Supporting Information

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SI Text

Replicating Viruses Are Less Sensitive to RNAi

As a complementary strategy to test whether replicating viruses are indeed less sensitive to RNAi compared with cellular transcripts, we tested antiviral silencing directed by siRNAs derived from artificial double-stranded RNA (dsRNA) in *drh-1* mutants. As illustrated in Fig. S10*A*, *gfp* dsRNA feeding before inducing FR1fp replication in *drh-1* mutant would produce *gfp*-specific siRNAs to target the FR1fp viral RNAs for RNAi. We reasoned that if replicating viruses are less sensitive to RNAi compared with cellular transcripts, FR1fp silencing should not occur in this setup. Indeed, as shown in Fig. S10*B*, similar to that in single or double mutants corresponding to *rde-1* and *rde-4*, the accumulation of FR1fp transcripts in *drh-1* null mutants was not affected by *gfp* dsRNA feeding, which completely destroyed the *gfp* transcripts. Please note, antiviral silencing remained defective in the *drh-1* null mutants despite production of FR1fp-derived siRNAs (1). Clearly, replicating viruses are sensitive to heterologous siRNAs, as the FR1fp replication can be markedly inhibited in worms feeding on the *gfp* dsRNA-producing food compared with that in worms feeding on regular OP50 food (Fig. S10*C*). These results, together with the results presented in Fig. 5, suggest that compared with cellular transcripts, replicating viruses are less sensitive to RNAi.

SI Materials and Methods

Genetics. The genotype for *drh-1* allele *ok3495* was confirmed by PCR, using primer pairs 5′TAATGCTTGTTGCTCATCCG3′ and 5′ ACACGCAACGCAGTTTTATT 3′. The genotype of *drh-3* mutants were confirmed through genomic DNA sequencing. The *drh-3* worm stock was maintained at 19 °C. When used for loss of function analyses, the *drh-3* mutants were incubated at 25 °C.

Plasmid Constructs and Transgenic Worms. The construction of FR1fp was described previously. The construct for *gfp* feeding RNAi was developed by inserting the full-length coding sequence of enhanced green fluorescence protein (GFP) into the multiple cloning site of L4440 (2). The HA-tagged Dicer-related RNA helicase 1 (DRH-1) variants were generated by fusion PCR, and the resulting constructs were confirmed by DNA sequencing.

Transgenic animals were generated through gonadal microinjection of plasmid constructs after standard protocol with minor modification. Briefly, the target plasmid constructs, each at a final concentration of 10 ng/μL, were mixed with the 2-log DNA ladder (New England Biolabs Inc.) at a final concentration of 120 ng/μL and the reporter plasmid Pmyo-2::mCherry at a final concentration of 40 ng/μL for injection into target worms. The generation of corresponding chromosomal integrants and an assay for viral replication were carried out as described previously (3).

Dicer-Related RNA Helicase 1 (DRH-1) Function Rescue Assay. Our DRH-1 function rescue assay used ectopic expression of the candidate gene in *drh-1* null mutants (*tm1329*) carrying a nuclear transgene corresponding to the FR1gfp replicon. The FR1gfp replicon was created by replacing the coding sequence of the B2 RNAi suppressor with GFP coding sequence (1). As a result, the replication of FR1gfp is suppressed in wild-type worms but will be restored in RNA silencing defective mutants, thereby producing green fluorescence with the intensity proportional to the viral replication level. The *sur-5* promoter was adopted for the expression of the DRH-1 domain variants, as it is known to be active in most of the worm cells (4). A construct that directs mCherry expression in worm pharynx was used as reporter construct in the DRH-1 function rescue assay. Our assay began with microinjection of a DRH-1 rescue construct into the gonad of *drh-1* mutants carrying the FR1gfp replicon, together with the mCherry construct, which was used as reporter for the DRH-1 transgenes. As most of the transgenic F1 worms generated through gonad injection randomly pass the transgenes, in the form of extrachromosomal array, onto only some of their next generations, those worms in the population that are free of the transgene and do not show red fluorescence in the pharynx serve as an internal negative control. Thus, on heat induction, which will initiate FR1gfp replication, green fluorescence will be produced in worms containing the extrachromosomal arrays corresponding to DRH-1 domains variants that are unable to rescue DRH-1 function.

1. 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(2): e1000286.
2. Timmons L, Fire A (1998) Specific interference by ingested dsRNA. *Nature* 395(6705):854.
3. Lu R, et al. (2005) Animal virus replication and RNAi-mediated antiviral silencing in Caenorhabditis elegans. *Nature* 436(7053):1040–1043.
4. Gu T, Orita S, Han M (1998) Caenorhabditis elegans SUR-5, a novel but conserved protein, negatively regulates LET-60 Ras activity during vulval induction. *Mol Cell Biol* 18(8):4556–4564.

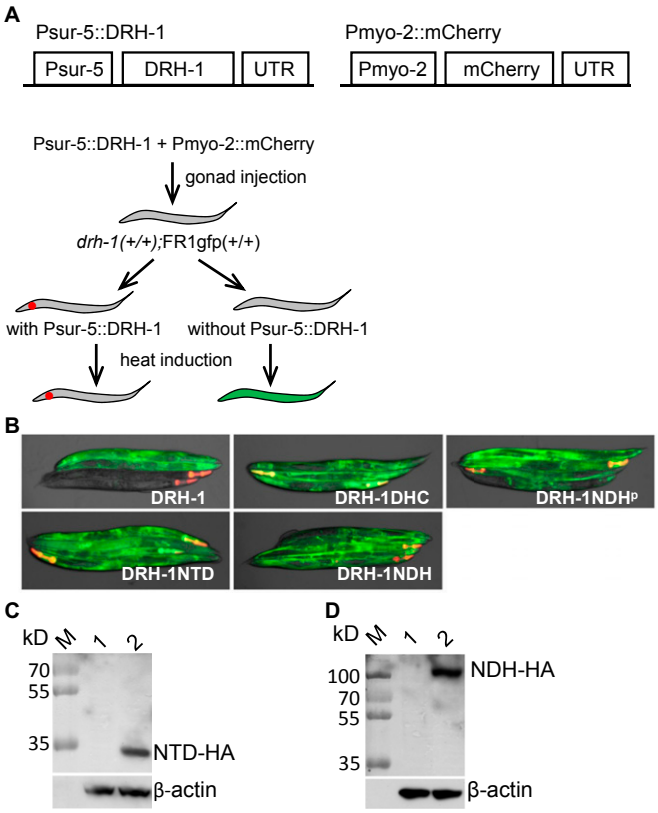

Fig. S1. Both the N-terminal domain and CTD of DRH-1 are indispensable for antiviral RNAi. (*A*, *Upper*) Schematic structure of the plasmid constructs Psur-5::DRH-1 and Pmyo-2::mCherry. Pmyo-2, the promoter for the endogenous gene *myo-2*; Psur-5, the promoter of the endogenous gene *sur-5*; UTR, the 3′ end untranslated region of the endogenous gene *unc-54*. (*Lower*) Schematic strategy of DRH-1 function rescue assay. Red dots indicate red fluorescence in the pharynx tissue. Green color represents green fluorescence produced by replicative FR1gfp. (*B*) Visualization of green fluorescence in *drh-1* mutants (*tm1329*) carrying the FR1gfp replicon transgene and the extrachromosomal arrays corresponding to wild-type DRH-1 or its derivatives, as indicated. Expression of mCherry in pharynx tissue directed by the *myo-2* promoter serves as visual mark of the transgenes. (*C* and *D*) Western blot detection of HA-tagged DRH-1 and its derivatives in corresponding transgenic worms. M, molecular weight references. 1, nontransgenic N2 worms; 2, N2 worms transgenic for HA-tagged DRH-1 or its derivatives as indicated. NDH-HA, HA-tagged DRH-1NDH; NTD-HA, HA-tagged DRH-1NTD. The expression of β-actin was detected and used as equal loading controls.

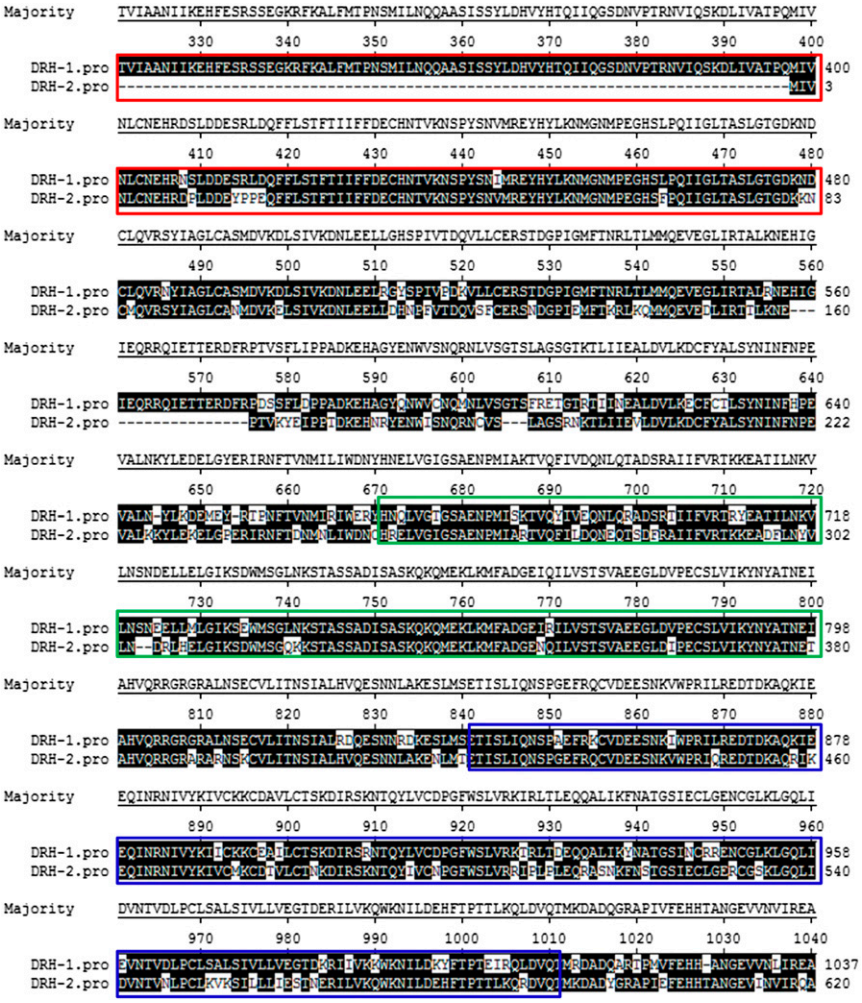

Fig. S2. DRH-1 and DRH-2 share high-level sequence homology. Shown here is the sequence alignment between DRH-2 and DRH-1, as generated by Clustal W. Sequence marked with a red box represents the conserved DEAD-box domain. Sequence marked with a green box represents the conserved helicase C-terminal domain. Sequence marked with a blue box represents the conserved C-terminal regulatory domain of retinoic acid inducible gene I (RIG-I).

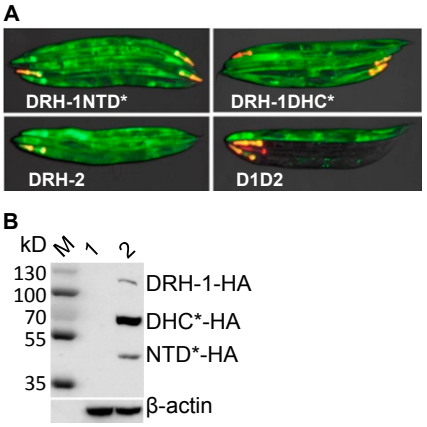

Fig. S3. The predicted domains of DRH-2 were functional in a fusion protein with the NTD of DRH-1. (*A*) Visualization of green fluorescence in *drh-1* mutants (*tm1329*) carrying both FR1gfp nuclear transgene and extrachromosomal arrays corresponding to wild-type DRH-1, putative DRH-2, or their domain variants, as indicated. See Fig. S1*B* for experimental details. (*B*) Western blot detection of HA-tagged DRH-1, DRH-1NTD*, and DRH-1DHC* in transgenic worms generated through coinjection of three plasmid constructs corresponding to HA-tagged DRH-1, DRH-1DHC*, and DRH-1NTD*, respectively. M, molecular weight references. 1, nontransgenic N2 worms; 2, N2 worms transgenic for HA-tagged DRH-1, DRH-1NTD*, and DRH-1DHC*. DHC*-HA, HA-tagged DRH-1DHC*; DRH-1-HA, HA-tagged DRH-1; NTD*-HA, HA-tagged DRH-1NTD*. The expression of β-actin was detected and used as equal loading controls.

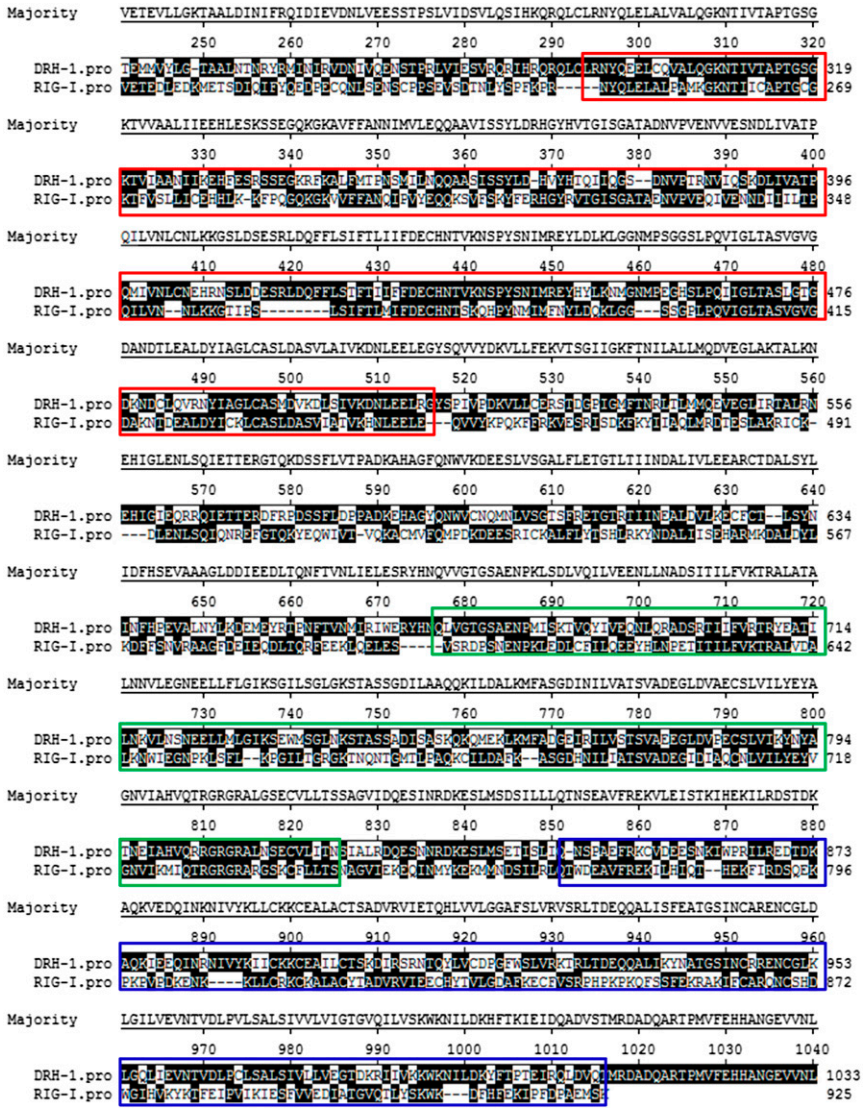

Fig. S4. DRH-1 and RIG-I share significant sequence homology within the RNA helicase domain, including the DEAD-box subdomain and the helicase C-terminal subdomain, and the CTD domain. Shown here is the sequence alignment between DRH-1 and RIG-I as generated by Clustal W. The sequence marked with a red box represents the conserved DEAD-box domain. The sequence marked with a green box represents the conserved helicase C-terminal. The sequence marked with a blue box represents the conserved C-terminal regulatory domain of RIG-I.

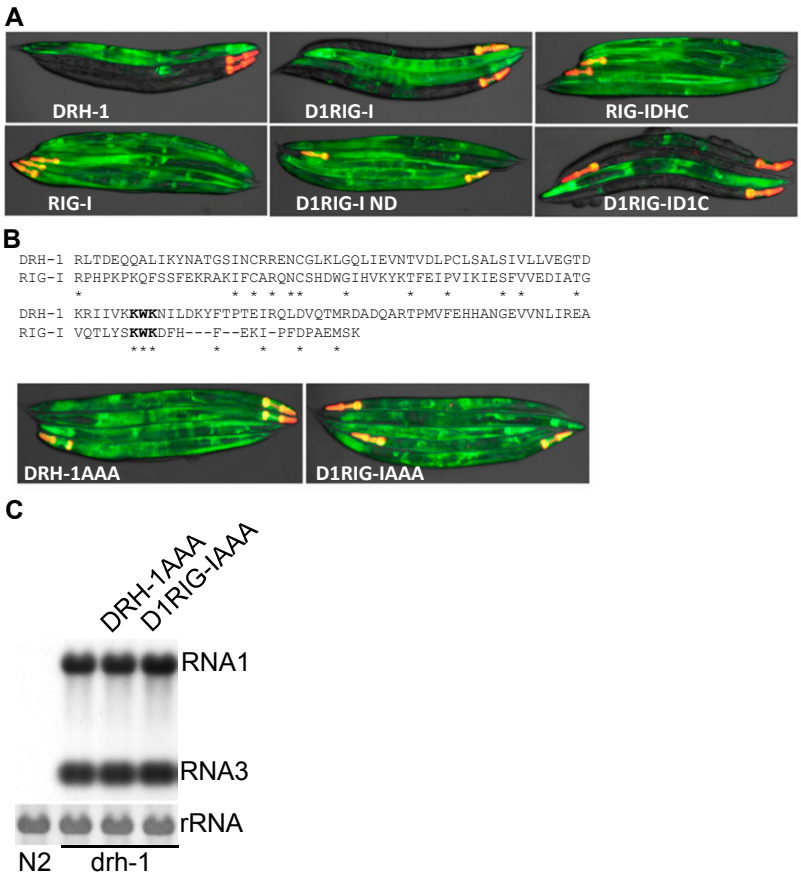

Fig. S5. The helicase and CTD domains of RIG-I were functional in a fusion protein with the NTD of DRH-1. (*A*) Visualization of green fluorescence in *drh-1* mutants carrying both FR1gfp replicon transgene and extrachromosomal arrays corresponding to wild-type RIG-I or its domain variants, as indicated. See Fig. S1*B* for experimental details. (*B*, *Upper*) Sequence alignment between the C-terminal sequences of DRH-1 and RIG-I. The KWK motifs are bold. (*Lower*) Visualization of green fluorescence in *drh-1* null mutants (*tm1329*) carrying both FR1gfp replicon transgene and extrachromosomal arrays corresponding to DRH-1AAA or D1RIG-IAAA. D1RIG-IAAA, a D1RIG-I variant that contains the K982A, W983A, and K984A point mutations; DRH-1AAA, a DRH-1 variant that contains K988A, W989A, and K990A point mutations. (*C*) Accumulation of FR1gfp transcripts detected by Northern blot in *drh-1* mutants carrying heat-inducible transgenes corresponding to DRH-1AAA or D1RIG-IAAA.

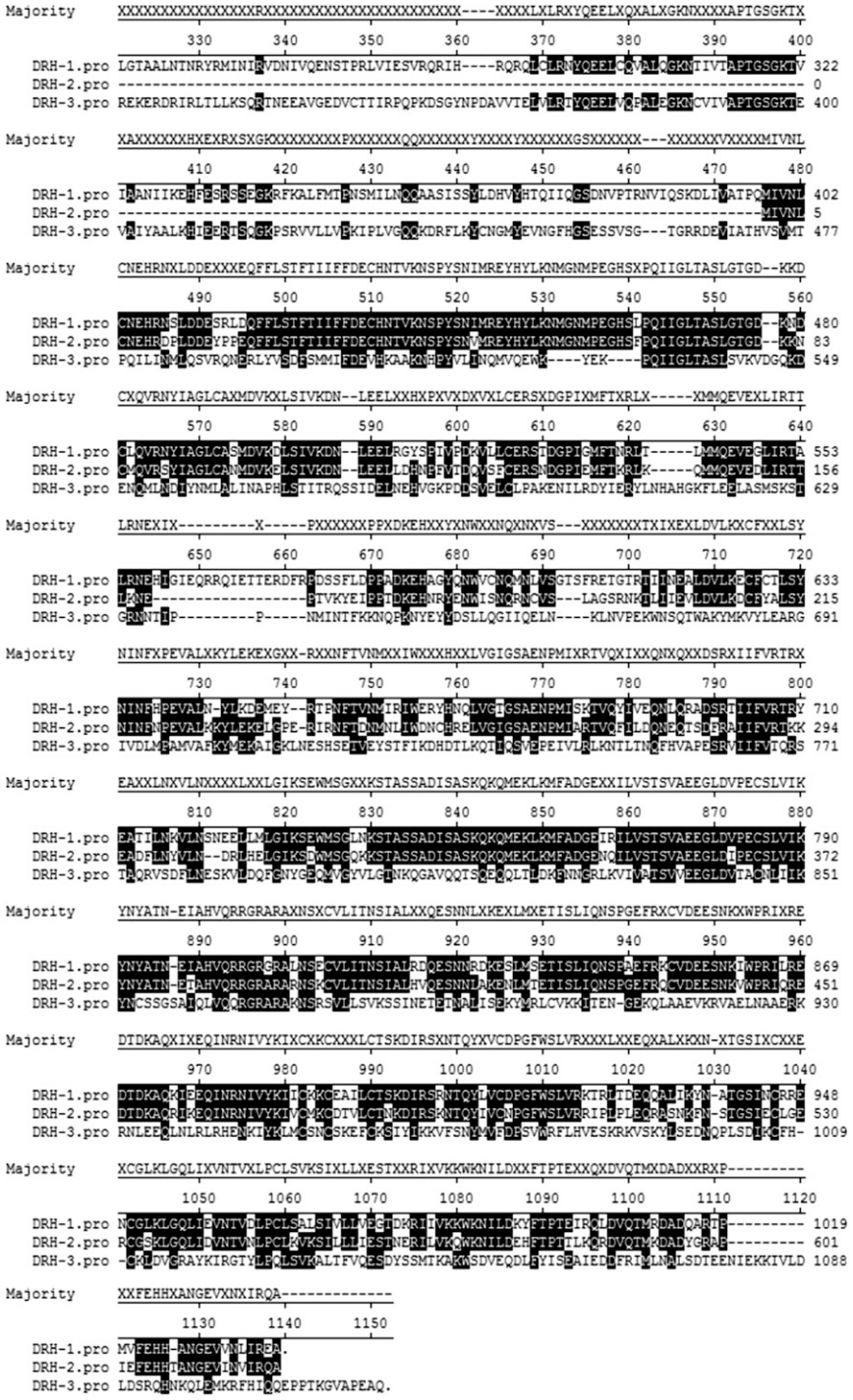

Fig. S6. DRH-3 contains more divergent sequences compared with DRH-1 and DRH-2. Shown here is the sequence comparison between DRH-1, DRH-2, and DRH-3. Clustal W was used for the sequence alignment.

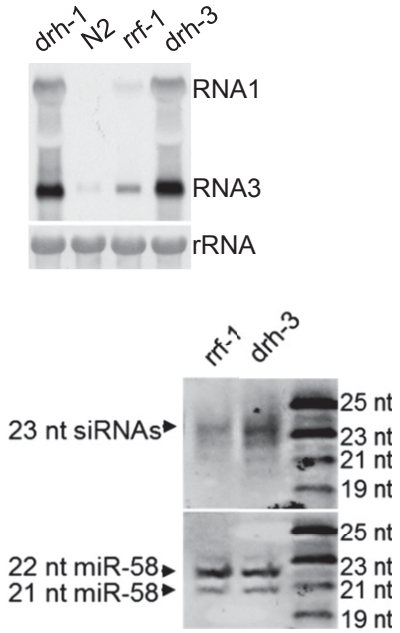

Fig. S7. DRH-3 regulates antiviral RNAi by a mechanism distinct from DRH-1. (*Upper*) Accumulation of FR1gfp transcripts detected by Northern blot in wild-type N2 worms and genetic mutants defective in *drh-1*, *rrf-1*, or *drh-3*. (*Lower*) Accumulation of FR1gfp-derived siRNAs detected by Northern blot in *rrf-1* and *drh-3* mutants. The accumulation of miR-58 was detected by Northern blot and used as both a size reference and an equal loading control.

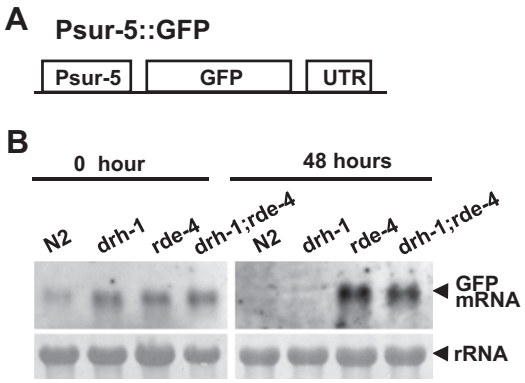

Fig. S8. DRH-1 is not required for long dsRNA-triggered RNAi. (*A*) Structure of the Psur-5::GFP transgene. Psur-5, the promoter of the worm gene *sur-5*. GFP, the coding sequence of enhanced green fluorescence protein; UTR, the 3′ end untranslated region of the worm gene *unc-54*. (*B*) Northern blot detection of *gfp* transcripts in response to *gfp* dsRNA ingestion in different worm strains as indicated. The accumulation of *gfp* transcripts was detected at 0 h (*Left*) and 48 h (*Right*) after *gfp* dsRNA ingestion as indicated. The full-length *gfp* cDNA was used to prepare probes for hybridization. Methylene blue-stained ribosomal RNA serves as an equal loading control.

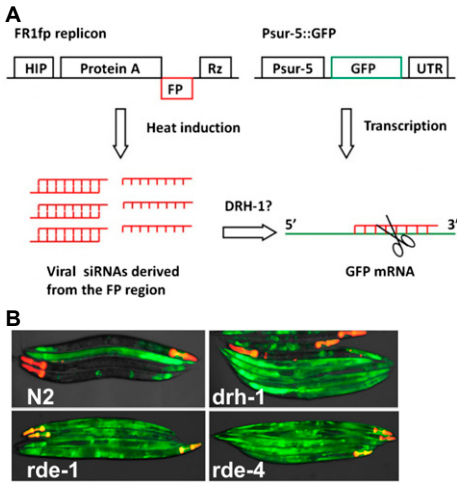

Fig. S9. DRH-1 is dispensable for virus-triggered RNAi targeting cellular transcripts. (*A*) Schematic of experiment strategy used to test whether DRH-1 is required for the silencing of cellular transcripts mediated by viral siRNAs. FP, the 3′ end half of the GFP coding sequence; HIP, heat-inducible promoter; Protein A, the replicase of Flock house virus; Rz, self-cleaving ribozyme sequence derived from hepatitis D virus, which functions to remove all nonviral sequence at the 3′ end of the FR1fp primary transcripts. (*B*) Visualization of *gfp* silencing triggered by replicating FR1fp in different genetic backgrounds, as indicated. All worm strains carry the same Psur-5::GFP transgene and FR1fp replicon transgene, which uses mCherry expressed in pharynx as a visual mark.

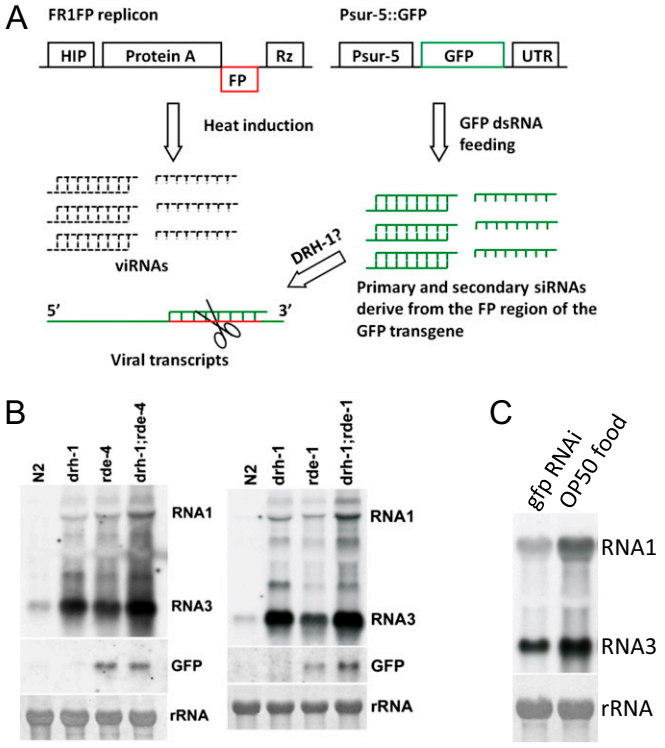

Fig. S10. Replicating viruses are less sensitive to long dsRNA-triggered RNAi compared with cellular transcripts. (*A*) Schematic of the strategy used to test whether replicating viruses are less sensitive to long dsRNA-triggered RNAi compared with cellular transcripts. (*B*) Accumulation of FR1fp transcripts in response to heat induction in worm strains as indicated. All worm strains used in this test contain the same Psur-5::GFP and FR1fp transgenes and have been fed on HT115 food expressing the *gfp* dsRNA. The FR1fp transcripts were detected using probes derived from the FP region of FR1fp. The GFP transcripts were detected using probes corresponding to the 5′ half of the GFP coding sequence that does not overlap with the FP region of FR1fp. (*C*) Heterologous siRNAs are capable of mediating virus silencing in *Caenorhabditis elegans*. Transgenic N2 strain carrying the Psur-5::GFP and the FR1fp nuclear transgenes was used in this test. Shown here is the accumulation of FR1fp transcripts in response to heat induction after the worms have been fed on OP50 food or HT115 food expressing *gfp* dsRNA.