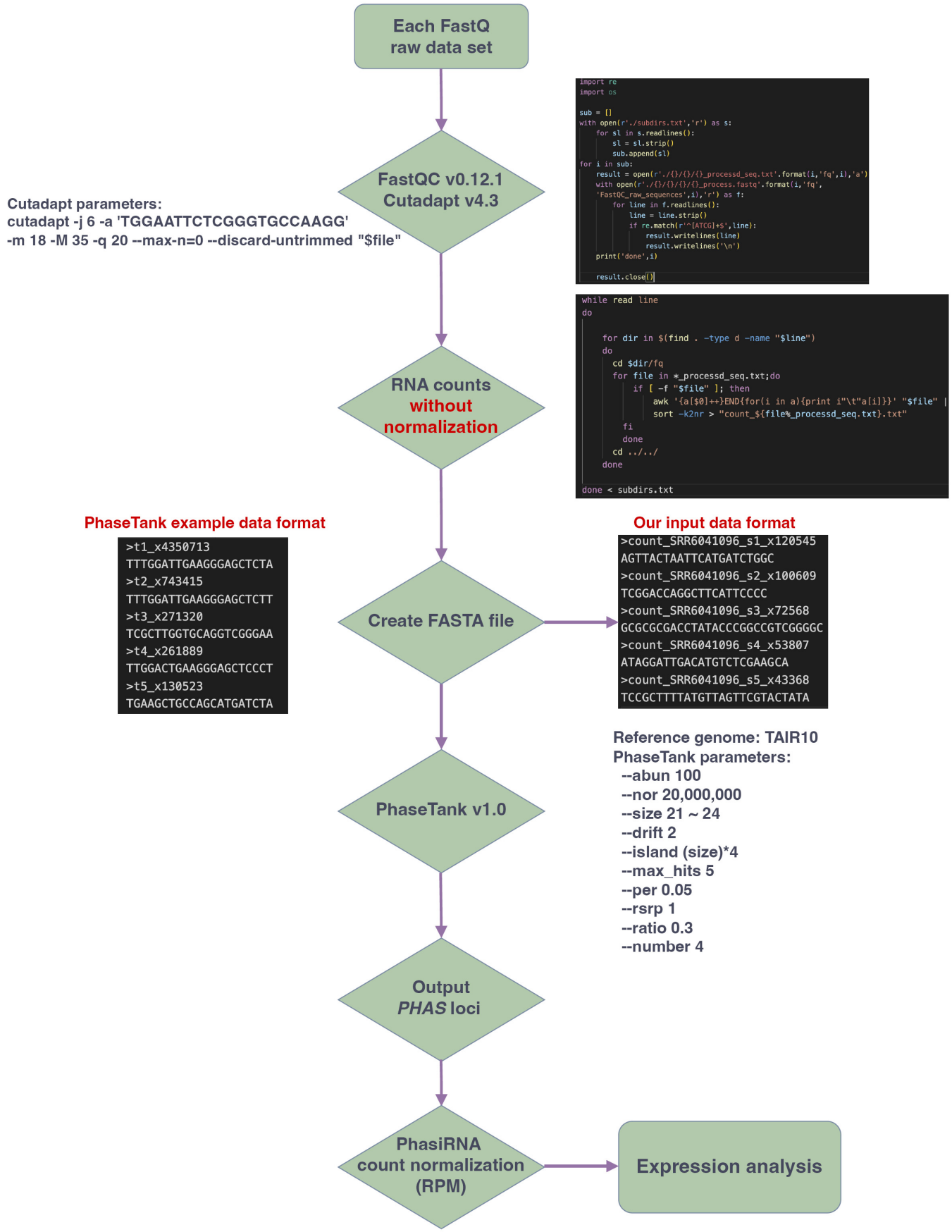


Figure S1 The workflow of PhaseTank-based phasiRNA locus predictions, including data format, analytical steps, and parameters.

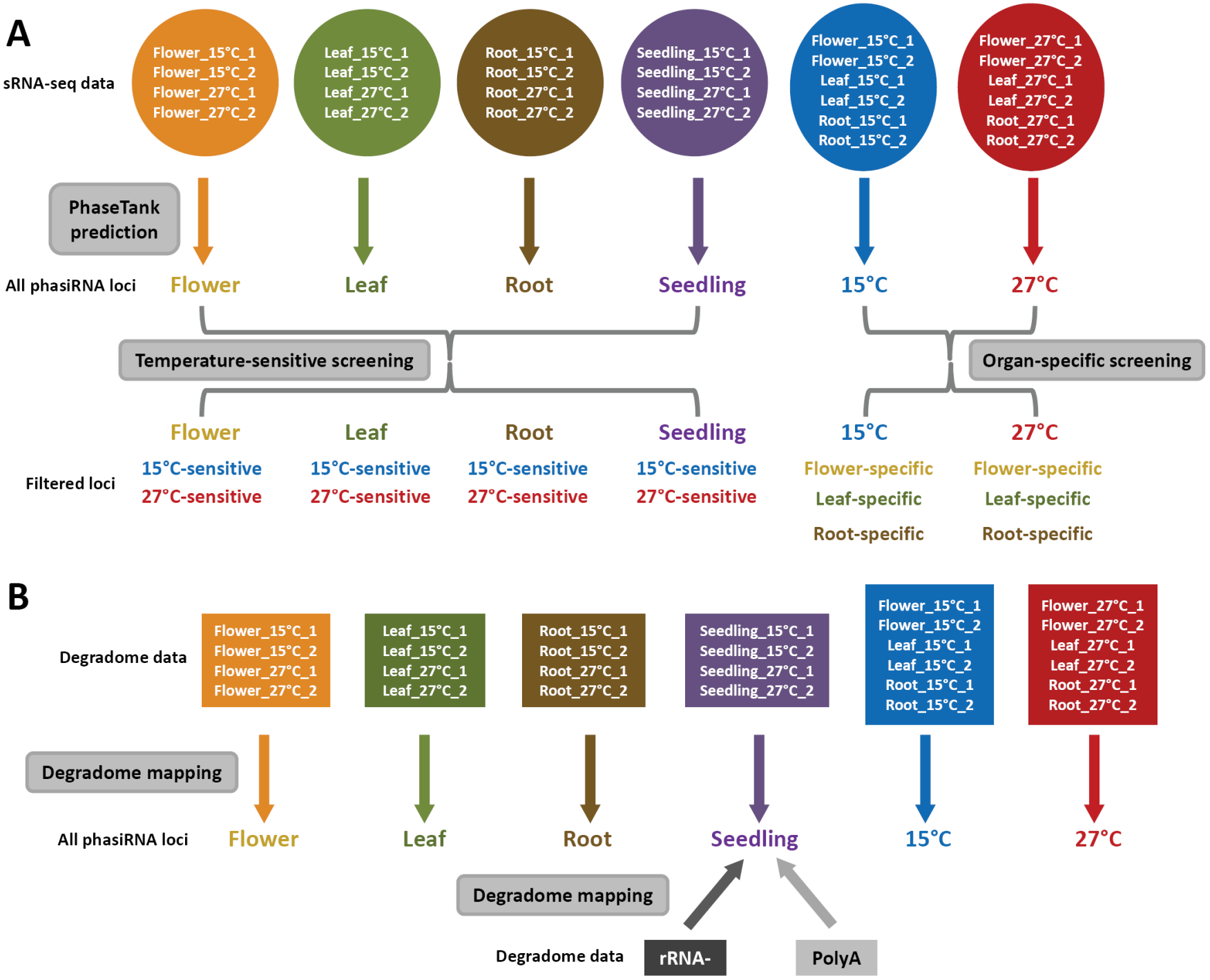

A
sRNA-seq data
Flower_15°C_1
Flower_15°C_2
Flower_27°C_1
Flower_27°C_2
Leaf_15°C_1
Leaf_15°C_2
Leaf_27°C_1
Leaf_27°C_2
Root_15°C_1
Root_15°C_2
Root_27°C_1
Root_27°C_2
Seedling_15°C_1
Seedling_15°C_2
Seedling_27°C_1
Seedling_27°C_2
Flower_15°C_1
Flower_15°C_2
Leaf_15°C_1
Leaf_15°C_2
Root_15°C_1
Root_15°C_2
Flower_27°C_1
Flower_27°C_2
Leaf_27°C_1
Leaf_27°C_2
Root_27°C_1
Root_27°C_2
PhaseTank prediction
All phasiRNA loci
Flower
Leaf
Root
Seedling
15°C
27°C
Temperature-sensitive screening
Organ-specific screening
Flower
Leaf
Root
Seedling
15°C
27°C
Filtered loci
15°C-sensitive
27°C-sensitive
15°C-sensitive
27°C-sensitive
15°C-sensitive
27°C-sensitive
15°C-sensitive
27°C-sensitive
Flower-specific
Leaf-specific
Root-specific
Flower-specific
Leaf-specific
Root-specific
B
Degradome data
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_15°C_1
Flower_15°C_2
Leaf_15°C_1
Leaf_15°C_2
Root_15°C_1
Root_15°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
Degradome mapping
All phasiRNA loci
Flower
Leaf
Root
Seedling
15°C
27°C
Degradome mapping
Degradome data
rRNA-
PolyA

Figure S2 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.

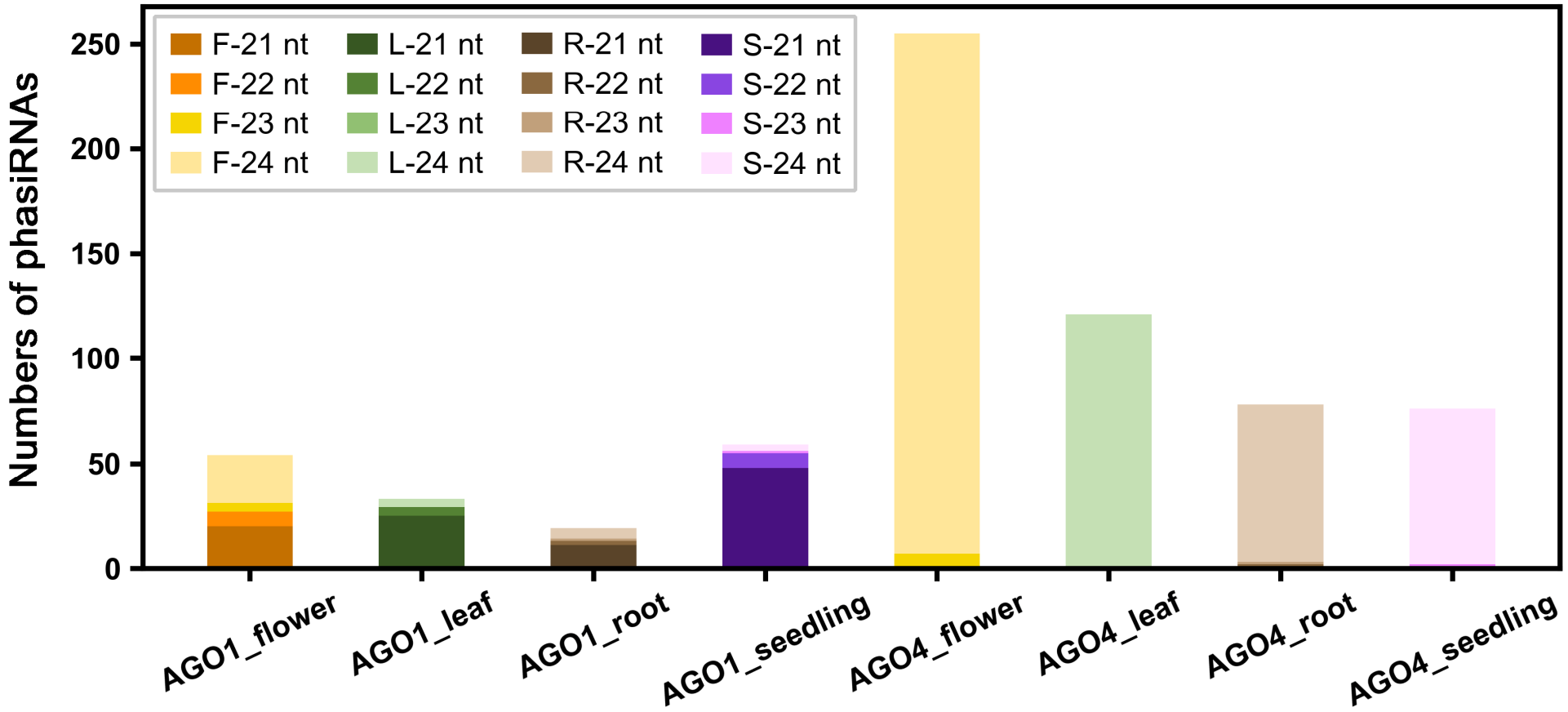


Figure S3 Numbers of the AGO-enriched phasiRNAs identified from different tissues (F: flower, L: leaf, R: root, and S: seedling).

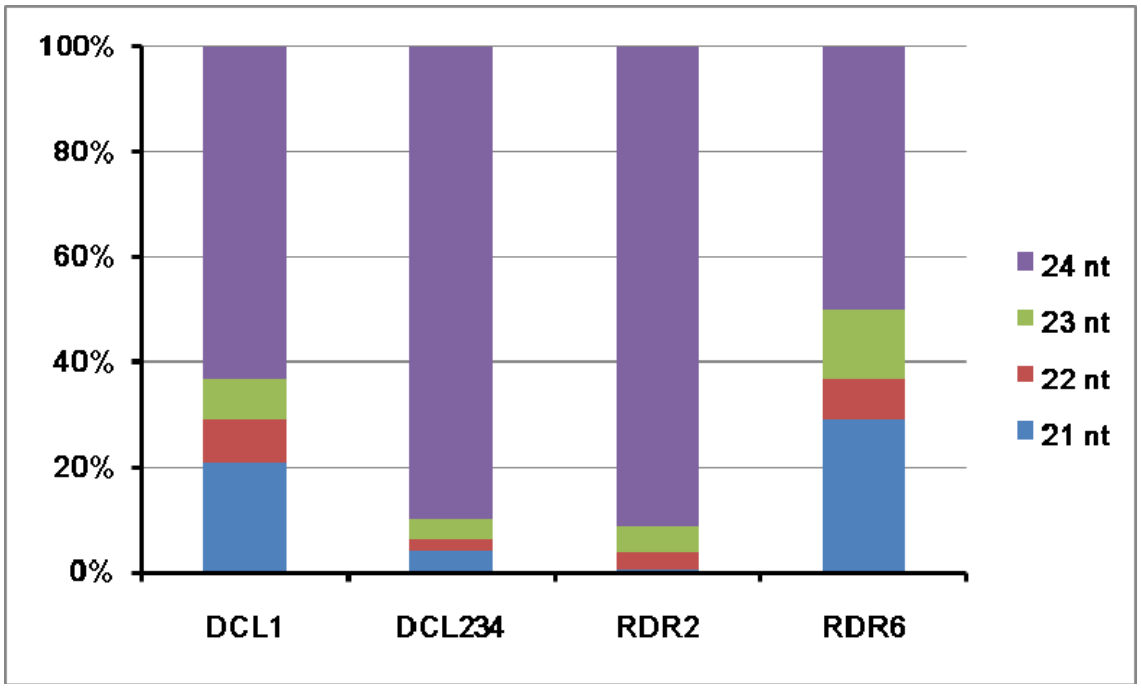
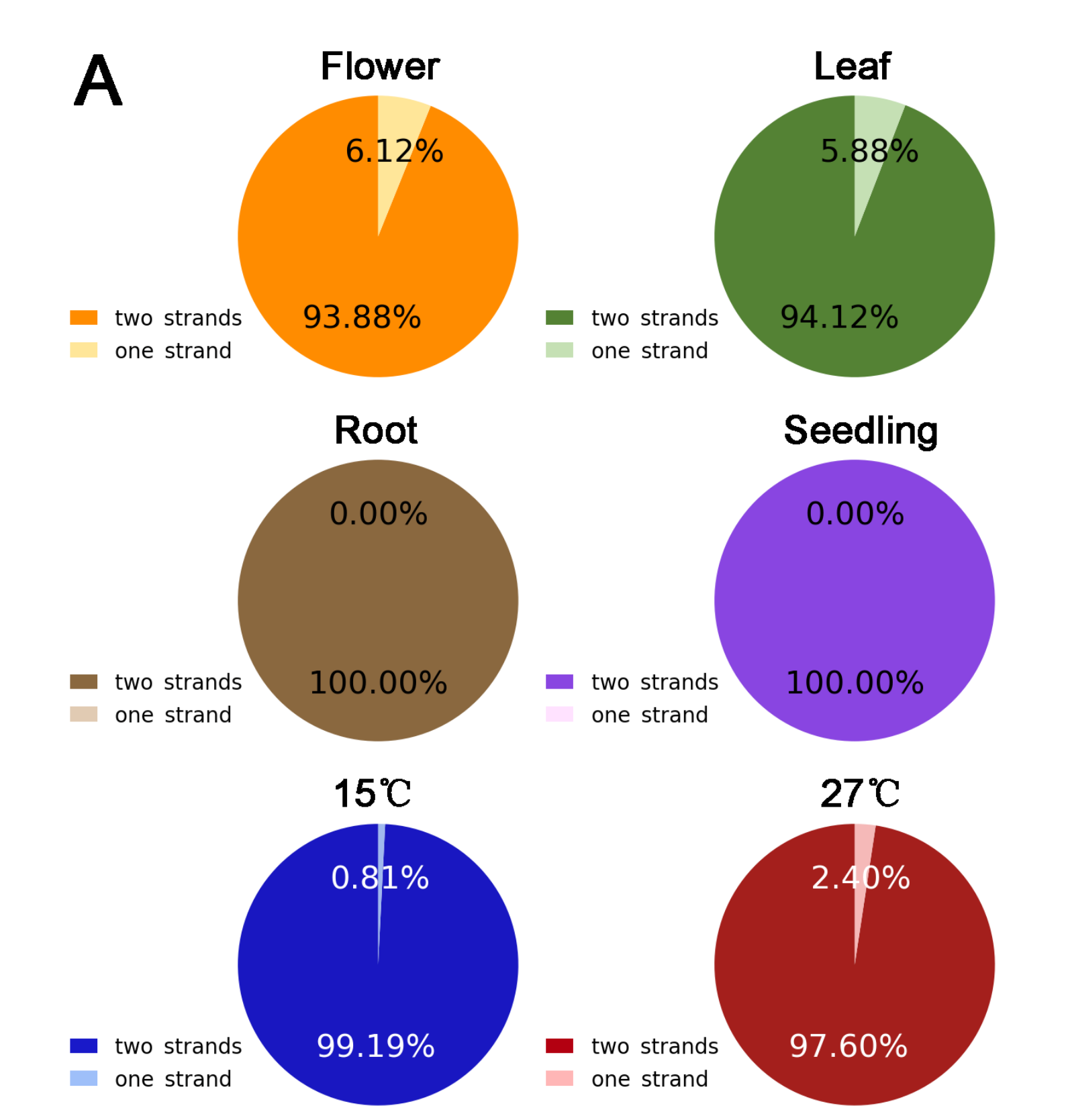


Figure S4 Statistics of the RDR/DCL-dependent phasiRNAs identified in flowers of *Arabidopsis*.


A
Flower
6.12%
93.88%
two strands
one strand
Leaf
5.88%
94.12%
two strands
one strand
Root
0.00%
100.00%
two strands
one strand
Seedling
0.00%
100.00%
two strands
one strand
15℃
0.81%
99.19%
two strands
one strand
27℃
2.40%
97.60%
two strands
one strand

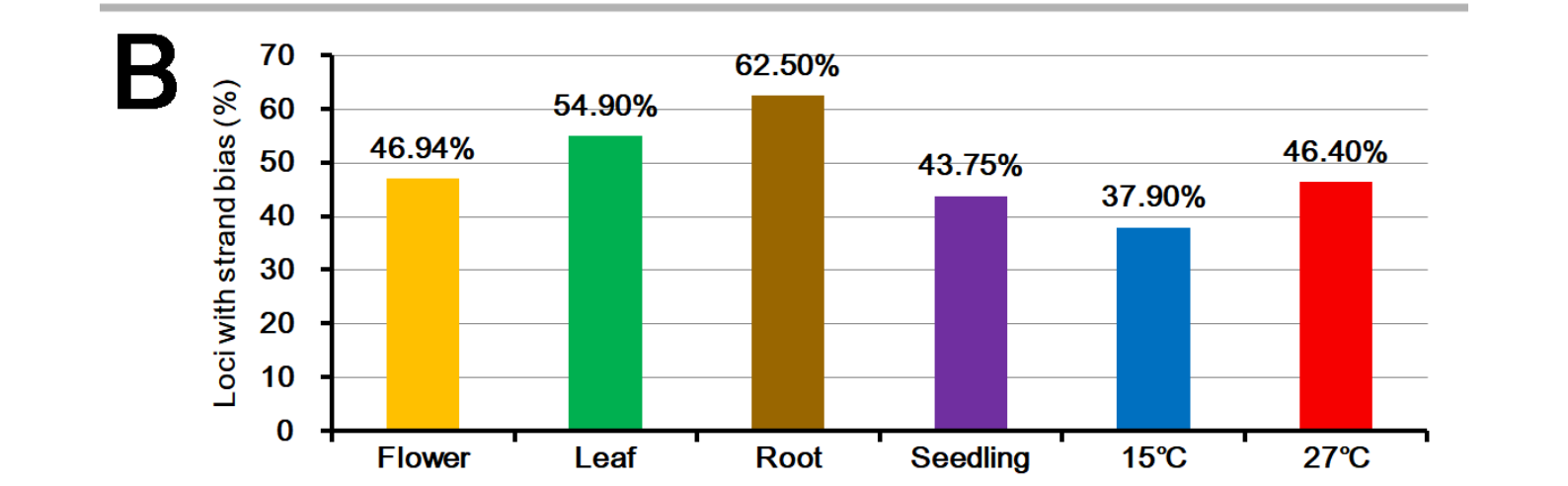

B
Loci with strand bias (%)
70
60
50
40
30
20
10
0
46.94%
54.90%
62.50%
43.75%
37.90%
46.40%
Flower
Leaf
Root
Seedling
15℃
27℃

Figure S6 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.

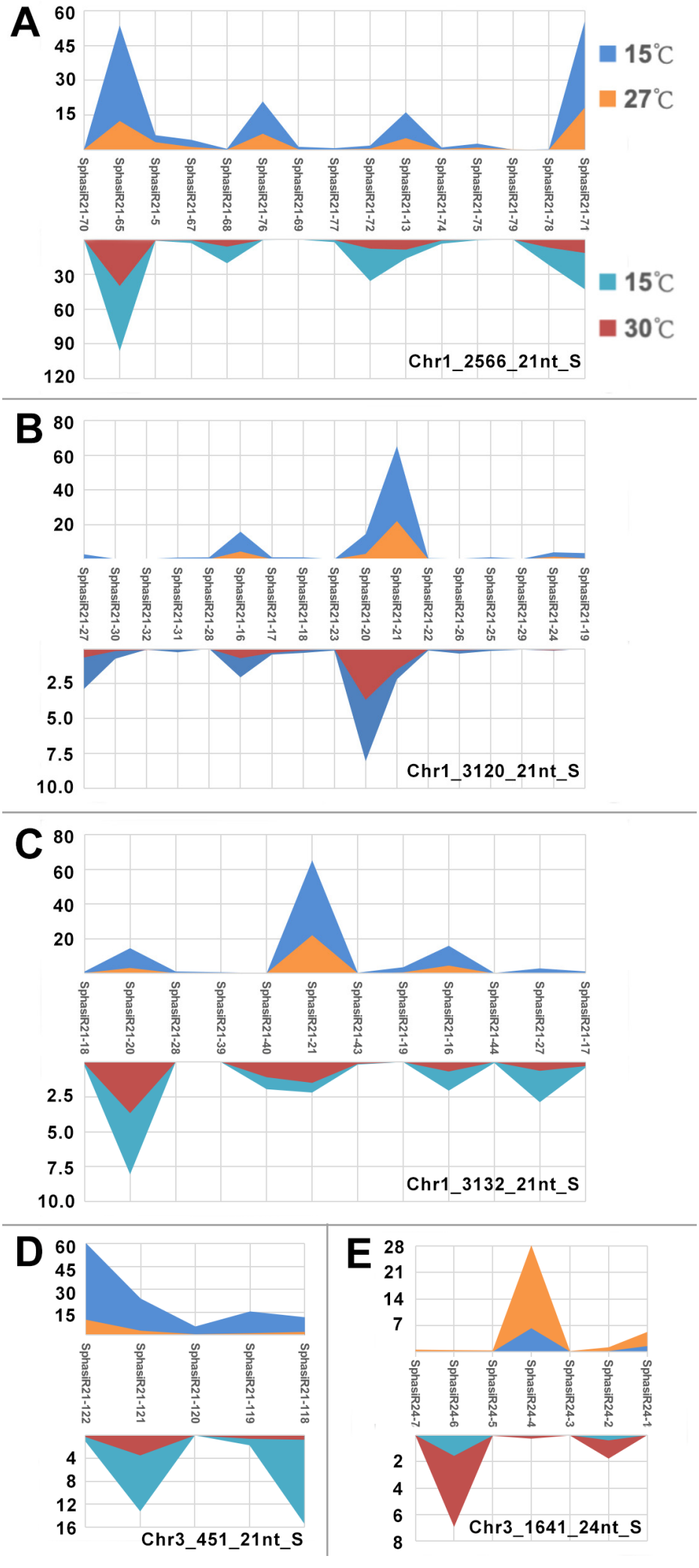

A
60
45
30
15
15℃
27℃
SphasiR21-70
SphasiR21-65
SphasiR21-5
SphasiR21-67
SphasiR21-68
SphasiR21-76
SphasiR21-69
SphasiR21-77
SphasiR21-72
SphasiR21-13
SphasiR21-74
SphasiR21-75
SphasiR21-79
SphasiR21-78
SphasiR21-71
30
60
90
120
15℃
30℃
Chr1_2566_21nt_S
B
80
60
40
20
SphasiR21-27
SphasiR21-30
SphasiR21-32
SphasiR21-31
SphasiR21-28
SphasiR21-16
SphasiR21-17
SphasiR21-18
SphasiR21-23
SphasiR21-20
SphasiR21-21
SphasiR21-22
SphasiR21-26
SphasiR21-25
SphasiR21-29
SphasiR21-24
SphasiR21-19
2.5
5.0
7.5
10.0
Chr1_3120_21nt_S
C
80
60
40
20
SphasiR21-18
SphasiR21-20
SphasiR21-28
SphasiR21-39
SphasiR21-40
SphasiR21-21
SphasiR21-43
SphasiR21-19
SphasiR21-16
SphasiR21-44
SphasiR21-27
SphasiR21-17
2.5
5.0
7.5
10.0
Chr1_3132_21nt_S
D
60
45
30
15
SphasiR21-122
SphasiR21-121
SphasiR21-120
SphasiR21-119
SphasiR21-118
4
8
12
16
Chr3_451_21nt_S
E
28
21
14
7
SphasiR24-7
SphasiR24-6
SphasiR24-5
SphasiR24-4
SphasiR24-3
SphasiR24-2
SphasiR24-1
2
4
6
8
Chr3_1641_24nt_S

Figure S7 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.

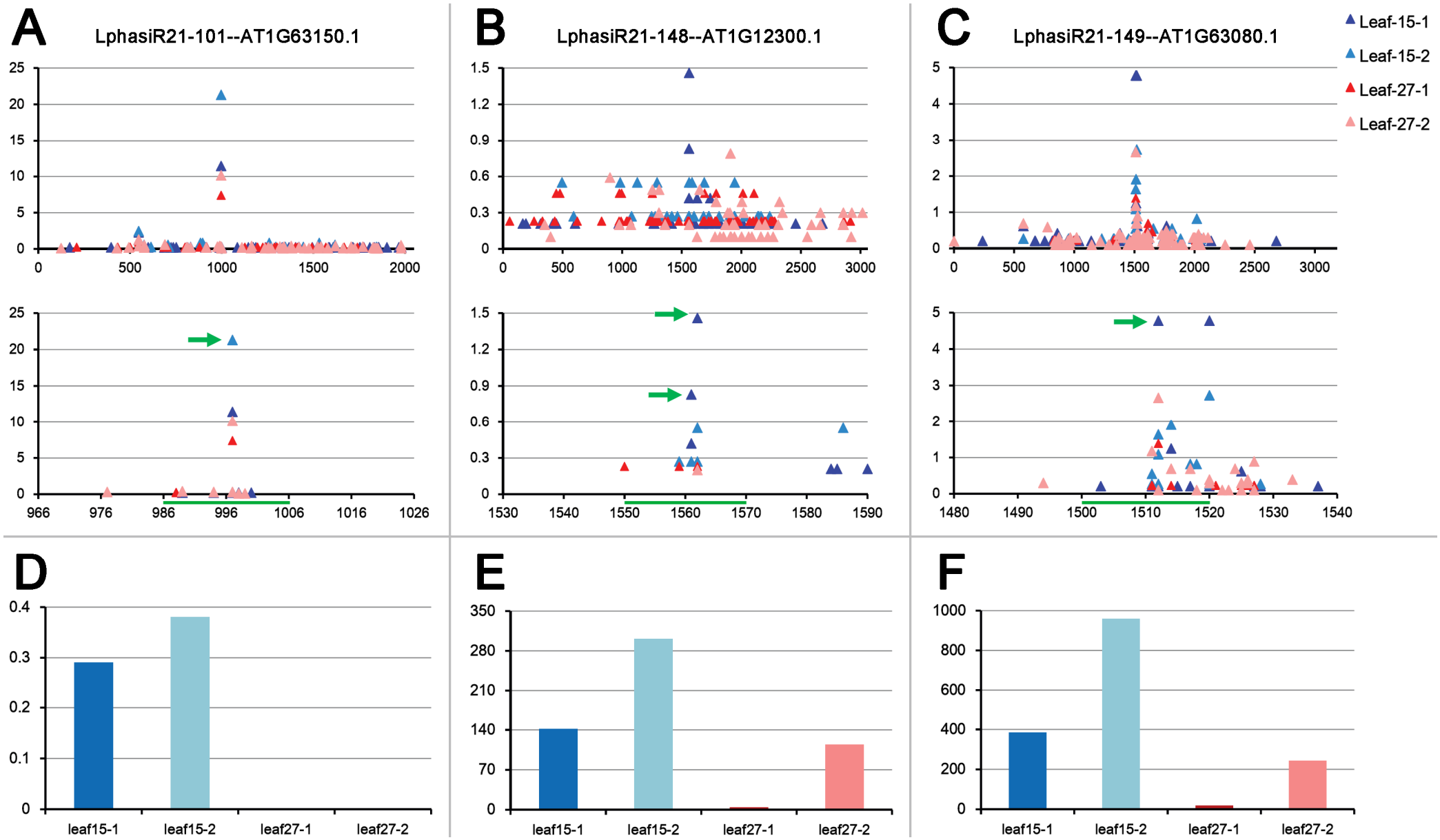
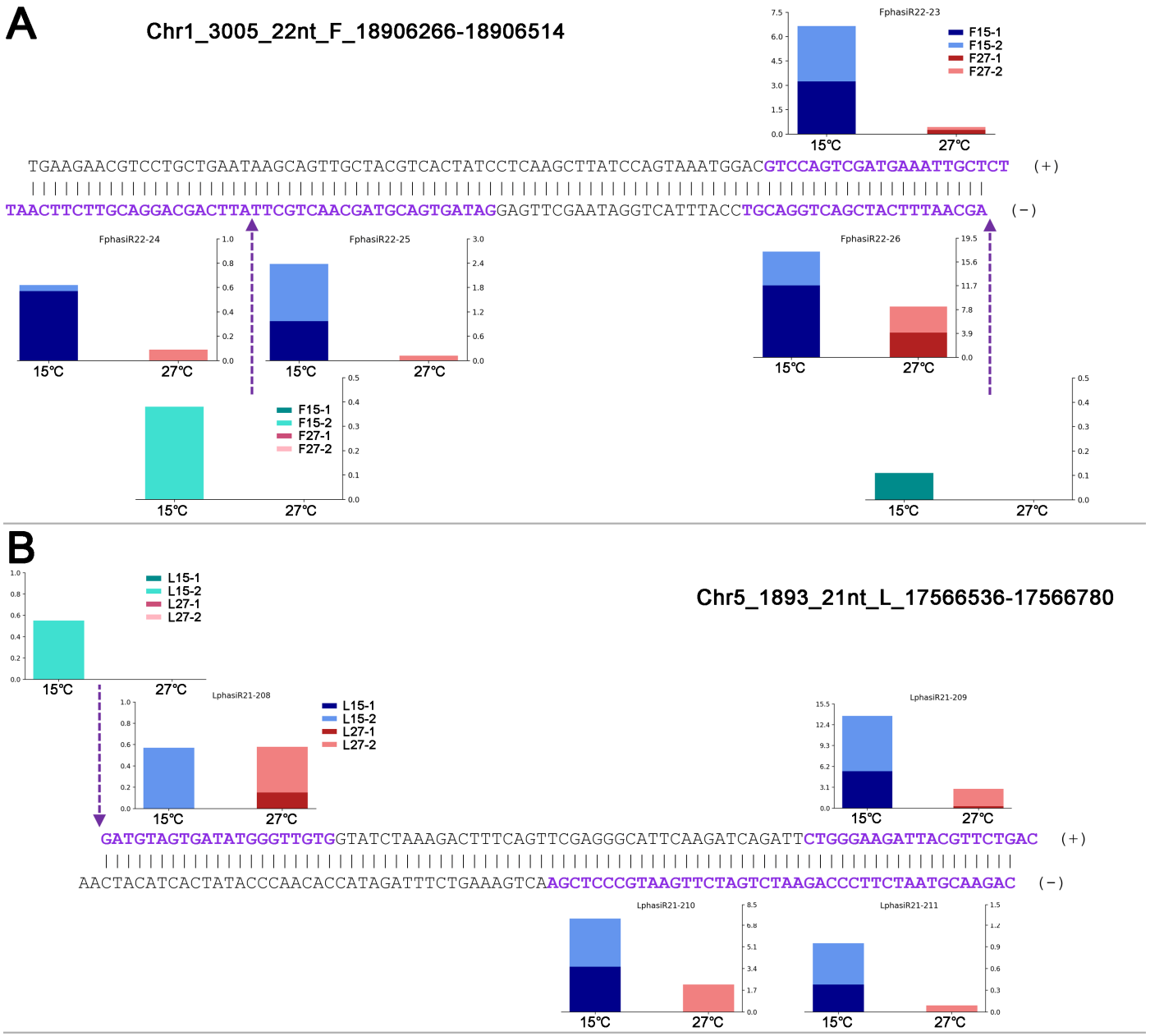


Figure S8 Temperature-sensitive phasiRNAs with a temperature-specific regulatory mode. (A) AT1G63150.1 (transcribed from a ta-siRNA locus) was targeted by LphasiR21-101. (B) AT1G12300.1 (encoding a pentatricopeptide repeat protein involved in mitochondrial RNA processing) was targeted by LphasiR21-148. (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
FphasiR22-23
F15-1
F15-2
F27-1
F27-2
15℃
27℃
TGAAGAACGTCCTGCTGAATAAGCAGTTGCTACGTCACTATCCTCAAGCTTATCCAGTAAATGGACGTCCAGTCGATGAAATTGCTCT (+)
TAACTTCTTGCAGGACGACTTATTCGTCAACGATGCAGTGATAGGAGTTCGAATAGGTCATTTACCTGCAGGTCAGCTACTTTAACGA (-)
FphasiR22-24
FphasiR22-25
FphasiR22-26
B
L15-1
L15-2
L27-1
L27-2
Chr5_1893_21nt_L_17566536-17566780
LphasiR21-208
LphasiR21-209
GATGTAGTGATATGGGTTGTGGTATCTAAAGACTTTCAGTTCGAGGGCATTCAAGATCAGATTCTGGGAAGATTACGTTCTGAC (+)
AACTACATCACTATACCCAACACCATAGATTTCTGAAAGTCAAGCTCCCGTAAGTTCTAGTCTAAGACCCTTCTAATGCAAGAC (-)
LphasiR21-210
LphasiR21-211

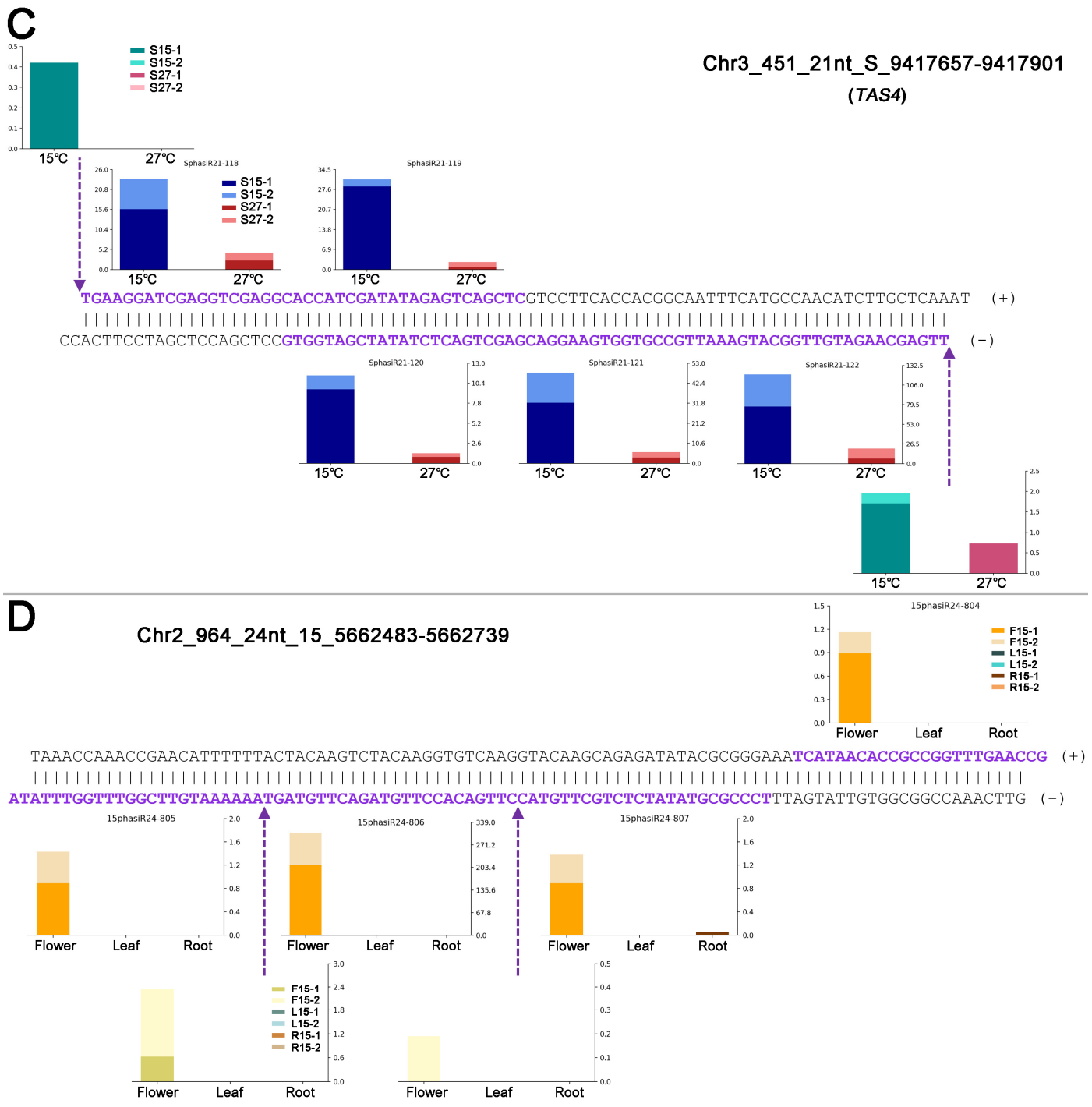

C
Chr3_451_21nt_S_9417657-9417901
(TAS4)
S15-1
S15-2
S27-1
S27-2
15℃
27℃
SphasiR21-118
SphasiR21-119
SphasiR21-120
SphasiR21-121
SphasiR21-122
TGAAGGATCGAGGTCGAGGCACCATCGATATAGAGTCAGCTCGTCCTTCACCACGGCAATTTCATGCCAACATCTTGCTCAAAT (+)
CCACTTCCTAGCTCCAGCTCCGTGGTAGCTATATCTCAGTCGAGCAGGAAGTGGTGCCGTTAAAGTACGGTTGTAGAACGAGTT (-)
D
Chr2_964_24nt_15_5662483-5662739
15phasiR24-804
F15-1
F15-2
L15-1
L15-2
R15-1
R15-2
Flower
Leaf
Root
TAAACCAAACCGAACATTTTTTACTACAAGTCTACAAGGTGTCAAGGTACAAGCAGAGATATACGCGGGAAATCATAACACCGCCGGTTTGAACCG (+)
ATATTTGGTTTGGCTTGTAAAAAATGATGTTCAGATGTTCCACAGTTCCATGTTCGTCTCTATATGCGCCCTTTAGTATTGTGGCGGCCAAACTTG (-)
15phasiR24-805
15phasiR24-806
15phasiR24-807

Figure S9 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.