Supporting Information

Li et al. 10.1073/pnas.1118282109

SI Materials and Methods

Transgenic tobacco lines. The TG34::nta-amiR:RDR6 (Fig. S3), *N-CFP*^{T2T1} and *N-CFP*^{t2t1} tobacco transgenic lines were generated in tobacco cv. SR1 by *Agrobacterium*-mediated leaf disc transformation. Parental tobacco lines of TG34::nta-RNAi:DCL2, DCL4 were described previously (1).

RNA isolation, sRNA northern blot analysis. Total RNA was extracted from leaf tissue using the TRIzol reagent (Invitrogen) according to the manufacture's protocol. Northern blot hybridization analysis and sRNA quantification were performed as described (1).

1. Kuang H, et al. (2009) Identification of miniature inverted-repeat transposable elements (MITEs) and biogenesis of their siRNAs in the Solanaceae: new functional implications for MITEs. *Genome Res* 19(1):42–56.

miRNA cleavage assays. Cleavage assays were conducted using the GeneRacer kit (Invitrogen) as described in the product manual.

Small RNA libraries and sequencing. Small RNA libraries were made according the Illumina sRNA-seq protocol and sequenced using Illumina GA at the V.J. Coates Genomic Sequencing Laboratory, UC Berkeley.

Bioinformatics. Bioinformatic tools linked to Solanaceae sequence databases are available at the webserver *Solanaceae* miRNA/tasiRNA Analysis Resources and Tools (SoMART) (http://somart.ist.berkeley.edu/).

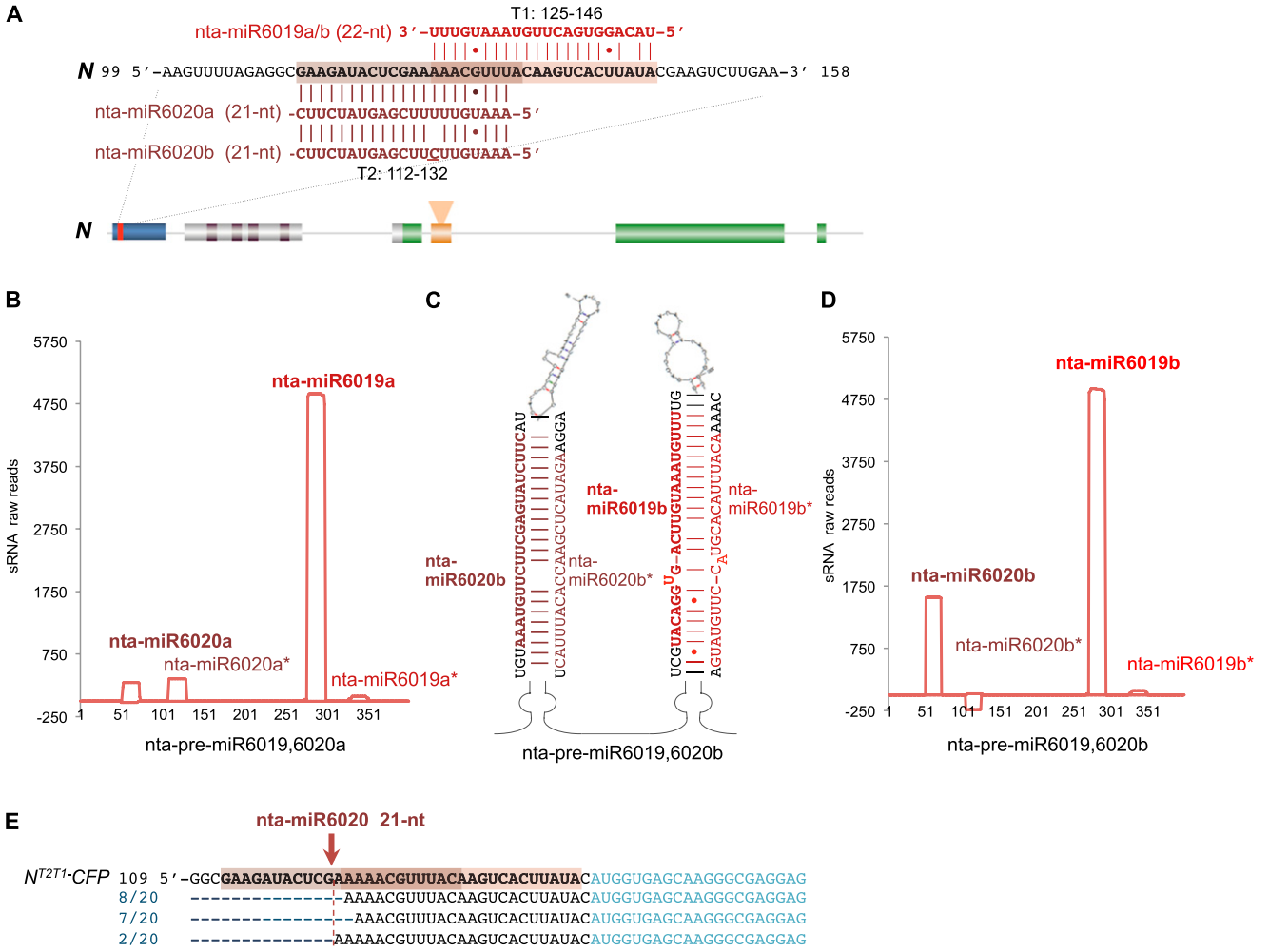

Fig. S1. nta-miR6019b, nta-miR6020b, nta-premiR6019,6020b secondary structure, sRNA mapping, and cleavage product sequences of *N-CFP* sensors. (*A*) The sequences of 22-nt nta-miR6019a/b, 21-nt nta-miR6020a, and nta-miR6020b are indicated in red and dark red, respectively. The nta-miR6019a/b and nta-miR6020a/b target sequences in *N*, T1 (125–136 bp) and T2 (112–132 bp) respectively, are indicated in bold black and are shaded. The *N* gene (GenBank U15605) map is described in Fig. 1. (*B*) sRNA number (raw reads, *y* axis) and distribution on the predicted nta-premiR6019,6020a precursor (*x* axis; Table S2) with nta-miR6019a, nta-miR6019a* and nta-miR6020a, nta-miR6020b* indicated. (*C*) The predicted fold-back structure of nta-premiR6019,6020b with nta-miR6019b and nta-miR6019b* (red) and nta-miR6020a and nta-miR6020a* (dark red) indicated. (*D*) sRNA number (raw reads, *y* axis) and distribution on the predicted nta-premiR6019,6020b precursor (*x* axis; Table S2) with nta-miR6019b, nta-miR6019b* and nta-miR6020b, nta-miR6020b* indicated. (*E*) nta-miR6020 cleavage (dark red arrow) of *N-CFP*. Sequence of *N-CFP* with nta-miR6019 and nta-miR6020 target sequences are indicated by bold black and shaded, aligned with *N-CFP* cleavage product sequences, indicated by black.

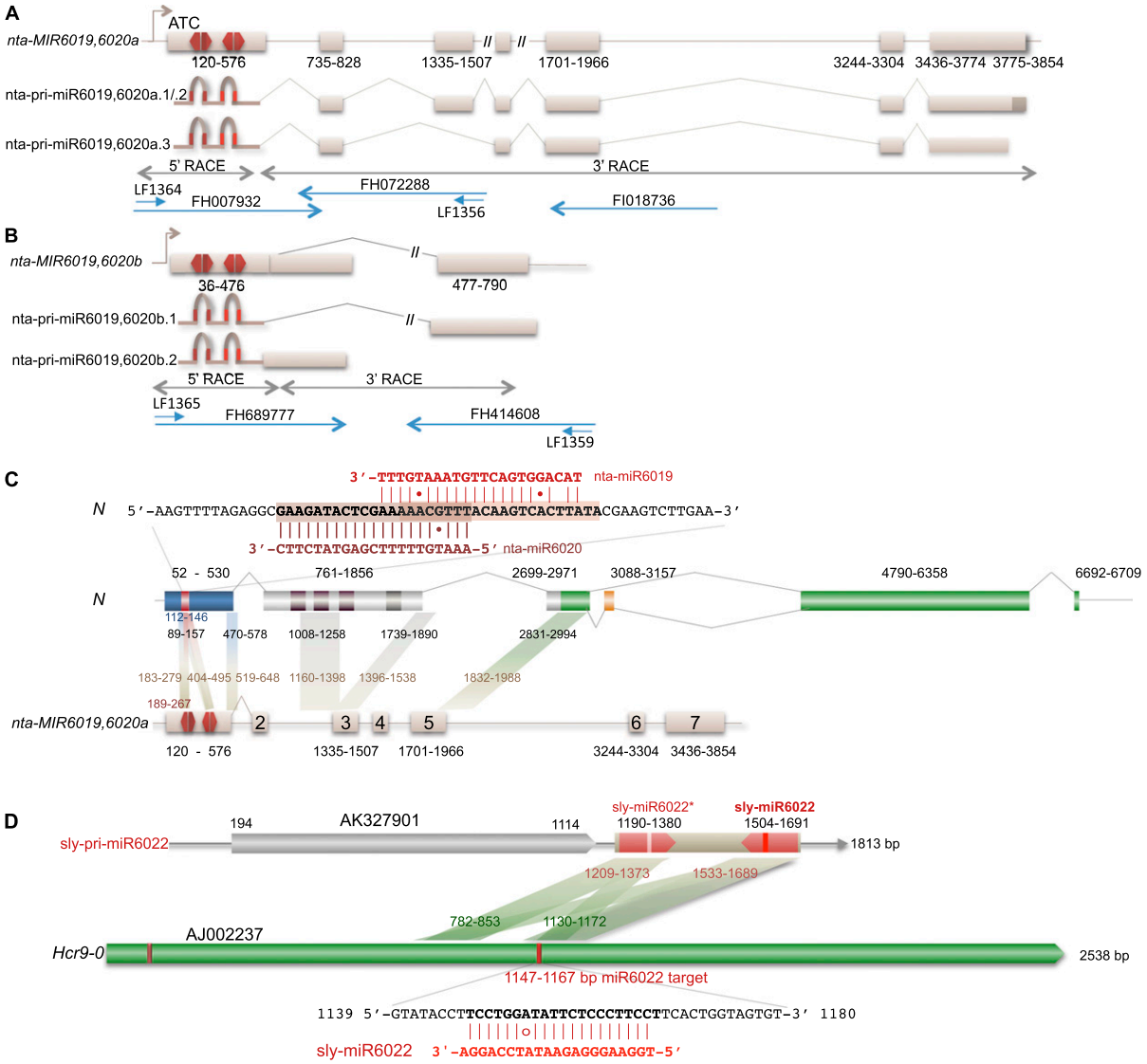

Fig. S2. (*A*) Maps of *nta-MIR6019,6020a* precursor locus and nta-pri-miR6019,6020a transcripts. nta-pri-miR6019,6020a sequences were determined by sequencing 5′ and 3′ RACE products of tobacco RNA. Primers used for 5′ and 3′ RACE are in Table S1. The progenitor *nta-MIR6019,6020a* sequence was determined by sequencing gaps in genomic DNA identified by alignment of 5′ and 3′ RACE products with available tobacco genome sequence. Sequences of *nta-MIR6019,6020a* and nta-pri-miR6019,6020a.1/0.2 are provided in Dataset S1. (*B*) Maps of *nta-MIR6019,6020b* and nta-pri-miR6019,6020b.1/0.2. Sequences determined as described in Fig. S2*A* and available in Dataset S1. (*C*) *N* and *nta-MIR6019,6020a* sequence similarity. (*Top*) The sequences of 22-nt nta-miR6019a and 21-nt nta-miR6020a and target sequences in *N* are indicated as described in Fig. 1. (*Middle*) Map of the *N*-gene is shown as described in Fig. S1 with coordinates indicated in black above exons. (*Bottom*) Map of *nta-MIR6019,6020a* as described in Fig. S2*A* with coordinates indicated in black below exons. Shading between *N* and *nta-MIR6019,6020a* maps indicates regions of sequence similarity between *N* and *nta-MIR6019,6020a*. The coordinates of sequences with similarity in *N* are shown in blue below *N* exons, and homologous sequences in *nta-MIR6019,6020a* are indicated in tan above the *nta-MIRR6019,6020a* exons. (*D*) *Hcr9-0* and sly-pri-miR6022 sequence similarity. (*Top*) Map sly-pri-miR6022 as described in Fig. S5 with coordinates indicated in black above ORF and sly-premiR6022. (*Middle*) Shading between sly-pri-miR6022 and *Hcr9-0* maps indicates regions of sequence similarity between sly-pri-miR6022 and *Hcr9-0*. The coordinates of sly-pri-miR6022 sequences with similarity to *Hcr9-0* are shown in red below inverted repeats of sly-premiR6022 (shaded red) and homologous *Hcr9-0* sequences are indicated in green above *Hcr9-0*. (*Bottom*) The target sequence of sly-miR6022 (red) in *Hcr9-0* (black) is indicated as described in Fig. S5.

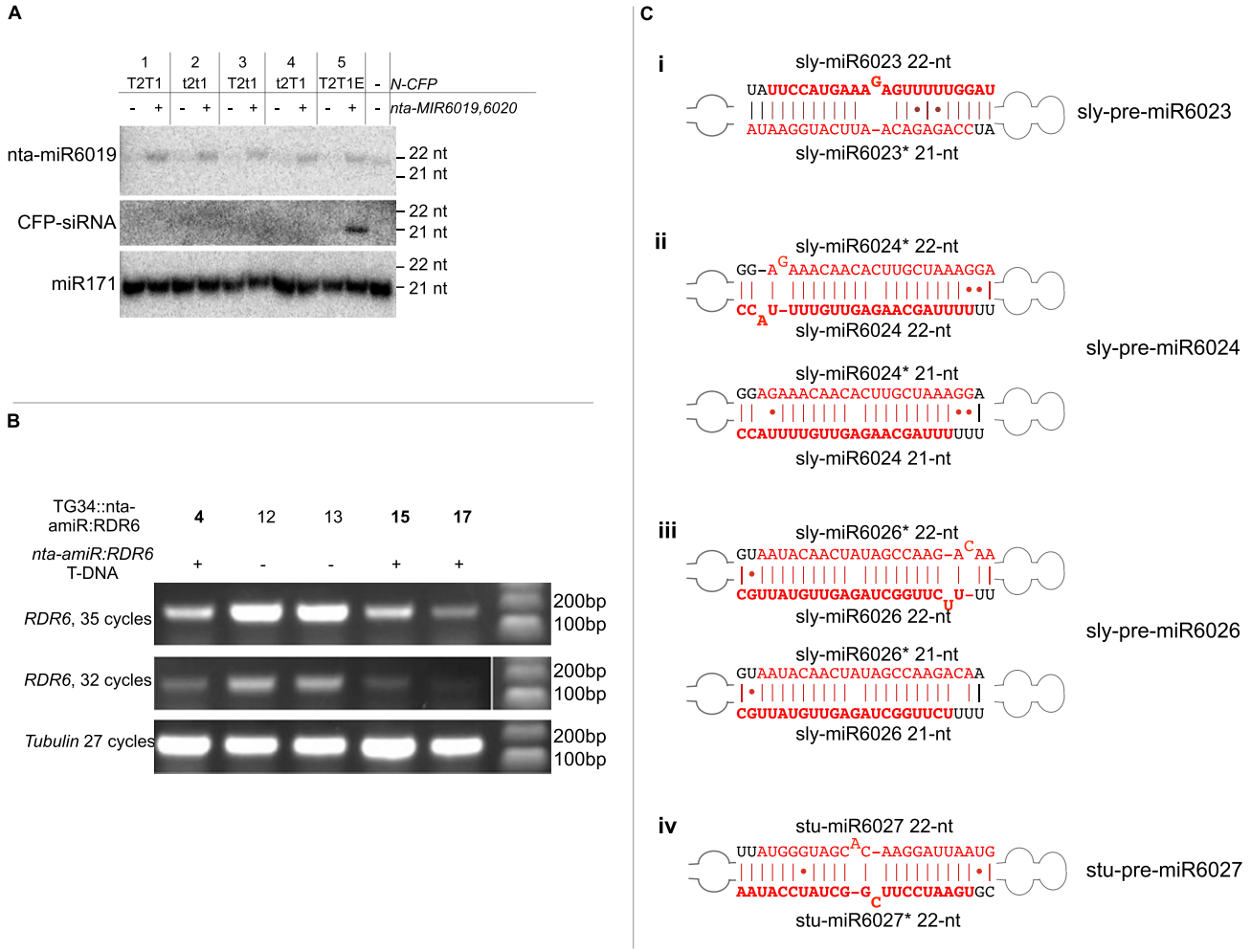

Fig. S3. (*A*) Northern blot hybridization analyses of sRNA isolated from *N. benthamiana* coinfiltrated with *N-CFP* sensors and *35S::nta-MIR6019,6020. N-CFP* sensors in each sample is indicated in lanes 1–5. Samples coinfiltrated with *35S::nta-MIR6019,6020* are indicated by "+". Hybridization probes are indicated on left of each image. (*B*) Reduced expression of *RDR6* in TG34::nta-amiR:RDR6 transgenic lines. TG34::nta-amiR:RDR6 positive plants were produced from a cross between SR1::nta-amiR:RDR6 and TG34. Positive (+) F_1 progeny (samples, 4, 15, and 17) were identified by using *nta-amiR:RDR6* T-DNA–specific primers (Table S1). Semiquantitative RT-PCR was performed by using RNA extracted from F_1 progeny to quantify *RDR6* expression using primers DP193 and DP194 (Table S1), and a *Tubulin* reference (primers LF1051 and LF1052) as an internal control. Two control plants (samples 12 and 13) negative for T-DNA (-) were also included as controls. *RDR6* expression is reduced in all three plants carrying the *nta-amiR:RDR6* T-DNA (4, 15, 17) compared levels in control plants (12, 13). Transgenic *nta-amiR:RDR6* tobacco lines were generated in tobacco cv. SR1 by *Agrobacterium*-mediated leaf disk transformation. (*C*) Predicted premiRNA secondary structures. (*i*) Secondary structure of sly-premiR6023 with sly-miR6023 and sly-miRNA* sequences indicated. (*ii*) Two alternative secondary structures for sly-pre-miR6024 with 21- or 22-nt sly-miR6024 and sly-miR6024* indicated. (*iii*) Two alternative secondary structures for sly-premiR6026 with 21- or 22-nt sly-miR6026 and sly-miR6026* indicated. (*iv*) Secondary structure of stu-premiR6027 with 22-nt stu-miR6027 and stu-miR6027* indicated. The indicated miRNA and miRNA* sequences were detected by using the bioinformatic pipeline (SoMART, http://somart.ist.berkeley.edu).

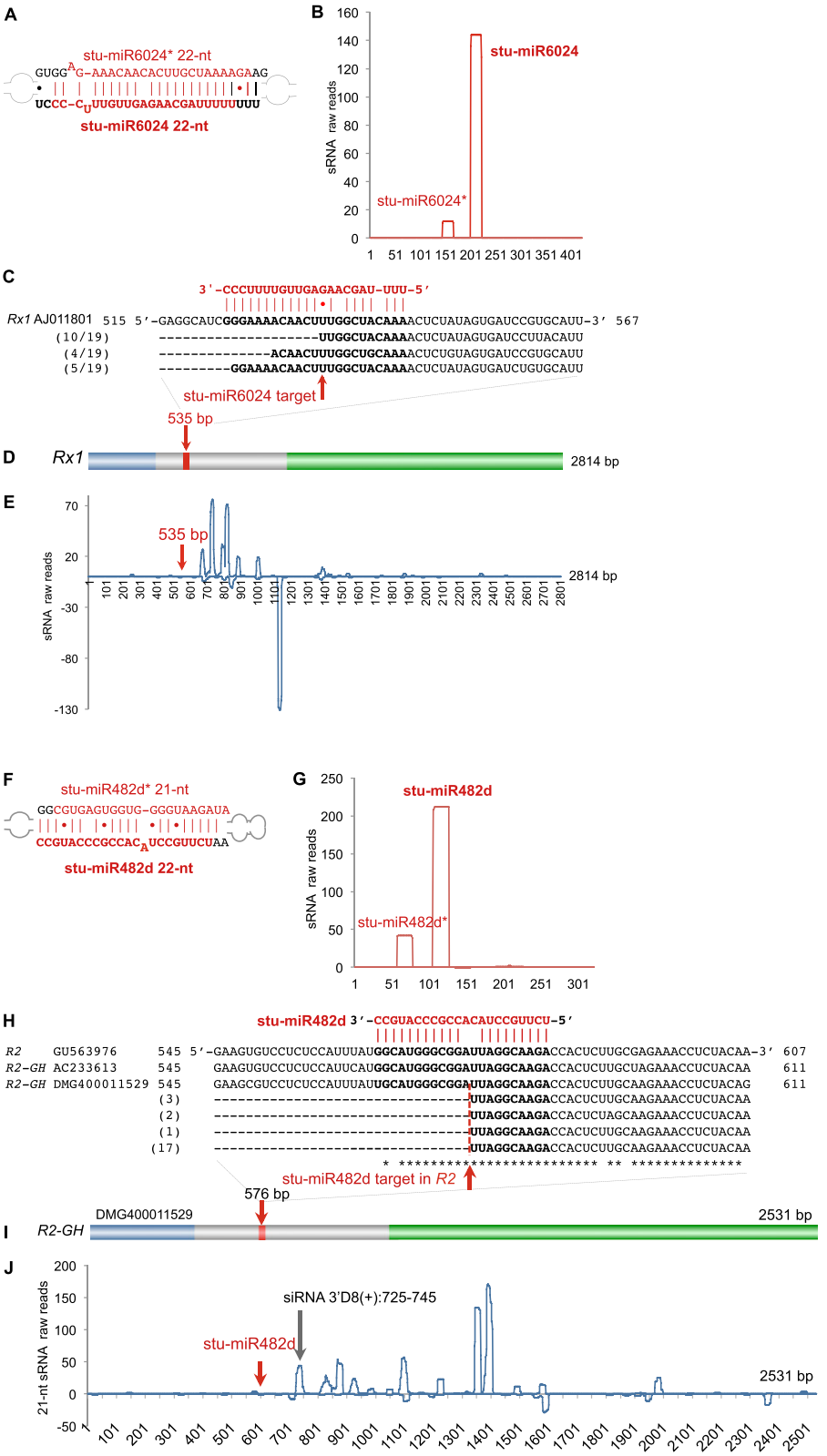

Fig. S4. Predicted prestu-miRNAs secondary structures, sRNA maps, *R-gene* cleavage, and secondary siRNAs of stu-miR6024 and stu-miR482d. (*A*) The secondary structure of the stu-premiR6024 with stu-miR6024 and stu-miR6024* sequences indicated. (*B*) sRNA number (raw reads, *y* axis) and distribution on stu-premiR6024 with locations of mapped stu-miR6024 and stu-miR6024* indicated (*x* axis; Table S2). (*C*) Alignment of 19 cleavage product sequences with *Rx1* sequences. Red arrow indicates the predicted stu-miR6024 cleavage site. (*D*) Map of *Rx1* with CC, NBS, and LRR domain coding regions shown in blue, gray, and green, respectively. Red arrow indicates the predicted stu-miR6024 cleavage site. (*E*) Number (raw reads, *y* axis) and distribution of *Rx1* 21-nt secondary siRNAs indicated by blue line on *Rx1* map (*x* axis). (*F*) The secondary structure of stu-premiR482d with stu-miR482d and stu-miR482d* sequences indicated. (*G*) sRNA number (raw reads, *y* axis) and distribution on stu-premiR482d (*x* axis; Table S2) with locations stu-miR482d and stu-miR482d* indicated. (*H*) Alignment of

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23-cleavage product sequences with late blight *R2* gene of *Solanum schenckii* (GU563976) and *Solanum tuberosum* (AC233613) and *Solanum phureja* (DMG400011529) *R2*-gene homologs (*R2-GH*). The red arrow indicates the predicted stu-miR482d cleavage site. (*I*) Map of *R2* homolog DMG400011529 with CC, NBS, and LRR domains shown in blue, gray, and green, respectively. The miR482d-targeted region is indicated by the red line. (*J*) Number (raw reads, *y* axis) and distribution of 21-nt secondary siRNAs on *R2-GH* (DMG400011529) indicated by blue line. Location of secondary siRNA 3′D8(+) (725-745) in-phase with stu-miR482d cleavage is indicated (gray arrow).

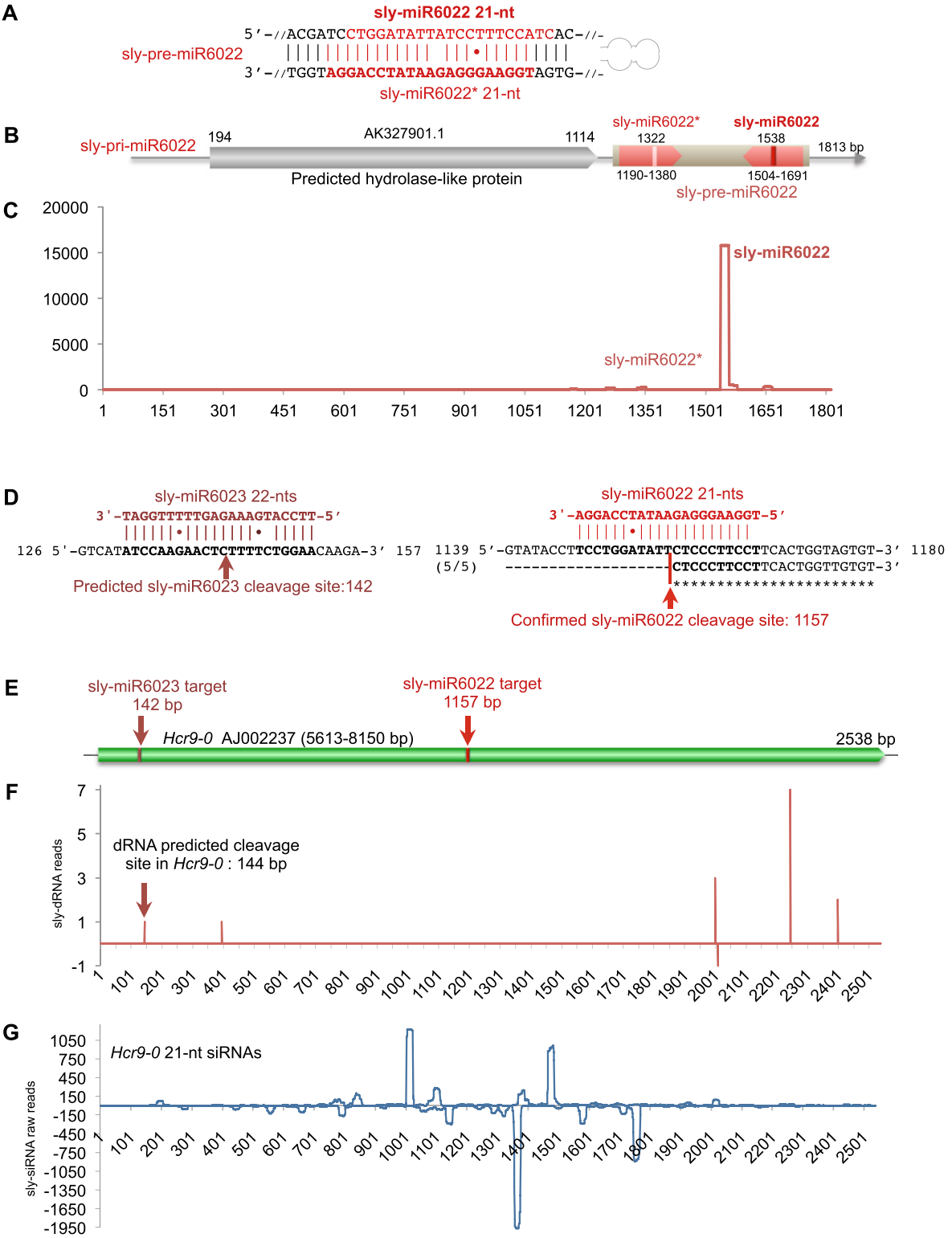

Fig. S5. Tomato *Cf9* homolog, *Hcr9-0,* is cleaved by sly-miR6022 and sly-miR6023 and produces secondary 21-nt siRNAs. (*A*) The secondary structure of the sly-premiR6022 with sly-miR6022 and sly-miR6022* sequences indicated. (*B*) Map of the expressed sly-pri-miR6022 gene (full-length tomato cDNA AK327901) with coordinates of a predicted hydrolase-like protein ORF and the sly-premiR6022 region located in the 3′ UTR indicated. (*C*) Number (raw reads, *y* axis) and distribution of sRNAs on sly-pri-miR6022 (AK327901, *x* axis) with the locations of mapped sly-miR6022 and sly-miR6022* indicated. (*D*) Sequences of the predicted cleavage site of sly-miR6023 (*Left*) and confirmed cleavage site of sly-miR6022 (*Right*) on tomato *Cf9* homolog, *Hcr9-0,* (coordinates 1–2538 in figure correspond to coordinates 5613–8150 of AJ002237). (*E*) Map of *Hcr9-0* with miRNA target sites indicated. (*F*) The number and distribution of tomato degradome RNAs (dRNA library D51, SoMART at http://somart.ist.berkeley.edu) mapped to tomato *Hcr9-0*. The 5′ terminus of a dRNA mapped to position 144 bp (indicated by dark red arrow) suggests cleavage at the predicted sly-miRNA6023 target site. The *y* axis indicates the number of dRNA raw reads in the sly-dRNA library mapped to *Hcr9-0* with 90% identity at the indicated positions (*x* axis). (*G*) The number of 21-nt siRNAs (raw reads SLY1 library, http://somart.ist.berkeley.edu, *y* axis) mapped to *Hcr9-0* (coordinates, *x* axis) indicated by the blue line.

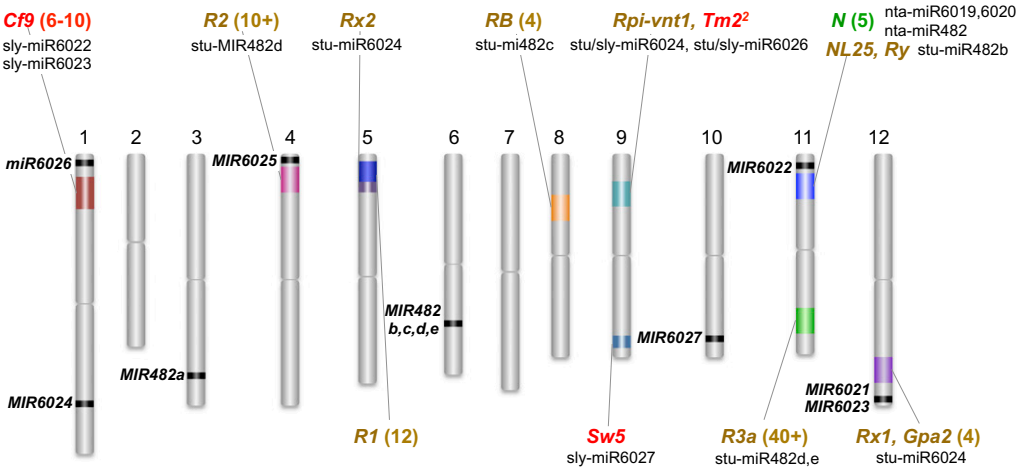

Fig. S6. Summary of Solanaceae *R*-genes and miRNAs. The approximate locations of syntenic *R*-gene regions in potato, tomato, and tobacco genomes are indicated in shaded rectangles with *R*-gene loci names for each indicated (not to scale). The locations of miRNA progenitor loci (*MIR*) are indicated by black horizontal bars, and their names are indicated in black bold. The location(s) of *nta-MIR6019,6020a/b* have not been determined. The miRNAs targeting the different *R*-genes are shown in black below or to the right of the *R*-gene names. *R* genes in different *Solanaceae* are color-coded: tobacco, green; potato, brown; tomato, red.

Other Supporting Information Files

Table S1 (DOCX)
Table S2 (DOCX)
Dataset S1 (DOCX)
Dataset S2 (DOCX)