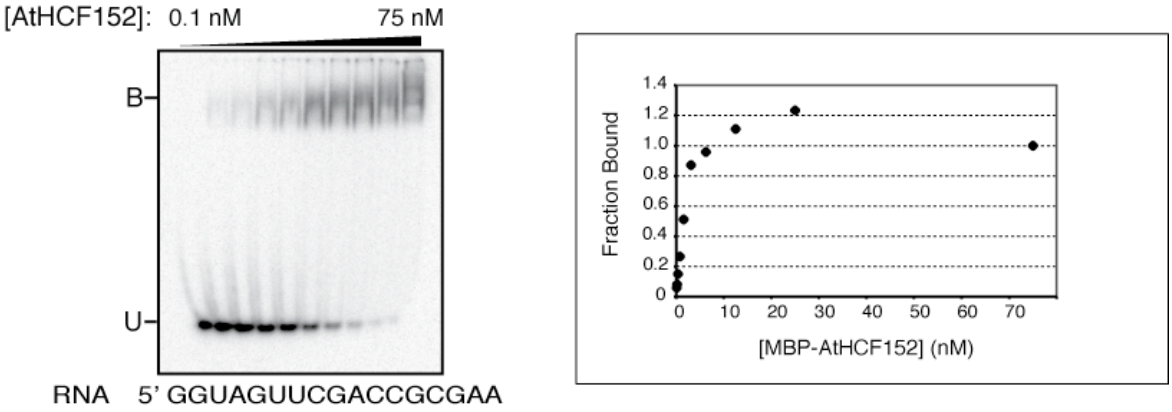
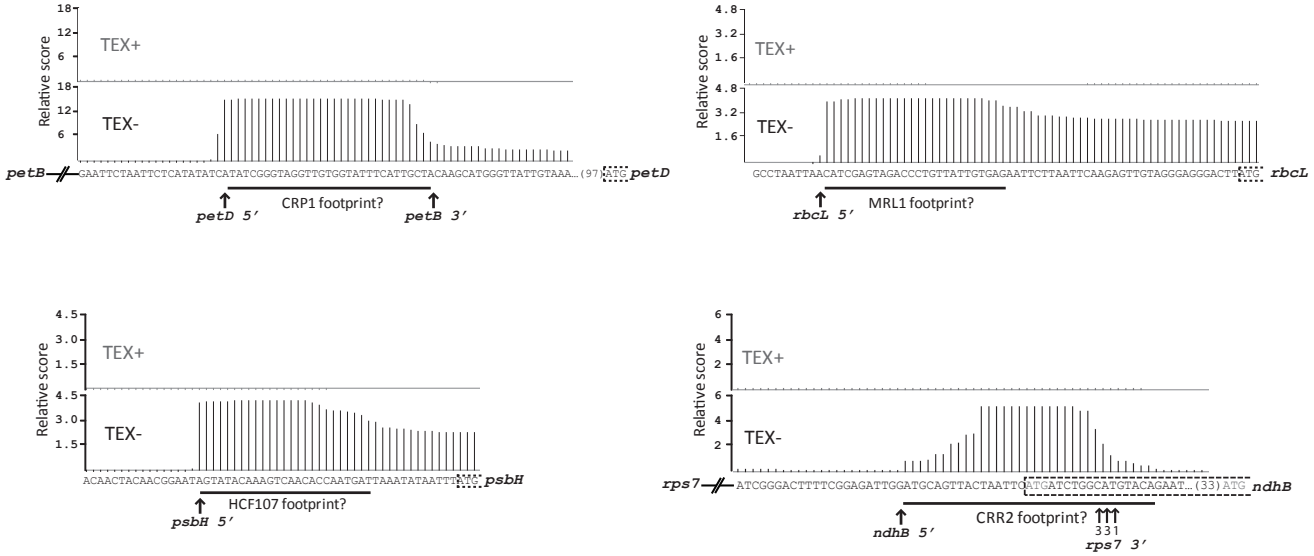
Supplementary Figure 1.
RNA binding curve to estimate affinity of MBP-AtHCF152 for its binding site. Binding reactions were performed with an RNA oligonucleotide harboring the AtHCF152 binding site and protein at the concentrations indicated. Bound (B) and unbound (U) RNAs were separated by native gel electrophoresis. These results show that the K_d under these conditions is ~ 1 nM, the concentration at which half maximal binding is observed.

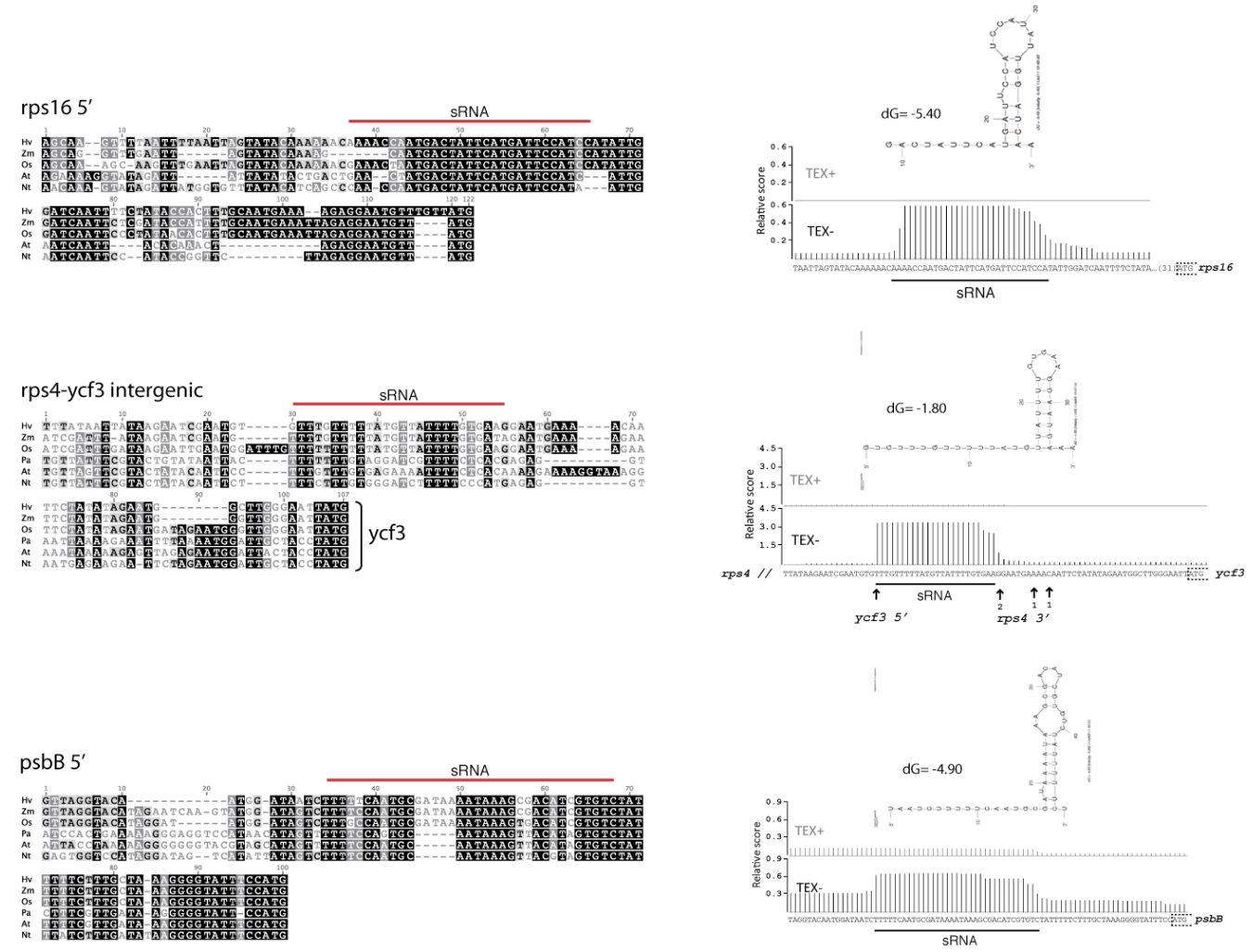


Supplementary Figure 2.
Barley transcriptome data documenting sRNAs at sites of action of genetically-characterized PPR proteins for which binding sites have not been demonstrated. RNA termini that fail to accumulate in the absence of the indicated PPR protein are indicated. Both the annotated and newly proposed start codon for *ndhB* are shown. The processed *ndhB* 5'-ends are heterogeneous; the most upstream end was arbitrarily labeled as the 5'-end. *rps7* 3'-ends were mapped by 3'-RACE and are annotated with the number of clones representing each position.

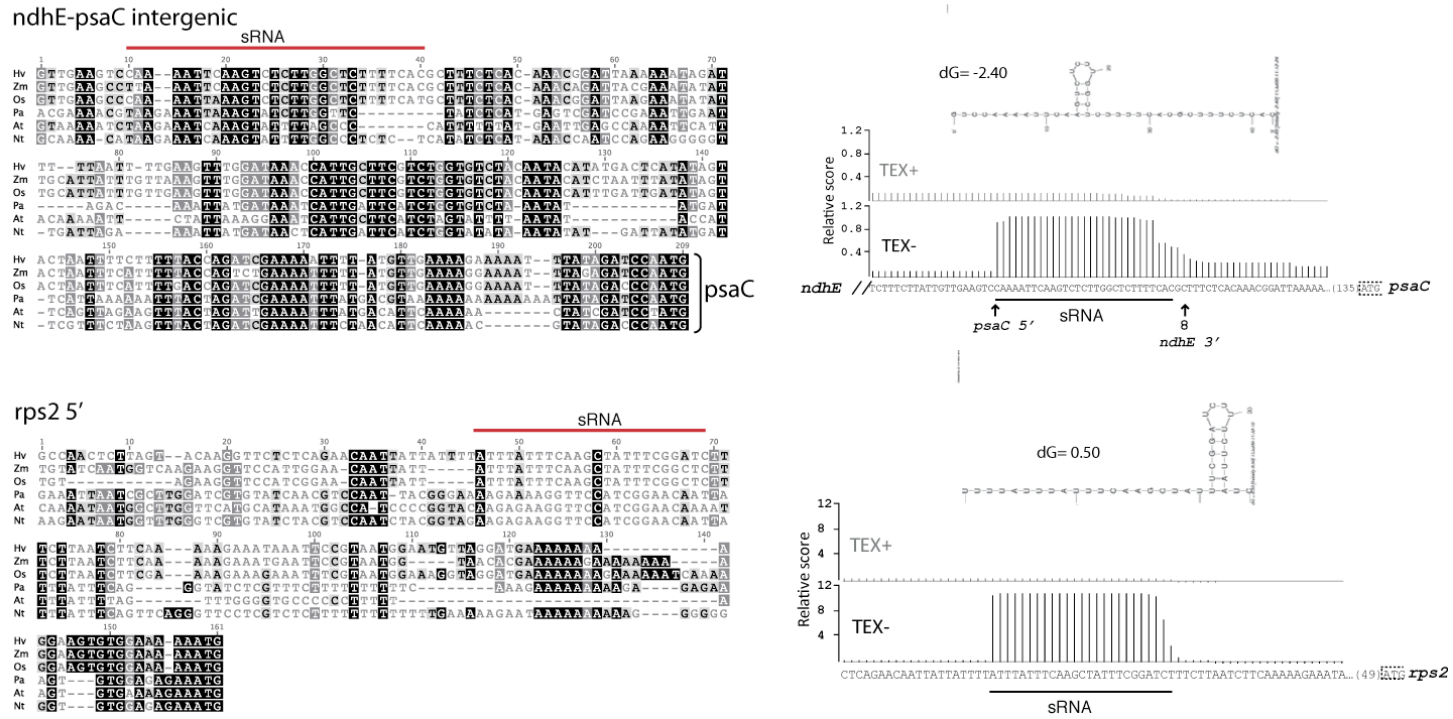


Supplementary Figure 3.
Supporting data for sRNAs that correlate with processed 5'-termini and summarized in Table 2. The aligned sequences come from barley (Hv), maize (Zm), rice (Os), poplar (Pa), Arabidopsis (At), and tobacco (Nt). The most stable secondary structure predicted by Mfold is shown for each sRNA. 3'-termini in several of the regions depicted were mapped by 3'-RACE; these are marked by vertical arrows under the sequence histograms, annotated with the number of clones representing each position.
(A) sRNAs mapping in 5'-UTRs.
(B) sRNAs matching processed 5'-termini that map within upstream open reading frames. Sequence alignments are not shown because these sequences are subject to additional constraints. The secondary structures of the *psbC* and *ndhK* 5'-UTRs (panels to the right) show that the sequences at the immediate 5'-end (i.e. matching the sRNA) are predicted to bind the downstream ribosome binding region if not sequestered by a protein.
(C) sRNA mapping at the 5'-end of the second of two fragments of *rps12*-intron 1.

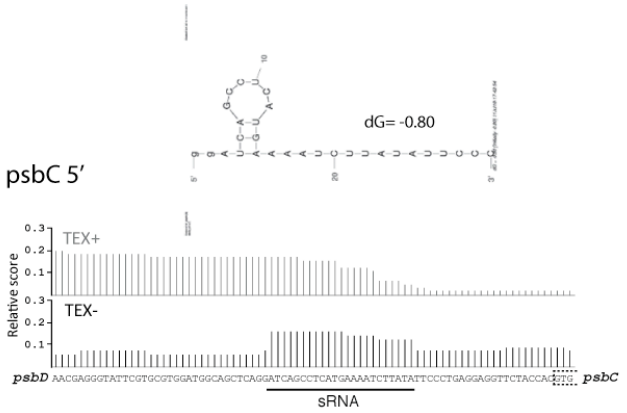
Supplementary Figure 3A part 1

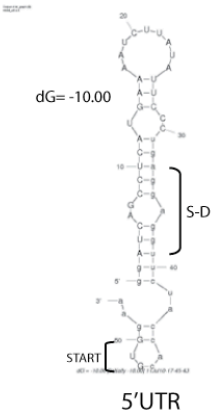


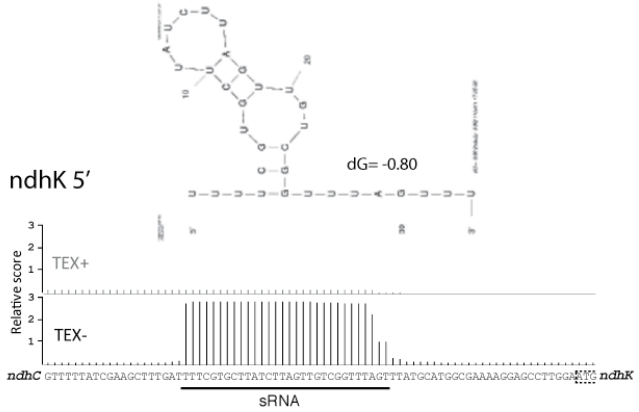
Supplementary Figure 3A part 2

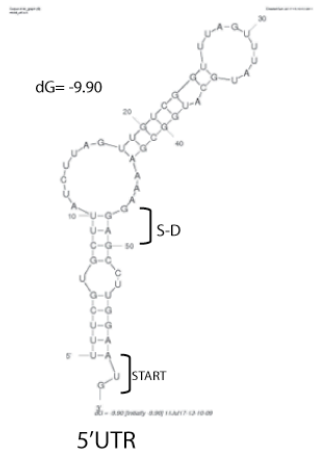


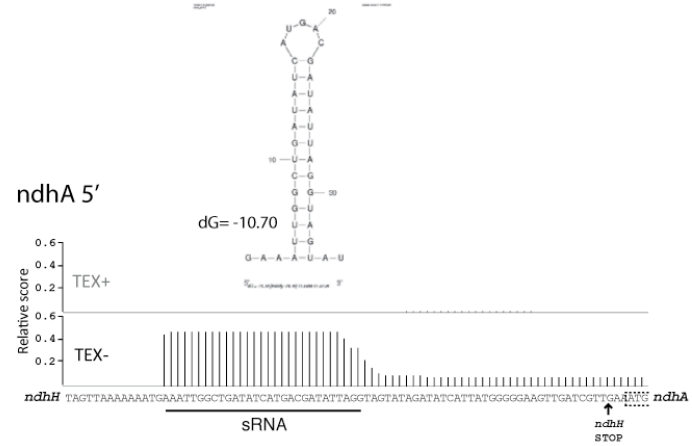
Supplementary Figure 3B



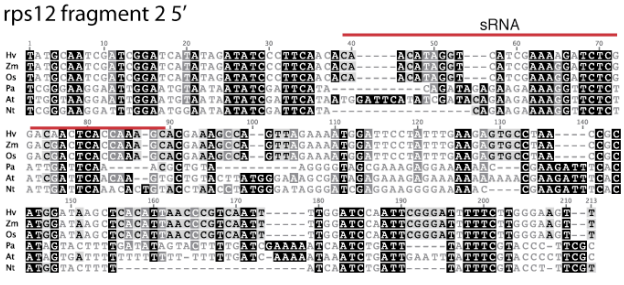


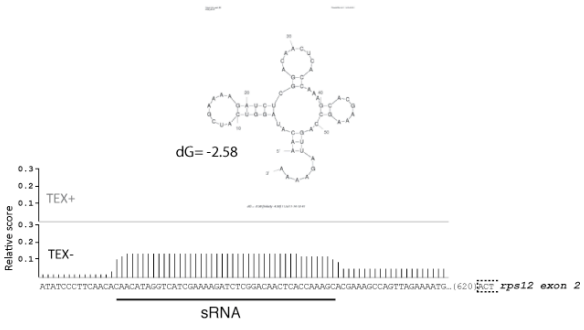






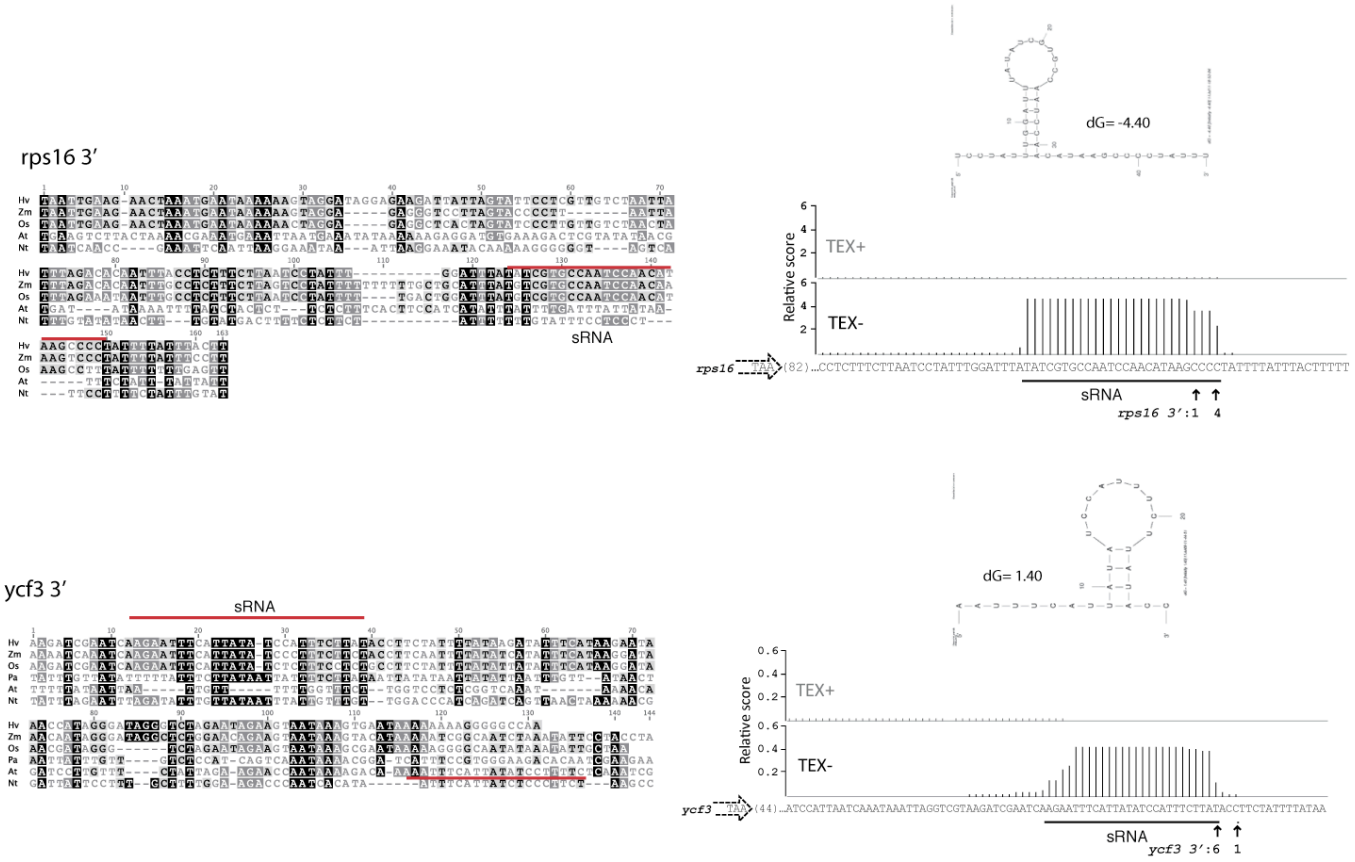
Supplementary Figure 3C



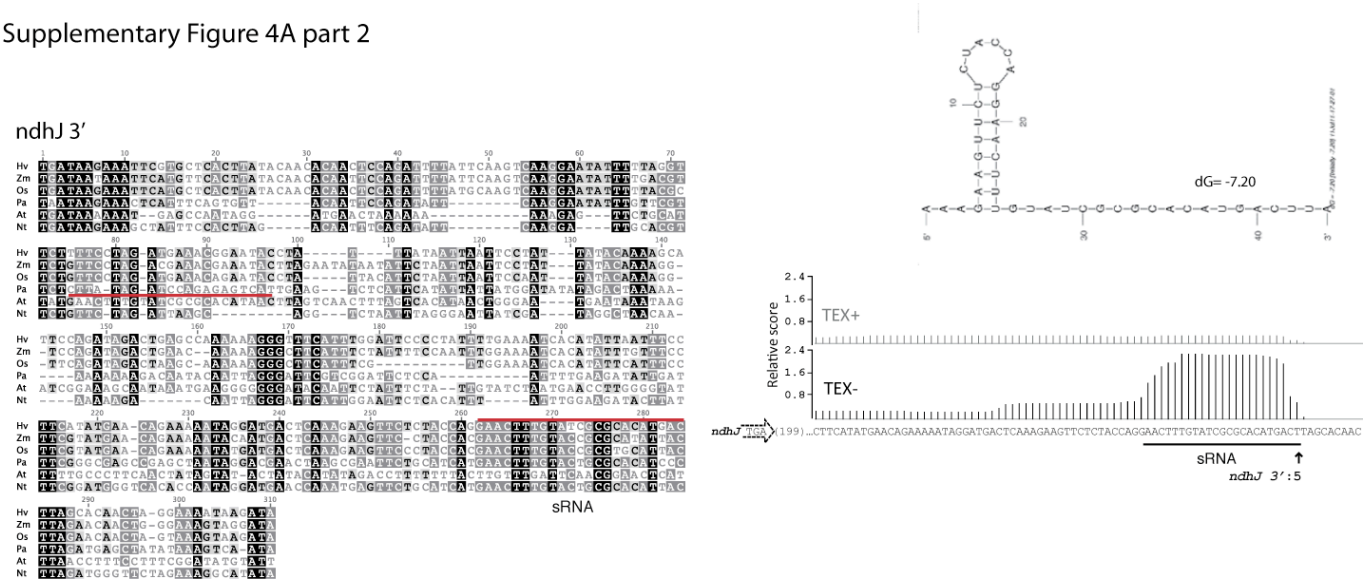


Supplementary Figure 4.
Supporting data for sRNAs that map in 3'-UTRs and summarized in Table 3. The aligned sequences come from barley (Hv), maize (Zm), rice (Os), poplar (Pa), Arabidopsis (At), and tobacco (Nt). The most stable secondary structure predicted by Mfold is shown for each sRNA. 3'-termini mapped by 3'-RACE are marked with vertical arrows, and annotated with the number of clones corresponding to each position.
(A) sRNAs proposed to be binding sites for PPR-like proteins that stabilize 3'-termini. The Arabidopsis *ndhJ* and *ycf3* sRNAs are underlined separately because they are conserved in sequence but not position with those in barley.
(B) sRNAs mapping in 3'-UTRs for which the evidence is less strong of a role as a 3'-stabilizing element.

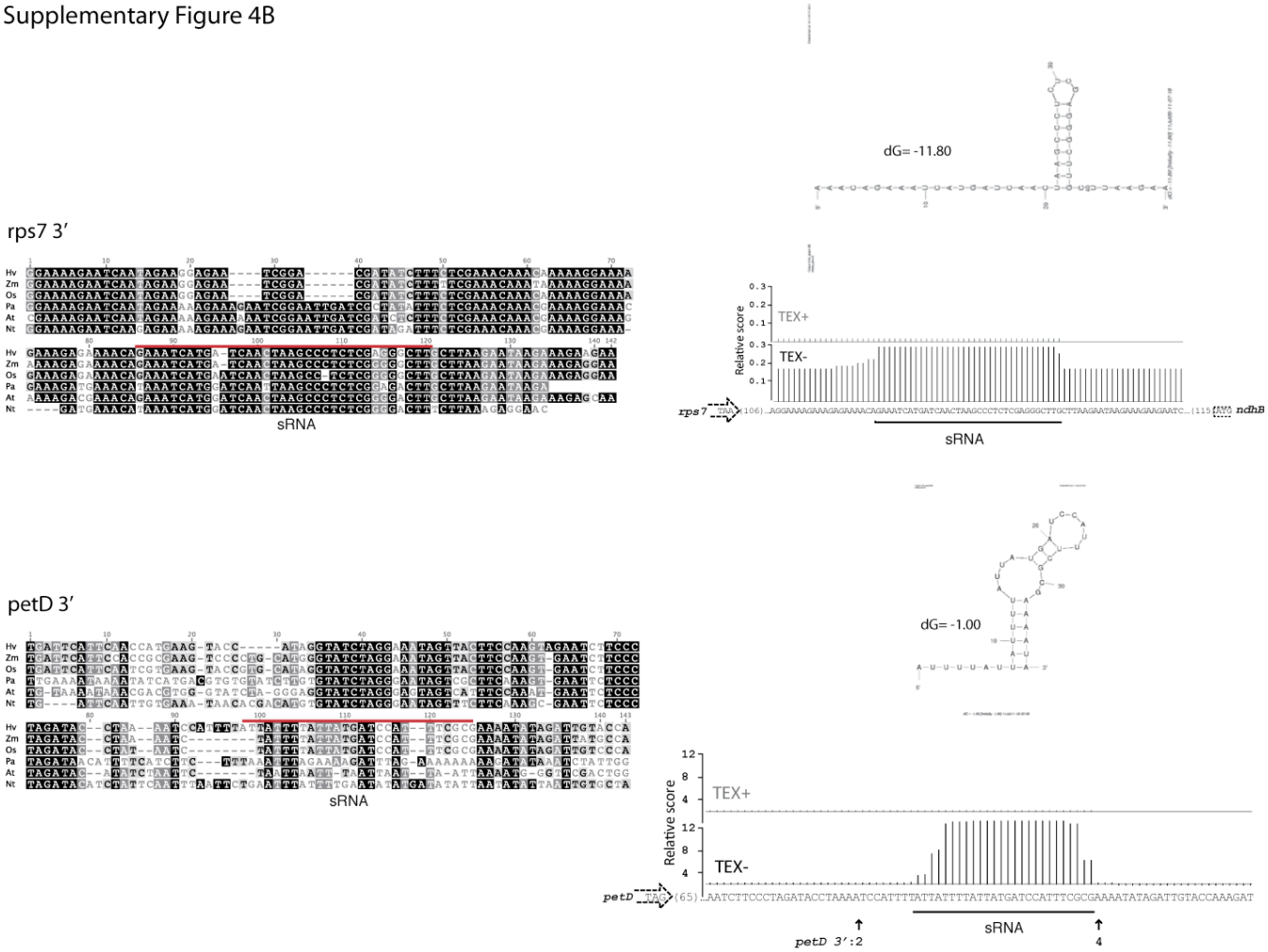
Supplementary Figure 4A part 1



Supplementary Figure 4A part 2

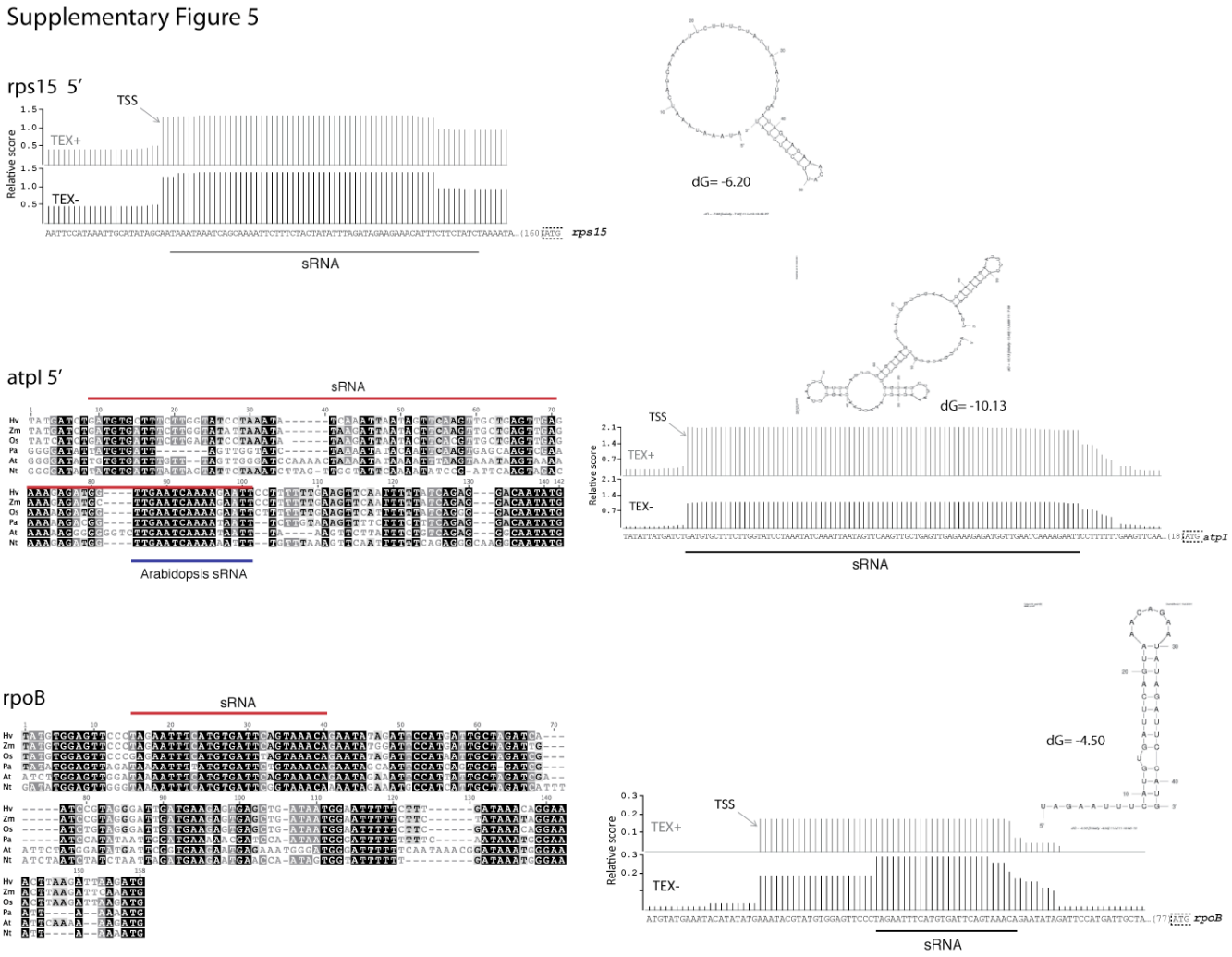


Supplementary Figure 4B



Supplementary Figure 5.

Supporting data for sRNAs that map near transcription start sites (summarized in Table 4). Alignments and RNA structure analyses are as described for Supplementary Figure 4.



Supplementary Table 1. Ends mapped by circular RT-PCR (cRT-PCR) and primer extension (PE) in *Zea mays*.

genes	Primer	Primer sequence	5'end	3'end	clones [1]
psbK cRT-PCR	psbK_FP psbK_RP	5' GGATGTTATGCCTGTCATACCTGTACTC 3' 5' GGCGAAGAAAAAGCTAGTCGGACAAAG 3'	(6989)- TGATCATTACATGGAATT	(7428)- CCAAAAAAAAAAAATGGAT	9
psbB cRT-PCR	psbB_FP psbB_RP	5' CAGACTTGGATGCTCAAGTGGAATTTGG 3' 5' GCATTATATGCACCGAAAGCAATCGACC 3'	(70648)- TTCCAATGCGATAAAA	(72363)- GGTGTGGAAGTTATAATT	8
atpI cRT-PCR	atpI_FP atpI_RP	5' TGCAACGTTAGCCGCAGCCTATATAG 3' 5' GTGTTGCCCTACTTCTACACCCGAT 3'	(32702)- GATGTGATTTCTTGGTAT	(33730)- TATATGCATTCAGGGGGG	7
psbC cRT-PCR	psbC_FP psbC_RP	5' ATGCAGGAAGAGCCCGAGCTGCT 3' 5' TGGTTTCTTGGTCACGACCAGCTAA 3'	(10074)- CAGCTCAGGATCAGCCTC	(11565)- CCATCTAGCCGAGCCATT	10
ndhA cRT-PCR	ndhA_FP ndhA_RP	5' CCCAGGATGAGAATGGATCAGTTATTAA 3' 5' GGGTAGAATCCATATAAGTCCGTAGATT 3'	(116524)- AAATTGGCTGATATCATG	(114292)- AGAGAAAGAAACATACC	10
clpP cRT-PCR	clpP_FP1 clpP_FP2 clpP_RP	5' CGCGAAATGATCACAAGGGTT 3' 5' GAAGACATGGAAAGGGATGTTT 3' 5' CGCAACGAATCTCTTGACCTAA 3'	Described in Figure 1		
rps12 PE	Rps12_RP	5' CCTTAAGCGCGGCCGATTTTCTAGC 3'			

[1] Number of clones out of 10 in which the ends mapped to the underlined 5' and 3' sequence.

Supplementary Table 2. Primers used for 3' RACE analysis of barley chloroplast 3' termini.

Name	***Sequence (5'-3')***	***Target***	***Description***
3R_1	CACCACTTCCCGTTCGACTTG	*rrn16*	cDNA synthesis, 1st PCR rrn16 (linker) specific primer
3R_2	CCGTTCGACTTGCATGTGTTA		2nd PCR rrn16 (linker) specific primer
PZ	GCTGAAGCCGCTATTGGACT	*ndhE*	1st PCR target specific primer
R39	TTGGCTAATTCGAAATCGTCTG		2nd PCR target specific primer
R27	AATTGCGGAAGCTTGGTTTG	*ycf3*	1st PCR target specific primer
R28	GCGCTTACTCCGGGAAATTA		2nd PCR target specific primer
R29	TGATAATCATCCGCGCCTTA	*ndhJ*	1st PCR target specific primer
R30	TTTTCCTAGATGAAACGGAATACCT		2nd PCR target specific primer
R31	TTTCCTTGAAAAGGGTGCTCA	*rps16*	1st PCR target specific primer
R32	AAGGGTGCTCAACCGACAAG		2nd PCR target specific primer
R33	GCCCAGTAGCTACGACCGTTT	*petD*	1st PCR target specific primer
R34	TGGAGCAACACTACCCATTGA		2nd PCR target specific primer
R35	TCCTTCCGTCCCAGATTGTT	*trnQ*	1st PCR target specific primer
R36	TGGGTTTAAATAAATTGGATCTTGG		2nd PCR target specific primer
R37.1	CTCGGGGGTTCCCTAGTTCT	*rps4*	1st PCR target specific primer
R38	TTCGTGTCCAAGCAGAAGGA		2nd PCR target specific primer
R42	TGGAAATAGCATGGAATAAGGTTTG	rps7	1st PCR target specific primer
R43	CCTATTCATGGGGATTCCGTA		2nd PCR target specific primer
R44	GTCAGCAGACGAAGCCAAAG	*clpP*	1st PCR target specific primer
R45	TCCGGAAATGTTTAAGGATTGG		2nd PCR target specific primer
M13_rev	GAGCGGATAACAATTTCACACAGG	pGEM®T	Sequencing of 3'-RACE products cloned into pGEM®T vector