

Title

Sensitized piRNA reporter identifies multiple RNA processing factors involved in piRNA-mediated gene silencing

Author List

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This supplementary information includes:

Figure S1. piRNA reporter screening strategy.

Figure S2. Loss of piRNAs following *snp-4*, *ints-1*, and *dic-1* knockdown.

Figure S3. NPP-1/6/7/9 and CSR-1 pathway disruption activates the sensitized piRNA reporter.

Figure S4. HRDE-1 does not share NRDE-3's nuclear localization signal.

Supplementary Table 1. Full list of candidates screened using piRNA reporter.

Figure S1. piRNA reporter screening strategy.

A) A model depicts the cellular context of biogenesis and effector steps in piRNA silencing.

Figure S2. Loss of piRNAs following *snp-4*, *ints-1*, and *dic-1* knockdown.

A) Scatterplots show the expression of mature type I piRNAs in the indicated RNAi knockdowns.

B) Scatterplots show the expression of type I piRNA precursors in the indicated RNAi knockdowns.

C) A histogram shows the distribution of type I and type II piRNA precursor sequence lengths in cap-enriched sequencing libraries from *ints-1* and empty vector RNAi treated worms.

D) A model shows the proposed action of the Integrator complex at piRNA loci.

Figure S3. NPP-1/6/7/9 and CSR-1 pathway disruption activates the sensitized piRNA reporter.

A) GFP expression in type II piRNA reporter in indicated RNAi knockdowns. Asterisks indicate significant reporter activation, see Methods for details.

B) GFP expression in type I piRNA reporter in indicated RNAi knockdowns. Asterisks indicate significant reporter activation, see Methods for details.

C) Percentage of worms with dispersed GFP::CSR-1 (top) or GFP::PRG-1 (bottom) granules in the indicated RNAi knockdowns. Asterisks indicate $p < 0.05$ in one-tailed Student's t-test.

D) Percentage of germline nuclei with nucleoli that contain NPP-7 following the indicated RNAi knockdowns. Representative fluorescent micrographs show mCherry::NPP-7 localization from indicated treatments. Treatments that also activate the piRNA reported are marked with an asterisk.

Figure S4. HRDE-1 does not share NRDE-3's nuclear localization signal.

- A) GFP expression in type II piRNA reporter in indicated RNAi knockdowns.
- B) A selection from the Smith-Waterman local alignment of HRDE-1 and NRDE-3 amino acid sequences that includes the NRDE-3 nuclear localization signal.

Supplementary Table 1. Full list of candidates screened using piRNA reporter.

- B) A model depicts the cellular context of biogenesis and effector steps in piRNA silencing.

Figure S1

A

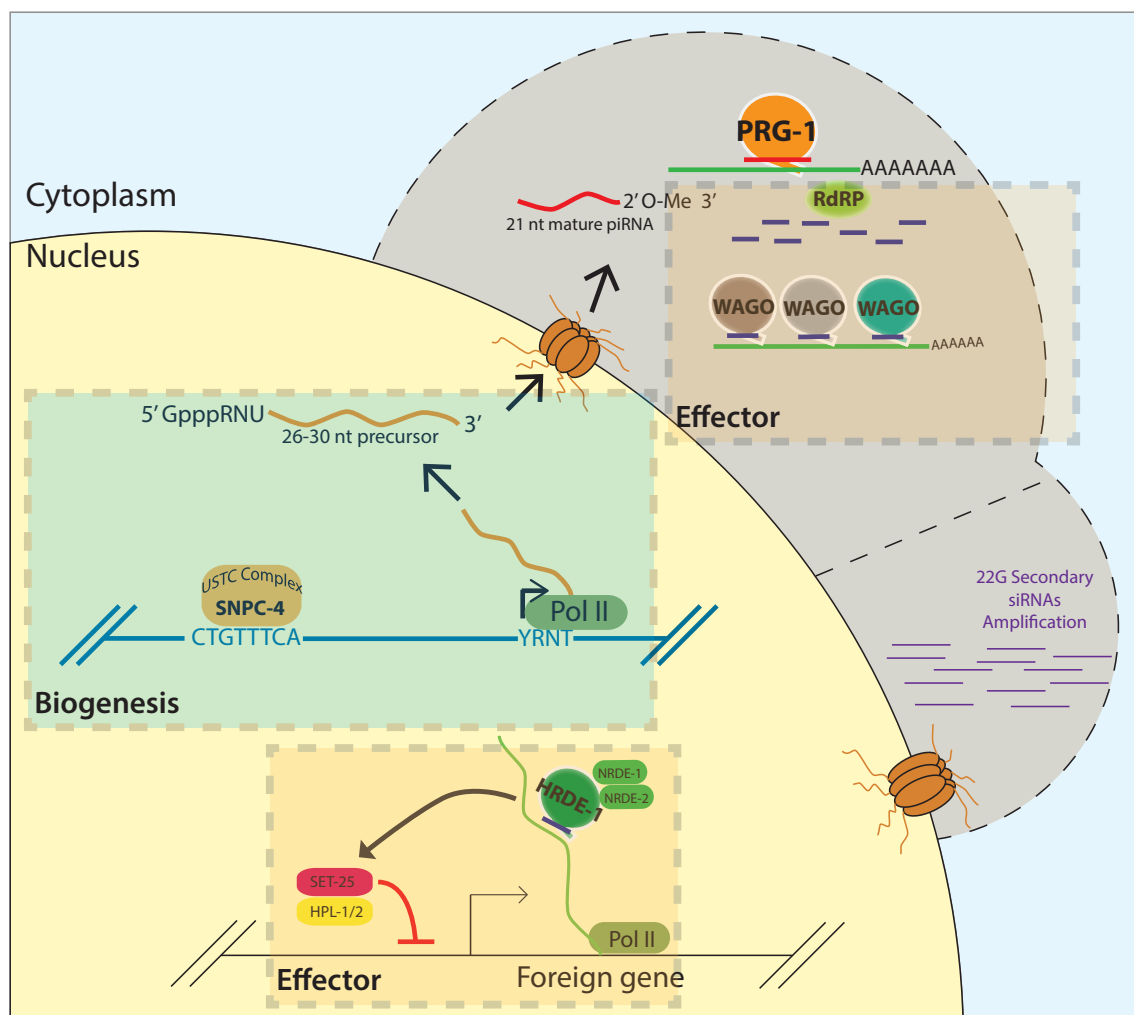


Figure S2

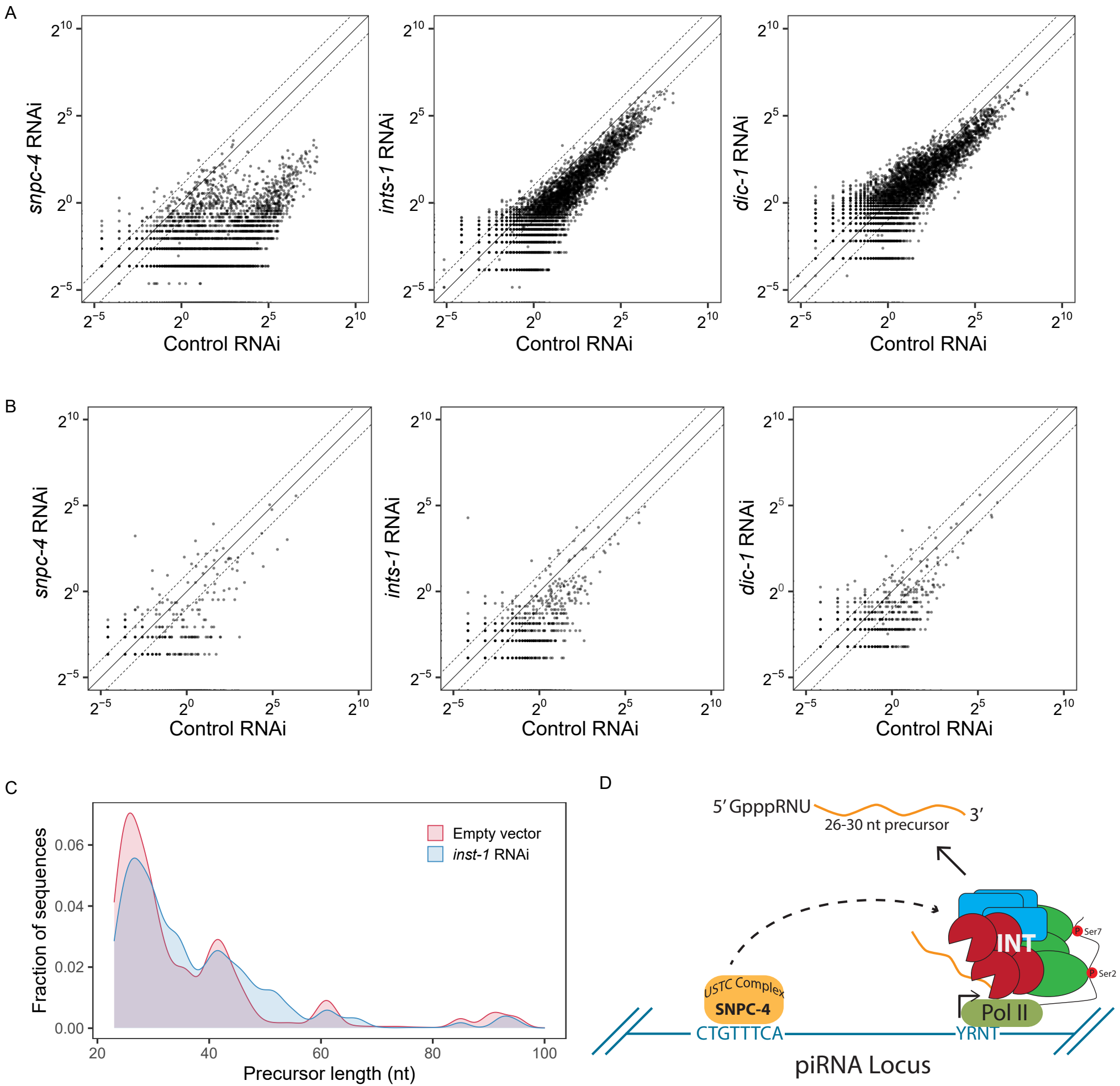


Figure S3

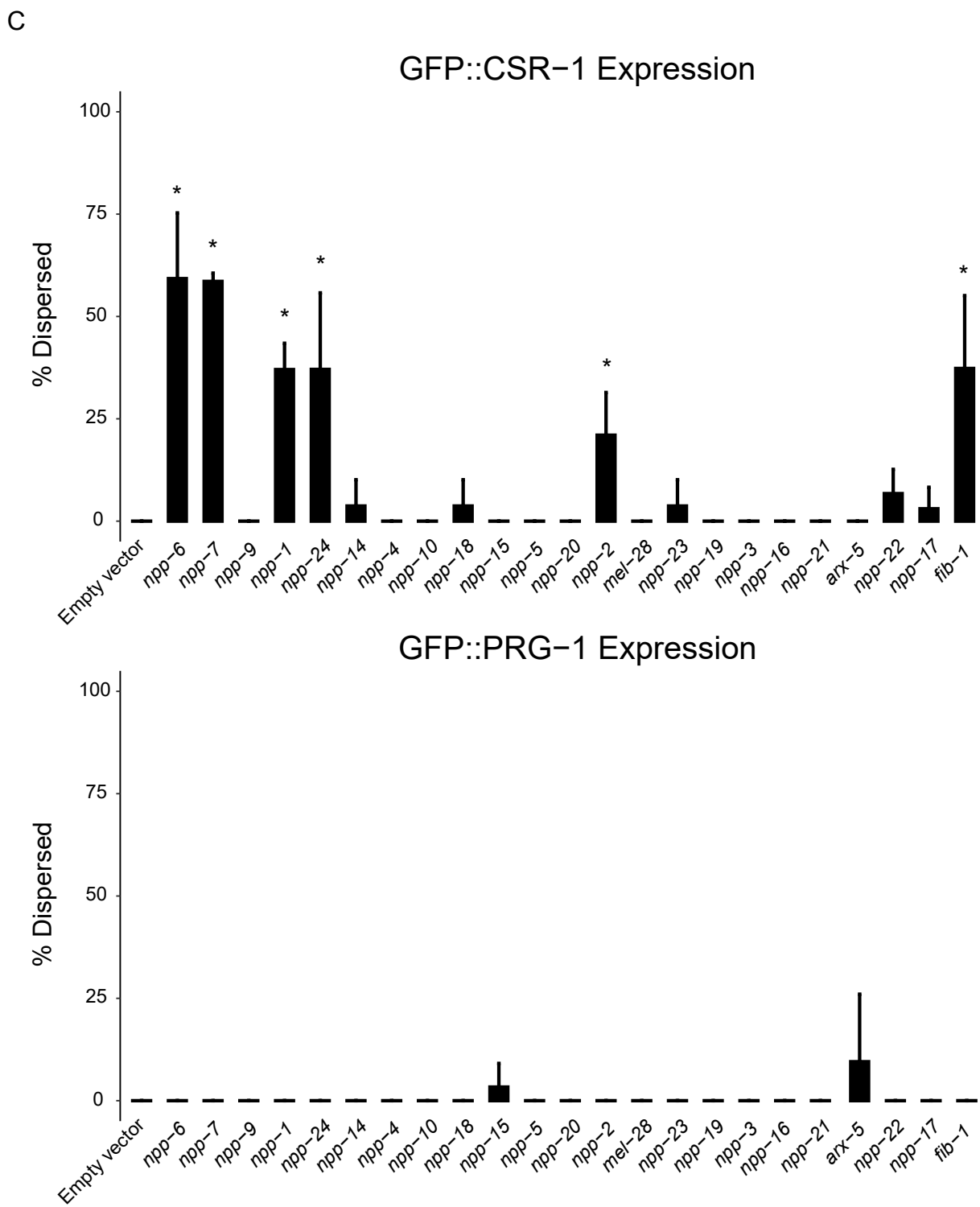
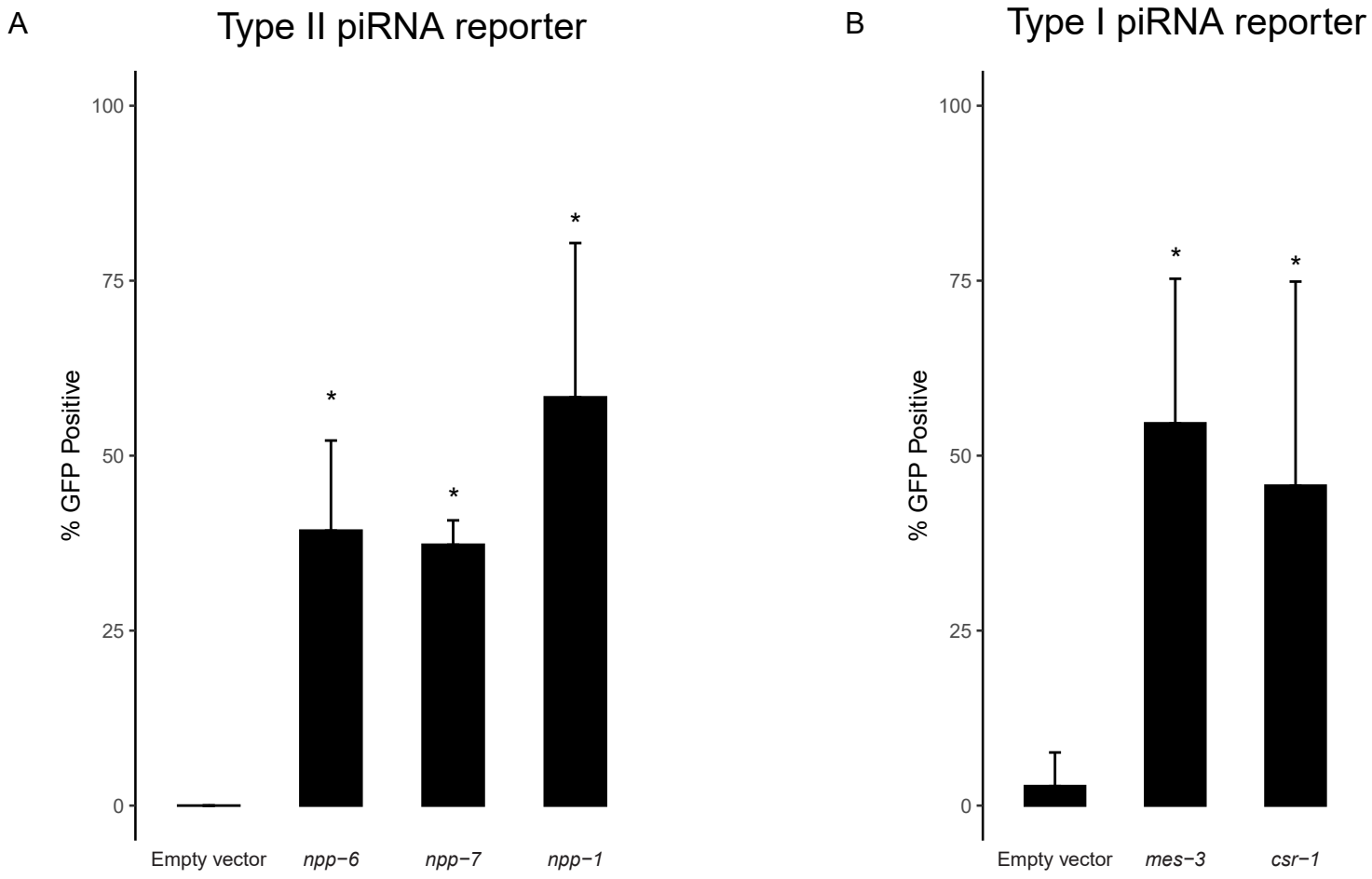
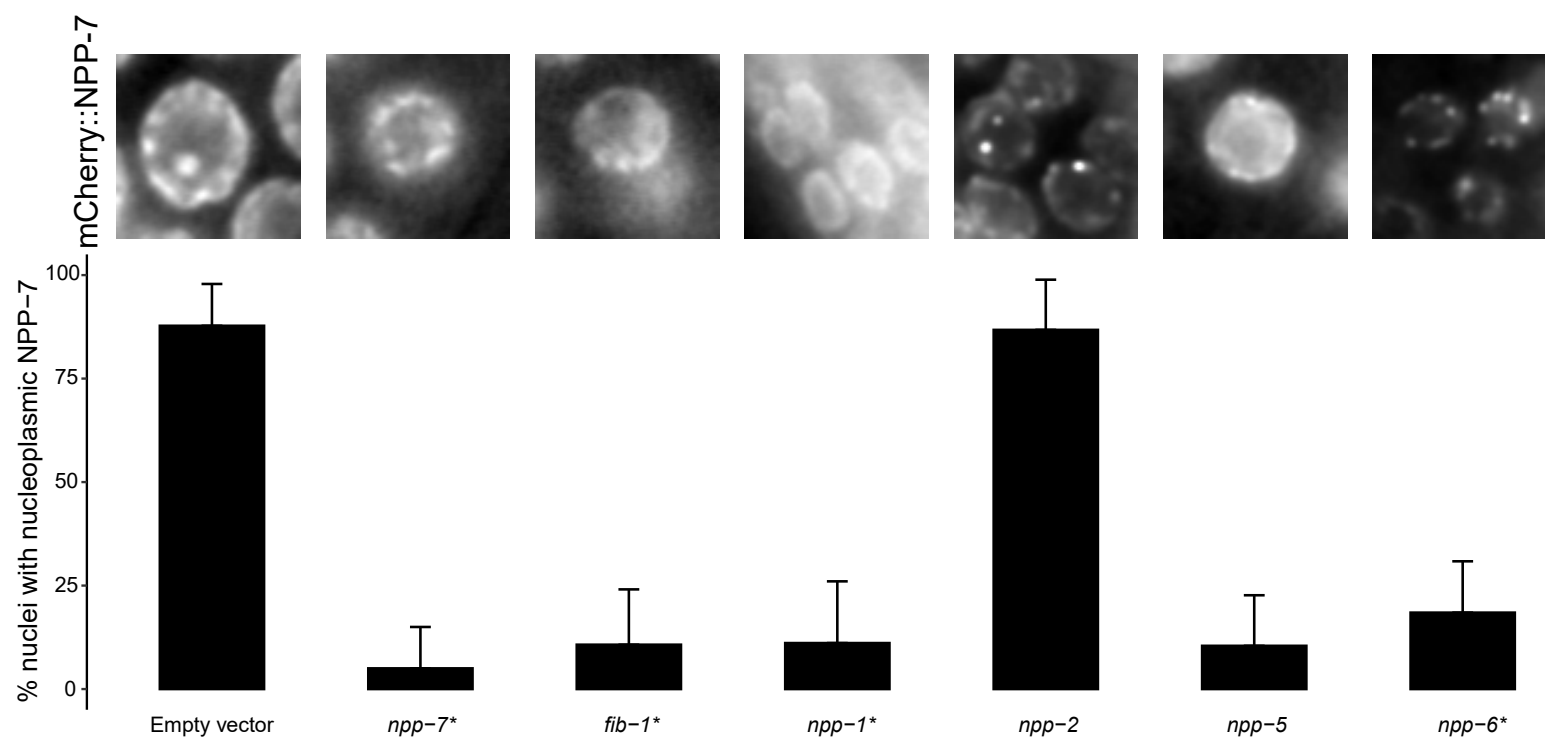


Figure S3, continued

D



* = also activated piRNA reporter

Figure S4

