories	DGE methods: number of genes / number of genes in the GO group (p-value)	AN	Average p-value
GO:0006629: lipid metabolic process	edgeR/TMM: 19/592 (1.79e-05) edgeR/TC: 22/592 (0.0004238) edgeR/RLE: 16/592 (8.847e-05) edgeR/upper: 7/343 (0.009761)	16	1.77E-04
GO:0006633: fatty acid biosynthetic process	edgeR/TMM: 5/69 (0.00087) edgeR/TC: 5/69 (0.006144) edgeR/RLE: 5/69 (0.0004101) edgeR/upper: 5/69 (0.001461) DESeq: 2/69 (0.006187)	5	0.00275
GO:0009611: response to wounding	edgeR/TMM: 5/22 (3.054e-06) edgeR/TC: 5/22 (2.732e-05) edgeR/RLE: 5/22 (1.351e-06) edgeR/upper: 5/22 (4.486e-07) DESeq: 3/22 (6.617e-06)	5	7.76E-06
GO:0032787: monocarboxylic acid metabolic process	edgeR/TMM: 5/96 (0.003758) edgeR/RLE: 5/96 (0.001836) edgeR/upper: 5/96 (0.000681)	5	0.002091
GO:0044283: small molecule biosynthetic process	edgeR/TMM: 8/217 (0.002388) edgeR/RLE: 7/217 (0.003716) edgeR/upper: 7/217 (0.001046)	7	0.002383
GO:0046394: carboxylic acid biosynthetic process	edgeR/TMM: 8/189 (0.0009954) edgeR/RLE: 7/189 (0.001713) edgeR/upper: 7/189 (0.000462)	7	0.0010568
GO:0055114: oxidation-reduction process	edgeR/TMM: 29/1645 (0.004426) edgeR/RLE: 25/1645 (0.00631) edgeR/upper: 21/1645 (0.006762)	25	0.005833
Mock NIK-OX – mock WT (UP-regulated)			
Enriched Categories	DGE methods: number of genes / number of genes in the GO group (p-value)	AN	
GO:0046148: pigment biosynthetic process	edgeR/TMM: 3/28 (0.006671) edgeR/TC: 3/28 (0.004615) edgeR/RLE: 3/28 (0.0075) edgeR/upper: 3/28 (0.003001)	3	0.0038946
Mock NIK-OX – mock WT (DOWN-regulated)			
Enriched Categories	DGE methods: number of genes / number of genes in the GO group (p-value)	AN	Average p-value
GO:0009611: response to wounding	edgeR/TMM: 3/22 (0.001621) edgeR/TC: 3/22 (0.003048) edgeR/RLE: 3/22 (0.001937) edgeR/upper: 3/22 (0.002832)	3	0.0023595
GO:0043648: dicarboxylic acid metabolic process	edgeR/TMM: 3/27 (0.002961) edgeR/TC: 3/27 (0.005512) edgeR/RLE: 3/27 (0.003528) edgeR/upper: 3/27 (0.005128)	3	0.0042822
GO:0044262: cellular carbohydrate	edgeR/TMM: 7/214 (0.00841) edgeR/TC: 8/214 (0.00833) edgeR/upper: 8/214 (0.007165)	8	0.007968

metabolic process			
Mock T474D – mock WT (UP-regulated)			
<i>Enriched Categories</i>	<i>DGE methods: number of genes / number of genes in the GO group (p-value)</i>	<i>AN</i>	<i>Average p-value</i>
GO:0006629: lipid metabolic process	edgeR/TMM: 47/592 (0.007967) edgeR/RLE: 46/592 (0.003633) edgeR/upper: 45/592 (0.007517) baySeq: 45/343 (5.446e-06)	46	0.00478
GO:0009765: photosynthesis, light harvesting	edgeR/TMM: 6/33 (0.008713) edgeR/upper: 6/33 (0.006678) DESeq: 5/33 (0.005769) baySeq: 8/33 (0.001161)	7	0.00558
GO:0009607: response to biotic stimulus	edgeR/TMM: 9/58 (0.009423) edgeR/upper: 8/58 (0.007652) baySeq: 10/58 (0.004553)	9	0.007209
GO:0044283: small molecule biosynthetic process	edgeR/TMM: 21/217 (0.009161) edgeR/RLE: 20/217 (0.008819) edgeR/upper: 21/217 (0.004946)	21	0.007642
GO:0051247: positive regulation of protein metabolic process	edgeR/TMM: 3/7 (0.00502) edgeR/RLE: 3/7 (0.004125) edgeR/upper: 3/7 (0.004283)	3	0.004476
Mock T474D – mock WT (DOWN-regulated)			
<i>Enriched Categories</i>	<i>DGE methods: number of genes / number of genes in the GO group (p-value)</i>	<i>AN</i>	<i>Average p-value</i>
GO:0006260: DNA replication	edgeR/TMM: 12/87 (0.001556) edgeR/RLE: 12/87 (0.003899) edgeR/upper: 12/87 (0.00235)	12	0.0026
GO:0006270: DNA replication initiation	edgeR/TMM: 2/2 (0.002646) edgeR/TC: 2/2 (0.007579) edgeR/RLE: 2/2 (0.003304) edgeR/upper: 2/2 (0.002919)	2	0.004112
GO:0006275: regulation of DNA replication	edgeR/TMM: 2/2 (0.002646) edgeR/TC: 2/2 (0.007579) edgeR/RLE: 2/2 (0.003304) edgeR/upper: 2/2 (0.002919) DESeq: 2/2 (0.00171)	2	0.00363
GO:0006323: DNA packaging	edgeR/TMM: 12/77 (0.0005285) edgeR/RLE: 12/77 (0.001394) edgeR/upper: 12/77 (0.0008159)	12	0.004509
GO:0006333: chromatin assembly or disassembly	edgeR/TMM: 12/80 (0.0007529) edgeR/TC: 15/80 (0.003447) edgeR/RLE: 12/80 (0.001953) edgeR/upper: 12/80 (0.001154)	13	0.001826
GO:0006334: nucleosome assembly	edgeR/TMM: 12/77 (0.0005285) edgeR/RLE: 12/77 (0.001394) edgeR/upper: 12/77 (0.0008159)	12	0.000913
GO:0006412: translation	edgeR/TMM: 53/579 (2.923e-05) edgeR/TC: 110/579 (1.131e-15) edgeR/RLE: 67/579 (2.465e-08) edgeR/upper: 59/579 (1.62e-06) DESeq: 51/579 (2.342e-07)	68	4.69E-07
GO:0006629: lipid metabolic process	edgeR/TMM: 56/592 (6.552e-06) edgeR/TC: 82/592 (1.355e-05) edgeR/RLE: 61/592 (5.595e-06) edgeR/upper: 58/592 (6.822e-06) DESeq: 43/592 (0.0002239)	60	0.000645
GO:0007018: microtubule-based movement	edgeR/TMM: 14/60 (1.519e-06) edgeR/TC: 19/60 (3.965e-07) edgeR/RLE: 16/60 (1.667e-07) edgeR/upper: 15/60 (4.582e-07) DESeq: 11/60 (2.969e-05)	15	4.07E-04
GO:0009059: macromolecule biosynthetic process	edgeR/TC: 250/2200 (1.178e-06) edgeR/RLE: 156/2200 (0.00198) edgeR/upper: 145/2200 (0.004376)	184	6.45E-03
GO:0009611: response to wounding	edgeR/TMM: 7/22 (8.009e-05) edgeR/TC: 7/22 (0.001975) edgeR/RLE: 7/22 (0.0001609) edgeR/upper: 7/22 (0.0001092) DESeq: 7/22 (1.977e-05)	7	0.00251
GO:0009765: photosynthesis,	edgeR/TMM: 9/33 (3.03e-05) edgeR/TC: 9/33 (0.001575) edgeR/RLE: 9/33 (7.225e-05) edgeR/upper: 9/33 (4.459e-05) DESeq: 9/33 (5.25e-06)	9	0.00237

light harvesting			
GO:0015995: chlorophyll biosynthetic process	edgeR/TMM: 4/13 (0.003425) edgeR/RLE: 4/13 (0.005109) edgeR/upper: 4/13 (0.00409)	4	0.000421
GO:0022607: cellular component assembly	edgeR/TMM: 17/153 (0.002249) edgeR/RLE: 17/153 (0.006862) edgeR/upper: 17/153 (0.003725)	17	0.00428
GO:0034622: cellular macromolecular complex assembly	edgeR/TMM: 17/131 (0.0003895) edgeR/TC: 21/131 (0.004471) edgeR/RLE: 17/131 (0.001345) edgeR/upper: 17/131 (0.0006802)	18	0.001721
GO:0044249: cellular biosynthetic process	edgeR/TMM: 163/2634 (0.004014) edgeR/TC: 296/2634 (2.09e-07) edgeR/RLE: 187/2634 (0.0005531) edgeR/upper: 172/2634 (0.002566)	205	0.00178
GO:0071824: protein-DNA complex subunit organization	edgeR/TMM: 12/77 (0.0005285) edgeR/RLE: 12/77 (0.001394) edgeR/upper: 12/77 (0.0008159)	12	9,13E-04
GO:0071840: cellular component organization or biogenesis	edgeR/TMM: 39/488 (0.004133) edgeR/TC: 62/488 (0.001511) edgeR/RLE: 42/488 (0.005659) edgeR/upper: 40/488 (0.005529)	46	0.00421
GO:1901576: organic substance biosynthetic process	edgeR/TMM: 173/2717 (0.0007624) edgeR/TC: 60/517 (0.002539) edgeR/RLE: 43/517 (0.003325) DESeq: 139/2717 (0.002492)	104	0.00228

*GSEA - Gene set enrichment analysis

**DGE – Differential gene expression

***AN – Average number

Table S2. Resistance protein-related genes differentially expressed in infected T474D (infected T474D-mock T474D)

Up-regulated resistance protein-related genes				
ITAG	LOG2FC	Q-VALUE	GO	DESCRIPTION
Solyc02g032050.1.1	1.620578758	0.040756495		Disease resistance-responsive (dirigent-like protein) family protein
Solyc05g042090.1.1	4.907459511	5.24E-06	GO:0043531,GO:0006915,GO:0005524	NB-ARC domain-containing disease resistance protein
Solyc07g008590.1.1	1.572643749	0.005472267	GO:0005515	disease resistance family protein / LRR family protein
Solyc07g008620.1.1	2.208276596	2.04E-06	GO:0005515	disease resistance family protein / LRR family protein
Solyc08g081780.1.1	1.470947445	0.002867545		Disease resistance-responsive (dirigent-like protein) family protein
Solyc12g044190.1.1	3.942547883	1.63E-12	GO:0043531,GO:0006915,GO:0005524	LRR and NB-ARC domains-containing disease resistance protein
Solyc12g097010.1.1	1.982314928	0.001087781	GO:0005515	disease resistance protein (TIR-NBS-LRR class), putative
Down-regulated resistance protein-related genes				
ITAG	LOG2FC	Q-VALUE	GO	DESCRIPTION
Solyc05g054340.2.1	-1.752069826	0.000112318	GO:0043531,GO:0006915,GO:0005524	NB-ARC domain-containing disease resistance protein
Solyc07g061940.2.1	-1.119795309	4.23E-03	GO:0000287,GO:0030976,GO:0003824	chlorsulfuron/imidazolinone resistant 1

Table S3. Gene Enrichment Analysis*					
(edgeR TMM T474D infected – T474D mock)					
Biological Process	Total	Downregulated genes	P-value	Upregulated genes	P-value2
ITAG 2.3 (Number of genes)	34727	758		1115	
Response to Salicylic Acid (GO:0009751)	404	6	0.87703	9	0.903966
Defense response to pathogen (GO:0042742 + GO:0050832)	1038	14	0.981838	36	0.34073
Defense response to virus (GO:0051607)	115	1	0.921305	2	0.887503
Virus induced gene silencing (GO:0009616)	198	1	0.987503	6	0.613786
Resistance like proteins	450	2	9.99E-01	7	0.990446

* Data were retrieved from <http://amigo.geneontology.org/amigo/term/GO:0009607> (Reponse to biotic stress GO:0009607). These groups are presented at : Source ITAG 2.3

** Statistical test based on hypergeometric distribution probability with significance of < 0.01

Response to Salicylic Acid (GO:0009751)

Up-regulated genes:

9

<i>ITAG</i>	<i>log2FC</i>	<i>q-value</i>	<i>Functional Description</i>
Solyc10g008930.1.1	5.415038	1.99E-09	Thioredoxin superfamily protein
Solyc09g006010.2.1	1.60671	0.016183	pathogenesis-related gene 1
Solyc03g044710.2.1	3.150635	0.000397	phospholipase A 2A
Solyc12g009770.1.1	1.685177	0.049657	receptor like protein 33
Solyc09g074270.2.1	2.016299	0.040922	alpha/beta-Hydrolases superfamily protein
Solyc03g095770.2.1	1.483342	2.15E-05	WRKY DNA-binding protein 70
Solyc08g062930.1.1	1.371314	0.040922	calmodulin-binding family protein
Solyc12g008960.1.1	2.706816	0.01327	calmodulin-binding family protein
Solyc06g073080.2.1	4.773136	0.013878	2-oxoglutarate (2OG) and Fe(II)-dependent oxygenase superfamily protein

Down-regulated genes:

6

<i>ITAG</i>	<i>log2FC</i>	<i>q-value</i>	<i>Functional Description</i>
Solyc07g044860.2.1	-1.773859	0.022822	photosystem II subunit P-1
Solyc04g076280.2.1	-1.483044	0.000125	K-box region and MADS-box transcription factor family protein
Solyc06g066370.2.1	-1.151764	0.003015	WRKY DNA-binding protein 33
Solyc04g079240.2.1	-2.216969	0.015538	phospholipase A 2A
Solyc02g065400.2.1	-1.336943	0.023129	photosystem II subunit O-2
Solyc11g072540.1.1	-1.239099	0.005653	Transducin/WD40 repeat-like superfamily protein

Defense response to pathogen (GO:0042742 + GO:0050832)

Up-regulated genes:

36

<i>ITAG</i>	<i>log2FC</i>	<i>q-value</i>	<i>Functional Description</i>
Solyc01g088400.2.1	2.55705	2.19E-08	Fatty acid hydroxylase superfamily
Solyc10g008930.1.1	5.415038	1.99E-09	Thioredoxin superfamily protein
Solyc08g082850.2.1	1.300149	0.001843	general control non-repressible 3
Solyc11g022590.1.1	4.206634	3.18E-30	kunitz trypsin inhibitor 1
Solyc09g006010.2.1	1.60671	0.016183	pathogenesis-related gene 1
Solyc03g044710.2.1	3.150635	0.000397	phospholipase A 2A

Solyc03g122140.2.1	2.157982	0.024809 Aldolase-type TIM barrel family protein
Solyc01g006320.2.1	2.558596	0.014784 Late embryogenesis abundant (LEA) hydroxyproline-rich glycoprotein family
Solyc06g074030.1.1	2.07484	7.25E-05 Polynucleotidyl transferase, ribonuclease H-like superfamily protein
Solyc06g084090.2.1	4.159211	0.002042 histone H2A 11
Solyc03g095770.2.1	1.483342	2.15E-05 WRKY DNA-binding protein 70
Solyc09g092580.2.1	6.39079	7.31E-05 cytochrome P450, family 83, subfamily B, polypeptide 1
Solyc12g096520.1.1	2.734469	0.013099 HOPW1-1-interacting 2
Solyc08g062930.1.1	1.371314	0.040922 calmodulin-binding family protein
Solyc12g008960.1.1	2.706816	0.01327 calmodulin-binding family protein
Solyc07g066470.2.1	2.115155	0.000707 hydroxymethylbilane synthase
Solyc06g073080.2.1	4.773136	0.013878 2-oxoglutarate (2OG) and Fe(II)-dependent oxygenase superfamily protein
Solyc04g014900.2.1	2.261185	0.000214 Leucine-rich receptor-like protein kinase family protein
Solyc01g088400.2.1	2.55705	2.19E-08 Fatty acid hydroxylase superfamily
Solyc10g008930.1.1	5.415038	1.99E-09 Thioredoxin superfamily protein
Solyc09g006010.2.1	1.60671	0.016183 pathogenesis-related gene 1
Solyc03g044710.2.1	3.150635	0.000397 phospholipase A 2A
Solyc07g008590.1.1	1.572644	0.005472 disease resistance family protein / LRR family protein
Solyc07g008620.1.1	2.208277	2.04E-06 disease resistance family protein / LRR family protein
Solyc09g083440.2.1	1.655611	0.003274 Serine protease inhibitor, potato inhibitor I-type family protein
Solyc09g084480.2.1	3.754167	2.17E-28 Serine protease inhibitor, potato inhibitor I-type family protein
Solyc05g055540.1.1	1.917008	0.049714 Major facilitator superfamily protein
Solyc10g055810.1.1	1.398374	1.58E-08 basic chitinase
Solyc10g068350.1.1	1.594934	0.00285 basic chitinase
Solyc10g074460.1.1	2.699916	0.000222 basic chitinase
Solyc06g035960.2.1	1.793104	0.010737 AMP-dependent synthetase and ligase family protein
Solyc03g095770.2.1	1.483342	2.15E-05 WRKY DNA-binding protein 70
Solyc09g074270.2.1	2.016299	0.040922 alpha/beta-Hydrolases superfamily protein
Solyc08g062930.1.1	1.371314	0.040922 calmodulin-binding family protein
Solyc12g008960.1.1	2.706816	0.01327 calmodulin-binding family protein
Solyc06g073080.2.1	4.773136	0.013878 2-oxoglutarate (2OG) and Fe(II)-dependent oxygenase superfamily protein

Down-regulated:

<i>ITAG</i>	<i>log2FC</i>	<i>q-value</i>	<i>Functional Description</i>
Solyc07g044860.2.1	-1.773859	0.022822	photosystem II subunit P-1
Solyc12g011330.2.1	-1.275852	0.046707	Signal transduction histidine kinase, hybrid-type, ethylene sensor
Solyc04g079240.2.1	-2.216969	0.015538	phospholipase A 2A
Solyc06g066370.2.1	-1.151764	0.003015	WRKY DNA-binding protein 33
Solyc01g099780.2.1	-2.066435	0.000181	translationally controlled tumor protein
Solyc02g065400.2.1	-1.336943	0.023129	photosystem II subunit O-2
Solyc04g012160.2.1	-1.346069	0.000802	Protein kinase superfamily protein
Solyc04g080960.2.1	-1.618068	1.01E-08	Papain family cysteine protease
Solyc04g078140.2.1	-1.161166	0.013082	cytochrome B5 isoform D
Solyc03g115820.2.1	-1.342818	1.17E-05	D-ribulose-5-phosphate-3-epimerase
Solyc07g044860.2.1	-1.773859	0.022822	photosystem II subunit P-1
Solyc04g079240.2.1	-2.216969	0.015538	phospholipase A 2A
Solyc06g066370.2.1	-1.151764	0.003015	WRKY DNA-binding protein 33
Solyc08g079230.1.1	-2.886709	0.001757	Bifunctional inhibitor/lipid-transfer protein/seed storage 2S albumin superfamily protein

Defense response to virus (GO:0051607)

Up-regulated genes:

2

<i>ITAG</i>	<i>log2FC</i>	<i>q-value</i>	<i>Functional Description</i>
Solyc10g006200.2.1	1.587426	0.029986	dicer-like 1
Solyc03g044710.2.1	3.150635	0.000397	phospholipase A 2A

Total number of Up-regulated genes: 1115

Total number of the group: 115

Hipergeometric probabilist for gene enrichment: 0.887503

Down-regulated genes:

1

<i>ITAG</i>	<i>log2FC</i>	<i>q-value</i>	<i>Functional Description</i>
Solyc04g079240.2.1	-2.216969	0.015538	phospholipase A 2A

Total number of Up-regulated genes: 758

Total number of the group: 115

Hipergeometric probabilist for gene enrichment: 0.921305

Virus induced gene silencing (GO:0009616)

Up-regulated:

6

<i>ITAG</i>	<i>log2FC</i>	<i>q-value</i>	<i>Functional Description</i>
Solyc10g006200.2.1	1.587426	0.029986	dicer-like 1
Solyc06g065450.2.1	2.753475	3.31E-06	forkhead-associated (FHA) domain-containing protein
Solyc08g082040.2.1	2.067544	0.026745	Protein of unknown function (DUF3133)
Solyc04g015030.2.1	3.168099	3.03E-18	farnesylated protein 3
Solyc04g076850.2.1	0.591435	0.00391	indole-3-acetic acid inducible 9
Solyc04g076850.2.1	0.591435	0.00391	indole-3-acetic acid inducible 9

Down-regulated:

1

<i>ITAG</i>	<i>log2FC</i>	<i>q-value</i>	<i>Functional Description</i>
Solyc07g062300.2.1	-2.533176	0.024733	NHL domain-containing protein

Resistance protein-related genes

Up-regulated:

7

<i>ITAG</i>	<i>log2FC</i>	<i>q-value</i>	<i>Functional Description</i>
Solyc02g032050.1.1	1.620579	0.040756	Disease resistance-responsive (dirigent-like protein) family protein
Solyc05g042090.1.1	4.90746	5.24E-06	NB-ARC domain-containing disease resistance protein
Solyc07g008590.1.1	1.572644	0.005472	disease resistance family protein / LRR family protein
Solyc07g008620.1.1	2.208277	2.04E-06	disease resistance family protein / LRR family protein
Solyc08g081780.1.1	1.470947	0.002868	Disease resistance-responsive (dirigent-like protein) family protein
Solyc12g044190.1.1	3.942548	1.63E-12	LRR and NB-ARC domains-containing disease resistance protein
Solyc12g097010.1.1	1.982315	0.001088	disease resistance protein (TIR-NBS-LRR class), putative

Total number of Up-regulated genes: 1115

Total number of the group: 450

Hipergeometric probabilist for gene enrichment: 0.990446

Down-regulated:

2

<i>ITAG</i>	<i>log2FC</i>	<i>q-value</i>	<i>Functional Description</i>
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Solyc05g054340.2.1	-1.75207	0.000112	NB-ARC domain-containing disease resistance protein
Solyc07g061940.2.1	-1.119795	0.004234	chlorsulfuron/imidazolinone resistant 1

Total number of Up-regulated genes: 758

Total number of the group: 450

Hipergeometric probabilist for gene enrichment: 0.999491

Table S4. Functional overlap DE defense-related genes up-regulated in CLN2777 (resistant) by 3, 5, and 7 dpi and in T474D infected by 10 dpi

ITAG	LOG2FC	Q-VALUE	GO	DESCRIPTION	FUNCTIONAL TERM
Solyc12g006910.1.1	1.437488357	0.000256891		cadmium tolerance 1	cadmium tolerance
Solyc06g065570.2.1	1.099197794	0.003977388	GO:0008080,GO:0016747,GO:0008152	Acyl-CoA N-acyltransferases (NAT) superfamily protein	acyltransferase
Solyc08g078120.1.1	1.119007505	0.012176808		Acyl-CoA N-acyltransferase with RING/FYVE/PHD-type zinc finger protein	acyltransferase
Solyc09g008520.2.1	1.292201253	3.46E-02	GO:0005515	Acyl-CoA N-acyltransferase with RING/FYVE/PHD-type zinc finger domain	acyltransferase
Solyc11g017250.1.1	1.751107855	0.002179764	GO:0016746,GO:0008152,GO:0008415	2-oxoacid dehydrogenases acyltransferase family protein	acyltransferase
Solyc06g074030.1.1	2.074839924	7.25E-05	GO:0005634	Polynucleotidyl transferase, ribonuclease H-like superfamily protein	ribonuclease
Solyc04g011430.2.1	2.132076671	0.034795837	GO:0016881	ubiquitin carrier protein 7	ubiquitin
Solyc09g017980.1.1	2.641368759	0.038145499	GO:0004221,GO:0006511	ubiquitin-specific protease 20	ubiquitin
Solyc10g081500.1.1	1.288849101	9.39E-05	GO:0005515,GO:0005198	protein kinases;ubiquitin-protein ligases	ubiquitin
Solyc12g099030.1.1	0.891122674	0.010381067	GO:0005515,GO:0003735,GO:0006412,GO:0005622,GO:0005840	ubiquitin 6	ubiquitin
Solyc11g013110.1.1	1.451913705	0.001383335	GO:0016491,GO:0016706,GO:0055114	flavonol synthase 1	flavonol synthase 1
Solyc02g088830.2.1	1.546356738	0.022099189		hydroxyproline-rich glycoprotein family protein	hydroxyproline-rich glycoprotein
Solyc10g078160.1.1	1.187143132	0.048966799		hydroxyproline-rich glycoprotein family protein	hydroxyproline-rich glycoprotein
Solyc08g080620.1.1	3.108395737	1.23201E-09		osmotin 34	osmotin
Solyc05g006730.2.1	2.355051524	0.018046248	GO:0005515	Glutathione S-transferase family protein	Glutathione S-transferase
Solyc03g115120.1.1	2.264334786	1.91E-02	GO:0031072	DNAJ heat shock N-terminal domain-containing protein	heat shock
c04g005900.2.1	1.560832185	0.040388262	GO:0005515	F-box/RNI-like superfamily protein	F-box