## Rogers et al. Supplemental Figure 3



Supplemental Figure 3. Production of piRNAs upon Piwi tethering is independent of the  $\lambda$ N-Piwi fusion construct, as well as the reporter sequence and genomic location. A) Tethering of  $\lambda$ N-FLAG-Piwi to the mKate2-4xBoxB, or to the Tubulin-GFP-BoxB reporter, results in piRNA production from the reporter. In the left graph, RPMs of small RNA-seq libraries from size selected total RNA from ovaries are shown. RPMs were calculated for reads mapping to the reporter specifically or non-specifically (allowed to map to the DM3 genome) normalized to total reads mapping to the DM3 genome. Size selected or 5'U reads mapping to the reporter were normalized to corresponding reads mapping to the DM3. On the right, RPMs calculated for reads specifically mapping to 42AB, normalized to reads mapping to the DM3 genome are shown for the same dataset. B) Recruitment of Piwi to the Tubulin-GFP-BoxB reporter results in piRNAs mapping along the entire reporter sequence. Shown are profiles for all reads (black) and reporter-specific reads (red) mapping to the DM3 genome, and profiles for 5'U 23-29nt reads mapping to the reporter, normalized to 5'U 23-29nt reads mapping to the DM3 genome. As a control, neither  $\lambda$ N-GFP nor  $\lambda$ N-FLAG tethering resulted in piRNAs.

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