

Journal of Experimental Botany, Global and local perturbation of the tomato microRNA pathway by a trans-activated DICER-LIKE 1 mutant

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Supplementary figures

Fig. S1 Sequence analysis of the tomato DCL1 homolog. (A) Scheme illustration of the gene, mRNA and protein structure of *SIDCL1*. In the gene, exons are represented by white boxes, introns and putative promoter and terminator sequences are shaded in black and dark grey, respectively. The location of the fragment used for silencing is indicated (*SIDCL1IR*). In the mRNA, 5' and 3' UTR sequences as determined by RACE are shaded in light grey and the predicted Watson-Crick pairing between sly-miR162 and target *SIDCL1* sequence is shown. Below, scheme of the predicted SIDCL1 protein structure compared to *Arabidopsis* (At) and rice (Os) DCL1s. The scheme shows in each protein the relative location of identified domains. (B) Accumulation of *SIDCL1* transcript in different tissues of tomato cv. Heinz based on published RNA-seq data from the tomato genome consortium 2012, Supplementary Table S1. Data are means of normalized expression of two independent biological samples. MG - mature green; Br - breaker.

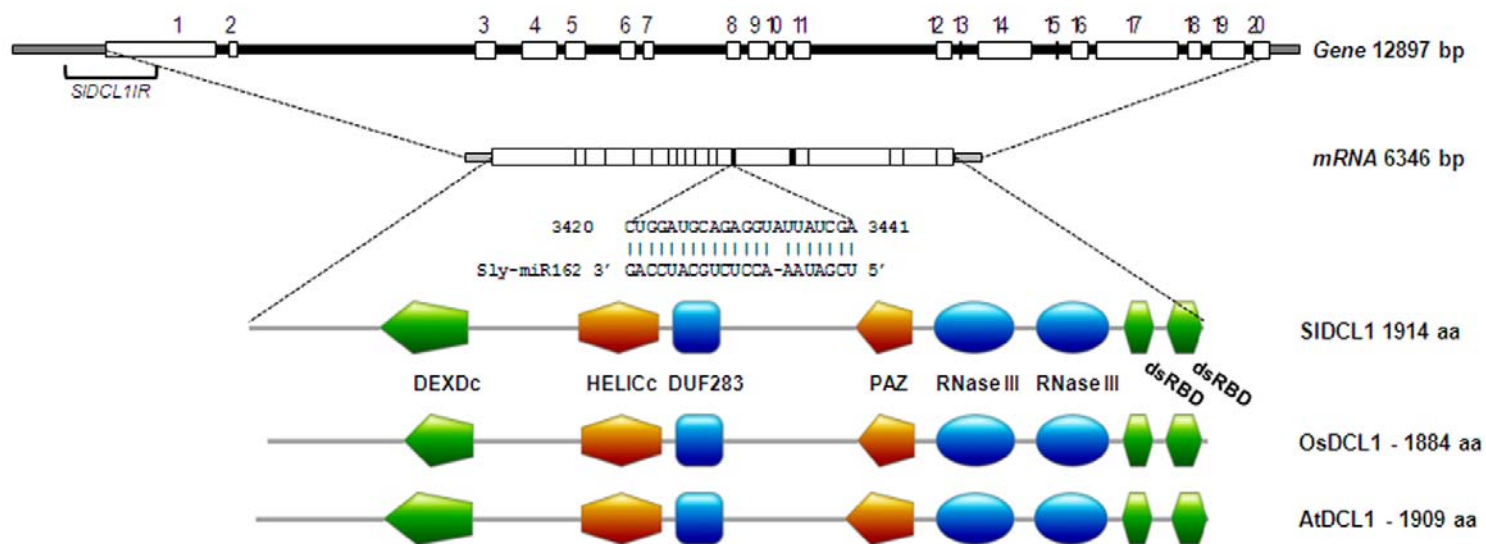
Fig. S2 Effect of *SIDCL1* silencing on *SIDCL2*-derived siRNAs accumulation. Schematic of *35S*>>*SIDCL1IR* and control siRNAs accumulation at indicated *SIDCL2*. The normalized abundances of siRNAs were plotted relative to their mRNA sequence as a function of the positions of their 5' ends. Values above and below zero indicate siRNAs

matching the plus and minus strand, respectively. The miR6026 cleavage site is indicated. Bar = 21 nts.

Fig. S3 Additional phenotypes of *FIL*>>*SIDCL1IR* plants. From top to bottom, control (*FIL:LhG4*) and *FIL*>>*SIDCL1IR* 7 DAS seedlings, the width of the pot is 7 cm; Representative 30 DAS cotyledons; Representative 42 DAS plants, white arrowheads mark the position of representative axillary meristems, which are dormant and active in *FIL:LhG4* and *FIL*>>*SIDCL1IR* plants, respectively, asterisks mark leaves that were cut to enable better visualization of the nodes. Inset shows representative cotyledonary axillary meristem outgrowth at a higher magnification. Scale bar = 1 cm.

Fig. S4 Quantitative analysis of *SIDCL1* levels in transactivated flowers. QRT-PCR analysis of *SIDCL1* expression levels in (A) Less than 1 mm *API*>>*SIDCL1IR* buds (B) isolated *AP3*>>*SIDCL1IR* petals from 10 mm buds. *TIP41* and *CAC* genes were used as reference genes in A and B, respectively. Error bars indicate \pm SD of three biological replicates, each measured in triplicate. Asterisks indicate significant difference as determined by Student's *t* test ($P \leq 0.01$).

A



B

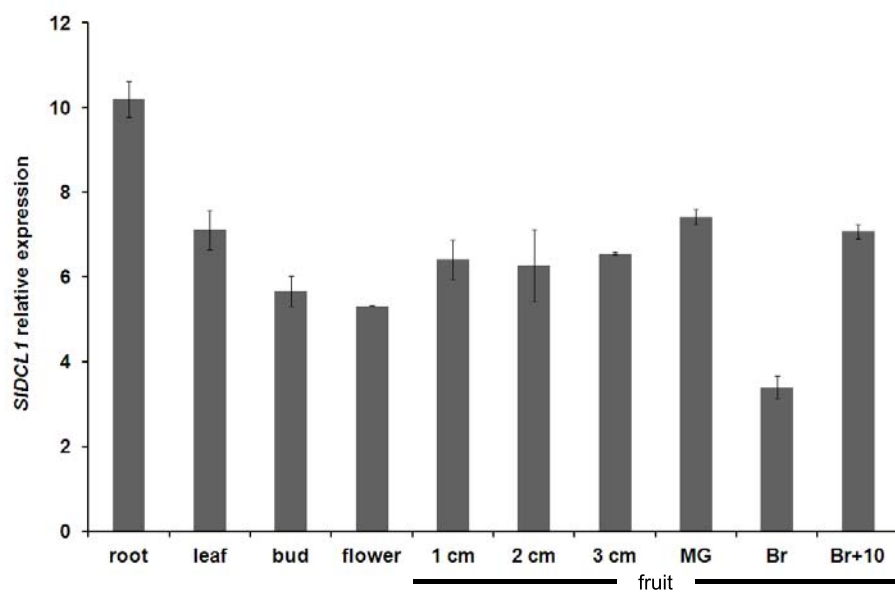


Figure S1

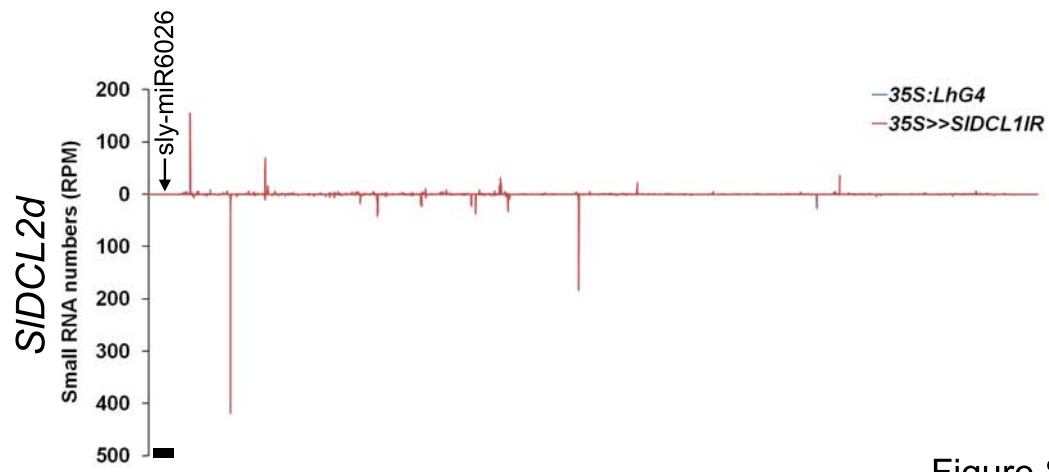
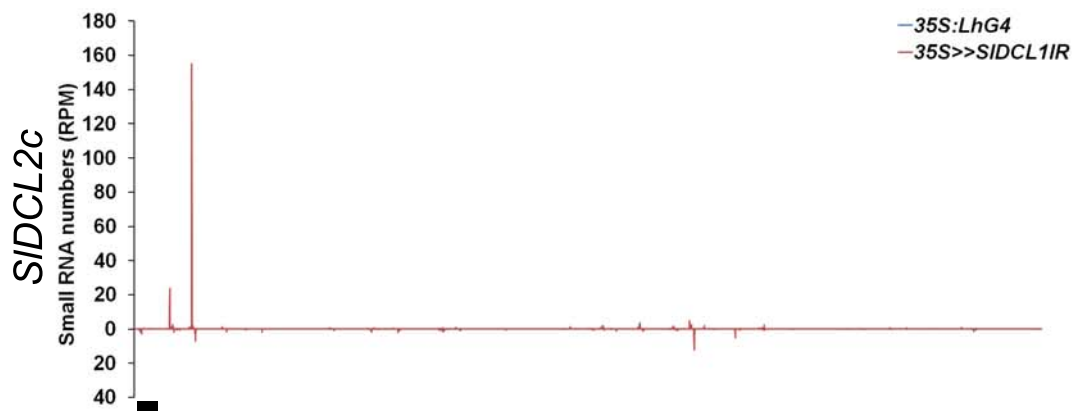
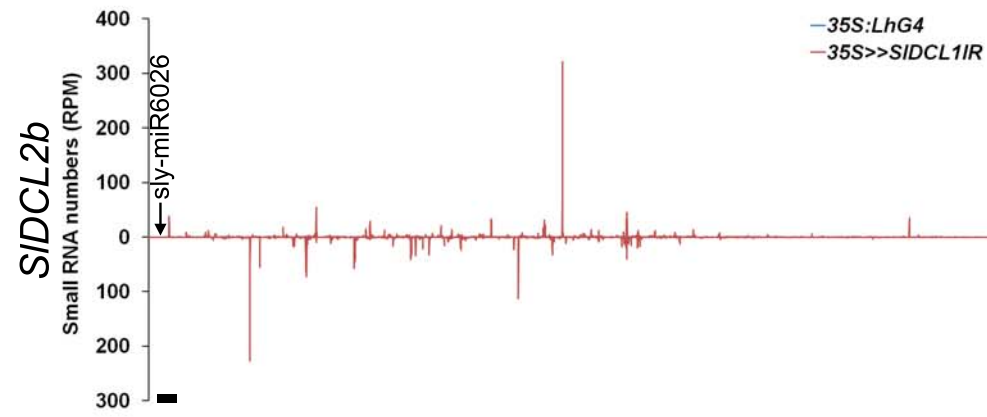


Figure S2

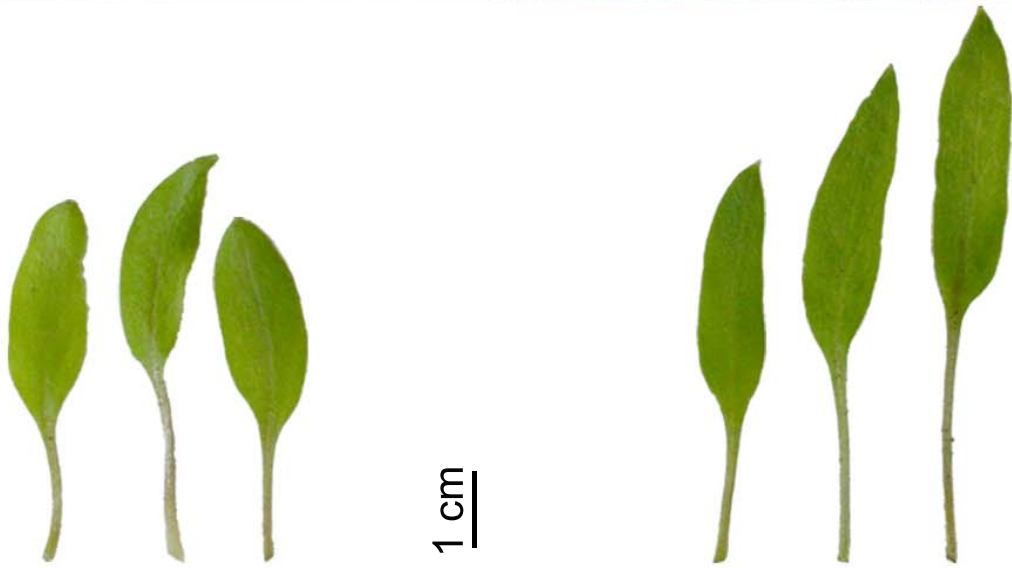
A

FIL:LhG4

FIL>>SIDCL1IR



B



C



Figure S3

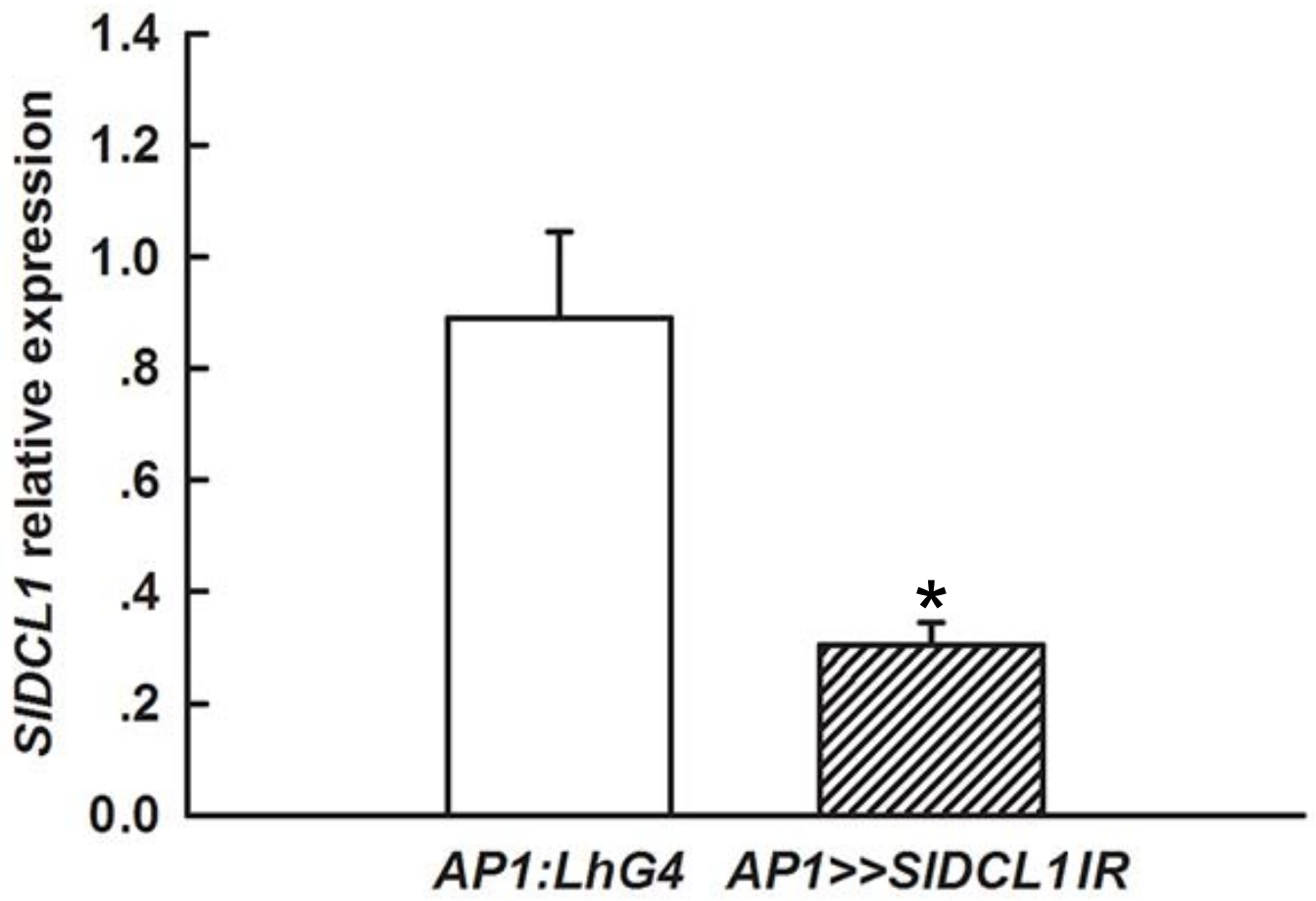
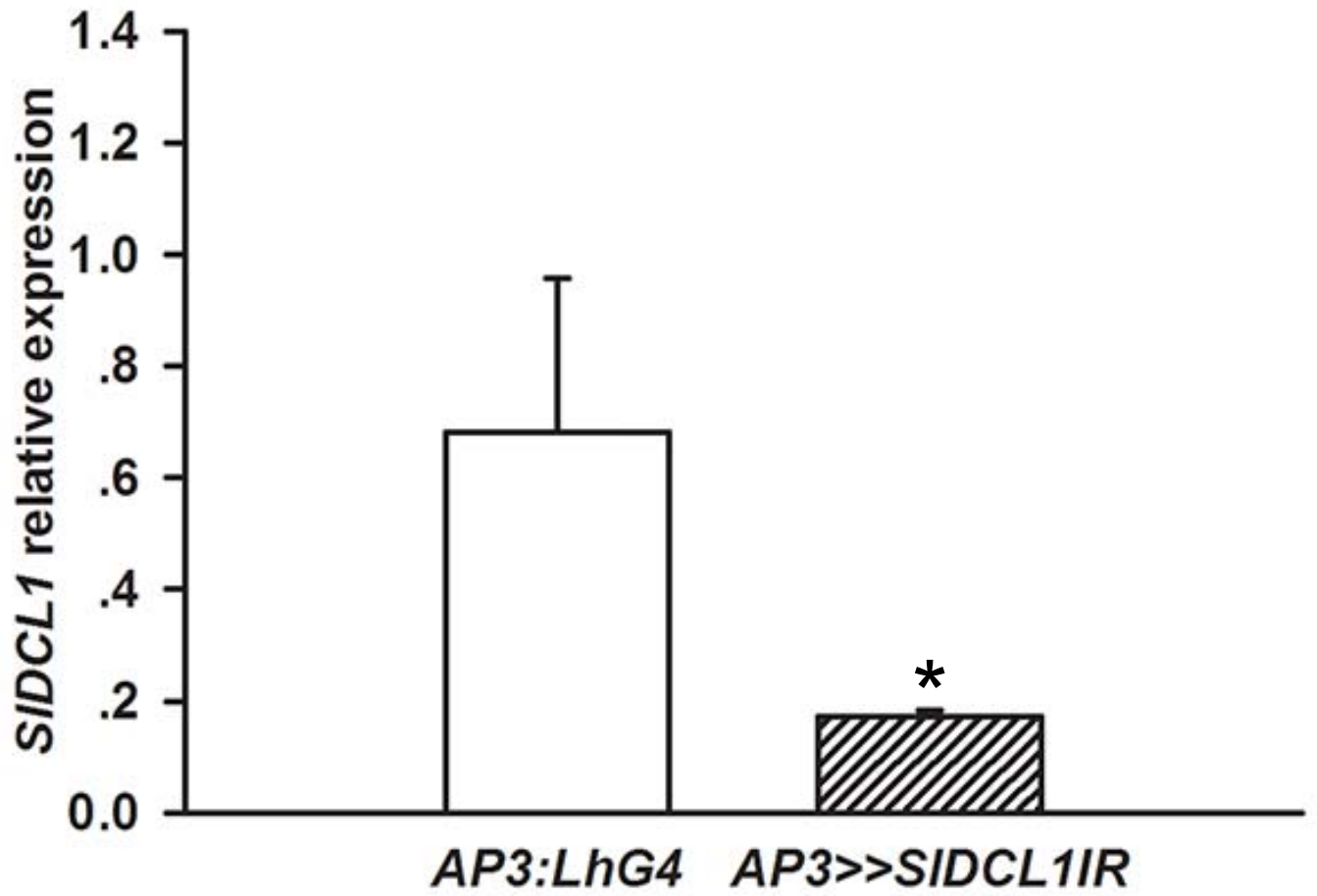
A**B**

Figure S4

Supplementary Table S1. Primers and probes used in this study

Primer ID	Primer sequence (5' - 3') ^{abc}	Remarks
GeneRacer-5'	CGACTGGAGCACGAGGACACTGA	5' and 3' RACE
GeneRacer-5'-nested	GGACACTGACATGGACTGAAGGAGTA	
GeneRacer-3'	GCTGTCAACGATACGCTACGTAACG	
GeneRacer-3'-nested	CGCTACGTAAACGGCATGACAGTG	
S1DCL1-5'-RACE	TTCAGCAGCGTCTGATAGAAAC	
S1DCL1-5'-RACE-nested	AAAGCCAAGGATTCCGCGAGCAGCTCTTCTA	
S1DCL1-3'-RACE	TGTGGCCCGTTGTCCTTGCAAATTC	
S1DCL1-3'-RACE-nested	TCAAGAGTCTCATCCGGACCCAATTGAT	
S1DCL2a_F_28	CTGCTGGCAAACATCCTCTT	
S1DCL2d_R_280	GTCGGTATGCAACATCAGAGC	
S1DCL2b-5'-RACE	GCATCATCAGAGCATCTCCTTGCTGAGTCACTA	
S1DCL2b-5'-RACE-nested	TGCAAATGGCAAAGGATCAGCAGAGACTA	
S1DCL1IRRNA_R	<u>CCC<u>AAGCTTGAATTC</u>CTCATAAGCATCAATCCAAT</u>	S1DCL1 RNAi construct
S1DCL1IRRNA_F	<u>ACGCGT<u>CGACTG</u>CAGCAGATATTAAGTCCGAAAGATCA</u>	
S1DCL1IRRNA_F	GGTTGCTAGGTCACAGGAGCA	
S1DCL1IRRNA_R	CAGCTTCAGTGGACTCTCTGGA	
S1DCL1_F_XhoI	<u>CCGCTCGAGGGTTGCTAGGTCACAGGAGCA</u>	
S1DCL1_F_898	GTGGTCTGTTGGTGAAGAGAT	S1DCL1 sequencing
S1DCL1_F_2481	AGTCAAGAAATGCGAACAGGTC	
S1DCL1_F_3227	GGATTTTGTTCGGAAAGGAT	
S1DCL1_F_4019	TGCAGCTCACTCTGGAAGA	
S1DCL1_R_1892	CCTCTAGCTCCAGCATCACA	
S1DCL1_R_2685	TATAATCTGATCCGGGCTTACG	
S1DCL1_R_6168	TTCAGCAGCGTCTGATAGAAAC	
pFlap_intron_F	AATTTCTTGTTCGGATCCTCATA	
OCS_rev	GAAACCGGGTAAGGATCT	Transgene identification
qRT-SGN-U592620_F	TTCAGGCCTCTGAACATATTGCT	<i>SIHAM</i>
qRT-SGN-U592620_R	CAACTGCAGAGCCTCCTTTGATA	
qRT-Solyc03g115850_F	CCACCATTGACAGATTTCATCG	
qRT-Solyc03g115850_R	GGTGAACGAAGTCGGAAGAG	NAC domain protein (<i>Solyc03g115850.2</i>)
qRT-S1ARF3_F	AACTACATTTCTCCCTTCCAG	Real time primers
qRT-S1ARF3_R	TCACAACAACACCTGCTAC	
qRT-S1ARF4_F	CGAAAGAACCATCTACTCC	Real time primers
qRT-S1ARF4_R	AAAGCCTCTCCAACCTCAAC	
qRT-S1ARF10_F	CAGGTCCAGCAGTCCCTTTCT	Real time primers
qRT-S1ARF10_R	CGCTGGAACCTTGGTGGTAA	
qRT-TIP41_F	ATGGAGTTTGTGAGTCTCTGTC	Real time primers
qRT-TIP41_R	GCTGCGTTTCTGGCTTAGG	
qRT-S1DCL1_F	TCGAAGGACCCATCTTAACTG	Real time primers
qRT-S1DCL1_R	CTATTGGCCCTCTGAAGACAAG	
qRT-S1DCL2a_F	CGGCTTCCGAAGAAGGTA	Real time primers
qRT-S1DCL2a_R	TTCAGCACTAGTCAAGAA	
qRT-S1DCL2b_F	TGCAGGACATATTTCTGTGGTC	Real time primers
qRT-S1DCL2b_R	TGAACATCCAAGCCCTCTTC	
qRT-S1DCL2d_F	GCTCAACTCTAAATTTGGATTCTCTG	Real time primers
qRT-S1DCL2d_R	AACTCTTTGAGTGTCTTCAGTGT	
qRT-S1DCL3_F	TTGCCACTGATGTGGTTGAG	Real time primers
qRT-S1DCL3_R	TCCCTCTGCTCTTGTGTTCC	
qRT-S1DCL4_F	AGTGAAGCTCAGATGGATGATG	Real time primers
qRT-S1DCL4_R	GCAGAAATACTCGTCTGTGTTG	
qRT-S1CAC F	CCTCCGTTGTGATGATGTAACCTGG	Real time primers
qRT-S1CAC R	ATTGGTGGAAAGTAACATCAT	
sly-miR160a_rc	TGGCATACAGGGAGCCAGGCA	sly-miR160a probe
sly-miR164_rc	TGCACGTGCCCTGCTTCTCCA	sly-miR164 probe
sly-miR171_rc	GATATTGGCAGGCTCAATCA	sly-miR171 probe
sRNA_id_1941824_rc	AGAGACACACTTATACTATACTAA	<i>TAPIR</i> probe
sly-miR390_rc	GGTGTATCCCTCTGAGCTT	sly-miR390 probe
sRNA_ID1897365_rc	AGTCTTTGCTCTAAGGCCACA	TAS5 probe
sRNA_ID477247_rc	GGCCCAAAGACAACATGAAGT	TAS4 probe

^aSequences corresponding to restriction enzyme sites are underlined.

Supplementary Table S2. Summary of small RNA profiling

	<i>35S:LhG4-1</i>	<i>35S:LhG4-2</i>	<i>35S>>SIDCL1IR-1</i>	<i>35S>>SIDCL1IR-2</i>
Raw Reads ^a	4,975,066	5,619,906	3,907,268	4,519,122
Genome-matched reads	3,487,457	4,008,933	2,730,156	3,223,011
Ratio ^b	70	71.3	70	71.3
t/r/sn/snoRNA	1,047,049	1,143,490	839,552	1,115,895
Ratio ^c	30.0	28.5	30.8	34.6
Available small RNA reads ^d	2,440,408	2,865,443	1,890,604	2,107,116
Unique sRNA sequences	899,019	1,139,600	854,896	923,666
Total hits on genome ^e	9,503,296	11,811,550	9,090,523	9,438,606

^aTotal acquired sequencing data from Illumina Hiseq 200 analyzer (18-26 nucleotide), 5' and 3' adaptors have been trimmed.

^bThe ratio represents the percentage of genome-matched reads to the raw reads.

^c The ratio represents the percentage of t/r/sn/snoRNAs reads to the genome-matched reads.

^dThe reads excluding the t/r/sn/snoRNAs and unmapped reads.

^e Sum of the hits of all the available unique small RNA sequence on related tomato genome.

Supplementary Table S3. Summary of small RNA annotations

	<i>35S:LhG4-1</i>	<i>35S:LhG4-2</i>	<i>35S>>SIDCLIIR-1</i>	<i>35S>>SIDCLIIR-2</i>
Available small RNA reads ^a	2,440,408	2,865,443	1,890,604	2,107,116
RNAi loci associated	175	17	6,601	7,136
Unannotated	1,574,705	1,864,450	1,363,737	1,466,600
cDNA associated	287,865	326,764	237,490	291,913
miRNA associated	252,851	313,790	72,018	101,023
Pre-miRNA associated	173,586	182,012	76,723	95,713
Repeat associated	151,226	178,410	134,035	144,731

^a The reads excluding the t/r/sn/snoRNAs and unmapped reads (clean).