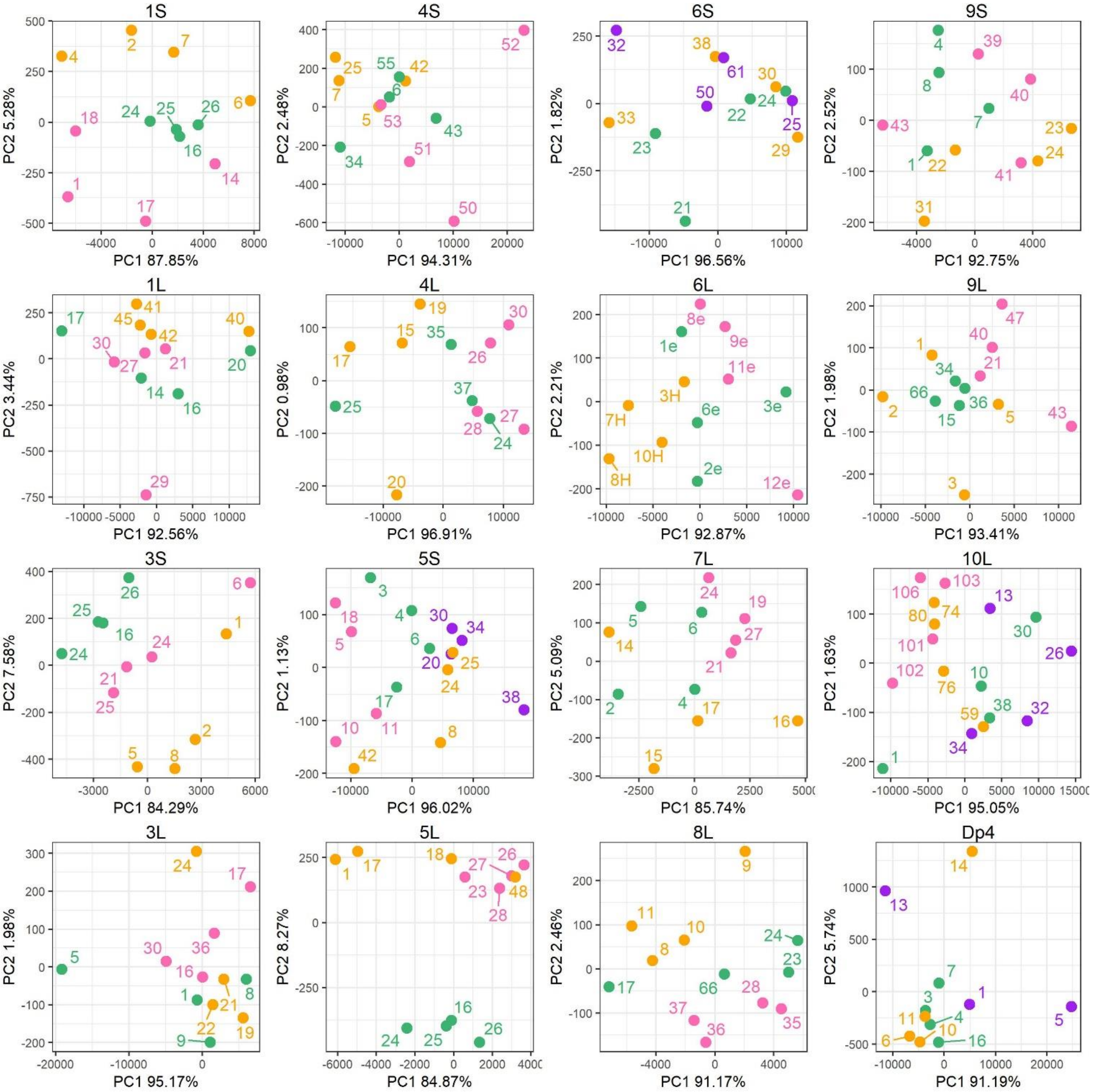
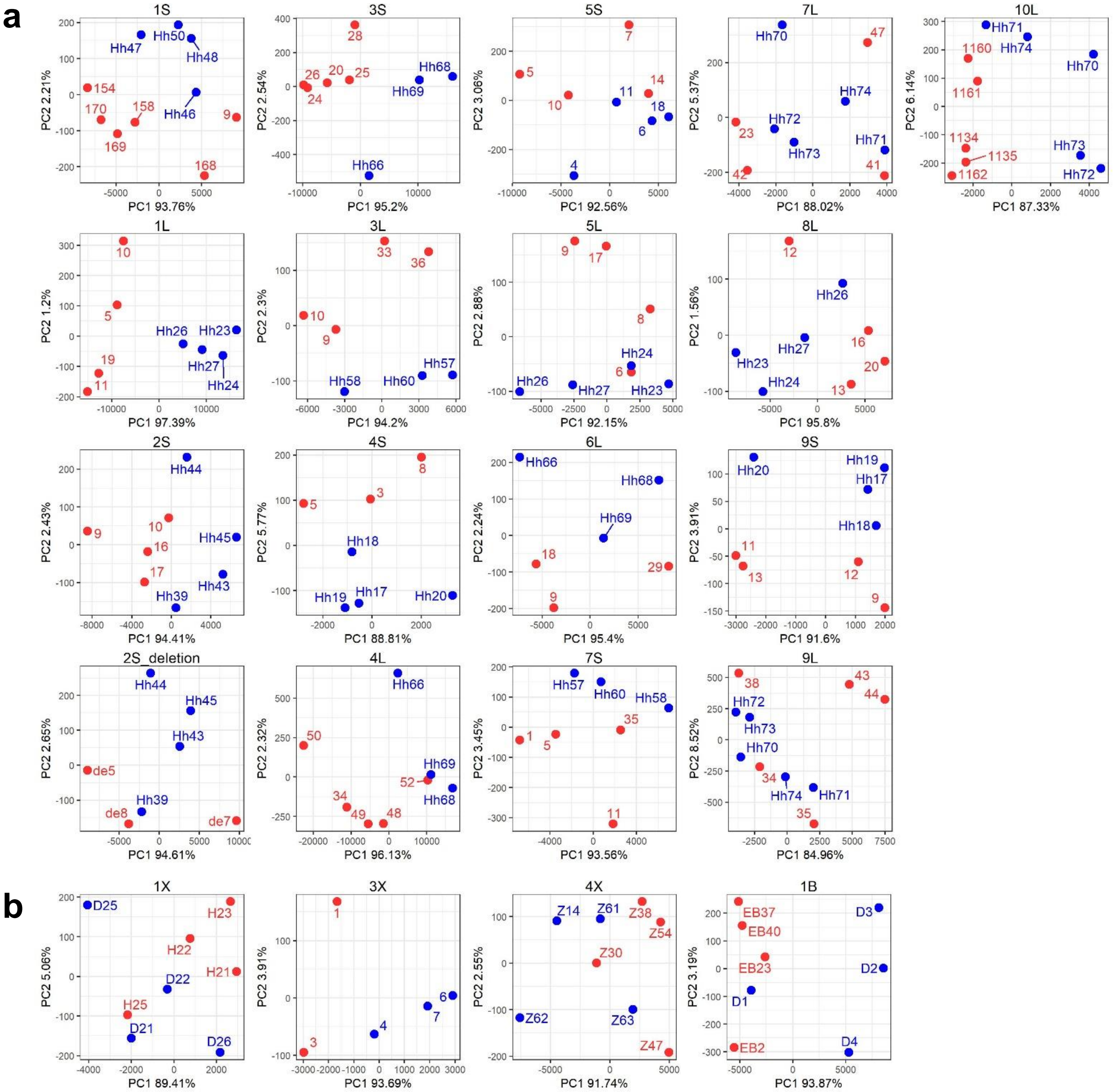
# Dosage-sensitive miRNAs trigger modulation of gene expression during genomic imbalance in maize

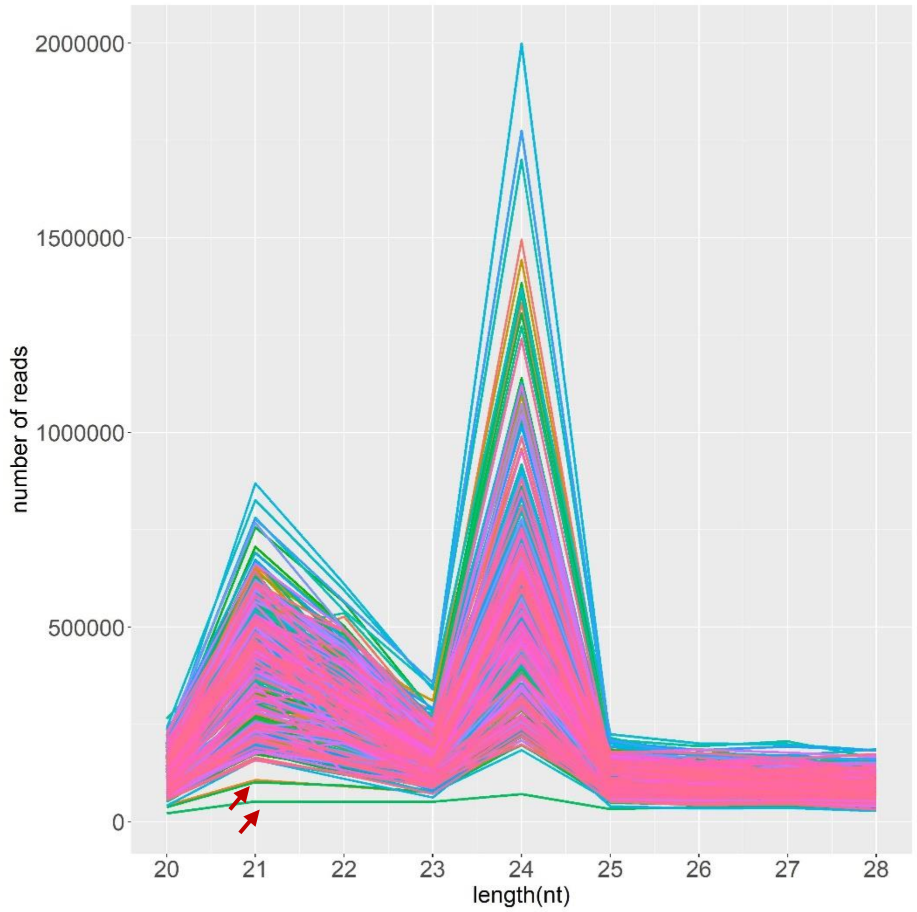
Shi *et al.*



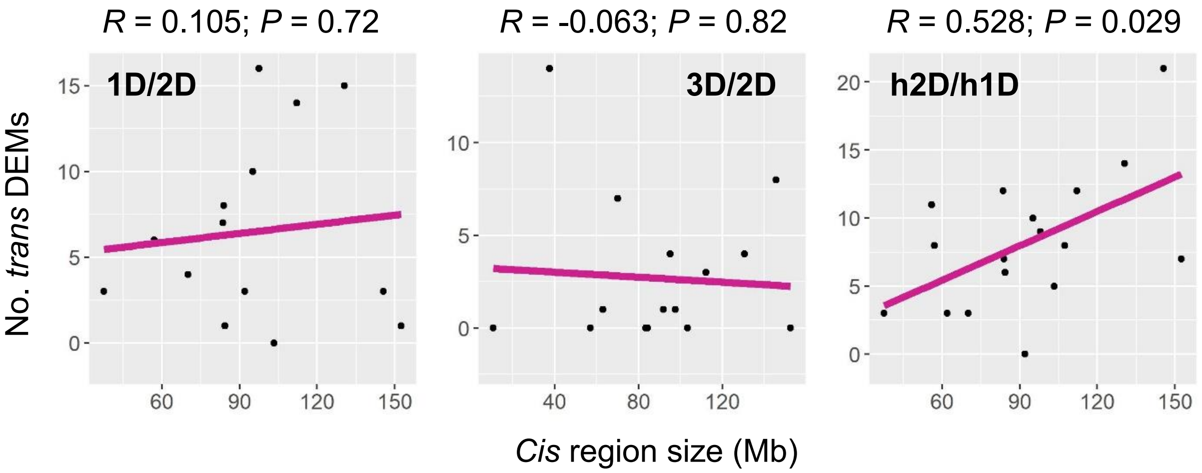
**Supplementary Fig. 1. PCA plots of diploid aneuploids, including monosomies, trisomies, tetrasomies, and the corresponding diploid control.** PCA plots were generated with normalized read counts. Data points of biological replicates from the same experimental or control group were labeled with their sample IDs (Supplementary Data 1). Each panel represents biological replicates from the experimental and control groups. Each group is labeled with a different color (1D, pink; 2D, green; 3D, orange; 4D, purple).



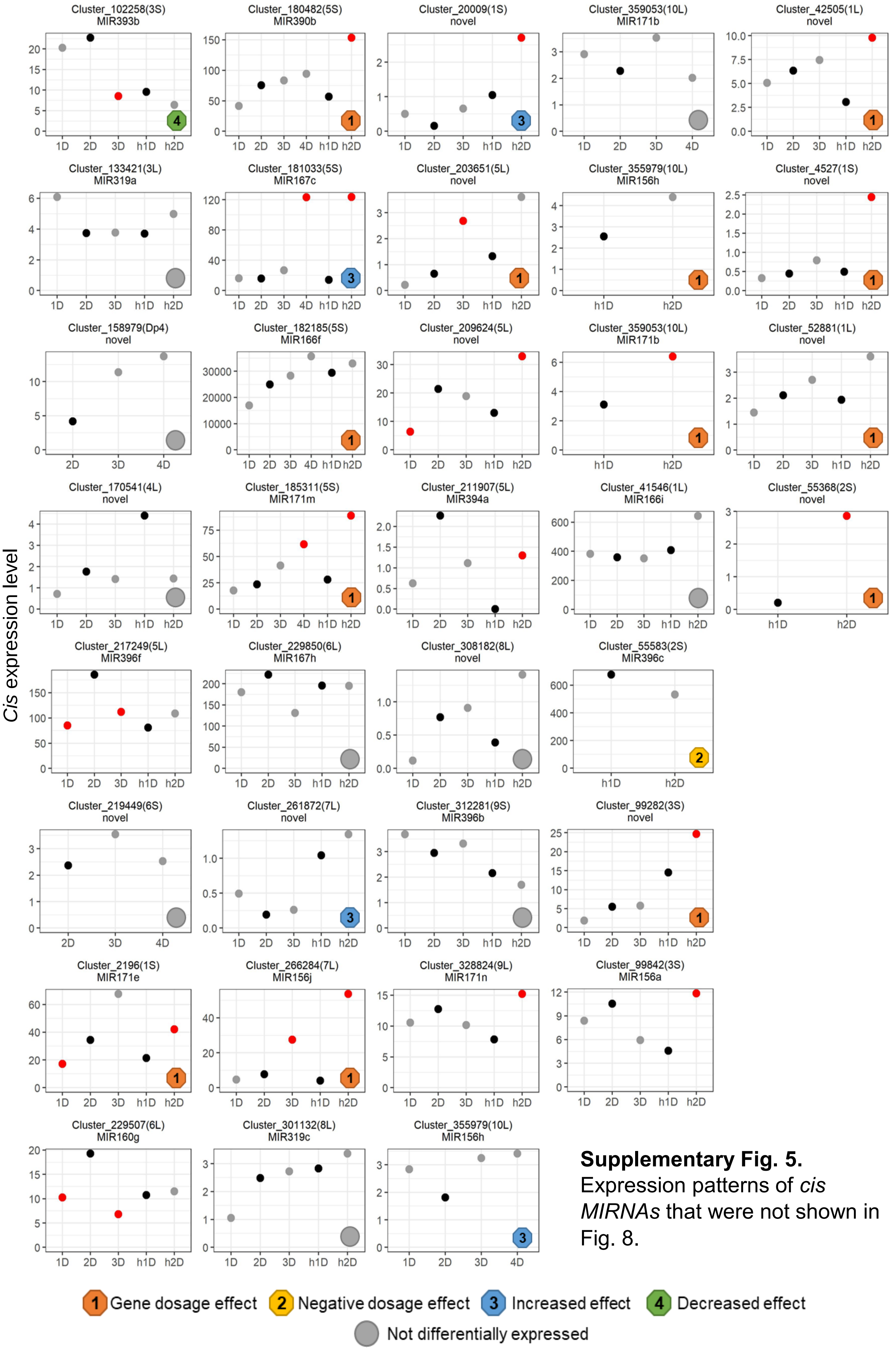
**Supplementary Fig. 2. PCA plots of haploid aneuploids, polyploids, and diploids with one B chromosome.** PCA plots were generated as described in Supplementary Fig. 1. **a** PCA plots of disomies (red) and the corresponding haploid control (blue). **b** PCA plots of polyploids and diploids with one B chromosome compared to diploids. The control group is labeled in blue (diploids) while the experimental group is labeled in red.



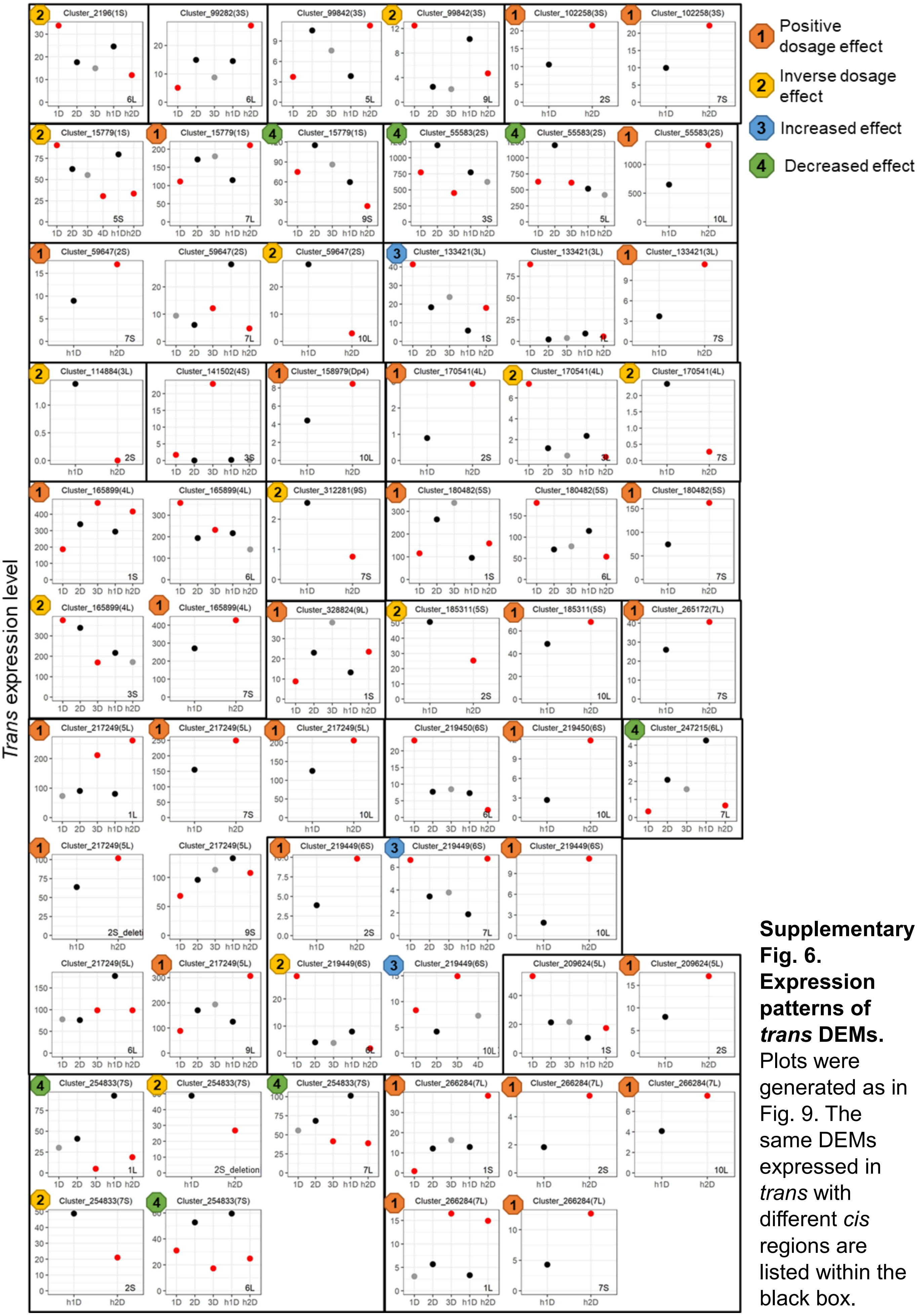
**Supplementary Fig. 3. Read length distribution of sRNA libraries.** Read length distribution of sRNA libraries after structural RNAs had been removed. Libraries that did not show distinct peaks at 21 and 24 nt are pointed by red arrows and were thus excluded from further analysis.



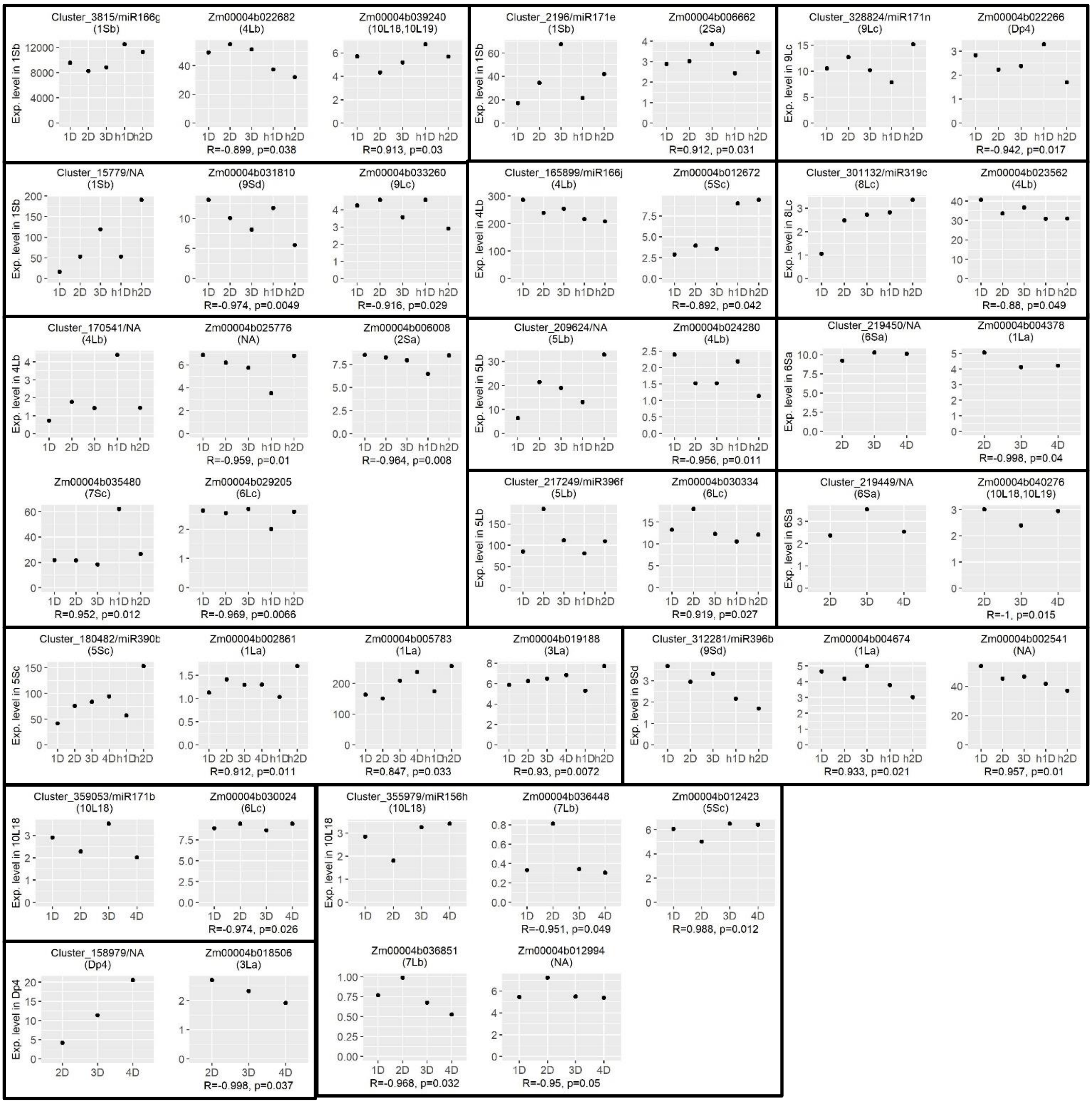
**Supplementary Fig. 4. Correlation between the number of *trans* DEMs and the corresponding size of the *cis* region.** The size of the *cis* region was computed in previous studies [1,2]. The number of DEMs was calculated as in Supplementary Data 3. *P*-value (*P*) represents the significance of Pearson correlation (two-sided; confidence intervals, 95%; no adjustment made for multiple comparisons), whereas *R* denotes the Pearson correlation coefficient.



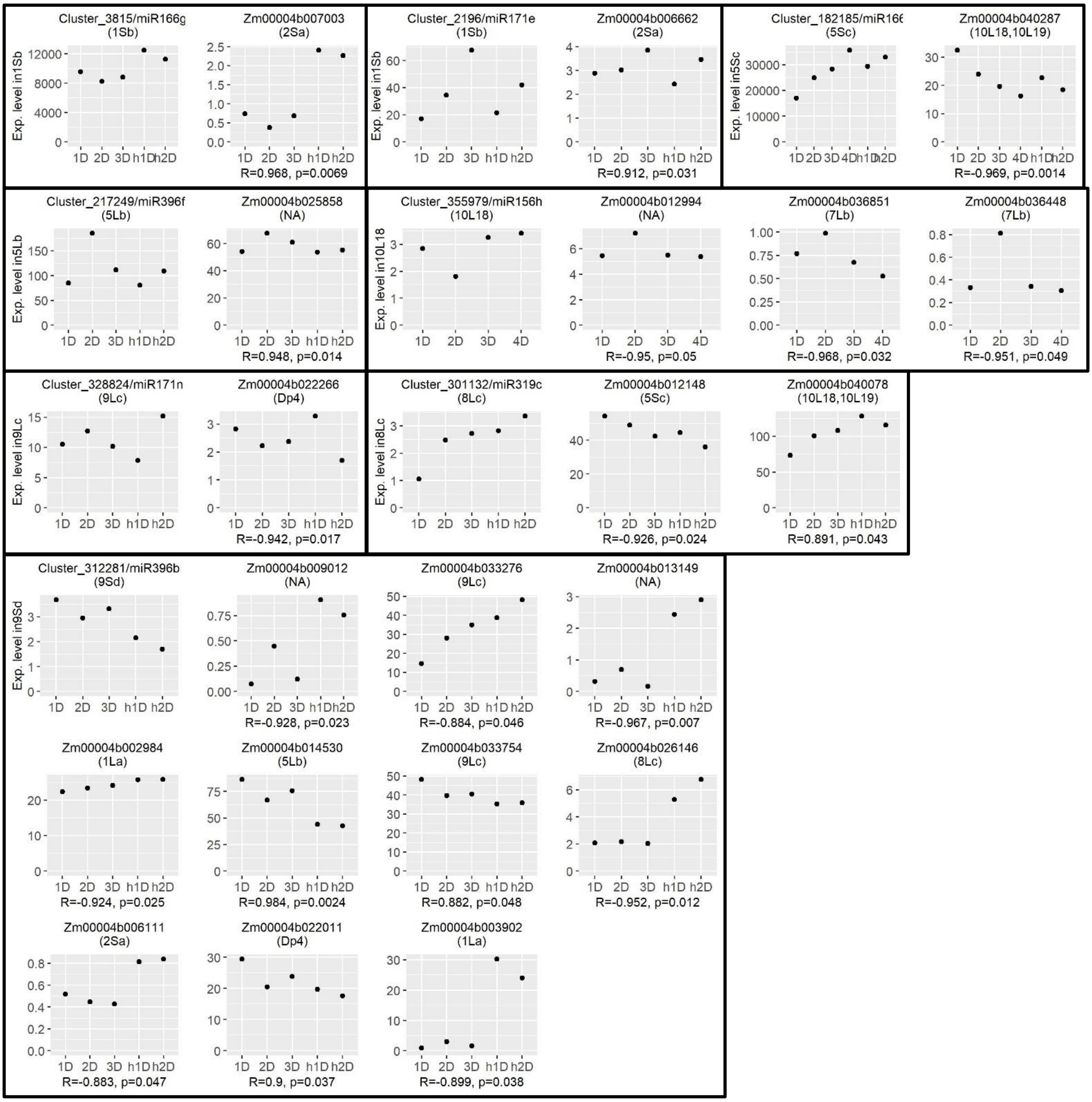
**Supplementary Fig. 5.** Expression patterns of *cis MIRNAs* that were not shown in Fig. 8.



**Supplementary Fig. 6. Expression patterns of *trans* DEMs.** Plots were generated as in Fig. 9. The same DEMs expressed in *trans* with different *cis* regions are listed within the black box.



**Supplementary Fig. 7. Expression patterns of *cis* miRNAs and their *trans* targets predicted by psRNATarget.** The x-axis refers to the dosage of the chromosomal region as in the y-axis label, whereas the y-axis designates the expression level (rpm) of each *MIRNA* in *cis* or each predicted target in *trans* (normalized read counts) corresponding to the genotype on the x-axis. The *cis* location of each *MIRNA* or each target is depicted within parentheses. *R*, Pearson correlation coefficient between expression levels of each *MIRNA* and its corresponding target. p, *P*-values for Pearson correlation (two-sided; confidence intervals, 95%; no adjustment made for multiple comparisons).



**Supplementary Fig. 8. Expression patterns of *cis* miRNAs and their *trans* targets predicted by degradome sequencing plotted as in Supplementary Fig. 7.**

**Supplementary Table 1. The proportion of *trans* DEGs out of expressed *trans* genes (% *trans* DEGs) and that of *trans* DEMs out of expressed *trans* miRNAs (% *trans* DEMs) in each comparison.** Data for differential gene expression were from previous studies [1,2]. Numbers in bold and underlined refer to the greater proportion out of % *trans* DEMs and % *trans* DEGs.

| | 1D/2D | | 3D/2D | | 4D/2D | | h2D/h1D | |
|---|---|---|---|---|---|---|---|---|
| *cis* region | % *trans* DEG | % *trans* DEM | % *trans* DEG | % *trans* DEM | % *trans* DEG | % *trans* DEM | % *trans* DEG | % *trans* DEM |
| 10L18 | 24.4% | **37.2%** | **19.7%** | 2.3% | **11.7%** | 7.0% | | |
| 10L19 | | | | | | | **47.8%** | 19.1% |
| 1La | **18.9%** | 6.8% | **26.1%** | 13.6% | | | 26.4% | **47.7%** |
| 1Sb | 37.4% | **40.5%** | **17.2%** | 10.8% | | | 11.8% | **36.8%** |
| 2Sa_deletion | | | | | | | **17.7%** | 7.0% |
| 2Sa | | | | | | | **26.2%** | 19.0% |
| 3La | **11.1%** | 2.4% | **6.2%** | 0.0% | | | **25.2%** | 16.3% |
| 3Sb | **39.5%** | 7.5% | **36.3%** | 35.0% | | | **20.3%** | 6.7% |
| 4Lb | **9.9%** | 2.4% | **7.1%** | 0.0% | | | **33.3%** | 13.0% |
| 4Sa | **12.9%** | 0.0% | **4.0%** | 0.0% | | | **12.7%** | 10.6% |
| 5Lb | **41.1%** | 11.1% | **43.2%** | 19.4% | | | **21.6%** | 7.3% |
| 5Sc | 5.8% | **20.0%** | **2.2%** | 0.0% | 9.3% | **15.0%** | **24.8%** | 17.9% |
| 6Lc | 12.1% | **35.0%** | **13.3%** | 7.5% | | | **28.4%** | 25.0% |
| 6Sa | | | **1.1%** | 0.0% | **2.3%** | 0.0% | | |
| 7Lb | 4.0% | **23.3%** | 4.1% | **9.3%** | | | **33.8%** | 22.7% |
| 7Sc | | | | | | | 10.7% | **25.0%** |
| 8Lc | 1.3% | **6.8%** | 2.0% | **2.3%** | | | **11.0%** | 0.0% |
| 9Lc | 5.8% | **15.6%** | **4.1%** | 0.0% | | | **40.1%** | 25.0% |
| 9Sd | **9.6%** | 13.3% | **5.7%** | 0.0% | | | 11.3% | **17.4%** |
| Dp4 | | | 2.0% | **2.3%** | **5.1%** | 0.0% | | |

**Supplementary Table 2. Normalized read counts and *P*-values from DME analysis of all expressed *cis* miRNAs.** DME analysis was performed by edgeR. Y, FDR < 0.05; N, FDR ≥ 0.05. Grey shades refer to data that is not available.

| Cluster name | *Cis* region | Normalized read counts (rpm) | | | | | | Statistically significant (Y/N) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1D | 2D | 3D | 4D | h1D | h2D | 1D/2D | 3D/2D | 4D/2D | h2D/h1D |
| Cluster_41546 | 1La | 382.50 | 359.52 | 352.40 | | 408.34 | 644.09 | N | N | | N |
| Cluster_42505 | 1La | 5.07 | 6.33 | 7.44 | | 3.07 | 9.79 | N | N | | Y |
| Cluster_52881 | 1La | 1.45 | 2.11 | 2.70 | | 1.94 | 3.61 | N | N | | N |
| Cluster_10766 | 1Sb | 2.48 | 6.42 | 16.77 | | 2.22 | 4.79 | Y | Y | | Y |
| Cluster_15779 | 1Sb | 16.19 | 53.18 | 119.32 | | 52.83 | 191.00 | Y | Y | | Y |
| Cluster_20009 | 1Sb | 0.51 | 0.15 | 0.66 | | 1.05 | 2.71 | N | N | | Y |
| Cluster_2196 | 1Sb | 17.14 | 34.48 | 67.62 | | 21.39 | 41.99 | Y | N | | Y |
| Cluster_3815 | 1Sb | 9578.26 | 8241.52 | 8827.19 | | 12524.63 | 11276.97 | N | N | | N |
| Cluster_4527 | 1Sb | 0.33 | 0.45 | 0.80 | | 0.50 | 2.44 | N | N | | Y |
| Cluster_55368 | 2Sa | | | | | 0.20 | 2.87 | | | | Y |
| Cluster_55583 | 2Sa | | | | | 677.16 | 533.60 | | | | N |
| Cluster_59647 | 2Sa | | | | | 14.72 | 5.47 | | | | Y |
| Cluster_133421 | 3La | 6.09 | 3.73 | 3.77 | | 3.70 | 4.99 | N | N | | N |
| Cluster_102258 | 3Sb | 20.28 | 22.74 | 8.56 | | 9.59 | 6.42 | N | Y | | N |
| Cluster_99282 | 3Sb | 1.89 | 5.49 | 5.74 | | 14.51 | 24.68 | N | N | | Y |
| Cluster_99842 | 3Sb | 8.37 | 10.55 | 5.92 | | 4.59 | 11.85 | N | N | | Y |
| Cluster_165899 | 4Lb | 286.58 | 238.70 | 253.90 | | 216.52 | 207.89 | N | N | | N |
| Cluster_170541 | 4Lb | 0.72 | 1.76 | 1.42 | | 4.40 | 1.44 | N | N | | N |
| Cluster_203651 | 5Lb | 0.22 | 0.65 | 2.68 | | 1.32 | 3.61 | N | Y | | N |
| Cluster_209624 | 5Lb | 6.41 | 21.42 | 18.90 | | 13.03 | 32.93 | Y | N | | Y |
| Cluster_211907 | 5Lb | 0.62 | 2.26 | 1.12 | | 0.00 | 1.30 | N | N | | Y |
| Cluster_217249 | 5Lb | 85.13 | 185.72 | 111.84 | | 80.90 | 108.90 | Y | Y | | N |
| Cluster_180482 | 5Sc | 41.61 | 75.47 | 83.43 | 94.13 | 56.92 | 153.36 | N | N | N | Y |
| Cluster_181033 | 5Sc | 16.24 | 15.79 | 26.93 | 123.26 | 14.04 | 123.65 | N | N | Y | Y |
| Cluster_182185 | 5Sc | 16990.12 | 24977.63 | 28304.42 | 35703.11 | 29384.64 | 32944.01 | N | N | N | N |
| Cluster_185311 | 5Sc | 17.64 | 23.62 | 41.54 | 61.64 | 28.15 | 88.88 | N | N | Y | Y |
| Cluster_229507 | 6Lc | 10.28 | 19.30 | 6.83 | | 10.77 | 11.49 | Y | Y | | N |
| Cluster_229850 | 6Lc | 179.99 | 221.78 | 131.21 | | 195.61 | 195.55 | N | N | | N |
| Cluster_219449 | 6Sa | | 2.36 | 3.55 | 2.53 | | | | N | N | |
| Cluster_219450 | 6Sa | | 9.21 | 10.31 | 10.11 | | | | N | N | |
| Cluster_261872 | 7Lb | 0.50 | 0.19 | 0.26 | | 1.04 | 1.34 | N | N | | N |
| Cluster_265172 | 7Lb | 19.31 | 20.80 | 26.48 | | 21.05 | 24.93 | N | N | | N |
| Cluster_266284 | 7Lb | 4.60 | 7.72 | 27.55 | | 4.08 | 53.74 | N | Y | | Y |
| Cluster_273394 | 7Lb | 1.43 | 4.88 | 9.62 | | 5.16 | 17.41 | Y | Y | | Y |
| Cluster_254833 | 7Sc | | | | | 93.45 | 42.21 | | | | Y |
| Cluster_301132 | 8Lc | 1.06 | 2.49 | 2.73 | | 2.83 | 3.37 | N | N | | N |
| Cluster_308182 | 8Lc | 0.12 | 0.77 | 0.91 | | 0.39 | 1.41 | N | N | | N |
| Cluster_328824 | 9Lc | 10.53 | 12.73 | 10.16 | | 7.84 | 15.22 | N | N | | Y |
| Cluster_312281 | 9Sd | 3.69 | 2.95 | 3.32 | | 2.16 | 1.70 | N | N | | N |
| Cluster_355979 | 10L18 | 2.84 | 1.81 | 3.25 | 3.41 | | | N | N | N | |
| Cluster_359053 | 10L18 | 2.91 | 2.28 | 3.53 | 2.02 | | | N | N | N | |
| Cluster_355979 | 10L19 | | | | | 2.55 | 4.40 | | | | N |
| Cluster_359053 | 10L19 | | | | | 3.11 | 6.39 | | | | Y |
| Cluster_158979 | Dp4 | | 4.19 | 11.36 | 20.58 | | | | N | N | |
| Cluster_161059 | Dp4 | | 2.06 | 1.94 | 1.94 | | | | N | N | |

**Supplementary Table 3. GO Enrichment analysis on *trans* mRNA targets of *cis* miRNAs, identified in psRNATarget prediction and/or degradome sequencing data analysis.** Expression levels of these mRNA targets are significantly correlated with those of miRNAs.

| **GO biological process complete** | Zea mays - REFLIST (39385) | input (233) | input (expected) | input (over/ under) | input (fold Enrichment) | input (FDR) |
|---|---|---|---|---|---|---|
| **protein refolding (GO:0042026)** | 58 | 6 | 0.34 | + | 17.49 | 0.0048 |
| **chaperone cofactor-dependent protein refolding (GO:0051085)** | 71 | 6 | 0.42 | + | 14.29 | 0.0142 |
| 'de novo' posttranslational protein folding (GO:0051084) | 71 | 6 | 0.42 | + | 14.29 | 0.0142 |
| 'de novo' protein folding (GO:0006458) | 81 | 6 | 0.48 | + | 12.52 | 0.0289 |
| **developmental process (GO:0032502)** | 882 | 18 | 5.22 | + | 3.45 | 0.0164 |
| regulation of RNA biosynthetic process (GO:2001141) | 2761 | 44 | 16.33 | + | 2.69 | 4.69E-06 |
| **regulation of transcription, DNA-templated (GO:0006355)** | 2761 | 44 | 16.33 | + | 2.69 | 4.69E-06 |
| regulation of nucleic acid-templated transcription (GO:1903506) | 2761 | 44 | 16.33 | + | 2.69 | 4.69E-06 |
| regulation of nucleobase-containing compound metabolic process (GO:0019219) | 2981 | 45 | 17.63 | + | 2.55 | 1.53E-05 |
| regulation of RNA metabolic process (GO:0051252) | 2923 | 44 | 17.29 | + | 2.54 | 2.52E-05 |
| regulation of cellular macromolecule biosynthetic process (GO:2000112) | 3037 | 45 | 17.96 | + | 2.5 | 3.98E-05 |
| regulation of macromolecule biosynthetic process (GO:0010556) | 3043 | 45 | 18 | + | 2.5 | 4.09E-05 |
| regulation of cellular biosynthetic process (GO:0031326) | 3071 | 45 | 18.17 | + | 2.48 | 4.75E-05 |
| regulation of biosynthetic process (GO:0009889) | 3078 | 45 | 18.21 | + | 2.47 | 4.96E-05 |
| regulation of gene expression (GO:0010468) | 3383 | 46 | 20.01 | + | 2.3 | 0.000256 |
| regulation of nitrogen compound metabolic process (GO:0051171) | 3499 | 47 | 20.7 | + | 2.27 | 0.00024 |
| regulation of primary metabolic process (GO:0080090) | 3541 | 47 | 20.95 | + | 2.24 | 0.000467 |
| regulation of cellular metabolic process (GO:0031323) | 3589 | 46 | 21.23 | + | 2.17 | 0.0013 |
| regulation of macromolecule metabolic process (GO:0060255) | 3854 | 49 | 22.8 | + | 2.15 | 0.000787 |
| regulation of metabolic process (GO:0019222) | 3959 | 49 | 23.42 | + | 2.09 | 0.00204 |
| regulation of cellular process (GO:0050794) | 5014 | 57 | 29.66 | + | 1.92 | 0.00225 |
| regulation of biological process (GO:0050789) | 5556 | 63 | 32.87 | + | 1.92 | 0.000531 |
| biological regulation (GO:0065007) | 6277 | 69 | 37.13 | + | 1.86 | 0.000453 |
| biological_process (GO:0008150) | 22488 | 168 | 133.02 | + | 1.26 | 0.0063 |
| Unclassified (UNCLASSIFIED) | 16901 | 65 | 99.98 | - | 0.65 | 0 |

**Supplementary Table 4. GO Enrichment analysis on *trans* mRNA targets of *cis* miRNAs, identified in both psRNATarget prediction and degradome sequencing data analysis.** Expression levels of these mRNA targets are significantly correlated with those of miRNAs.

| **GO biological process complete** | Zea mays - REFLIST (39385) | input (33) | input (expected) | input (over/ under) | input (fold Enrichment) | input (FDR) |
|---|---|---|---|---|---|---|
| **transcription, DNA-templated (GO:0006351)** | 400 | 10 | 0.34 | + | 29.84 | 2.18E-09 |
| nucleic acid-templated transcription (GO:0097659) | 411 | 10 | 0.34 | + | 29.04 | 2.83E-09 |
| RNA biosynthetic process (GO:0032774) | 423 | 10 | 0.35 | + | 28.22 | 3.74E-09 |
| **developmental process (GO:0032502)** | 882 | 10 | 0.74 | + | 13.53 | 4.24E-06 |
| nucleobase-containing compound biosynthetic process (GO:0034654) | 917 | 10 | 0.77 | + | 13.02 | 6.13E-06 |
| heterocycle biosynthetic process (GO:0018130) | 1157 | 10 | 0.97 | + | 10.32 | 5.44E-05 |
| aromatic compound biosynthetic process (GO:0019438) | 1170 | 10 | 0.98 | + | 10.2 | 6.03E-05 |
| organic cyclic compound biosynthetic process (GO:1901362) | 1320 | 10 | 1.11 | + | 9.04 | 0.000185 |
| regulation of RNA biosynthetic process (GO:2001141) | 2761 | 18 | 2.31 | + | 7.78 | 1.43E-09 |
| **regulation of transcription, DNA-templated (GO:0006355)** | 2761 | 18 | 2.31 | + | 7.78 | 1.43E-09 |
| regulation of nucleic acid-templated transcription (GO:1903506) | 2761 | 18 | 2.31 | + | 7.78 | 1.43E-09 |
| regulation of RNA metabolic process (GO:0051252) | 2923 | 18 | 2.45 | + | 7.35 | 3.73E-09 |
| regulation of nucleobase-containing compound metabolic process (GO:0019219) | 2981 | 18 | 2.5 | + | 7.21 | 5.19E-09 |
| regulation of cellular macromolecule biosynthetic process (GO:2000112) | 3037 | 18 | 2.54 | + | 7.07 | 7.09E-09 |
| regulation of macromolecule biosynthetic process (GO:0010556) | 3043 | 18 | 2.55 | + | 7.06 | 7.33E-09 |
| RNA metabolic process (GO:0016070) | 1861 | 11 | 1.56 | + | 7.06 | 0.000433 |
| regulation of cellular biosynthetic process (GO:0031326) | 3071 | 18 | 2.57 | + | 7 | 8.55E-09 |
| regulation of biosynthetic process (GO:0009889) | 3078 | 18 | 2.58 | + | 6.98 | 8.88E-09 |
| regulation of nitrogen compound metabolic process (GO:0051171) | 3499 | 19 | 2.93 | + | 6.48 | 5.77E-09 |
| regulation of primary metabolic process (GO:0080090) | 3541 | 19 | 2.97 | + | 6.4 | 7.12E-09 |
| regulation of gene expression (GO:0010468) | 3383 | 18 | 2.83 | + | 6.35 | 4.3E-08 |
| regulation of cellular metabolic process (GO:0031323) | 3589 | 19 | 3.01 | + | 6.32 | 9.03E-09 |
| regulation of macromolecule metabolic process (GO:0060255) | 3854 | 19 | 3.23 | + | 5.88 | 3.16E-08 |
| regulation of metabolic process (GO:0019222) | 3959 | 19 | 3.32 | + | 5.73 | 5.05E-08 |
| cellular nitrogen compound biosynthetic process (GO:0044271) | 2091 | 10 | 1.75 | + | 5.71 | 0.0119 |
| cellular macromolecule biosynthetic process (GO:0034645) | 2159 | 10 | 1.81 | + | 5.53 | 0.0157 |
| macromolecule biosynthetic process (GO:0009059) | 2223 | 10 | 1.86 | + | 5.37 | 0.0203 |
| gene expression (GO:0010467) | 2475 | 11 | 2.07 | + | 5.3 | 0.00712 |
| regulation of cellular process (GO:0050794) | 5014 | 20 | 4.2 | + | 4.76 | 3.03E-07 |
| nucleic acid metabolic process (GO:0090304) | 2894 | 11 | 2.42 | + | 4.54 | 0.0316 |
| regulation of biological process (GO:0050789) | 5556 | 20 | 4.65 | + | 4.3 | 1.93E-06 |
| biological regulation (GO:0065007) | 6277 | 21 | 5.26 | + | 3.99 | 1.97E-06 |
| Unclassified (UNCLASSIFIED) | 16901 | 9 | 14.16 | - | 0.64 | 0 |

## Supplementary references

1. Shi, X. *et al.* Genomic imbalance determines positive and negative modulation of gene expression in diploid maize. *Plant Cell* **33**, 917–939 (2021).
2. Yang, H. *et al.* Predominantly inverse modulation of gene expression in genomically unbalanced disomic haploid maize. *Plant Cell* **33**, 901–916 (2021).