Supporting Information

Wu et al. 10.1073/pnas.0911353107

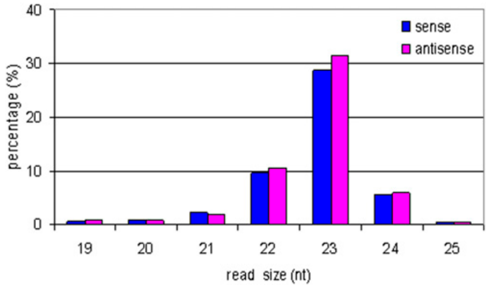

Fig. S1. Size distribution of positive- and negative-strand viral siRNAs cloned and sequenced from RNAi-defective 1 (*rde-1*) mutant *C. elegans* animals carrying a self-replicating recombinant RNA1 of FHV, FR1gfp [Lu R, Yigit E, Li WX, Ding SW (2009) An RIG-I-Like RNA helicase mediates antiviral RNAi downstream of viral siRNA biogenesis in Caenorhabditis elegans. PLoS Pathog 5:e1000286]. The *rde-1* encodes an AGO essential for dsRNA-induced RNAi and for the biogenesis of secondary siRNAs. The recombinant RNA1 of FHV does not express the RNAi suppressor B2 but expresses enhanced GFP in the position of B2.

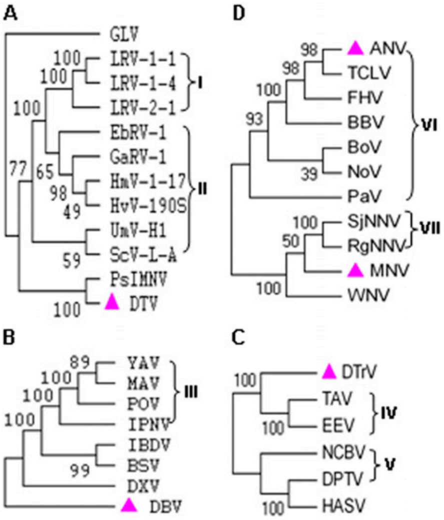

Fig. S2. Phylogenetic analysis of the five previously undescribed viruses in the viral family *Totiviridae* (*A*), *Birnaviridae* (*B*), *Tetraviridae* (*C*), and *Nodaviridae* (*D*) by the Clustal W method. Viral RdRP was used in *A*–*C*, whereas CP was used in *D*. Trees were calculated by using the neighbor-joining method, and the reliability of each branch was evaluated with bootstrap (1,000 times repeat). The viruses discovered in this study are indicated by a pink arrowhead. Viruses in the officially recognized genera are indicated as follows: (I) *Leishmaniavirus*, (II) *Totivirus*, (III) *Aquabirnavirus*, (IV) *Betatetravirus*, (V) *Omegatetravirus*, (VI) *Alphanodavirus*, and (VII) *Betanodavirus*. Abbreviations of virus names are as follows: Black beetle virus (BBV), Boolarra virus (BoV), Nodamura virus (NoV), Pariacato virus (PaV), Striped Jack nervous necrosis virus (SjNNV), Redspotted grouper nervous necrosis virus (RgNNV), and Wuhan nodavirus (WNV). *Giardia lamblia* virus (GLV), Leishmania RNA virus 1-1 (LRV-1-1), Leishmania RNA virus 1-4 (LRV-1-4), Leishmania RNA virus 2-1 (LRV-2-1), *Eimeria brunetti* RNA virus 1 (EbRV-1), *Gremmeniella abietina* RNA virus L1 (GaRV-1), *Helicobasidium mompa* no.17 virus (HmV-1-17), *Helminthosporium victoriae* virus 190S (HvV-190S), *Ustilago maydis* virus ^{1}H (UmV-^{1}H), *Saccharomyces cerevisiae* virus L-A (ScV-L-A), Yellowtail ascites virus (YAV), Marine birnavirus (MAV), *Paralichthys olivaceus* birnavirus (POV), Infectious pancreatic necrosis virus (IPNV), Blotched snakehead virus (BSV), *Thosea asigna* virus (TAV), *Euprosterna elaeasa* virus (EEV), *Nudaurelia capensis* beta virus (NCBV), *Dendrolimus punctatus* tetravirus (DPTV), and *Helicoverpa armigera* stunt virus (HASV).

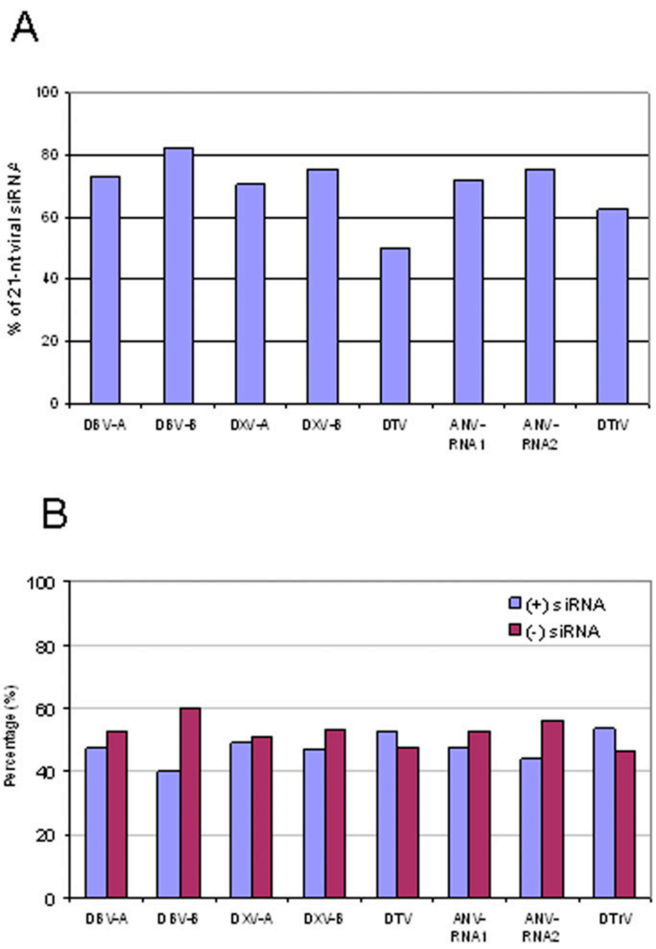

Fig. S3. Features of the 21-nt viral siRNAs sequenced from S2-GMR cells. (*A*) The 21-nt siRNA species was the most abundant of the viral small RNAs with a length of 18–28 nucleotides for all five viruses. (*B*) Approximately equal ratios of (+) and (–) 21-nt siRNAs for all five viruses detected.

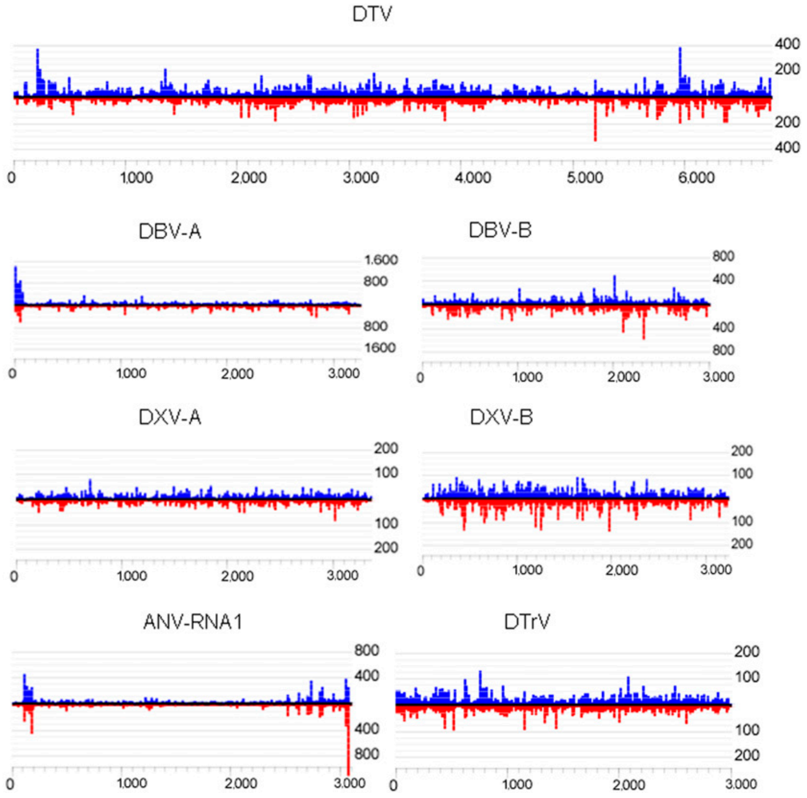

Fig. S4. Reads of perfectly matched 21-nt viral siRNAs sequenced from S2-GMR cells were plotted to the positive (blue) and negative (red) strands of each viral genome RNA segment (20-nt windows).

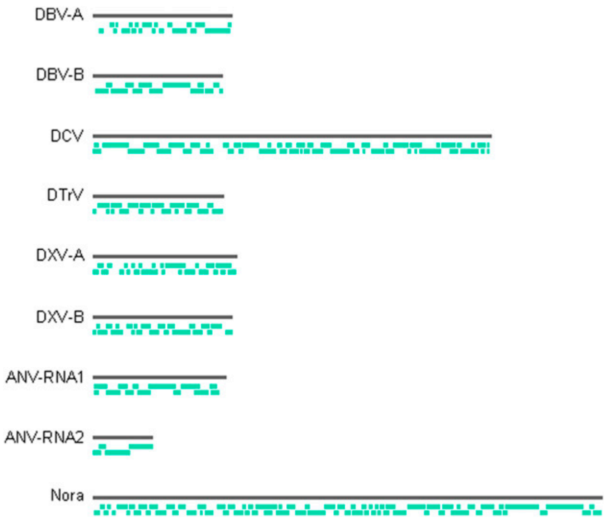

Fig. S5. Virus discovery in OSS cells by viral genome assembly from sequenced viral piRNAs of 25–30 nucleotides in length after viral siRNAs were removed.