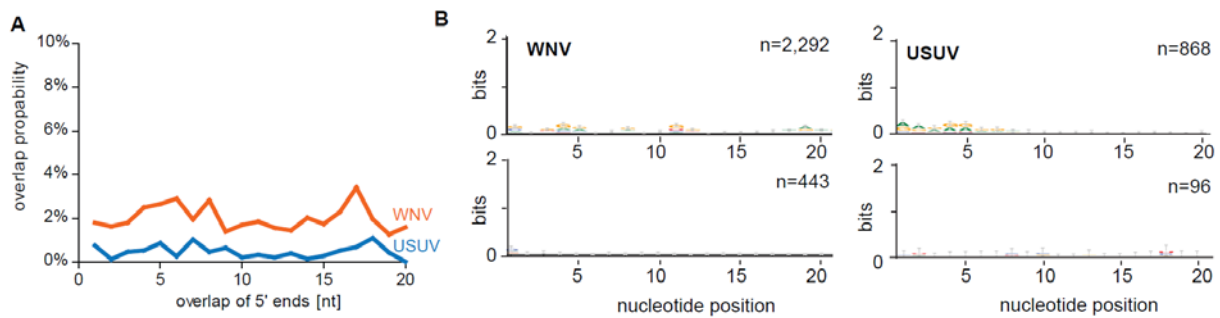


## Supplemental Material



### Supplemental Figure S1. WNV and USUV are not targeted by the piRNA pathway.

Ping-pong amplification of vpiRNA causes a specific sequence signature [33, 36, 37]. Specifically, vpiRNAs derived from the opposite strands tend to show a 10-nt overlap. Furthermore, the antisense vpiRNAs usually start with uridine, whereas vpiRNAs from the sense strand have a bias for adenine at position 10. **(A)** Neither WNV (orange line) nor USUV (blue line) derived 25-30 nt small RNAs show an enrichment for 10 nt overlap. The overlap probability has been determined as specified [29, 43]. **(B)** Nucleotide biases for 25-30 nts viral small RNAs from the positive strand (upper panels) and negative strands (lower panels) for the indicated virus. Neither WNV (left panels) or USUV (right panels) have a strong nucleotide bias that would be indicative for Ping-pong amplification. n is the number of reads used to create the sequence logo.