Supplementary Information Table S1: Top Ranking Fragments in PPR5 RIP-chip assays

		PPR5 IP		OEC IP		Comparison: log2 PPR5 ratio-log2 OEC ratio	
Fragment Name	Fragment number[a]	Median Log2 Ratio (E)[b]	*n*[b]	Median Log2 Ratio (E)[b]	*n*[b]	Relative Enrichment ($E_{PPR5} - E_{OEC}$)	P[c]
trnG-UCCex/int	**25**	**-1,85**	**10**	**-6,84**	**9**	**5,00**	**1,96E-08**
trnD-GUC	34	-3,97	8	-6,30	10	2,33	2,50E-06
trnV-GAC	197	-4,06	10	-5,94	10	1,87	2,73E-07
trnV-UACex2int	97	-3,24	9	-4,90	8	1,67	3,39E-02
trnG-UCCintex1/ORF69	**26**	**-4,36**	**7**	**-6,00**	**9**	**1,64**	**7,03E-03**
rpl23	171	-4,29	10	-5,81	9	1,52	1,53E-05
rps8	157	-4,04	6	-5,43	10	1,39	9,12E-02
rpoC1/C2	48	-4,33	10	-5,69	9	1,37	4,46E-07
rps2B	54	-3,39	7	-4,75	9	1,36	1,41E-04
petN3prime	37	-3,36	9	-4,71	10	1,36	1,94E-05
orf173-3'	180	-4,01	10	-5,30	10	1,29	4,42E-05
rpl16-int	162	-4,32	10	-5,56	6	1,24	6,75E-06
rpl2/rpl23	170	-4,21	10	-5,44	10	1,23	6,78E-06
psaC/ndhE/ndhG	236	-2,60	5	-3,82	9	1,23	1,16E-01
rpl14	159	-4,25	9	-5,39	8	1,13	3,07E-02
rpl33	127	-3,38	8	-4,51	10	1,12	2,42E-04
ORF69	**27**	**-4,17**	**6**	**-5,26**	**9**	**1,09**	**3,31E-02**
rrn16-3prime	200	-2,44	10	-3,53	8	1,09	4,25E-02
rps3	163	-4,34	9	-5,32	8	0,98	2,66E-02
ycf9	20	-3,94	9	-4,89	10	0,95	3,85E-03
trnT-GGU/trnE-UCC5'	30	-4,34	9	-5,19	9	0,85	1,06E-03
atpF1	61	-3,83	10	-4,64	9	0,81	3,04E-03
rpl20/rps12int15'	130	-3,61	10	-4,42	9	0,81	1,11E-02
trnL-UAG	227	-3,34	9	-3,78	9	0,44	5,85E-01
ycf3ex1/trnS5'	81	-4,17	10	-4,52	8	0,35	2,13E-01
trnI-CAU	173	-4,14	9	-4,39	8	0,24	1,71E-01
orf139	176	-3,44	8	-3,53	10	0,09	8,95E-01

Elements ranking in the top 10% for median normalized enrichment ratio (E) from wild-type stroma are ordered according to the magnitude of their relative enrichment in PPR5 versus OEC immunoprecipitations ($E_{PPR5} - E_{OEC}$). Fragments that map within or adjacent to the *trnG*-UCC locus are in boldface

[a] Fragments on the array are numbered according to chromosomal position. The nucleotide residues on each fragment are described in Array Express (accession number A-MEXP-164) and in Supplemental Table 1 from (5).

[b] E = median (log_2F635/F532) normalized across two replicate experiments with PPR5 antibodies and two control experiments (one with antibodies against OEC23 and one with antibodies against OEC16).
Replicate experiments constitute a total of n replicate spots with signal above background. Fragments for which at least three spots per array did not meet our background cutoff in the supernatant (F532) channel were not considered reliable and were not used to generate this table.

[c] P values were calculated with a t test (two-tailed, unequal variance) and represent the probability that there is no difference in enrichment from PPR5 and OEC immunoprecipitations.

Supplementary Information, Table S2: Oligonucleotides and probes used in this study

Fig.	Detection of	Probes / Primers [§]		Homolo-gous to	Pos 1[#]	Pos 2[#]
Fig. 1C	*ppr5*	AGCTGGTGCGCTCGCTGCT (ppr5-cDNAF3)	GGAGTAGATGCCGTTGTCAGC (ppr5-cDNAR3)	nucleus	/	/
	actin	GCCACTGATCCAGACACTGT	ATTCAGGTGATGGTGTGAGC	nucleus	/	/
Fig. 2B	*rrn16*	maize cp DNA fragment in clone prrn16-1, a Bluescript SKII- derivative; primers M13for and M13rev		plastid	95236	95553
	rrn23	AAGGCCCCTTAATGACCGCT	CGGCTCGAGGCATTTTCTCT	plastid	100098	100599
Fig. 3B	*trnG*-UCC probe A	GCCTTAAGTATATCATTTCA	GGGCTATCTAAATCCTAA	plastid	13942	14167
	trnG-UCC probe B	TCCGCTCTGTAGGGCCGAAAACT	GGTTTTGAATTAGAGA	plastid	13141	13341
	trnG-UCC probe C	CTTCGTTCCCTAATTTC	GCGGGTATAGTTTAGTGG	plastid	13505	14013
	trnG-UCC probe D	AGCGGGTAGCGGGAATC	GGGAACGAAGTAAG	plastid	13245	13514
	rpl23	CCGTATTATTAAGTATTCTC	TACTGGAACTCAGAGCATAG	plastid	84411	84750
	trnV-UACexint	ATTATGAAACAGAATATCCTG	TCTTGCAGCGGAACGATAGAGA	plastid	52711	53491
	rpl16 intron	AATGAAATGAGAAGCGTGCGA	CAACCTATTGCTTCGTATTGTCG	plastid	80002	80888
	trnV-GAC	TTGACTGTCCATAATAGTGC	AAACTCACATTAGGTTTAGG	plastid	94751	94949
Fig. 4D	*trnG*-UCC intron	TATCCAAAGACTAATTGAATTTGC	GAATGAAATTAGGGAACGAAGTA	plastid	13503	13828
	trnG-UCC exon 2	CATCGTTAGCTTGGAAGGCTAGG		plastid	13269	13291
	trnV-GAC	CTTCCACCACGTCAAGGTGACACTCTACCGC		plastid	94903	94873
	trnD-GUC	CGAACCCGCAGCTTCCGCCTTGACAGGGC		plastid	17110	17082
Fig. 5	*trnG*-UCC intron 5’	TATCCAAAGACTAATTGAATTTGC	GAATGAAATTAGGGAACGAAGTA	plastid	13503	13828
	trnG-UCC exon 2	CATCGTTAGCTTGGAAGGCTAGG		plastid	13269	13291
	trnG-UCC exon 1	CACACTTTTACCAAACTATACCCGCTAC		plastid	13986	14016
	trnG-UCC intron 3’	GTTATAGTCGACGTTGGTTGATTATTTTTGACGTCTCTAATTCAAAACCGAA CATGAAATTTTGATTTCATTCGGCTCCTTTATGGATATTCTCACCACT		plastid	13293	13392
Fig. 6	*cox1*	TTCATCTTCGGTGCCATTGC	CCTGCCAGTACCGGAAGTGA	mitoch.	354925	355484
	actin	GCCACTGATCCAGACACTGT	ATTCAGGTGATGGTGTGAGC	nucleus	/	/
	trnV-UAC	AGGGCTATAGCTCAGTTCGGT	TAGGGCTATACGGAT	plastid	53158	53834
	trnG-UCC	GCGGGTATAGTTTAGTGGTAAA	ATCGAACCCGCATCGTTAGC	plastid	13259	14013
	pDrive	acc. no. DQ996013		vector	/	/
	trnG-UCC exon 2	CATCGTTAGCTTGGAAGGCTAGG		plastid	13269	13291
	trnG-UCC gene unspliced	ATCGAACCCGCATCGTTAGC	GCGGGTATAGTTTAGTGGTAAA	plastid	13259	14013
Suppl. Fig. S2	*rps2*	GCCCCTTACATCTCGGCAAA	CATAATATATATCAATACCCTC	plastid	31951	32697
	rpl33	GTTCATGGCCAAGGGCAAAG	TGATCCAGAACCAGAAGAAG	plastid	66994	67470
Suppl. Fig. S3	*rpl16* intron	AATGAAATGAGAAGCGTGCGA	CAACCTATTGCTTCGTATTGTCG	plastid	80002	80888
	rpl16 exon	TTCCAGAAAGAAAAACCAGGGG	AATCCTGCCGAGGCAATCAT	plastid	79466	79871
	rpl33	GTTCATGGCCAAGGGCAAAG	TGATCCAGAACCAGAAGAAG	plastid	66994	67470
	trnD-GUC	CGAACCCGCAGCTTCCGCCTTGACAGGGC		plastid	17110	17082
	rpl14	TAAATTTAGGATTTTAAAGC	TGAAGATAAAAAACCCCTGG	plastid	78959	79500

if two primers are given per row, Pos 1 denotes starting position of primer one relative to position 1 on the plastid chromosome of maize (3). If only one primer or a clone insert is given, pos1 and pos2 denote the start and end-point of the primer /clone insert relative to position 1 on the plastid chromosome of maize. For *cox1* primers, the start position of the primer relative to the mitochondrial chromosome of maize is indicated (2).

[§] all primers are given in 5'-to-3' direction

Supplementary References

1. **Altschul, S. F., T. L. Madden, A. A. Schaffer, J. Zhang, Z. Zhang, W. Miller, and D. J. Lipman.** 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res **25:**3389-3402.
2. **Clifton, S. W., P. Minx, C. M. Fauron, M. Gibson, J. O. Allen, H. Sun, M. Thompson, W. B. Barbazuk, S. Kanuganti, C. Tayloe, L. Meyer, R. K. Wilson, and K. J. Newton.** 2004. Sequence and comparative analysis of the maize NB mitochondrial genome. Plant Physiol **136:**3486-3503.
3. **Maier, R. M., K. Neckermann, G. L. Igloi, and H. Kossel.** 1995. Complete sequence of the maize chloroplast genome: gene content, hotspots of divergence and fine tuning of genetic information by transcript editing. J Mol Biol **251:**614-28.
4. **Saitou, N., and M. Nei.** 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol **4:**406-425.
5. **Schmitz-Linneweber, C., R. Williams-Carrier, and A. Barkan.** 2005. RNA immunoprecipitation and microarray analysis show a chloroplast Pentatricopeptide repeat protein to be associated with the 5' region of mRNAs whose translation it activates. Plant Cell **17:**2791-2804.
6. **Thompson, J., D. Higgins, and T. Gibson.** 1994. Clustal W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucl Acids Res **22:**4673-4680.

Supplementary Figure Legends

Supplementary Information, Figure S1: Phylogenetic analysis of PPR5 homologs.

A) Neighbor joining distance tree of rice and Arabidopsis proteins that were found as best hits in BLAST (1) searches with the Arabidopsis PPR5 sequence as query. Arabidopsis protein phosphatase 5, a tetratricopeptide repeat protein was used as outgroup. Alignments were performed using the ClustalW algorithm (6) and the tree was constructed using the neighbour-joining algorithm (4). Bootstrap values were from 100 repetitions.

B) Alignment of PPR5 orthologs in maize (Zm), Rice (Os), and Arabidopsis (At). TargetP (Emanuelsson et al., 2000) and Predotar (Small et al., 2004) algorithms both predict that all three proteins are targeted to the chloroplast. The insertion site of the *Mu1* element in *ppr5-1* mutants is indicated by an arrowhead. PPR domains were identified at http://toolkit.tuebingen.mpg.de/tprpred and are indicated above the sequences by brackets. AtPPR5 corresponds to AGI locus At4g39620; OsPPR5 corresponds to TIGR locus Os02g51480. ZmPPR5 corresponds to GenBank accession EU037901.

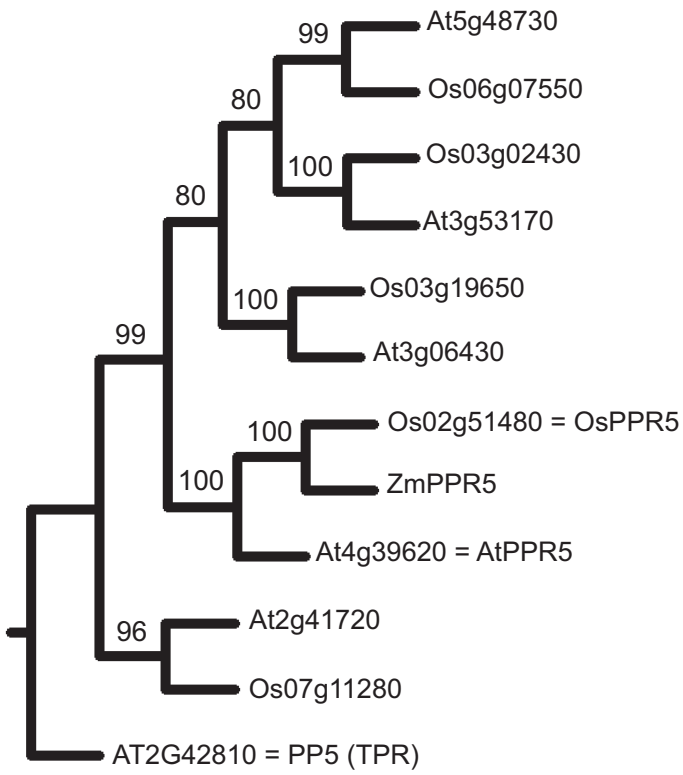
Supplementary Information, Figure S2: Slot-blot analysis of RNAs that were detected in RIP-chip experiments as potential minor partners of PPR5.

Blots shown here are the same as those shown in Figure 3B after stripping of the previous probe and reprobing with the probes indicated. One-sixth of the RNA recovered from each immunoprecipitation pellet (P) and one-twelfth of the RNA recovered from each supernatant (S) was applied to replicate slot blots.

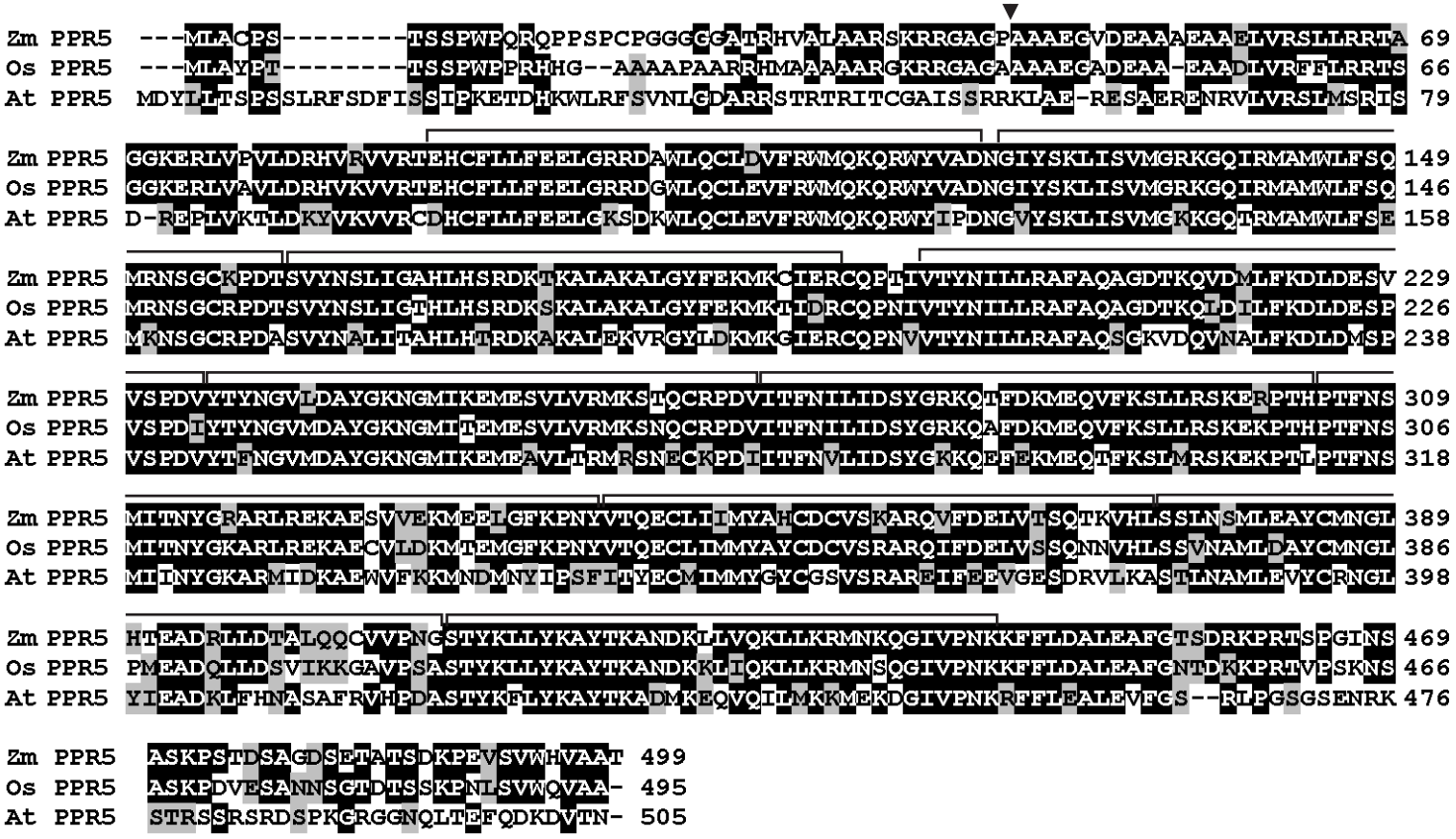
Supplementary Information, Figure S3: RNA gel blot hybridisation of RNAs that were detected in RIP-chip experiments as potential minor partners of PPR5 or that are positioned adjacent to such minor partners on the plastid chromosome.
RNAs from seedlings were size-fractionated on agarose gels, transferred to nylon-mebranes and hybridized with the probes indicated below. For *rpl16*, a large precursor RNA accumulating in WT is reduced in mutants (upper asterisk). A smaller intron-containing RNA increased in *ppr5* mutants (lower asterisk). The other hybridizations did not lead to the identification of major qualitative differences between wild-type and *ppr5* mutant RNA samples.

Supplemental Information, Figure S1, Beick et al.

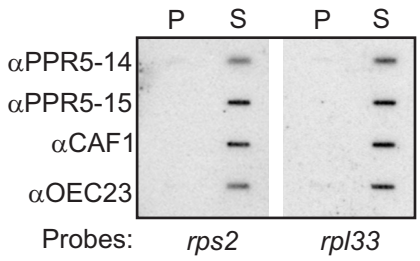
A



B



Supplemental Information, Figure S2, Beick et al.



Supplemental Information, Figure S3, Beick et al.