

## Supplementary Legends

Figure S1. Position of miRNA and long-miRNA sequences retrieved from ASRP and MPSS PLUS databases on their hairpin folded precursor.

(A) Position of long-miRNA sequences retrieved from the ASRP database on their precursor (sequences are summarized in Data S1B).

(B) Position of long-miRNA sequences retrieved from the MPSS PLUS database on their precursor (sequences are summarized in Supplementary Data S1C).

For each MIR gene for which a long-miRNA sequence was found, we give the name and the size of the canonical miRNA and its position is shown in red on the hairpin. We then give the size of each long-miRNA sequence and its position is shown in red on the hairpin.

Figure S2. Biogenesis of long-miRNAs also depends on DCL3 in rosette leaves.

RNA gel blot analysis of the genetic DCL requirement for biogenesis of long-miRNAs. SmRNAs (20 µg) from rosette leaves of *dcl* mutants and of Col-0 wild type were loaded on the gels. Probed miRNAs are indicated on the left side of the blots. The loading control is U6 snRNA. The upper and lower ticks to the right of each lane mark the positions of the 24-nt and 21-nt size markers, respectively and are colored red when miRNA species of that size class are detected. <sup>b</sup>miR165 stands for the miR165/miR166. <sup>1</sup>indicates the miRNAs encoded by a single *MIR* gene.

Figure S3. *In silico* predicted hairpin structures for the different IR loci from which smRNAs are probed in Figure 5.

Folding predictions were made using Mfold online at <http://frontend.bioinfo.rpi.edu/applications/mfold/cgi-bin/rna-form1.cgi> (1,2). *IR* gene name, genomic location, and predicted hairpin structure are given. Sequences of each hairpin and coordinates of the first and last nucleotides can be visualized by zooming in.

**Supplementary References**

1. Mathews, D.H., Sabina, J., Zuker, M. and Turner, D.H. (1999) Expanded sequence dependence of thermodynamic parameters improves prediction of RNA secondary structure. *J Mol Biol*, **288**, 911-940.
2. Zuker, M. (2003) Mfold web server for nucleic acid folding and hybridization prediction. *Nucleic Acids Res*, **31**, 3406-3415.

**Figure S1A:** Position of long-miRNA sequences retrieved from the ASRP database on their precursor

### MIR156a

20-nt



23-nt



### MIR156b

20-nt



23-nt



23-nt



### MIR156d

20-nt



24-nt



23-nt



### MIR157c

21-nt

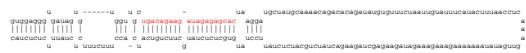


23-nt



### MIR157d

20-nt



23-nt



**MIR158a**

20-nt

24-nt

23-nt

23-nt

25-nt

25-nt

**MIR159a**

21-nt

23-nt

24-nt

**MIR159b**

21-nt

23-nt

25-nt

23-nt

**MIR159c**

21-nt

24-nt

**MIR319a**

20-nt



23-nt



24-nt



25-nt



24-nt



23-nt



23-nt



23-nt

**MIR319b**

20-nt



24-nt



23-nt



24-nt



23-nt

**MIR160a**

21-nt



23-nt



24-nt



24-nt



MIR160b

21-nt

c	c =	a	aaac	ag
gu <u>gc</u> u <u>gc</u> 	w <u>gg</u> c <u>u</u> 	c <u>u</u> g <u>u</u> a <u>u</u> g <u>cc</u> c 	ag 	au <u>gc</u> au <u>uu</u> 
ca <u>gu</u> u <u>ac</u> 	ac <u>u</u> g 	g <u>u</u> ca <u>u</u> g <u>gg</u> u <u>gg</u> 	uc 	u <u>ac</u> gu <u>uu</u> 
a	u	a	--ac	ac

23-nt

23-nt

C	C =	A	AAAC	AG		
gucgugc	wggcu	c	cugua <u>u</u> ccac	Ag	a <u>u</u> cgauuu	u
ca <u>u</u> acg	acu <u>g</u>	g	ca <u>u</u> ga <u>u</u> gg <u>u</u> gg	uc	u <u>ac</u> guuu	u
A	U	A	A	—AC	AC	

```

23-nt

      c   c =           a   aaaaC   ag
guuguugc wggccu c cuguaugccac ag   auucgauuu u
||| ||||| | | | | | | | | | | | | | | | | | | | | | |
caguaucg acuca g gacaaugccgggg uc   uacggauuu u
      a   u a           a   ---ac   ac

```

MIR160c

21-nt

c	c	ga ug auu	g
guaua <u>ucg</u>	gg <u>ccuu</u>	ug <u>uaugccac</u>	g g
ca <u>guau</u>	ac <u>ugagg</u>	ac <u>auugccgg</u>	c c
ca <u>guau</u>	ac <u>ugagg</u>	ac <u>auugccgg</u>	gg <u>ccuuuuuu</u>

24-nt

c	c	ga ug auu	g
guuuuugc	uggccucc	uguaauucacac	g g cccaaaaauu

a      a      ga gu ---      u

**23-nt**

MIR161

21-nt									
u	ga	gg	ac	uuuuu	c	c	u		
guuu	ucuc	uuuuuu	cgg	uuuuuu	ccccc	gaa	uuuuuu		
cgaa	gggg	aaaaaa	ggg	aaaaaa	cccccc	ccca	aaaaaa		
a	-	ag	--	----	a	a	c		

24-nt

## 23-nt

23-nt

23-nt

u	ga	gg	ac	uuuuu	c	c	u
gcuuu	ucuc	uuuuug	cag	ugcgu	gau	aaugca	
coss	aaaaa	aaaaau	ouu	acoua	cua	uuacou	

MIR162a

21-nt

24-nt

MIR164b

24-nt

a	ca	cauu	a	---	
gaagg	gaag	ggccacug	acuagcucau	uuua	ca

MIR164c

21-nt



23-nt



MIR165a

21-nt



23-nt



MIR167a

21-nt



23-nt



21-nt



MIR167b

21-nt



25-nt

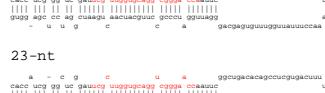


MIR168a

21-nt



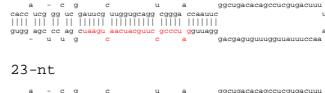
24-111



- 12



24-nt



**MIR168b**

**21-nt**

**23-nt**

**MIR169a**

**21-nt**

**24-nt**

**24-nt**

**23-nt**

**23-nt**

**23-nt**

**MIR169b**

**21-nt**

**23-nt**

**23-nt**

**24-nt**

**23-nt**

**23-nt**

**24-nt**

**23-nt**

**MIR169c**

21-nt



24-nt



23-nt

**MIR169f**

21-nt



25-nt



24-nt

**MIR169g**

21-nt



24-nt

**MIR169h**

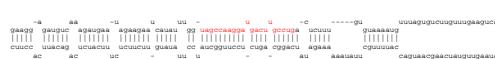
21-nt



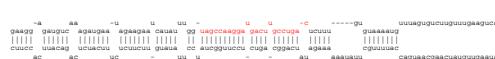
23-nt

**MIR169i**

21-nt



23-nt

**MIR169l**

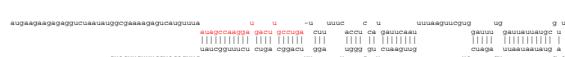
21-nt



24-nt



23-nt



**MIR169m**

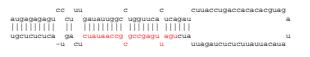
21-nt



24-nt

**MIR171a**

21-nt



23-nt



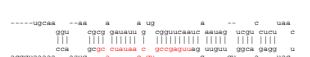
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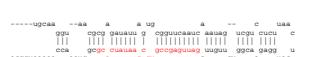
23-nt

**MIR171b**

21-nt



23-nt

**MIR171c**

21-nt



23-nt



24-nt



23-nt



25-nt



24-nt



MIR172b

20-nt



23-nt



23-nt



MIR173

22-nt



23=nt



23-nt



MIR390a

21-nt



24-nt



35-nt

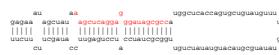


23-nt



MIR390b

31-nt

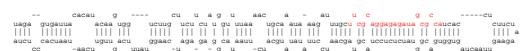


23-nt



**MIR391**

21-nt



24-nt



24-nt



24-nt

**MIR394a**

20-nt



24-nt

**MIR396a**

21-nt



23-nt



24-nt

**MIR396b**

21-nt



23-nt

**MIR397a**

21-nt



23-nt

**MIR397b**

21-nt



23-nt



**MIR399b**

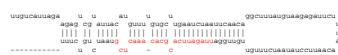
21-nt



24-nt

**MIR403**

21-nt



24-nt



23-nt



25-nt



23-nt

**MIR408**

21-nt



23-nt

**MIR447a**

21-nt



23-nt



25-nt

**MIR447b**

21-nt



23-nt

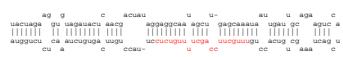


25-nt

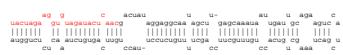


**MIR773**

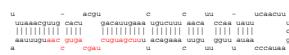
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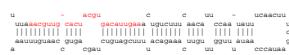
24-nt

**MIR775**

21-nt



24-nt



24-nt



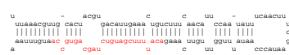
24-nt



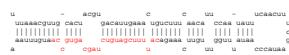
23-nt



25-nt



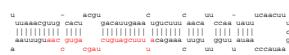
24-nt



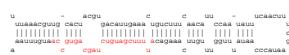
25-nt



24-nt



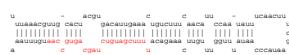
23-nt



25-nt



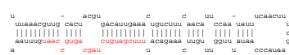
23-nt



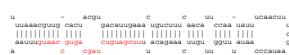
24-nt



23-nt

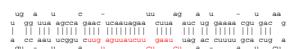


25-nt

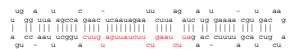


**MIR776**

21-nt



24-nt



23-nt

**MIR780**

21-nt



24-nt



23-nt



24-nt



23-nt



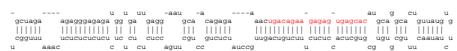
24-nt



**Figure S1B:** Position of long-miRNA sequences retrieved from the MPSS PLUS database on their precursor

### MIR156b

20-nt



23-nt



25-nt



### MIR156c

21-nt



23-nt



23-nt



### MIR157c

21-nt

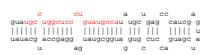


23-nt



### MIR160a

21-nt



24-nt



### MIR163

24-nt



26-nt



### MIR165a

21-nt



23-nt



**MIR166a**

21-nt



23-nt

**MIR167a**

21-nt



23-nt

**MIR167b**

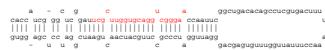
21-nt



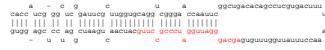
23-nt

**MIR168a**

21-nt



23-nt

**MIR169a**

21-nt



24-nt



25-nt

**MIR169g**

21-nt



24-nt



**MIR169h**

21-nt



23-nt

**MIR169i**

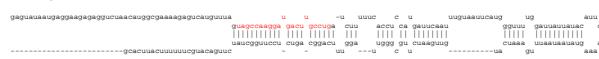
21-nt



23-nt

**MIR169j**

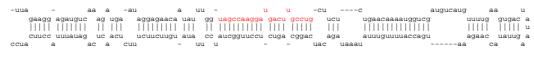
21-nt



23-nt

**MIR169k**

21-nt



23-nt

**MIR169n**

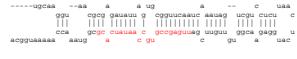
21-nt



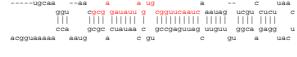
23-nt

**MIR171b**

21-nt



24-nt

**MIR319b**

20-nt



24-nt

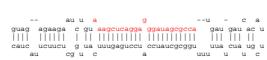


**MIR390a**

21-nt



23-nt



24-nt

**MIR390b**

21-nt



23-nt



24-nt

**MIR391**

21-nt



24-nt

**MIR393a**

21-nt



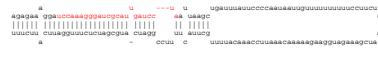
24-nt

**MIR393b**

21-nt



24-nt

**MIR394a**

20-nt



24-nt



**MIR775**

21-nt

23-nt

24-nt

**MIR783**

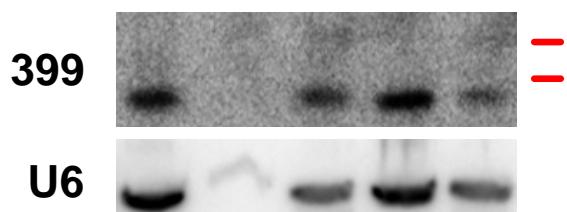
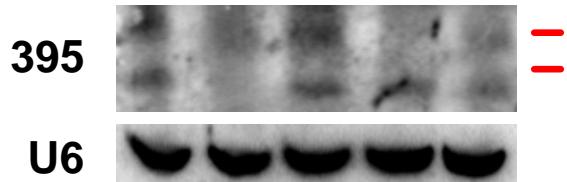
21-nt

23-nt

24-nt

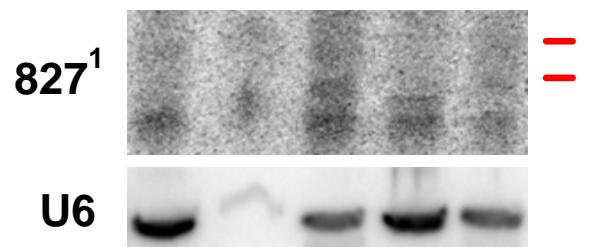
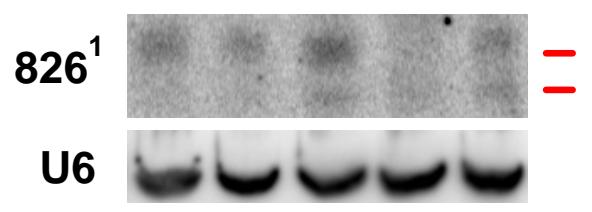
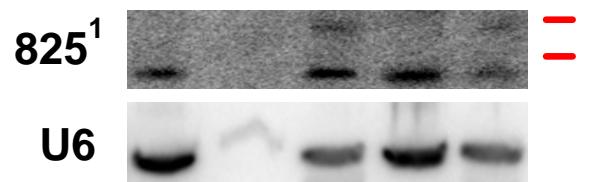
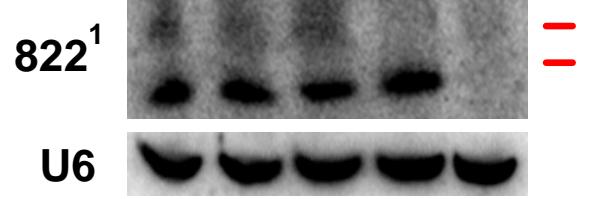
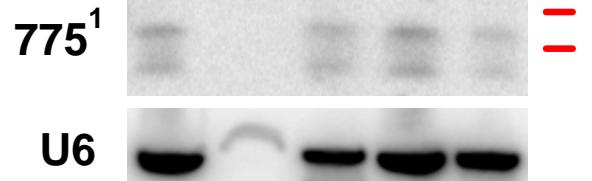
## Rosette L

Col-0 dcl1-9 dcl2-5 dcl3-1 dcl4-2



## Rosette L

Col-0 dcl1-9 dcl2-5 dcl3-1 dcl4-2



NameGenomic LocationPredicted Hairpin

IR1

between At5g55010 and At5g55020



IR2

between At1g67480 and At1g67490



IR3

between At5g03550 and At5g03555



IR4

overlaps At4g21366 and At4g21370



IR5

1.968.912  
1.965.271

spans At3g06435

Table SI. Changes in the distribution of the size of miRNA sequences<sup>a</sup> in leaf and inflorescence.

miRNA length (nt)	Normalized Nr. reads <sup>a</sup>		miRNA Class	Ratio reads Inf/Leaf
	Leaf	Inflorescences		
20	1881	5591		
21	67002	61471	Canonical	0.99
22	2085	3430		
23	158	365		
24	67	331	Long	2.97
25	11	6		

<sup>a</sup> Based on 71,294 leaf reads and 36,104 inflorescence reads of smRNA sequences derive from *MIR* genes in the datasets of (14) and (17). *MIR163*-derived smRNAs were excluded.

Data were normalized for equal numbers of reads obtained with the two organs.

Table SII. Distribution of miRNAs sequences on IR loci\*.

	Tissue datasets	Nr. unique miRNAs	Nr. reads	% matching both strands	% of Single Hit matching	
					Forward strand	Reverse strand
<b>IR1</b>	Leaves	31	93	2.2	3.3	<b>96.7</b>
	Inflorescences	56	140	0.7	2.2	<b>97.8</b>
<b>IR2</b>	Leaves	4	10	0.0	0.0	<b>100.0</b>
	Inflorescences	36	289	0.0	0.3	<b>99.7</b>
<b>IR3</b>	Leaves	18	96	0.0	1.0	<b>99.0</b>
	Inflorescences	15	126	0.0	0.0	<b>100.0</b>
<b>IR4</b>	Leaves	5	5	80.0	0	<b>100.0</b>
	Inflorescences	61	96	95.8	0	<b>100.0</b>
<b>IR5</b>	Leaves	794	3180	92.6	2.5	<b>97.5</b>
	Inflorescences	2090	4746	85.0	9.3	<b>90.7</b>

\* Analysis of datasets of (14) and (17).