

**A**

sRNA-seq data

PhaseTank prediction

All phasiRNA loci

Flower  
15°C-sensitve  
27°C-sensitve

Leaf  
15°C-sensitve  
27°C-sensitve

Root  
15°C-sensitve  
27°C-sensitve

Seedling  
15°C-sensitve  
27°C-sensitve

Temperature-sensitive screening

Organ-specific screening

Filtered loci

Flower-specific  
Leaf-specific  
Root-specific

Flower\_15°C\_1  
Flower\_15°C\_2  
Flower\_27°C\_1  
Flower\_27°C\_2

Leaf\_15°C\_1  
Leaf\_15°C\_2  
Leaf\_27°C\_1  
Leaf\_27°C\_2

Root\_15°C\_1  
Root\_15°C\_2  
Root\_27°C\_1  
Root\_27°C\_2

Seedling\_15°C\_1  
Seedling\_15°C\_2  
Seedling\_27°C\_1  
Seedling\_27°C\_2

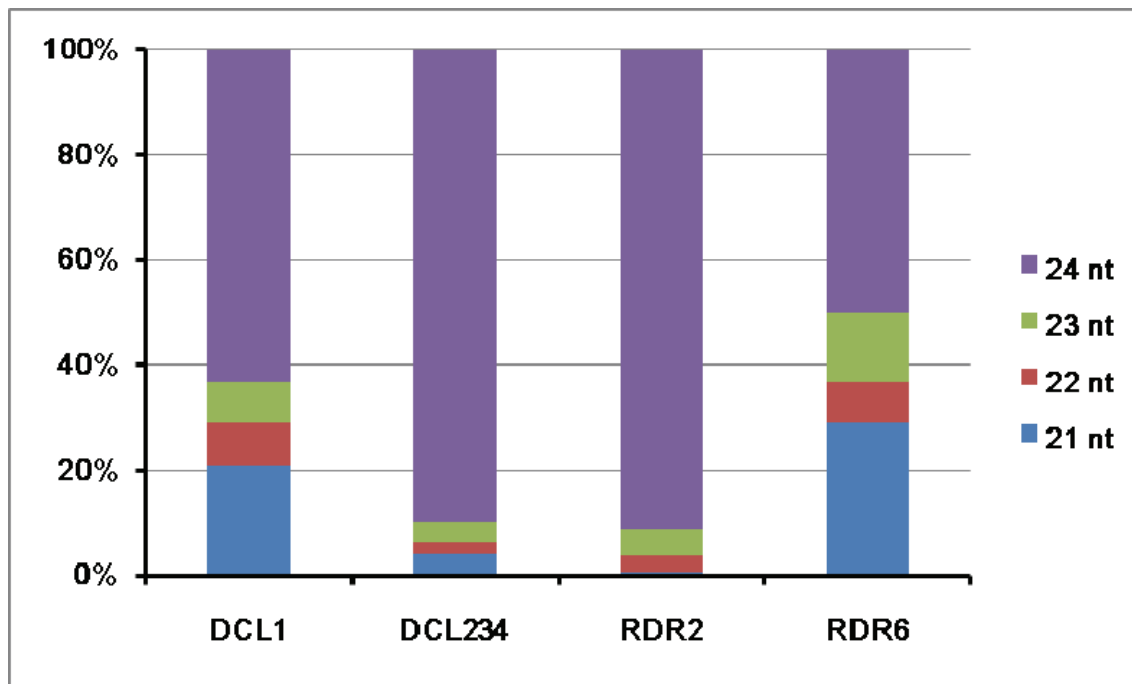
Flower\_27°C\_1  
Flower\_27°C\_2  
Leaf\_27°C\_1  
Leaf\_27°C\_2  
Root\_27°C\_1  
Root\_27°C\_2

15°C

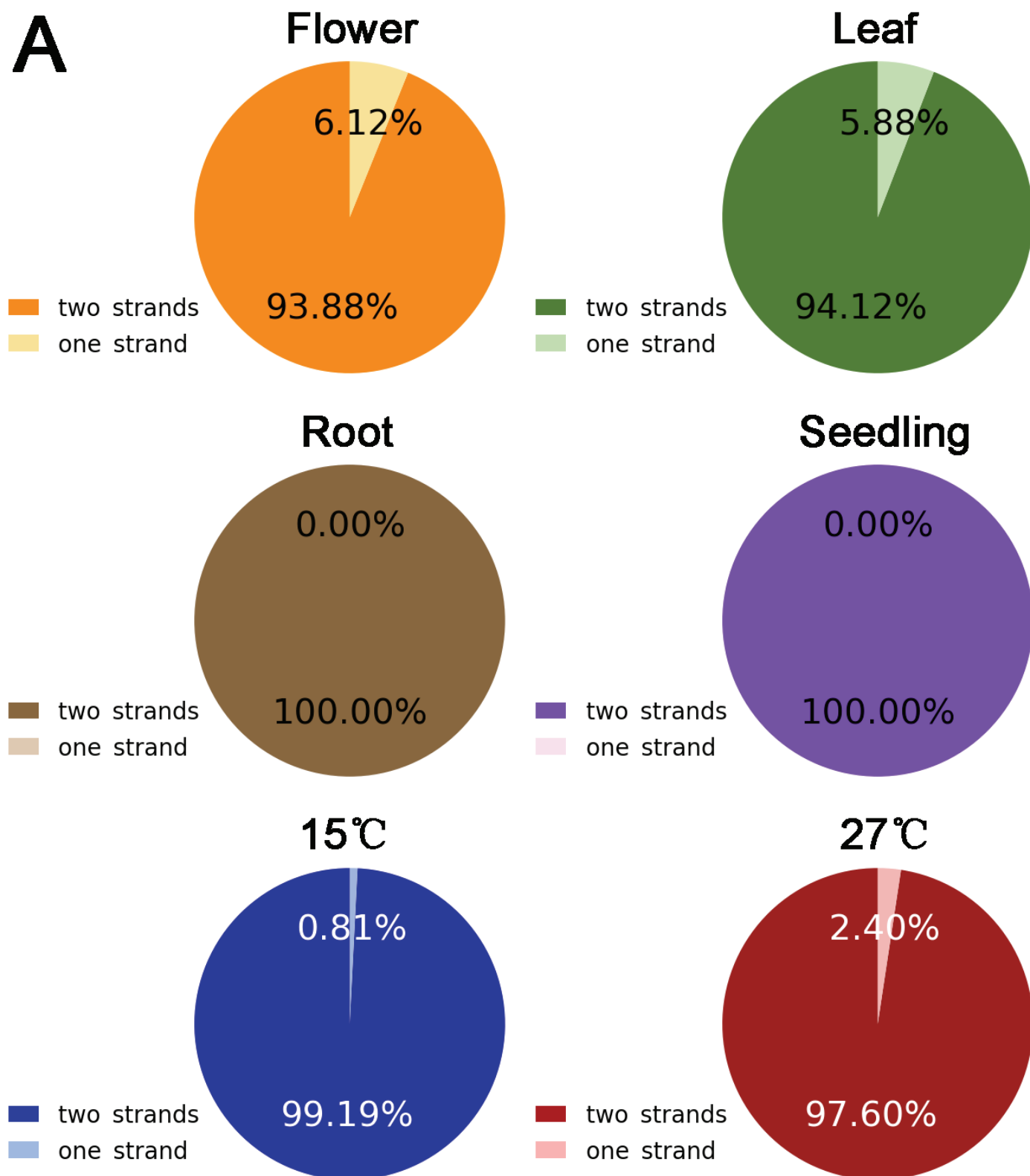
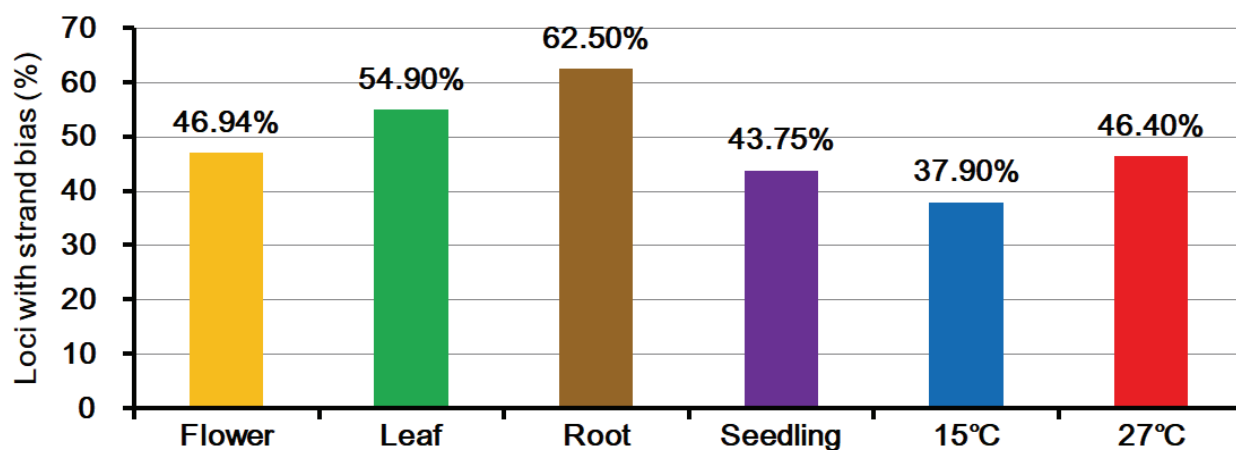
27°C

[illegible]

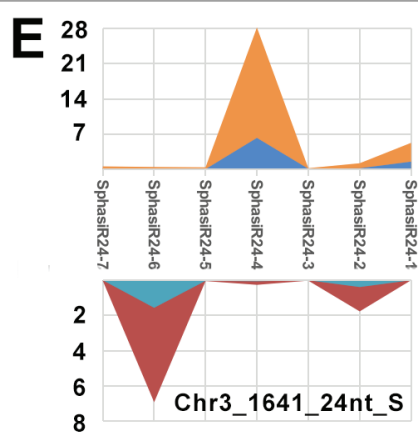
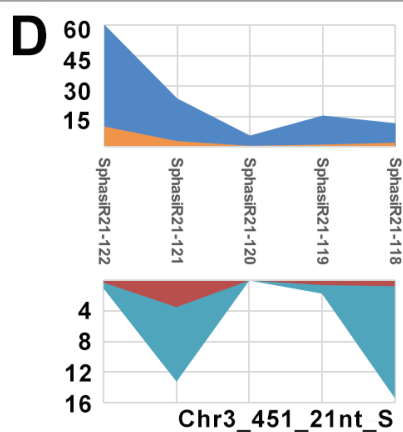
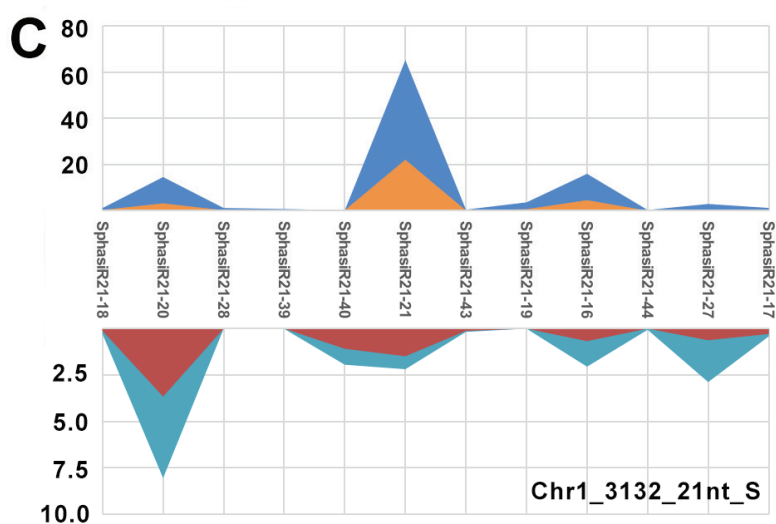
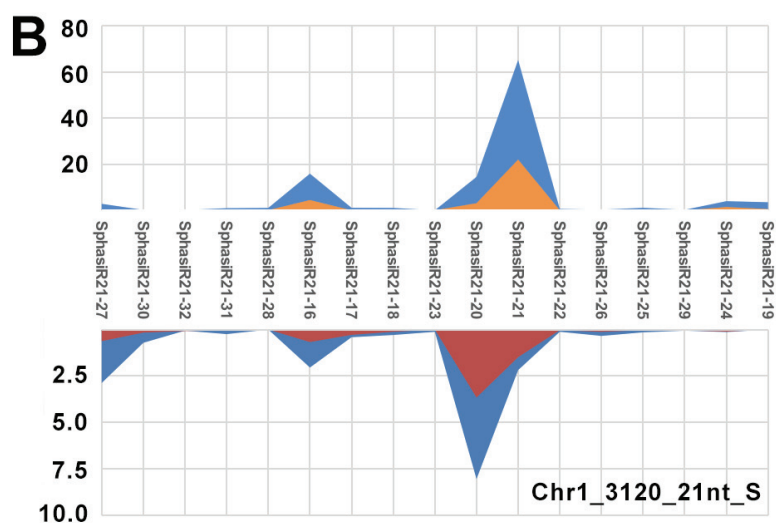
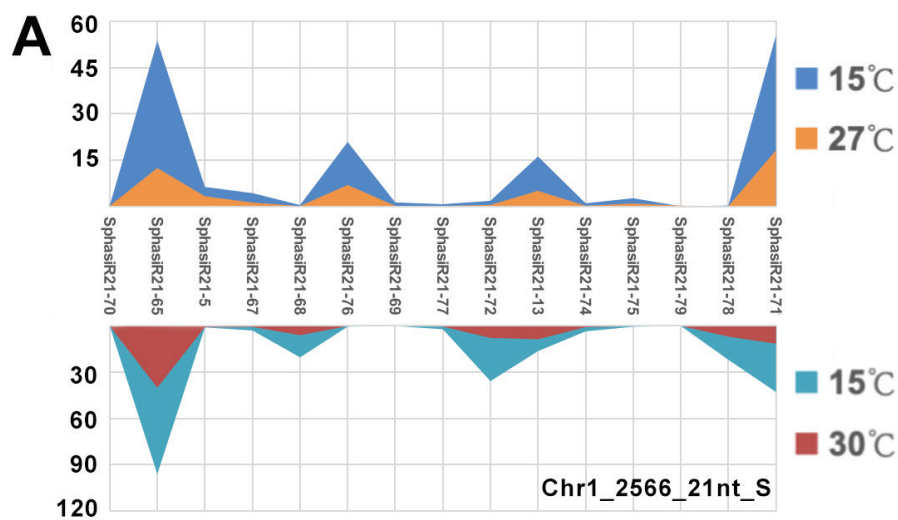
**Figure S1** Graphic presentation of the workflows for phasiRNA locus analyses in *Arabidopsis*. (A) The workflow for genome-wide phasiRNA locus prediction and temperature-sensitive/organ-specific locus identification. (B) The workflow for degradome data-based analysis of the processing signals on the phasiRNA loci. See Table S1 for the detailed list of the sRNA-seq and degradome-seq data used in this study. See “Materials and methods” for the details of locus prediction and temperature-sensitive/organ-specific screening.



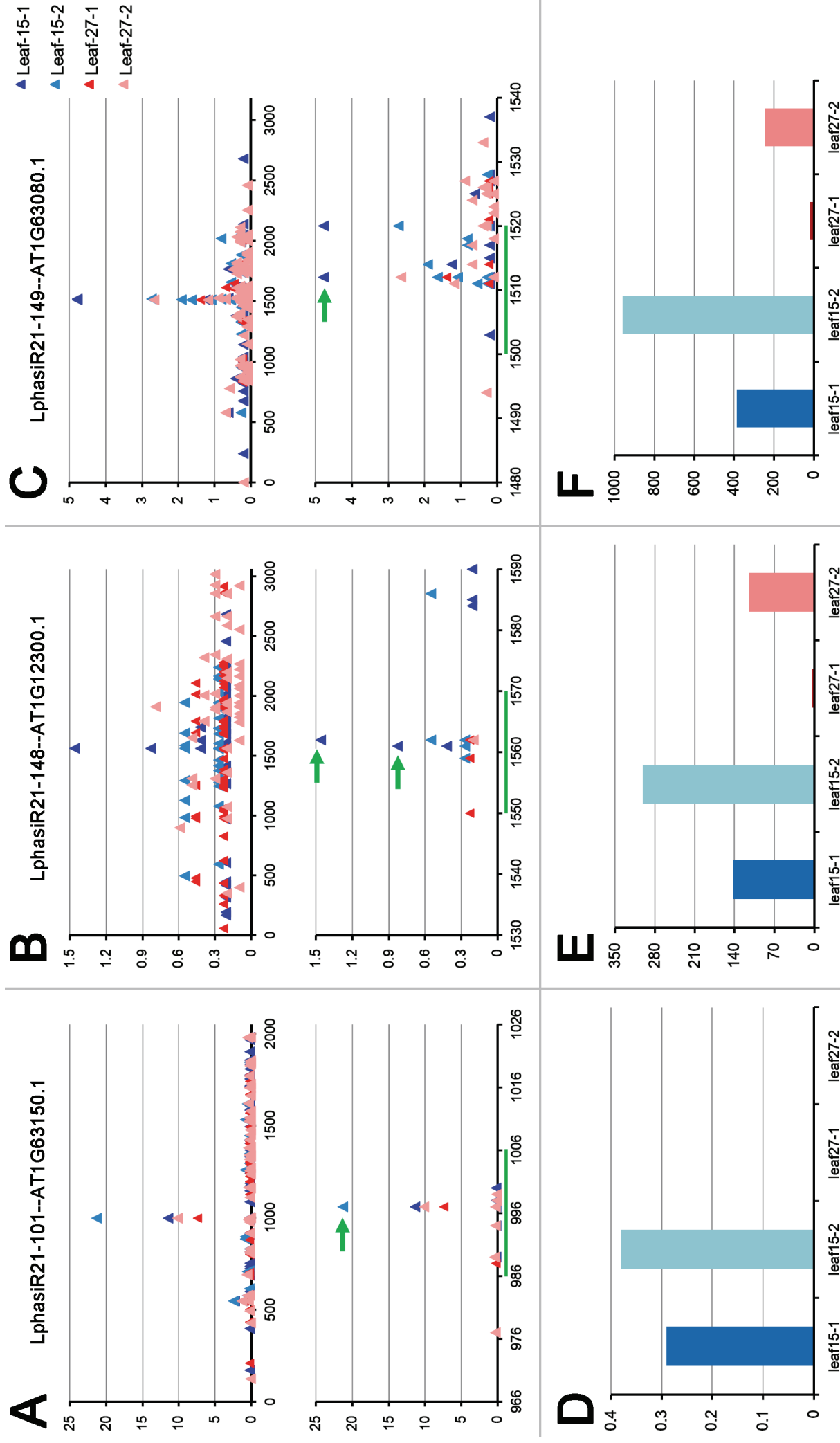
**Figure S2** Statistics of the RDR/DCL-dependent phasiRNAs identified in flowers of *Arabidopsis*.

**A****B**

**Figure S3** Strand bias of the loci for phasiRNA production. (A) Statistics of the temperature-sensitive or organ-specific loci with a double (“two strands”)- or single (“one strand”)-stranded mode for phasiRNA production. “Flower”, “Leaf”, “Root” and “Seedling” represent the results of the temperature-sensitive loci identified in the four different tissues respectively. “15°C” and “27°C” represent the results of the organ-specific loci identified under the two temperature treatments. (B) Percentages of the temperature-sensitive or organ-specific loci with significant strand bias for phasiRNA production. “Significant strand bias” was defined for a locus as the number of phasiRNAs detected on one strand was two times or more than the other strand.



**Figure S4** Validation of the temperature-sensitive phasiRNA loci in *Arabidopsis* seedlings. (A) Chr1\_2566\_21nt\_S overlapped with *TAS1b* (*AT1G50055*) is highly induced under the low temperature treatments. (B) Chr1\_3120\_21nt\_S overlapped with the *TAS* candidate identified in flowers (Chr1-3582-21nt-F) is highly induced under the low temperature treatments. (C) Chr1\_3132\_21nt\_S overlapped with *AT1G63150* (an annotated ta-siRNA locus) is highly induced under the low temperature treatments. (D) Chr3\_451\_21nt\_S overlapped with *TAS4* (*AT3G25795*) is highly induced under the low temperature treatments. (E) Chr3\_1641\_24nt\_S is activated under the high temperature treatments. For each area chart, the *x* axis lists all of the detected phasiRNAs within the locus. The *y* axis measures the averaged expression levels of the phasiRNAs in RPM (reads per million). For the public data sets (the upper chart of each panel), the averaged RPM values were calculated from two biological replicates. For the 15°C and 30°C treatments (the lower chart of each panel), the averaged RPM values were calculated from three biological replicates.



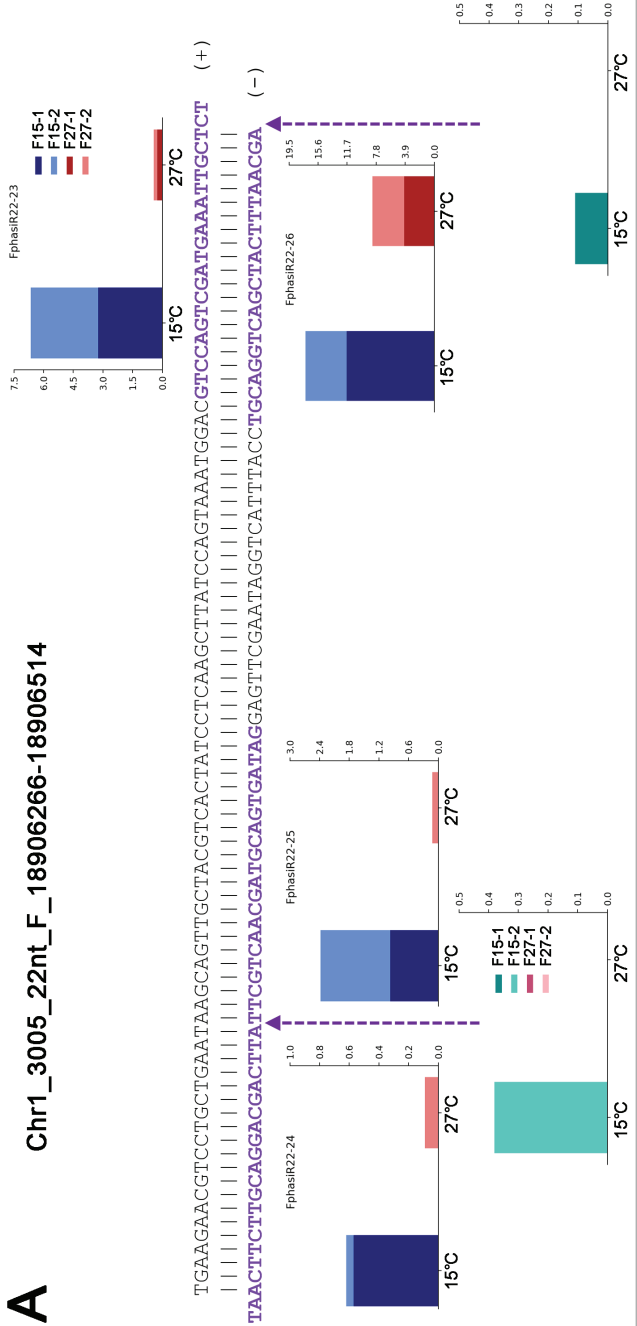
**Figure S5** Temperature-sensitive phasiRNAs with a temperature-specific regulatory mode. (A) AT1G63150.1 (transcribed from a ta-siRNA locus) was targeted by LphasiR21-101. (B) AT1G12300.1 (encoding a pentatricopeptide repeat protein involved in mitochondrial RNA processing) was targeted by LphasiR21-148. (C) AT1G63080.1 (transcribed from a ta-siRNA locus) was targeted by LphasiR21-149. From (A) to (C), the upper panels present the global distribution patterns of



the degradome signals along the full-length target transcripts, and the lower panels focus on the local degradome signals surrounding the target sites (marked by green lines) of the phasiRNAs. For all panels, the *x* axes indicate the position on the target transcripts, and the *y* axes measure the intensities of the degradome signals. The strongest signals mapped to the expected cleavage sites are marked by green arrows. (D) Expression of LphasiR21-101 at 15°C and 27°C. (E) Expression of LphasiR21-148 at 15°C and 27°C. (F) Expression of LphasiR21-149 at 15°C and 27°C. From (D) to (F), the *y* axes measure the phasiRNA levels in RPM (reads per million). All of the three phasiRNAs are 21-nt ones identified from leaves. There are two biological replicates for the 15°C and 27°C treatments, respectively.

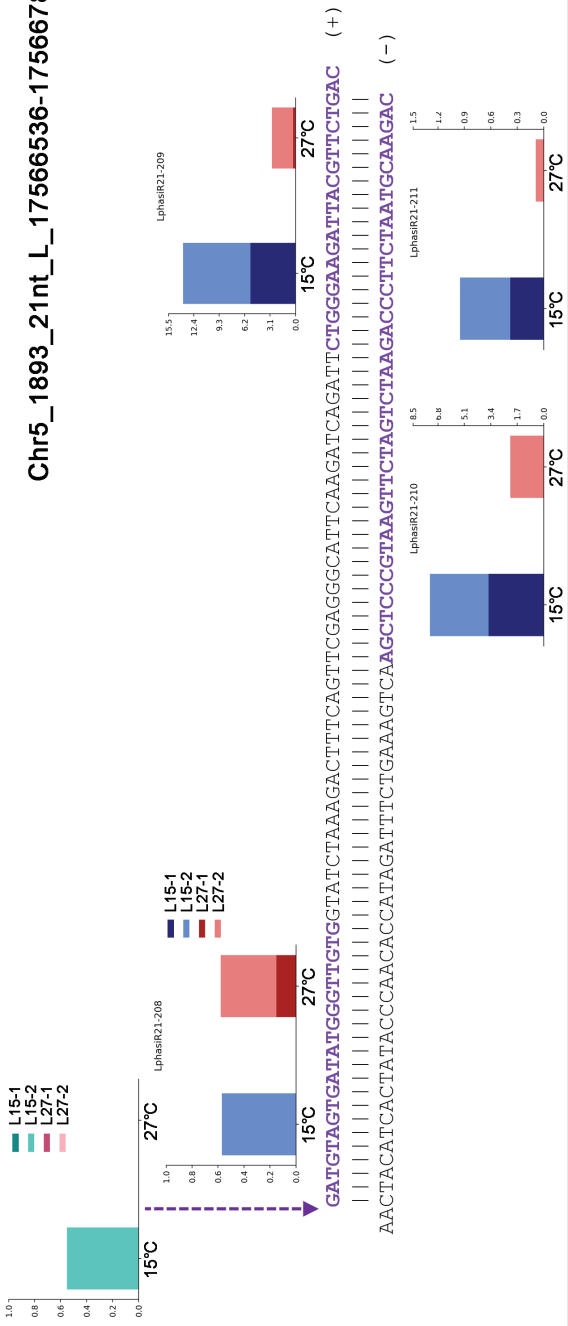
A

Chr1\_3005\_22nt\_F\_18906266-18906514



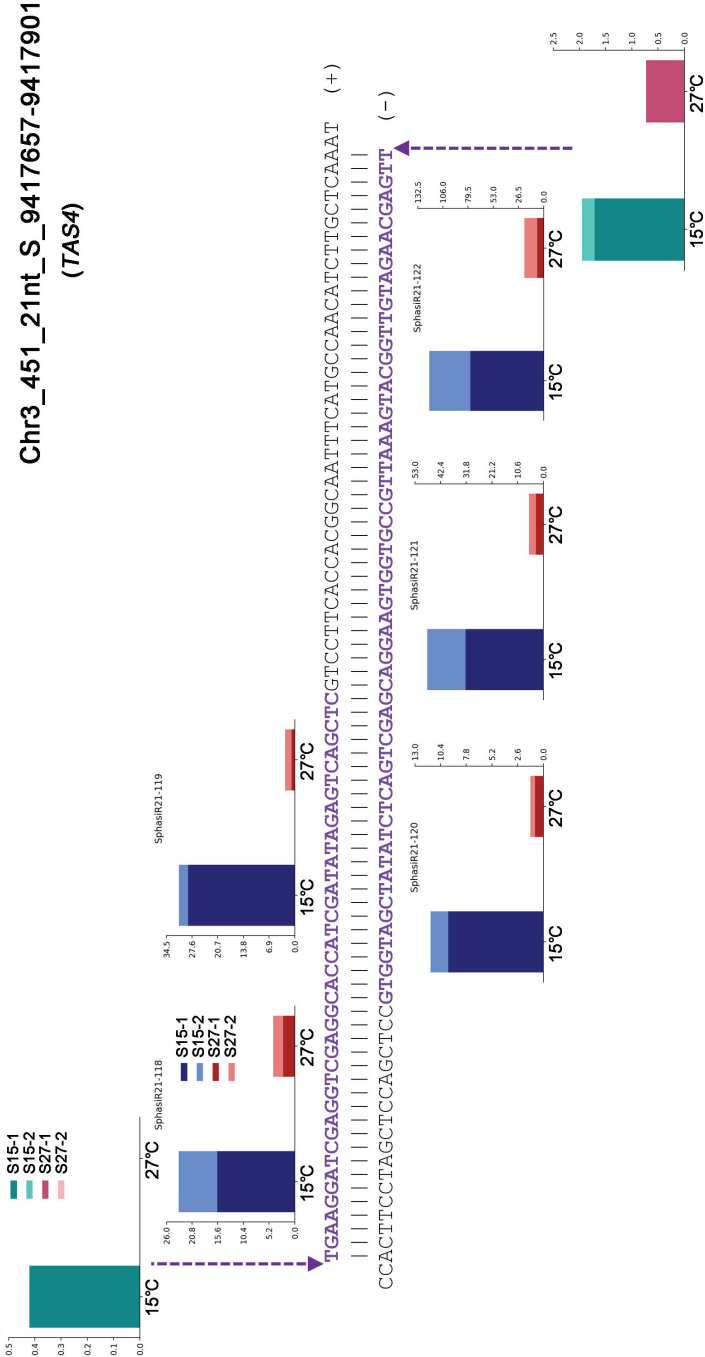
Chr5\_1893\_21nt\_L\_17566536-17566780

B



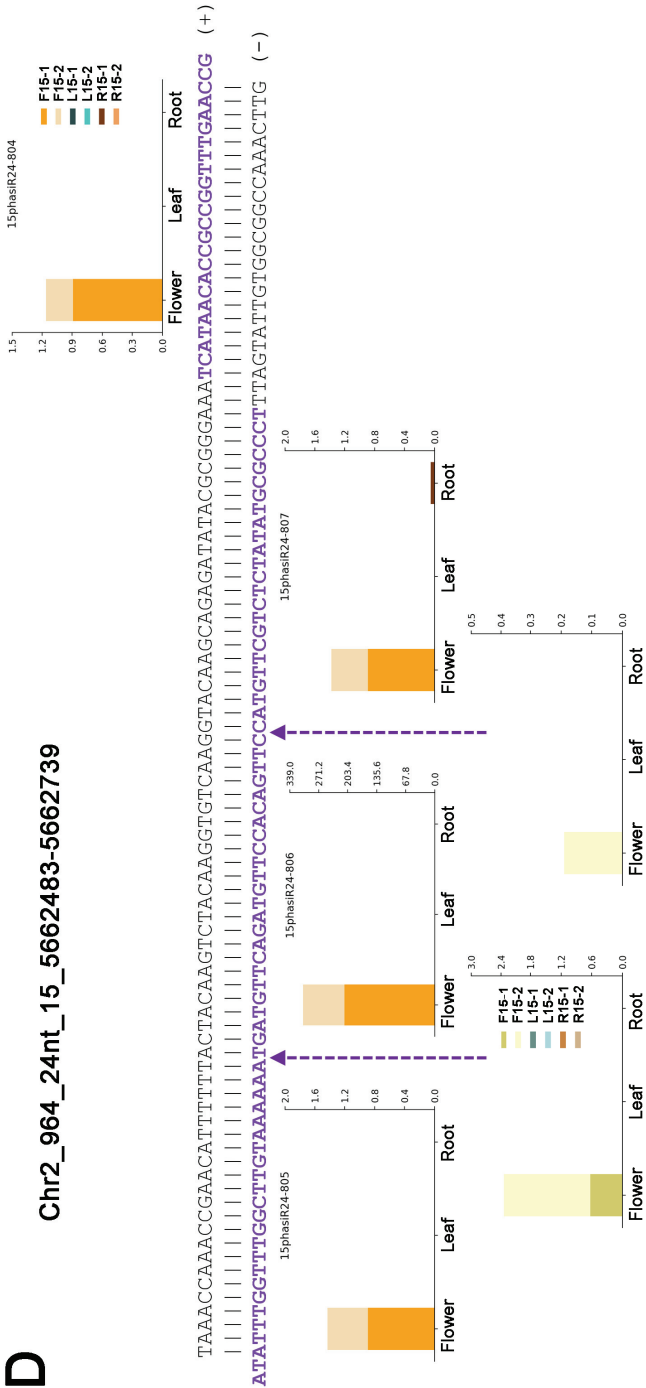
C

Chr3\_451\_21nt\_S\_9417657-9417901  
(TAS4)



D

Chr2\_964\_24nt\_15\_5662483-5662739



**Figure S6** Examples indicating the high correlation between the levels of phasiRNAs and their processing signal intensities. (A) The phasiRNAs produced from the locus Chr1\_3005\_22nt\_F (identified from flowers; ranging from 18,906,266 to 18906,514 on chromosome 1) are expressed at higher levels under 15°C (refer to the histograms marked by different phasiRNA IDs; the phasiRNA sequences are highlighted by purple; similarly hereinafter). Accordingly, the degradome signals mapped to the processing sites are stronger under 15°C (refer to the histograms with the purple dotted arrows indicating the processing sites, similarly hereinafter). For all histograms, the y axes measure the RPM (reads per million) levels, similarly hereinafter. (B) Most of the phasiRNAs produced from Chr5\_1893\_21nt\_L (identified from leaves; ranging from 17,566,536 to 17,566,780 on chromosome 5) are expressed at higher levels under 15°C. Accordingly, the degradome signal mapped to the processing site is stronger under 15°C. (C) The phasiRNAs produced from Chr3\_451\_21nt\_S (identified from seedlings; ranging from 9,417,657 to 9,417,901 on chromosome 3) are expressed at higher levels under 15°C. Accordingly, the degradome signals mapped to the processing sites are stronger under 15°C. Note: Chr3\_451\_21nt\_S overlaps with *TAS4* (*AT3G25795*). (D) The phasiRNAs produced from Chr2\_964\_24nt\_15 (identified under 15°C; ranging from 5,662,483 to 5,662,739 on chromosome 2) are expressed at higher levels in flowers. Accordingly, the degradome signals mapped to the processing sites are stronger in flowers. For both phasiRNA and degradome levels, two biological replicates of each sample were summed in RPM for histogram presentations. F15: flowers at 15°C; F27: flowers at 27°C; L15: leaves at 15°C; L27: leaves at 27°C; R15: roots at 15°C; R27: roots at 27°C; S15: seedlings at 15°C; S27: seedlings at 27°C.