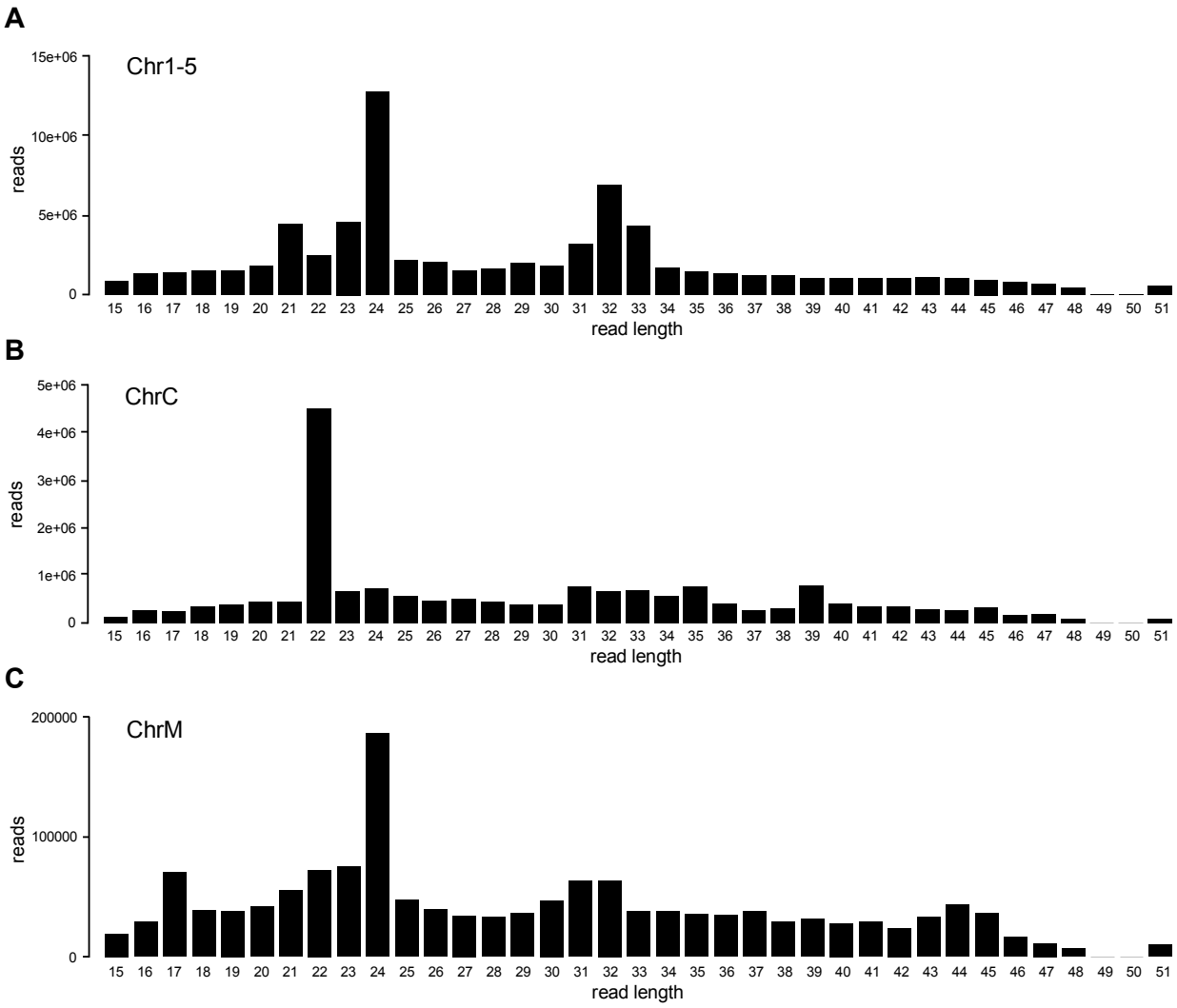
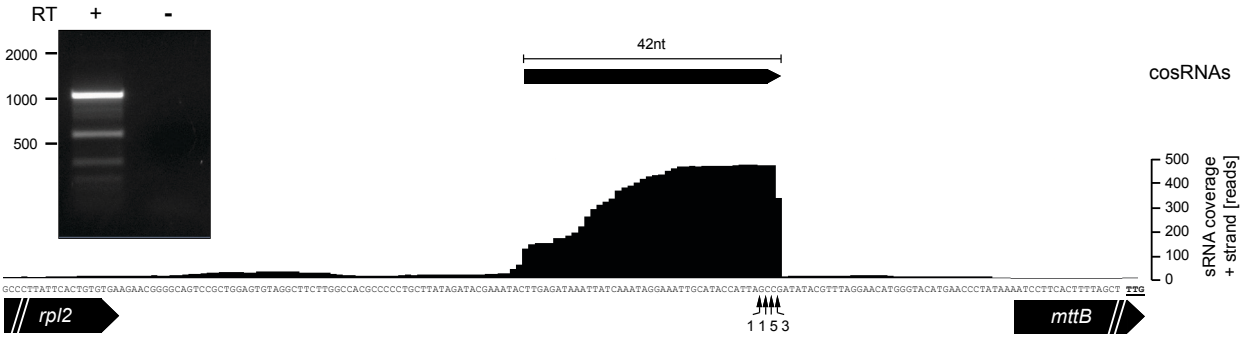
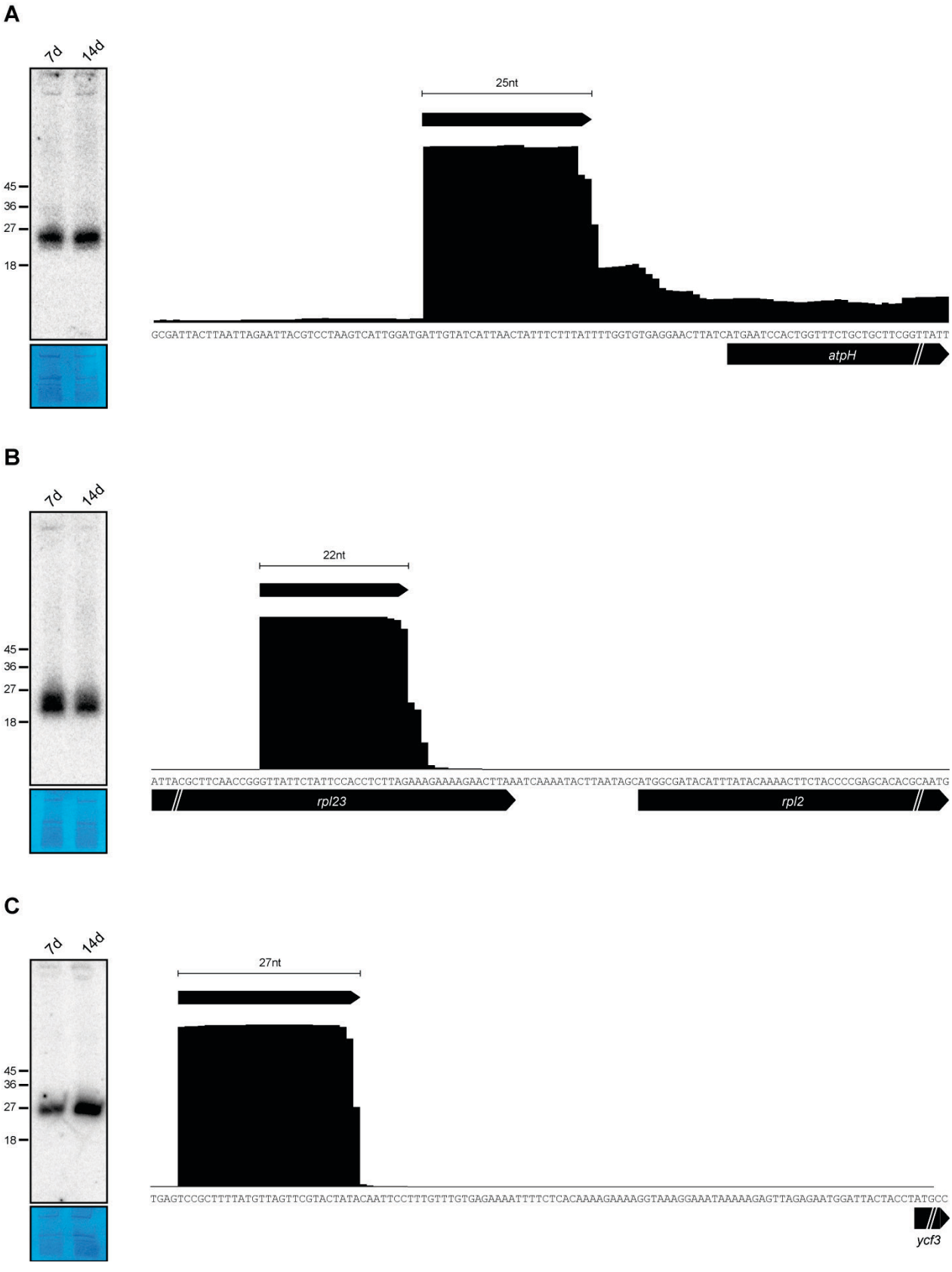


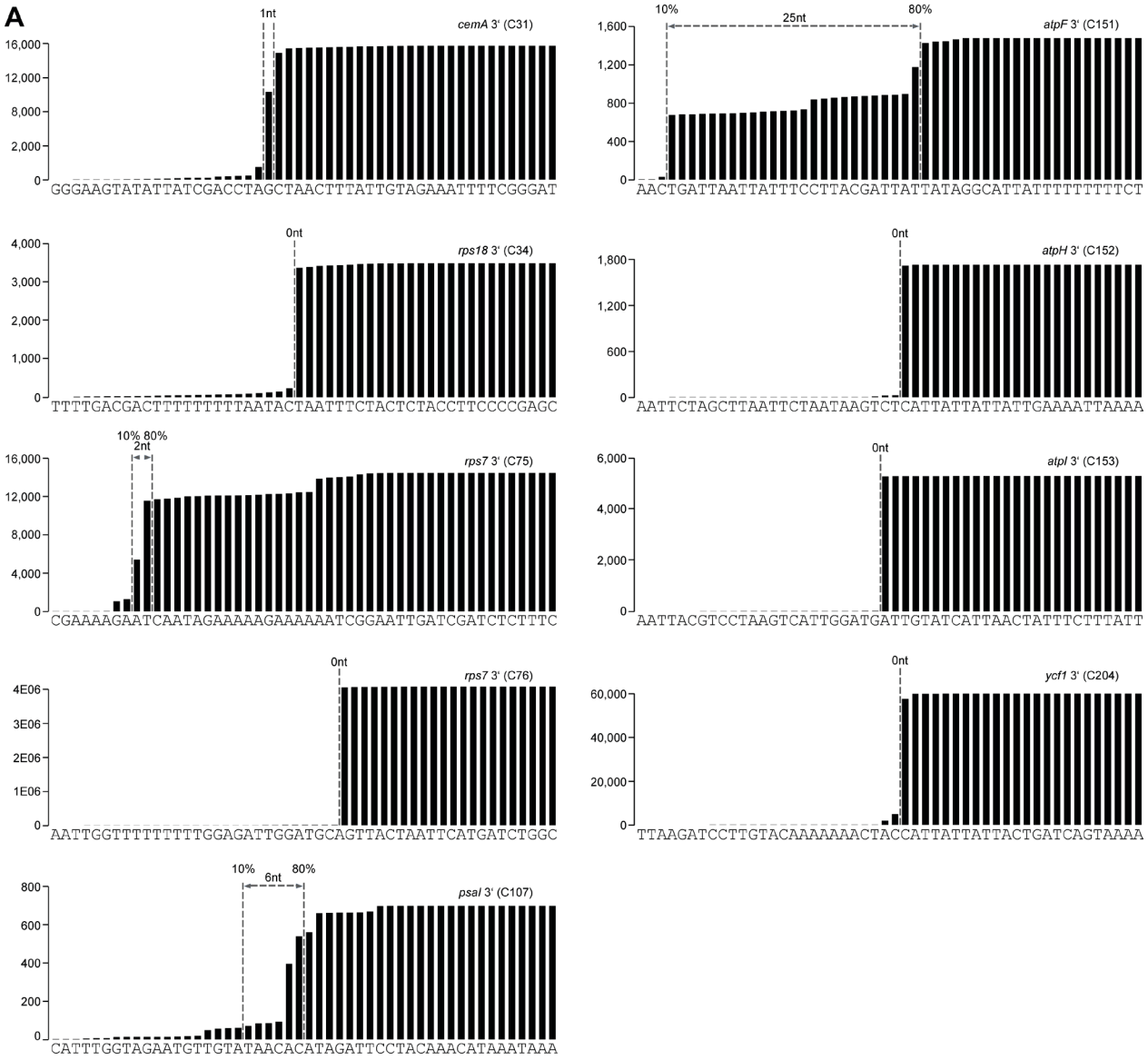

**Supplementary Figure S1: Read distribution of sRNAs that map to nuclear and organelle genomes.** sRNAs from eight different individual Arabidopsis Col-0 lines (20) were mapped using nuclear, mitochondrial, and plastid genomes as reference sequences (NCBI: JF729201, TAIR10; 22). Mapping was performed using bowtie (23), allowing for a maximum of two mismatches. The number of mapped reads is plotted against the length of the sRNA. sRNAs that map to the nuclear chromosomes (A), the plastid (B) and mitochondrial genome (C) are displayed.

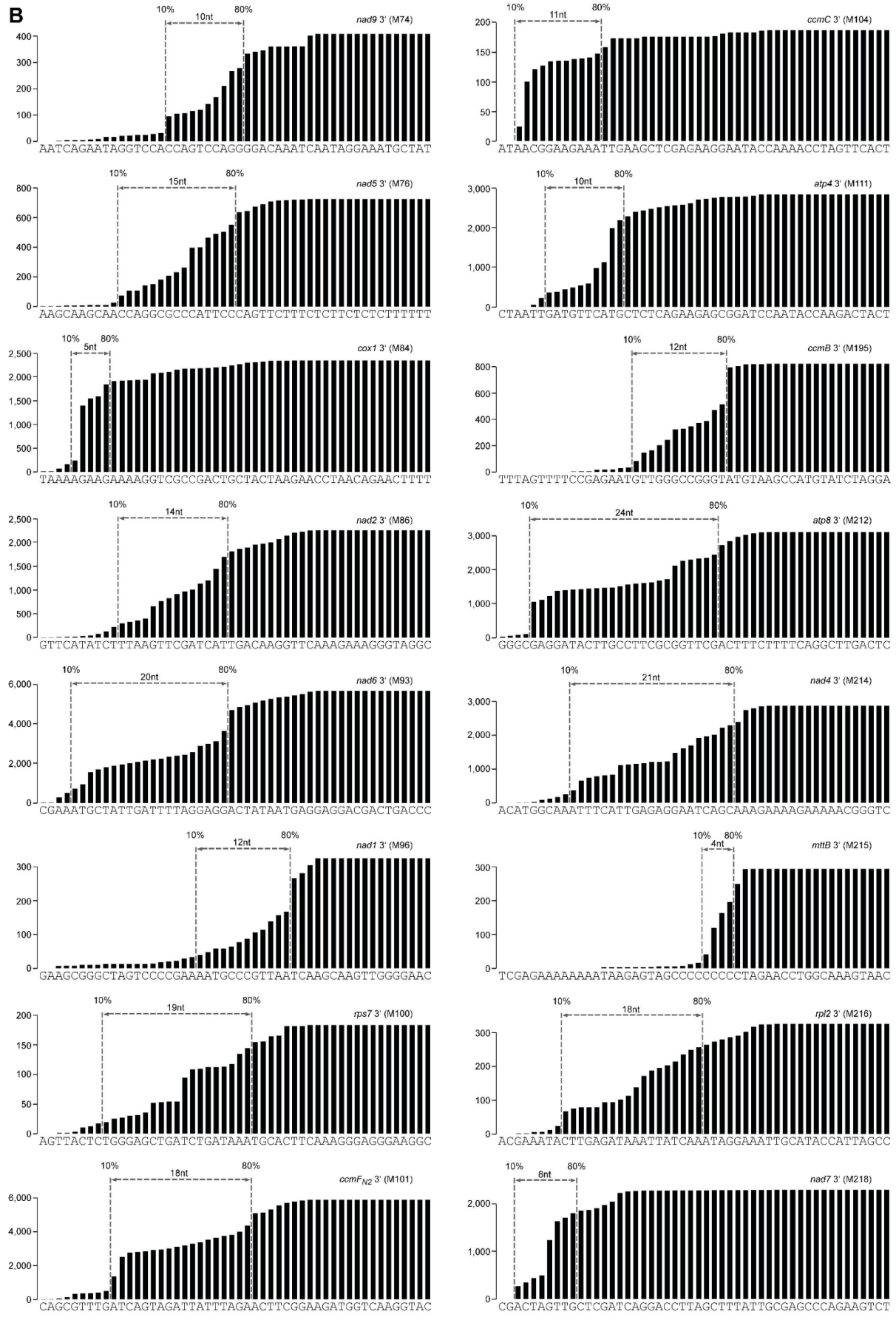



**Supplementary Figure 2: Identification of a monocistronic *rpl2* transcript.** Total Arabidopsis RNA was ligated to a 3'-blocked DNA oligonucleotide selectively at the 3′-end, reverse-transcribed using an oligonucleotide-specific primer, and amplified by PCR using combinations of gene-specific and oligonucleotide-specific primers. The amplification products were separated on an agarose gel (left side). PCR products were gel-purified, cloned, and sequenced. Amplification of a sample without reverse transcriptase (-RT) was included as a control for nonspecific amplification of DNA. The intergenic region between *rpl2* and *mttB* is shown, with arrows indicating TAIR10 annotations. The sRNA coverage is shown in black above the genome sequence. The sRNA identified is marked with an arrow. The number of 3'-ends obtained by cloning the 1-kb band is shown below the genome sequence. Only individual ligation events, as judged from analyses of the barcode included in the 3' adapter oligonucleotide, were counted. The sequence of a potential alternative startcodon (67) is underlined.

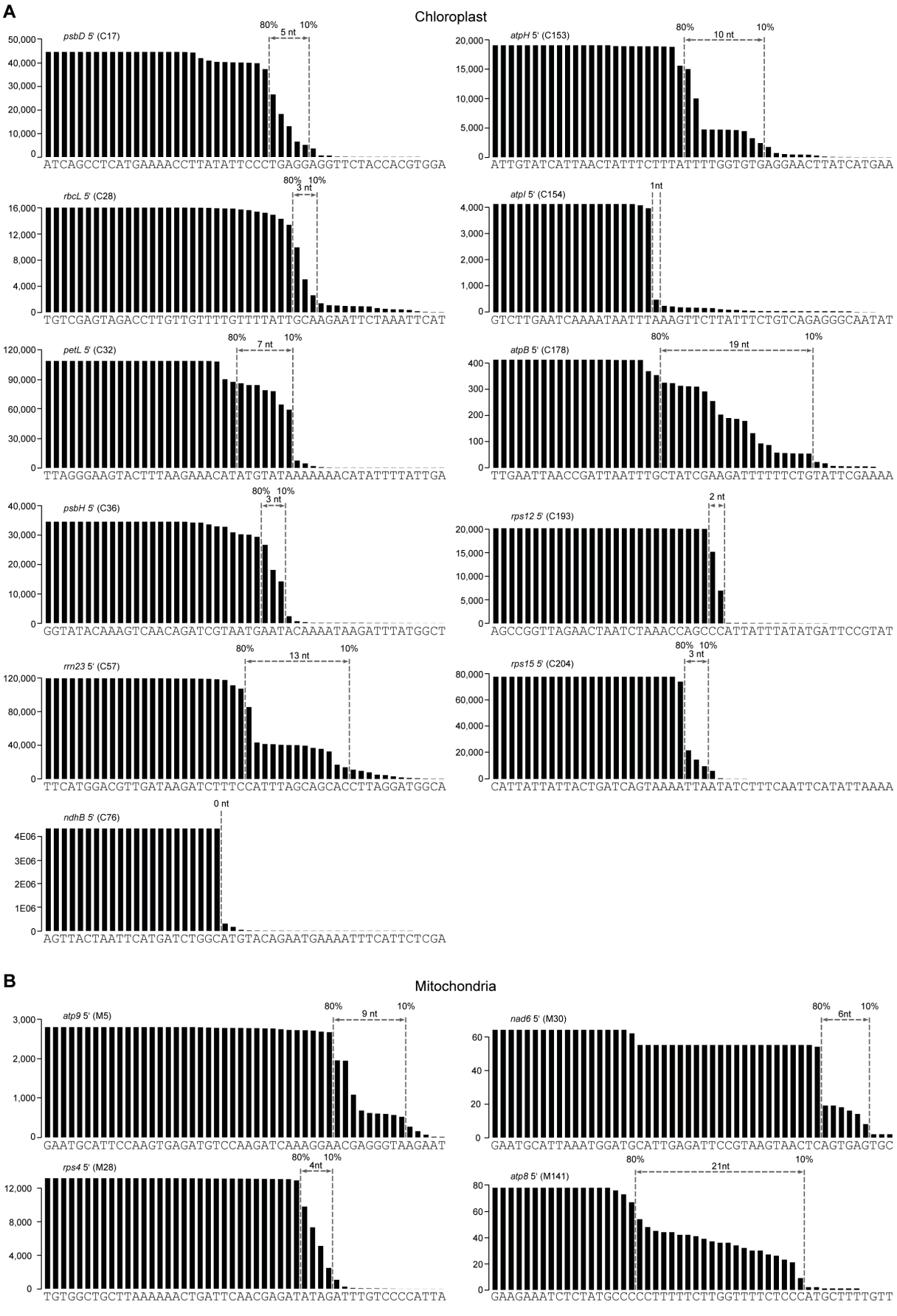

A
7d
14d
45
36
27
18
25nt
GCGATTACTTAATTAGAATTACGTCCTAAGTCATTGGATGATTGTATCATTAACTATTTCTTTATTTTGGTGTGAGGAACTTATCATGAATCCACTGGTTTCTGCTGCTTCGGTTATT
atpH
B
7d
14d
45
36
27
18
22nt
ATTACGCTTCAACCGGGTTATTCTATTCCACCTCTTAGAAAGAAAAGAACTTAAATCAAAATACTTAATAGCATGGCGATACATTTATACAAAACTTCTACCCCGAGCACACGCAATG
rpl23
rpl2
C
7d
14d
45
36
27
18
27nt
TGAGTCCGCTTTTATGTTAGTTCGTACTATACAATTCCTTTGTTTGTGAGAAAATTTTCTCACAAAAGAAAAGGTAAAGGAAATAAAAAGAGTTAGAGAATGGATTACTACCTATGCC
ycf3

**Supplementary Figure S3: Detection of plastid cosRNAs by RNA gel blot analysis.** Total RNA (20 µg) from 7- and 14-day-old seedlings was separated on denaturing polyacrylamide gels, and three cosRNAs were detected using $^{32}$P-end-labeled oligonucleotide probes after transfer to a nylon membrane. Sizes of marker oligonucleotides are indicated beside the radiograph. A methylene blue-stained membrane after transfer is shown as a loading control. The right side of each panel shows the location of the sRNA with respect to neighboring genes. The sRNA coverage is shown in black. The sizes of cosRNAs are shown above arrows, indicating the identified cosRNAs. Detection of the cosRNAs *atpH* 5' (A), *rpl2* 5' (B), and *ycf3* 5' (C). The cosRNA *atpH* 5' likely represents the footprint of the Arabidopsis homolog of maize PPR10.

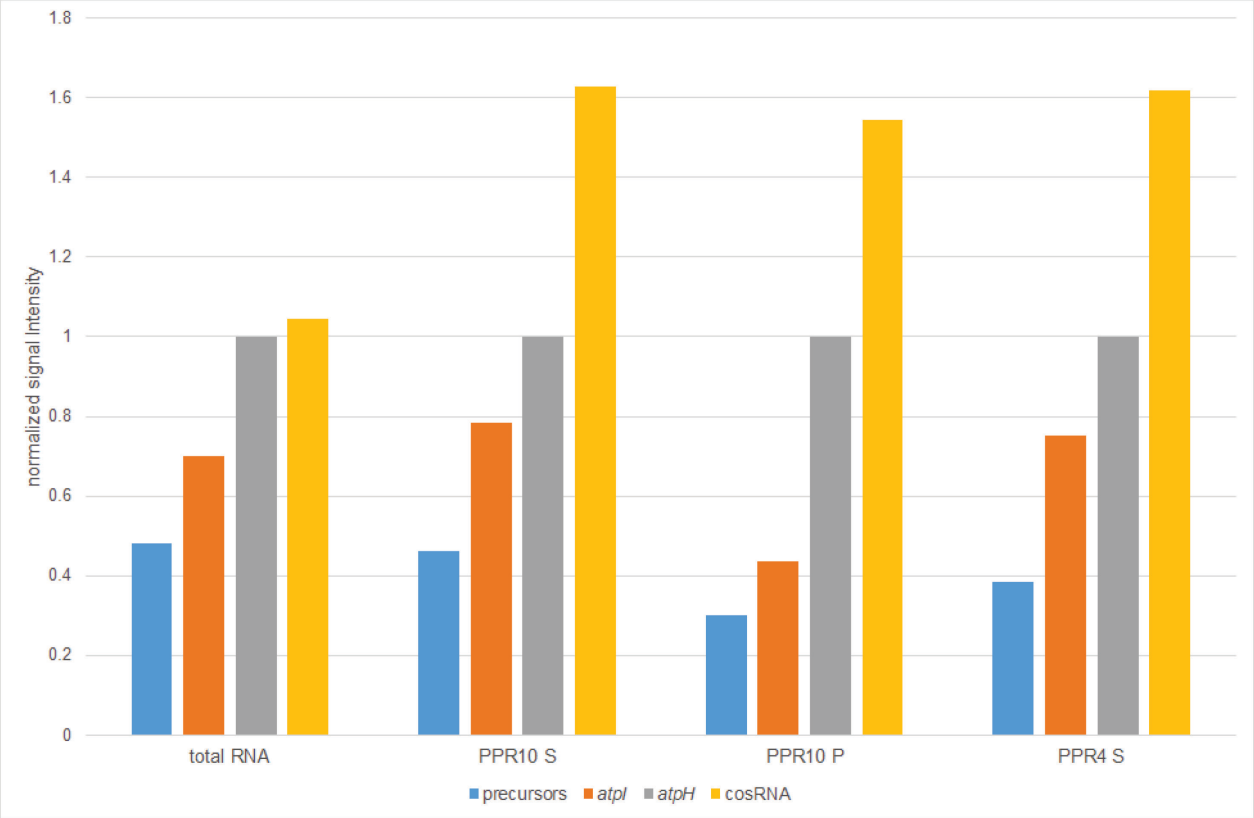

A
1nt
cemA 3' (C31)
16,000
12,000
8,000
2,000
0
GGGAAGTATATTATCGACCTAGCTAACTTTATTGTAGAAATTTTCGGGAT
10%
25nt
80%
atpF 3' (C151)
1,600
1,200
800
400
0
AACTGATTAATTATTTCCTTACGATTATTATAGGCATTATTTTTTTTTCT
0nt
rps18 3' (C34)
4,000
3,000
2,000
1,000
0
TTTTGACGACTTTTTTTTTAATACTAATTTCTACTCTACCTTCCCCGAGC
0nt
atpH 3' (C152)
1,800
1,200
600
0
AATTCTAGCTTAATTCTAATAAGTCTCATTATTATTATTGAAAATTAAAA
10% 80%
2nt
rps7 3' (C75)
16,000
12,000
8,000
4,000
0
CGAAAAGAATCAATAGAAAAAGAAAAAATCGGAATTGATCGATCTCTTTC
0nt
atpI 3' (C153)
6,000
4,000
2,000
0
AATTACGTCCTAAGTCATTGGATGATTGTATCATTAACTATTTCTTTATT
0nt
rps7 3' (C76)
4E06
3E06
2E06
1E06
0
AATTGGTTTTTTTTTGGAGATTGGATGCAGTTACTAATTCATGATCTGGC
0nt
ycf1 3' (C204)
60,000
40,000
20,000
0
TTAAGATCCTTGTACAAAAAAACTACCATTATTATTACTGATCAGTAAAA
10%
6nt
80%
psaI 3' (C107)
800
600
400
200
0
CATTTGGTAGAATGTTGTATAACACATAGATTCCTACAAACATAAATAAA


B
10%
10nt
80%
nad9 3' (M74)
AATCAGAATAGGTCCACCAGTCCAGGGGACAAATCAATAGGAAATGCTAT
10%
11nt
80%
ccmC 3' (M104)
ATAACGGAAGAAATTGAAGCTCGAGAAGGAATACCAAAACCTAGTTCACT
10%
15nt
80%
nad5 3' (M76)
AAGCAAGCAACCAGGCGCCCATTCCCAGTTCTTTCTCTTCTCTCTTTTTT
10%
10nt
80%
atp4 3' (M111)
CTAATTGATGTTCATGCTCTCAGAAGAGCGGATCCAATACCAAGACTACT
10%
5nt
80%
cox1 3' (M84)
TAAAAGAAGAAAAGGTCGCCGACTGCTACTAAGAACCTAACAGAACTTTT
10%
12nt
80%
ccmB 3' (M195)
TTTAGTTTTCCGAGAATGTTGGGCCGGGTATGTAAGCCATGTATCTAGGA
10%
14nt
80%
nad2 3' (M86)
GTTCATATCTTTAAGTTCGATCATTGACAAGGTTCAAAGAAAGGGTAGGC
10%
24nt
80%
atp8 3' (M212)
GGGCGAGGATACTTGCCTTCGCGGTTCGACTTTCTTTTCAGGCTTGACTC
10%
20nt
80%
nad6 3' (M93)
CGAAATGCTATTGATTTTAGGAGGACTATAATGAGGAGGACGACTGACCC
10%
21nt
80%
nad4 3' (M214)
ACATGGCAAATTTCATTGAGAGGAATCAGCAAAGAAAAGAAAAACGGGTC
10%
12nt
80%
nad1 3' (M96)
GAAGCGGGCTAGTCCCCGAAAATGCCCGTTAATCAAGCAAGTTGGGGAAC
10%
4nt
80%
mttB 3' (M215)
TCGAGAAAAAAAAATAAGAGTAGCCCCCCCCCCTAGAACCTGGCAAAGTAAC
10%
19nt
80%
rps7 3' (M100)
AGTTACTCTGGGAGCTGATCTGATAAATGCACTTCAAAGGGAGGGAAGGC
10%
18nt
80%
rpl2 3' (M216)
ACGAAATACTTGAGATAAATTATCAAATAGGAAATTGCATACCATTAGCC
10%
18nt
80%
ccmF$_{N2}$ 3' (M101)
CAGCGTTTGATCAGTAGATTATTTAGAACTTCGGAAGATGGTCAAGGTAC
10%
8nt
80%
nad7 3' (M218)
CGACTAGTTGCTCGATCAGGACCTTAGCTTTATTGCGAGCCCAGAAGTCT

**Supplementary Figure S4: 5' Sharpness of cosRNAs overlapping transcript 3'-ends.** sRNA coverage at 3'-ends of chloroplast (A) and mitochondria (B) transcript ends mapped previously (13, 33, 46, 69). The coverage is shown in number of reads. Only alignments that end at the dominant 3'-end of the cosRNA are shown and used for the analysis of coverage decrease. The decrease in cosRNA coverage at the 5'-end was measured as the number of nucleotides needed to decrease maximal coverage from 80% to 10% (dashed lines).


A
Chloroplast
psbD 5' (C17)
80%
5 nt
10%
ATCAGCCTCATGAAAACCTTATATTCCCTGAGGAGGTTCTACCACGTGGA
atpH 5' (C153)
80%
10 nt
10%
ATTGTATCATTAACTATTTCTTTATTTTGGTGTGAGGAACTTATCATGAA
rbcL 5' (C28)
80%
3 nt
10%
TGTCGAGTAGACCTTGTTGTTTTGTTTTATTGCAAGAATTCTAAATTCAT
atpI 5' (C154)
1nt
GTCTTGAATCAAAATAATTTAAAGTTCTTATTTCTGTCAGAGGGCAATAT
petL 5' (C32)
80%
7 nt
10%
TTAGGGAAGTACTTTAAGAAACATATGTATAAAAAAACATATTTTATTGA
atpB 5' (C178)
80%
19 nt
10%
TTGAATTAACCGATTAATTTGCTATCGAAGATTTTTTCTGTATTCGAAAA
psbH 5' (C36)
80%
3 nt
10%
GGTATACAAAGTCAACAGATCGTAATGAATACAAAATAAGATTTATGGCT
rps12 5' (C193)
2 nt
AGCCGGTTAGAACTAATCTAAACCAGCCCATTATTTATATGATTCCGTAT
rrn23 5' (C57)
80%
13 nt
10%
TTCATGGACGTTGATAAGATCTTTCCATTTAGCAGCACCTTAGGATGGCA
rps15 5' (C204)
80%
3 nt
10%
CATTATTATTACTGATCAGTAAAATTAATATCTTTCAATTCATATTAAAA
ndhB 5' (C76)
0 nt
AGTTACTAATTCATGATCTGGCATGTACAGAATGAAAATTTCATTCTCGA
B
Mitochondria
atp9 5' (M5)
80%
9 nt
10%
GAATGCATTCCAAGTGAGATGTCCAAGATCAAAGGAACGAGGGTAAGAAT
nad6 5' (M30)
80%
6nt
10%
GAATGCATTAAATGGATGCATTGAGATTCCGTAAGTAACTCAGTGAGTGC
rps4 5' (M28)
80%
4nt
10%
TGTGGCTGCTTAAAAAACTGATTCAACGAGATATAGATTTGTCCCCATTA
atp8 5' (M141)
80%
21nt
10%
GAAGAAATCTCTATGCCCCCTTTTTCTTGGTTTTCTCCCATGCTTTTGTT

**Supplementary Figure S5: 3' Sharpness of cosRNAs overlapping transcript 5'-ends.** sRNA coverage at 5'-ends of chloroplast (A) and mitochondria (B) transcript ends mapped previously (13, 33, 46, 68–73). The coverage is shown in number of reads. Only alignments that start at the dominant 5'-end of the cosRNA are shown and used for the analysis of coverage decrease. The decrease in cosRNA coverage at the 3'-end was measured as the number of nucleotides needed to decrease maximal coverage from 80% to 10% (dashed lines).




**Supplementary Figure S6:** Quantification of the bands in the RNase protection assay (Figure 5). Individual bands corresponding to polycistronic precursors, processed *atpI*, processed *atpH* and the cosRNA in the radiographs were quantified using ImageLab software (BioRad) and normalized to the signal for the processed *atpH* mRNAs. The values were adjusted for the content of radiolabeled Us. S stands for supernatant fractions of the immunoprecipitations, P for pellet fractions.