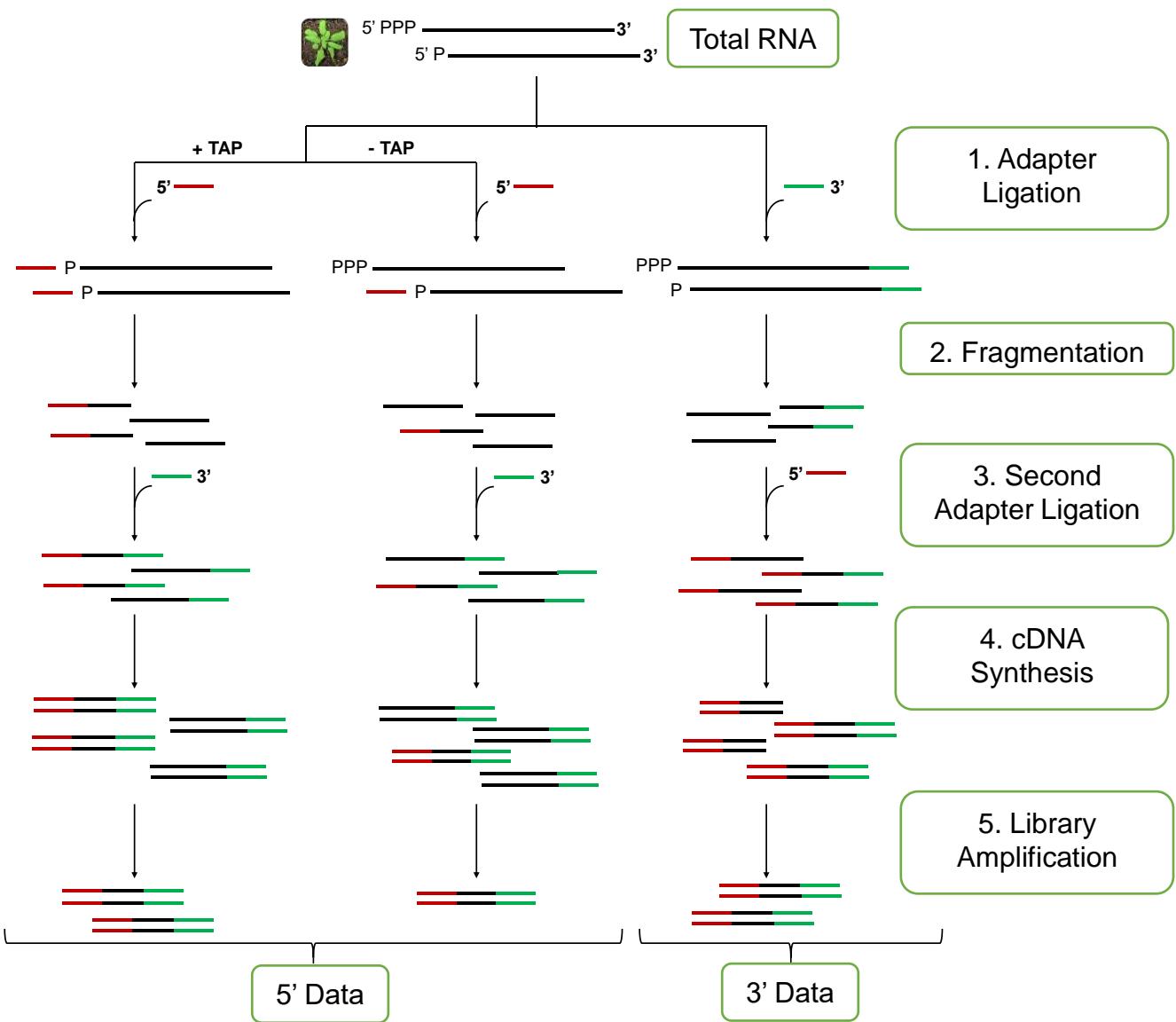


Supplementary Figure S1: Terminome-Seq strategy

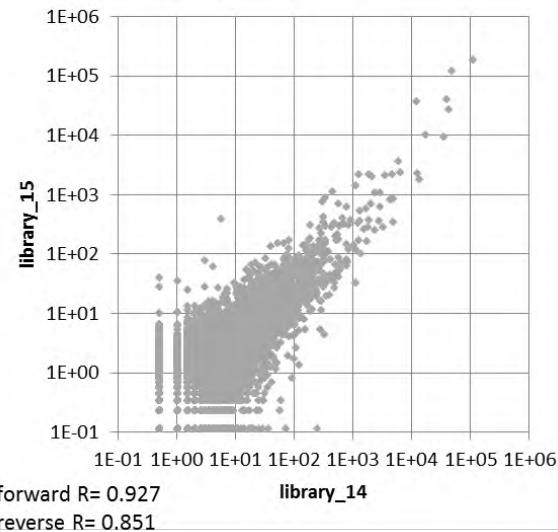
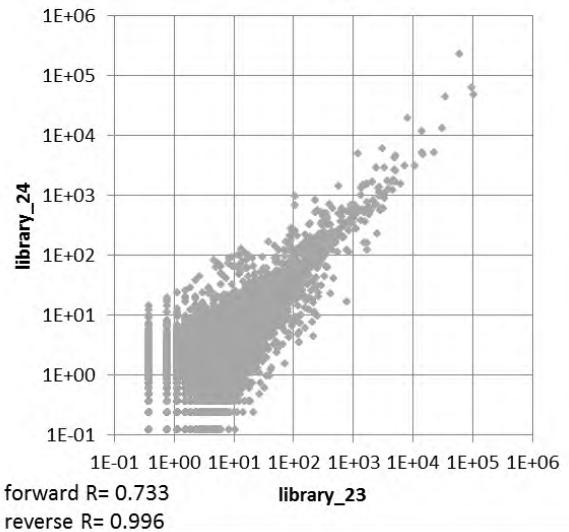
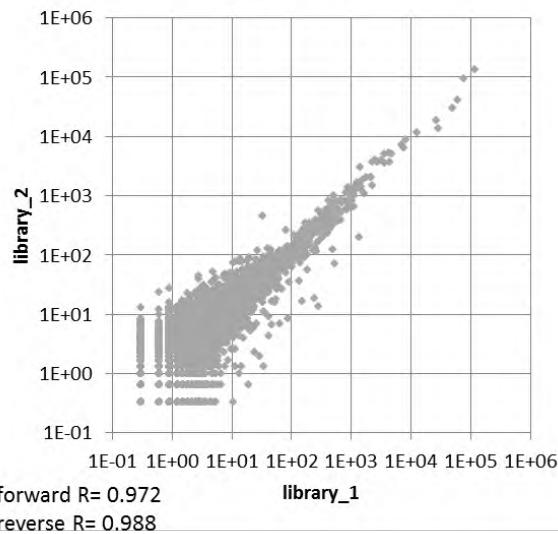
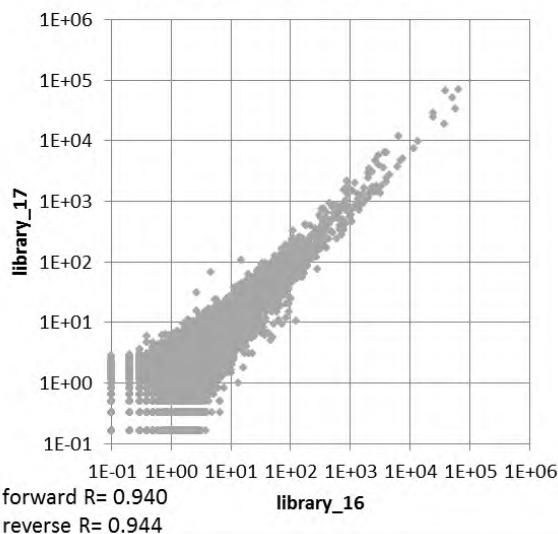
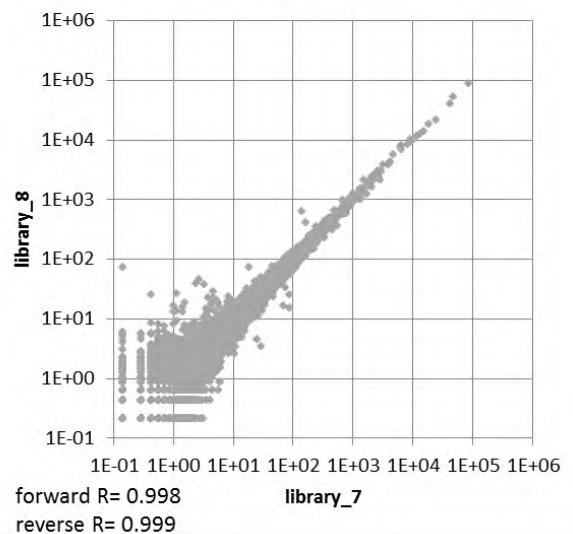
Total leaf RNA was used to create three different RNA-Seq libraries. For 5' end analysis, optional TAP treatment was completed and a 5' adapter was ligated, followed by fragmentation and ligation of a 3' adapter. For 3' end analysis, the 3' adapter was ligated first. The first adapter ligation prior to fragmentation ensures that all cDNAs amplified for library sequencing represent an end naturally present in the RNA population. Technical details are in the Materials and Methods section.



Supplementary Figure S1

Supplementary Figure S2: Reproducibility between replicates for A) WT and B) *pnp1-1* Terminome-Seq

Replicates of the indicated library samples were graphed based on RPM for a given end. Correlation coefficients for each strand are given to the bottom left of each graph.

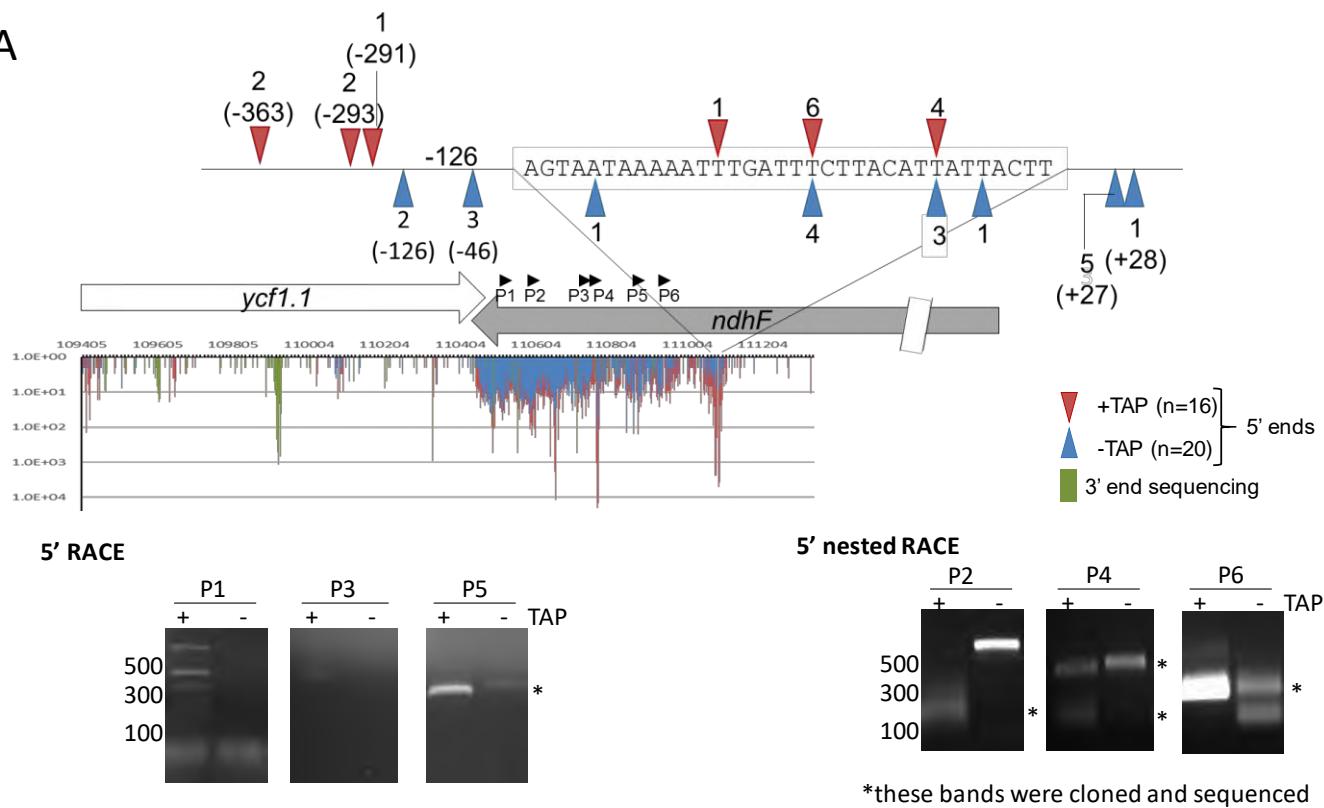
A**WT_5'ends_no_TAP****WT_5'ends_TAP****WT_3'ends****B****pnp1-1_5'ends_no_TAP****pnp1-1_3'ends****Supplementary Figure S2**

Supplementary Figure S3: Identifying and validating TSS using Terminome-Seq

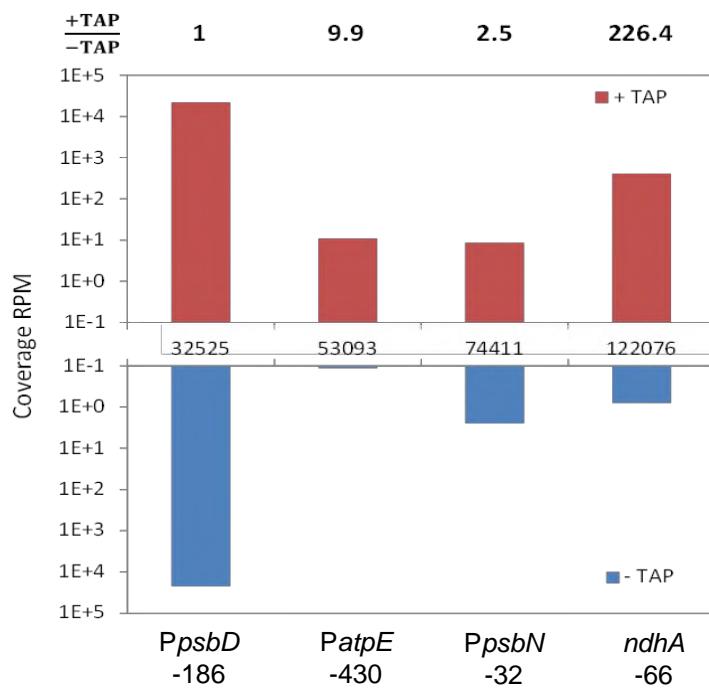
A) TSS within *ndhF* were mapped using 5' RACE. The overlapping *ycf1.1* and *ndhF* gene models are shown with Terminome-Seq results for the minus strand below (see key for color scheme). Black arrowheads represent the 3' primers used for 5' RACE, for which the corresponding stained gels of the PCR reactions are shown.

B) Comparison between +TAP coverage (red) vs -TAP coverage (blue) for selected 5' ends. The derived ratio used to determine TSS status is indicated above the graph. Genomic positions 53093 and 74411 have been described as *PatpE* -430 (1, 2) and *PpsbN* -32 (3), respectively, but do not reach the $\frac{+TAP}{-TAP} > 10$ threshold. Genomic position 32525 previously described as *PpsbD* -186 (4, 5) is likely to be a processed end. Genomic position 122076, previously described as the *ndhA* -66 processed 5' end (6), gives a strong indication of being a TSS.

A



B

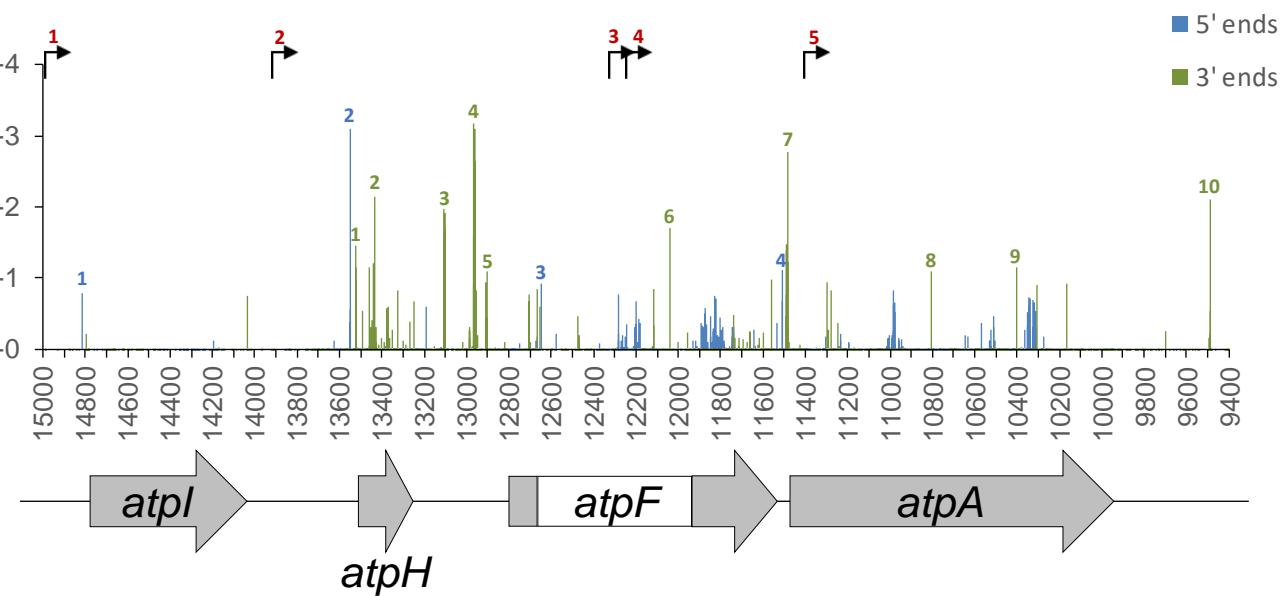


Supplementary Figure S3

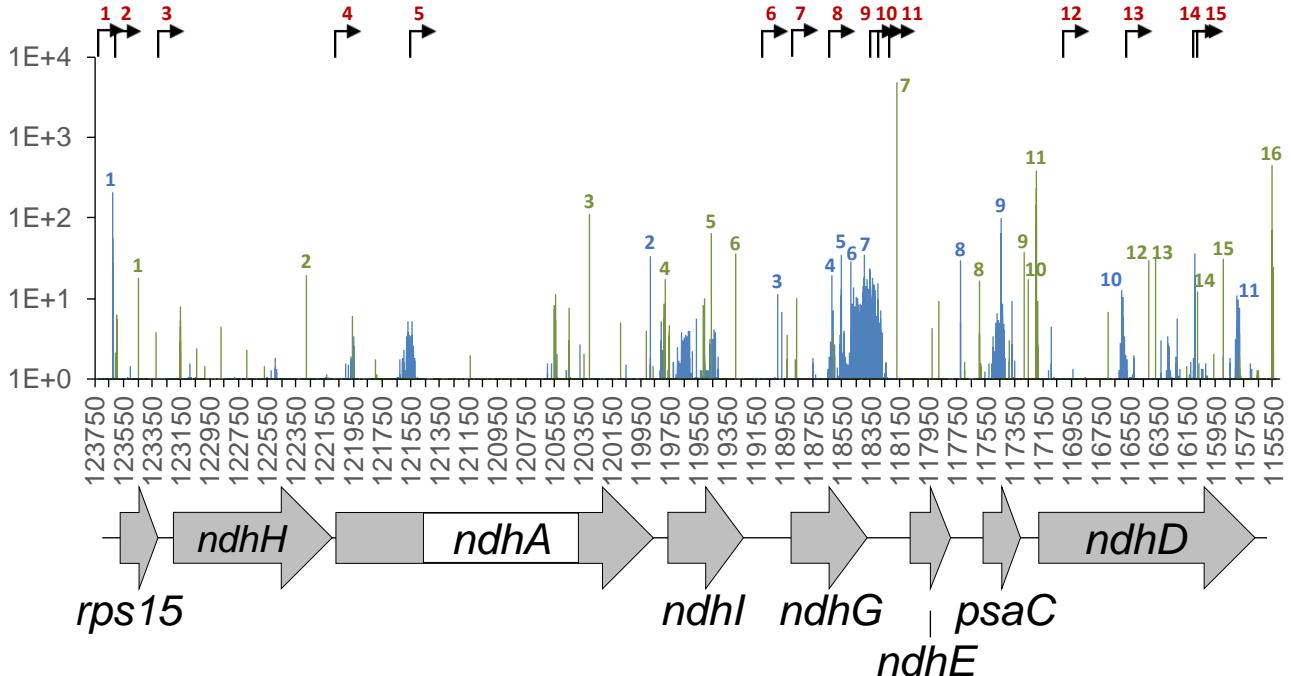
Supplementary Figure S4: Terminome-Seq coverage of the *atpI/H/F/A* (A) and *ndhH/A/I/G/E/psaC/ndhD* (B) gene clusters

The corresponding gene models are shown below, with exons in gray and introns in white. –TAP 5' ends are in blue and 3' ends are in green; bent arrows represent TSS inferred from +TAP data. Numbered peaks and promoters refer to features listed in Supplementary Table S4.

A *atpI/H/F/A* operon



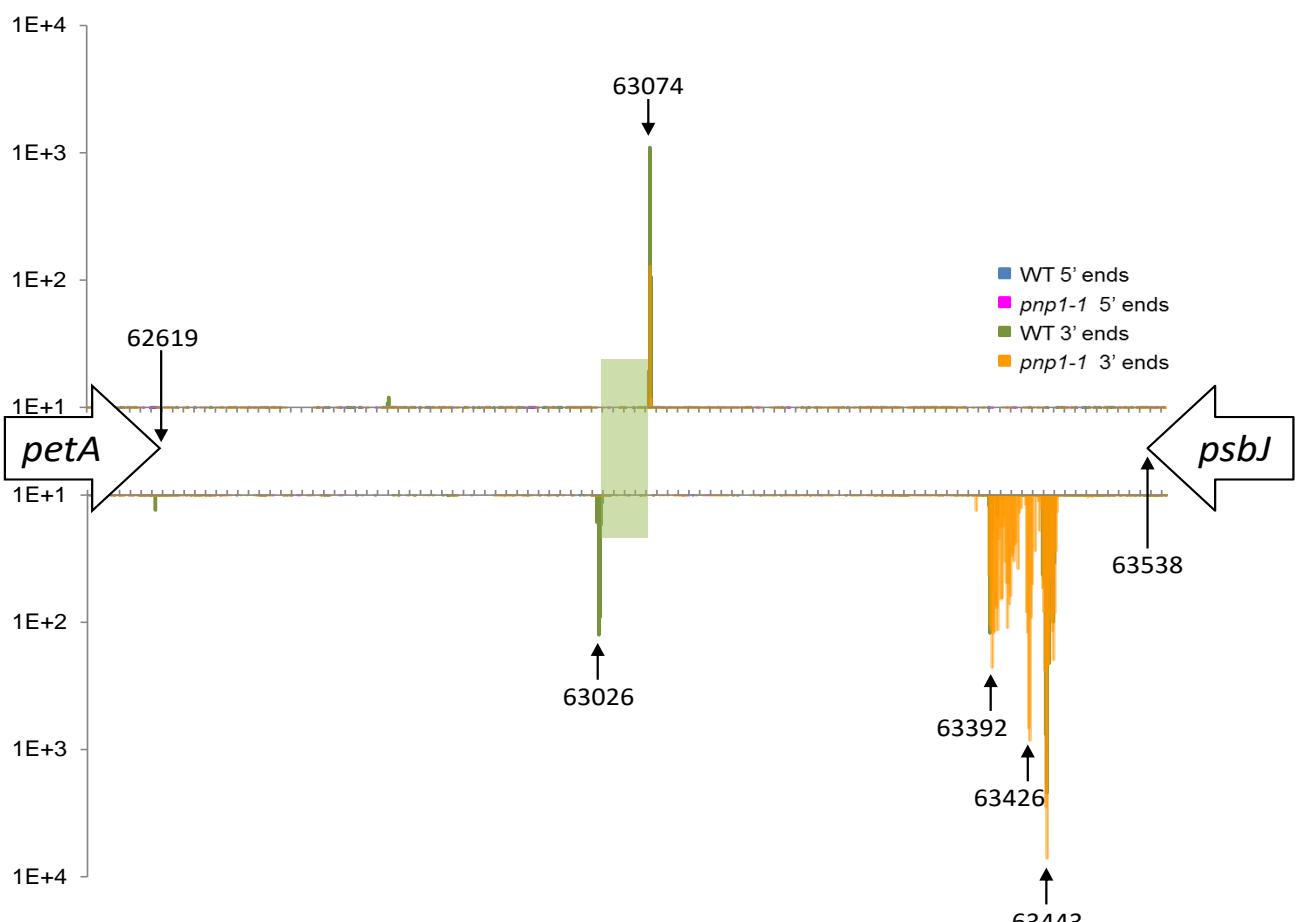
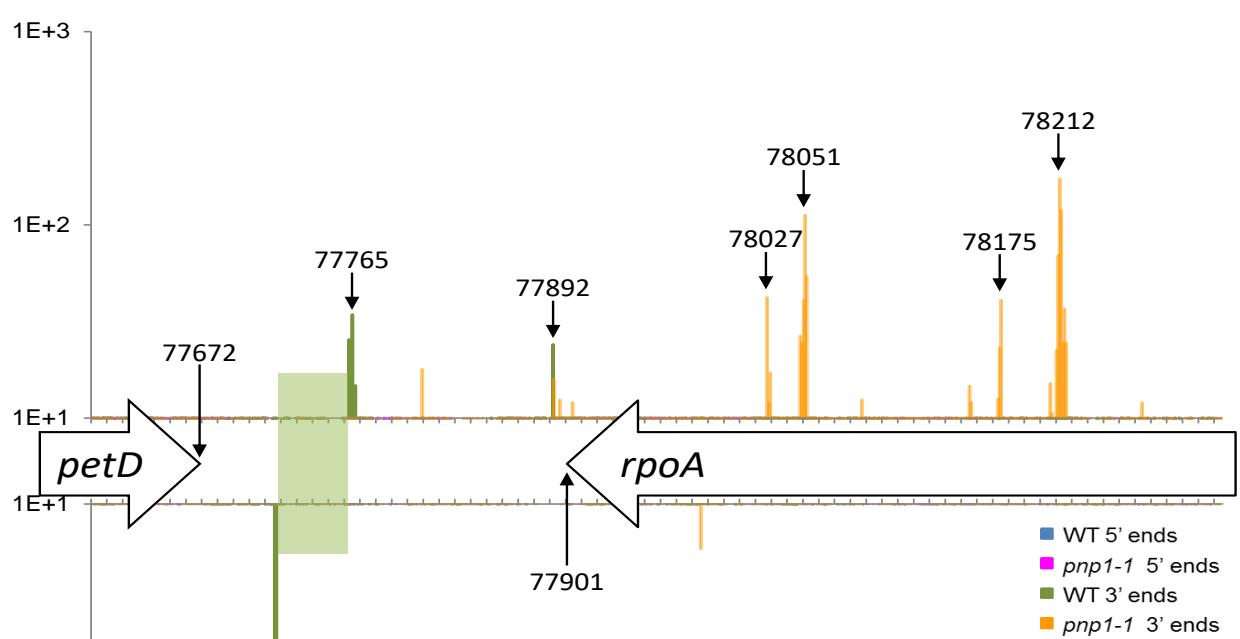
B *rps15/ndhH/A/I/G/E/psaC/ndhD* operon



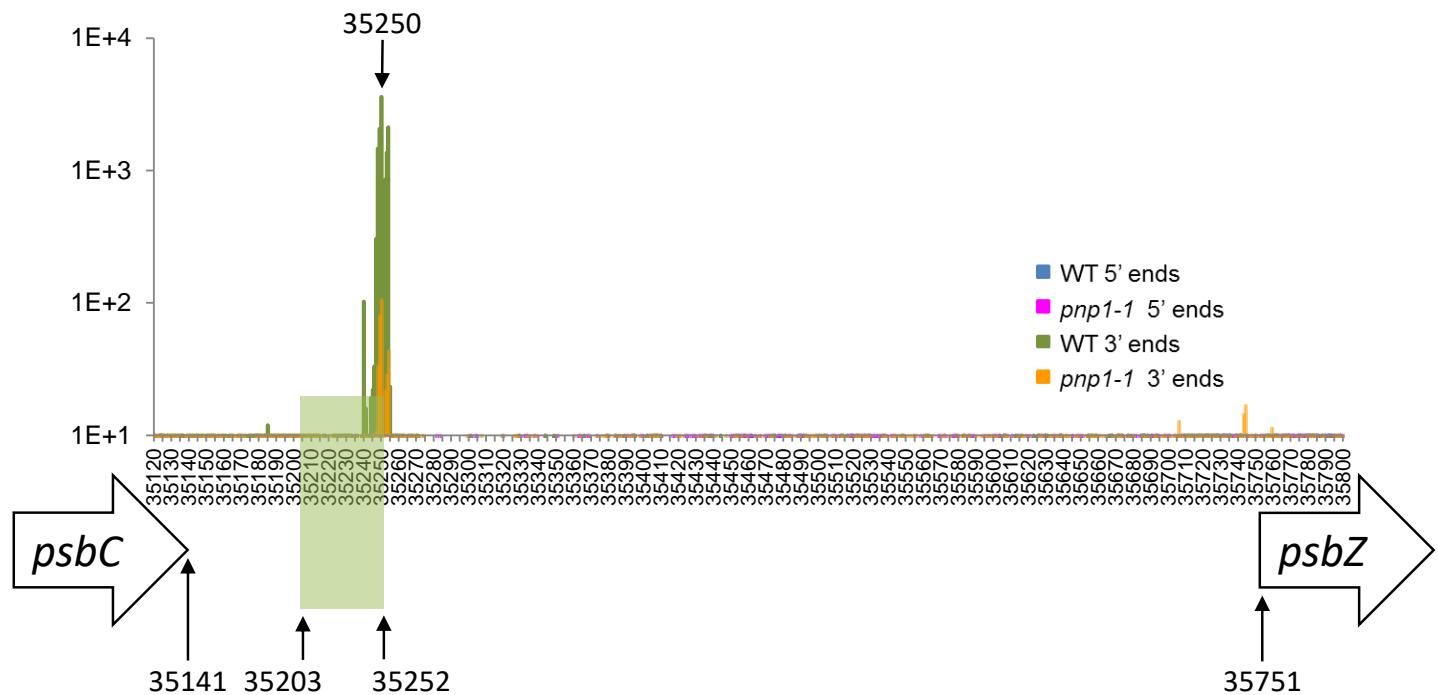
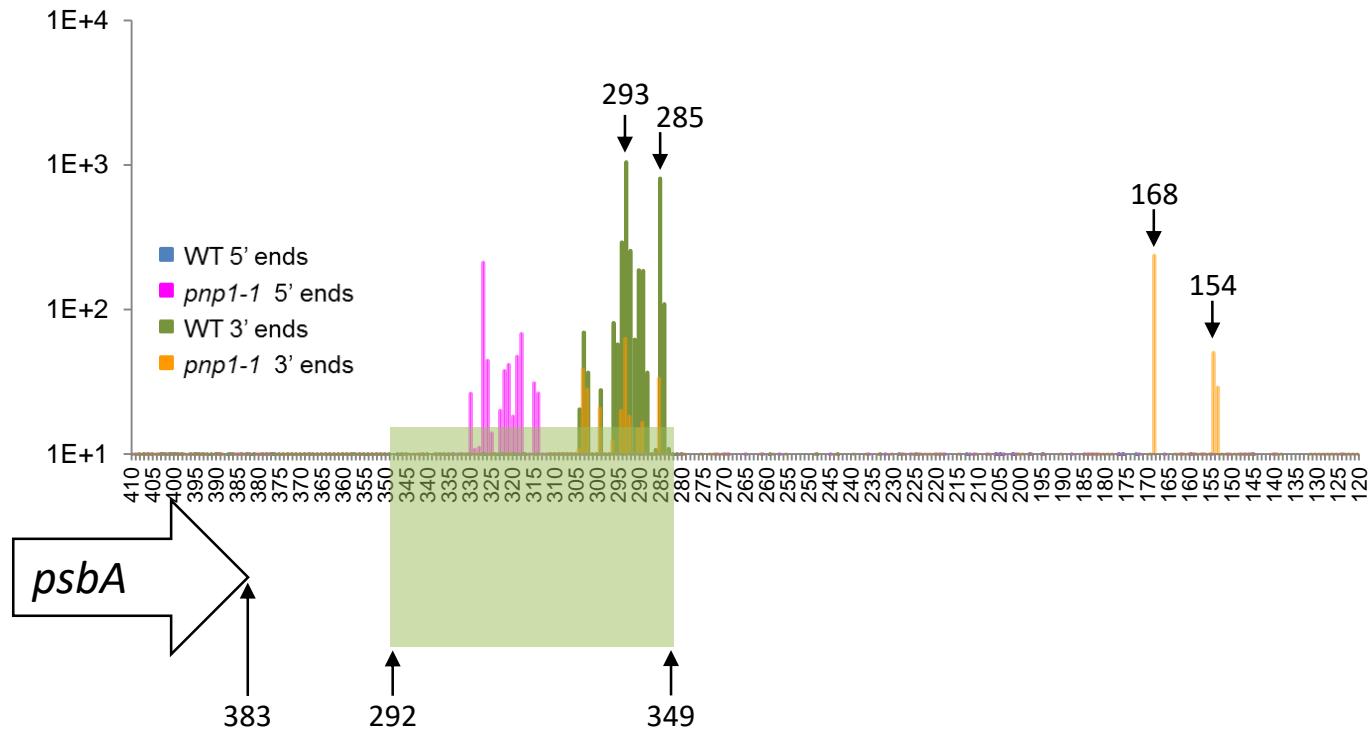
Supplementary Figure S4

Supplementary Figure S5: Transcript termini at secondary structures

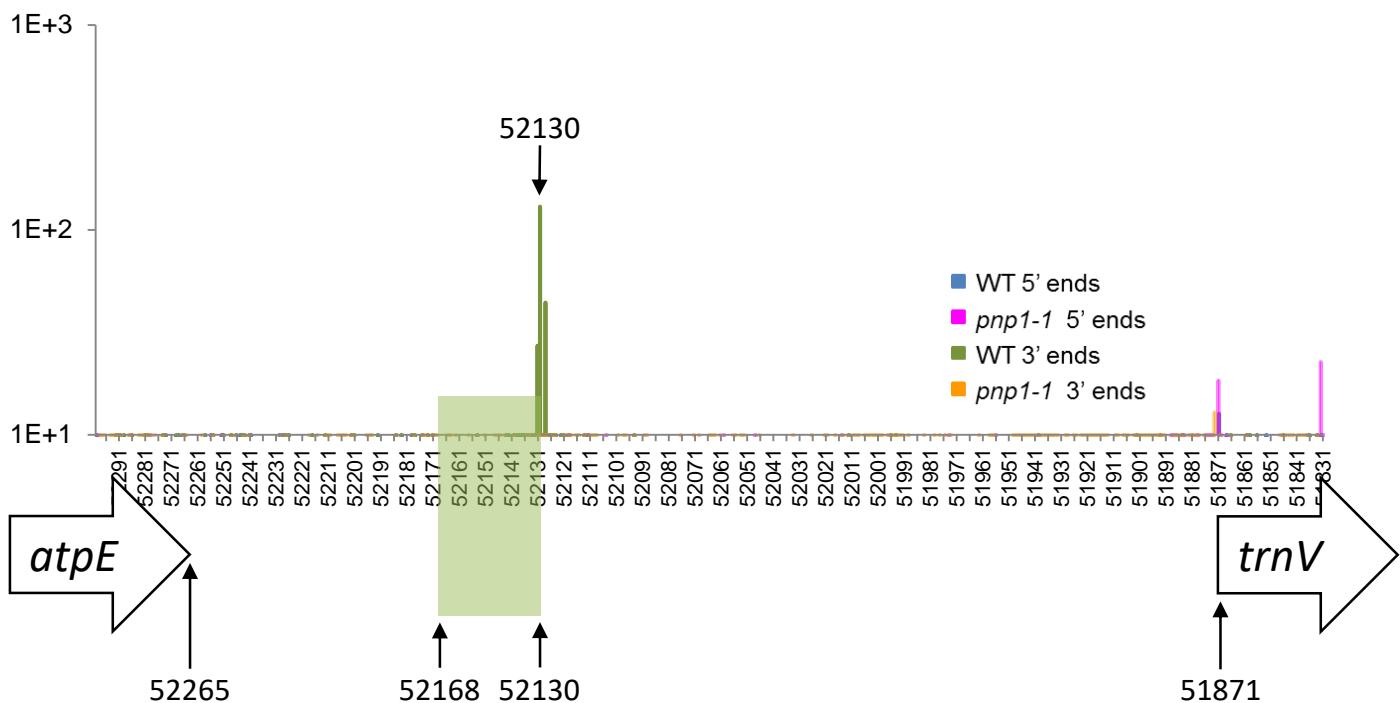
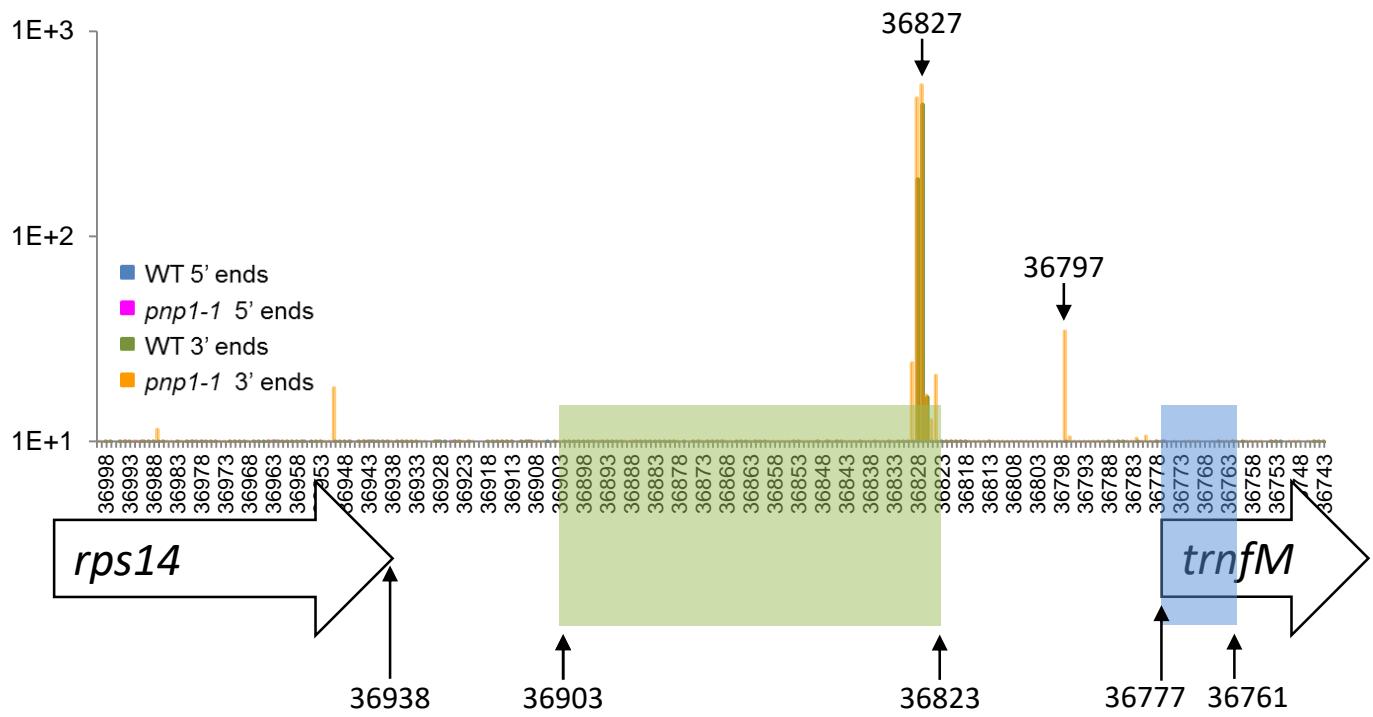
Terminome-Seq coverage around predicted secondary structures in the WT and PNPase mutant. Gene models are represented as open arrows and color coding of ends is provided in an inset. Genome positions where ends accumulate are indicated with black arrows. The stem-loops matching described smRNA are highlighted in green, and an RBP footprint is in blue.



Supplementary Figure S5



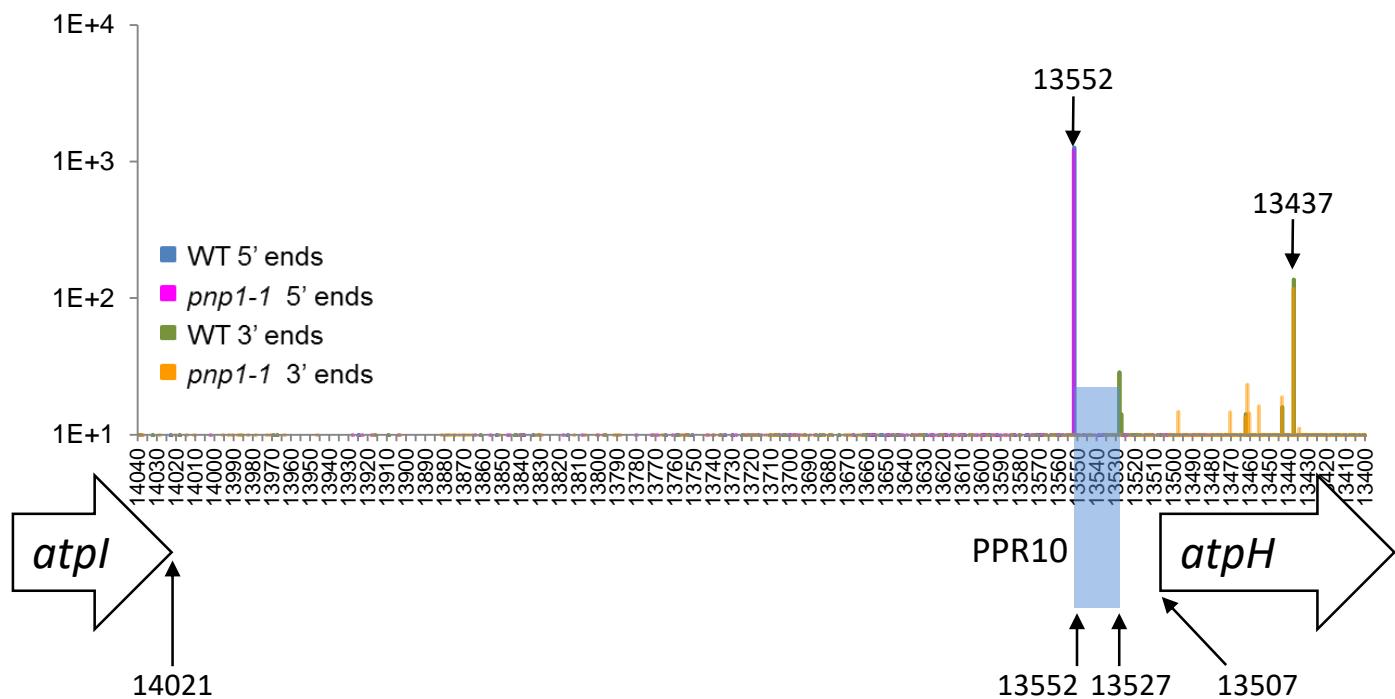
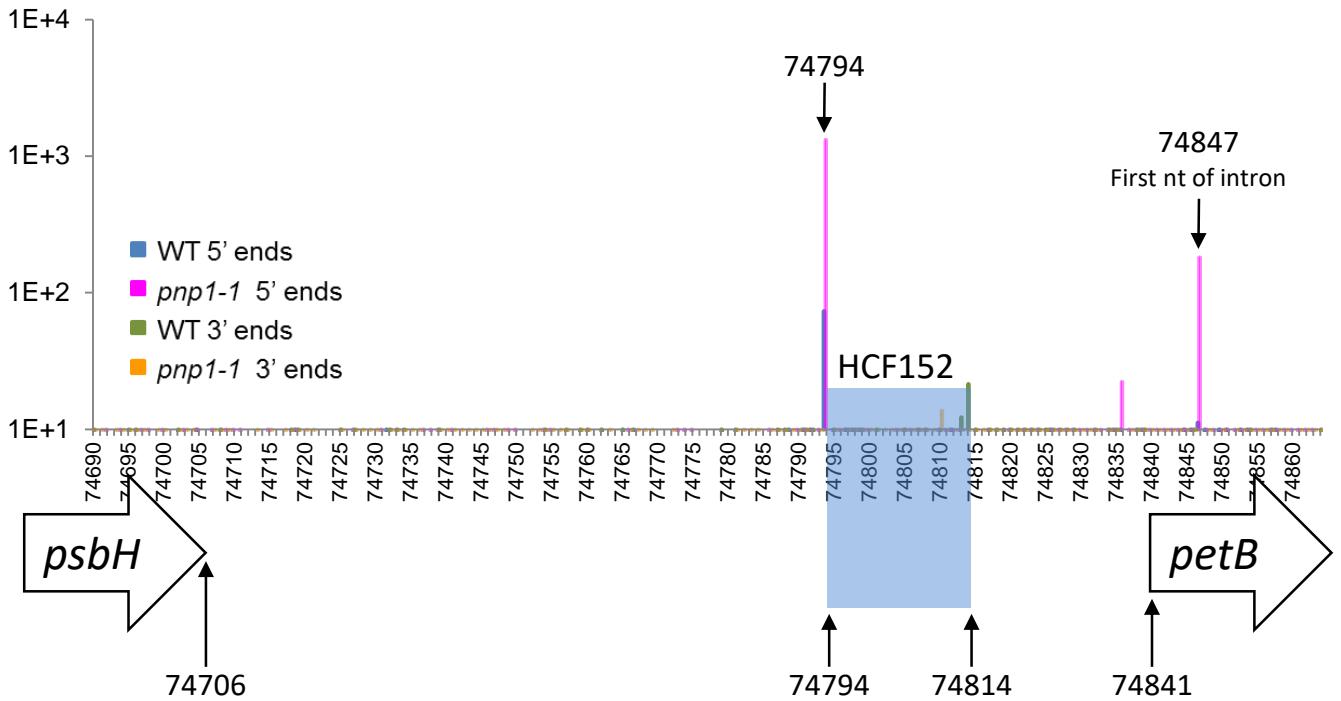
Supplementary Figure S5



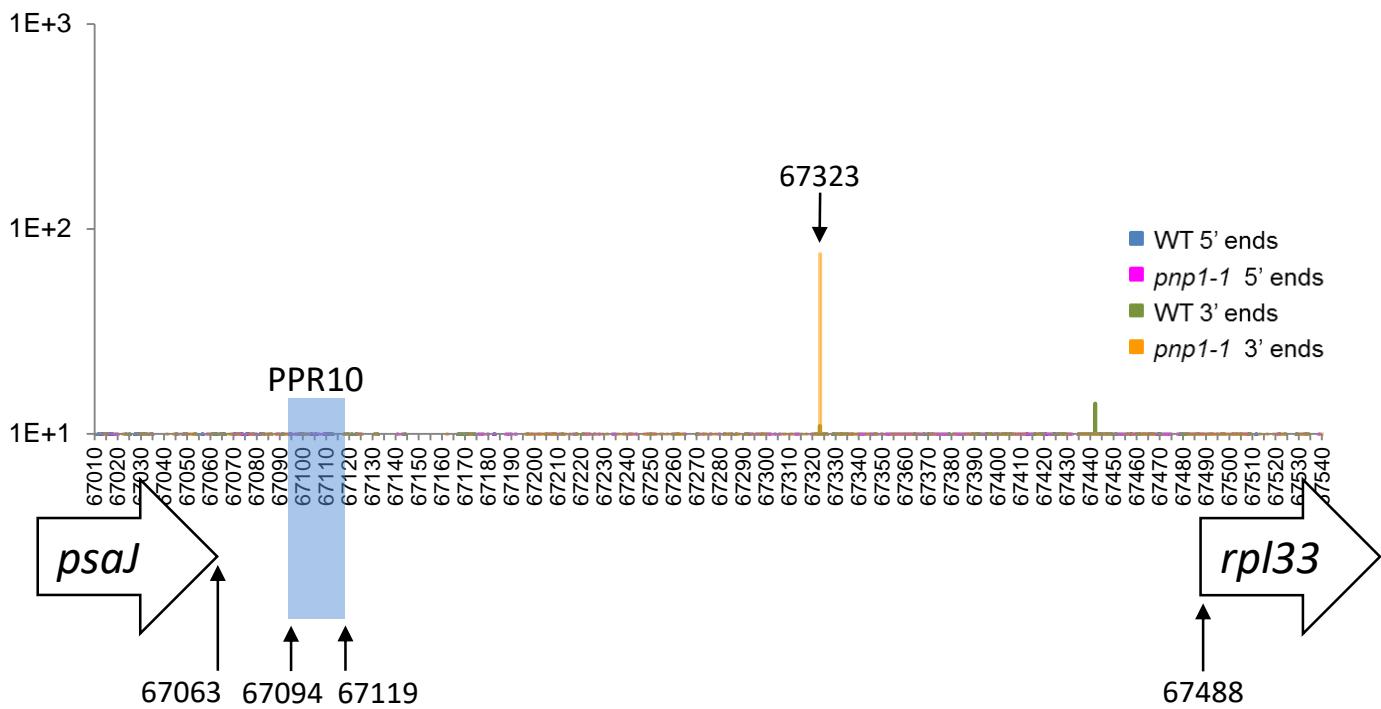
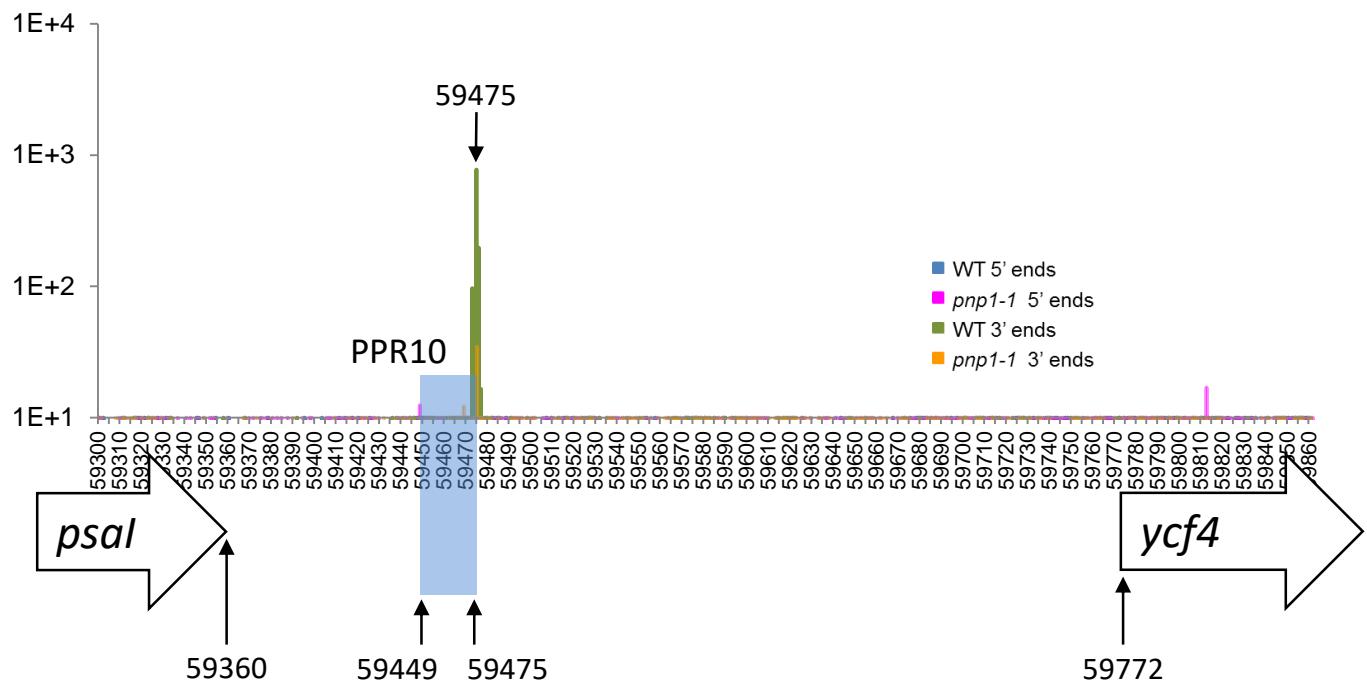
Supplementary Figure S5

Supplementary Figure S6: Transcript termini surrounding known and putative RBP sites

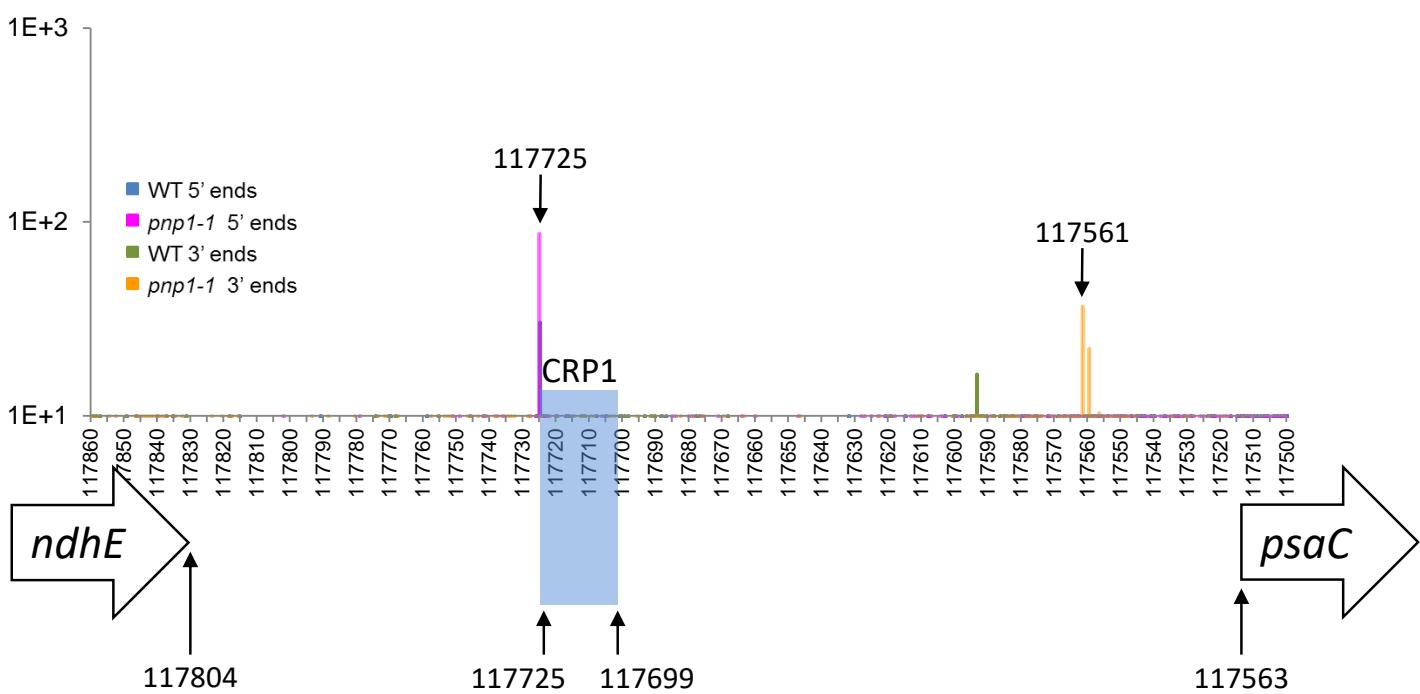
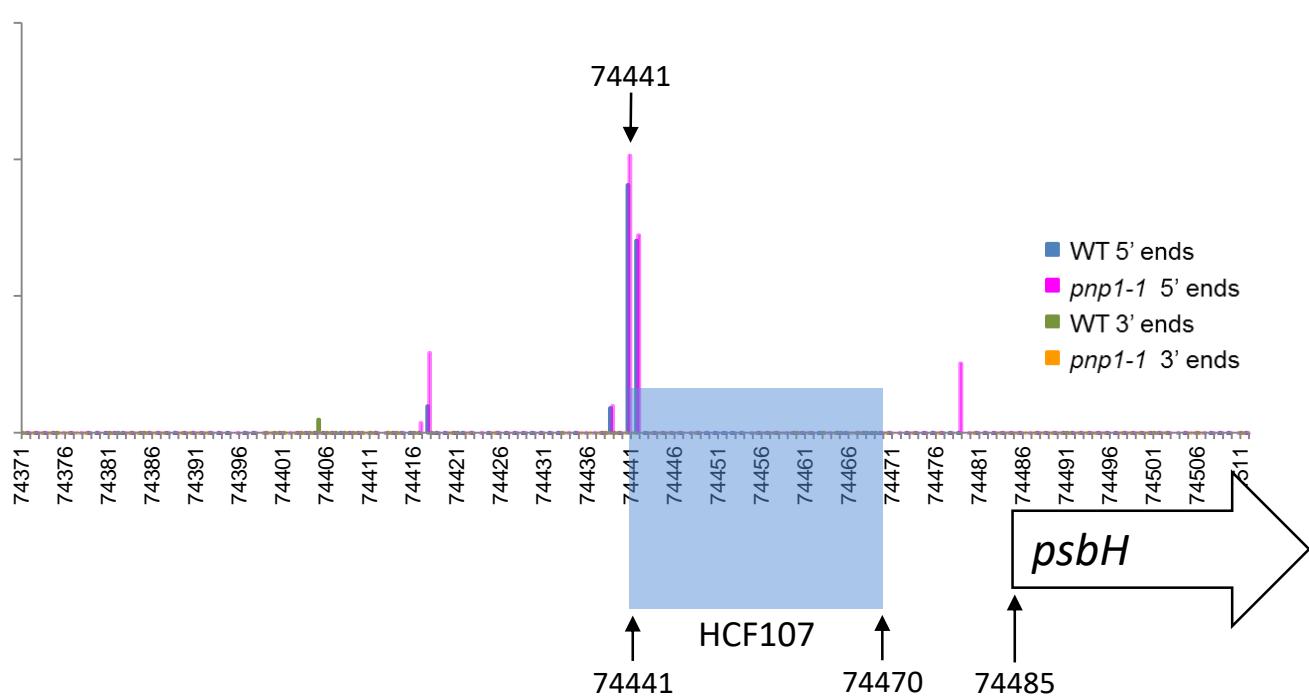
Terminome-Seq coverage near binding sites for HCF152, PPR10, HCF107, CRP1, HCF145, RAP, SOT1, PGR3, PPR5, PPR38, EMB175 and CRR2 is indicated for WT and the PNPase mutant (*pnp1-1*). Gene models are represented as open arrows and color coding of ends is provided in an inset. Genome positions where ends accumulate are indicated with black arrows. RBP footprints matching described smRNAs are highlighted in blue.

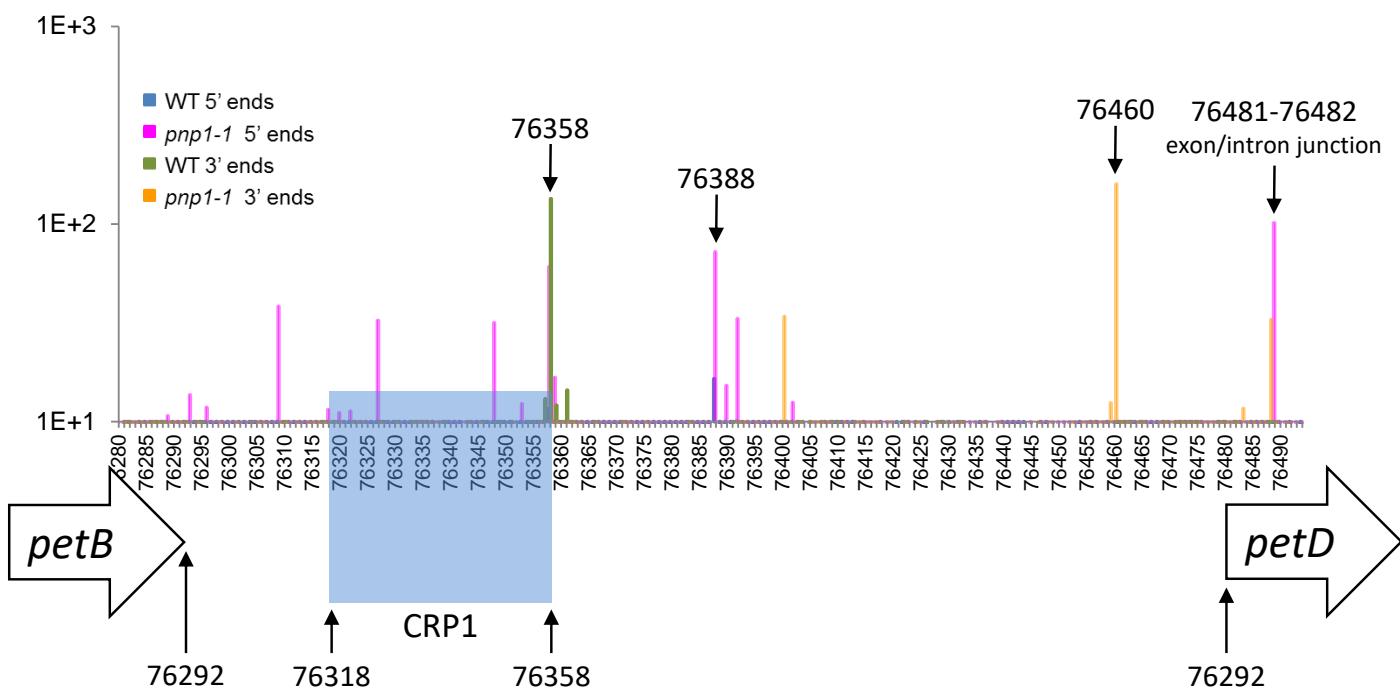
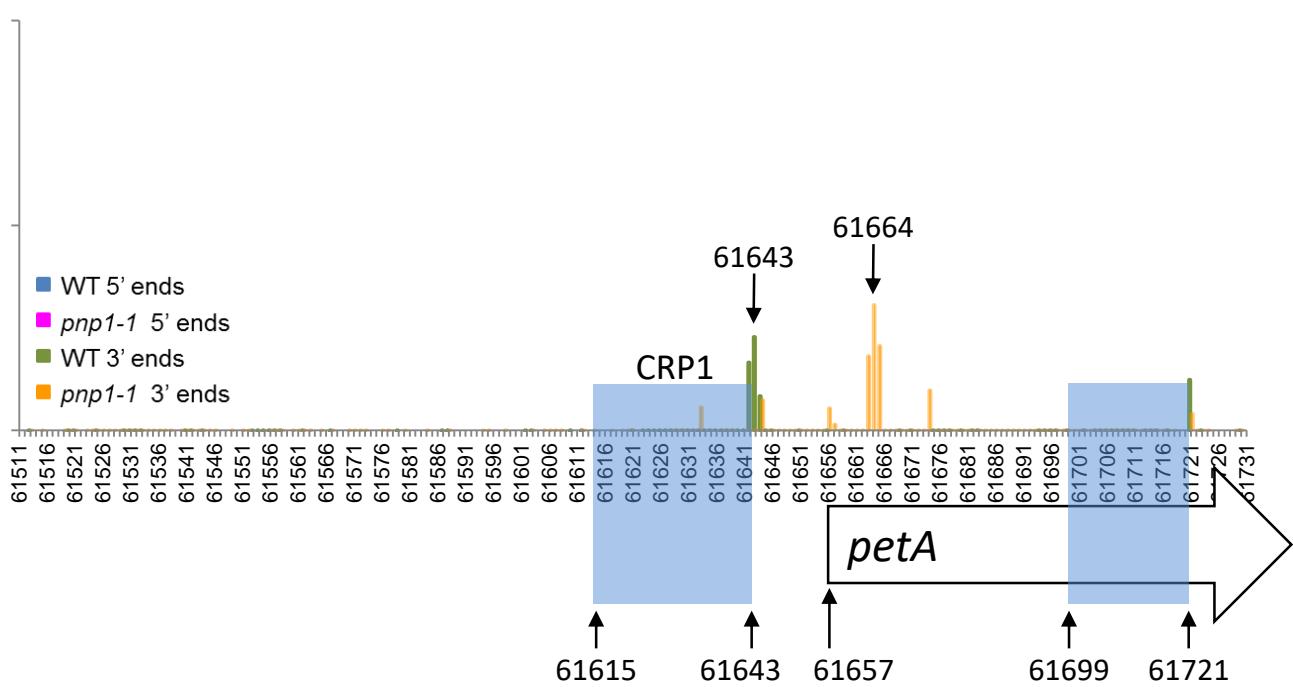


Supplementary Figure S6

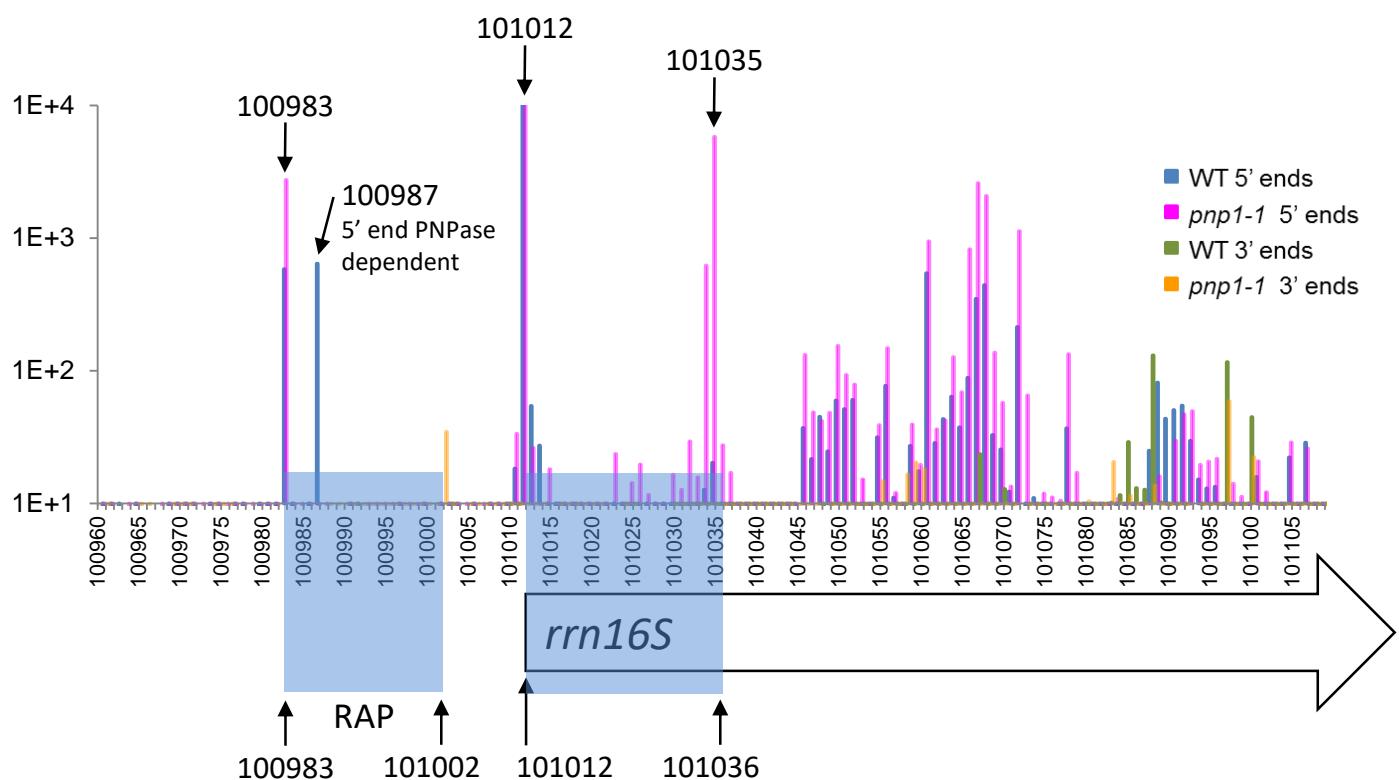
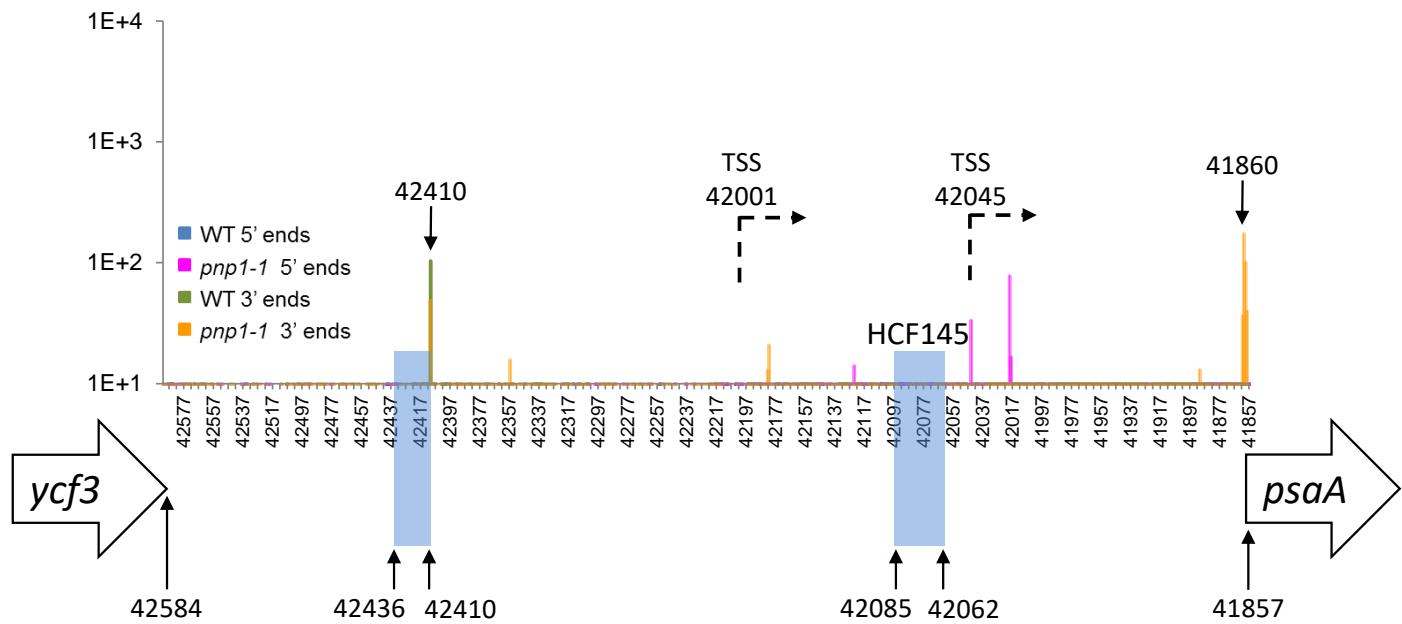


Supplementary Figure S6

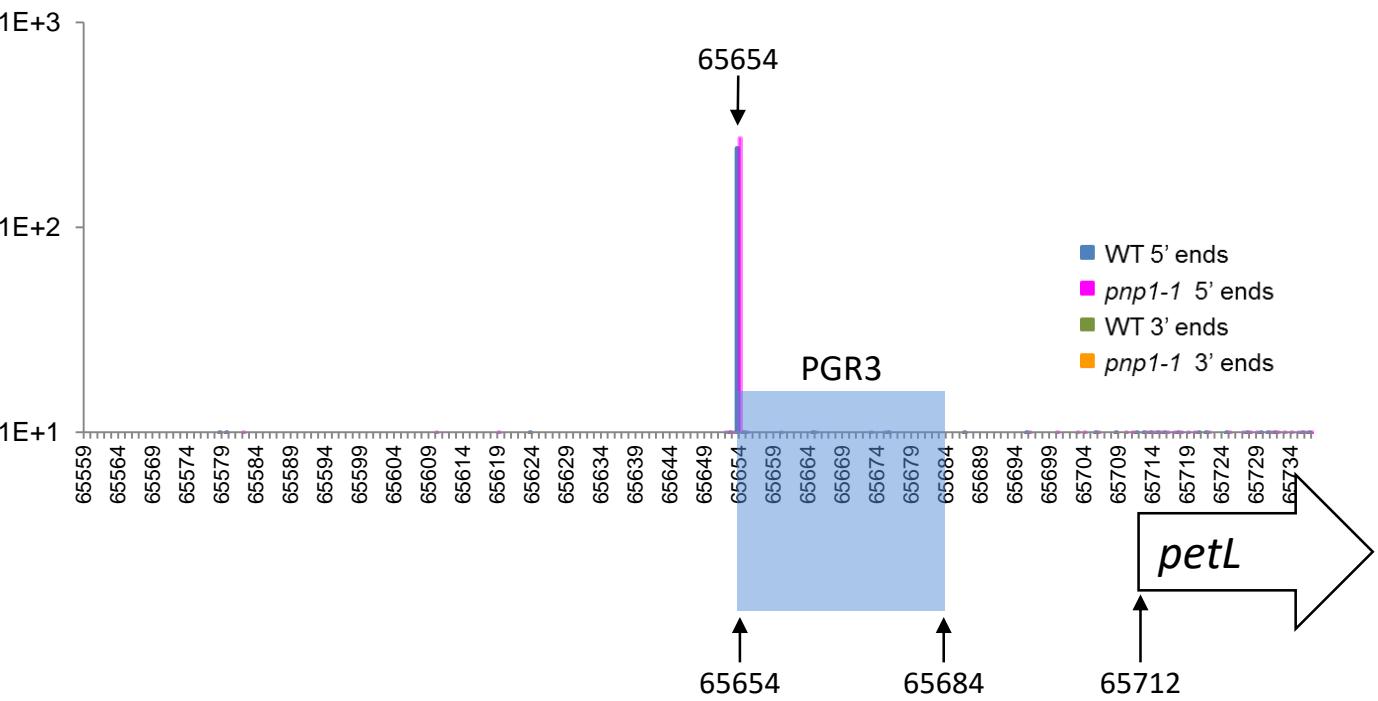
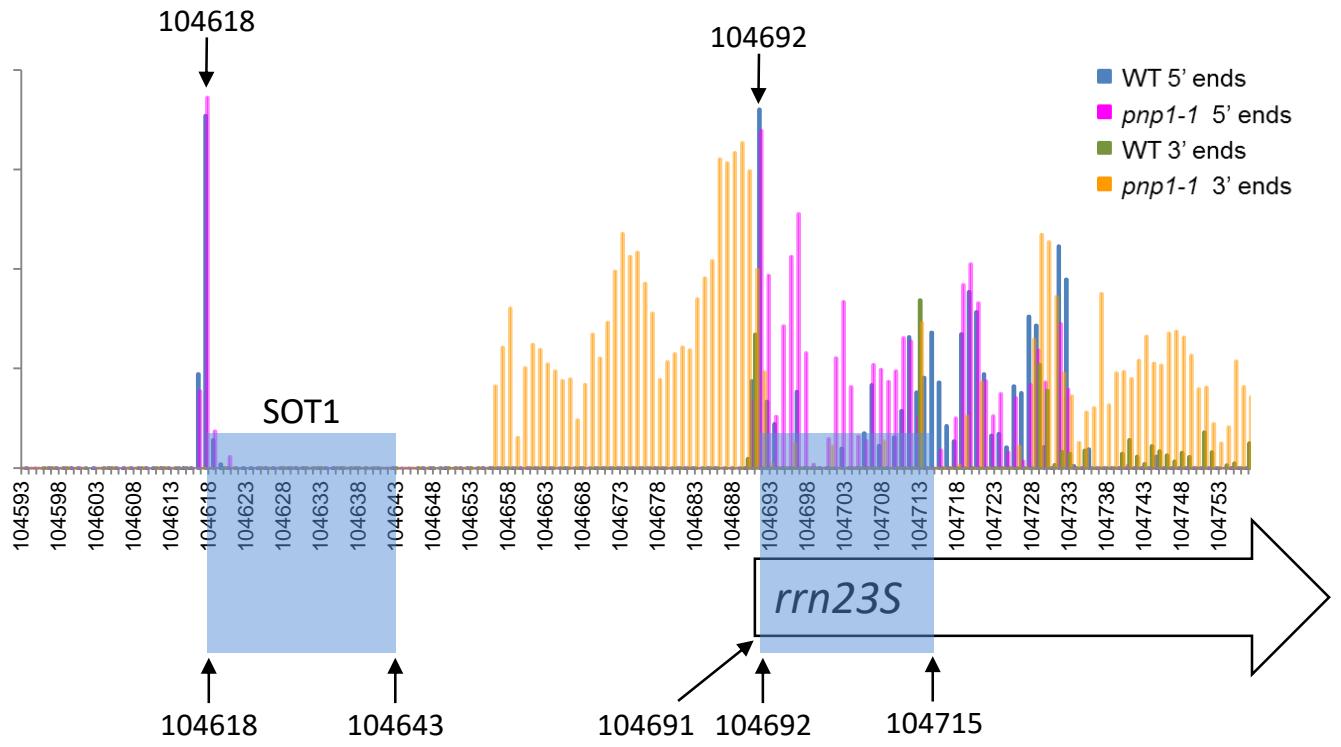




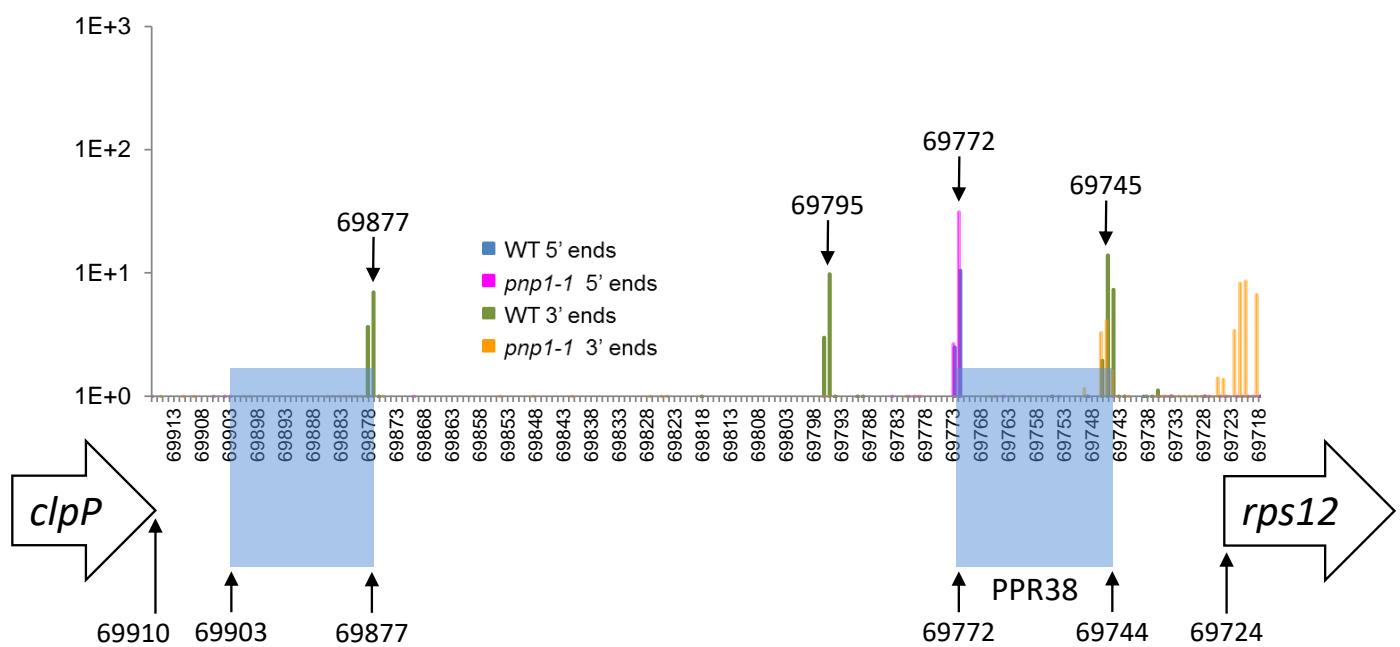
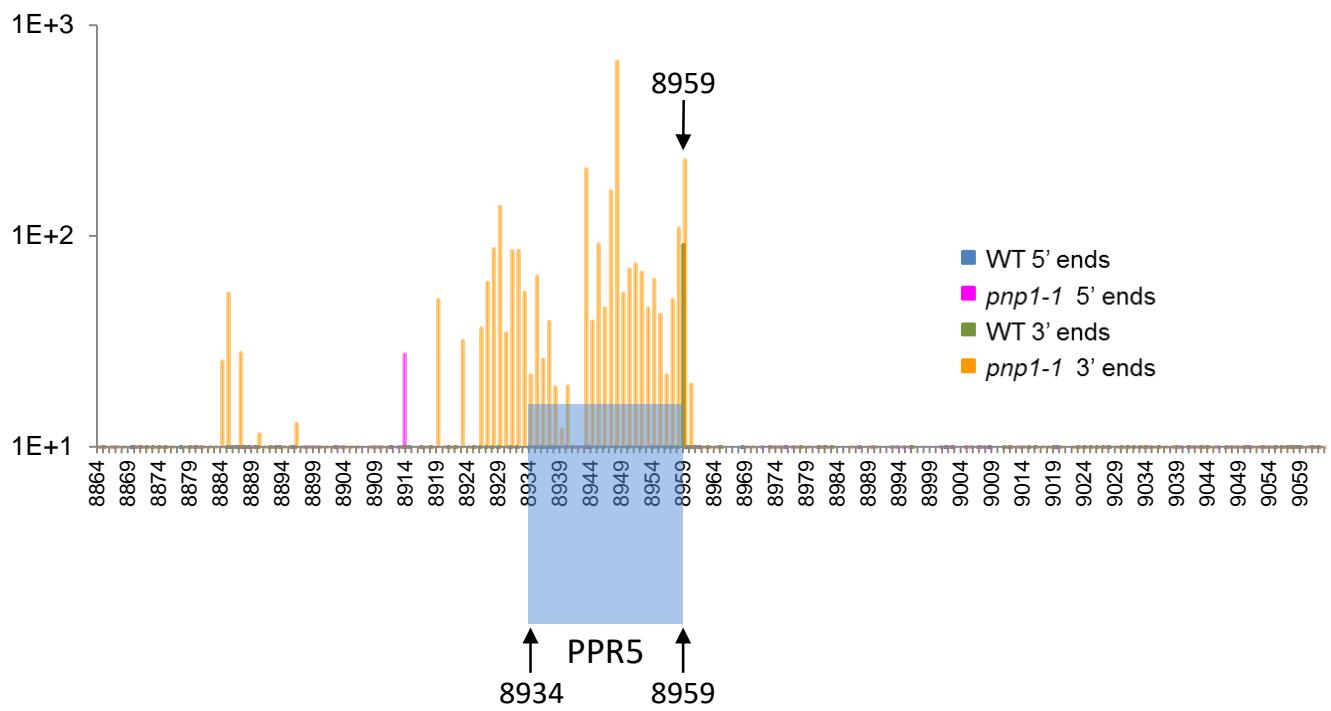
Supplementary Figure S6



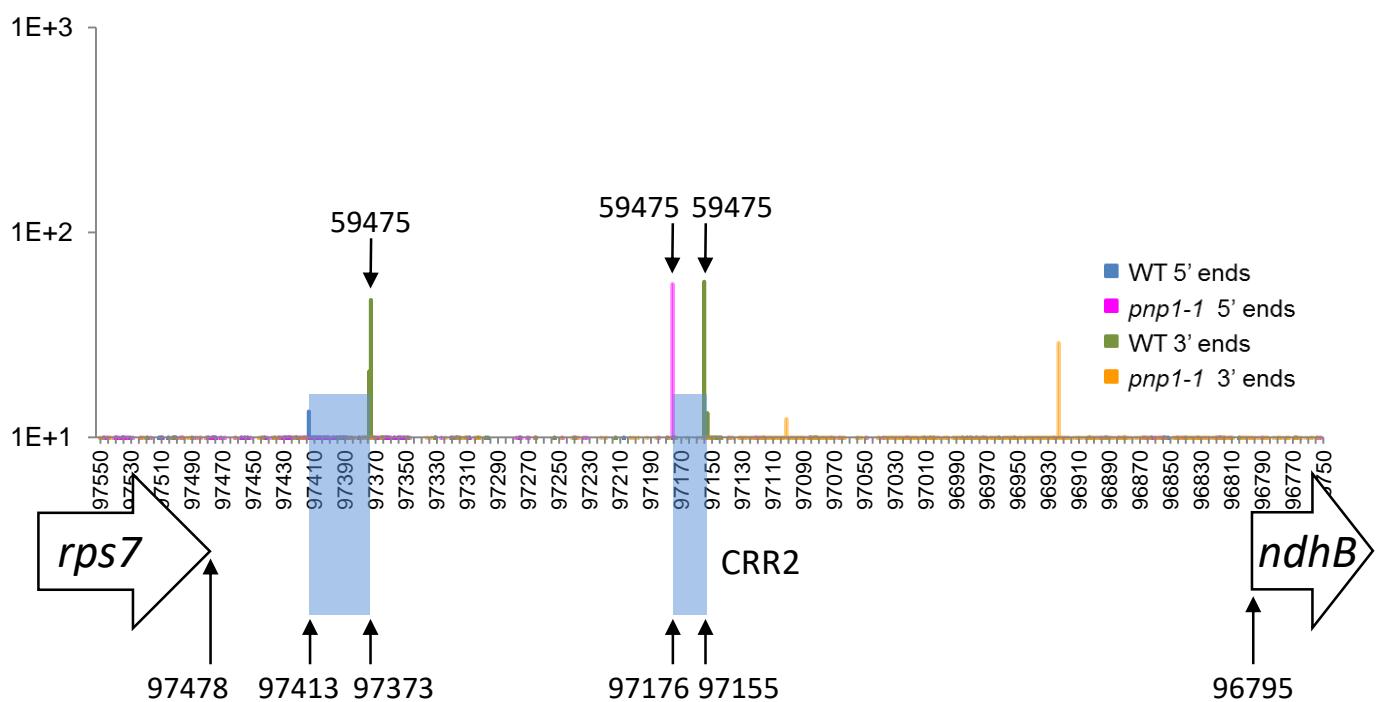
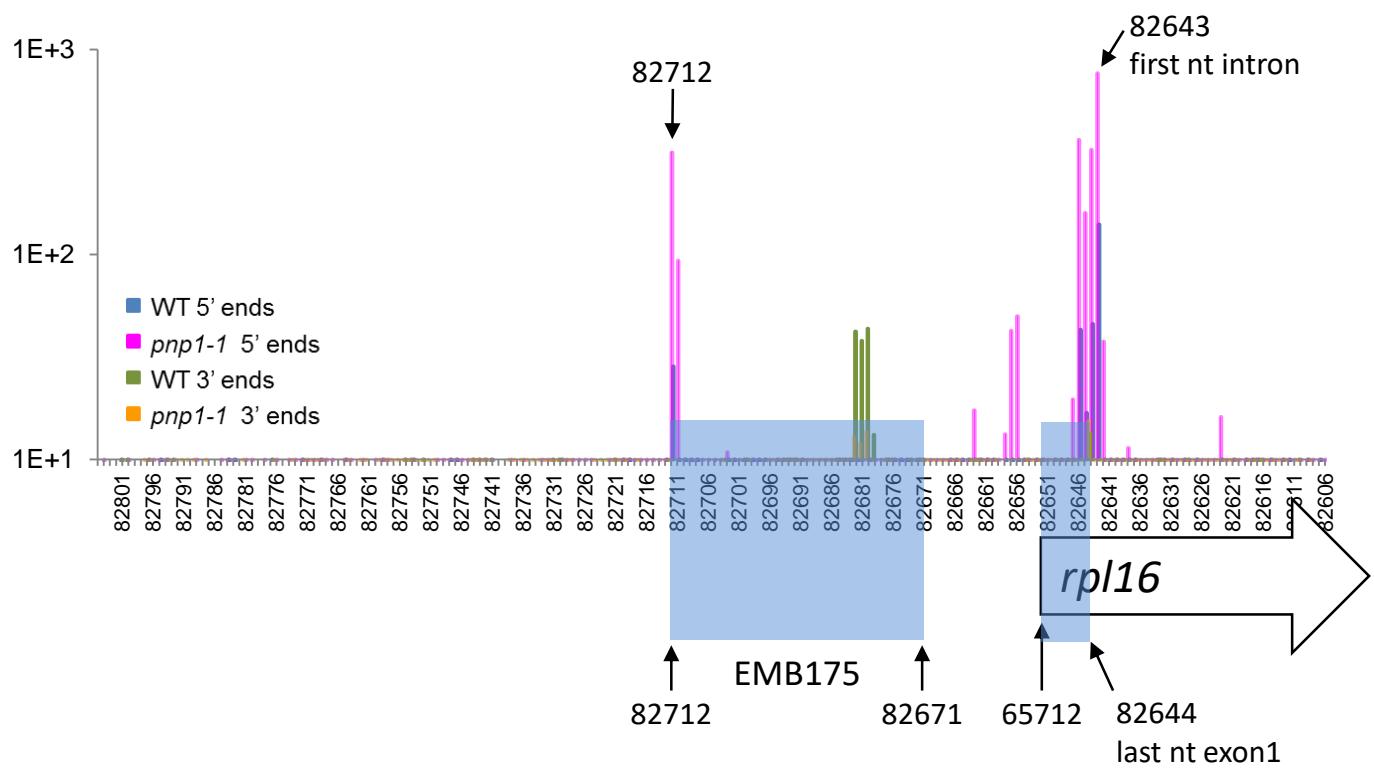
Supplementary Figure S6



Supplementary Figure S6



Supplementary Figure S6

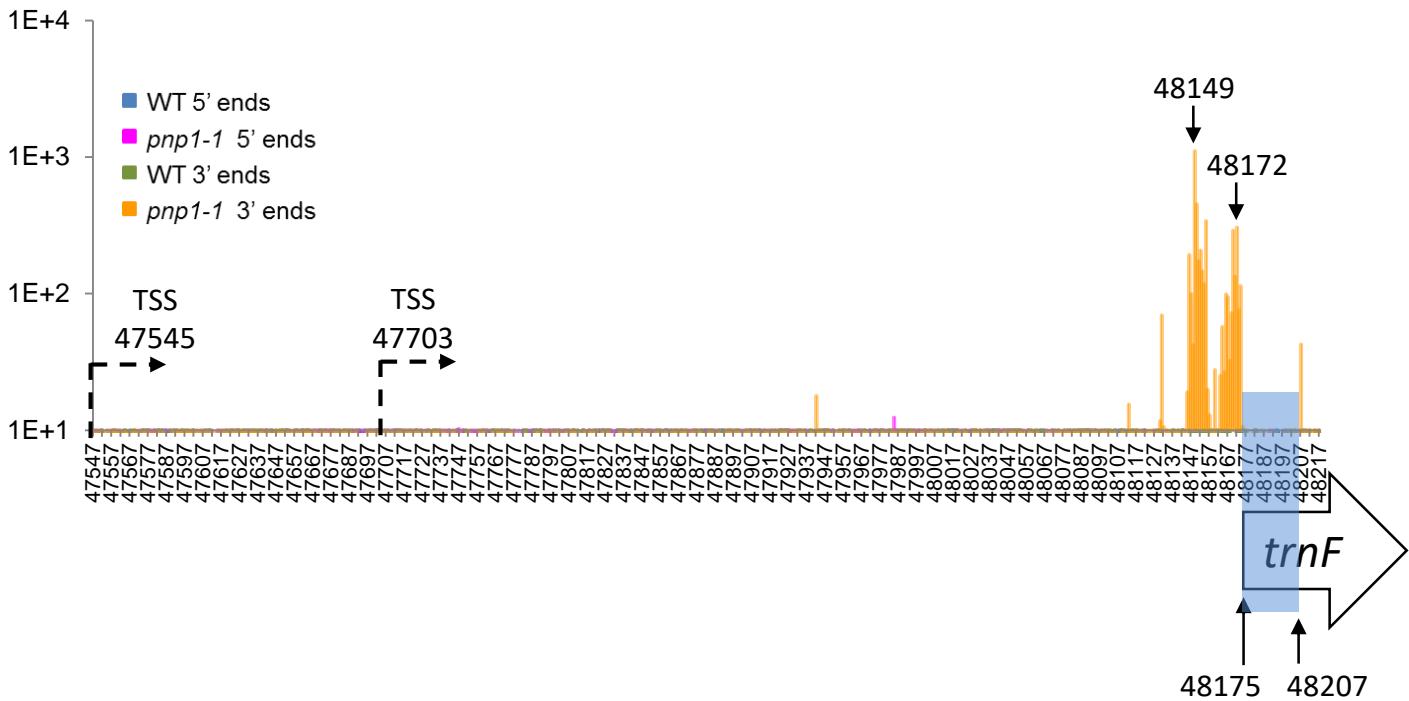


Supplementary Figure S6

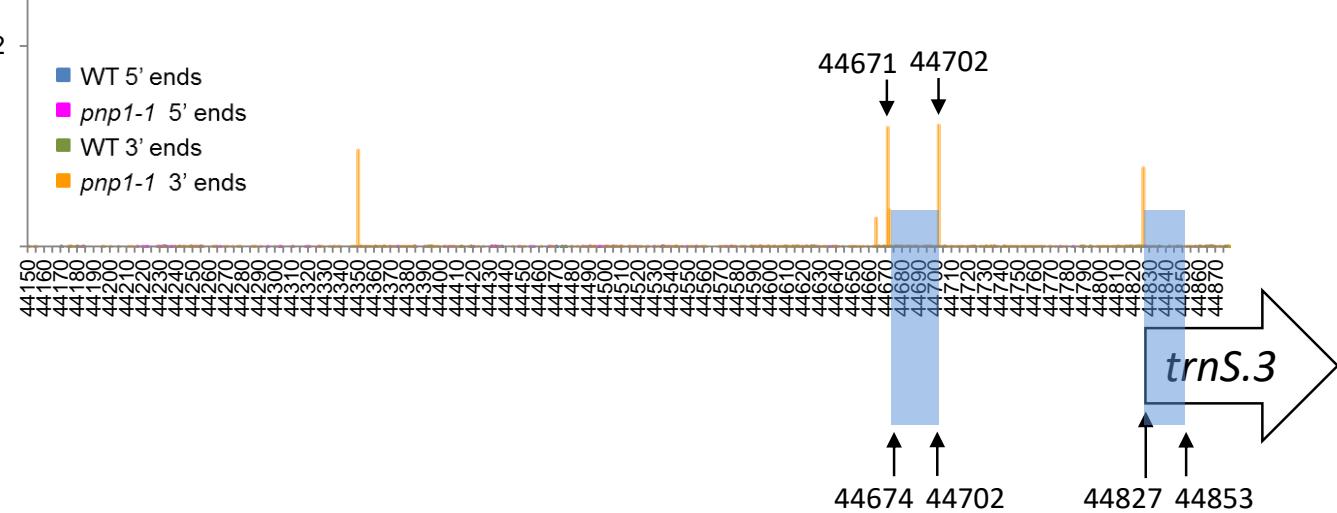
Supplementary Figure S7: Transcript termini upstream of tRNAs

Comparison of Terminome-Seq coverage for WT and *pnp1-1* in selected tRNA-encoding regions. Gene models are represented as open arrows and color coding of ends is provided in an inset. Genome positions where ends accumulate are indicated with black arrows, and smRNA locations are highlighted in blue. TSS for tRNAs are indicated by bent arrows. Additional data, for *trnG*, is shown in Supplementary Figure S8.

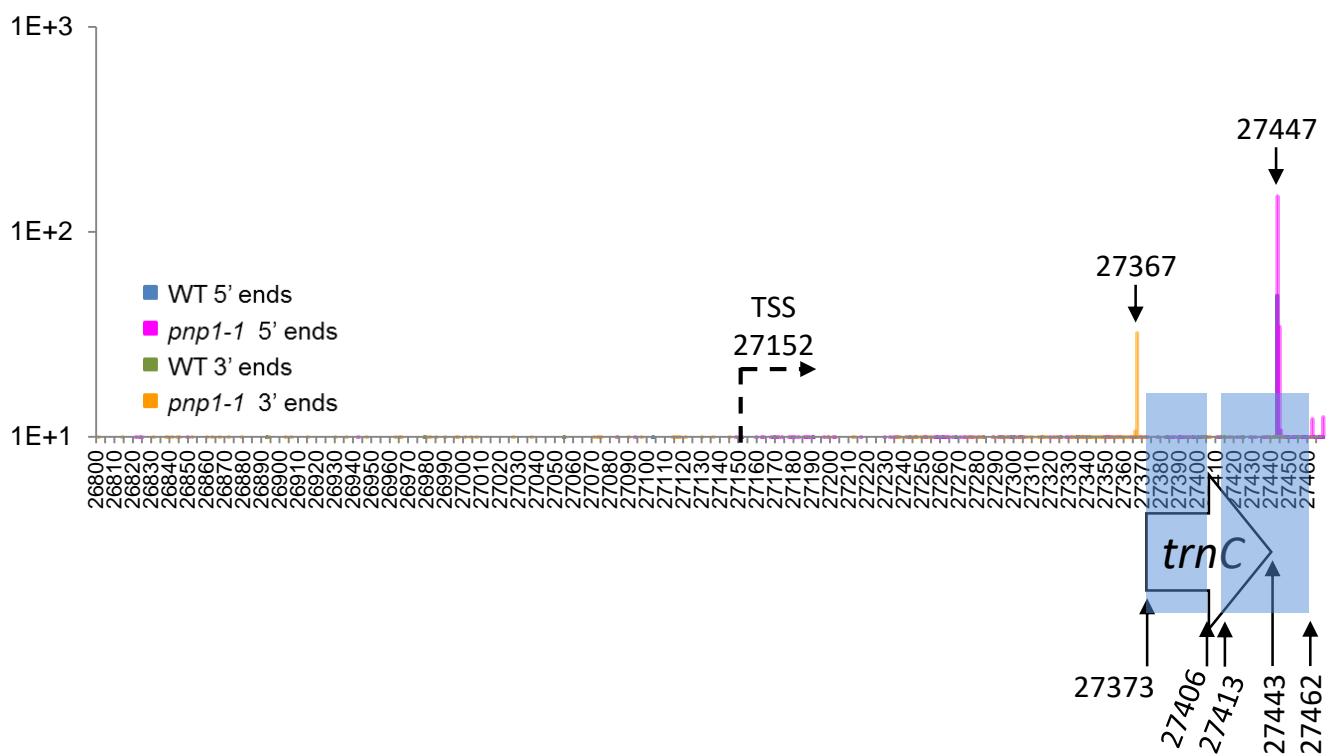
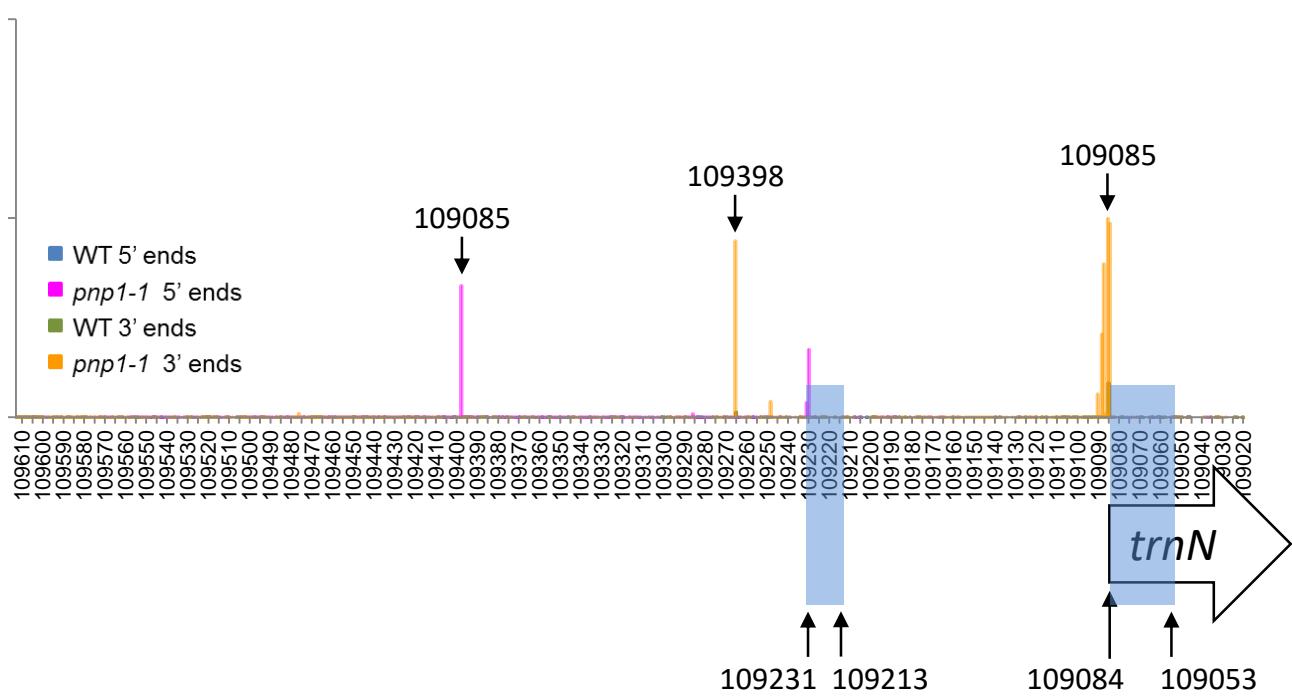
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- pnp1-1* 5' ends
- WT 3' ends
- pnp1-1* 3' ends



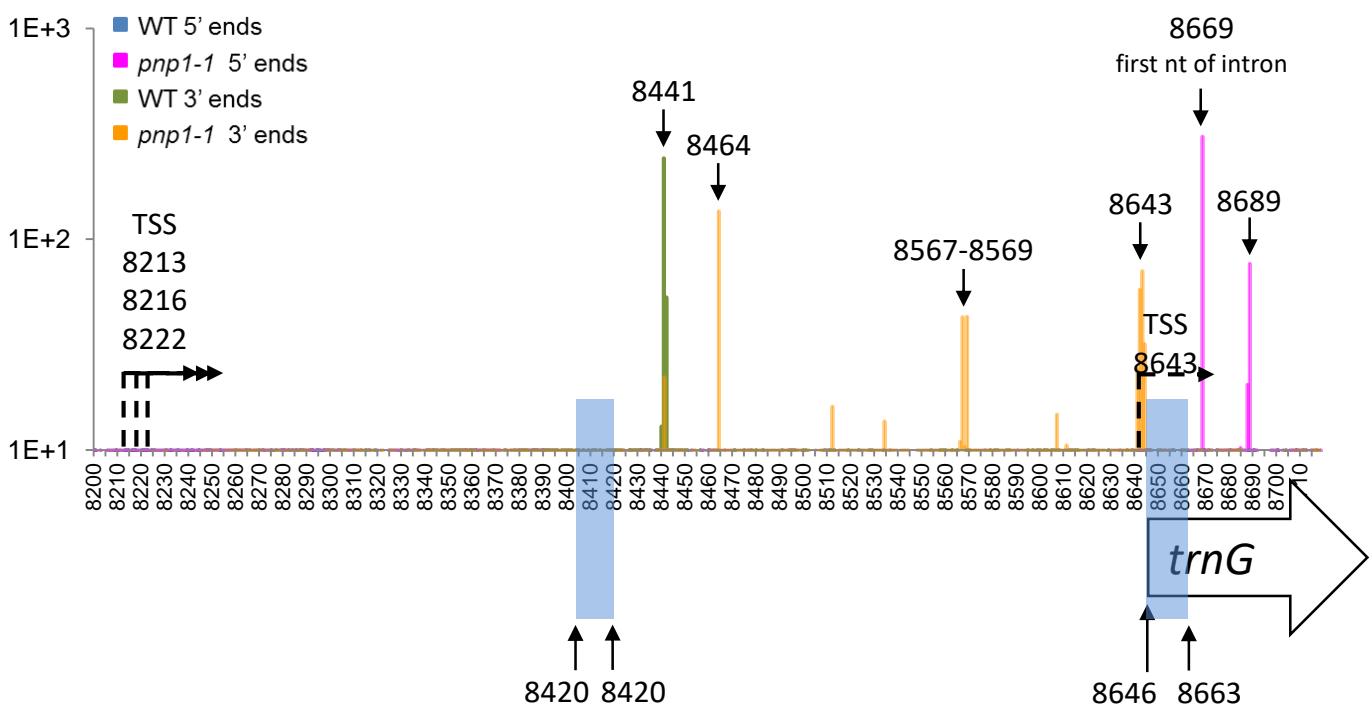
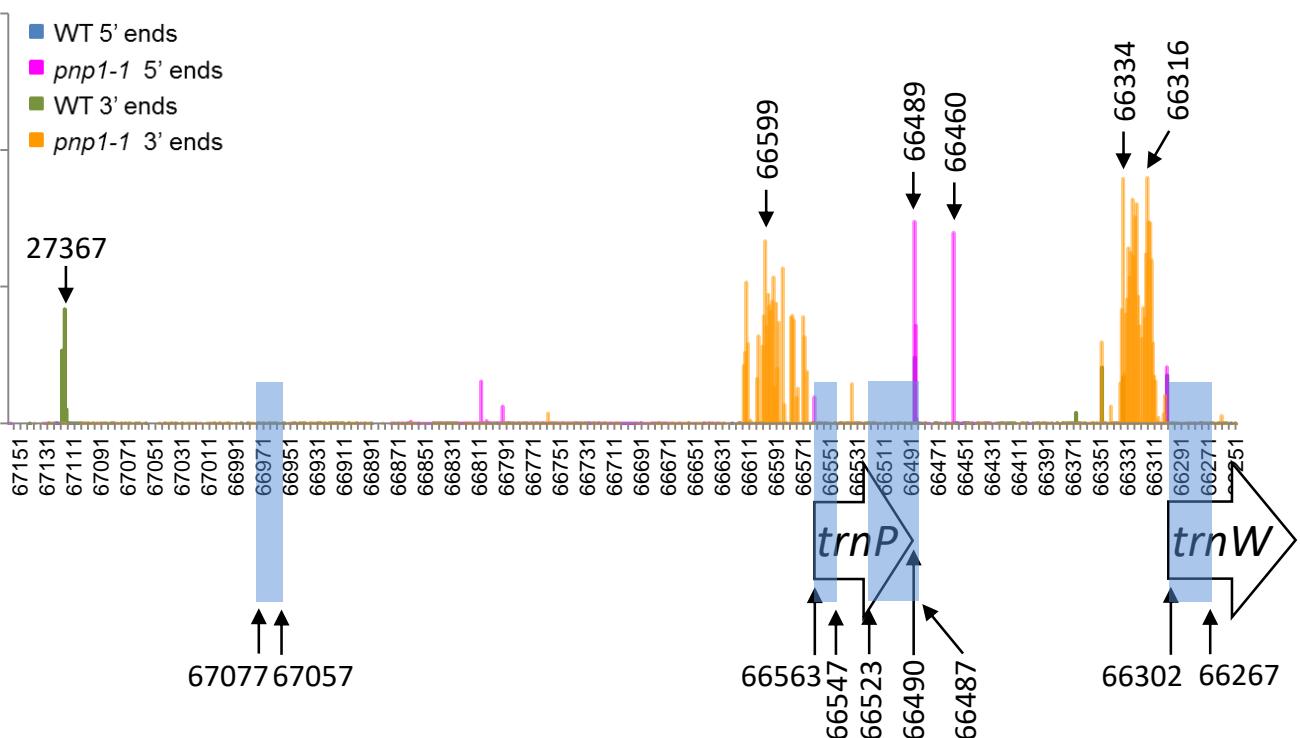
- WT 5' ends
- pnp1-1* 5' ends
- WT 3' ends
- pnp1-1* 3' ends



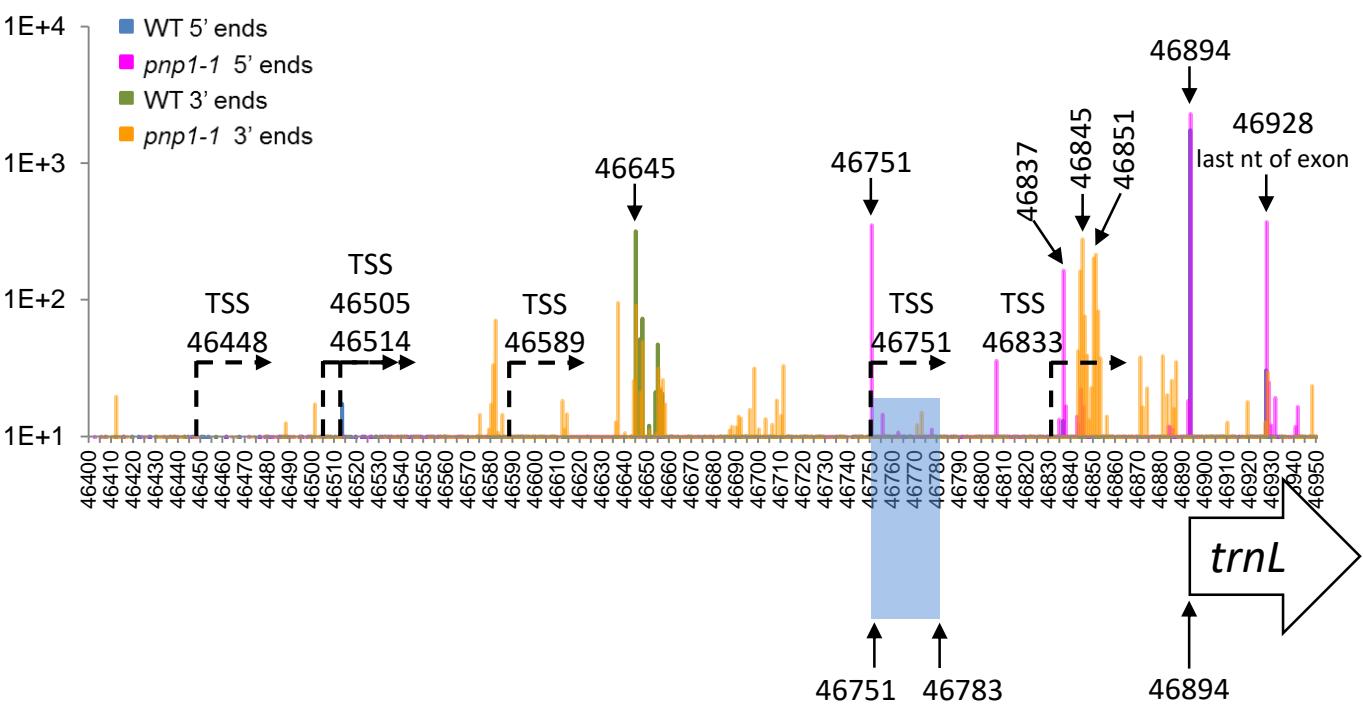
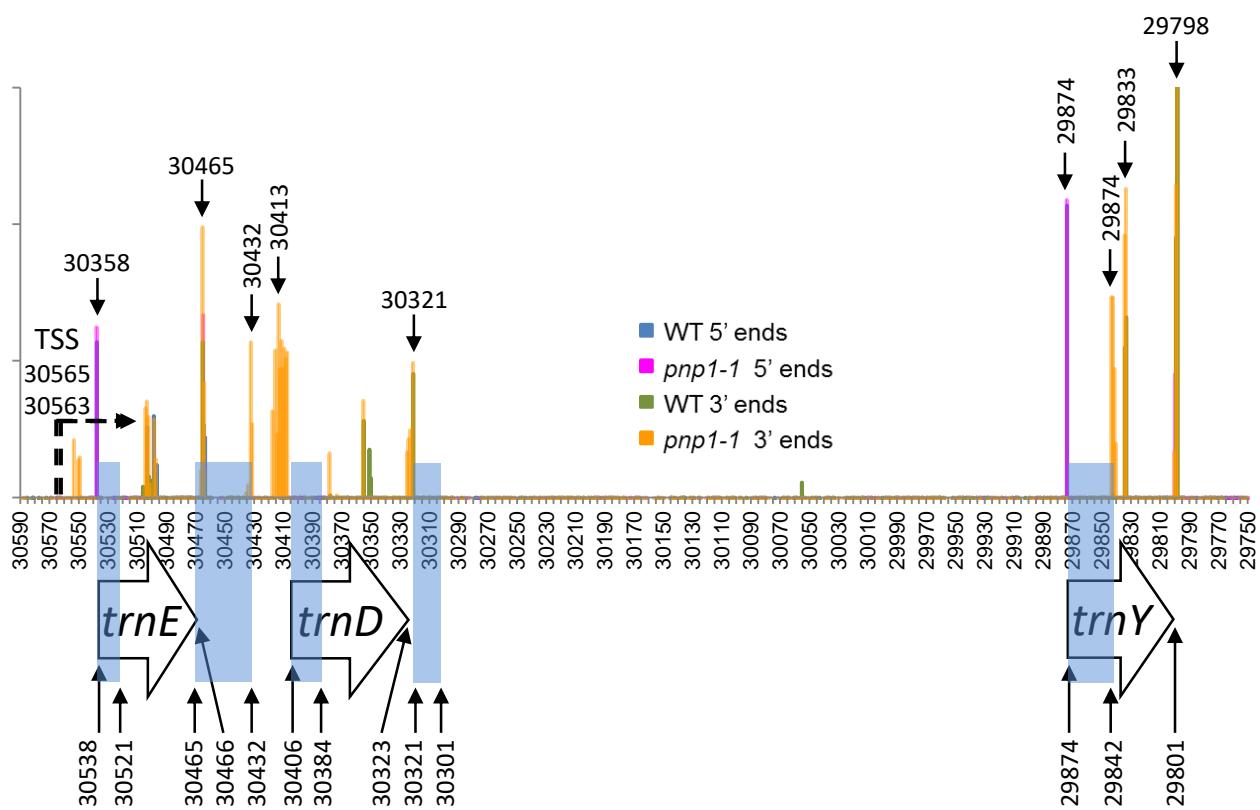
Supplementary Figure S7



Supplementary Figure S7

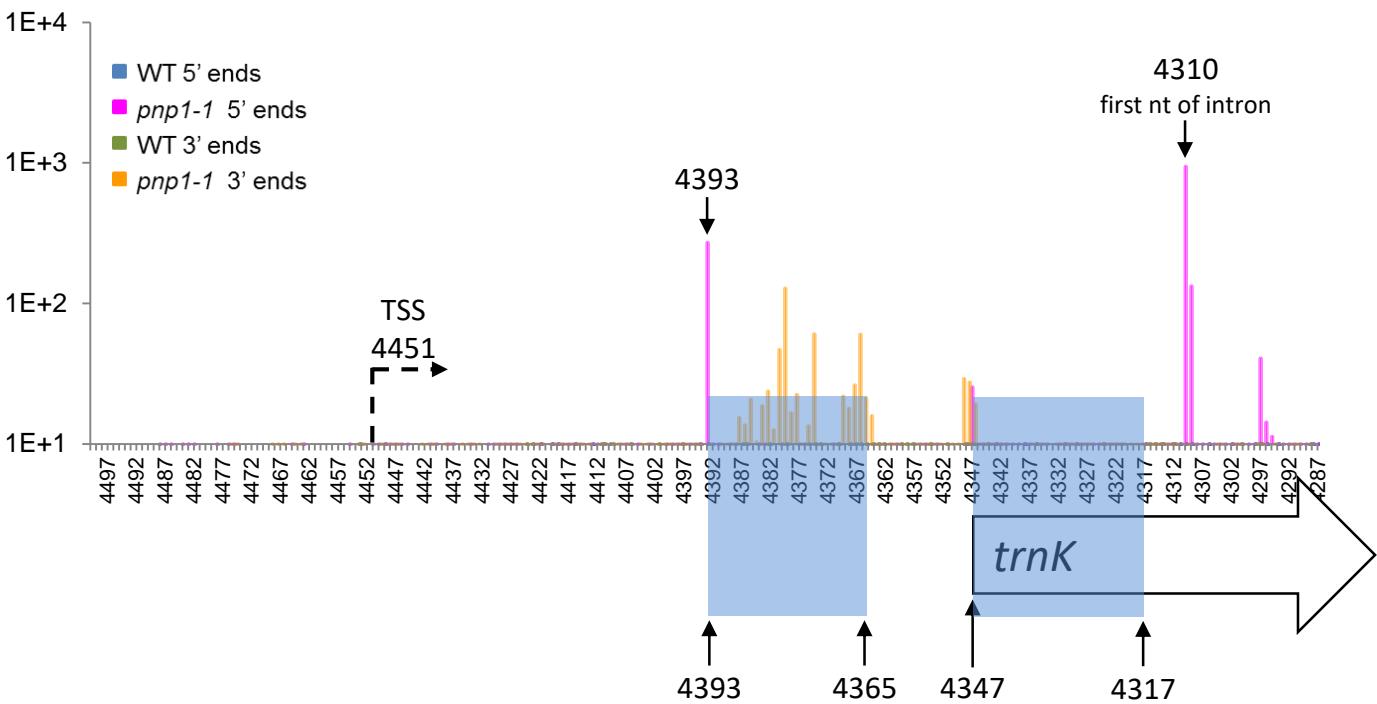


Supplementary Figure S7



Supplementary Figure S7

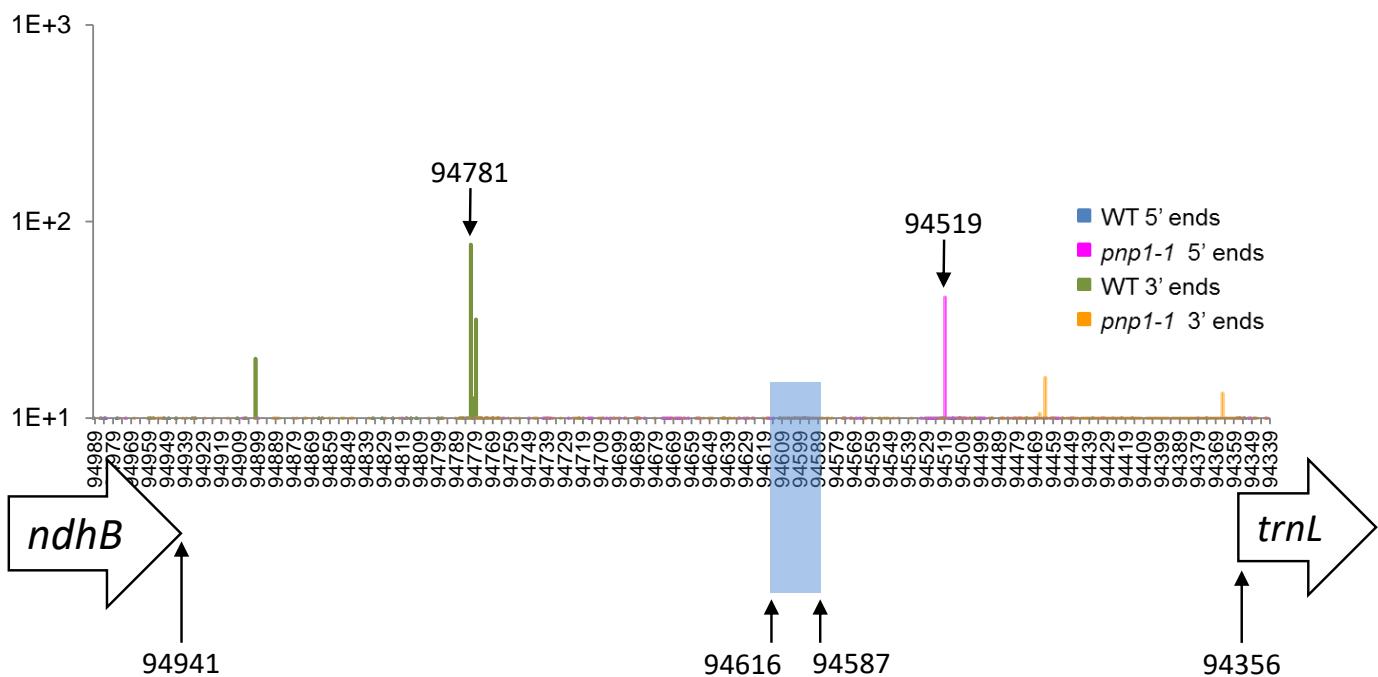
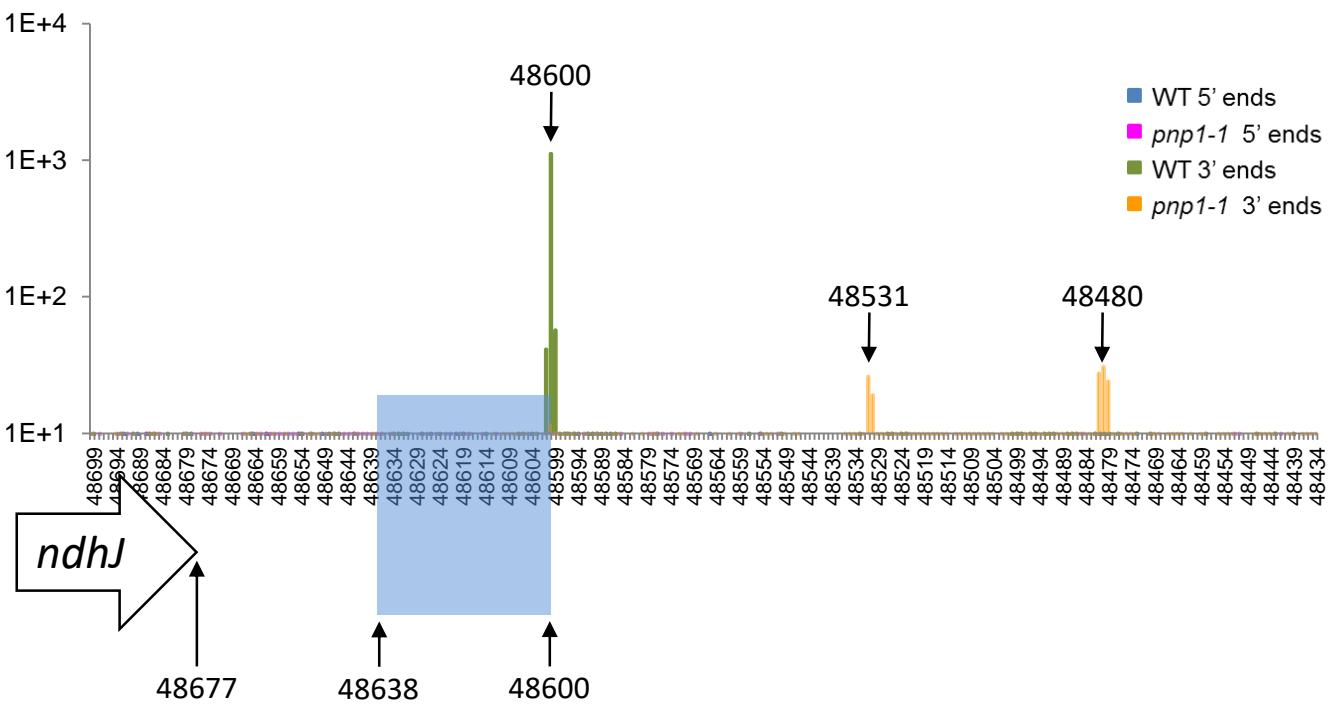
- WT 5' ends
- pnp1-1* 5' ends
- WT 3' ends
- pnp1-1* 3' ends



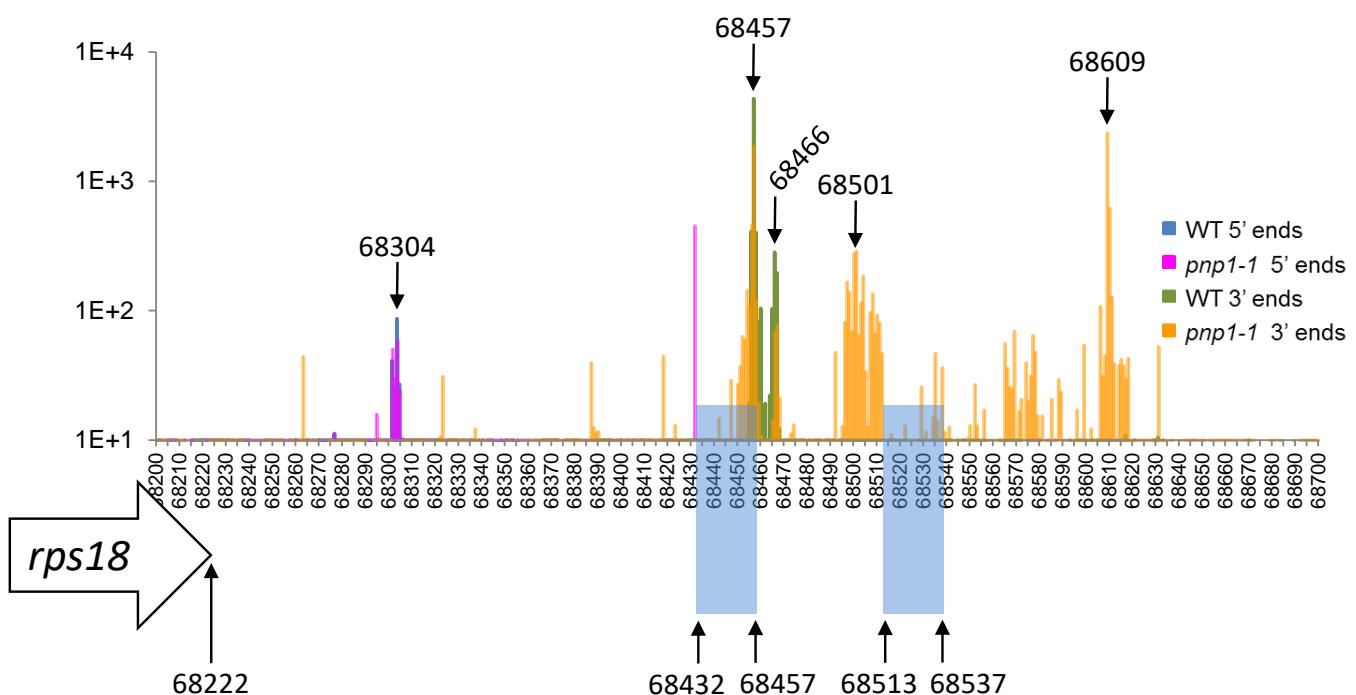
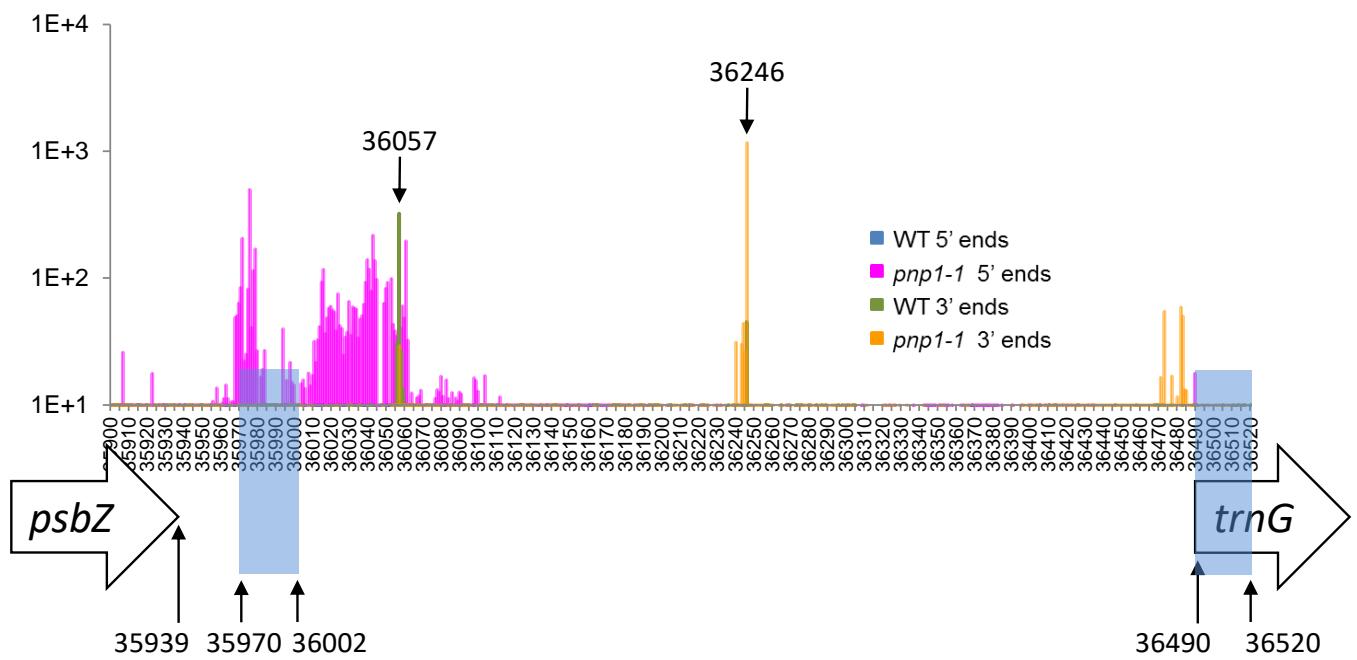
Supplementary Figure S7

Supplementary Figure S8: Transcript termini in intergenic regions

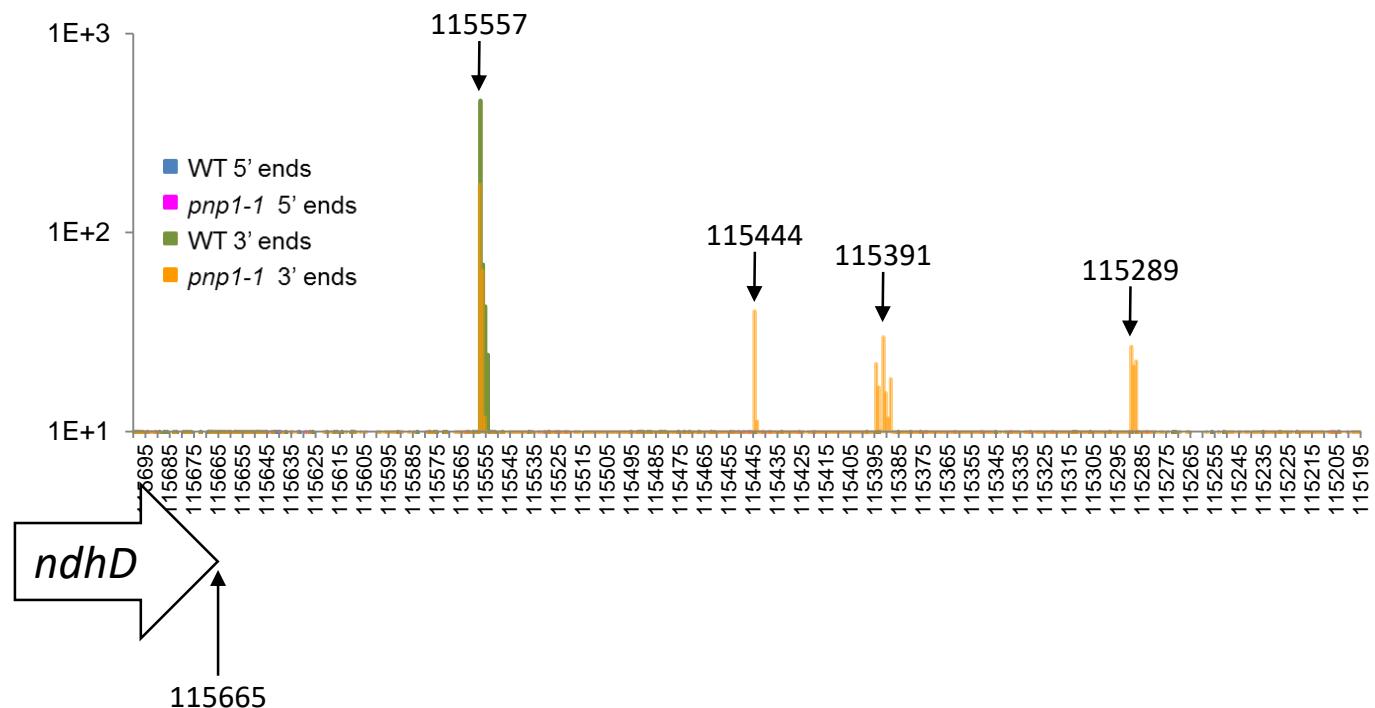
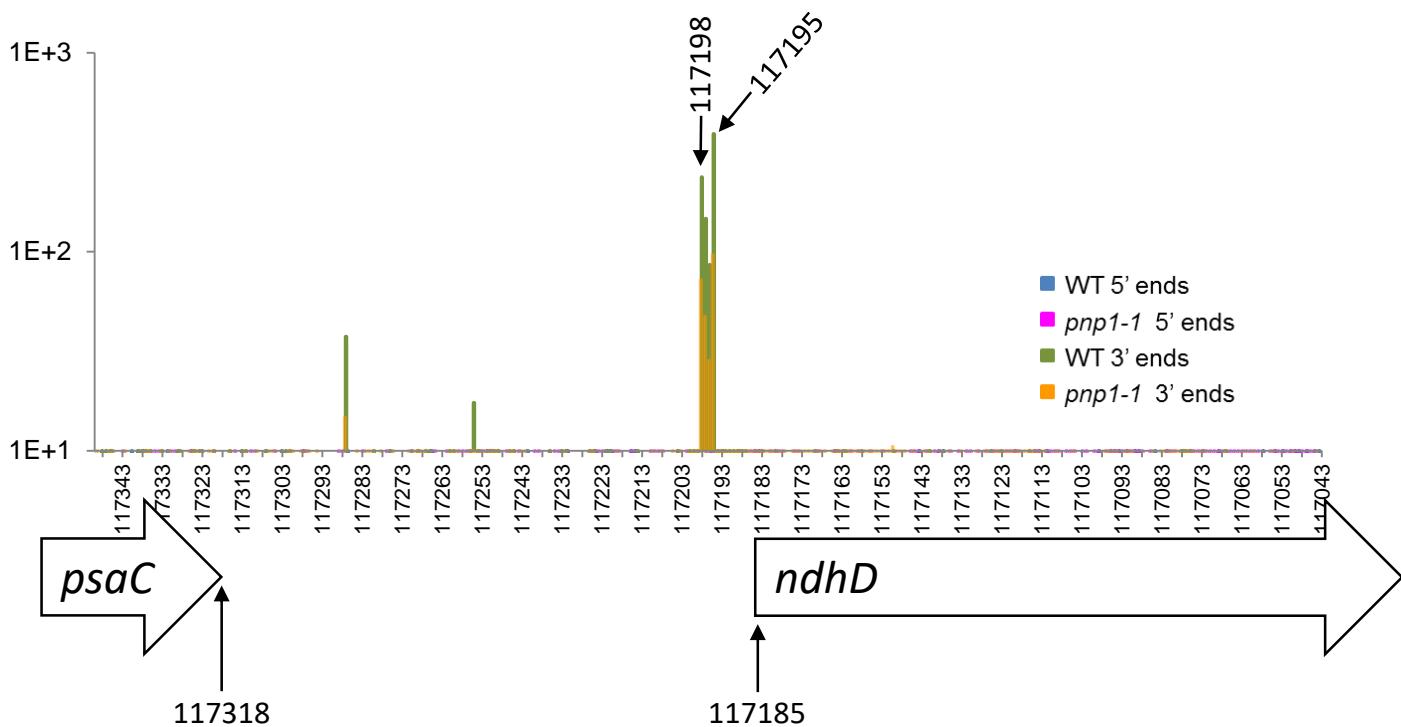
Comparison of Terminome-Seq coverage for WT and *pnp1-1* in selected intergenic regions. Gene models are represented as open arrows and color coding of ends is provided in an inset. Genome positions where ends accumulate are indicated with black arrows, and smRNA locations are highlighted in blue.



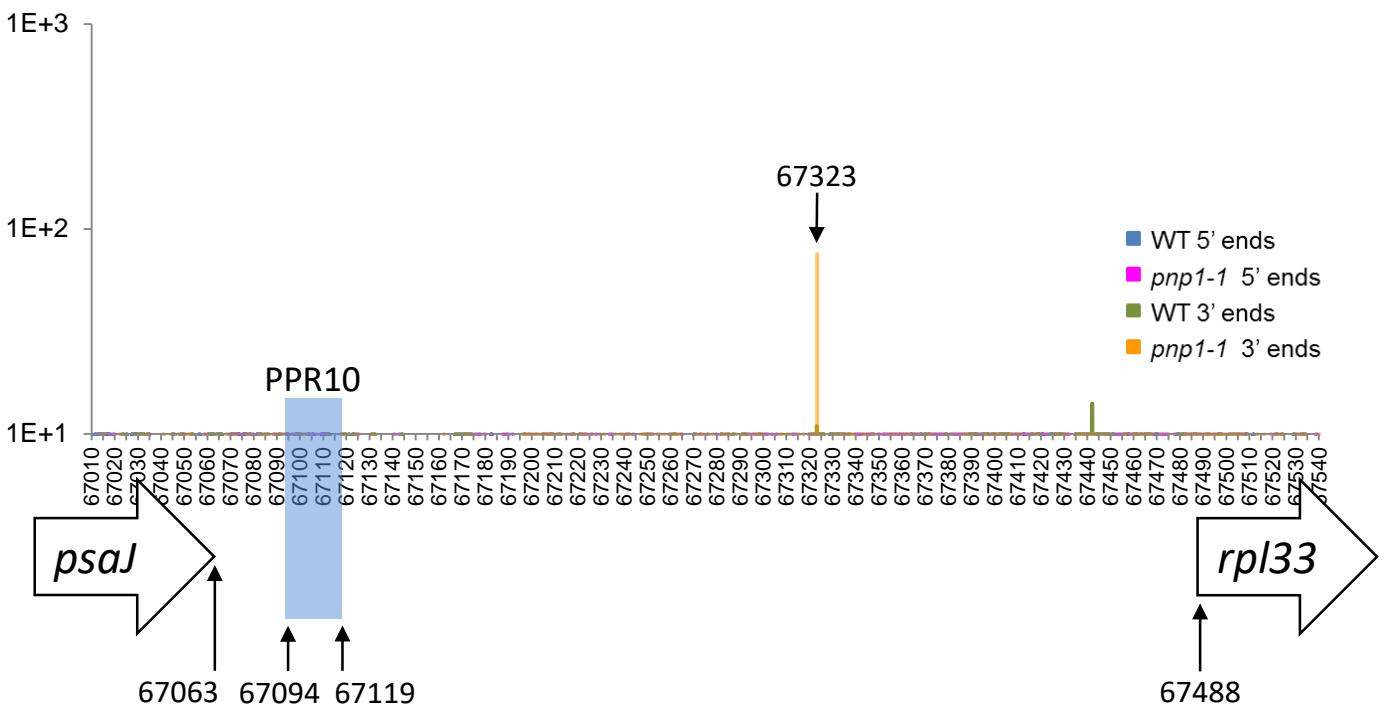
Supplementary Figure S8



Supplementary Figure S8



Supplementary Figure S8



Supplementary Figure S8

SUPPLEMENTARY FIGURES REFERENCES

1. Kapoor,S., Wakasugi,T., Deno,H. and Sugiura,M. (1994) An atpE-specific promoter within the coding region of the atpB gene in tobacco chloroplast DNA. *Curr. Genet.*, **26**, 263–8.
2. Ghulam,M.M., Courtois,F., Lerbs-Mache,S., Merendino,L. and Merendino,L. (2013) Complex Processing Patterns of mRNAs of the Large ATP Synthase Operon in Arabidopsis Chloroplasts. *PLoS One*, **8**, e78265.
3. Zghidi,W., Merendino,L., Cottet,A., Mache,R. and Lerbs-Mache,S. (2007) Nucleus-encoded plastid sigma factor SIG3 transcribes specifically the psb N gene in plastids. *Nucleic Acids Res.*, **35**, 455–464.
4. Hanaoka,M., Kanamaru,K., Takahashi,H. and Tanaka,K. (2003) Molecular genetic analysis of chloroplast gene promoters dependent on SIG2, a nucleus-encoded sigma factor for the plastid-encoded RNA polymerase, in *Arabidopsis thaliana*. *Nucleic Acids Res.*, **31**, 7090–8.
5. Hoffer,P.H. and Christopher,D.A. (1997) Structure and blue-light-responsive transcription of a chloroplast psbD promoter from *Arabidopsis thaliana*. *Plant Physiol.*, **115**, 213–22.
6. Zoschke,R., Watkins,K.P., Miranda,R.G. and Barkan,A. (2016) The PPR-SMR protein PPR53 enhances the stability and translation of specific chloroplast RNAs in maize. *Plant J.*, **85**, 594–606.
7. Liere,K., Kestermann,M., Müller,U. and Link,G. (1995) Identification and characterization of the *Arabidopsis thaliana* chloroplast DNA region containing the genes psbA, trnH and rps19'. *Curr. Genet.*, **28**, 128–30.
8. Shen,Y., Danon,A. and Christopher,D.A. (2001) RNA binding-proteins interact specifically with the *Arabidopsis* chloroplast psbA mRNA 5' untranslated region in a redox-dependent manner. *Plant Cell Physiol.*, **42**, 1071–8.
9. Zhelyazkova,P., Sharma,C.M., Forstner,K.U., Liere,K., Vogel,J. and Borner,T. (2012) The Primary Transcriptome of Barley Chloroplasts: Numerous Noncoding RNAs and the Dominating Role of the Plastid-Encoded RNA Polymerase. *24*, 123–136.
10. Meng,B.-Y., Wakasugi,T. and Sugiura,M. (1991) Two promoters within the psbK-psbI-trnG gene cluster in tobacco chloroplast DNA. *Curr. Genet.*, **20**, 259–264.