Figure S1 - page 1

Eudicots













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Monocots









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Figure S1. The RNA secondary structure of Bowtie-validated MIR2275 homologs. Secondary structures of loci were predicted via mFOLD. If the potential MIR2275 homolog formed a stem-and-loop structure, it is shown above. The red nucleotides denote the mature MIR2275 homolog in the secondary structure. Phalli Oropetium Osativa Bdistachyon Pvirgatum Acoerulea Sitalica Sviridis Zmays Acomosus Mdomestica vesca Ppersica Lusitatissimum Cclementina Csinesis Ptrichocarpa Mesculenta Spurpurea Vinifera Dcarota Phalli Oropetium Osativa Bdistachyon Pvirgatum Acoerulea Sitalica Sviridis Zmays Acomosus Moomestica vesca Ppersica Lusitatissimum Cclementina Csinesis Ptrichocarpa Mesculenta Spurpurea Vinifera Dcarota والمراجع miRNA Phalli Oropetium

57 55 58 63 57 61 58 61 61 67 73 80 GGGDGG - TTGAATTTGAGAATTGGATGGAACCAAATCTTG- - TTTGOGAA- - - - AGOGAGG CAAGACTT 141 127 117 131 119 119 112 119 132 120 127 133 125 135 122 138 129 135 151

 miRNA

 GGITTICCTCCAATATCTCA GITCAGCTG

 GGITTICCTCCAATATCTCA GITCAGCTGITCAGA

 AGACAGTGATT

 AGACAGTGAT

 AGACAGTGAACAACATCCTCGGTCAAGTAGCAAC

 GGITTICCTCCAATATCTCA

 CCCAATATCTCA

 AGTTAGTCAATTCTCA

 AGTTAGTCAAACATTGACACTGCCC

 AGTTTCCTCCAATATCTCA

 AGTTTCCTCCAATATCTCA

 CCCAATATCTCA

 AGTTTCCTCCAATATCTCA

 GTTTTCCTCCAATATCTCA

 CCTCCAATATCTCA

 GTTTTCCTCCAATATCTCA

 CCTCCAATATCTCA

 GTTTCCTCCAATATCTCA

 CCTCCAATATCTCA

 GGTTTCCTCCAATATCTCA

 GTTTCCTCCAATATCTCA

 GGTTTCCTCCAATATCTCA

 GGTTTCCTCCAATATCTCA

 GGTTTCCTCCAATATCTCA

 GGTTTCCTCCAATATCTCA

 GGTTTCCTCCAATATCTCA

 GGTTTCCTCCAATATCTCA

 GGTTTCCTCCAATATCTCA

 GCCACCTAA 189 193 189 202 190 186 209 193 197 Lusitatissimum Cclementina 208 196 210 209 214 225 . . 270.

Figure S2. Alignment of MIR2275 homologs.

Osätiva

Bdistachyon Pvirgatum

Acoerulea Sitalica Sviridis

Zmays Acomosus

vesca Ppersica

Csinesis Ptrichocarpa Mesculenta

Spurpurea Vinifera

Dcarota

Mdomestica Evesca

Sequences corresponding to miR2275 wih flanking 200 bp islands were extracted from genomes of the above-listed genomes and aligned via ClustalX2. Only the 100th to 340th nucleotide of each sequence (including gaps) are shown. miR2275 and miR2275* in each species are shown.



Figure S3. Determination of phase score cutoffs using three different algorithms (a) Small RNA accumulation at 27 known 21 nt PHAS loci (Table S1) was analyzed using the PHAS-Test algorithm from Guo et al. (2015), which is used by ShortStack. Variables and formula are shown. Red line represents the cut-off used in this study to determine if a locus was phased or not; scores above the red line were considered 'phased'.

(b) Same as in panel a except for the PHAS-Test algorithm from Dotto et al. (2014).

(c) Same as in panel a except for the PHAS-Test algorithm from Zheng et al. (2014); scores below the red-line were considered 'phased'.



Figure S4. Phase scores of 27 previously known A. thaliana phased siRNA loci from the indicated algorithms. Cutoff values for called 'phasing' in our study are shown in red.



Figure S5. Genome browser snapshots of the three Arabidopsis thaliana PHAS-Test passing loci.

(a) Alignments and read size distribution of Locus_13139. Red reads represent positive-strand mapped genes, while blue reads are those that mapped to the negative strand. Numbers in the brackets are the range of coverage shown in Reads per Million.

(b) Same as a, except for Locus_17614.

(c) Same as a, except for Locus_32524.



Locus_13139



Locus_17614



Locus_32524



TAS2

Figure S6. The three PHAS-Test passing loci have inconsistent phase register frequencies. Frequencies of phase registers where calculated in eight publicly available wild-type inflorescence libraries from A. thaliana (the accession numbers are listed in the grey boxes). TAS2 is shown for comparison. Phase register frequencies are calculated via the following formula: (number of reads "in phase"/ total number of reads at locus)*100.



Figure S7. The three putative 24 nt PHAS loci show AGO-loading profiles that are distinct from TAS loci.

Small RNAs from immunoprecipitation protein were aligned to the A. thaliana (TAIR10) genome. Numbers indicate the ratio of sRNA accumulation between immunoprecipitated and total libraries (in RPMs).

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Figure S9. rdr6-dependent, 24 nt-dominated loci' characteristics compared to other 24nt-dominated loci.

(a) The overlap between rdr6-dependent, 24 nt-dominated loci with genes and transposons was calculated as in Figure 3a. Numbers at the top indicate the count in each category.

(b) The proportion of multi-mapping reads produced at rdr6-dependent, 24 nt-dominated loci compared to other 24 nt-dominated loci. Numbers at the top indicate the count in each category.

(c) Same as panel b except showing small RNA accumulation (in RPM). Amount in each category is the same as panel b.

(d) Same as panel b except showing length (in nts). Amount in each category is the same as panel b.



Figure S10. Several 24 nt-dominated small RNA loci pass PHAS-detection algorithms in four other eudicots.

Venn diagram shows numbers of 24 nt-dominated loci that were called 'phased' by the indicated algorithms. Species examined is shown above the graphs.

Chr.	Start	Stop	Locus Name	Source
Chr2	11721539	11722468	TAS1a	(Vazquez et al., 2004)
Chr1	18549204	18550042	TAS1b	(Allen et al., 2005)
Chr2	16537288	16538277	TAS1c	(Allen et al., 2005)
Chr2	16539384	16540417	TAS2	(Allen et al., 2005)
Chr3	5861491	5862437	TAS3a	(Montgomery et al., 2008)
Chr5	20134200	20134786	TAS3b	(Howell et al., 2007)
Chr5	23394005	23394500	TAS3c	(Howell et al., 2007)
Chr3	9415004	9422587	TAS4	(Rajagopalan et al., 2006)
Chr1	23299057	23300958	PPR-At1g62910	(Ronemus et al., 2006)
Chr1	23412730	23415149	PPR-At1g63130	(Ronemus et al., 2006)
Chr1	23306534	23308683	PPR-At1g62930	(Ronemus et al., 2006)
Chr1	23387631	23390816	PPR-At1g63080	(Ronemus et al., 2006)
Chr1	23507320	23509053	PPR-At1g63400	(Ronemus et al., 2006)
Chr1	23419396	23421579	PPR-At1g63150	(Ronemus et al., 2006)
Chr1	23385324	23387167	PPR-At1g63070	(Ronemus et al., 2006)
Chr1	23489840	23491519	PPR-At1g63330	(Ronemus et al., 2006)
Chr1	23176930	23179248	PPR-At1g62590	(Ronemus et al., 2006)
Chr5	15555156	15558732	TIR-NBS-LRR-At5g38850	(Howell et al., 2007)
Chr1	4368760	4371293	AFB3	(Si-Ammour et al., 2011)
Chr5	16638370	16641728	ATCHX18	(Howell et al., 2007)
Chr1	17886098	17892586	AGO1	(Axtell et al., 2006)
Chr3	23273116	23276375	TIR1	(Si-Ammour et al., 2011)
Chr4	1404887	1407139	AFB1	(Si-Ammour et al., 2011)
Chr3	9867845	9870640	AFB2	(Si-Ammour et al., 2011)
Chr5	15757717	15758109	SLG	(Chen et al., 2007)
Chr4	8380848	8383496	CC-NBS-LRR-At4g14610	(Zhai et al., 2011)
Chr4	8146345	8152131	MET2	(Chen et al., 2010)

Table S1. List of 25 known 21 nt PHAS loci in A. thaliana.

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Accession Number	Genotype 3'	Adapter (First 8 nts)	Source
GSM2825283	Wild-type Replicate 1	TGGAATTC	(Polydore & Axtell, 2018)
GSM2825284	Wild-type Replicate 2	TGGAATTC	(Polydore & Axtell, 2018)
GSM2825285	Wild-type Replicate 3	TGGAATTC	(Polydore & Axtell, 2018)
GSM2825286	rdr1-1/2-1/6-15 Replicate 1	TGGAATTC	(Polydore & Axtell, 2018)
GSM2825287	rdr1-1/2-1/6-15 Replicate 2	TGGAATTC	(Polydore & Axtell, 2018)
GSM2825288	rdr1-1/2-1/6-15 Replicate 3	TGGAATTC	(Polydore & Axtell, 2018)
GSM1533527	Wild-type Replicate 1	TGGAATTC	(Groth et al., 2014)
GSM1533528	Wild-type Replicate 2	TGGAATTC	(Groth et al., 2014)
GSM1533529	Wild-type Replicate 3	TGGAATTC	(Groth et al., 2014)
GSM1533542	<i>dcl3</i> Replicate 1	TGGAATTC	(Groth et al., 2014)
GSM1533543	dcl3 Replicate 2	TGGAATTC	(Groth et al., 2014)
GSM1533544	dcl3 Replicate 3	TGGAATTC	(Groth et al., 2014)
GSM1845210	Wild-type Replicate 1	AGATCGGA	(Elvira-Matelot et al., 2016)
GSM1845211	Wild-type Replicate 2	AGATCGGA	(Elvira-Matelot et al., 2016)
GSM1845212	Wild-type Replicate 3	AGATCGGA	(Elvira-Matelot et al., 2016)
GSM1845222	<i>dcl2-1/3-1/4-2t</i> Replicate 1	AGATCGGA	(Elvira-Matelot et al., 2016)
GSM1845223	<i>dcl2-1/3-1/4-2t</i> Replicate 2	AGATCGGA	(Elvira-Matelot et al., 2016)
GSM1845224	<i>dcl2-1/3-1/4-2t</i> Replicate 3	AGATCGGA	(Elvira-Matelot et al., 2016)
GSM1087973	Wild-type Replicate 1	TCGTATGC	(Jeong et al., 2013)
GSM1087974	Wild-type Replicate 2	TCGTATGC	(Jeong et al., 2013)
GSM1087975	dcl1-7 Replicate 1	TCGTATGC	(Jeong et al., 2013)
GSM1087976	dcl1-7 Replicate 2	TCGTATGC	(Jeong et al., 2013)
GSM1377370	Wild-type Replicate 1	TGGAATTC	(Li et al., 2014)
GSM1377371	Wild-type Replicate 2	TGGAATTC	(Li et al., 2014)
GSM1377372	nrpd1-3 Replicate 1	TGGAATTC	(Li et al., 2014)
GSM1377373	nrpd1-3 Replicate 2	TGGAATTC	(Li et al., 2014)
GSM1377376	rdr2-1 Replicate 1	TGGAATTC	(Li et al., 2014)

Table S2. A. thaliana small RNA libraries used in this study

GSM1377377	rdr2-1 Replicate 2	TGGAATTC	(Li et al., 2014)
GSM2102962	Wild-type Replicate 1	TGGAATTC	(Panda et al., 2016)
GSM2102963	Wild-type Replicate 2	TGGAATTC	(Panda et al., 2016)
GSM2102965	rdr6-15 Replicate 1	TGGAATTC	(Panda et al., 2016)
GSM2102462	rdr6-15 Replicate 2	TGGAATTC	(Panda et al., 2016)
GSM893112	Wild-type Replicate 1	CACTCGGG	(Lee et al., 2012)
GSM893113	Wild-type Replicate 2	CACTCGGG	(Lee et al., 2012)
GSM893114	Wild-type Replicate 3	CACTCGGG	(Lee et al., 2012)
GSM893115	nrpb1-1 (nrpe) Replicate 1	CACTCGGG	(Lee et al., 2012)
GSM893116	nrpb1-1 (nrpe) Replicate 2	CACTCGGG	(Lee et al., 2012)
GSM893117	nrpb1-1 (nrpe) Replicate 3	CACTCGGG	(Lee et al., 2012)
GSM1668899	Wild-type Replicate 1	TGGAATTC	(Zhai et al., 2015)
GSM1668905	Wild-type Replicate 2	TGGAATTC	(Zhai et al., 2015)

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