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**Supplemental Material**: *C. elegans* ADARs antagonize silencing of cellular dsRNAs by the antiviral RNA interference pathway

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**Supplemental Inventory:**

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**Supplementary References** – References in Supplemental Material that are not included in primary References section.

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**Supplemental Fig. S14** – Cas9 targeting schemes used to generate \( \Delta EER \) mutations.

**Supplemental Fig. S15** – Additional data on bursting and brood size phenotypes of \( adr-1;adr-2;rrf-3 \) triple mutant lines, not shown in Figure 4.

**Supplemental Fig. S16** – Analyses of EER properties for three group of EAGs.

**Supplemental Fig. S17** – Differential expression analysis and GSEA of \( adr-1(uu49);adr-2(uu28);rrf-3(uu56);rde-1(uu51) \) and \( adr-1(uu49);adr-2(uu28);rrf-3(uu56);rde-4(uu53) \) quadruple mutant embryos compared to \( adr-1(uu49);adr-2(uu28);rrf-3(uu56) \) triple mutants.

**Supplemental Table S1** – Strains used in this study

**Supplemental Table S2** – Primers used in qRT-PCR analyses.

**Supplemental Table S3** – Primers used for sgRNA synthesis and genotyping of new mutations generated for this study.

**Supplemental Table S4** – Mutations generated by CRISPR protocols for this study.

**Supplemental File S1** – List of EERs, with genomic properties and siRNA abundance measurements.

**Supplemental File S2** – Lists of circRNAs, ADAR-modulated RNA loci, and 21U/piRNA loci that overlap EERs.

**Supplemental File S3** – Table describing EER-EAG expression correlation across developmental stages.
Supplemental File S4 – List of EAGs with associated EER-23H siRNA abundance and expression data.

Supplemental File S5 – Differential gene expression in \textit{adr-1(uu49);adr-2(uu28);rrf-3(uu56)} triple mutant embryos and related strains.

Supplemental File S6 – EAG enriched GO categories.

Supplemental File S7 – Gene set enrichment analysis comparing \textit{adr-1(uu49);adr-2(uu28);rrf-3(uu56);rde-1(uu51)} and \textit{adr-1(uu49);adr-2(uu28);rrf-3(uu56);rde-4(uu53)} quadruple mutants to \textit{adr-1(uu49);adr-2(uu28);rrf-3(uu56)} triple mutants.

Supplemental Materials and Methods

\textit{EER annotation}

Tables of annotated \textit{C. elegans} (ce10/WS220) protein coding genes, introns, 3’ UTRs, 5’ UTRs, ncRNAs, and pseudogenes and their genomic coordinates were downloaded from UCSC Genome Browser in .bed format (https://genome.ucsc.edu/). We merged ncRNA and pseudogene annotations into a single “ncRNA” annotation. The bedtools2 (https://github.com/arq5x/bedtools2) application annotateBed defined the number of overlapping bases shared between EERs and annotated features. To annotate EERs within or further than 1kb from genes, we combined annotations of protein-coding genes and ncRNAs, and then extended the start and stop coordinates 1000 nt away from gene boundaries.

\textit{EER overlap with other annotated regions}

The USeq application IntersectRegions was used to overlap EERs with .bed files for \textit{C. elegans} circRNAs (circBase.org) or ADAR-modulated RNA loci (from Wu et al. 2011) and calculate enrichment significance by \(X^2\) approximation. A .bed file of genomic regions covered
by at least 5 reads in combined developmental RNAseq datasets (i.e. genomic space with sufficient coverage to define EERs) was used with parameters “-r” and “-n 10000” to make 10,000 randomized expressed regions used to approximate the likelihood of random intersection with circRNAs or ADAR-modulated RNA loci.

**Annotation of EER-associated genes (EAGs)**

EAGs were defined as genes containing one or more EERs within the gene on the same strand or else the closest gene within 1kb of an EER on the same strand. Using gene tables for protein coding genes and pseudogenes downloaded from UCSC (see Methods), we used the bedtools application intersectBed to identify genes overlapping EERs, and the USeq application FindNeighboringGenes, to find the closest gene to EERs within 1kb.

**Small RNAseq data preparation and analysis**

Mixed-stage 5’P-dependent small RNAseq datasets were downloaded from Gene Expression Omnibus record GSE28888. Small RNAseq 5’P-independent datasets of wildtype, adr-1(gv6);adr-2(gv42), adr-1(gv6);adr-2(gv42);rde-1(ne219), and adr-1(gv6);adr-2(gv42);rde-4(ne299) embryos and L4 larvae from Wu et al. (2011) were provided by Diane Wu and Andrew Fire. Small RNAseq reads were aligned to the *C. elegans* CE10/WS220 genome with Novoalign, parameters: -o SAM -a ATCTCGTATGCGTCTTCTGCTTG -r All). We used the USeq application SamTranscriptomeParser (parameters -n 1000000 –a 30) to parse alignments into bam files. For uniquely mapping reads, we parsed reads with the samtools application view (parameters –F 4 –q 10). Small RNA libraries were normalized to the total number of mapped reads in each alignment file. For Supplemental Fig. S11, reads mapping sense or antisense to EERs were extracted with the bedtools2 application intersectBed
Reich et al. (https://github.com/arq5x/bedtools2) and analyzed by custom bash scripts. Repetitively mapped reads were used for relative siRNA abundance plots shown in Supplemental Fig. S12.

**Viability assays**

To quantify development of *adr-1;adr-2* mutants, we modified the bursting assay described in Materials and Methods as follows. After adults were removed and eggs laid on Day 0 were counted, embryos were allowed to hatch and mature for 24 hrs, after which unhatched eggs were counted. 64-70 hrs after egg lay, worms on the plate were scored and counted as L3-adults or L1-L2 larvae. If the sum of unhatched eggs, L1-L2 larvae, and L3-adults was less than the initial egg count, the difference was added to the subset of unhatched eggs. All steps and incubations were performed at 20°C.

**Recombinant Cas9 purification**

To express Cas9 in *E. coli*, we used a human codon-optimized *Streptococcus pyogenes* Cas9 gene with N-terminal HA-SV40, NLS, and TEV protease site cloned into pET28b with N- and C-terminal 6xHis tags (a generous gift of Dr. Jin-Soo Kim, Seoul National University). pET28b-Cas9-N3T (KanR) was transformed into BL21(DE3) cells. A 500 mL culture of LB-Kan (50 mg/L Kan) was inoculated with a 5 mL overnight culture and grown at 37°C to an OD of 0.4-0.5. We added IPTG to 0.5 mM and induced Cas9 expression for 4 hrs at 25°C.

Cells were pelleted, resuspended in lysis buffer (50 mM NaH₂PO₄ pH 8.0, 10 mM imidazole, 300 mM NaCl, 10% glycerol, 1 mM β-mercaptoethanol), and lysed by homogenization followed by sonication. Clarified lysate was batch bound to Qiagen Ni-NTA agarose resin for 45 min at 4°C. Resin was washed 3x in 1 M NaCl, 1x in 300 mM NaCl, and protein was eluted with 125 mM imidazole. Protein was dialyzed into 20 mM HEPES pH 7.5,
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150 mM KCl, 1 mM DTT, 10% glycerol, and concentrated to 8 mg/mL. Protein was stored long term in 25% glycerol at -80°C.

sgRNA design and synthesis

We used the Broad Institute sgRNA Design Tool (http://portals.broadinstitute.org/gpp/public/analysis-tools/sgrna-design-v1) to identify S. pyogenes Cas9 target sites in regions of interest. We chose guide sequences with a 5’G (to facilitate T7 RNA polymerase in vitro transcription) scoring >0.1 and BLASTed them against the C. elegans genome, eliminating candidates with <4 mismatches that contained the NGG protospacer adjacent motif needed for cleavage.

For each CRISPR/Cas9 mutation, we designed two guide RNAs. For whole-gene disruptions, we selected sgRNAs situated ~1 kb or more apart to induce large deletions. To delete the EERs in ccb-1 and egl-8, we chose guide RNAs to target Cas9 to sequences flanking each EER within the intron far enough from 5’ and 3’ splice sites and consensus splicing signals to prevent disruption. The EER-containing intron of efa-6 was too repetitive to delete without likely off-target mutations, so we directed Cas9 to cleave within the efa-6 exonic sequences flanking the intron, and provided a single-stranded DNA template to replace the entire intron with a 60 nt synthetic intron lacking the predicted double-stranded structure (Materials and Methods; Supplemental Fig. S14C; Supplemental Table S3).

Primers of the form TAATACGACTCACTATA-N_{19-22}-GTTTTAGAGCTAGAAATAG, where N_{19-22} represented the guide target sequence, were used with the reverse primer CRISPR_sgRNA_R1 (Supplemental Table S3) to amplify the sgRNA transcription template from the plasmid DR274 (Hwang et al. 2013). In vitro transcription reactions were incubated overnight at 37°C in 100 μl containing 10 μg PCR template, 20 U T7 RNA polymerase, 3 mM
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ATP, 3 mM UTP, 3 mM CTP, 3 mM GTP, 40 mM Tris pH 7.8, 2 mM spermidine, 15 mM DTT, and 15 mM MgCl₂. Reactions were DNase-treated 1 hr at 37°C, and RNA was extracted in phenol-chloroform, ethanol precipitated, and resuspended in 10 mM Tris, 1 mM EDTA to a concentration of ~4 μg/μl.

**Imaging GFP::NRDE-3 localization**

L3 worms of each genotype were anesthetized in M9 buffer containing 5 mM levamisole and mounted on 2% agarose pads. Images in Figure 4A and Supplemental Fig. S15A were taken using a QImaging Retiga 2000R camera on a fluorescent Zeiss Axioskop 2 MOT microscope.

**Gene Ontology (GO) analysis**

A background list of genes was determined by calculating all genes overlapping a .bed file of genomic regions covered by at least 5 reads in our developmental RNAseq experiment (i.e. all genes with sufficient coverage to define an EER). The lists of background genes and EAGs (see Supplemental File S3) were provided to the GOMiner web interface (https://discover.nci.nih.gov/gominer/htgm.jsp) to calculate enriched GO categories with a False Discovery Rate < 0.05 (Zeeberg *et al.* 2003; Zeeberg *et al.* 2005).

**Gene set enrichment analysis (GSEA)**

Gene sets for 877 biological process high quality GO annotations were downloaded from http://www.go2msig.org/cgi-bin/prebuilt.cgi?taxid=6239, to which were added gene sets of 298 Orsay virus-induced genes and 231 EAGs significantly downregulated in *adr-1;adr-2;rrf-3*
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mutants relative to wildtype (Fig. 5B). GSEA software was downloaded from http://software.broadinstitute.org/gsea/index.jsp. Poly(A)+ RNAseq rlog-normalized expression values outputted by DESeq2 were used for analysis. Default parameters were used for GSEA, with the exception that we used permutation type “gene_sets,” since “phenotype” permutation recommends seven samples per condition. Note that we also included the full set of 965 EAGs, but GSEA was unable to calculate normalized enrichment scores for a gene set this large.

**Supplementary References**

Hwang WY, Fu Y, Reyon D, Maeder ML, Tsai SQ, Sander JD, Peterson RT, Yeh JR, Joung JK. 2013. Efficient genome editing in zebrafish using a CRISPR-Cas system. *Nat Biotechnol* **31**: 227-229.

Zeeberg BR, Feng W, Wang G, Wang MD, Fojo AT, Sunshine M, Narasimhan S, Kane DW, Reinhold WC, Lababidi S et al. 2003. GoMiner: a resource for biological interpretation of genomic and proteomic data. *Genome Biol* **4**: R28.

Zeeberg BR, Qin H, Narasimhan S, Sunshine M, Cao H, Kane DW, Reimers M, Stephens RM, Bryant D, Burt SK et al. 2005. High-Throughput GoMiner, an 'industrial-strength' integrative gene ontology tool for interpretation of multiple-microarray experiments, with application to studies of Common Variable Immune Deficiency (CVID). *BMC Bioinformatics* **6**: 168.
**Supplemental Figure S1.** Model of *C. elegans* Dicer-dependent siRNA pathways.

**LEFT:** The antiviral RNAi pathway (Ashe et al. 2013; Guo et al. 2013) acts on viral dsRNA produced during viral genome replication by a viral RNA-dependent RNA polymerase (RdRP; sense strand, blue; antisense strand, red). In Step 2 of this pathway, DCR-1, the dsRNA binding protein RDE-4, and the Dicer-related helicase DRH-1, act together to produce double-stranded siRNAs with 5' phosphates and an overhang at the 3' end, as expected for a Dicer cleavage product. Each strand of the viral siRNA is 23 nts with a 5' nucleotide that is more often A, C, or U than G (23H siRNAs, see main text). Primary 23H siRNAs are loaded into the Argonaute RDE-1 to promote the production of secondary siRNAs (Step 4) by the *C. elegans* RdRP RRF-1. Secondary siRNAs are predominantly 22 nt long, contain a 5’ triphosphorylated guanosine (22G siRNAs), and are antisense to target RNAs. Factors required in Steps 4 and 5 of the antiviral pathway are inferred to be the same as for the 26G pathway, but roles for some
factors have not been tested (noted by asterisks). RIGHT: In a poorly understood manner, certain mRNAs are targeted for silencing by the 26G endogenous siRNA pathway (Vasale et al. 2010; Thivierge et al. 2012; Billi et al. 2014). In contrast to the antiviral pathway, Steps 1 and 2 likely occur concomitantly (Blumenfeld and Jose, 2016), with synthesis of dsRNA by the RdRP RRF-3 followed closely by its cleavage by DCR-1. RDE-4, DRH-3 and ERI-1, 3 and 5 are also required for Steps 1 and 2 to produce 26 nt siRNAs, which have a 5’ monophosphorylated guanosine (26G siRNAs) and are primarily antisense to the mRNA. 26G siRNAs are loaded into tissue-specific Argonautes (ERGO-1 or ALG-3 or ALG-4, see main text) that promote production of secondary 22G siRNAs, by a complex containing the RdRP RRF-1 or EGO-1 (in the soma and germline, respectively). For both pathways, loading and silencing (Step 5) involves additional Argonautes (WAGO and SAGO).
Supplemental Figure S2. Flowchart outlining the bioinformatics pipeline used to identify Editing-Enhanced Regions, adapted from Whipple et al. (2015). Additional details are provided in Materials and Methods.
Supplemental Figure S3. Tukey boxplots show expression in each developmental stage of (A) embryo-specific EERs, (B) early larval-specific EERs, (C) late larval-specific EERs, and (D) young adult-specific EERs. For all panels, expression values are calculated from input RNAseq samples only. Note for all plots that quartile boundaries with value of 0 are set at 0.1 FPKM due to the logarithmic scale.
**Supplemental Figure S4.** Genomic locations of all 1523 EERs. Vertical black lines indicate EER locations, while the total number of EERs on each chromosome are shown to the right.

| Chromosome | #EERs |
|------------|-------|
| Chr I      | 443   |
| Chr II     | 191   |
| Chr III    | 322   |
| Chr IV     | 273   |
| Chr V      | 244   |
| Chr X      | 50    |
Supplemental Figure S5. EER repeat content. (A) Classification of EER sequences by the % of EER nucleotides that overlapped annotated RepeatMasker repetitive sequences. (B) Classification of the ten most abundant transposon classes represented in EER sequences.
Supplemental Figure S6. EER genomic annotation and predicted structure stabilities. (A) The percent of nucleotides from random regions or EERs from each set of developmental stages that overlap specified genomic annotations. (B) UNAFold-predicted folding free energies ($\Delta G$) of sequences from EERs (blue) or length-matched expressed random regions (red) plotted against length. A slope test on the linear regressions of each set of regions shows they are significantly different ($p<0.0001$).
Supplemental Figure S7. EERs display expression patterns distinct from random regions. (A) Distributions of EER abundance and length-matched expressed random region (Random) abundance in each stage of development measured. Solid black lines report median abundance. (B-D) Tukey boxplots show log₂(abundance fold-change) of all EERs and random regions in the transitions between each developmental stage and the subsequent stage. ns: not significant; ****: p < 0.0001; Mann-Whitney U test.
Supplemental Figure S8. EER editing events per stage. Plot shows the average number of A-to-G changes observed in reads covering all EER editing sites (edited >1% and <99%) in each developmental stage for each treatment (three biological replicates each). Error bars reflect standard deviation of three RNAseq replicates; *: $p<0.05$, **: $p<0.01$; Student’s T-test.
Supplemental Figure S9. Viability and development of progeny in three independent *adr-1;adr-2* mutant strains. Shown are the fraction of embryos laid on Day 0 that were found on Day 3 as L3-adults (green), L1-L2 larvae (blue), or which were lost or failed to hatch (red). Error bars represent standard deviation over n=3 assays; **: \( p<0.01 \); ****: \( p<0.0001 \), significance determined by two-way ANOVA with Tukey’s multiple comparisons correction, asterisk colors indicate the categories compared between genotypes.
Supplemental Figure S10. EERs are more abundant than ADAR-modulated RNA loci in wildtype animals. Tukey boxplots show distributions of ADAR-modulated RNA loci (ARL) and EER abundance (FPKM) in input RNAseq samples from each developmental stage (E: Early, L: Late, Y: Young). ****: $p < 0.0001$; Mann-Whitney U test.
**Supplemental Figure S11.** EER-22G siRNAs in wildtype and *adr-1(gv6);adr-2(gv42)* mutant animals. Sense (positive) and antisense (negative) EER-mapped siRNAs in wildtype and *adr-1(gv6);adr-2(gv42)* embryo and L4 stage 5’P-independent small RNAseq samples from Wu et al. (2011) are plotted by length (nt) and 5’ nt (see color key in top right panels). Small RNA reads were aligned either to allow reads to map in multiple locations (repetitively mapped) or to only include reads that mapped to a single location (uniquely mapped).
5’P-independent siRNAs antisense to EERs

Embryo

\[
\log_2(\text{adr-1,adr-2-siRNAs})
\]

\[
\log_2(\text{adr-1,adr-2-siRNAs})
\]

L4

\[
\log_2(\text{adr-1,adr-2-siRNAs})
\]

\[
\log_2(\text{adr-1,adr-2-siRNAs})
\]

Embryo L4

\[
\log_2(\text{adr-1,adr-2-siRNAs})
\]

\[
\log_2(\text{adr-1,adr-2-siRNAs})
\]
Supplemental Figure S12. EER-22G siRNAs require rde-1 and rde-4 for embryonic accumulation in adr-1;adr-2 double mutant animals. In all panels, abundance of siRNAs antisense to 1523 EERs (black line) or control random regions (dotted grey line) are plotted as log$_2$ ratio of siRNA reads in one genotype over siRNA reads in the control genotype. For top panels, EER-22G siRNA enrichment in adr-1(gv6);adr-2(gv42) double mutants are plotted relative to wildtype in (A) embryo or (B) L4 larval stages. In middle panels, EER-22G siRNAs in adr-1(gv6);adr-2(gv42);rde-1(ne219) triple mutants are plotted relative to adr-1(gv6);adr-2(gv42) double mutants in (C) embryo or (D) L4 larval stages. In the two bottom panels, EER-22G siRNA abundance in adr-1(gv6);adr-2(gv42);rde-4(ne299) triple mutants is plotted relative to adr-1(gv6);adr-2(gv42) double mutants in (E) embryo or (F) L4 larval stages.
Supplemental Figure S13. EAG expression is decreased in *adr-1;adr-2* double mutant strains. Plots show EAG expression, determined by qRT-PCR, in three independent sets of *adr-1;adr-2* deletions. Each EAG was normalized to the geometric mean of four control genes (*y45f10d.4, cdc-42, pmp-3*, and *ama-1*). Plot shows mean expression relative to the expression of the wildtype strain in each experiment (n=8); error bars represent SD.
Supplemental Figure S14. Cas9 targeting schemes used to generate three ΔEER mutations. Genome browser views of (A) ccb-1, (B) egl-8, and (C) efa-6 are shown with the location of EERs (green) in each gene and the editing frequency (orange) of edited adenosines in all developmental stages combined. Regions targeted for cleavage by Cas9 are marked with arrows. For (A) and (B), deleted sequences are shown in red. A schematic of the HDR template and recombination pattern (red crossed lines) are shown for efa-6(ΔEER) in (C). See Supplemental Table S4 for specific information on each mutation.
Supplemental Figure S15. Synthetic phenotypes of adr-1;adr-2;rrf-3 mutants are consistent across multiple alleles and two assays. (A) A representative burst $adr-1(uu49);adr-2(uu28);rrf-3(uu56)$ adult. Scale bar = 100 μm. (B) Bursting assay of $adr-1;adr-2;rrf-3$ triple mutant strains and parent strains using multiple independently-derived deletions of each gene. Allele designations for each gene are listed in the order of the genes listed below them (i.e. the first listed allele corresponds to the first listed gene below it). See Supplemental Table S4 for specific information on $uu$ alleles; $n \geq 6$ assays. (C) Brood sizes of the lines used in (B); $n \geq 6$ assays. (D) Brood sizes of $adr-1;adr-2;rrf-3$ rescue strains characterized in Fig. 5D; $n \geq 6$ assays. Individual broods are shown as dots. In all panels, error bars represent SD.
Supplemental Figure S16. Characteristics of EERs associated with misregulated EAGs. (A) The number of introns or UTRs that overlap EERs in each gene (EER features; gray circles) are plotted for EAG categories shown in Fig. 5C-E. Note that we counted strand-specific unannotated regions <1kb from a gene as a UTR, but only if an annotated UTR did not also overlap an EER. Horizontal lines plot the average #EER features per gene and error bars show SD. Mann-Whitney U test: **, p < 0.01; ***, p < 0.001. (B) Tukey boxplots show the lengths of EERs associated with EAGs in each category. Mann-Whitney U test: *, p < 0.05. (C) Annotation of EER nucleotides associated with EAGs in each category.
Supplemental Figure S17. EAG and Orsay virus-induced gene misregulation in *adr-1(uu49);adr-2(uu28);rrf-3(uu56)* triple mutants partly depends on *rde-1* and *rde-4*. Volcano plots show the fold change and significance of gene expression changes in (A) *adr-1(uu49);adr-2(uu28);rrf-3(uu56);rde-1(uu51)* quadruple mutant embryos and (B) *adr-1(uu49);adr-2(uu28);rrf-3(uu56);rde-4(uu53)* quadruple mutant embryos relative to *adr-1(uu49);adr-2(uu28);rrf-3(uu56)* triple mutant embryos. Since the vast majority of gene expression changes had $-10\log_{10}(\text{adjusted p-value})$ close to 0, most genes are not shown. Horizontal dotted line represents adjusted p-value cutoff of 0.05, where genes above this line were considered significantly differentially expressed. GSEA enrichment plots for 231 EAGs downregulated in *adr-1;adr-2;rrf-3* to wildtype show expression enrichment in (C) *adr-1;adr-2;rrf-3;rde-1* and (D) *adr-1;adr-2;rrf-3;rde-4* embryos compared to *adr-1;adr-2;rrf-3*. Vertical black lines (center of each plot) indicate the position of EAGs in a gene list sorted highest to lowest by expression in *adr-1;adr-2;rrf-3;rde-1* (or *adr-1;adr-2;rrf-3;rde-4*) relative to expression in *adr-1;adr-2;rrf-3*, with the most upregulated genes to the left and most downregulated genes to the right. Green traces show the enrichment score, described in Subramanian *et al.* (2005), calculated for the EAG gene set. (E-F) Orsay virus-induced gene enrichment plots are shown for the same genotype comparisons as in (C-D).
Supplemental Table S1. Strains used in this study. Citations are listed in the References section of the main text.

| Strain | Genotype | Citation |
|--------|----------|----------|
| Bristol N2 | Wildtype | (Brenner 1974) |
| BB4    | adr-1(gv6);adr-2(gv42) | (Tonkin et al. 2002) |
| BB21   | adr-1(tm668);adr-2(ok735) | (Hundley et al. 2008) |
| BB204  | ccb-1(uu35) | This study |
| BB234  | efa-6(uu46; Δefa-6 intron 8 + 60 nt syntron) | This study |
| BB235  | egl-8(uu47) | This study |
| BB239  | adr-1(uu49);adr-2(uu28) | This study |
| BB242  | adr-1(uu49);adr-2(uu28);rde-1(uu51) | This study |
| BB244  | adr-1(uu49);adr-2(uu28);rde-4(uu53) | This study |
| BB245  | adr-1(uu49);adr-2(uu28);ccb-1(uu35) | This study |
| BB246  | adr-1(uu49);adr-2(uu28);efa-6(uu46; Δefa-6 intron 8 + 60 nt syntron) | This study |
| BB247  | adr-1(uu49);adr-2(uu28);egl-8(uu47) | This study |
| BB250  | rrf-3(uu56) | This study |
| BB251  | rrf-3(uu57) | This study |
| BB259  | adr-1(uu49);adr-2(uu28);ggIs1[nrde-3p::3xFlag::gfp::nrde-3 CDS + unc-119(+)] | This study |
| BB260  | adr-1(uu49);adr-2(uu28);rrf-3(pk1426) | This study |
| BB261  | adr-1(uu49);adr-2(uu28);rrf-3(uu56) | This study |
| BB265  | adr-1(uu49);adr-2(uu28);rrf-3(uu57) | This study |
| BB266  | adr-1(uu49);rrf-3(uu56) | This study |
| BB267  | adr-2(uu28);rrf-3(uu56) | This study |
| BB270  | adr-1(uu49);adr-2(uu28);rrf-3(uu56);rde-1(uu51) | This study |
| BB272  | adr-1(uu49);adr-2(uu28);rrf-3(uu56);rde-4(uu53) | This study |
| BB273  | adr-1(uu49);adr-2(uu28);rrf-3(uu56);drh-1(uu60) | This study |
| BB277  | rrf-3(uu56); ggIs1[nrde-3p::3xFlag::gfp::nrde-3 CDS + unc-119(+)] | This study |
| BB278  | adr-1(uu49);adr-2(uu28);rrf-3(uu56);ggIs1[nrde-3p::3xFlag::gfp::nrde-3 CDS + unc-119(+)] | This study |
| BB279  | adr-1(uu49);adr-2(uu28);rrf-3(uu56);nrde-3(uu64) | This study |
| BB280  | adr-1(uu49);adr-2(uu28);rrf-3(uu56);rrf-1(uu65) | This study |
| BB283  | adr-1(uu49);adr-2(uu28);ergo-1(uu68) | This study |
| BB286  | adr-1(uu49);adr-2(uu28);rrf-3(uu56);set-25(uu66) | This study |
| BB288  | ergo-1(uu68) | This study |
| BB289  | adr-1(uu49);adr-2(uu28);rrf-3(uu56);ergo-1(uu68) | This study |
| NL2099 | rrf-3(pk1426) | (Simmer et al. 2002) |
| YY178  | ggIs1[nrde-3p::3xFlag::gfp::nrde-3 CDS + unc-119(+)] | (Guang et al. 2008) |
### Supplemental Table S2. Primers used for qRT-PCR analysis.

| Primer name         | Sequence                                      |
|---------------------|-----------------------------------------------|
| ama1_RTPCR_F1       | GTCAATGATGGGACATCGTGTC                       |
| ama1_RTPCR_R1       | GTGATGAGTGTCTCGGCAACC                       |
| Y45F10D4_RTPCR_F1   | CGAGAAACCCGCGAAATGTCGGA                     |
| Y45F10D4_RTPCR_R1   | CGGTTGCCAGGAAGATGAGGCG                      |
| cdc42_RTPCR_F1      | AGCCATCTGCGGCTCTCG                          |
| cdc42_RTPCR_R1      | GCAACCGCTTCGTTGGGCC                        |
| pmp3_RTPCR_F2       | TGGAATTGGTTTGACCAAATGC                     |
| pmp3_RTPCR_R2       | TTCAGCTCTTCCGGAAGTTCC                      |
| daf2_RTPCR_F1       | GCTACTATACGCTGACTCTC                       |
| daf2_RTPCR_R1       | TTGTGTAAATGCGGTAGGTCTC                     |
| hmr1_RTPCR_F1       | TGAGTCATCCATTTTGAGGCTG                     |
| hmr1_RTPCR_R1       | TGAGTCCATCCCGGTAGGCTG                      |
| efa6_RTPCR_F1       | GTGATCCAGATTCCAGGCTG                       |
| efa6_RTPCR_R1       | TCTGTGTAAGGTTAGGAACGC                     |
| mdt17_RTPCR_F1      | GAACCTCGAGTGAGAAGGTCTC                     |
| mdt17_RTPCR_R1      | CTGATTAGGGATTGACGTC                       |
| ceh100_RTPCR_F1     | ACCAGAAACGGAAGAATCC                      |
| ceh100_RTPCR_R1     | GGAACCCATCCGCTCATCTGG                     |
| ergo1_RTPCR_F1      | TCCACACTCAAGGAATTCTC                       |
| ergo1_RTPCR_R1      | GTTCCGACTTCCCAGGAC                       |
| ogt1_RTPCR_F1       | CGTTGTGCTCCGATAAGGTC                      |
| ogt1_RTPCR_R1       | TCTACTCGACTCCATCATGCG                     |
| rhgf2_RTPCR_F1      | CCACTCGAGTGTATGGAAGG                      |
| rhgf2_RTPCR_R1      | GAGCTTCGATGCTGTAGGTCTC                    |
| ccb1_RTPCR_F1       | ACTCGAATTTCAGTATCATCAG                    |
| ccb1_RTPCR_R1       | CCACGGTTTATACTTGGCTC                      |
| egl8_RTPCR_F1       | CAGACGTGTCTTTCAAGGAC                      |
| egl8_RTPCR_R1       | TTGGACACGACGATATCTCC                     |
Supplemental Table S3. Primers used for CRISPR/Cas9 gene targeting and genotyping. For primers encoding sgRNA transcription templates used for gene disruptions, sgRNA target sequences are shown in lowercase letters.

| Primer name                  | Sequence                                                                 |
|------------------------------|--------------------------------------------------------------------------|
| CRISPR_sgRNA_R1             | AAA AGC ACC GAC TCG GTG CCA C                                            |
| T7_sgRNA_ccb1_Int5_A        | TAATACGACTCACTATAGtgatcttttgagaggaagTTTTTAGAGCTA GAAATAG                |
| T7_sgRNA_ccb1_Int5_B        | TAATACGACTCACTATAgtaagaccccccaagtgagaaTTTTTAGAGCT AGAAATAG              |
| ccb1_Ext5_F1                | GAT GCC AAG AAG TGG ATC ACG                                              |
| ccb1_Ext6_R1                | CTT TTG TTG GAG GCG TCG TAA C                                            |
| ccb1_Int5_F2                | TTG CAG TGC AAG ACG ATT ACC                                              |
| T7_sgRNA_efa6_Ex8           | TAATACGACTCACTATAgctgtcattcggagagtctgtggtTTTTAGAGCT AGAAATAG            |
| T7_sgRNA_efa6_Ex9           | TAATACGACTCACTATAgctgtcattcggagagtctgtggtTTTTAGAGCT AGAAATAG            |
| efa6_Int8_F1                | AAC ACC ATT CCC TAG TGA GTG                                              |
| Syntron_sense               | GTA AGT TTA AAC AGT TCG GTA CTA ACT AAT CCA TGG ACG GTA AAA AAT ACT AAT CCA TAG TTA TTA AAT TTT CAG |
| efa6_Ex8_Synt_F1            | GGA TTT CTT ATG CGA AAA TAT GTT AGA GAa ACc GAc GGT GGA AAG AGT AAG TTT AAA CAG |
| efa6_Ex9_Synt_R1            | CGA AGA CGA GCG TAT ACC ATT CTC CAg gaG CGA CGT CCG AAT GGA GCT GAA AAT TTA AAG |
| T7_egl8_Int11_sgRNA_A_F1    | TAATACGACTCACTATAgggcagcagccaacacccataTTTTAGAGCTAGAAATAG                |
| T7_egl8_Int11_sgRNA_B_F1    | TAATACGACTCACTATAgggagttacaggaaatacaaaTTTTAGAGCTAGAAATAG                |
| egl8_Ext11_F1               | CCA AGG AAA ACG ACG AAG CAC                                              |
| egl8_Ext12_R1               | GCG AAA ATC CGC TCC TCT TC                                               |
| egl8_Int11_F1               | TCG AAA ATG TGG GAA ATG CTC                                              |
| T7_sgRNA_adr2_5p_A          | TAATACGACTCACTATAggaacaaaaagtccacatgTTTTAGAGCT AGAAATAG                 |
| T7_sgRNA_adr2_3p_B          | TAATACGACTCACTATAggtgattcggagagtctttatctTTTTAGAGCTAGAAATAG             |
| adr2_5p_F2                  | GTTCACTAGTCGATGGTGCTC                                                   |
| adr2_3p_R2                  | AATCACATGGGTCACTGATGC                                                   |
| adr2_WT_R1                  | ACAGTTCCTCACCACAAAGTCG                                                  |
| T7_adr1_Start_sgRNA_A       | TAATACGACTCACTATAggtaatcttttgactacgaaaTTTTAGAGCT AGAAATAG              |
| T7_adr1_Ext11_sgRNA         | TAATACGACTCACTATAgggcggttgtagttatgtcagtaaTTTTAGAGCTAGAAATAG            |
| adr1_5p_F1                  | GTGTCATCTAAAGAAGGCTGGAG                                                 |
| adr1_Ext12_R1               | CGAAAGCAGCAAGAGGTGAAG                                                   |
| T7_rde1_5p_sgRNA_A          | TAATACGACTCACTATAggacatgtttcatcacttttgTTTTAGAGCTAGAAATAG              |
| Gene     | sgRNA Name | Sequence                                                                 |
|----------|------------|---------------------------------------------------------------------------|
| rde1     | T7_rde1_Ex10_sgRNA_B | TAATACGACTCACTATAaggaattgtgaaccctacatcGTTTTAGAGCATAAGAAATAG           |
|          | rde1_5p_F1 | AGA GTG GTT CTG CAA ACA CG                                                 |
|          | rde1_WT_F2 | CTA CGT GTT AGT CAT GAT GAG C                                               |
|          | rde1_Ex11_R1 | CTA GCA GAG AGA AAA GCA AGT C                                              |
| T7_rde4  | T7_rde4_Ex1_sgRNA_A | TAATACGACTCACTATAaggtactagaagaggtgctaGT TT TAGAGCTAG AAATAG           |
|          | T7_rde4_Ex4_sgRNA_B | TAATACGACTCACTATAaggtcttgagaagactagacgcGT TT TAGAGCTAG AAATAG           |
|          | rde4_Ex1_F1 | AAG CGT TTT CGG TGG ATC AG                                                 |
|          | rde4_WT_F2 | AAG ACG GTA TCG AAT CTC TGG                                                 |
|          | rde4_Ex4_R1 | ACA AGC ACA CTG TTT AGC AGC                                                 |
| T7_rrf3  | T7_rrf3_sgRNA_A | TAATACGACTCACTATAgcctcaaatctcgcatacgagGT TT TAGAGCTAAGAAATAG           |
|          | T7_rrf3_Ex8_sgRNA_C | TAATACGACTCACTATAgttgaacctgacattgaaggGT TT TAGAGCTAAGAAATAG           |
|          | rrf3_Ex4_F1 | CGA TTG CGA TTG GAA ACT GC                                                 |
|          | rrf3_WT_R1 | GCA CGT TTC CAT ATT GAG AAC C                                               |
|          | rrf3_Ex9_R3 | TTG TGA TCC TTT TGT AGC                                                   |
| T7_drh1  | T7_drh1_sgRNA_A | TAATACGACTCACTATAgccttctgctggagagcagagGT TT TAGAGCTAG AAATAG           |
|          | T7_drh1_sgRNA_B | TAATACGACTCACTATAgttatcttctctcaggattcgGT TT TAGAGCTAAGAAATAG           |
|          | drh1_Ex4_F1 | GTC TCC TAC GCT TCA TTG AAC                                                 |
|          | drh1_WT_R1 | GCA GTT CCT AAA TAG ACC ATC                                                 |
|          | drh1_Ex18_R2 | ACT TCA ATC AAC TGA CCA AGC                                                 |
| T7_sgRNA_nrde3 | T7_sgRNA_nrde3_Ex1_A | TAATACGACTCACTATAggtatctcttcagaagatgtgtgtGT TT TAGAGC TAAATAG       |
|          | T7_sgRNA_nrde3_Ex11_B | TAATACGACTCACTATAgttcattgcatttagatgtgtGT TT TAGAGC TAAATAG       |
|          | nrde3_5p_F1 | CAT TCC TTT GCT GTG CGA CTG                                                 |
|          | nrde3_3p_R1 | CAA GTG AAA TCC CTG GTA AAC C                                               |
|          | nrde3_WT_R2 | CGA CCT CCA AGA GAT CCA TGC                                                 |
| T7_rrf1  | T7_rrf1_sgRNA_A | TAATACGACTCACTATAggtctcttcgaataagatgtgtgtGT TT TAGAGC TAAATAG       |
|          | T7_rrf1_sgRNA_B | TAATACGACTCACTATAggtcattgcatttagatgtgtgtGT TT TAGAGC TAAATAG       |
|          | rrf1_Ex_F1 | ACG GTT CGA TTG TGA TTG GAG                                                 |
|          | rrf1_Ex17_R2 | GAT TTG CTC CAC CAA TTT TC GC                                               |
|          | rrf1_WT_F2 | CAA CGG ACA ACT CAG GGT TAG                                                 |
| T7_set25 | T7_set25_Ex2_sgRNA_A | TAATACGACTCACTATAggtcattgcatttagatgtgtgtGT TT TAGAGC TAAATAG       |
|          | T7_set25_Ex7_sgRNA_B | TAATACGACTCACTATAgactcattgcatttagatgtgtgtGT TT TAGAGC TAAATAG       |
|          | set25_Ex1_F1 | TAC AGA AGC GAC AGC ATC TC                                                   |
|          | set25_Ex7_R1 | CAT TTG ACA CTC GAC CGT TTC                                                  |
|                | Sequence                                                                 |
|----------------|--------------------------------------------------------------------------|
| set25_WT_R2    | ATC TCC TGC GAT TGC TTT GAG                                              |
| T7_ergo1_Ex1_sgRNA_A | TAATACGACTCACTATAGggtatgtcgaactaaaccaGTGTTAGAGCTAAGAAATAG             |
| T7_ergo1_Ex6_sgRNA_B  | TAATACGACTCACTATAggagagttcatagatcagtcacacGTGTTAGAGCTAGAAATAG         |
| ergo1_Ex1_F1  | GGA CAA TCG CTA CGA TGA TCG                                              |
| ergo1_Ex6_R1  | CAC GTA TCG TGA AGC ACA TAG                                               |
| ergo1_WT_F2   | TCG ACG TTT CTC ATC CAT CG                                               |
**Supplemental Table S4**: Novel mutations generated by CRISPR/Cas9 for this study.

| Mutation            | Coordinates (ce10/WS220) | Nature of mutation | Inserted sequence                                                                 |
|---------------------|---------------------------|--------------------|----------------------------------------------------------------------------------|
| *adr-2(uu28)*       | chrIII:7230936-7232861    | -1926 nt deletion  |                                                                                 |
| *ccb-1(uu35; ΔEER)* | chrI:3644035-3644883      | -849 nt deletion   |                                                                                 |
| *efa-6(uu46; Δefa-6 intron 8 + 60 nt syntron)* | chrIV:12607422-12609861 | +97/-2440 nt insertion/deletion | AACCGACGTTGGAAAGAGTAAGTTTAAACAGTTTCTGTTAATCTAACTATCCATGGACATAGATATCTT   |
|                     |                           |                    | TAAATTTTCAGCTCCATTGGGCAGTCGCTCC                                                  |
| *egl-8(uu47; ΔEER)* | chrV:30802-34717          | -3916 nt deletion  |                                                                                 |
| *adr-1(uu49)*       | chrI:7773430-7777062      | +1/-3633 nt insertion/deletion | T                                                                                 |
| *rde-1(uu51)*       | chrV:9988461-9991606      | -3146 nt deletion  |                                                                                 |
| *rde-4(uu53)*       | chrIII:10217478-10218402 | -925 nt deletion   |                                                                                 |
| *rrf-3(uu56)*       | chrI:8163812-8165364      | -1553 nt deletion  |                                                                                 |
| *rrf-3(uu57)*       | chrI:8163805-8165366      | +22/-1562 nt insertion/deletion | GTATTCTGGTGCCACCAGACA                                                             |
| *drh-1(uu60)*       | chrIV:6608226-6612649     | +54/-4424 nt insertion/deletion | CATGTATACAATTTGGGAAAAAGCAGCTGCTTTGGAGAGAGAGAAGATACCTGGG                           |
| *nrde-3(uu64)*      | chrX:372267-376618        | +64/-4352 nt insertion/deletion | CACATGTGACCATGTGCAGAGAAGAAGAAGAGTACCTGGG                                      |
| *rrf-1(uu65)*       | chrI:7645169-7648452      | +20/-3284 nt insertion/deletion | TCACTTACACTATATTGGAA                                                             |
| *set-25(uu66)*      | chrIII:13287014-13292728 | +15/-5715 nt insertion/deletion | GCTGTGACCCAGCT                                                                  |
| *ergo-1(uu68)*      | chrV:1009051-1016719      | +1/-7669 nt insertion/deletion | T                                                                                 |