

## Supplemental Figure Legends:

**Supplemental Figure S1.** Global sRNA profiles for the species shown in Fig. 1.

**Supplemental Figure S2.** Presence of viral-derived small RNAs (sRNAs) in *Orobanchae* sRNA libraries compared to *Arabidopsis* mock and cucumber mosaic virus (CMV)-infected sRNA libraries. Values shown are the average between multiple sRNA libraries (2 bioreplicates for *Orobanchae*, CMV-mock/control and CMV-infected) with error bars representing the standard deviation between bioreplicates.

**Supplemental Figure S3.** Comparison of microRNA conservation between *Cuscuta*, *Arabidopsis*, maize and rice. **A.** Number of members in each microRNA (miRNA) family indicated in *Arabidopsis*, maize, rice and *Cuscuta*. **B.** Comparison of highly conserved miRNA families (conserved between more than 7 Solanaceae species, 33 miRNA families), not conserved miRNA families (conserved in less than 7 Solanaceae species, 138 miRNA families) and *Cuscuta* exclusive miRNA families (7 miRNA families). Whiskers extend to 5th and 95th percentile. Data points outside of those percentiles are represented as circles.

**Supplemental Figure S4.** Principal component analysis of the sRNA libraries produced from different *Cuscuta* tissues restricted to miRNAs.

**Supplemental Figure S5.** Size distribution of individual microRNA (miRNA) families for each *Cuscuta* sRNA library produced in this study.

**Supplemental Figure S6.** Size and 5' nucleotide distribution for each replicate of the *C. campestris* sRNA libraries shown in Fig. 3B and C.

**Supplemental Figure S7:** Heat map showing differential expression of Argonaute (AGO) proteins in young and emerged haustoria. AGO proteins were identified in the genome of *C. campestris* (version r.032, Vogel et al. 2018) based on their sequence-based annotation. Their expression is shown as fold-changes ( $\log_2$ ) in gene expression rates. Fold changes with a p-value > 0.05 were considered significant and are color-coded with a linear color scale from yellow (-3) to blue (+3). Non-significant

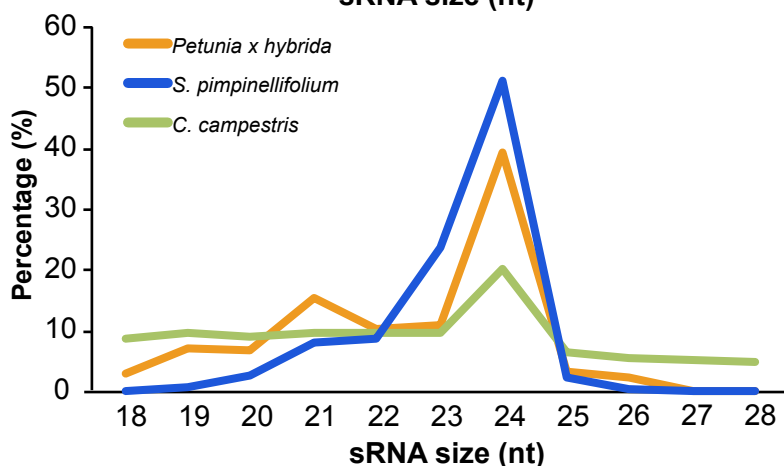
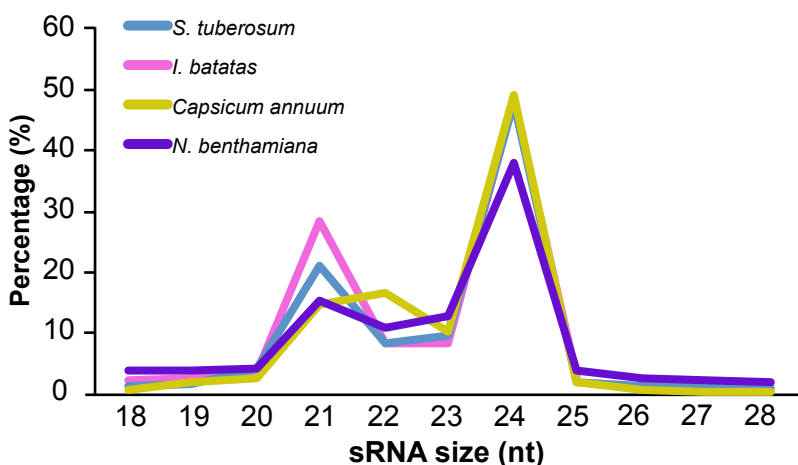
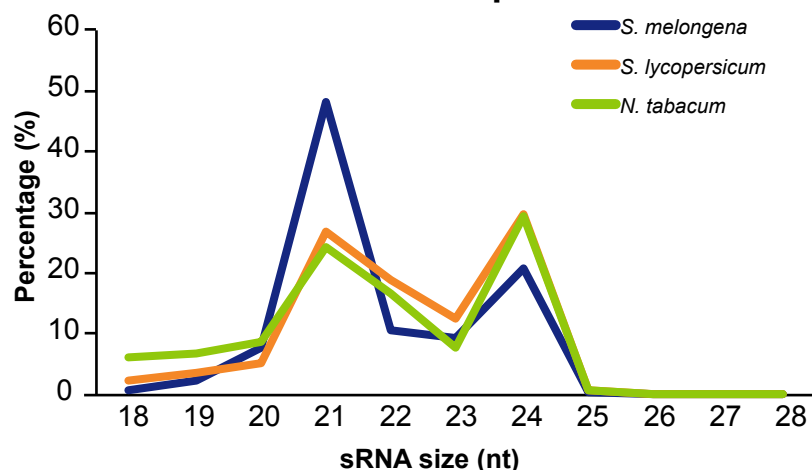
values are not shown. To indicate the overall expression level, reads per kilobase per million (RPKM) for the niS control stage are shown (in italics, as average of three replicates). Expression values are from Bawin et al. (2022) and correspond to the niS (stems), SWE (prehaustoria) and PEN (emerged haustoria) stages.

**Supplemental Figure S8.** Secondary structure of new microRNAs identified in this study. RNA secondary structures of all pre-microRNA (pre-miRNA) sequences identified in the study are shown. Color scale indicates base-pair probabilities, with the maximum value (1) being colored in red, while the lowest value (0) is colored in purple. Position of the mature miRNA and miRNA\* sequences along the pre-miRNA secondary structure are indicated with a black and grey line respectively.

**Supplemental Figure S9.** Identification of miRNA-targeted mRNAs in *Cuscuta* through degradome sequencing. **A.** Principal component analysis of the PARE/degradome libraries produced from different *C. campestris* tissues. **B.** Table showing PARE-identified genes targeted by conserved and new miRNAs in haustorial tissues. Presence of the target site in different bioreplicates is indicated and the percentage of reads that map to a 20 nt window relative to the cleavage position compared to the number of total reads mapping to the targeted transcript. In the table the gene identification number (Gene ID) for *Cuscuta* genome version r.032 is shown, together with a brief description of the gene function (Description), the miRNA mediating its cleavage (miRNA), the cleavage position (C), the number of replicates in which the cleavage site was observed (R, which is indicated by a grey-color scale in the table legend) and the presence of the cleavage event in the prehaustoria (Pre) or emerged haustoria (Emr) degradome libraries. The amount of degradome reads present at the target site (within a 20 bp window) is indicated with a color scale as indicated on the right side of the table, with red indicating up to 90% and blue indicating less than 10% of the total degradome reads of the transcript accumulating within the 20bp window defined by the cleavage position.

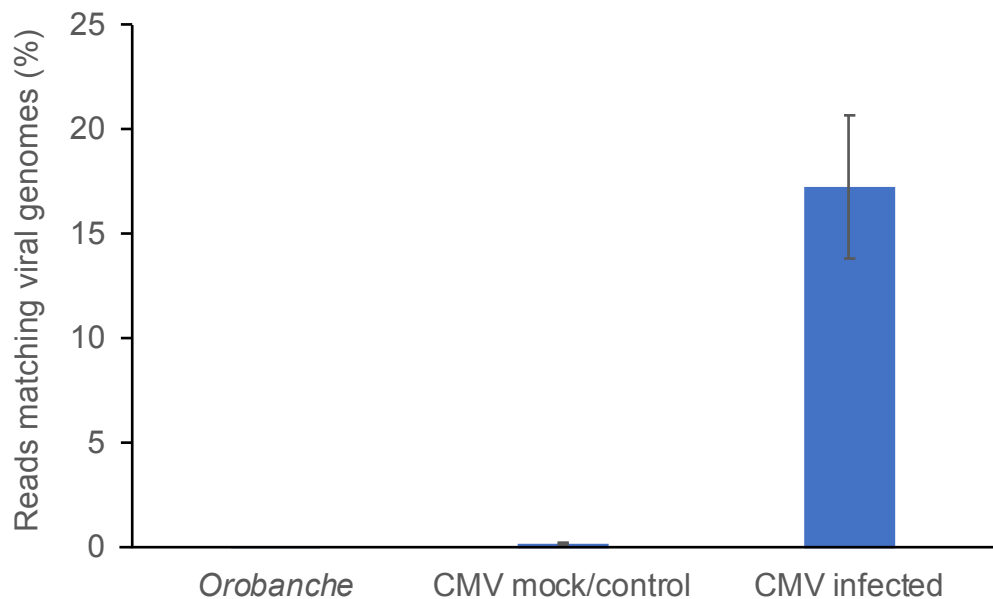
# Supplemental Figure S1

## Global sRNA profiles



**Supplemental Figure S1.** Global sRNA profiles for the species shown in Fig. 1.

## Supplemental Figure S2



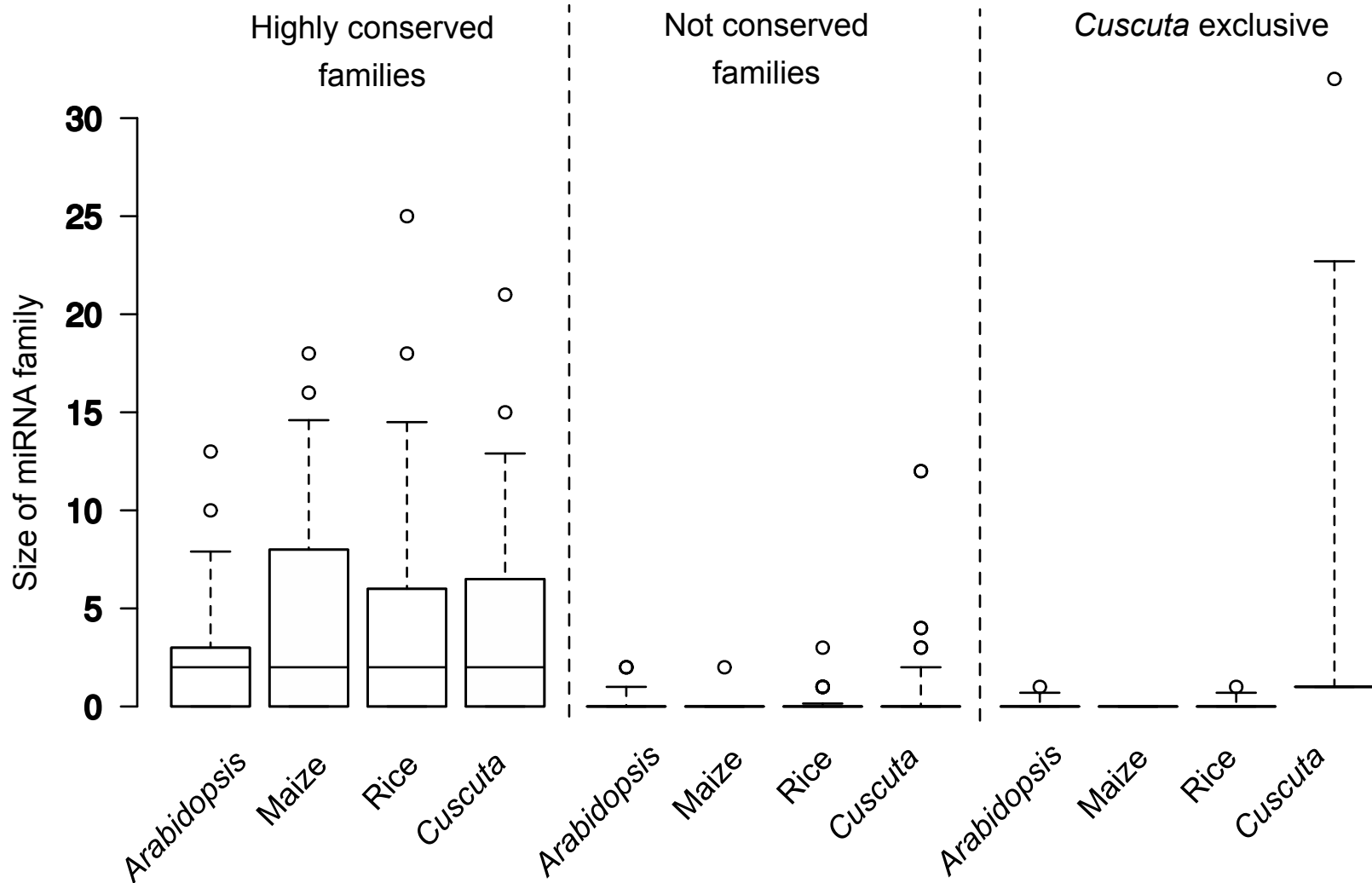
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# Supplemental Figure S3

A.

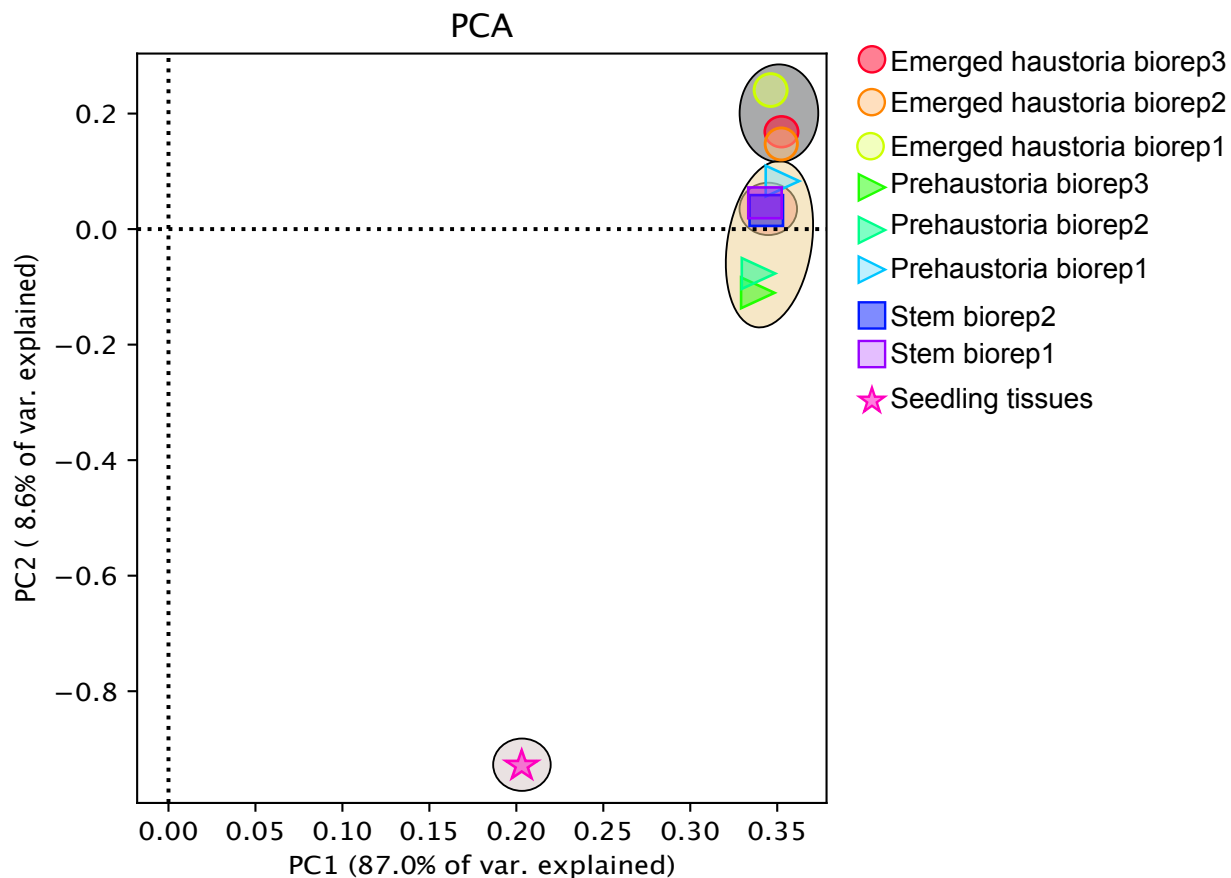
|                       |         | Arabidopsis | Maize | Rice | Cuscuta |
|-----------------------|---------|-------------|-------|------|---------|
| Common to all species | 156/157 | 14          | 12    | 12   | 24      |
|                       | 165/166 | 9           | 14    | 13   | 15      |
| Common to 9 species   | 159     | 3           | 11    | 6    | 1       |
|                       | 160     | 3           | 7     | 6    | 10      |
|                       | 164     | 3           | 8     | 6    | 6       |
|                       | 167     | 4           | 10    | 10   | 2       |
|                       | 168     | 2           | 2     | 2    | 4       |
|                       | 169     | 13          | 18    | 18   | 5       |
|                       | 171     | 3           | 14    | 9    | 8       |
|                       | 172     | 5           | 5     | 4    | 8       |
|                       | 319     | 3           | 4     | 2    | 12      |
|                       | 390     | 2           | 2     | 1    | 2       |
|                       | 393     | 2           | 3     | 2    | 1       |
|                       | 396     | 2           | 8     | 8    | 6       |
|                       | 397     | 2           | 2     | 2    | 2       |
|                       | 398     | 3           | 2     | 2    | 1       |
|                       | 399     | 6           | 10    | 11   | 0       |
|                       | 408     | 1           | 2     | 1    | 3       |
|                       | 530     | 0           | 0     | 1    | 0       |
|                       | 845     | 2           | 0     | 0    | 12      |
|                       | 894     | 0           | 0     | 0    | 0       |
|                       | 1863    | 0           | 0     | 3    | 0       |
|                       | 2916    | 0           | 0     | 0    | 0       |
|                       | 5139    | 0           | 0     | 0    | 2       |
|                       | 6478    | 0           | 0     | 0    | 9       |
|                       | 8175    | 1           | 0     | 0    | 0       |
|                       | 1134    | 0           | 0     | 0    | 0       |
|                       | 1511    | 0           | 0     | 0    | 12      |
|                       | 5049    | 0           | 0     | 0    | 2       |
|                       | 5141    | 0           | 0     | 0    | 2       |
|                       | 6173    | 0           | 0     | 0    | 4       |
|                       | 7782    | 0           | 0     | 0    | 0       |
|                       | 162     | 2           | 1     | 2    | 0       |
|                       | 395     | 6           | 16    | 25   | 0       |
|                       | 403     | 1           | 0     | 0    | 0       |
|                       | 482     | 0           | 1     | 0    | 0       |
|                       | 3627    | 0           | 0     | 0    | 0       |
|                       | 3711    | 0           | 0     | 0    | 0       |
|                       | 5072    | 0           | 0     | 1    | 0       |
|                       | 394     | 2           | 2     | 1    | 0       |
|                       | 477     | 0           | 0     | 0    | 0       |
|                       | 827     | 1           | 1     | 1    | 0       |

B.



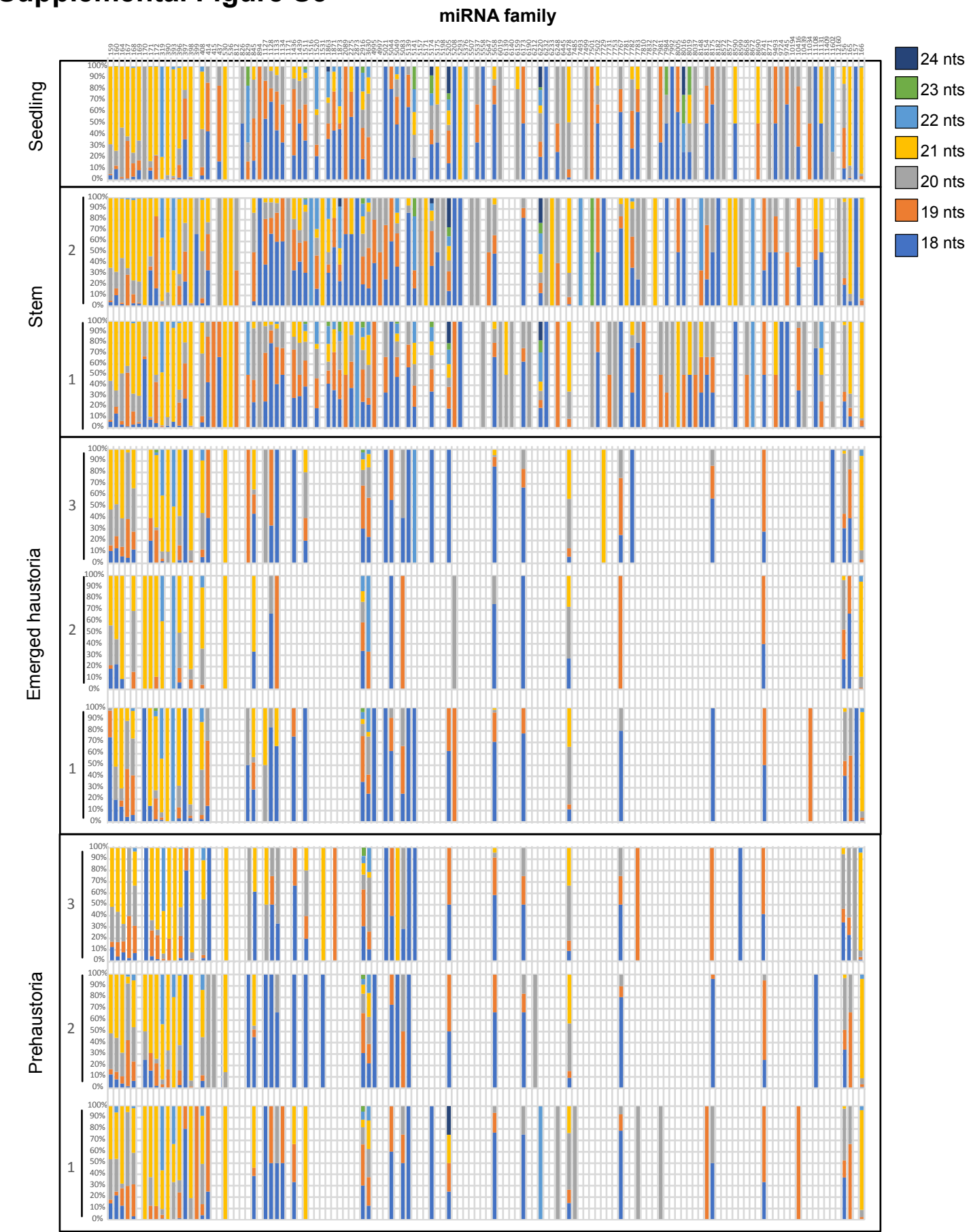
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## Supplemental Figure S4



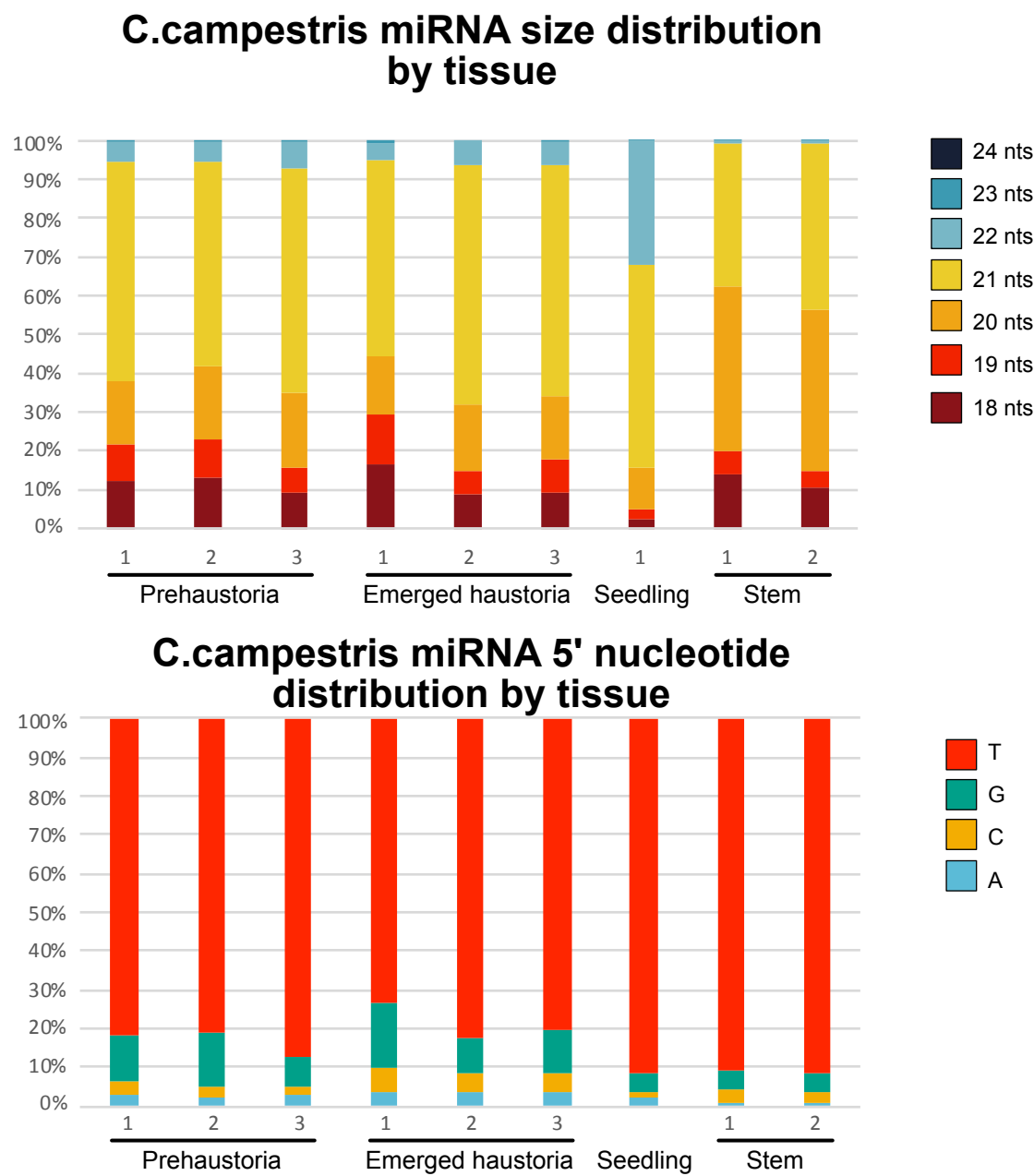
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Supplemental Figure S5



**Supplemental Figure S5.** Size distribution of individual microRNA (miRNA) families for each *Cuscuta* sRNA library produced in this study.

Supplemental Figure S6

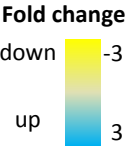


**Supplemental Figure S6.** Size and 5' nucleotide distribution for each replicate of the *C. campestris* sRNA libraries shown in Fig. 3B and C.



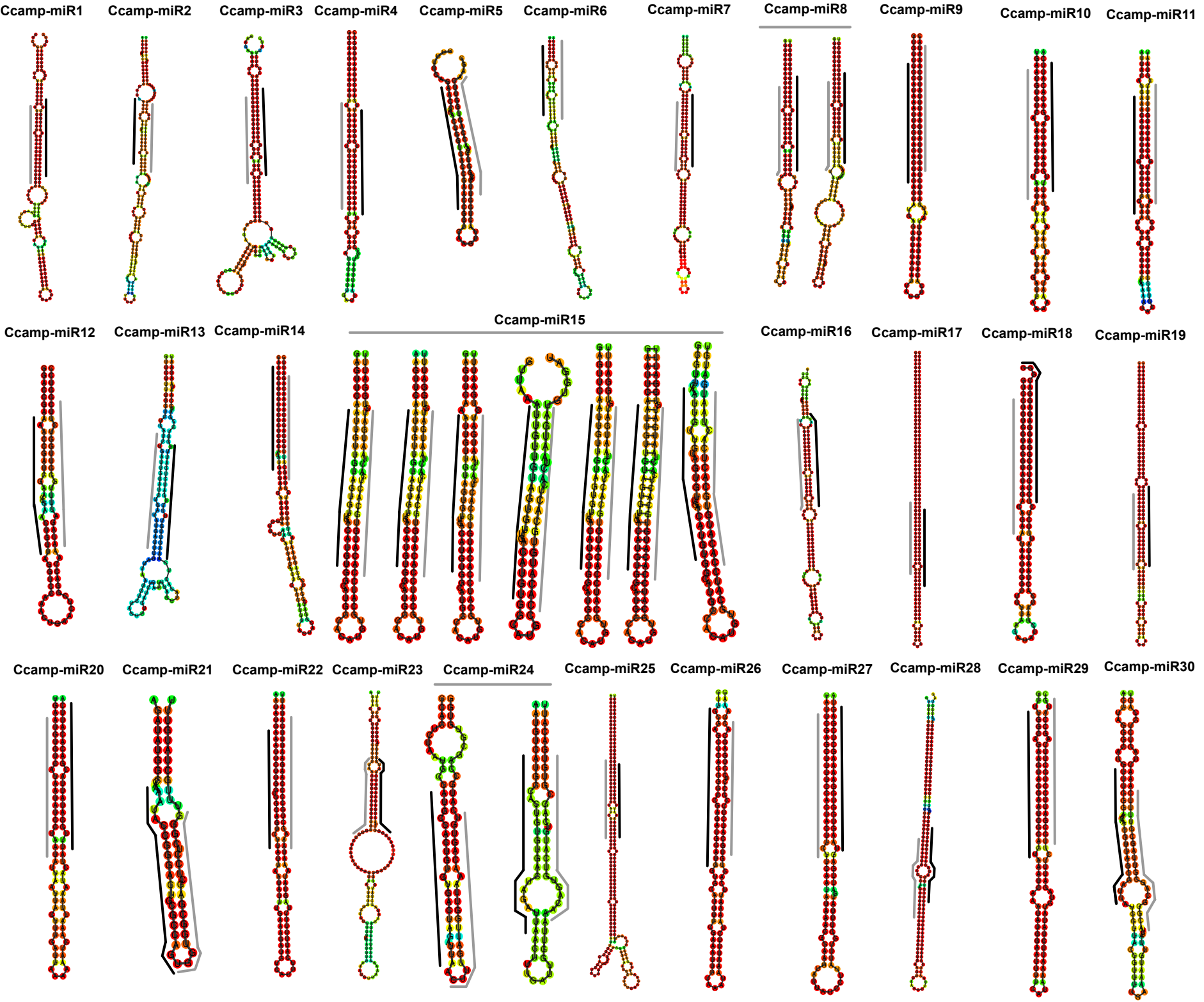
Supplemental Figure S7

| <i>Cuscuta campestris</i><br>Identifier | pre-<br>haustoria vs<br>stem [logFC] | emerged<br>haustoria vs<br>stem [logFC] | RPKM in stems<br>[avg. (st-dev)] | Protein<br>name | Mapman<br>v.4 bincode | Description or function  |
|---|--------------------------------------|---|----------------------------------|-----------------|-----------------------|--|
| Cc004803.t1                             |                                      |   | 29.33 (5)                        | AGO10           | 16.9.2.7              | miRNA recruiting factor (AGO) of RNA-induced silencing complex (RISC) assembly |
| Cc008841.t1                             | 0.61                                 | 0.01                                    | 66.84 (12.6)                     | AGO1            | 16.9.2.7              | miRNA recruiting factor (AGO) of RNA-induced silencing complex (RISC) assembly |
| Cc021107.t1                             | 0.83                                 | 0.11                                    | 67.67 (19)                       | AGO1            | 16.9.2.7              | miRNA recruiting factor (AGO) of RNA-induced silencing complex (RISC) assembly |
| Cc044782.t1                             | 0.41                                 | 0.52                                    | 20.67 (2.5)                      | AGO10           | 16.9.2.7              | miRNA recruiting factor (AGO) of RNA-induced silencing complex (RISC) assembly |
| Cc013394.t1                             | -0.82                                | -0.11                                   | 15.62 (2.8)                      | AGO2            | 12.5.1.15.2           | siRNA-binding factor (AGO2) of non-canonical RdDM pathway                      |
| Cc018519.t1                             | -1.17                                | -0.27                                   | 49.7 (6.8)                       | AGO2            | 12.5.1.15.2           | siRNA-binding factor (AGO2) of non-canonical RdDM pathway                      |
| Cc017068.t1                             | 0.51                                 | 0.58                                    | 77.43 (23.9)                     | AGO4            | 12.5.1.13             | siRNA-integrating factor (AGO)   |
| Cc028339.t1                             | 0.65                                 | 0.75                                    | 68.94 (20.6)                     | AGO4            | 12.5.1.13             | siRNA-integrating factor (AGO)   |
| Cc017553.t1                             | 1.05                                 | 0.63                                    | 15.3 (5.8)                       | AGO5            | 35.1                  | Protein argonaute MEL1 OS=Oryza sativa subsp. Japonica                         |
| Cc032321.t1                             | 2.74                                 | 1.43                                    | 3.38 (1.67)                      | AGO5            | 35.1                  | Protein argonaute MEL1 OS=Oryza sativa subsp. Japonica                         |
| Cc037224.t1                             | 0.99                                 | -0.31                                   | 1.64 (0.19)                      | AGO6            | 12.5.1.13             | siRNA-integrating factor (AGO)   |
| Cc008056.t1                             | 1.67                                 | 0.56                                    | 1.27 (0.53)                      | AGO6            | 12.5.1.13             | siRNA-integrating factor (AGO)   |
| Cc022959.t1                             | 1.28                                 | 1.30                                    | 0.48 (0.25)                      | AGO7            | 16.5.1.6              | regulatory protein (AGO7) of transacting siRNA pathway                         |
| Cc045134.t1                             | 2.09                                 | 1.75                                    | 0.69 (0.17)                      | AGO7            | 16.5.1.6              | regulatory protein (AGO7) of transacting siRNA pathway                         |



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Supplementary Figure 8

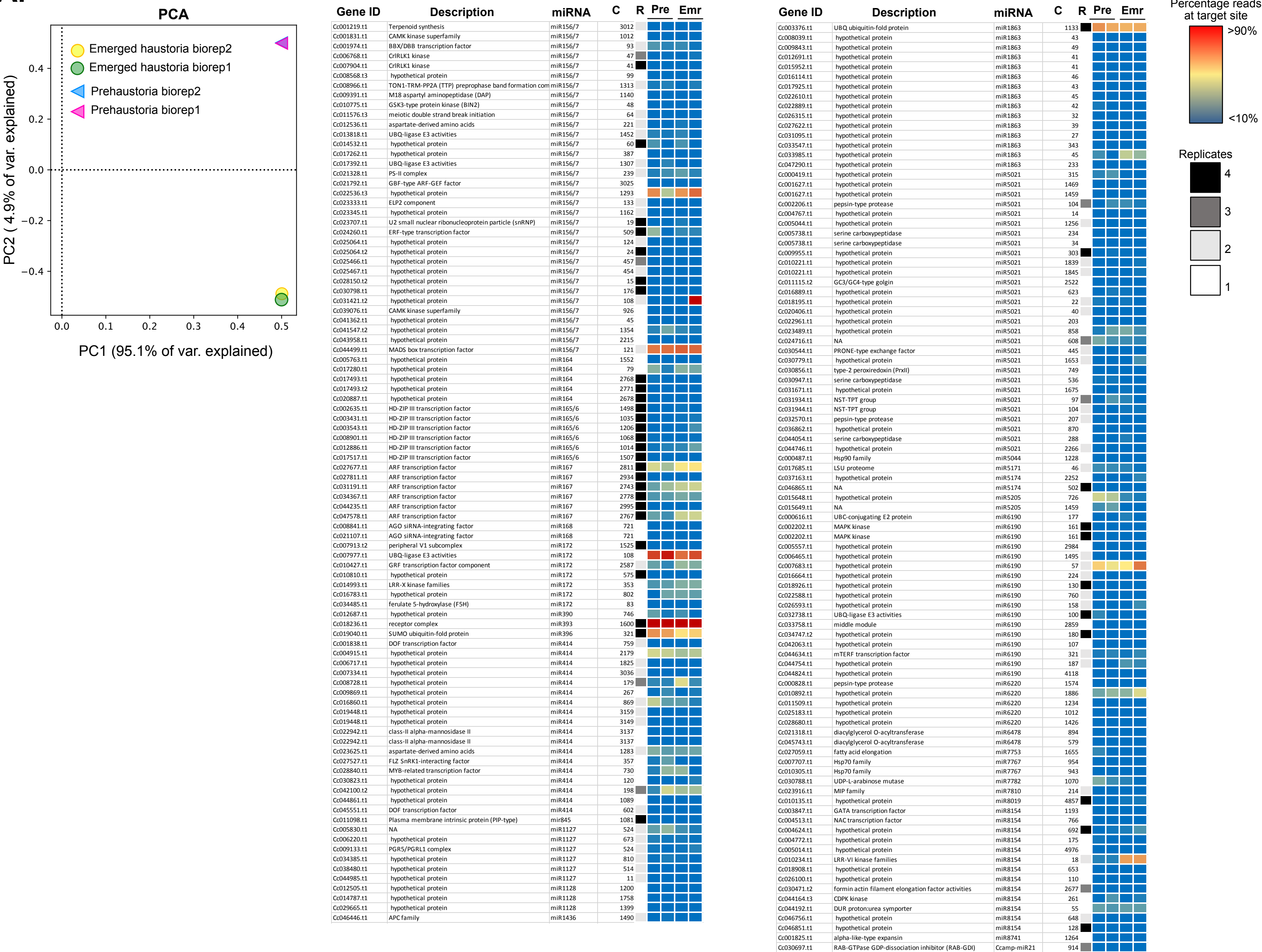


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# Supplementary Figure 9

A.

B.



**Supplemental Figure S9.** Identification of miRNA-targeted mRNAs in *Cuscuta* through degradome sequencing. **A.** Principal component analysis of the PARE/degradome libraries produced from different *C. campestris* tissues. **B.** Table showing PARE-identified genes targeted by conserved and new miRNAs in haustorial tissues. Presence of the target site in different bioreplicates is indicated and the percentage of reads that map to a 20 nt window relative to the cleavage position compared to the number of total reads mapping to the targeted transcript. In the table the gene identification number (Gene ID) for *Cuscuta* genome version r.032 is shown, together with a brief description of the gene function (Description), the miRNA mediating its cleavage (miRNA), the cleavage position (C), the number of replicates in which the cleavage site was observed (R, which is indicated by a grey-color scale in the table legend) and the presence of the cleavage event in the prehaustoria (Pre) or emerged haustoria (Emr) degradome libraries. The amount of degradome reads present at the target site (within a 20 bp window) is indicated with a color scale as indicated on the right side of the table, with red indicating up to 90% and blue indicating less than 10% of the total degradome reads of the transcript accumulating within the 20bp window defined by the cleavage position.