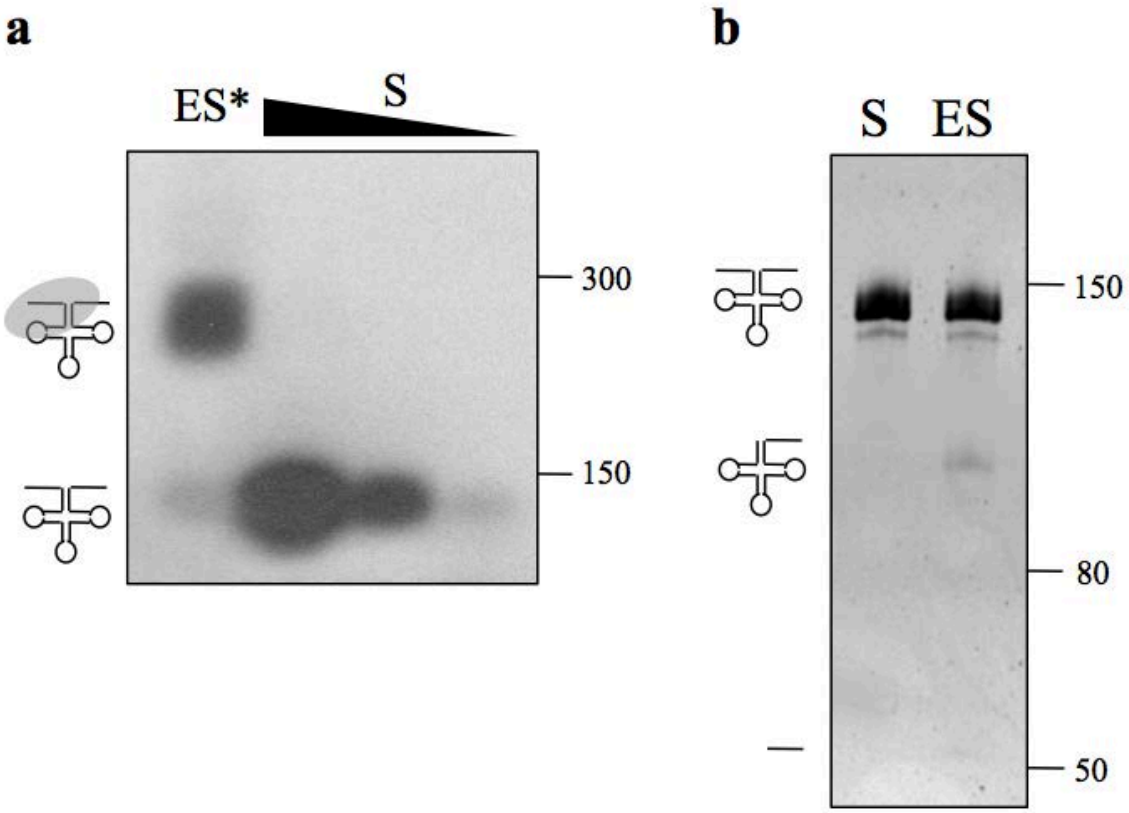
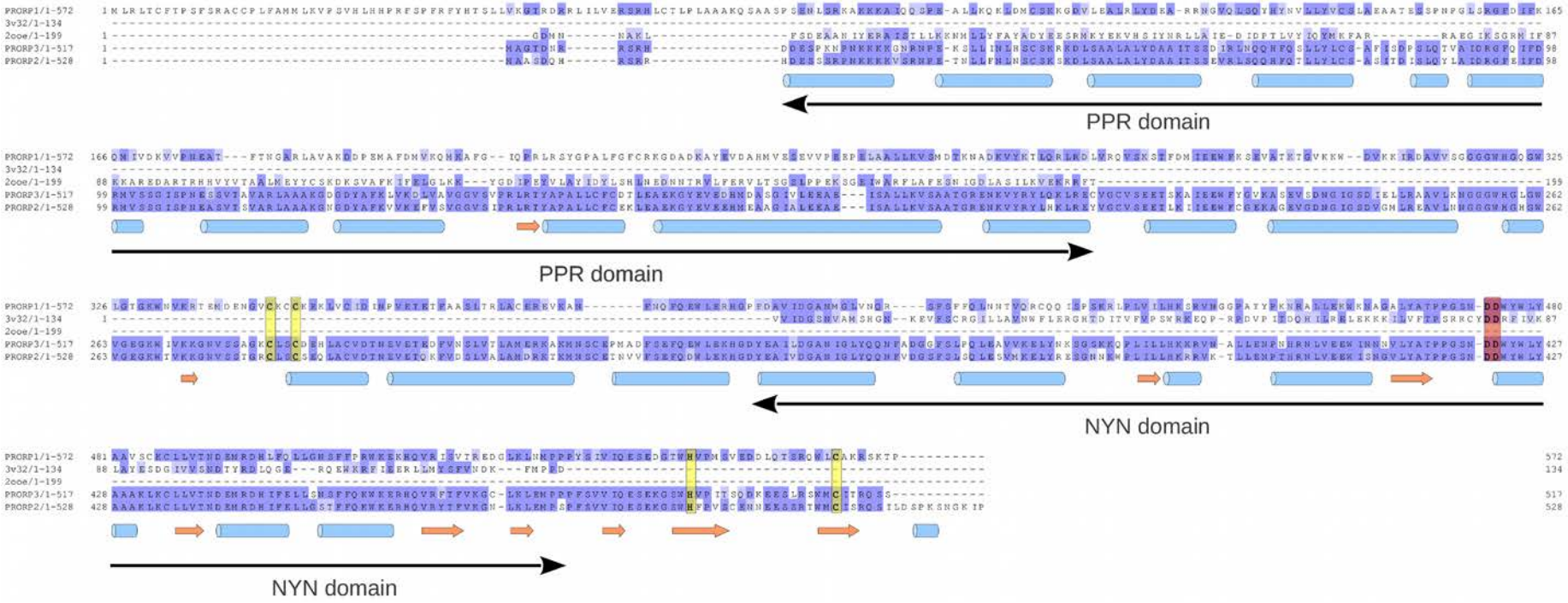
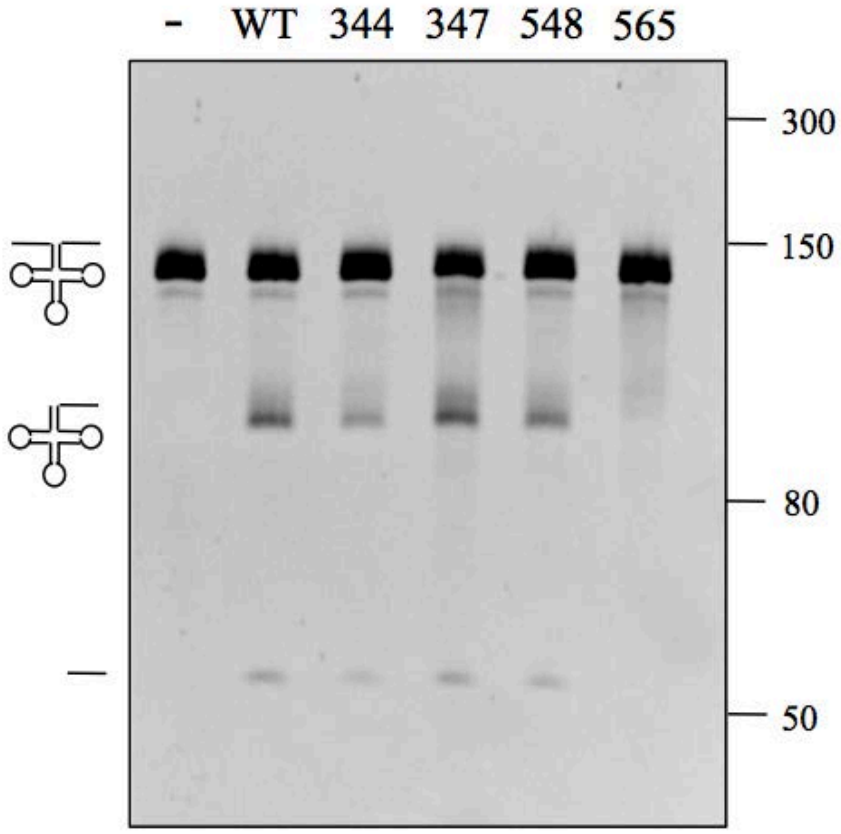


Supplementary Figure S1. **Analysis of the PRORP / tRNA complex used in the footprinting analysis.** (**a**) The crosslinked PRORP / tRNA complex (ES*) was analysed on a denaturing polyacrylamide gel together with 1, 0.1 and 0.01 mg of free tRNA substrate (S). The ES* sample revealed two bands corresponding to the higher molecular weight crosslinked complex and to residual free substrate. Band quantification showed that only 1% of the total signal was present as free substrate. (**b**) In order to show that the PRORP / tRNA complex analysed during footprinting experiments corresponds to a functional complex, a PRORP / tRNA sample in footprinting buffer (ES) was taken just prior to the crosslink step, incubated 15' at RT and analysed by denaturing PAGE. This revealed that RNase P cleavage had taken place and thus showed that the protein / tRNA complex used in the footprint analysis is an active complex. Molecular weight markers are indicated in nucleotides



Supplementary Figure S2 Alignment of PRORP 1-2-3 with template structures identified by Phyre2[24]. The best solution for the N-terminal PPR domain was the TPR domain of the murine cleavage stimulation factor (PDBid 2ooe; sequence identity of 10% with PRORP2; Phyre2 confidence score: 99.0%). The best solution for the C-terminal NYN domain was the catalytic domain of MCPIP1 RNase (PDB id 3v32; sequence identity with PRORP2: 25%; Phyre2 confidence score: 99.9%). Secondary structure predictions for PRORP2 are represented (α-helices in blue, β-strands in orange). Zinc coordinating residues are highlighted in yellow and conserved catalytic aspartates in red.



Supplementary Figure S3. **RNase P activity of zinc binding mutants.** Cleavage assays were carried out with PRORP proteins mutated at positions involved in zinc binding. Reactions were performed without protein (-), with wild type PRORP1 (WT), with point mutants C344A (344), C347A (347), H548A (548) and C565A (565). Apart from mutant 565, which also has the most reduced zinc content, all the other PRORP mutants had unaffected RNase P activity. Molecular weight markers are indicated in nucleotides.

Supplementary Table S1. Oligonucleotides used for mutagenesis of tRNA and PRORP sequences.

Precursors of *Arabidopsis thaliana* mitochondrial tRNA Cys	
ptrnCm_G1C_FW	GGTGGCGGGTTT**C**GCTAGGTAACAT
ptrnCm_G1C_RV	ATGTTACCTAGC**G**AAACCCGCCACC
ptrnCm_G18A_FW	GGCTAGGTAACATAAT**A**GAAATGTATCGGACTGC
ptrnCm_G18A_RV	GCAGTCCGATACATTTC**T**ATTATGTTACCTAGCC
ptrnCm_G18C_FW	GGCTAGGTAACATAAT**C**GAAATGTATCGGACTG
ptrnCm_G18C_RV	CAGTCCGATACATTTC**G**ATTATGTTACCTAGCC
ptrnCm_G19A_FW	GGCTAGGTAACATAATG**A**AAATGTATCGGACTGC
ptrnCm_G19A_RV	GCAGTCCGATACATTT**T**CATTATGTTACCTAGCC
ptrnCm_G19C_FW	GCTAGGTAACATAATG**C**AAATGTATCGGACTGC
ptrnCm_G19C_RV	GCAGTCCGATACATTT**G**CATTATGTTACCTAGC
ptrnCm_G19U_FW	GGCTAGGTAACATAATG**T**AAATGTATCGGACTGC
ptrnCm_G19U_RV	GCAGTCCGATACATTT**A**CATTATGTTACCTAGCC
ptrnCm_C56A_FW	CTGTAATGACGGTT**A**GACTCCGTCCTTGG
ptrnCm_C56A_RV	CCAAGGACGGAGTC**T**AACCGTCATTACAG
ptrnCm_C56G_FW	CTGTAATGACGGTT**G**GACTCCGTCCTTG
ptrnCm_C56G_RV	CAAGGACGGAGTC**C**AACCGTCATTACAG
ptrnCm_G57A_FW	CTGTAATGACGGTTC**A**ACTCCGTCCTTGGC
ptrnCm_G57A_RV	GCCAAGGACGGAGT**T**GAACCGTCATTACAG
ptrnCm_G57C_FW	CTGTAATGACGGTTC**C**ACTCCGTCCTTGGC
ptrnCm_G57C_RV	GCCAAGGACGGAGT**G**GAACCGTCATTACAG
ptrnCm_C72G_FW	TCCGTCCTTGGC**G**TACACCTTCATG
ptrnCm_C72G_RV	CATGAAGGTGTA**C**GCCAAGGACGGA
ptrnCm_MAC_FW	ACCGTCATTATACATTTCCATTATGTTACCTAGCC
ptrnCm_MAC_RV	TGGAAATGTATAATGACGGTTCGACTCCGT
ptrnCm_MDAC_FW	CCGTCATTATACCTAGCCAAACCCGCCAC
ptrnCm_MDAC_RV	GGCTAGGTATAATGACGGTTCGACTCCGT

***Arabidopsis thaliana* PRORP1 cDNA**	
AtPRORP1_C344A_FW	CAGAGATGGATGAGAATGGTGTA**GC**TAAATGTTGCAAAGAGAAGCTTG
AtPRORP1_C344A_RV	CAAGCTTCTCTTTGCAACATTTA**GC**TACACCATTCTCATCCATCTCTG
AtPRORP1_C347A_FW	GGATGAGAATGGTGTATGTAAATGT**GC**CAAAGAGAAGCTTGTTTGTATTGAT
AtPRORP1_C347A_RV	ATCAATACAAACAAGCTTCTCTTTG**GC**ACATTTACATACACCATTCTCATCC
AtPRORP1_H548A_FW	CTGAAGATGGAACCTGG**GC**TGTACCAATGAGCGTAG
AtPRORP1_H548A_RV	CTACGCTCATTGGTACA**GC**CCAGGTTCCATCTTCAG
AtPRORP1_C565A_FW	CATCAAGGCAATGGTTA**GC**CGCAAAAAGATCCAAAAC
AtPRORP1_C565A_RV	GTTTTGGATCTTTTTGCG**GC**TAACCATTGCCTTGATG

Point mutations are indicated in bold.