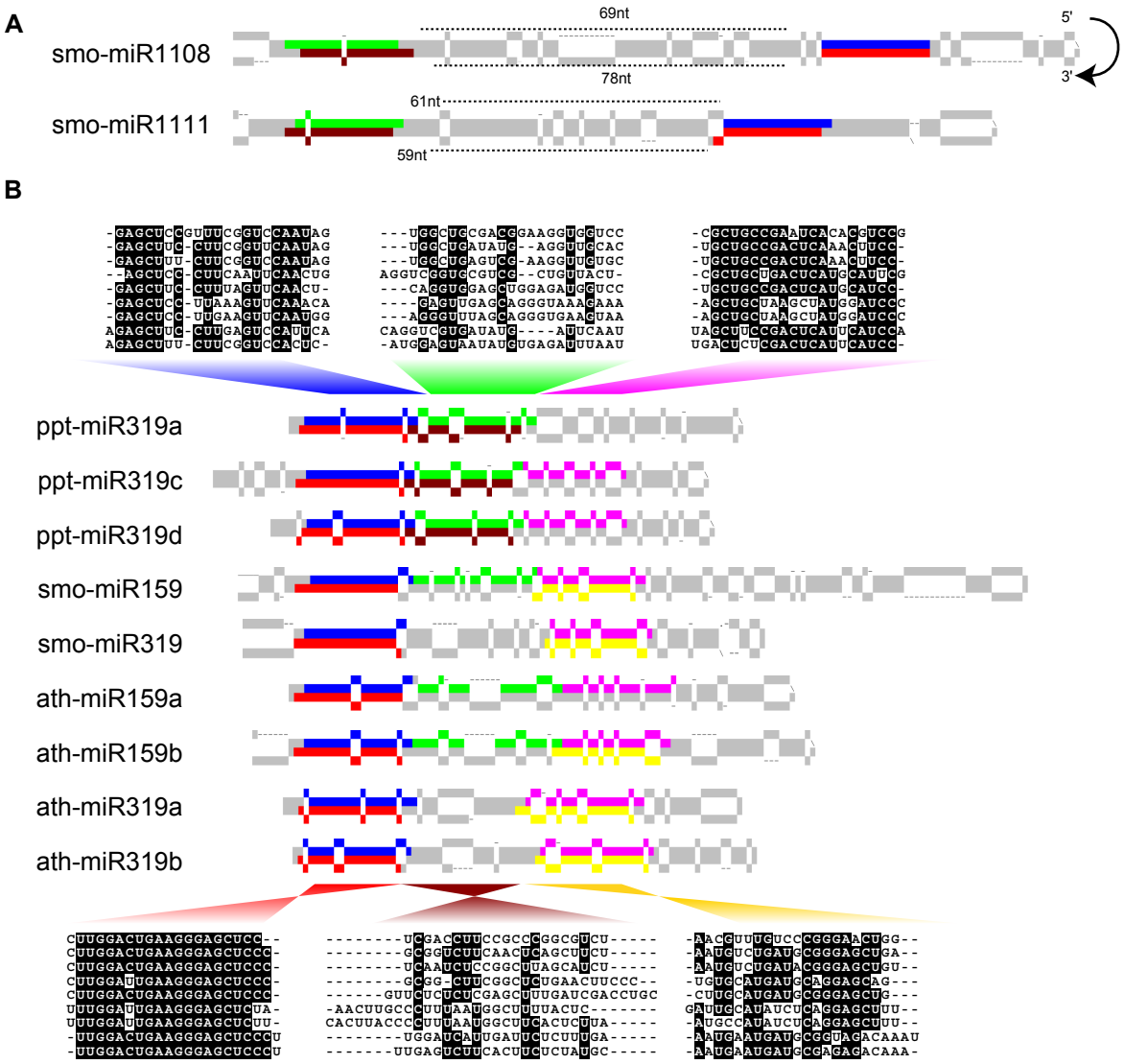

Supplementary Figure 1. Alternative miRNA production.
(A) Schematics of *S. moellendorffii* miRNA stem-loops from which multiple miRNA/miRNA* duplexes were sequenced. Curved arrow indicates 5' to 3' direction, which is the same in all of the schematics. Red: miRNA, blue: miRNA*, green and brown: alternative miRNA/miRNA* duplex.
(B) Conserved production of alternative miRNA/miRNA* duplexes in the miR159/319 family. Schematics of the indicated stem-loops from *P. patens* (ppt), *S. moellendorffii* (smo) and *A. thaliana* (ath) are colored according to the observation of additional reads from these loci. ClustalW alignments of the small RNAs from these nine loci are shown, 5' to 3', with identical residues of six or more loci shaded black. Red: miR159/319, blue: miR159*/319*, green and brown: alternative miRNA/miRNA* duplex #1, purple and yellow: alternative miRNA/miRNA* #2. *A. thaliana* data from (Rajagopalan et al., 2006) This conservation of both sequence identity and of expression prompted a search for potential targets of these two alternative miRNAs in *A. thaliana* and *P. patens*. Using refined miRNA target prediction methods (Allen et al., 2005; Rajagopalan et al., 2006), the alternative miR159/319 miRNAs from the 5' arm (colored magenta in Figure 1B) of *A. thaliana* were predicted to target nine nearly identical Ulp1 protease homologs (At1g25886, At1g27780, At1g34740, At2g14770, At3g24390, At3g42730, At4g03300, At4g05280, and At5g36860), while the alternative miR159/319 miRNAs from the 3' arm (colored yellow in Figure 1B) were predicted to target two distinct UBP-like ubiquitin-specific protease genes (At4g17895 and At5g46740). Targets of the *P. patens* alternative miR319 miRNAs were much more difficult to predict using standard parameters, but a single ubiquitin-specific protease homolog (Phypa1_1155889) was predicted as a target of the 5' alternative ppt-miR319cd sequence. These predicted targets of alternative miR159/319 miRNAs in both moss and flowering plants were all predicted to function in the removal of ubiquitin or small ubiquitin-like modifiers (e.g. SUMO) from substrate proteins. We were unable to detect cleavage products for any of the *A. thaliana* or *P. patens* potential targets of the alternative miR159/319 miRNAs using RNA-ligase mediated 5' rapid amplification of cDNA ends (RLM 5'-RACE; Kasschau et al., 2003). Thus, further experimentation will be required to conclude that these alternative miR159/miR319 miRNAs are functional.

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