

Expanded View Figures

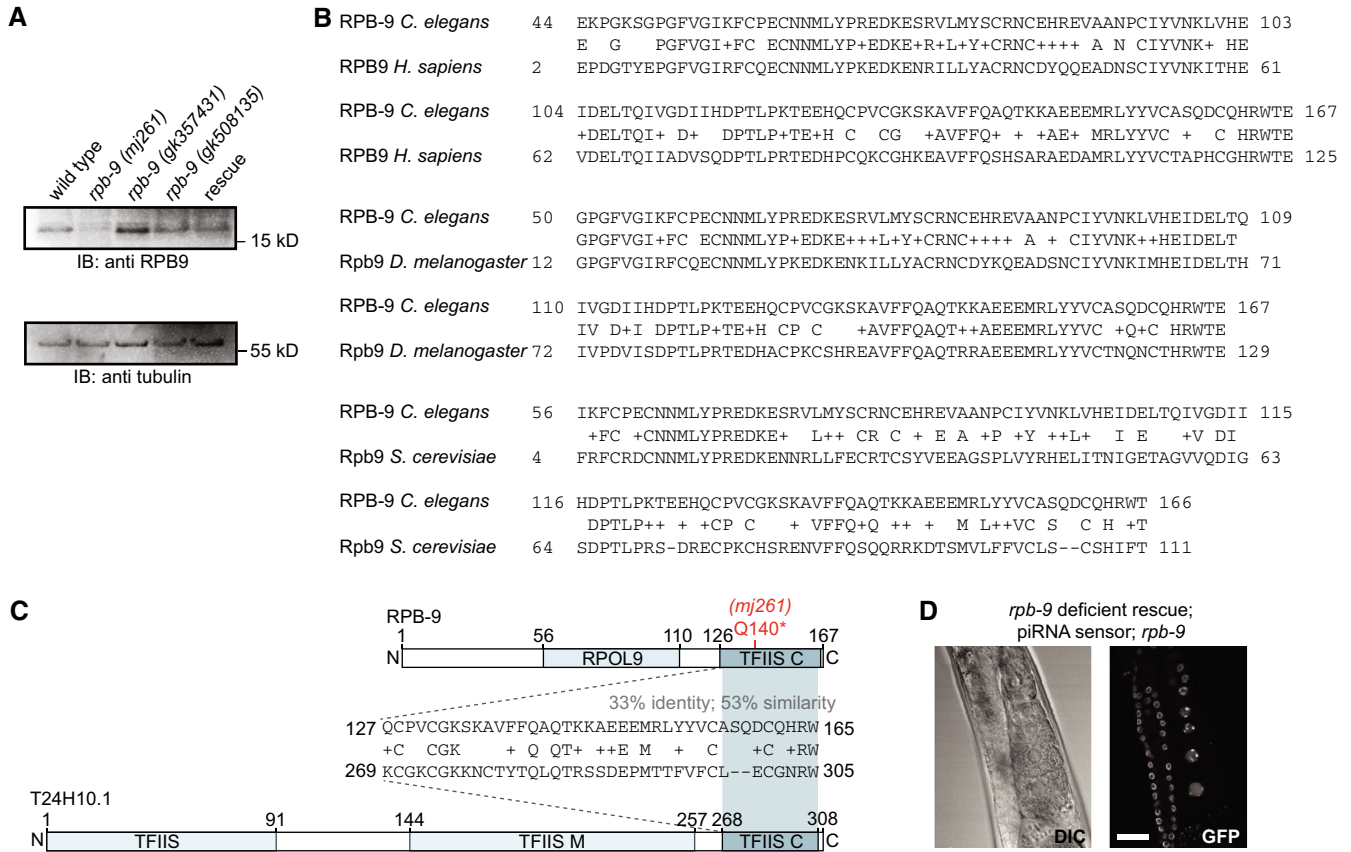
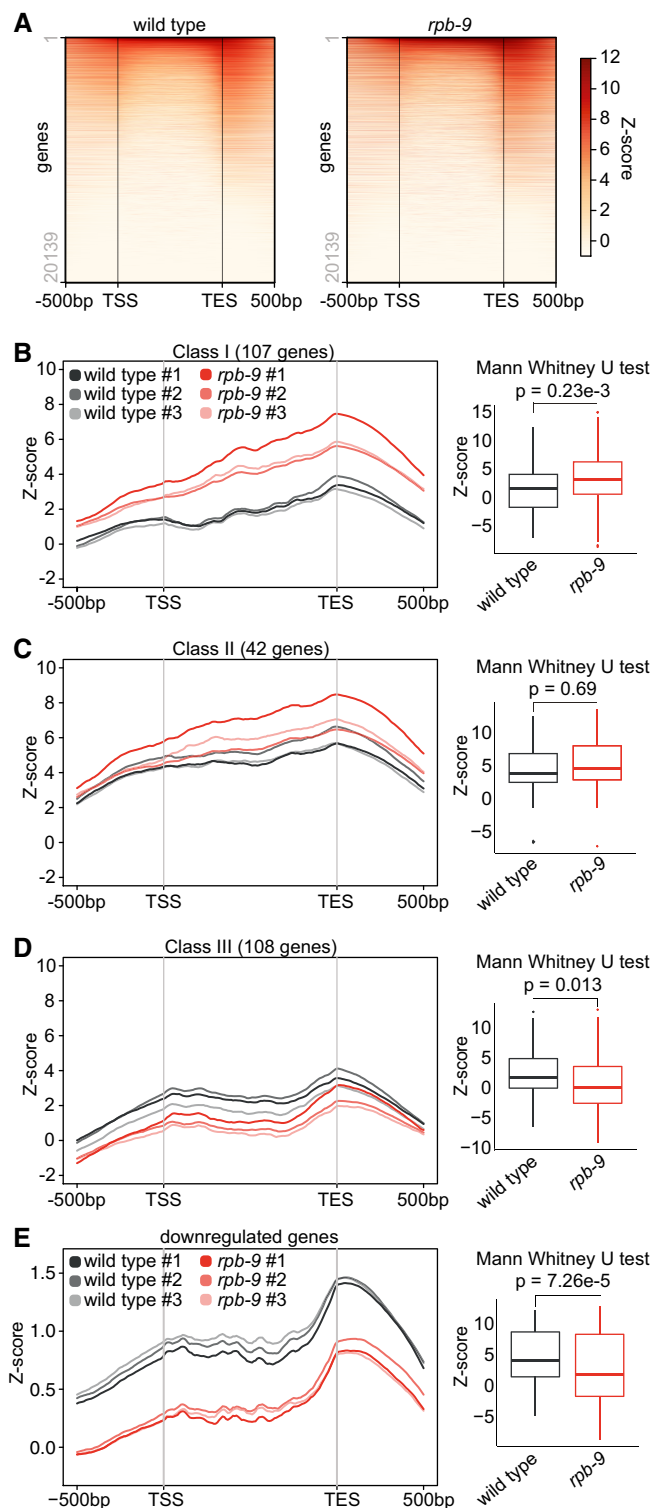


Figure EV1. Related to Fig 1.

- A Western blot quantification of RPB-9 expression in wild-type, *rpb-9* (*mj261*), and *rpb-9* rescue animals. *rpb-9* alleles *gk508135* and *gk357431* (Thompson *et al*, 2013) are additional controls that do not show piRNA sensor desilencing.
- B RPB-9 domain conservations. Alignments of the RPB-9 protein to its *H. sapiens*, *D. melanogaster* and *S. cerevisiae* homologs. Positive (NCBI BlastP) amino acids are indicated by “+”.
- C Comparison of RPB-9 and the *Caenorhabditis elegans* TFIIS homolog T24H10.1 with alignment of the common TFIIS C domain. The introduced RPB-9 Q140* mutation is highlighted in red. Positive (NCBI BlastP) amino acids are indicated by “+”.
- D Representative DIC and fluorescence microscopy images of piRNA sensor expression in deficient rescue *rpb-9* (*mj261*; *mjSi89*) animals. Scale bar = 20 μ m.

Source data are available online for this figure.

**Figure EV2. Related to Fig 4.**

- A Analysis of differential RNA Pol II binding (RPB-1/AMA-1 ChIP-seq) over all genes in *rpb-9* (*mj261*) mutants compared with wild type.
- B–D Analysis of RNA Pol II binding at upregulated genes in *rpb-9* (*mj261*) mutants, as defined in Appendix Fig S2B. Class I: upregulated genes with increased RNA Pol II binding (B); class II: upregulated genes with invariant RNA Pol II binding (C); class III: upregulated genes with reduced RNA Pol II binding (D). $n = 3$ biological replicates are shown per each genotype (RNA-sequencing filtering from total RNA “Ribo-Zero” RNA-seq libraries). Central bands represent the median, boxes represent the 25th and 75th percentiles, and whiskers represent the lowest and highest values, excluding outliers.
- E Analysis of RNA Pol II binding at downregulated genes in *rpb-9* (*mj261*) mutants ($n = 3$), as defined in Fig 3C.

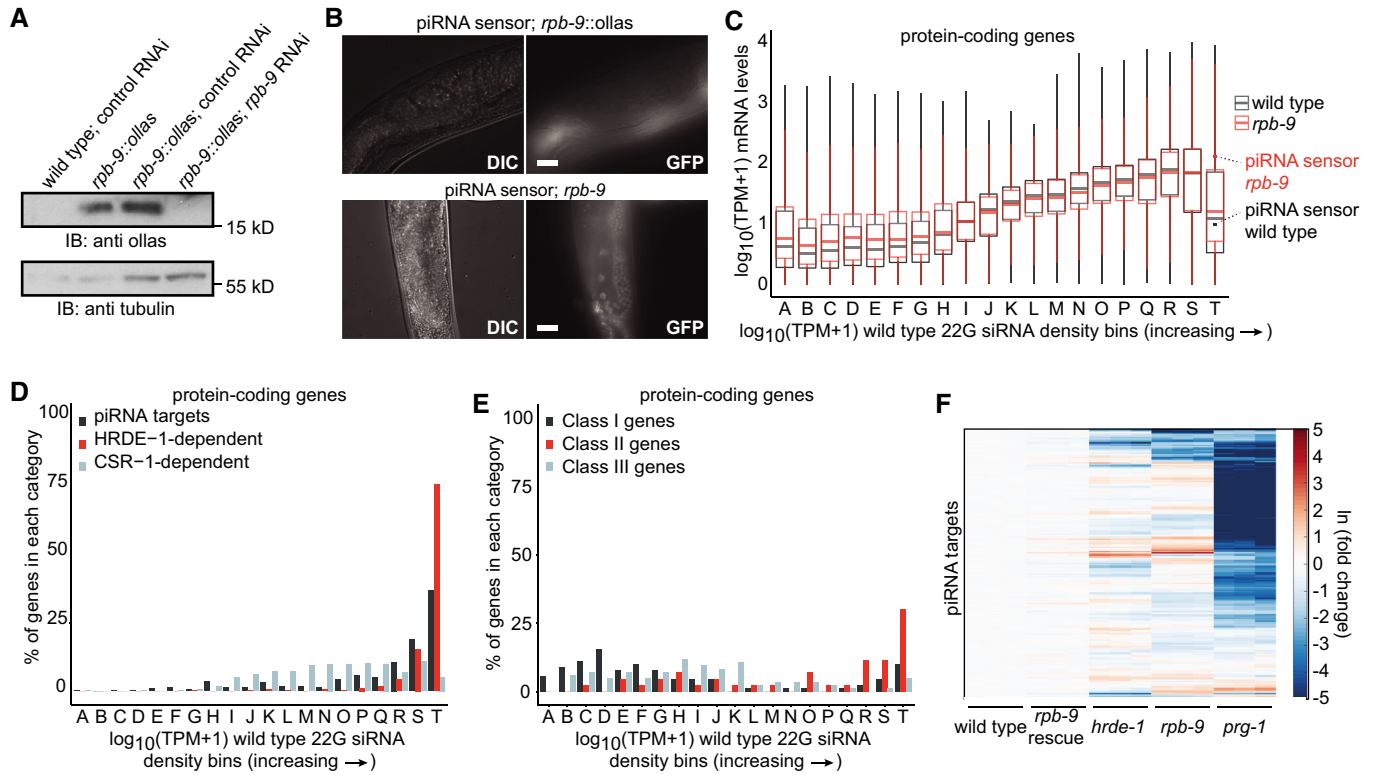


Figure EV3. Related to Fig 5.

A Western blot quantification of the amount of RPB-9 protein in the *rpb-9::ollas* line by Western blot. *rpb-9* RNAi samples are shown for control.
 B Representative DIC and fluorescence microscopy images of piRNA sensor expression in *rpb-9::ollas* (*mj604*). *rpb-9* (*mj261*) is shown for control. Scale bar = 20 μ m.
 C Transcriptome binning according to increasing 22G siRNA density in wild-type animals (gray, $n = 3$). Mean normalized *rpb-9* (*mj261*) mRNA reads (red, $n = 3$) are overlaid with mean normalized wild-type mRNA reads (gray) (total RNA Ribo-Zero RNA-seq libraries). The piRNA sensor transcript is highlighted in red (*rpb-9* (*mj261*)) and gray (wild type). Central bands represent the median, boxes represent the 25th and 75th percentiles, and whiskers represent the lowest and highest values, excluding outliers.
 D Distribution of piRNA targets (as defined in Ref. (Bagijn et al, 2012)) and of HDRE-1- and CSR-1-dependent 22G siRNAs across bins as defined in (C).
 E Distribution of class I, class II, and class III genes (as defined in Fig EV2C–E) across bins as defined in (C).
 F Cluster analysis of 22G siRNAs mapping to piRNA target genes in wild-type, *rpb-9* (*mj261*), *rpb-9* rescue (*mj261*; *mjSi70*), *prg-1* (*n4357*), and *hrde-1* (*tm1200*) animals (5^l-independent small RNA libraries).

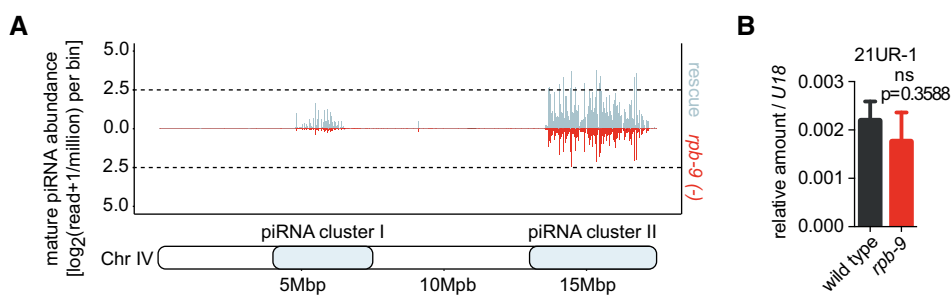
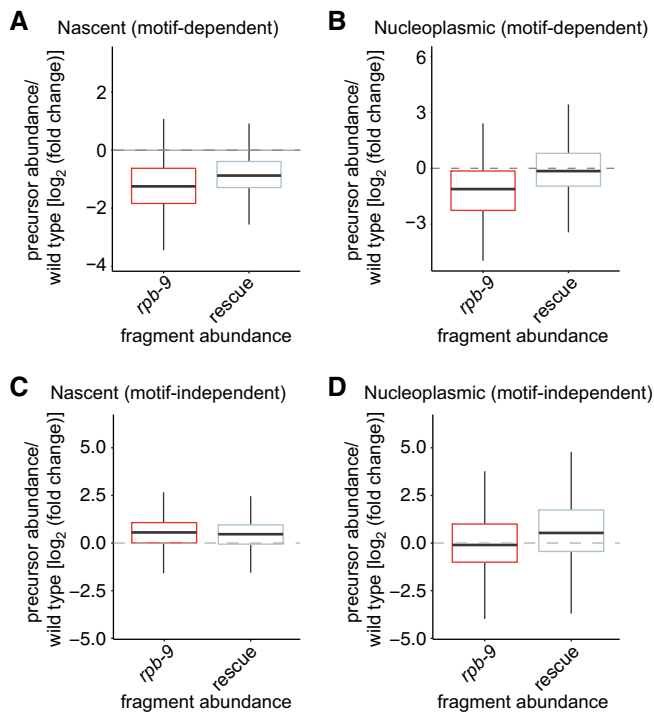


Figure EV4. Related to Fig 6.

A Mature piRNA expression along chromosome IV coordinates (motif-dependent piRNA clusters I and II) in *rpb-9* (*mj261*) and *rpb-9* rescue (*mj261*; *mjSi70*) animals.
 B RT-qPCR quantification of mature piRNA 21UR-1 levels in wild-type and *rpb-9* (*mj261*) animals. $n = 3$ biological replicates, mean and SD shown (two-tailed t-test).

**Figure EV5. Related to Fig 7.**

A Motif-dependent nascent piRNA precursor abundance in *rpb-9* (*mj261*) and rescue (*mj261; mjSi70*) animals relative to wild type ($n = 2$).

B Motif-dependent nucleoplasmic piRNA precursor abundance in *rpb-9* (*mj261*) and rescue (*mj261; mjSi70*) animals relative to wild type ($n = 2$).

C Motif-independent nascent piRNA precursor abundance in *rpb-9* (*mj261*) and rescue (*mj261; mjSi70*) animals relative to wild type ($n = 2$).

D Motif-independent nucleoplasmic piRNA precursor abundance in *rpb-9* (*mj261*) and rescue (*mj261; mjSi70*) animals relative to wild type ($n = 2$). Central bands represent the median, boxes represent the 25th and 75th percentiles, and whiskers represent the lowest and highest values, excluding outliers.