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Supplementary Information for

Enhanced resistance to bacterial and oomycete pathogens by short tandem target mimic RNAs in tomato

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Supplementary Methods

Plant strains and growth conditions. Tomato (*Solanum lycopersicum*) cultivars M82 were raised from seeds in compost (Levington^{TM} M3) and maintained in a growth room with 16/8h light/dark periods at 22°C (day) and 18°C (night), with 60% relative humidity, at a light intensity of 300 µmol photons $m^{-2} \cdot s^{-1}$. Agrobacterium tumefaciens-mediated stable transformation of tomato plants were performed based on published work (1).

Cloning and vector construction. The STTMs vector construction was done based on a previous report (2). In brief, a long (110bp) DNA oligo containing two mimic sequences separated by a spacer was designed and cloned into a pENTR L1L2 vector (Invitrogen). The insert in this plasmid (pENTR-STTM) was then LR recombined into pGWB402 destination vector containing a *2X35S* promoter driving the expression of the insert, and kanamycin-resistant marker (*NOS promoter:NPTII:NOS terminator*) for selection. All constructs were confirmed by Sanger sequencing.

Small RNA northern blot. Small RNA detection was performed using the northern blot technique. In brief, 5 µg of total RNA per sample were prepared in 10 µl, added equal volume of 2X loading buffer (95% deionized formamide, 18 mM EDTA, 0.025% SDS, xylene cyanol FF, bromophenol blue), and heated at 65°C for 5 min. Then placed in ice for 1 min and loaded and run in a 15% polyacrylamide 7 M urea gel, using 0.5X TBE running buffer. RNA was then transferred to a positively charged nylon membrane (Amershan Hybond-N+, GE Healthcare^{TM}) using overnight capillary system: gels were soaked for 10 min in 20X SSC, then placed on a clean glass plate, membrane on top, 2 pieces of 3MM paper soaked in 20X SSC, and finally 3-5cm of thick paper on top. Another glass plate was put on top and 1kg of weight over this plate. RNA was cross-linked two times per side with 0.12 J of UV light in a Stratalinker® (Agilent^{TM}). Oligonucleotides and Locked Nucleic Acid (LNA^{TM}; by Exiqon) probes radiolabelled with γ^{32} P-ATP were hybridized in ULTRAhyb-Oligo buffer (Thermo Fisher Scientific^{TM}) for 12 hours at 40°C or 2 hours at 57°C, respectively. Then washed three times with 2X SSC, 0.2% SDS. Phosphoimager plates (Fujifilm) were exposed and then imaged with a Typhoon 8610 (Molecular Dynamics).

Target prediction. All tomato miRNA mature sequences were downloaded from miRBase (v21) (3). MicroRNAs targeting genes were predicted by psRNATarget v1 (Dai and Zhao, 2011). Cut-off values were established based on the optimal scores reported in previous studies (4). Tomato NLR sequences were retrieved from a previous study (5) and curated using the ITAG3.2 annotation.

Degradome (PARE) analysis. Parallel analysis of RNA ends (PARE) was performed using the software sPARTA (6). The analysis was done on publicly available datasets from the tomato degradome data of leaf samples (7).

sRNAseq analysis. Small RNAs libraries were prepared using the NEBNext ® Small RNA Library Prep (New England Biolabs). In brief, three biological replicates each of 1 month old tomato leaf RNA were prepared using 1 µg of total RNA per sample. After preparation, size selection of libraries was performed using BluePippin 3% agarose cassettes (Sage Science). Each library was barcoded, pooled, and sequenced using a single NextSeq 500/550 High Output Kit v2 (75 cycles). Sequences were demultiplex, and trimmed and filtered using Trim Galore! (Babraham Bioinformatics) with default parameters and reads were concordantly aligned to the Heinz genome SL3.00 version using Bowtie v1.2.0 with modifiers -v 1 -m 50 –best –strata. Identification of sRNA loci and differential expression was performed using the segementSeq package and baySeq respectively (8, 9).

***Phytophthora infestans* infections.** The *Phytophthora infestans* strain in this study is 88069 (10). Cultures were stored in liquid nitrogen and grown on rye sucrose medium. Infection assays were performed on detached tomato leaves, measuring lesion sizes. In brief, four well developed leaves per plant and four plants per condition were detached from four-week-old plants and placed on water-saturated paper in a tray. Spore suspensions of P. infestans were prepared by rinsing two-week-old plates covered with mycelium with cold water and incubating the sporangiophore at 4°C for 1-2 hours. After release of zoospores, the concentration was adjusted to approximately $5 \cdot 10^4$ spores $\cdot ml^{-1}$. *P. infestans* were spot-inoculated on the abaxial side of the leaf, by placing six 10 µl droplets on various locations right and left of the midvein. The trays were covered and incubated at room temperature at constant light a photoperiod. Disease assessments were performed daily from 3 to 7 days post inoculation (dpi) under blue light using a DarkReader ® Transilluminator (Clare Chemical Research) and a Nikon COOLPIX P520. Lesion diameters were measured using ImageJ software, followed by statistical analysis and plotting in R.

***Pseudomonas syringae* infections.** The *Pseudomonas syringae pv. tomato* strain used in this study is DC3000, which is a pathogen of tomato developed in 1986 as rifampicin-resistant derivative of Pst DC52. Cultures were stored in liquid nitrogen and grown on King's B medium. Infection assays were performed in planta, inoculating mature tomato leaves and measuring bacterial growth based on previous work (11). Statistical analysis and plotting were done in R.

RNAseq analysis. RNAseq libraries were prepared using the Truseq® mRNA HT kit (Illumina). In brief, total RNA from three different conditions (young: 3 weeks old leaves, old: 6 weeks old leaves, and infected: 3 day post inoculation of detached leaves with *P. infestans*) with six biological replicates each of 3 week old, and 6 week old tomato leaves, RNA were prepared using 1 µg of total RNA per sample. PolyA bead selection and strand-specific RNA-seq libraries were made and indexed according to manufacturer instructions. Finalized libraries were sequenced as a pool on one lane of a NextSeq 500/550 High Output Kit v2

(75+75 cycles). Sequences were de-multiplex, and trimmed and filtered using Trim Galore! (Babraham Bioinformatics) with default parameters. Trimmed reads were pseudo-aligned to ITAG3.2 transcriptome using Kallisto (12), with the parameter -b 100. Differential expression was performed on kallisto-estimated counts using the Bioconductor package limma (13). For visual representations and analysis, abundances were reported as quantile-normalized transcripts per million (TPM). Processing, analysis and plotting were done in R.

Phylogenetic analysis. BLASTN analyses were performed using genomic sequences of tomato genes and miRNA precursor sequences against the genomes of all plant model organisms and all available genome assemblies of major Solanaceae species. The threshold expectation value was defined at 10^{-3} to filter out any spurious hits. Any hits were then manually curated.

Supplementary images

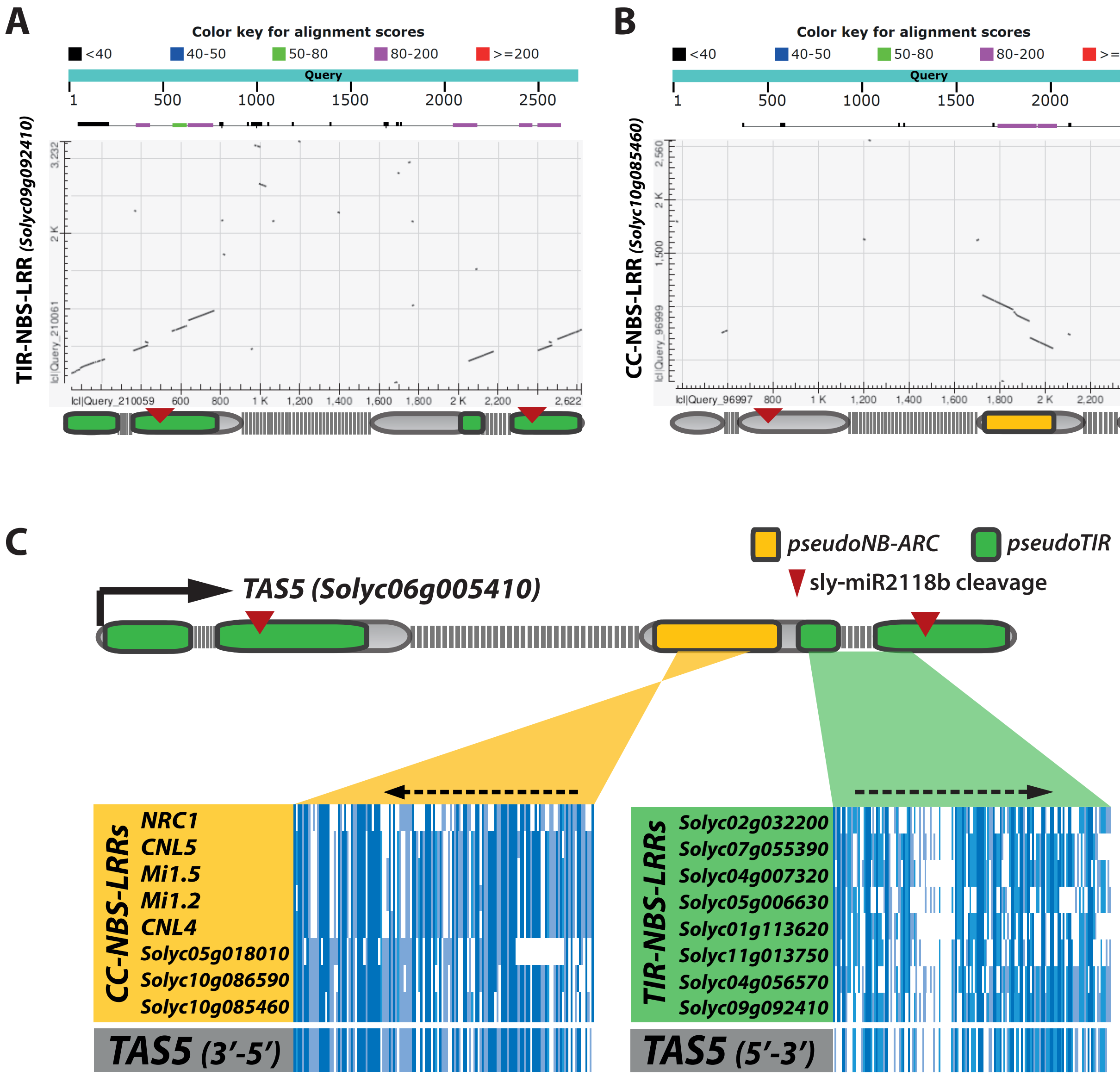

Fig. S1. Sequence similarity between NLR domains and TAS5. BLAST (Basic Local Alignment Search Tool) summary and dot plot matrix of TAS5 versus (A) the closest TNL and (B) the closest CNL in the tomato genome. Gene diagram of TAS5 locus is placed underneath the matrix indicates in (A) similarity to a TIR domain in green and (B) similarity to a NB-ARC in yellow. (C) Regions within exons with most significant sequence similarity with known NLR domains are highlighted in yellow and green. Nucleotide sequence alignment of these regions and known tomato NLRs are shown below. The degree of conservation for each nucleotide along the region is represented by the colour, with a dark blue denoting a high level of conservation and a light blue denoting a low level. Dotted arrows indicate direction of the sequence similarity.

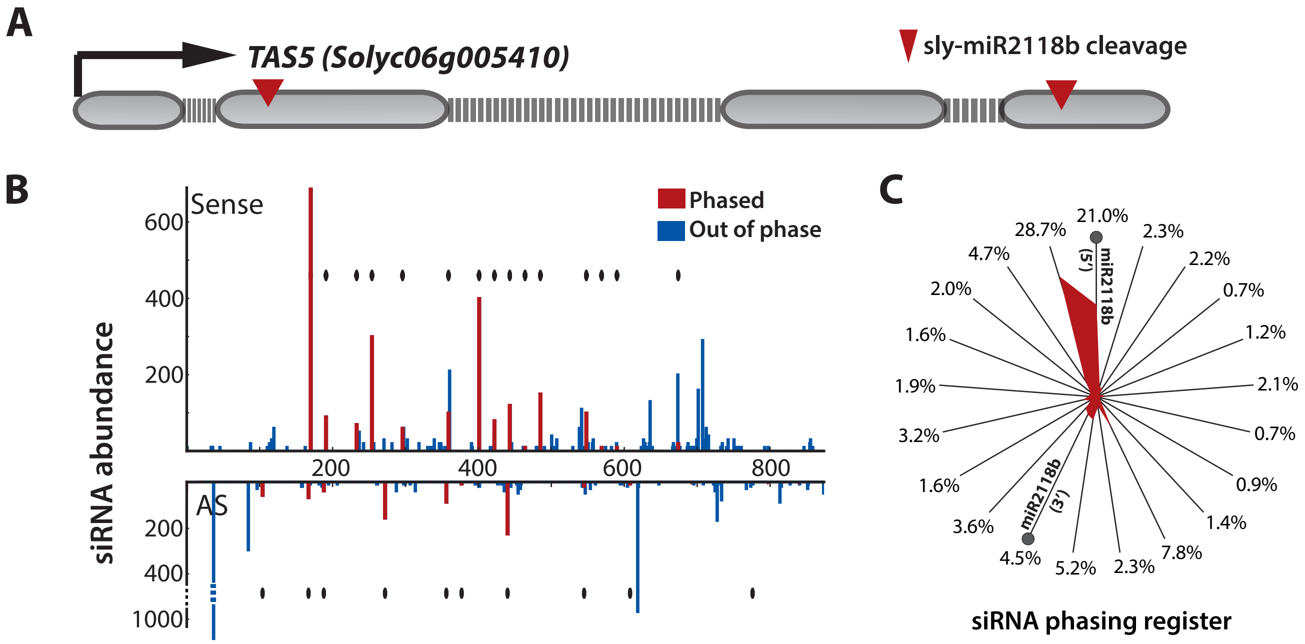

Fig. S2. TAS5 is a phasiRNA-producing locus. (A) Gene diagram of TAS5 locus. A 2.7kb region containing 4 exons (grey boxes) and 3 introns (grey dotted lines). The arrow on the left indicates transcription start site and direction. (B) Number of sequenced small RNAs with 5' residues at each position between the cleavage sites of the TAS5 transcript. Red bars indicate phased sequences while blue indicate out of phased. Red diamonds indicate that expected phased siRNA is present in the sample. (C) Distribution of the phasing of small RNAs at the TAS5 locus. Each spoke of wheel the represents 1 of the 21 possible registers, with the percentage of small RNAs mapping plotted as distance from the centre (correction of 2-nt 3' overhangs of DCL cleavage was applied when assigning register in the anti-sense strand). The specific registers predicted from 5' and 3' cleavage sites of miR2118b are indicated with grey circles.

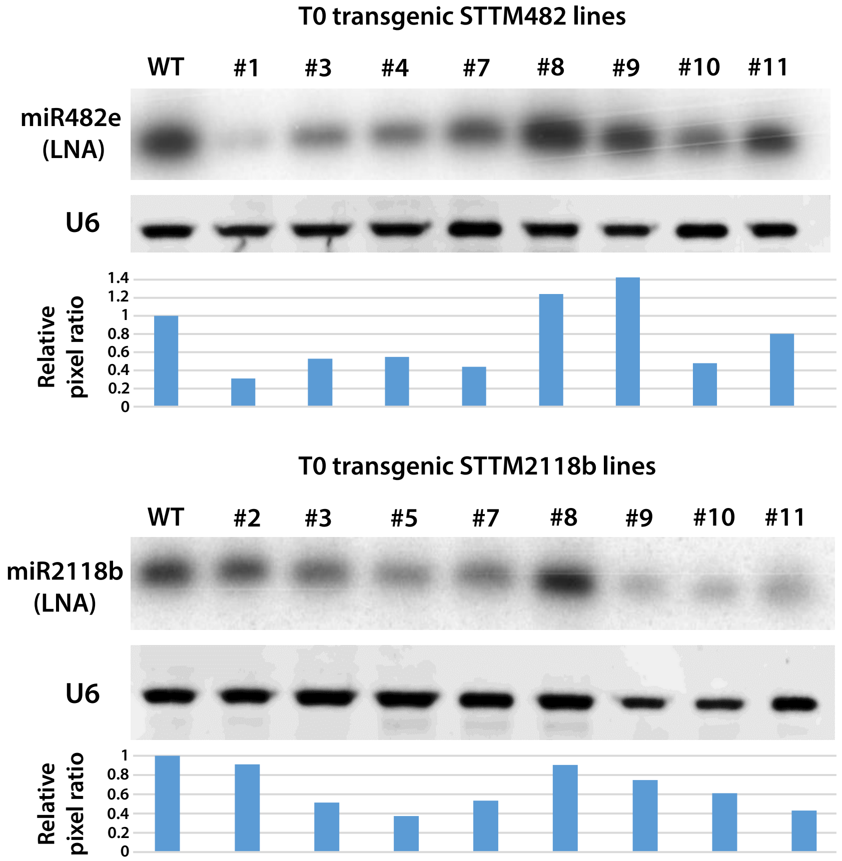

Fig. S3. Levels of target miRNA sequestration in first generation STTM transgenic lines. RNA gel blot analysis of tomato transgenic mimic lines. Upper line shows miRNA blotted with highly specific locked nucleic acid (LNA) probes. Lower image shows the same blot hybridized with U6, as a loading control. Barplot indicates relative pixel ratio of miRNA signal vs U6 signal.

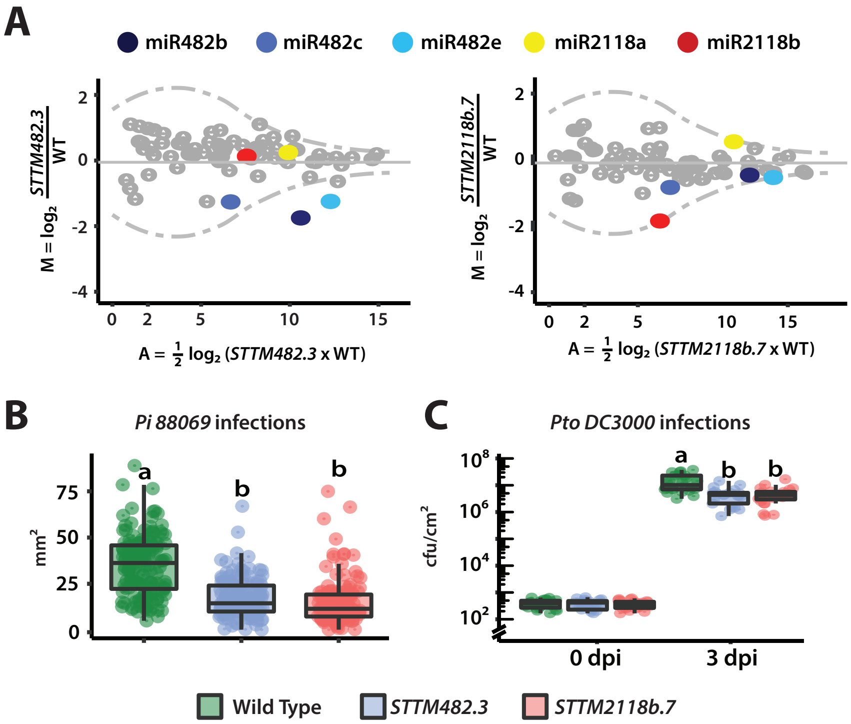

Fig. S4. Effects were consistent across independent STTM lines. (A) MA plot showing fold changes of miRNAs in STTM lines. Tomato mature miRNA sequences were extracted from miRBASE. The blue dots indicate miR482, yellow is miR2118a and red is miR2118b; grey indicate other miRNAs. The dotted line represents a Poisson distribution with 1 % significance values at the top and bottom of the range, applying the 0 correction (if nreads=0;+1). sRNA reads are normalized to the whole library with reads per million (nRPM) and presented as the mean from three biological replicates. (B) Boxplot and leaf images of lesion size in WT and mimicry lines. Statistically significant differences were determined using one-way ANOVA test followed by Tukey HSD at 95% confidence limits. (C) Boxplot of bacterial population in WT and STTM lines leaves infected with *Pseudomonas syringae pv. tomato DC3000*. Bacterial counts at 0 and 3 days post leaf infiltration. Statistically significant differences were determined using ANOVA test followed by Tukey HSD at 95% confidence limits.

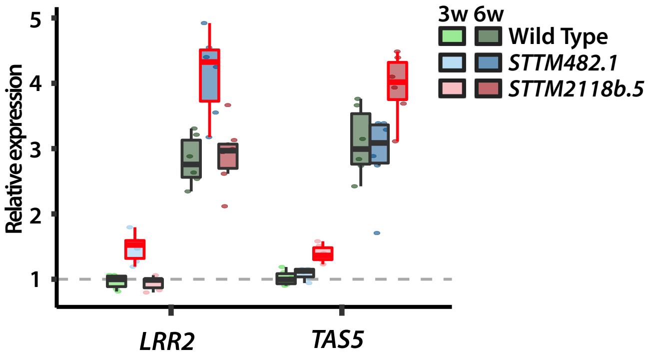

Fig. S5. qRT-PCR analysis validates RNA-seq results for miR482 and miR2118b targets. Quantitative PCR analysis for the abundance of target mRNAs LRR2 and TAS5 in 3 and 6 week old leaf tissue (n=6). Expression values were adjusted to tomato housekeeping gene EXP and shown relatively to WT values. Statistically significant differences were explored using two-way ANOVA test followed by Tukey HSD at 95% confidence limits.

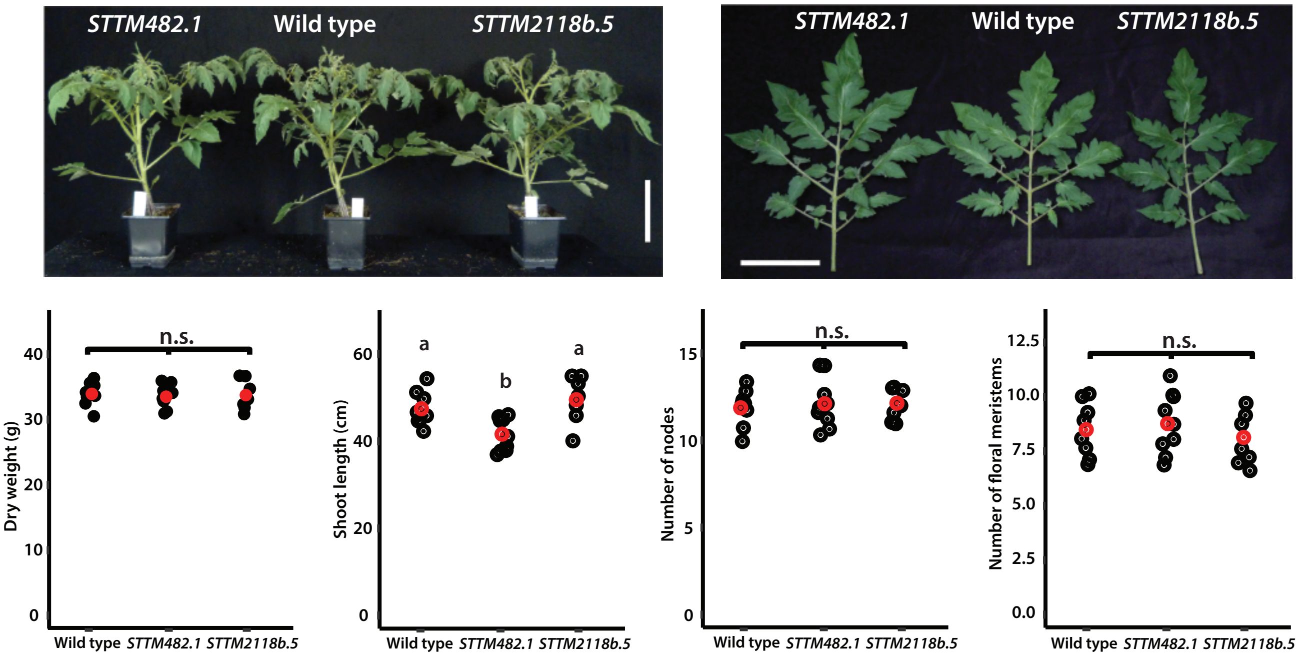

Fig. S6. Growth remains majorly unaffected in STTM transgenic lines. (Top) representative images of leaves and whole plants. White scale bars represent 10cm. (Bottom) Dot plots representing differences in shoot dry weight, length, number of nodes, and number of floral meristems. Black dots represent individual plants, and red dots represent means of all biological replicates (n=8). Images and data were collected at 8 week after germination. Statistically significant differences were determined using a two-way ANOVA test followed by Tukey HSD at 95% confidence limits.

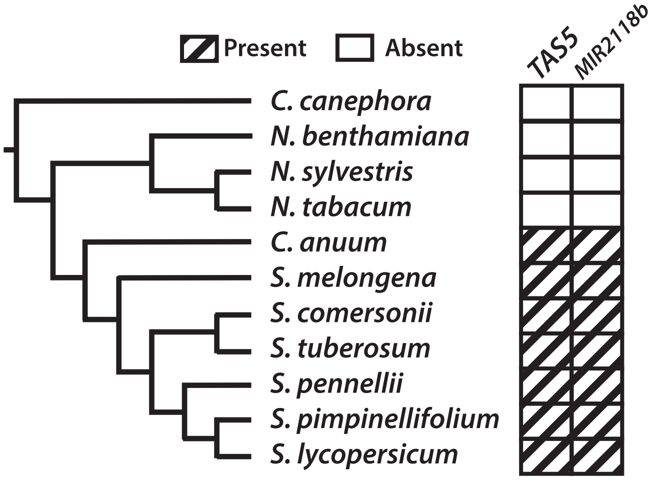

Fig. S7. TAS5 and MIR2118b are only present in Solanum species. Diagram summarising the presence or absence of genomic sequences matching TAS5 and MIR2118b in Solanaceae and a close relative. A close sequenced relative of Solanaceae, *Coffea canephora*, was included as an outgroup.

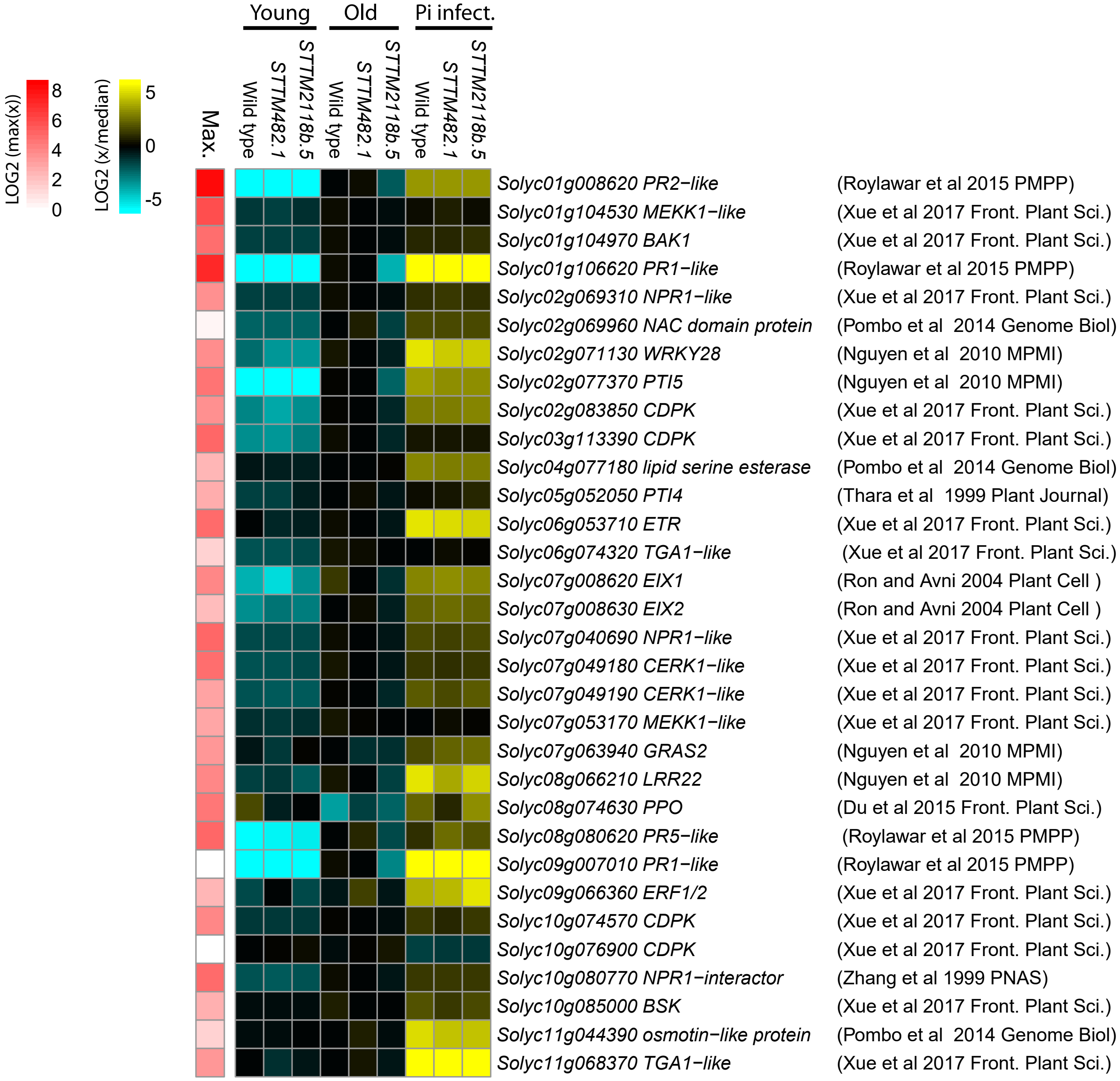

Fig. S8. Known non-NLR defense-related genes remain unaffected in STTM lines. Heatmap of the RNAseq for the non-NLR PTI/ETI-related genes. RNAseq abundances are presented in transcript per million (TPM) and Log2 fold changes between STTM lines and WT across conditions. The list was elaborated based on published work in tomato (14–21).

Supplementary tables

Table S1. Summary of identified members of the miR482/2118 family in tomato. All available nomenclatures of miR482/2118 members in tomato in the different studies, present in the current literature.

Sequence	This work	miRBase	Zhang et al. 2016 (22)	DeVries et al. 2015 (23)	Shivaprasaed et al. 2012 (24)	Karlova et al. 2013 (25)	Notes
UUUCCAAUUCCACCCAUUCCUA	mi2118a	sly miR482a	miR482a	miR482	miR482	miR-Y	-
UUUCCUAUUCCACCCAUGCCAA	mi2118b	sly-miR482d	miR482d	miR482g	-	-	-
UCUUGCCUACACCGCCCAUGCC	miR482b	sly-miR482b	miR482b	miR482a	miR482a	miR482*	-
UCUUUCCUACUCCUCCCAUACC	miR482e	sly-miR482e	miR482e	miR482f	miR482f		-
UCUUGCCAAUACCGCCCAUUCC	miR482c	sly-miR482c	miR482c	miR482b	miR482b		-
UUACCAAUUCCACCCAUUCCUA	-	-	-	miR482h	-	-	No evidence of expression
UCUUUCCUACUCCUCCCCUACC	-	-	-	-	miR482c	-	No evidence of expression or presence of the sequence in the genome
UCUUUCCUACUCCUCCCAUGCC	-	-	-	-	miR482e	-	
UCUUUCCUACUCCUCCCAUCCC	-	-	-	-	miR482d	-	

Table S2. Summary of all predicted targets of miR482/2118 members.

miRNA	Target	Score	UPE	Posit	Target description
mi2118a	Solyc11g008140	1	11.101	1224	Pectate lyase
mi2118a	Solyc01g102920	2	14.713	635	Disease resistance protein (TIR-NBS-LRR class)
mi2118a	Solyc04g007320	2	21.199	1110	Disease resistance protein (TIR-NBS-LRR class)
mi2118a	Solyc01g020371	2.5	20.058	349	GRF zinc finger family protein
mi2118a	Solyc03g116360	2.5	15.966	2405	Regulator of chromosome condensation (RCC1) family protein
mi2118a	Solyc04g024950	2.5	15.862	4	MATH domain/coiled-coil protein
mi2118a	Solyc04g049780	2.5	13.961	70	Retrovirus-related Pol polyprotein from transposon TNT 1-94
mi2118a	Solyc04g053070	2.5	17.989	278	DNA topoisomerase
mi2118a	Solyc06g009533	2.5	18.082	77	Kinase family protein
mi2118a	Solyc06g062440	2.5	22.729	593	Disease resistance protein
mi2118a	Solyc10g007065	2.5	17.785	580	Phenylalanyl-tRNA synthetase alpha chain
mi2118a	Solyc01g066020	3	25.484	656	disease resistance protein (TIR-NBS-LRR class)
mi2118a	Solyc01g087200	3	19.95	515	Disease resistance protein
mi2118a	Solyc01g090860	3	8.404	782	Nucleotidyltransferase family protein
mi2118a	Solyc02g030100	3	17.13	3745	Vacuolar protein sorting-associated protein 54
mi2118a	Solyc02g030105	3	17.13	2139	Vacuolar protein sorting-associated protein 54
mi2118a	Solyc02g091890	3	6.22	4704	myb-like protein X
mi2118a	Solyc03g083130	3	16.198	1745	gamma-irradiation and mitomycin c induced 1
mi2118a	Solyc05g009750	3	17.541	164	NBS-LRR resistance protein-like protein
mi2118a	Solyc08g005510	3	18.753	1512	disease resistance protein (TIR-NBS-LRR class)
mi2118a	Solyc09g090390	3	18.271	1709	2-oxoglutarate-dependent dioxygenase AOP2
mi2118a	Solyc10g050115	3	5.699	891	Transposon Ty3-I Gag-Pol polyprotein
mi2118a	Solyc11g011350	3	19.87	1381	disease resistance protein (TIR-NBS-LRR class)
mi2118a	Solyc12g009450	3	19.542	727	Disease resistance protein (CC-NBS-LRR class) family
mi2118a	Solyc12g056490	3	23.311	324	WD40 repeat-containing protein
mi2118a	Solyc01g094520	3.5	20.797	978	F-box/kelch-repeat protein
mi2118a	Solyc01g112260	3.5	30.089	316	Phosphoenolpyruvate carboxylase
mi2118a	Solyc02g064680	3.5	19.849	1125	Calcium-transporting ATPase
mi2118a	Solyc02g090860	3.5	19.613	1387	Phenylalanyl-tRNA synthetase alpha chain
mi2118a	Solyc03g025190	3.5	21.744	803	anthocyanin permease
mi2118a	Solyc03g112630	3.5	16.495	3497	Sec14p-like phosphatidylinositol transfer family protein
mi2118a	Solyc04g011960	3.5	15.189	521	Disease resistance protein (CC-NBS-LRR class) family
mi2118a	Solyc04g011980	3.5	16.151	521	Disease resistance protein (CC-NBS-LRR class) family
mi2118a	Solyc04g011990	3.5	15.575	1077	Disease resistance protein (NBS-LRR class) family
mi2118a	Solyc04g012000	3.5	17.228	221	NBS-coding resistance gene analog
mi2118a	Solyc04g012010	3.5	18.466	541	Disease resistance protein (NBS-LRR class) family
mi2118a	Solyc05g010240	3.5	11.485	3748	Chaperonin-60 beta subunit
mi2118a	Solyc06g061215	3.5	20.712	170	Proteinase inhibitor II
mi2118a	Solyc06g069390	3.5	24.527	1442	D-aminoacyl-tRNA deacylase
mi2118a	Solyc07g063430	3.5	13.261	643	Peroxisomal membrane (Mpv17/PMP22) family protein
mi2118a	Solyc08g066500	3.5	13.834	554	Homeobox leucine-zipper protein
mi2118a	Solyc09g005290	3.5	20.812	668	Nbs-lrr resistance protein
mi2118a	Solyc09g091990	3.5	18.213	665	Kinase family protein
mi2118a	Solyc10g087013	3.5	18.136	838	Cytochrome P450
mi2118a	Solyc12g056960	3.5	13.791	484	Glucan 1
mi2118a	Solyc01g008800	4	14.87	1561	disease resistance protein (TIR-NBS-LRR class)
mi2118a	Solyc01g111160	4	17.893	181	far-red elongated hypocotyls 3
mi2118a	Solyc02g081870	4	16.621	267	Pleiotropic drug resistance ABC transporter
mi2118a	Solyc02g093340	4	15.426	1078	RNA-binding (RRM/RBD/RNP motifs) family protein
mi2118a	Solyc03g111140	4	18.294	766	Malate synthase
mi2118a	Solyc03g115740	4	10.662	2443	Xyloglucan alpha-1
mi2118a	Solyc05g008340	4	23.996	791	Core-2/I-branching beta-1
mi2118a	Solyc05g009470	4	14.478	2663	Alpha-glucosidase
mi2118a	Solyc06g009533	4	20.01	1085	Kinase family protein
mi2118a	Solyc06g065820	4	14.049	582	Ethylene Response Factor H.1
mi2118a	Solyc06g068700	4	21.249	2627	Calreticulin/calnexin
mi2118a	Solyc07g008950	4	20.753	2523	Methionyl-tRNA synthetase family protein
mi2118a	Solyc07g008955	4	20.753	2631	Unknown protein

mi2118a	Solyc07g041030	4	22.468	277	DNA topoisomerase
mi2118a	Solyc08g013900	4	19.494	4686	Plant regulator RWP-RK family protein
mi2118a	Solyc08g082000	4	17.749	789	Homeobox-leucine zipper HOX24
mi2118a	Solyc09g007710	4	26.176	986	Disease resistance protein (TIR-NBS-LRR class) family
mi2118a	Solyc09g075010	4	4.321	683	HSP20-like chaperones superfamily protein
mi2118a	Solyc11g045350	4	19.839	3532	Plant regulator RWP-RK family protein
mi2118a	Solyc11g062220	4	19.961	5265	Zinc finger CCCH domain-containing protein 44
mi2118b	Solyc06g005410	1	15.878	592	TAS5
mi2118b	Solyc06g005410	1	15.569	1639	TAS5
mi2118b	Solyc02g032650	2	15.284	796	Disease resistance protein (TIR-NBS-LRR class)
mi2118b	Solyc01g105340	2.5	20.142	810	Chaperone protein DnaJ
mi2118b	Solyc01g113620	2.5	16.895	806	Disease resistance protein (TIR-NBS-LRR class) family
mi2118b	Solyc04g009110	2.5	18.119	572	Nbs-lrr resistance protein
mi2118b	Solyc04g009130	2.5	20.332	584	Nbs-lrr resistance protein
mi2118b	Solyc04g009290	2.5	18.898	572	Disease resistance protein
mi2118b	Solyc04g026110	2.5	22.415	491	Disease resistance family protein
mi2118b	Solyc05g009750	2.5	17.541	164	NBS-LRR resistance protein-like protein
mi2118b	Solyc08g075630	2.5	26.52	743	NBS-LRR resistance protein
mi2118b	Solyc08g076000	2.5	25.842	851	NBS-LRR resistance protein
mi2118b	Solyc01g102920	3	14.713	635	Disease resistance protein (TIR-NBS-LRR class)
mi2118b	Solyc02g030290	3	19.46	281	Nbs-lrr resistance protein
mi2118b	Solyc02g037540	3	23.811	479	Disease resistance protein
mi2118b	Solyc06g062440	3	22.729	592	Disease resistance protein
mi2118b	Solyc08g075640	3	24.051	836	NBS-LRR resistance protein
mi2118b	Solyc09g075010	3	16.92	611	HSP20-like chaperones superfamily protein
mi2118b	Solyc09g098100	3	18.265	2059	CC-NBS-LRR_Solyc09g098100
mi2118b	Solyc01g110000	3.5	16.414	2336	Beta-galactosidase
mi2118b	Solyc02g036270	3.5	16.852	679	Disease resistance protein (NBS-LRR class) family
mi2118b	Solyc03g123630	3.5	23.333	1670	pectin methylesterase
mi2118b	Solyc04g009120	3.5	22.379	599	Nbs-lrr resistance protein
mi2118b	Solyc04g025820	3.5	20.87	312	Disease resistance protein
mi2118b	Solyc04g025840	3.5	20.843	491	Disease resistance family protein
mi2118b	Solyc05g008070	3.5	21.429	530	Disease resistance protein
mi2118b	Solyc05g014030	3.5	21.401	738	Regulator of chromosome condensation (RCC1) family protein
mi2118b	Solyc06g007780	3.5	21.021	2776	Nuclear transport factor 2 (NTF2)
mi2118b	Solyc07g039400	3.5	22.502	491	Disease resistance protein
mi2118b	Solyc07g039420	3.5	22.458	566	Disease resistance protein (NBS-LRR class) family
mi2118b	Solyc08g067060	3.5	24.14	674	Pentatricopeptide repeat superfamily protein
mi2118b	Solyc08g074250	3.5	15.378	545	Disease resistance protein (CC-NBS-LRR class) family
mi2118b	Solyc11g008140	3.5	11.101	1224	Pectate lyase
mi2118b	Solyc12g006040	3.5	21.275	662	NBS-LRR protein
mi2118b	Solyc01g105775	4	23.141	221	Carbonic anhydrase
mi2118b	Solyc01g111100	4	18.433	635	Neutral invertase
mi2118b	Solyc02g079310	4	16.167	387	Equilibrative nucleoside transporter family protein
mi2118b	Solyc02g079350	4	14.312	2490	Equilibrative nucleoside transporter family protein
mi2118b	Solyc02g083960	4	19.892	3334	2-oxoglutarate and Fe-dependent oxygenase-like protein
mi2118b	Solyc03g007330	4	22.962	983	ATP-dependent zinc metalloprotease FTSH protein
mi2118b	Solyc03g083430	4	21.732	1551	Splicing factor 3A subunit 3
mi2118b	Solyc04g005540	4	17.383	850	Disease resistance protein (NBS-LRR class) family
mi2118b	Solyc04g005550	4	21.364	868	Disease resistance protein (NBS-LRR class) family
mi2118b	Solyc04g071260	4	21.507	2316	Actin
mi2118b	Solyc05g014760	4	21.583	3126	Kinase family protein
mi2118b	Solyc05g018720	4	17.342	62	NBS-coding resistance protein
mi2118b	Solyc07g005770	4	24.094	539	Disease resistance protein
mi2118b	Solyc07g064700	4	25.463	1362	Bromodomain-containing protein
mi2118b	Solyc09g005120	4	18.535	483	DnaJ domain-containing protein
mi2118b	Solyc09g076010	4	8.678	370	Acyl-CoA N-acyltransferase
mi2118b	Solyc10g008230	4	19.11	548	Disease resistance protein
mi2118b	Solyc11g069020	4	20.947	497	Disease resistance protein
mi2118b	Solyc12g017800	4	23.624	1102	NBS-LRR class disease resistance protein
mi2118b	Solyc12g099060	4	23.521	644	Disease resistance protein

mi2118b	Solyc12g099940	4	15.941	2435	Acyl-CoA N-acyltransferase
miR482b	Solyc04g009070	0	25.758	211	Disease resistance family protein
miR482b	Solyc02g036270	1.5	12.267	681	Disease resistance protein (NBS-LRR class) family
miR482b	Solyc04g009120	1.5	21.183	601	Nbs-lrr resistance protein
miR482b	Solyc05g008070	1.5	21.303	532	Disease resistance protein
miR482b	Solyc04g025820	2	19.14	314	Disease resistance protein
miR482b	Solyc04g025840	2	19.226	493	Disease resistance family protein
miR482b	Solyc07g039420	2	23.494	568	Disease resistance protein (NBS-LRR class) family
miR482b	Solyc11g065780	2	21.191	786	Disease resistance protein
miR482b	Solyc12g017800	2	24.453	1104	NBS-LRR class disease resistance protein
miR482b	Solyc01g067165	2.5	20.018	529	Disease resistance protein (CC-NBS-LRR class) family protein
miR482b	Solyc04g009130	2.5	20.794	586	Nbs-lrr resistance protein
miR482b	Solyc04g009290	2.5	23.41	574	Disease resistance protein
miR482b	Solyc07g039400	2.5	22.497	493	Disease resistance protein
miR482b	Solyc10g054970	2.5	19.563	538	CCNBS gene
miR482b	Solyc10g054990	2.5	16.38	520	Disease resistance protein (NBS-LRR class) family
miR482b	Solyc10g055170	2.5	21.742	46	Disease resistance protein (CC-NBS-LRR class) family protein
miR482b	Solyc11g006530	2.5	18.804	526	Disease resistance protein
miR482b	Solyc11g006630	2.5	21.57	532	Disease resistance protein
miR482b	Solyc04g009240	3	27.611	565	Nbs-lrr resistance protein
miR482b	Solyc04g009250	3	28.375	577	Nbs-lrr resistance protein
miR482b	Solyc04g009660	3	28.833	550	Nbs-lrr resistance protein
miR482b	Solyc04g009690	3	27.03	562	Nbs-lrr resistance protein
miR482b	Solyc07g005770	3	23.779	541	Disease resistance protein
miR482b	Solyc08g005440	3	21.814	627	NBS-LRR disease resistance protein
miR482b	Solyc10g051050	3	21.243	929	Disease resistance protein
miR482b	Solyc11g020090	3	20.197	103	Disease resistance protein
miR482b	Solyc11g020100	3	23.736	1110	Disease resistance protein
miR482b	Solyc11g069925	3	24.835	622	Disease resistance protein
miR482b	Solyc01g113620	3.5	16.593	808	Disease resistance protein (TIR-NBS-LRR class) family
miR482b	Solyc02g032650	3.5	15.288	798	Disease resistance protein (TIR-NBS-LRR class)
miR482b	Solyc02g070730	3.5	25.498	412	NBS-LRR resistance protein
miR482b	Solyc04g005540	3.5	15.547	852	Disease resistance protein (NBS-LRR class) family
miR482b	Solyc04g005550	3.5	18.848	870	Disease resistance protein (NBS-LRR class) family
miR482b	Solyc04g009110	3.5	17.68	574	Nbs-lrr resistance protein
miR482b	Solyc04g056746	3.5	16.29	687	Pentatricopeptide repeat-containing protein
miR482b	Solyc05g006630	3.5	29.979	1034	disease resistance protein (TIR-NBS-LRR class)
miR482b	Solyc05g007170	3.5	22.989	5882	Disease resistance protein
miR482b	Solyc06g072000	3.5	20.074	307	P-loop containing nucleoside triphosphate hydrolases protein
miR482b	Solyc07g044790	3.5	21.329	1223	Pvr4
miR482b	Solyc07g044797	3.5	21.329	286	CC-NBS-LRR disease resistance protein
miR482b	Solyc07g049700	3.5	24.47	544	Disease resistance protein
miR482b	Solyc09g065560	3.5	14.452	1794	Sulfate transporter
miR482b	Solyc11g006520	3.5	23.607	819	Disease resistance protein
miR482b	Solyc11g006640	3.5	26.45	532	Disease resistance protein
miR482b	Solyc11g068360	3.5	22.83	622	Disease resistance protein
miR482b	Solyc11g069620	3.5	21.64	706	Disease resistance protein
miR482b	Solyc12g044180	3.5	19.352	526	CC-NBS-LRR disease resistance protein
miR482b	Solyc12g044190	3.5	18.858	526	Disease resistance protein (CC-NBS-LRR class) family protein
miR482b	Solyc12g044200	3.5	18.45	1186	Disease resistance protein (CC-NBS-LRR class) family protein
miR482b	Solyc02g037540	4	23.271	481	Disease resistance protein
miR482b	Solyc03g078300	4	20.201	726	Disease resistance protein
miR482b	Solyc04g009150	4	25.145	565	Nbs-lrr resistance protein
miR482b	Solyc04g026110	4	21.107	493	Disease resistance family protein
miR482b	Solyc04g048920	4	15.927	46	CC-NBS-LRR disease resistance protein
miR482b	Solyc05g012740	4	20.929	1585	Disease resistance protein
miR482b	Solyc07g053010	4	28.026	1171	NBS-LRR type disease resistance protein
miR482b	Solyc10g045050	4	14.193	268	Enolase
miR482b	Solyc10g051170	4	20.714	673	Disease resistance protein
miR482b	Solyc10g055050	4	17.252	379	CC-NBS-LRR disease resistance protein
miR482b	Solyc11g069990	4	23.911	1218	I2C5

miR482b	Solyc11g070020	4	23.911	1990	Disease resistance protein
miR482b	Solyc11g071423	4	25.798	229	Disease resistance protein
miR482b	Solyc11g071995	4	26.508	622	Disease resistance protein
miR482b	Solyc12g006040	4	20.75	664	NBS-LRR protein
miR482c	Solyc08g075630	1.5	24.851	745	NBS-LRR resistance protein
miR482c	Solyc08g076000	1.5	24.722	853	NBS-LRR resistance protein
miR482c	Solyc02g021140	2.5	7.72	4	Superoxide dismutase
miR482c	Solyc02g078280	3	11.484	1597	DNA ligase-like protein
miR482c	Solyc05g006630	3	29.979	1034	disease resistance protein (TIR-NBS-LRR class)
miR482c	Solyc06g076350	3	15.598	392	LePCL1
miR482c	Solyc11g011560	3	19.304	1085	PHD finger protein family
miR482c	Solyc11g065780	3	21.191	786	Disease resistance protein
miR482c	Solyc00g006530	3.5	25.591	771	Calmodulin-binding protein
miR482c	Solyc01g102660	3.5	18.543	218	Glutathione S-transferase
miR482c	Solyc02g032650	3.5	15.288	798	Disease resistance protein (TIR-NBS-LRR class)
miR482c	Solyc02g036270	3.5	12.267	681	Disease resistance protein (NBS-LRR class) family
miR482c	Solyc04g005540	3.5	15.547	852	Disease resistance protein (NBS-LRR class) family
miR482c	Solyc04g005550	3.5	18.848	870	Disease resistance protein (NBS-LRR class) family
miR482c	Solyc04g009110	3.5	17.68	574	Nbs-lrr resistance protein
miR482c	Solyc04g009150	3.5	25.145	565	Nbs-lrr resistance protein
miR482c	Solyc04g025160	3.5	19.754	340	ATPase
miR482c	Solyc04g026110	3.5	21.107	493	Disease resistance family protein
miR482c	Solyc04g080590	3.5	25.393	748	Proteasome subunit alpha type
miR482c	Solyc06g060360	3.5	21.646	1010	Adenine nucleotide alpha hydrolases-like superfamily protein
miR482c	Solyc06g083875	3.5	17.908	247	pollen Ole e I family allergen protein
miR482c	Solyc07g053200	3.5	12.314	923	Adenine nucleotide alpha hydrolases-like superfamily protein
miR482c	Solyc08g065740	3.5	23.005	475	Vacuolar processing enzyme
miR482c	Solyc08g068040	3.5	19.304	2071	zinc finger FYVE domain protein
miR482c	Solyc10g007200	3.5	11.289	503	Hexosyltransferase
miR482c	Solyc10g076440	3.5	18.053	475	Disease resistance protein
miR482c	Solyc11g017370	3.5	19.701	1910	Pentatricopeptide repeat-containing protein
miR482c	Solyc11g062010	3.5	20.533	9655	Chromatin structure-remodeling complex subunit snf21
miR482c	Solyc12g005230	3.5	13.933	4336	Breast carcinoma-amplified sequence 3
miR482c	Solyc12g019144	3.5	23.587	1538	RING/U-box superfamily protein
miR482c	Solyc01g097390	4	20.058	1141	NAD(P)-linked oxidoreductase superfamily protein
miR482c	Solyc01g103450	4	30.773	415	Heat shock protein 70
miR482c	Solyc02g005180	4	25.396	273	Sugar facilitator protein 2
miR482c	Solyc02g070730	4	25.498	412	NBS-LRR resistance protein
miR482c	Solyc02g078790	4	14.982	1601	Transcription factor jumonji domain protein
miR482c	Solyc02g080960	4	18.235	39	transmembrane protein
miR482c	Solyc03g097980	4	17.739	550	Guanine nucleotide-binding alpha-2 subunit
miR482c	Solyc03g113620	4	28.565	230	MYB transcription factor
miR482c	Solyc05g005460	4	23.644	2043	Quinone oxidoreductase-like protein
miR482c	Solyc05g018370	4	5.408	808	Leguminosin group485 secreted peptide
miR482c	Solyc06g062440	4	22.498	595	Disease resistance protein
miR482c	Solyc06g068210	4	6.629	2252	Protein FAR1-RELATED SEQUENCE 8
miR482c	Solyc07g052760	4	16.463	540	DNA-binding storekeeper protein-related transcriptional regulator
miR482c	Solyc07g055380	4	19.526	1969	Disease resistance protein (TIR-NBS-LRR class)
miR482c	Solyc07g055610	4	20.758	553	Disease resistance protein (TIR-NBS-LRR class)
miR482c	Solyc09g007830	4	14.32	265	Cytokinin riboside 5'-monophosphate phosphoribohydrolase
miR482c	Solyc11g010660	4	12.438	988	protein SGT1
miR482c	Solyc11g062010	4	21.236	9856	Chromatin structure-remodeling complex subunit snf21
miR482c	Solyc11g069830	4	21.579	309	ATPase ASNA1
miR482e	Solyc11g006530	1.5	18.804	526	Disease resistance protein
miR482e	Solyc11g006630	1.5	21.57	532	Disease resistance protein
miR482e	Solyc07g049700	2	24.47	544	Disease resistance protein
miR482e	Solyc01g067165	2.5	20.018	529	Disease resistance protein (CC-NBS-LRR class) family protein
miR482e	Solyc04g009070	2.5	25.758	211	Disease resistance family protein
miR482e	Solyc05g008070	2.5	21.303	532	Disease resistance protein
miR482e	Solyc06g074760	2.5	15.026	1529	Ring/U-Box superfamily protein
miR482e	Solyc10g054970	2.5	19.563	538	CCNBS gene

miR482e	Solyc10g054990	2.5	16.38	520	Disease resistance protein (NBS-LRR class) family
miR482e	Solyc10g055170	2.5	21.742	46	Disease resistance protein (CC-NBS-LRR class) family protein
miR482e	Solyc11g020100	2.5	23.736	1110	Disease resistance protein
miR482e	Solyc12g009450	2.5	19.509	729	Disease resistance protein (CC-NBS-LRR class) family
miR482e	Solyc12g017800	2.5	24.453	1104	NBS-LRR class disease resistance protein
miR482e	Solyc01g014840	3	16.059	649	disease resistance protein (TIR-NBS-LRR class)
miR482e	Solyc01g108460	3	19.975	5259	Carboxypeptidase
miR482e	Solyc04g009250	3	28.375	577	Nbs-lrr resistance protein
miR482e	Solyc04g009660	3	28.833	550	Nbs-lrr resistance protein
miR482e	Solyc04g009690	3	27.03	562	Nbs-lrr resistance protein
miR482e	Solyc05g032850	3	17.25	2848	evolutionarily conserved C-terminal region 2
miR482e	Solyc07g027020	3	15.263	914	Protein kinase family protein
miR482e	Solyc09g091050	3	15.779	833	Calcium-dependent lipid-binding (CaLB domain) family protein
miR482e	Solyc11g006520	3	23.607	819	Disease resistance protein
miR482e	Solyc11g006640	3	26.45	532	Disease resistance protein
miR482e	Solyc11g069925	3	24.835	622	Disease resistance protein
miR482e	Solyc12g006040	3	20.75	664	NBS-LRR protein
miR482e	Solyc01g008800	3.5	16.457	1564	disease resistance protein (TIR-NBS-LRR class)
miR482e	Solyc01g066020	3.5	26.023	658	disease resistance protein (TIR-NBS-LRR class)
miR482e	Solyc02g032200	3.5	18.488	769	Disease resistance protein (TIR-NBS-LRR class) family
miR482e	Solyc02g036270	3.5	12.267	681	Disease resistance protein (NBS-LRR class) family
miR482e	Solyc03g046207	3.5	24.478	703	Disease resistance protein (CC-NBS-LRR class) family protein
miR482e	Solyc04g005540	3.5	15.547	852	Disease resistance protein (NBS-LRR class) family
miR482e	Solyc04g005550	3.5	18.848	870	Disease resistance protein (NBS-LRR class) family
miR482e	Solyc04g009120	3.5	21.183	601	Nbs-lrr resistance protein
miR482e	Solyc04g074865	3.5	16.828	1400	Retrovirus-related Pol polyprotein from transposon TNT 1-94
miR482e	Solyc05g006630	3.5	29.979	1034	disease resistance protein (TIR-NBS-LRR class)
miR482e	Solyc07g005770	3.5	23.779	541	Disease resistance protein
miR482e	Solyc07g044790	3.5	21.329	1223	Pvr4
miR482e	Solyc07g044797	3.5	21.329	286	CC-NBS-LRR disease resistance protein
miR482e	Solyc10g051050	3.5	21.243	928	Disease resistance protein
miR482e	Solyc11g011350	3.5	20.579	1383	disease resistance protein (TIR-NBS-LRR class)
miR482e	Solyc11g020090	3.5	20.197	103	Disease resistance protein
miR482e	Solyc11g068360	3.5	22.83	622	Disease resistance protein
miR482e	Solyc11g069300	3.5	25.111	2436	Kinase family protein
miR482e	Solyc11g069620	3.5	21.64	706	Disease resistance protein
miR482e	Solyc11g069990	3.5	23.911	1218	I2C5
miR482e	Solyc11g070020	3.5	23.911	1990	Disease resistance protein
miR482e	Solyc11g071423	3.5	25.798	229	Disease resistance protein
miR482e	Solyc11g071995	3.5	26.508	622	Disease resistance protein
miR482e	Solyc12g005970	3.5	15.572	493	Disease resistance protein (CC-NBS-LRR class) family
miR482e	Solyc12g044180	3.5	19.352	526	CC-NBS-LRR disease resistance protein
miR482e	Solyc12g044190	3.5	18.858	526	Disease resistance protein (CC-NBS-LRR class) family protein
miR482e	Solyc12g044200	3.5	18.45	1186	Disease resistance protein (CC-NBS-LRR class) family protein
miR482e	Solyc01g087200	4	19.546	517	Disease resistance protein
miR482e	Solyc01g100310	4	14.841	947	Calmodulin-binding protein
miR482e	Solyc02g032650	4	15.288	798	Disease resistance protein (TIR-NBS-LRR class)
miR482e	Solyc02g073574	4	17.186	583	Disease resistance protein
miR482e	Solyc02g084450	4	17.186	2110	Disease resistance protein
miR482e	Solyc03g078300	4	20.201	727	Disease resistance protein
miR482e	Solyc04g011590	4	30.314	622	Amino acid transporter
miR482e	Solyc04g011960	4	15.415	523	Disease resistance protein (CC-NBS-LRR class) family
miR482e	Solyc04g011980	4	16.137	523	Disease resistance protein (CC-NBS-LRR class) family
miR482e	Solyc04g011990	4	15.741	1079	Disease resistance protein (NBS-LRR class) family
miR482e	Solyc04g012000	4	17.872	223	NBS-coding resistance gene analog
miR482e	Solyc04g012010	4	18.376	543	Disease resistance protein (NBS-LRR class) family
miR482e	Solyc04g048920	4	15.927	46	CC-NBS-LRR disease resistance protein
miR482e	Solyc08g079150	4	16.89	22	SAUR-like auxin-responsive protein family
miR482e	Solyc09g007710	4	25.33	989	Disease resistance protein (TIR-NBS-LRR class) family
miR482e	Solyc10g055050	4	17.252	379	CC-NBS-LRR disease resistance protein
miR482e	Solyc11g065780	4	21.191	786	Disease resistance protein

miR482e	Solyc12g005520	4	23.107	121	Disease resistance protein (CC-NBS-LRR class) family
miR482e	Solyc12g096920	4	17.72	544	Disease resistance protein (CC-NBS-LRR class) family protein

Table S3. Target prediction for all siRNA-producing NLRs. Summary of sNLs, with their gene id, class of NLR protein based on the phylogen genetic analysis of a previous study (5) and not in the presence of representative domains, total counts for 21-nt sRNAs (nRPM), and targeting scores for each individual microRNA (red indicates stronger targeting prediction). *TAS5* (bottom) is added for reference.

General information			Target Prediction				
Gene_ID	**NLR class**	**sRNA prod.**	**miR482b**	**miR482e**	**miR482c**	**miR2118a**	**miR2118b**
Solyc11g065780	CNL	1159.4	2	3	4	-	-
Solyc05g008070	CNL	569.4	1.5	2.5	-	-	3.5
Solyc02g036270	CNL - LRR1	514.6	1.5	3.5	3.5	-	3.5
Solyc04g005540	CNL - LRR2	303.2	3.5	3.5	3.5	-	4
Solyc11g071995	CNL	259.4	4	3.5	-	-	-
Solyc04g005550	CNL	129.6	3.5	3.5	3.5	-	4
Solyc10g051050	CNL	128.0	3	3.5	-	-	-
Solyc09g064610	CNL	77.7	-	-	-	-	-
Solyc01g008800	TNL	74.7	-	3.5	-	4	-
Solyc08g007630	CNL	70.0	-	-	-	-	-
Solyc05g009630	CNL	66.9	-	-	-	-	-
Solyc11g069990	CNL	65.3	4	3.5	-	-	-
Solyc11g069620	CNL	64.9	3.5	3.5	-	-	-
Solyc11g069925	CNL	58.4	3	3	-	-	-
Solyc05g005330	CNL	48.2	-	-	-	-	-
Solyc11g068360	CNL	34.4	3.5	3.5	-	-	-
Solyc07g049700	CNL	25.0	3.5	2	-	-	-
Solyc11g071410	CNL	23.3	-	-	-	-	-
Solyc10g085460	CNL	19.9	-	-	-	-	-
Solyc11g011350	TNL	18.2	-	3.5	-	3	-
Solyc11g006640	CNL	17.8	3.5	3	-	-	-
Solyc12g044190	CNL	17.3	3.5	3.5	-	-	-
Solyc11g020100	CNL	17.3	3	2.5	-	-	-
Solyc12g044200	CNL	17.2	3.5	3.5	-	-	-
Solyc07g005770	CNL	15.5	3	3.5	-	-	4
Solyc09g018220	CNL	14.7	-	-	-	-	-
Solyc11g064770	CNL	14.3	-	-	-	-	-
Solyc02g032650	TNL	14.3	3.5	4	3.5	-	2
Solyc12g006040	CNL	13.7	4	3	-	-	3.5
Solyc08g076000	CNL	12.5	-	-	1.5	-	2.5
Solyc11g069660	CNL	12.0	-	-	-	-	-
Solyc09g098130	CNL	11.1	-	-	-	-	-
Solyc06g005410	TAS5	431.2	-	-	-	-	1 / 2

Table S4 Summary of degradome (PARE) signatures. Summary of validated degradome products for miR482/2118 members on the whole tomato transcriptome (p-value < 0.05). Peak category refers to PARE read abundances of that position correspond to (0) > 90th percentile, (1) > 75th, (2) > 50th percentile, (3) < 50th percentile of total PARE read signatures in the genome.

miRname	Target	Score	Cleavage Position	Reads	Proportion of reads in 10nt window	Peak category	Corrected p-value	Annotation
miR2118b/a	Solyc06g005410	1 / 4	513	62	1	0	0.0001/0.0058	TAS5
miR482b/c	Solyc02g036270	1.5 / 3	1017	12	1	2	0.0058/ 0.0155	LRR1
miR2118b	Solyc06g005410	2	2536	18	0.3	2	0.0061	TAS5
miR482c	Solyc04g005540	3	864	7	1	2	0.0155	LRR2
miR482e	Solyc11g013750	4	1778	9	1	2	0.0401	Disease resistance protein (NLR class) family
miR2118a	Solyc06g064550	7	6499	8	1	2	0.042	Aspartokinase-homoserine dehydrogenase
miR482c	Solyc06g062440	3.5	607	7	1	2	0.0475	Disease resistance protein
miR2118a	Solyc08g065220	7	4795	4	1	3	0.0487	Glycine decarboxylase p-protein

Table S5. Summary of phasing signatures. Top 20 phasing signatures in our sRNA dataset mapping to tomato genes. Colour code indicates when the gene is a predicted to be a preferential target of (blue) miR482 or (red) miR2118.

Gene ID	Start	End	phaseR score	Annotation
Solyc02g036270	278	3514	-26.9	🟦 LRR1
Solyc06g005410	361	1513	-24.6	🟥 TAS5
Solyc10g051050	938	2624	-22.9	🟦 Disease resistance protein (NLR class) family
Solyc09g074520	409	2264	-21.0	Transport inhibitor response 1 (TIR1)
Solyc05g008070	287	2479	-18.8	🟦 Disease resistance protein (NLR class) family
Solyc04g005540	431	3965	-18.3	🟦 LRR2
Solyc05g009630	701	2948	-17.4	Disease resistance protein (NLR class) family
Solyc11g069990	763	3397	-16.5	🟦 Disease resistance protein (NLR class) family
Solyc11g065820	2293	6253	-16.0	🟦 Disease resistance protein (NLR class) family
Solyc12g099870	1691	2072	-15.5	LRR RLK
Solyc11g011350	780	3629	-15.5	🟦 Disease resistance protein (NLR class) family
Solyc06g048960	518	3675	-15.3	Dicer-like 2a (DCL2a)
Solyc05g051230	2225	2876	-14.2	MOCS3-like
Solyc04g051190	154	1809	-14.2	P450 carotenoid β-hydrolase (CYP97A29)
Solyc11g071995	506	3586	-14.1	🟦 Disease resistance protein (NLR class) family
Solyc11g020100	957	2602	-14.0	🟦 Disease resistance protein (NLR class) family
Solyc01g058100	0	126	-13.9	NAD(P)H-quinone oxidoreductase subunit K
Solyc09g018220	562	2549	-13.5	Disease resistance protein (NLR class) family
Solyc02g032650	376	3091	-13.3	🟦 Disease resistance protein (NLR class) family
Solyc11g065790	12	409	-13.2	🟦 Disease resistance protein (NLR class) family

Table S6. Most significant differential sRNA loci in STTM lines. Genetic loci with differential accumulation of sRNAs (any size-class), with their gene id, annotation, log2 fold changes, direction of the change, and adjusted p-value (cut-off of 0.05). Colour code indicates when the gene is a predicted to be a preferential target of (blue) miR482 or (red) miR2118.

Differential sRNA loci between STTM482.1 and wild type

Gene ID	Annotation	Log2(FC)	Condition	adj. p-value
Solyc11g065780	Disease resistance protein (NLR class) family	1.596	WT > STTM482.1	2.3E-06
Solyc09g064610	Disease resistance protein (NLR class) family	3.255	WT > STTM482.1	3.2E-05
Solyc01g008790	Non specific phospholipase C	2.730	WT > STTM482.1	2.3E-04
Solyc01g067165	Disease resistance protein (NLR class) family	1.971	WT > STTM482.1	3.4E-04
Solyc01g008800	Disease resistance protein (NLR class) family	2.508	WT > STTM482.1	4.5E-04
Solyc04g017620	F-box family protein	2.065	WT > STTM482.1	5.4E-04
Solyc12g044190	Disease resistance protein (NLR class) family	1.924	WT > STTM482.1	9.4E-04
Solyc07g005770	Disease resistance protein (NLR class) family	2.787	WT > STTM482.1	1.4E-03
Solyc08g076000	Disease resistance protein (NLR class) family	2.336	WT > STTM482.1	1.8E-03
Solyc02g036270	Disease resistance protein (NLR class) family	1.921	WT > STTM482.1	2.3E-03
Solyc02g032650	Disease resistance protein (NLR class) family	2.140	WT > STTM482.1	3.6E-03
Solyc04g005550	Disease resistance protein (NLR class) family	2.306	WT > STTM482.1	7.1E-03
Solyc04g005540	Disease resistance protein (NLR class) family	1.751	WT > STTM482.1	1.7E-02
Solyc01g067147	Asterix-like protein	2.230	WT > STTM482.1	3.2E-02
Solyc01g100380	Calreticulin	1.322	WT < STTM482.1	1.2E-02
Solyc03g112330	U-box domain-containing kinase family protein	1.684	WT < STTM482.1	2.5E-02
Solyc03g112335	O-acyltransferase (WSD1-like) family protein	1.637	WT < STTM482.1	4.0E-02
Solyc09g097780	Glycine-rich protein	1.499	WT < STTM482.1	4.8E-02

Differential sRNA loci between MIM2118b and wild type

Gene ID	Annotation	Log2(FC)	Condition	adj. p-value
Solyc06g005410	TAS5	2.168	WT > STTM2118b.5	8.09E-05

Table S7. Summary of sNLs. Gene id, class of NLR protein based on the phylogenetic analysis of Andolfo et al. [2014]. Total counts for 21-nt sRNAs (nRPM) in wild type (WT), STTM482.1 and STTM2118b.5 lines. Log2 fold changes between WT and STTM lines (intensity of colour indicates stronger reduction). Summary of target prediction, with letters indicating the predicted targeting miRNA. Summary of RNAseq abundances in transcript per million (TPM) and Log2 fold changes between STTM lines and WT across conditions. SlyTAS5 (bottom) is added for additional reference.

General information		small RNA Analysis					Target Predictions		RNAseq Analysis								
		Total 21nt counts			Log2 difference				Avg Exp (TPM)			LOG2(STTM482.1 /WT)			LOG2(STTM2118b.5 /WT)		
Gene_ID	**NLR class**	**WT**	**STTM482.1**	**STTM2118b.5**	**S4.1/W**	**S2.5/W**	**miR482**	**miR2118**	**Young**	**Old**	**P. inf**	**Young**	**Old**	**P. inf**	**Young**	**Old**	**P. inf**
Solyc11g065780	CNL	1159.4	420.5	1225.5	-1.463	0.080	be c	-	0.0	0.0	0.0						
Solyc05g008070	CNL	569.4	578.4	577.9	0.023	0.021	**be**	b	5.1	6.4	7.4	-0.353	-0.395	-0.689	-0.349	-0.347	-0.178
Solyc02g036270	CNL - LRR1	514.6	124.2	545.1	-2.050	0.083	b ec	b	4.9	5.2	4.3	0.422	0.486	0.516	0.228	0.180	0.161
Solyc04g005540	CNL - LRR2	303.2	78.5	349.7	-1.949	0.206	bec	b	8.9	15.1	14.7	0.237	0.299	0.273	-0.064	0.087	-0.039
Solyc11g071995	CNL	259.4	338.2	291.3	0.382	0.167	be	-	6.1	11.2	6.6	0.167	0.052	0.400	-0.166	0.113	-0.061
Solyc04g005550	CNL	129.6	24.1	150.8	-2.429	0.219	bec	b	3.3	10.0	13.7	-0.140	-0.468	0.133	0.227	-0.622	-0.140
Solyc10g051050	CNL	128.0	131.0	146.7	0.034	0.197	b e	-	1.3	1.9	2.6	-0.163	-0.705	0.533	0.085	-0.767	-0.039
Solyc09g064610	CNL	77.7	7.9	83.9	-3.296	0.111	-	-	6.1	8.1	5.5	-0.230	-0.645	-0.172	0.045	-0.441	-0.197
Solyc01g008800	TNL	74.7	11.2	82.8	-2.738	0.148	e	a	1.2	8.2	12.9	0.009	-0.263	0.288	0.121	-0.848	0.067
Solyc08g007630	CNL	70.0	48.9	78.6	-0.518	0.167	-	-	5.1	9.4	2.3	0.137	-0.217	1.148	0.138	-0.151	0.281
Solyc05g009630	CNL	66.9	67.5	71.9	0.013	0.103	-	-	17.4	33.5	15.2	-0.044	-0.067	0.868	0.056	-0.154	0.756
Solyc11g069990	CNL	65.3	56.2	61.3	-0.217	-0.091	be	-	2.8	4.0	1.8	0.055	-0.192	1.019	0.231	0.056	0.445
Solyc11g069620	CNL	64.9	36.1	72.4	-0.845	0.158	be	-	3.7	6.9	2.4	0.193	0.068	1.390	-0.027	-0.208	0.622
Solyc11g069925	CNL	58.4	47.0	58.9	-0.314	0.012	**be**	-	3.2	6.7	4.0	0.170	0.085	1.009	-0.100	-0.122	0.496
Solyc05g005330	CNL	48.2	47.1	38.3	-0.032	-0.329	-	-	6.8	8.8	5.2	-0.285	-0.080	0.309	-0.175	0.095	0.099
Solyc11g068360	CNL	34.4	24.0	33.6	-0.523	-0.036	be	-	1.9	4.6	1.1	0.361	-0.113	1.133	0.252	0.294	0.283
Solyc07g049700	CNL	25.0	22.8	28.7	-0.129	0.200	b e	-	0.5	2.3	8.8	-0.664	-0.140	-0.205	0.470	-1.055	-0.168
Solyc11g071410	CNL	23.3	27.1	26.6	0.215	0.186	-	-	2.1	4.4	2.9	0.394	0.142	-0.087	-0.189	0.223	-0.098
Solyc10g085460	CNL	19.9	18.4	13.7	-0.114	-0.532	-	-	16.0	22.0	17.4	-0.089	0.025	0.829	0.036	-0.058	0.569
Solyc11g011350	TNL	18.2	21.7	25.8	0.252	0.504	e	a	1.0	1.7	2.1	-0.210	0.164	0.614	0.123	-0.242	0.587
Solyc11g006640	CNL	17.8	14.4	19.5	-0.305	0.132	b e	-	4.3	5.0	1.7	0.645	0.566	1.325	0.094	0.261	0.570
Solyc12g044190	CNL	17.3	4.4	17.9	-1.974	0.047	be	-	3.8	10.8	6.2	0.504	-0.090	0.868	-0.293	0.175	0.196
Solyc11g020100	CNL	17.3	16.1	15.8	-0.108	-0.128	**be**	-	1.3	8.1	4.9	0.290	-0.153	0.303	0.409	-0.374	-0.082
Solyc12g044200	CNL	17.2	5.1	17.3	-1.758	0.012	be	-	5.3	4.0	0.9	-0.215	-0.027	0.856	-0.061	0.536	1.031
Solyc07g005770	CNL	15.5	2.3	15.2	-2.780	-0.028	b e	b	1.2	1.2	0.4	-0.098	0.252	1.079	-0.204	0.565	0.832
Solyc09g018220	CNL	14.7	16.8	6.4	0.200	-1.196	-	-	1.0	1.5	2.3	-0.894	-0.150	0.341	-0.351	-0.580	-0.074
Solyc11g064770	CNL	14.3	5.9	16.6	-1.284	0.215	-	-	0.0	0.0	0.0						
Solyc02g032650	TNL	14.3	3.1	14.1	-2.214	-0.023	bec	**b**	0.7	7.0	10.5	1.264	0.372	0.882	1.0151	0.0349	0.799
Solyc12g006040	CNL	13.7	11.5	5.7	-0.258	-1.261	b e	b	0.3	1.0	5.4	0.308	-0.334	0.292	-0.1123	-0.4187	0.105
Solyc08g076000	CNL	12.5	2.2	12.2	-2.526	-0.033	**c**	**b**	0.9	1.1	1.4	-0.141	0.231	-0.017	-0.2096	-0.0822	0.123
Solyc11g069660	CNL	12.0	6.1	13.2	-0.977	0.132	-	-	3.5	2.0	0.2	-0.279	-0.828	0.929	0.0269	0.2589	0.208
Solyc09g098130	CNL	11.1	11.9	12.0	0.101	0.108	-	-	3.0	1.9	4.8	-0.554	-0.016	-0.602	-0.0318	-0.0852	-0.514
Solyc06g005410	TAS5	431.2	342.2	83.8	-0.334	-2.363	-	**b**	2.1	5.2	7.7	0.1841	-0.0324	0.016	0.4292	0.5832	0.759

Table S8. Table S8. Most differentially expressed genes in STTM lines. Genes with differential accumulation in at least two of the three conditions (adjusted p-value cut-off of 0.05), with gene id, direction of the change, summary of RNAseq abundances in transcript per million (TPM) in STTM lines and WT across conditions, and annotation. Predicted targets for miR482 or miR2118, respectively, are highlighted in red. (WT = wild type; S4 = STTM482.1; S2 = STTM2118b.5; Y = Young; O = Old; P = P.infestans infected).

Differentially expressed genes between STTM482.1 and wild type

GeneID	Dir.	Y.WT	Y.S4	O.WT	O.S4	P.WT	P.S4	Annotation
Solyc00g005000	WT<S4	0.02	0.00	1.50	3.55	0.05	0.61	Eukaryotic aspartyl protease family protein
Solyc01g010937	WT<S4	0.07	5.76	0.20	5.90	0.11	4.54	LOW QUALITY:BED zinc finger
Solyc01g017560	WT<S4	0.61	2.15	0.58	0.83	0.08	0.89	response regulator 11
Solyc01g028805	WT<S4	9.52	18.63	9.33	24.51	20.29	21.59	Cytochrome P450
Solyc01g081250	WT<S4	0.10	0.34	0.04	0.97	0.17	38.26	Glutathione s-transferase
Solyc01g087620	WT<S4	33.73	41.68	116.74	123.32	196.39	253.24	ubiquitin-like protein 5
Solyc01g100980	WT<S4	4.27	2.91	0.05	0.61	0.47	2.02	Pectin lyase-like superfamily protein
Solyc01g102850	WT<S4	1.47	5.73	20.18	38.84	6.77	16.28	Disease resistance protein
Solyc01g103110	WT<S4	7.55	8.56	13.29	16.51	3.18	4.45	Acyl-CoA N-acyltransferase
Solyc01g104350	WT<S4	5.78	7.63	17.55	20.06	11.67	12.50	Elongation factor 4
Solyc01g105310	WT<S4	1.60	2.93	6.57	13.62	2.40	8.22	metacaspase 2
Solyc01g109690	WT<S4	2.81	5.56	2.06	3.56	0.00	0.00	MYB transcription factor
Solyc02g014077	WT<S4	0.36	1.62	1.99	3.88	1.22	7.81	phosphoglucan
Solyc02g014690	WT<S4	0.00	0.29	0.06	2.11	0.00	0.44	Late embryogenesis abundant
Solyc02g030290	WT<S4	0.00	0.00	0.17	0.66	0.28	1.02	LOW QUALITY:Nbs-lrr resistance protein
Solyc02g036270	WT<S4	130.65	166.41	189.88	250.36	141.11	219.59	LRR1
Solyc02g036280	WT<S4	18.59	23.82	27.43	34.13	21.56	32.77	Pvr4
Solyc02g037640	WT<S4	0.36	1.08	1.13	3.02	0.70	1.93	LOW QUALITY:DNA helicase homolog
Solyc02g062580	WT<S4	3.01	4.10	3.13	4.20	1.73	1.97	Phosphatidic acid phosphatase 2
Solyc02g071750	WT<S4	0.38	0.94	12.66	15.55	10.64	15.30	RNA ligase/cyclic nucleotide phosphodiesterase
Solyc02g082060	WT<S4	1.29	2.98	21.85	23.96	9.54	15.59	PPPDE putative thiol peptidase
Solyc02g084037	WT<S4	0.74	1.61	1.36	3.21	1.04	2.35	stress response NST1-like protein
Solyc02g085120	WT<S4	8.51	8.13	0.87	2.80	0.69	2.98	Laccase
Solyc03g006580	WT<S4	19.93	26.27	36.65	40.28	33.65	34.81	LisH and RanBPM domains containing protein
Solyc03g112200	WT<S4	16.91	37.15	168.28	162.60	35.90	69.75	LOW QUALITY:Insulinase
Solyc03g117220	WT<S4	0.00	0.00	0.14	0.33	0.09	0.41	Malectin/receptor-like protein kinase
Solyc03g119700	WT<S4	65.04	80.98	88.20	112.84	39.06	53.54	ATP-dependent Clp protease adaptor protein ClpS
Solyc04g007800	WT<S4	9.37	33.40	176.34	189.09	27.06	64.91	Calcium-dependent lipid-binding
Solyc04g009860	WT<S4	0.06	1.55	12.65	18.29	37.23	47.17	2-oxoglutarate
Solyc04g025160	WT<S4	21.56	27.22	12.43	16.48	4.32	6.98	ATPase
Solyc04g025120	WT<S4	94.29	123.85	66.65	88.97	25.67	37.54	ATPase
Solyc04g049185	WT<S4	0.00	0.15	0.00	0.62	0.11	1.52	LOW QUALITY:BED zinc finger
Solyc04g051427	WT<S4	0.02	0.90	0.13	1.50	0.08	2.35	BED zinc finger
Solyc04g052890	WT<S4	0.08	1.41	0.00	1.33	0.00	0.00	SAUR-like auxin-responsive protein family
Solyc04g054157	WT<S4	0.00	0.10	0.01	0.26	0.03	0.66	BED zinc finger
Solyc04g054385	WT<S4	0.00	0.22	0.14	1.41	0.31	3.53	HAT family dimerisation domain containing protein
Solyc04g072050	WT<S4	4.05	3.90	4.32	6.10	4.22	5.46	Transmembrane protein
Solyc04g074490	WT<S4	17.65	19.05	52.56	74.32	90.10	122.37	F-box protein
Solyc04g079480	WT<S4	0.10	1.02	2.05	5.77	4.15	16.77	Serpin-like protein
Solyc04g080810	WT<S4	9.09	12.88	22.68	28.32	32.92	45.27	Cytokinin oxidase/dehydrogenase-like
Solyc04g081670	WT<S4	2.32	3.15	2.29	3.62	0.74	1.11	Vacuolar-processing enzyme
Solyc04g081700	WT<S4	0.47	5.00	2.07	16.81	2.70	25.40	Ribosomal protein S5/Elongation factor
Solyc04g082710	WT<S4	91.98	81.11	9.04	28.93	7.10	32.33	Cysteine protease
Solyc05g005130	WT<S4	3.33	5.33	14.64	16.24	14.12	19.99	Disease resistance protein
Solyc05g006850	WT<S4	5.29	13.09	51.11	72.09	35.99	65.07	Thioredoxin family protein
Solyc05g007240	WT<S4	23.11	37.81	43.31	57.83	17.02	16.91	P-loop nucleoside triphosphate hydrolase
Solyc05g013630	WT<S4	28.86	79.44	252.55	328.00	51.96	95.33	LOW QUALITY:CP-interacting protein-L
Solyc05g015847	WT<S4	0.00	0.54	0.21	1.49	0.46	5.90	BED zinc finger
Solyc05g021370	WT<S4	0.02	0.15	0.10	3.09	0.02	1.82	Lectin alpha chain
Solyc05g021373	WT<S4	0.00	0.02	0.00	0.30	0.00	0.29	ATP synthase epsilon chain
Solyc05g042126	WT<S4	0.00	1.92	0.20	1.04	0.23	1.38	BED zinc finger
Solyc05g051740	WT<S4	2.79	3.25	4.24	8.40	1.00	2.52	Prosystemin
Solyc05g053965	WT<S4	0.21	0.64	1.78	3.56	0.36	2.08	Amino acid transporter
Solyc05g054010	WT<S4	0.02	0.50	0.15	3.63	0.19	5.47	NBS-LRR resistance protein-like protein
Solyc06g008235	WT<S4	0.13	0.81	0.41	3.05	0.48	5.22	NDH-dependent cyclic electron flow 5
Solyc06g051835	WT<S4	0.00	0.31	0.20	0.43	0.03	0.40	BED zinc finger
Solyc06g054380	WT<S4	7.76	8.41	8.71	10.57	5.88	8.10	RNA binding protein
Solyc06g060110	WT<S4	59.36	116.11	549.41	432.76	373.75	592.50	amino acid transporter 2
Solyc06g062900	WT<S4	3.27	4.61	3.88	4.11	1.69	3.11	Transcription initiation factor TFIID subunit 12
Solyc06g068190	WT<S4	0.73	3.90	1.05	4.54	0.95	4.77	Ubiquitin system component Cue protein
Solyc06g069727	WT<S4	46.15	81.36	25.64	44.82	15.53	19.77	Thioredoxin-like protein
Solyc06g069760	WT<S4	23.54	23.05	23.56	34.67	6.89	10.78	Dof zinc finger protein 22
Solyc06g073180	WT<S4	94.25	97.10	29.65	46.97	36.69	59.01	CONSTANS interacting protein 1
Solyc06g075520	WT<S4	0.58	1.29	1.31	1.79	0.81	2.73	Dehydroascorbate reductase
Solyc06g075650	WT<S4	3.85	2.71	3.33	11.16	1.48	5.85	tonoplast intrinsic protein 1.2
Solyc06g076460	WT<S4	56.72	66.45	71.32	84.05	90.51	86.97	Transcription elongation factor B polypeptide 1
Solyc07g005750	WT<S4	1.02	1.86	3.68	5.55	4.45	6.00	F-box family protein
Solyc07g007725	WT<S4	0.00	0.08	0.00	0.09	0.01	0.04	Bifunctional inhibitor/lipid-transfer protein
Solyc07g025530	WT<S4	3.25	3.03	1.11	1.52	0.38	0.67	S-adenosyl-L-methionine-dependent methyltransferase
Solyc07g032493	WT<S4	0.00	0.45	0.00	1.50	0.27	3.80	BED zinc finger
Solyc07g032497	WT<S4	0.00	1.66	0.00	1.92	0.11	2.21	HAT family dimerisation domain containing protein
Solyc07g055450	WT<S4	0.00	0.02	0.01	0.11	0.01	0.17	Cytochrome P450
Solyc07g055460	WT<S4	0.04	5.14	2.64	53.57	5.33	69.56	Cytochrome P450
Solyc07g055480	WT<S4	0.00	0.06	0.03	0.63	0.28	1.47	Cytochrome P450
Solyc07g064560	WT<S4	2.40	6.19	8.84	17.79	9.72	26.51	AT hook
Solyc08g005550	WT<S4	0.03	0.16	0.01	0.01	0.03	0.13	alternative oxidase 1c
Solyc08g048560	WT<S4	0.00	1.69	0.00	3.43	0.00	0.85	Protein kinase protein / WD-40 repeat protein
Solyc08g044280	WT<S4	7.91	14.60	13.15	16.80	10.81	28.41	Adenine nucleotide alpha hydrolase-like domain kinase
Solyc08g061323	WT<S4	11.75	22.65	20.05	32.87	18.25	41.23	BED zinc finger
Solyc08g062220	WT<S4	65.65	302.63	445.59	529.02	69.48	166.43	Glycosyltransferase
Solyc08g062290	WT<S4	6.35	12.33	13.76	20.69	5.74	10.60	Light-independent protochlorophyllide reductase subunit B
Solyc08g065265	WT<S4	0.21	0.64	0.46	1.74	0.85	5.57	BED zinc finger
Solyc08g065947	WT<S4	0.00	1.04	0.26	1.20	0.08	1.06	HAT family dimerisation domain containing protein
Solyc08g068070	WT<S4	0.38	0.55	1.27	1.23	0.56	0.94	hemoglobin 3
Solyc08g077190	WT<S4	0.18	0.51	0.24	0.58	0.18	0.32	LOW QUALITY:MATE efflux family protein
Solyc08g083410	WT<S4	0.00	0.29	0.00	0.22	0.00	0.05	Cytochrome P450 family protein
Solyc08g079370	WT<S4	0.00	0.14	0.01	0.53	0.07	0.35	Cytochrome P450 family protein

Solyc08g080070	WT<S4	4.62	4.44	0.41	1.32	0.14	0.48	LOW QUALITY:transmembrane protein
Solyc08g081790	WT<S4	40.42	32.16	2.05	6.18	0.31	2.54	Dirigent protein
Solyc08g082120	WT<S4	29.73	129.44	913.02	1011.90	130.23	302.31	Methanol inducible protein
Solyc09g011030	WT<S4	14.51	20.07	15.45	27.18	7.81	18.11	Hsp70-binding protein 1
Solyc09g011990	WT<S4	0.24	3.78	1.30	6.62	8.59	9.42	Clade IV lectin receptor kinase
Solyc09g018610	WT<S4	0.00	0.68	0.00	4.39	0.00	2.36	Mitochondrial ATP synthase subunit G protein
Solyc09g056010	WT<S4	0.01	0.13	0.44	0.69	0.25	0.73	ACT-like protein tyrosine kinase family protein
Solyc09g056185	WT<S4	0.03	2.96	0.01	2.93	0.04	2.50	HAT family dimerisation domain containing protein
Solyc09g060100	WT<S4	18.96	20.14	4.93	9.09	3.97	7.33	transmembrane protein
Solyc09g064365	WT<S4	0.00	0.04	0.20	1.43	0.13	2.90	BED zinc finger
Solyc09g064742	WT<S4	0.38	2.05	1.19	8.32	1.41	12.07	root hair specific 16
Solyc09g064750	WT<S4	0.60	2.21	1.59	4.30	1.30	8.63	root hair specific 16
Solyc09g065883	WT<S4	0.00	0.08	0.00	0.60	0.15	1.68	Na+/H+ antiporter
Solyc09g072725	WT<S4	0.41	2.20	1.42	3.28	0.92	3.41	LOW QUALITY:BED zinc finger
Solyc09g074185	WT<S4	0.10	11.04	2.69	16.76	0.70	16.55	LOW QUALITY:BED zinc finger
Solyc09g089740	WT<S4	16.45	21.22	12.04	28.55	14.84	38.07	2-oxoglutarate
Solyc09g098380	WT<S4	0.06	0.16	0.05	4.50	1.23	6.62	Transmembrane amino acid transporter family protein
Solyc10g006600	WT<S4	5.02	6.07	15.40	15.89	9.74	12.31	Gluconokinase
Solyc10g018590	WT<S4	84.36	149.01	159.09	251.92	22.69	33.15	Plastid lipid-associated protein
Solyc10g018903	WT<S4	0.00	0.10	0.00	0.22	0.01	0.44	SPT4 homolog 2
Solyc10g050050	WT<S4	0.05	0.51	0.00	0.52	0.00	0.08	Nucleotide binding site-leucine rich repeat protein
Solyc10g054810	WT<S4	0.80	4.05	4.17	8.62	0.04	0.00	X-intrinsic protein 1.3
Solyc10g078670	WT<S4	0.00	0.07	0.00	0.08	0.07	0.22	BZIP transcription factor family protein
Solyc10g079600	WT<S4	9.83	10.66	6.05	13.53	2.13	5.46	Two-component response regulator
Solyc10g081770	WT<S4	18.30	25.43	54.31	87.06	8.20	21.72	Polyketide cyclase/dehydrase
Solyc11g017440	WT<S4	20.92	19.27	8.77	17.41	1.34	3.85	Gibberellin-regulated family protein
Solyc11g050938	WT<S4	0.00	1.34	0.25	9.69	2.11	29.50	Peroxisomal
Solyc11g065340	WT<S4	4.16	5.85	46.14	63.04	17.49	18.90	Magnesium-chelatase subunit H
Solyc11g072580	WT<S4	4.10	3.56	0.42	0.59	0.00	0.10	Major facilitator superfamily protein
Solyc12g010370	WT<S4	0.18	0.85	0.09	0.62	0.33	1.45	Ultrapetala
Solyc12g010755	WT<S4	0.80	3.82	0.41	2.77	1.49	6.55	Ultrapetala
Solyc12g015865	WT<S4	0.03	1.12	0.41	2.48	0.23	3.97	LOW QUALITY:BED zinc finger
Solyc12g021160	WT<S4	2.29	3.43	13.29	20.00	4.66	9.34	LOW QUALITY:cysteine-rich RLK
Solyc12g042340	WT<S4	0.13	0.21	0.10	1.29	0.25	1.10	JHL07K02.14 protein
Solyc12g042800	WT<S4	0.02	4.20	0.17	10.99	0.01	6.16	dsRNA-binding domain-like superfamily protein
Solyc12g049370	WT<S4	29.62	32.44	63.58	74.79	47.65	60.59	Nucleoside diphosphate kinase
Solyc12g087880	WT<S4	0.06	0.13	6.29	14.13	0.08	0.95	Purine permease-like protein
Solyc12g094700	WT<S4	20.22	16.89	1.86	4.87	0.59	3.25	Cysteine protease
Solyc01g098790	WT>S4	1.39	0.40	2.41	1.03	1.11	1.30	HSP20-like chaperones superfamily protein
Solyc01g103470	WT>S4	0.15	0.09	1.48	0.41	4.09	2.70	Cytosolic Fe-S cluster assembly factor nar-1
Solyc01g111880	WT>S4	0.46	0.42	0.64	0.17	0.84	0.17	MAP kinase kinase kinase 11
Solyc02g014840	WT>S4	0.34	0.10	0.71	0.19	0.17	0.19	F-box protein
Solyc02g084890	WT>S4	11.52	3.50	37.08	14.29	7.58	11.24	NBS-LRR disease resistance protein NBS50
Solyc02g089040	WT>S4	5.86	6.35	8.33	5.22	14.78	10.85	Unknown protein
Solyc02g089720	WT>S4	58.40	14.62	112.61	35.19	615.21	503.41	Glycoside hydrolase
Solyc02g089900	WT>S4	12.02	2.52	53.40	12.44	38.38	38.64	Kinase family protein
Solyc03g025340	WT>S4	41.58	30.80	193.39	141.50	116.73	122.60	Calcium-dependent lipid-binding family protein
Solyc03g036480	WT>S4	0.02	0.00	3.64	0.46	0.89	0.20	Phenylalanine ammonia-lyase
Solyc03g042560	WT>S4	0.08	0.09	121.76	9.07	21.30	2.90	Phenylalanine ammonia-lyase
Solyc03g044660	WT>S4	8.31	4.00	6.08	3.20	7.85	5.88	4-hydroxy-tetrahydrodipicolinate synthase
Solyc03g059490	WT>S4	32.70	21.47	51.00	35.24	48.99	54.31	Leucine rich repeat receptor protein kinase 2
Solyc03g093080	WT>S4	1.92	0.40	6.96	0.73	7.10	2.91	Xyloglucan endotransglucosylase/hydrolase
Solyc03g093110	WT>S4	1.77	0.30	3.07	0.25	3.13	0.78	xyloglucan endotransglucosylase-hydrolase
Solyc03g093120	WT>S4	3.37	0.32	16.34	1.23	18.57	5.90	xyloglucan endotransglucosylase-hydrolase
Solyc03g097170	WT>S4	11.39	3.94	2.36	1.64	8.23	0.96	Cinnamoyl-CoA reductase
Solyc03g120550	WT>S4	84.11	22.39	103.67	37.28	75.50	60.93	Major facilitator superfamily protein
Solyc04g074420	WT>S4	6.62	1.85	7.08	1.13	0.40	0.27	Phosphate-responsive 1 family protein
Solyc04g074430	WT>S4	96.24	23.12	67.78	9.78	12.26	3.29	Phosphate-responsive 1 family protein
Solyc04g077140	WT>S4	4.37	3.38	1.74	0.56	1.02	0.34	DUF1005 family protein
Solyc04g077610	WT>S4	24.61	12.69	46.07	21.78	13.32	20.67	U-box domain-containing family protein
Solyc04g078550	WT>S4	8.15	3.82	25.90	11.05	17.29	17.14	WRKY transcription factor 7
Solyc04g082270	WT>S4	1.29	0.70	0.22	0.29	0.69	0.32	DUF241 domain protein
Solyc05g005170	WT>S4	7.60	4.33	0.01	0.00	0.18	0.01	Pectin lyase-like superfamily protein
Solyc05g024230	WT>S4	0.76	0.04	1.11	0.09	2.54	0.74	ATP-dependent 6-phosphofructokinase
Solyc05g055730	WT>S4	17.18	5.00	0.74	0.67	15.15	3.25	Methylenetetrahydrofolate reductase
Solyc06g009810	WT>S4	11.82	5.81	8.79	4.90	7.45	3.18	Ethylene-responsive transcription factor
Solyc06g059740	WT>S4	0.13	0.18	7.34	3.05	7.68	3.14	Alcohol dehydrogenase
Solyc06g073050	WT>S4	41.02	19.57	104.71	49.63	96.66	109.81	NAC domain-containing protein
Solyc06g083720	WT>S4	4.05	2.14	0.02	0.00	0.07	0.01	Subtilisin-like protease
Solyc07g008240	WT>S4	0.37	0.77	1.90	0.43	0.18	0.04	Non-symbiotic hemoglobin 1
Solyc07g022920	WT>S4	15.34	4.76	26.72	7.67	22.56	6.40	BSD domain-containing family protein
Solyc08g008370	WT>S4	78.66	18.45	412.85	104.43	327.11	307.70	DCD
Solyc08g076320	WT>S4	30.87	25.83	42.18	13.57	43.79	27.38	Cellulose synthase
Solyc09g005080	WT>S4	2.71	1.21	12.38	6.40	21.81	22.18	verticillium wilt disease resistance 2
Solyc09g011690	WT>S4	24.33	12.16	45.46	24.46	9.62	10.89	WAS/WASL-interacting protein family member 2
Solyc09g059477	WT>S4	0.43	0.02	0.00	0.00	0.23	0.02	Transducin/WD40 repeat-like superfamily protein
Solyc10g011740	WT>S4	44.83	18.71	144.78	58.89	93.82	127.79	Ubiquitin-conjugating enzyme E2
Solyc11g005150	WT>S4	8.64	4.31	1.14	1.15	8.89	2.73	entensin X55687
Solyc11g007660	WT>S4	10.89	6.05	31.89	17.81	15.27	18.93	type-1 restriction enzyme mjaxp r protein
Solyc11g010390	WT>S4	0.89	0.98	10.17	3.64	28.83	13.26	LOW QUALITY:Arabinogalactan-protein
Solyc11g020230	WT>S4	71.99	16.32	297.22	78.68	61.82	66.47	Cytokinin-regulated kinase 1
Solyc12g006980	WT>S4	36.84	33.17	36.79	17.65	32.43	16.92	Leucine-rich repeat receptor-like protein kinase family

Differentially expressed genes between STTM2118b.5 and wild type

GeneID	**Dir.**	**Y.WT**	**Y.S2**	**O.WT**	**O.S2**	**P.WT**	**P.S2**	**Annotation**
Solyc00g005092	WT<S2	0.00	9.23	0.71	32.12	0.74	45.10	Serine/threonine phosphatase-like protein
Solyc00g005287	WT<S2	0.00	0.00	0.08	0.70	0.13	1.37	3-oxoacyl-[acyl-carrier-protein] synthase
Solyc00g010760	WT<S2	0.00	0.00	0.02	0.11	0.01	0.15	LOW QUALITY:DNA helicase homolog
Solyc01g060350	WT<S2	0.30	0.65	0.90	4.17	0.52	3.59	Mediator of RNA polII transcription subunit 33A
Solyc01g098630	WT<S2	8.46	22.36	5.95	11.00	1.88	2.85	Mitochondrial inner membrane translocase subunit Tim44
Solyc01g108800	WT<S2	0.17	0.25	0.22	0.51	0.13	0.37	3-hydroxyisobutyryl-CoA hydrolase-like protein
Solyc02g014077	WT<S2	0.36	1.37	1.99	5.98	1.22	6.94	phosphoglucan
Solyc02g037640	WT<S2	0.36	1.99	1.13	3.86	0.70	4.65	LOW QUALITY:DNA helicase homolog
Solyc02g094300	WT<S2	22.10	44.15	48.60	63.60	22.88	23.80	ACT domain-containing protein

Solyc03g116960	WT<S2	0.04	0.07	0.08	0.22	0.00	0.14	LOW QUALITY:Pentatricopeptide repeat
Solyc04g009190	WT<S2	0.60	1.32	1.36	2.76	1.96	4.20	UPF0664 stress-induced protein C29B12.11c
Solyc04g051780	WT<S2	27.63	36.36	40.03	70.75	24.71	50.43	Programmed cell death protein 2-like protein
Solyc04g071150	WT<S2	3.58	14.01	17.12	29.55	0.77	1.16	Cytochrome P450 family protein
Solyc05g021163	WT<S2	0.00	0.03	0.00	1.02	0.00	0.48	ubiquitin-conjugating enzyme 34
Solyc05g021180	WT<S2	0.00	0.18	0.00	5.49	0.00	2.92	Pyridoxal-5'-phosphate-dependent enzyme family protein
Solyc05g024190	WT<S2	0.39	9.76	1.46	11.11	0.64	4.85	Chlorophyll synthase
Solyc05g041770	WT<S2	0.08	0.06	0.81	3.02	0.81	5.32	Guanylate-binding family protein
Solyc05g053965	WT<S2	0.21	0.67	1.78	2.55	0.36	1.95	Amino acid transporter
Solyc05g054010	WT<S2	0.02	0.21	0.15	1.23	0.19	4.70	NBS-LRR resistance protein-like protein
Solyc05g055400	WT<S2	116.91	89.27	51.20	97.52	14.50	38.05	Cytochrome P450
Solyc06g005410	WT<S2	4.85	6.63	16.20	20.85	20.45	37.26	TAS5
Solyc06g008235	WT<S2	0.13	0.55	0.41	3.61	0.48	4.44	NDH-dependent cyclic electron flow 5
Solyc06g075520	WT<S2	0.58	2.09	1.31	1.95	0.81	2.11	Dehydroascorbate reductase
Solyc06g075513	WT<S2	0.13	0.41	0.27	0.48	0.10	0.36	Dehydroascorbate reductase
Solyc06g076540	WT<S2	0.00	0.11	0.03	0.33	0.02	0.45	Class I heat shock protein
Solyc07g026710	WT<S2	0.00	0.00	0.35	2.03	0.10	0.74	Transposon protein
Solyc07g055460	WT<S2	0.04	3.33	2.64	44.05	5.33	50.18	Cytochrome P450
Solyc07g063320	WT<S2	0.28	1.86	0.97	2.85	0.43	2.97	LanC-like protein 2
Solyc08g015680	WT<S2	0.00	0.76	0.00	0.81	0.00	1.50	Potassium transporter
Solyc08g044280	WT<S2	7.91	19.03	13.15	16.02	10.81	30.59	Adenine nucleotide alpha hydrolase-like domain kinase
Solyc08g023340	WT<S2	0.00	0.22	0.06	1.52	0.46	5.73	Lectin
Solyc08g022130	WT<S2	0.00	0.27	0.26	1.40	0.00	0.67	F-box-like/WD repeat-containing protein TBL1XR1
Solyc08g062220	WT<S2	65.65	368.55	445.59	663.20	69.48	149.57	Glycosyltransferase
Solyc08g062290	WT<S2	6.35	25.05	13.76	29.24	5.74	17.16	Light-independent protochlorophyllide reductase subunit B
Solyc08g065590	WT<S2	0.00	6.94	0.83	15.58	1.95	32.66	Vacuolar processing enzyme
Solyc08g065600	WT<S2	0.00	0.65	0.12	2.15	0.29	3.47	Vacuolar-processing enzyme
Solyc08g065720	WT<S2	0.01	0.06	0.00	0.19	0.00	0.30	Vacuolar processing enzyme
Solyc08g066920	WT<S2	0.12	0.26	0.33	0.70	0.16	0.83	ATP/ADP transporter
Solyc08g082545	WT<S2	0.05	0.19	0.10	0.25	0.01	0.06	LOW QUALITY:Peroxidase
Solyc09g011030	WT<S2	14.51	20.73	15.45	20.44	7.81	15.58	Hsp70-binding protein 1
Solyc09g011990	WT<S2	0.24	1.19	1.30	3.86	8.59	9.70	Clade IV lectin receptor kinase
Solyc09g018610	WT<S2	0.00	3.68	0.00	17.25	0.00	9.20	Mitochondrial ATP synthase subunit G protein
Solyc09g031528	WT<S2	0.24	0.13	1.34	4.42	1.59	6.83	3-oxoacyl-[acyl-carrier-protein] synthase
Solyc09g056185	WT<S2	0.03	0.64	0.01	0.88	0.04	0.49	HAT family dimerisation domain containing protein
Solyc09g066030	WT<S2	0.00	0.40	0.00	0.30	0.00	1.29	Homeobox leucine zipper protein
Solyc11g021360	WT<S2	11.36	51.96	23.05	73.35	7.78	12.61	Protease Do-like 7
Solyc12g005070	WT<S2	2.55	3.74	2.41	3.07	0.87	1.13	CASP-like protein
Solyc12g042800	WT<S2	0.02	0.13	0.17	1.53	0.01	0.59	dsRNA-binding domain-like superfamily protein
Solyc01g067030	WT>S2	11.23	6.17	13.41	6.83	7.36	7.66	F-box associated interaction domain-containing protein
Solyc02g088240	WT>S2	1.02	0.78	5.65	1.17	21.94	8.06	Phosphate transporter PHO1-like protein
Solyc03g007395	WT>S2	6.99	3.71	11.28	4.96	5.91	3.15	Photosystem I P700 chlorophyll a apoprotein A1
Solyc03g007400	WT>S2	0.45	0.22	1.01	0.39	0.54	0.20	Galactose oxidase/kelch repeat superfamily protein
Solyc03g025150	WT>S2	75.83	57.68	139.97	97.48	151.73	160.98	Dentin sialophosphoprotein-related
Solyc03g036480	WT>S2	0.02	0.00	3.64	0.12	0.89	0.18	Phenylalanine ammonia-lyase
Solyc03g036473	WT>S2	0.00	0.00	8.42	0.00	1.63	0.09	Phenylalanine ammonia-lyase
Solyc04g050220	WT>S2	5.97	3.09	6.00	1.70	3.29	1.01	F-box associated interaction domains-containing protein
Solyc05g040050	WT>S2	9.88	7.47	17.16	12.18	19.68	19.73	DnaJ domain-containing protein
Solyc05g052470	WT>S2	6.92	2.91	44.28	25.40	42.22	37.22	Ferritin
Solyc05g055730	WT>S2	17.18	7.77	0.74	1.05	15.15	5.44	Methylenetetrahydrofolate reductase
Solyc06g009170	WT>S2	6.69	3.06	61.28	41.04	53.43	48.01	Epoxide hydrolase
Solyc06g063090	WT>S2	1.18	0.92	3.92	1.59	31.57	21.92	alanine aminotransferase 2
Solyc08g077460	WT>S2	10.94	6.91	16.99	10.73	35.92	30.16	SBP
Solyc11g071770	WT>S2	14.77	10.26	25.75	17.13	36.76	38.65	Translation elongation factor
Solyc12g005060	WT>S2	0.25	0.02	2.04	0.93	1.61	1.76	ATP synthase gamma-subunit
Solyc12g036727	WT>S2	0.19	0.01	0.63	0.15	0.77	0.10	NADH-ubiquinone oxidoreductase chain 5

Table S9. Oligonucleotide sequences used in this study.

Experiment	Name	Sequence (5'-3'
Northern blot	*sly-miR168*	GTCCCGACCTGCACCAAGCGA
	sly-U6	GGCCATGCTAATCTTCTCTGTATCGTT
	sly-miR482e	GGTATGGGAGGAGTAGGAAAGA
	sly-miR482b (LNA)	GGCATGGGCGGTGTAGGCAAGA
	sly-miR2118a	TAGGAATGGGTGGAATTGGAAA
	sly-miR2118b (LNA)	TTGGCATGGGTGGAATAGGAAA
qRT-PCR	*TAS5_qPCR_1_F*	GGTTTGGTTCGGGTTGTTTA
	TAS5_qPCR_1_R	TCAACATTGCTTTCCCACTTT
	LRR2_qRT_B_F	CTAGCGAAGCGTGGTCTTGA
	LRR2_qRT_B_R	TGAGCACAAAAGAGTTGTAGCTT
	EXP_qPCR_F	GCTAAGAACGCTGGACCTAATG
	EXP_qPCR_R	TGGGTGTGCCTTTCTGAATG
RT-PCR	*mimicry_482_F*	AAGGCATGGGCGGCACTGT
	mimicry_482_R	GAATTCTCTTTCCTACTATGC
	mimicry_2118b_F	ACCAATTGGCATGGGTGATA
	mimicry_2118b_R	GAATTCTTTCCTATTCAGTCA
Mimicry cloning	*miR2118b_mimic_Fw*	CACCAATTGGCATGGGTGATAGAATAGGAAAGTTGTTGTTGTTATGGTCTAATTT... ...AAATATGGTCTAAAGAAGAAGAATTTGGCATGGGTGACTGAATAGGAAAGAATTC
	miR2118b_mimic_Rv	GAATTCTTTCCTATTCAGTCACCCATGCCAAATTCTTCTTCTTTAGACCATATTT... ...AAATTAGACCATAACAACAACAACTTTCCTATTCTATCACCCATGCCAATTGGTG
	miR482_mimic_Fw	CACCAAGGCATGGGCGGCACTGTAGGCAAGAGTTGTTGTTGTTATGGTCTAATTT... ...AAATATGGTCTAAAGAAGAAGAATGGTATGGGAGGCATAGTAGGAAAGAGAATTC
	miR482_mimic_Rv	GAATTCTCTTTCCTACTATGCCTCCCATACCATTCTTCTTCTTTAGACCATATTTAAA... ...TTAGACCATAACAACAACAACTCTTGCCTACAGTGCCGCCCATGCCTTGGTG

References

1. Van Eck J, Kirk DD, Walmsley AM (2006) Tomato (Lycopersicum esculentum). *Methods in molecular biology (Clifton, N.J.)* 343:459–73.
2. Yan J, et al. (2012) Effective small RNA destruction by the expression of a short tandem target mimic in Arabidopsis. *The Plant Cell* 24(2):415–427.
3. Kozomara A, Griffiths-Jones S (2013) miRBase: annotating high confidence microRNAs using deep sequencing data. *Nucleic acids research* 42(D1):D68—-D73.
4. Srivastava PK, Moturu TR, Pandey P, Baldwin IT, Pandey SP (2014) A comparison of performance of plant miRNA target prediction tools and the characterization of features for genome-wide target prediction. *BMC Genomics* 15(1):348.
5. Andolfo G, et al. (2014) Defining the full tomato NB-LRR resistance gene repertoire using genomic and cDNA RenSeq. *BMC Plant Biology* 14(1):120.
6. Kakrana A, Hammond R, Patel P, Nakano M, Meyers BC (2014) sPARTA: a parallelized pipeline for integrated analysis of plant miRNA and cleaved mRNA data sets, including new miRNA target-identification software. *Nucleic acids research* 42(18):e139—-e139.
7. Mohorianu I, et al. (2011) Profiling of short RNAs during fleshy fruit development reveals stage-specific sRNAome expression patterns. *The Plant Journal* 67(2):232–246.
8. Hardcastle TJ, Kelly KA, Baulcombe DC (2012) Identifying small interfering RNA loci from high-throughput sequencing data. *Bioinformatics* 28(4):457–463.
9. Hardcastle TJ, Kelly KA (2013) Empirical Bayesian analysis of paired high-throughput sequencing data with a beta-binomial distribution. *BMC Bioinformatics* 14(1):135.
10. Kamoun S, Van West P, Vleeshouwers VGAA, De Groot KE, Govers F (1998) Resistance of Nicotiana benthamiana to Phytophthora infestans Is Mediated by the Recognition of the Elicitor Protein INF1, Technical report.
11. Liu X, et al. (2015) Bacterial leaf infiltration assay for fine characterization of plant defense responses using the Arabidopsis thaliana-Pseudomonas syringae Pathosystem. *JoVE (Journal of Visualized Experiments)* (104):e53364—-e53364.
12. Bray NL, Pimentel H, Melsted P, Pachter L (2016) Near-optimal probabilistic RNA-seq quantification. *Nature Biotechnology* 34(5):525–527.
13. Ritchie ME, et al. (2015) limma powers differential expression analyses for rna-sequencing and microarray studies. *Nucleic acids research* 43(7):e47–e47.
14. Ron M, Avni A (2004) The receptor for the fungal elicitor ethylene-inducing xylanase is a member of a resistance-like gene family in tomato. *The Plant Cell* 16(6):1604–1615.
15. Roylawar P, Panda S, Kamble A (2015) Comparative analysis of baba and piriformospora indica mediated priming of defence-related genes in tomato against early blight. *Physiological and Molecular Plant Pathology* 91:88–95.
16. Xue DQ, et al. (2017) Transcriptome analysis of the cf-12-mediated resistance response to cladosporium fulvum in tomato. *Frontiers in plant science* 7:2012.
17. Thara VK, Tang X, Gu YQ, Martin GB, Zhou JM (1999) Pseudomonas syringae pv tomato induces the expression of tomato erebp-like genes pti4 and pti5 independent of ethylene, salicylate and jasmonate. *The Plant Journal* 20(4):475–483.
18. Zhang Y, Fan W, Kinkema M, Li X, Dong X (1999) Interaction of npr1 with basic leucine zipper protein transcription factors that bind sequences required for salicylic acid induction of the pr-1 gene. *Proceedings of the National Academy of Sciences* 96(11):6523–6528.
19. Pombo MA, et al. (2014) Transcriptomic analysis reveals tomato genes whose expression is induced specifically during effector-triggered immunity and identifies the epk1 protein kinase which is required for the host response to three bacterial effector proteins. *Genome biology* 15(10):1.
20. Du J, et al. (2015) Elicitin recognition confers enhanced resistance to <i>Phytophthora infestans</i> in potato. *Nat Plants* 1(4):15034.
21. Nguyen HP, et al. (2010) Methods to study pamp-triggered immunity using tomato and nicotiana benthamiana. *Molecular Plant-Microbe Interactions* 23(8):991–999.
22. Zhang Y, Xia R, Kuang H, Meyers BC (2016) The diversification of plant NBS-LRR defense genes directs the evolution of microRNAs that target them. *Molecular biology and evolution* 33(10):2692–2705.
23. De Vries S, Kloesges T, Rose LE (2015) Evolutionarily dynamic, but robust, targeting of resistance genes by the miR482/2118 gene family in the solanaceae. *Genome Biology and Evolution* 7(12):3307–3321.
24. Shivaprasad PV, et al. (2012) A microRNA superfamily regulates nucleotide binding site–leucine-rich repeats and other mRNAs. *The Plant Cell* 24(3):859–874.
25. Karlova R, et al. (2013) Identification of microRNA targets in tomato fruit development using high-throughput sequencing and degradome analysis. *Journal of experimental botany* 64(7):1863–1878.