Supplementary Figures and Methods

Supplementary Material and Methods

Primers used in this study

F48E8.4 Forward 5' - CTTCTAGTCCCGCCAAATTTATG - 3'

F48E8.4 Reverse 5' – CAGTTGAAGTTATTCCACGACCC - 3'

rncs-1 Forward 5' – ATTTTTTCCCGACAAAGATGGAACTCAAGGAT – 3'

rncs-1 Reverse 5' –

TGATTCAACATTTCAAAAACTTGTATTTTACATCTAAAACTATAAA – 3'

AL_SD_1 *adr-1(gv6)* forward: CAATGTCGCAAAACCAAATG

AL_OBN_132 *adr-1(gv6)* reverse: GAGATGTTCCATTGGCTCC

AL_SD_3 *adr-2(gv42)* forward: AAGGAAAGAACGCATTGGTG

AL_SD_4 *adr-2(gv42)* reverse: GTTTCTCAGCTCCAGGCATC

AL_SD_7 *adr-1(tm668)* forward: CCAGGGTTGGATCCTCTCGGTG

AL_SD_8 *adr-1(tm668)* reverse: GTCACGAAGAGCTTCACGAATGACC

AL_SD_6 *adr-2(ok735)* forward: AGCCTGAGCTCGCTTCCAATCTTCAAG

AL_SD_5 *adr-2(ok735)* reverse: CCCCCAGCTTACAGTAATCATCAGTTCTGCC

HH1944 :GTAATTTATTTGACTACGAAATGGATC

HH1945 :TCCAATTTGGTTTGTTTTGG

HH1948 :CTCTCGGCATATTTCCTCTATATTG

HH1949: TGTCCATAACCGAAGTTGTAGTTAG

HH1952 :AGGTAATTTATTTGACTACGAAATGGATC

HH1953 :TTATTTTGCGAAATTGTTGTTACG

HH1954: CGACTCCATCCAGATTGTG

HH1955 :GTTTCCTTAAATAATATTCAACTCCG

DNA and RNA Sanger sequencing

To obtain cDNA, extracted RNA (MirVana) was treated with DNase I (Ambion) and then a reverse transcriptase reaction was performed with SuperScriptIII (Invitrogen), using 6-mer random primers. DNA was extracted using Phire Tissue Direct PCR Master Mix (Thermo Scientific). The amplification products were directly sequenced by Sanger sequencing.

Isoforms validation

Presence of ADR-1 isoforms in different worm strains was assessed via PCR amplification of cDNA from adult worms of strains N2, *adr-1(gv6)*, *adr-2(gv42)*, *adr-1(gv6)*; *adr-2(gv42)*, *adr-1(tm668)*, *adr-2(ok735)*, and *adr-1(tm668)*; *adr-2(ok735)*. RNA was isolated from whole

worms using Trizol (Invitrogen) followed by DNase (Fisher Scientific, Hampton, NH) treatment, and purification using the RNeasy Extraction Kit (Qiagen, Hilden, Germany). cDNA was synthesized from 2ug of whole worm RNA using Superscript III(Invitrogen) along with random hexamers (Fisher Scientific) and oligo-dT (Fisher Scientific) primers. Amplification of the different isoforms was carried out using Platinum PFX DNA Polymerase and 3ul of cDNA from each strain. ADR-1 isoform C was amplified using primers HH1944 and HH1945. ADR-1 isoform E was amplified using primers HH1948and HH1949. ADR-1 isoform G was amplified using primers HH1952 and HH1953. ADR-2 was amplified using primers HH1954 and HH1955.

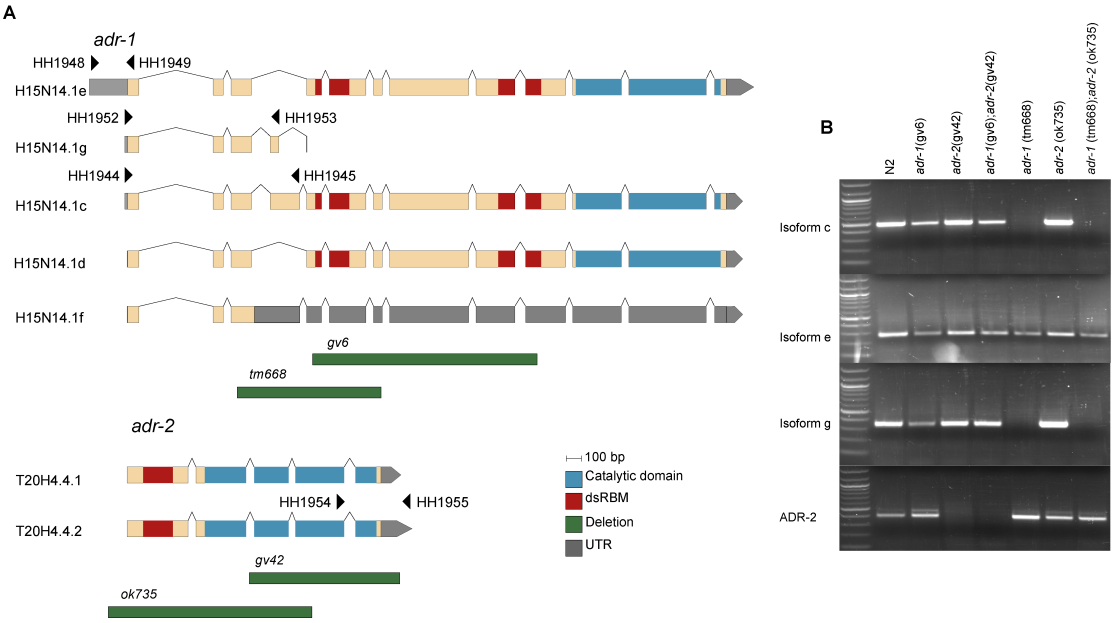
Chemotaxis Assay

Adult worms were used to assess chemotaxis behavior similarly to what was performed in (Deffit et al. 2017). Chemotaxis to benzaldehyde (1:1000 dilution in ethanol) and trimethylthiazole (1:10,000 dilution) was assessed and chemotaxis index determined using the formula in (Deffit et al. 2017) . Three replicate plates for each worm strain were used in each of the 5-9 biological replicates.

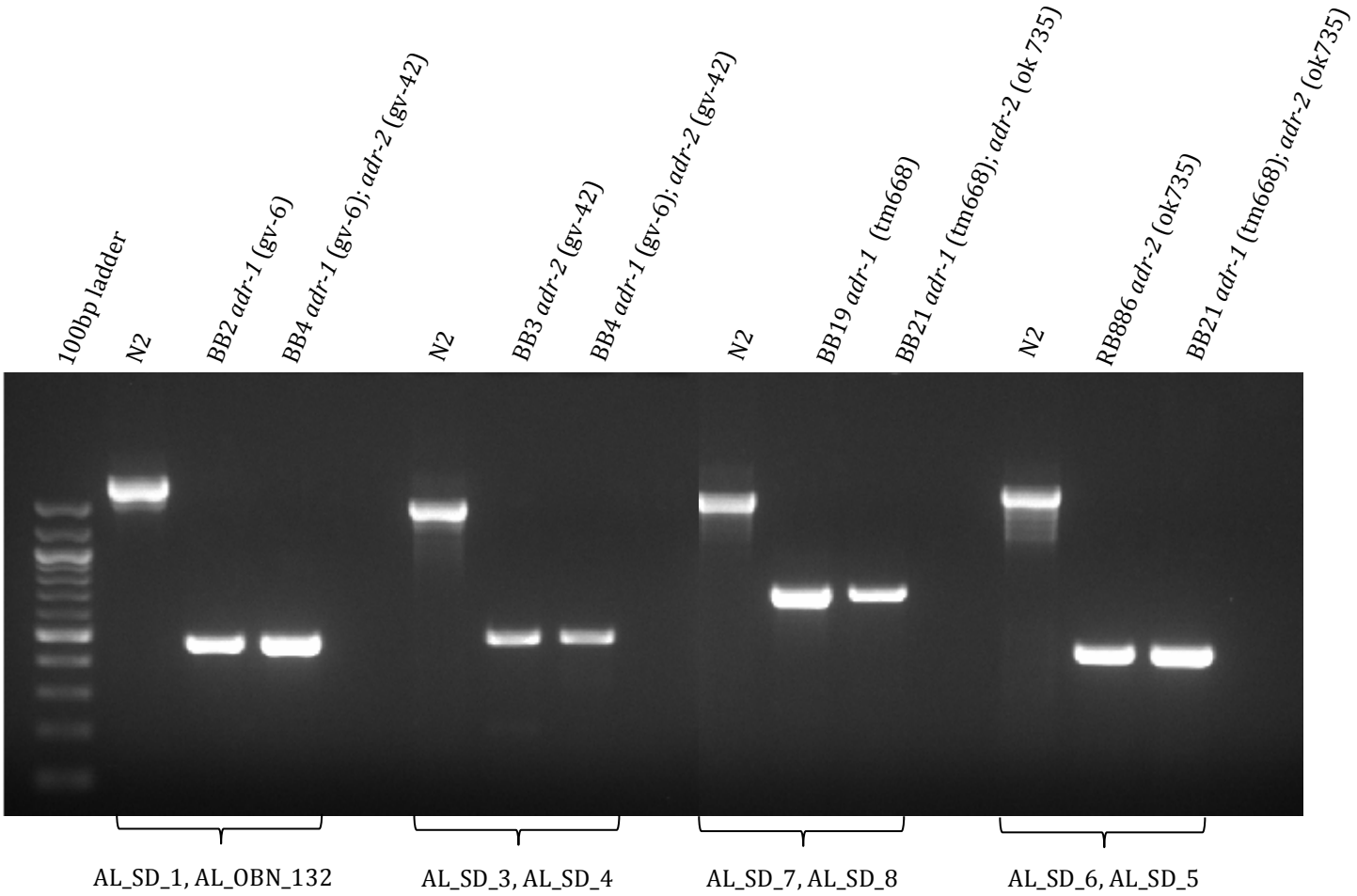
Western analysis

Plates of starved worms were chunked onto 15 cm plates and allowed to grow for 3 days, with additional food added at day 2 to prevent starvation. Worms were collected from NGM plates using 1X M9 buffer (0.04 M Na_2HPO_4, 0.02 M KH_2PO_4, 0.009 M NH_4Cl, 0.02 M NaCl), washed with extract buffer (50 mM HEPES [pH 7.4]; 70 mM K-Acetate, 5 mM Mg-Acetate, 0.05% NP-40, and 10% glycerol) and frozen at -80^{o}C. A cold motor and pestle were used to make worm lysates from the frozen pellets. The total protein concentration of the lysates was quantified using a Bradford assay (Sigma-Aldrich) and an equivalent amount of lysates from each strain were subjected to SDS-PAGE and immunoblotting with a custom ADR-2 antibody (described in (Deffit et al. 2017)) and an antibody to β-actin (Cell Signaling Technology).

Supplementary Figures



Supplementary Figure 1: Schematic view of *adr-1* and *adr-2* genes and their isoforms validation. A. *adr-1* and a*dr-2* genes are presented including known isoforms and the deletion strains used in this study. The location of the primers used to validate the isoforms is indicated (C) electrophoresis gel reveal the different isoforms of *adr-1* and *adr-2* genes. ADR-1 isoform C was amplified using primers HH1944 and HH1945. ADR-1 isoform E was amplified using primers HH1948 and HH1949. ADR-1 isoform G was amplified using primers HH1952 and HH1953. ADR-2 was amplified using primers HH1954 and HH1955.

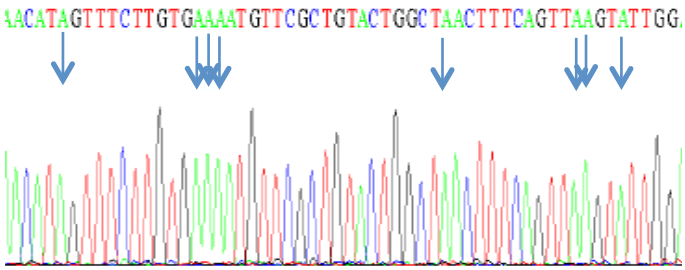


Supplementary Figure 2. Validation of ADAR strains. Gel presenting PCR to validate strains. Validation of alleles *adr-1(gv-6)* (with primers AL_SD_1, AL_OBN_132) (BB2, BB4 strains), *adr-2 (gv-42)* (with primers AL_SD_3, AL_SD_4) (BB3 and BB4 strains), *adr-1(tm668)* (with primers AL_SD_7, AL_SD_8) (BB19 and BB21 strains) and *adr-2(ok735)* (with primers AL_SD_6, AL_SD_5) (RB886 and BB21 strains).

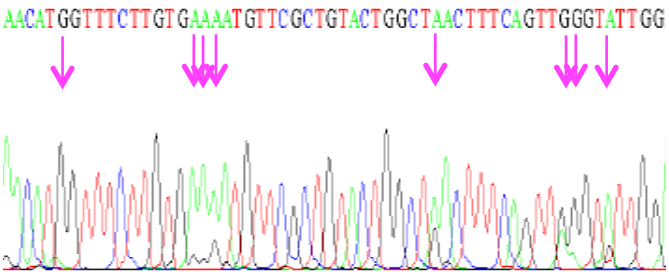
F48E8.4
chrIII: 5459498...5459554
Positions: 5459503, 5459514, 5459515, 5459516, 5459535, 5459546, 5459547, 5459550

A.

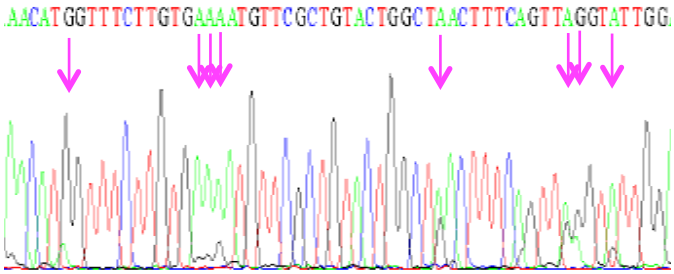
N2 genomic DNA



N2 embryo

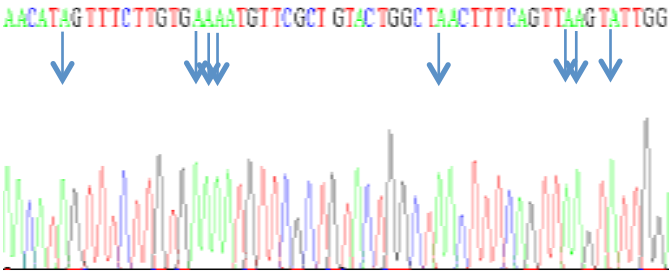


N2 L4

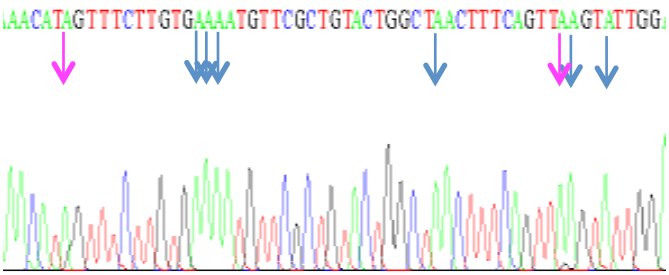


B.

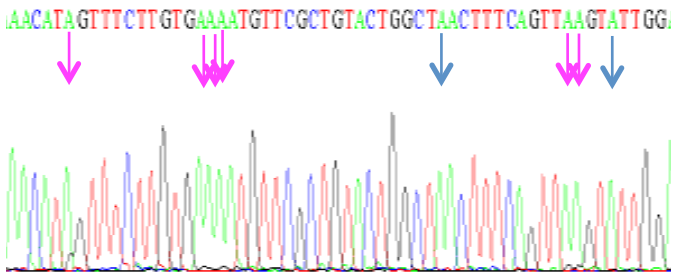
BB2 (adr-1) genomic DNA



BB2 (adr-1) embryo

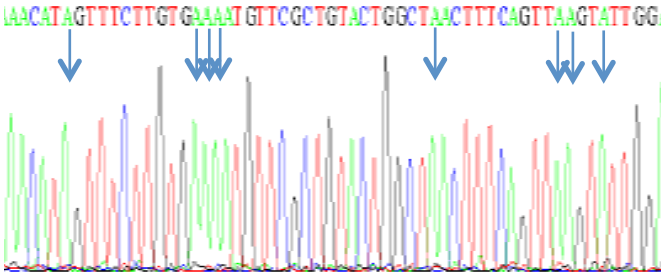


BB2 (adr-1) L4

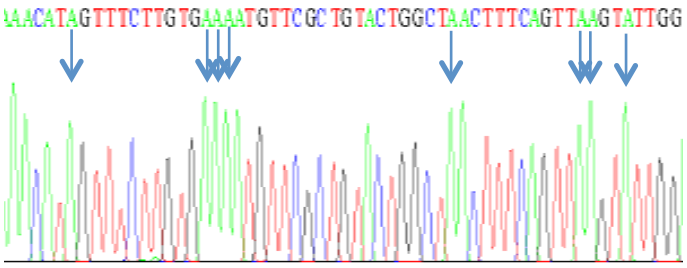


C.

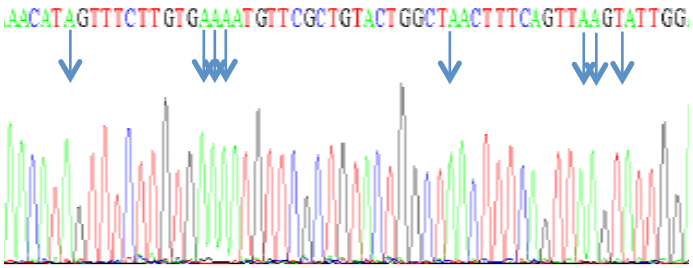
BB3 (adr-2) genomic DNA



BB3 (adr-2) embryo

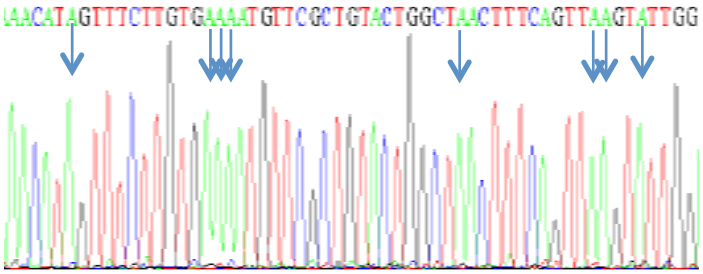


BB3 (adr-2) L4

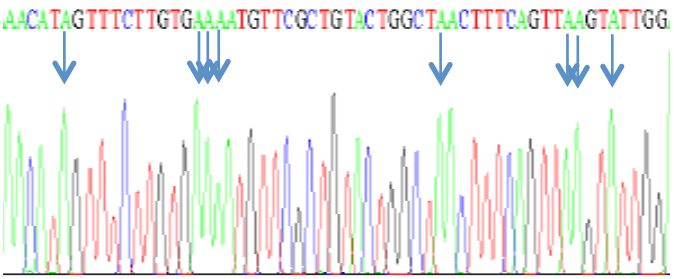


D.

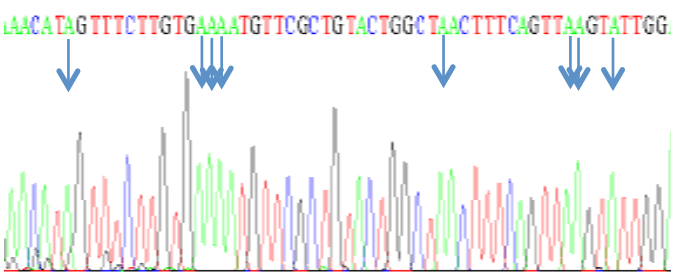
BB4 (adr-1, adr-2) genomic DNA



BB4 (adr-1, adr-2) embryo

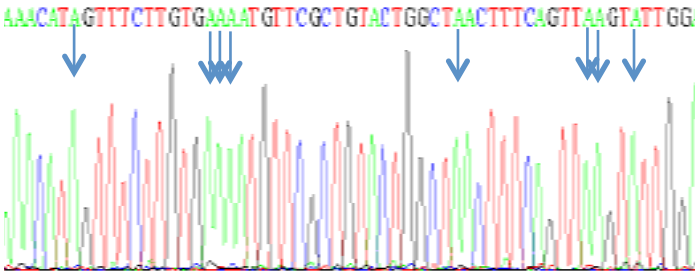


BB4 (adr-1, adr-2) L4

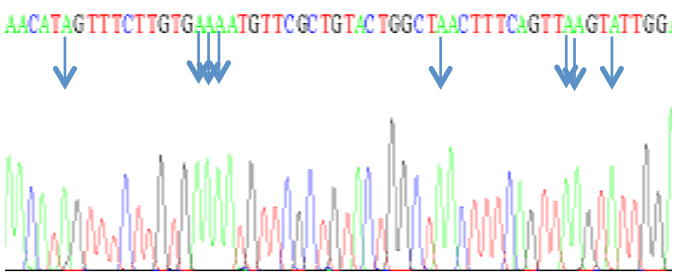


E.

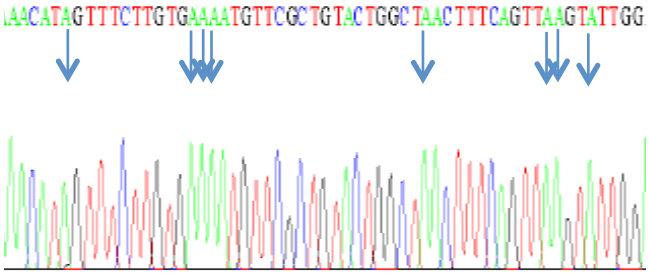
BB19 (adr-1) genomic DNA



BB19 (adr-1) embryo

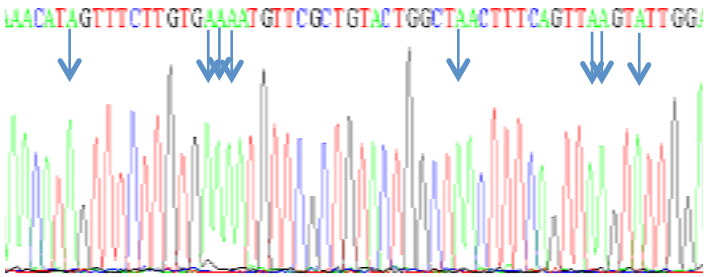


BB19 (adr-1) L4

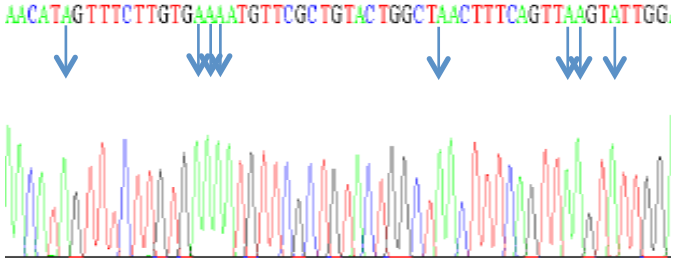


F.

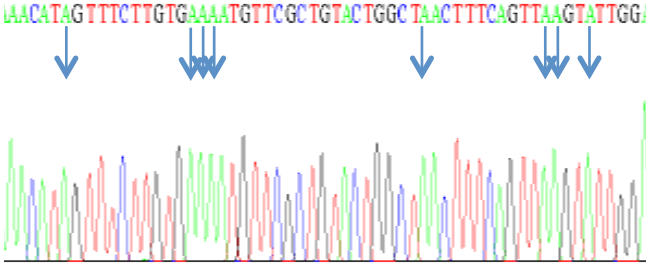
RB886 (adr-2) genomic DNA



RB886 (adr-2) embryo

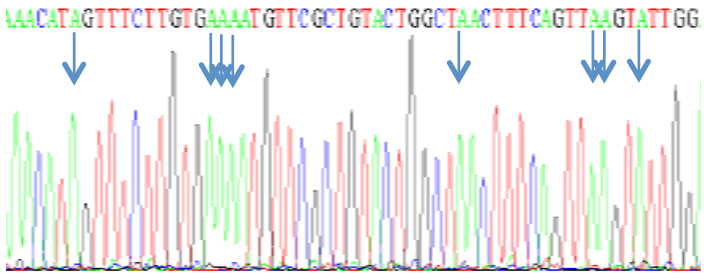


RB886 (adr-2) L4

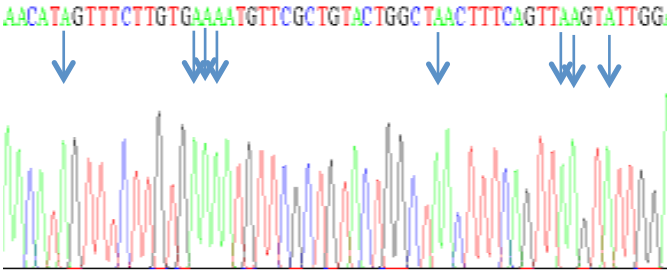


G.

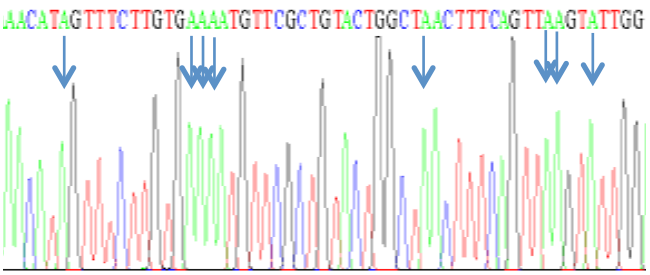
BB21 (adr-1, adr-2) genomic DNA



BB21 (adr-1, adr-2) embryo



BB21 (adr-1, adr-2) L4

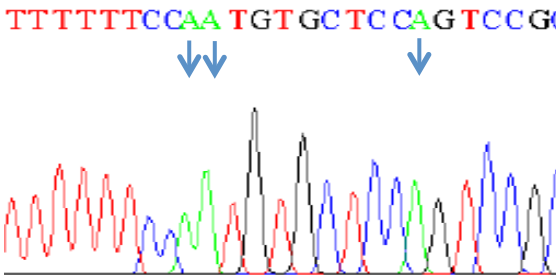


Supplementary Figure 3. *F48E8.4* gene is not edited in ADAR mutants. Sanger sequencing of *F48E8.4* genomic DNA and mRNA from embryo stage and from L4 stage. A. N2 B. BB2 (adr-1) C. BB3 (adr-2) D. BB4 (adr-1, adr-2) E. BB19 (adr-1) F. RB886 (adr-2) G. BB21 (adr-1 adr-2). Blue arrows indicate that no editing was observed, magenta arrows indicate edited site.

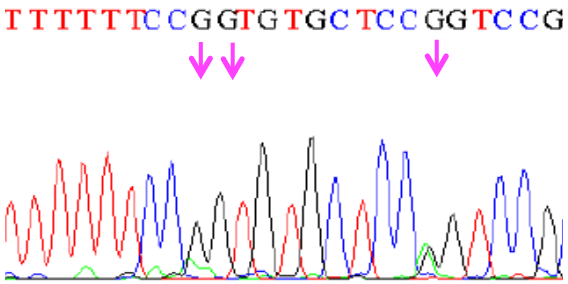
rncs-1
X: 1,012,083..1,012,106
Positions: 1,012,091, 1,012,092, 1,012,101

A.

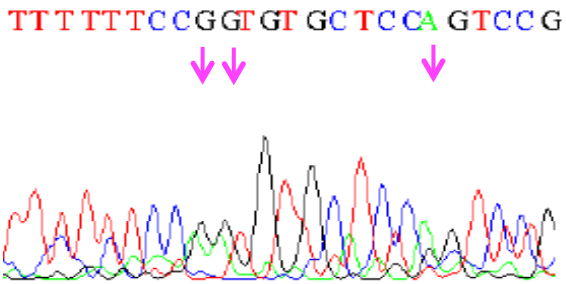
N2 genomic DNA



N2 embryo

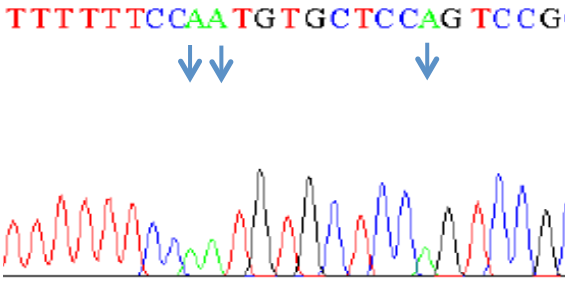


N2 L4

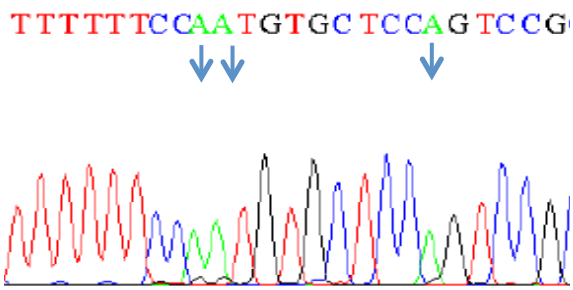


B.

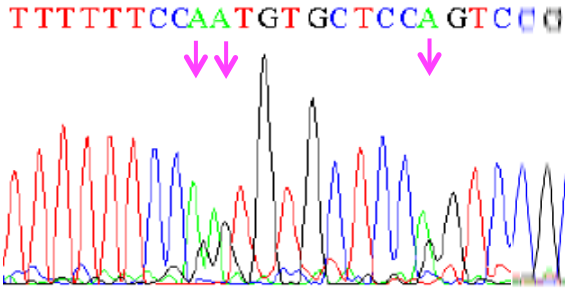
BB2 (adr-1) genomic DNA



BB2 (adr-1) embryo

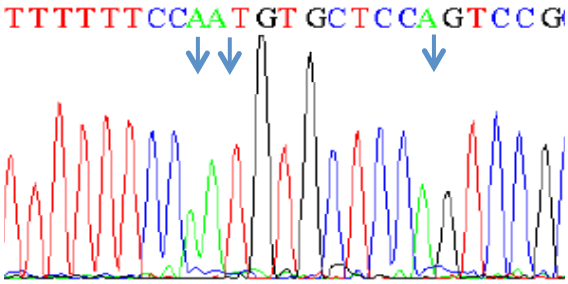


BB2 (adr-1) L4

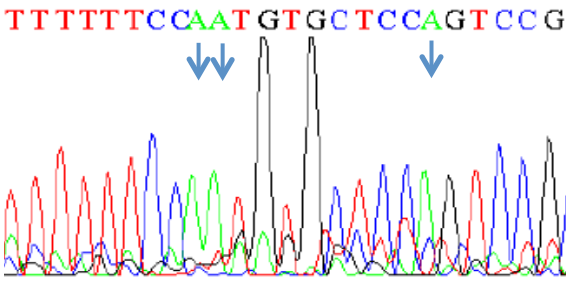


C.

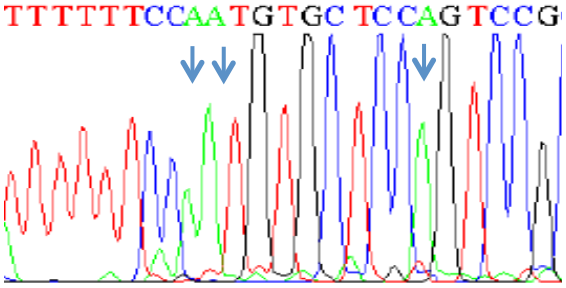
BB3 (adr-2) genomic DNA



BB3 (adr-2) embryo

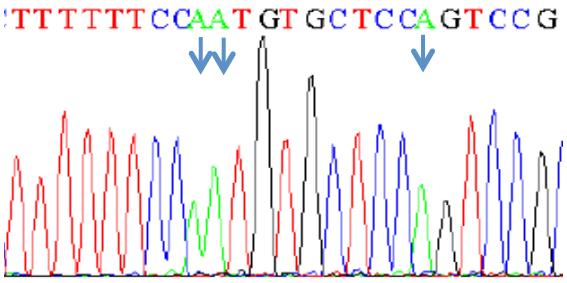


BB3 (adr-2) L4

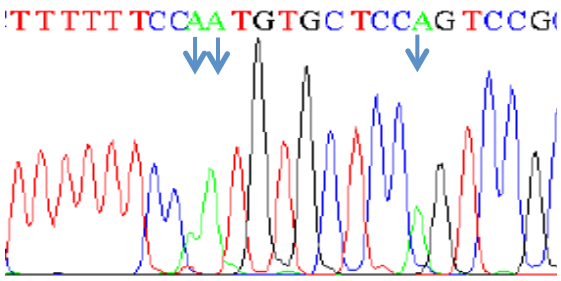


D.

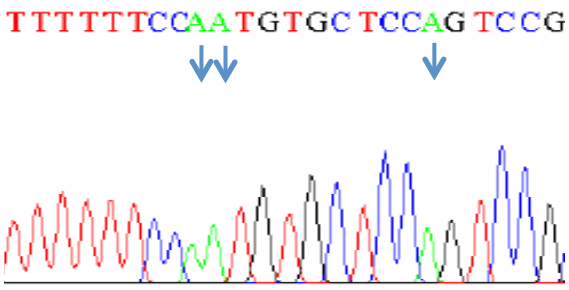
BB4 (adr-1, adr-2) genomic DNA



BB4 (adr-1, adr-2) embryo

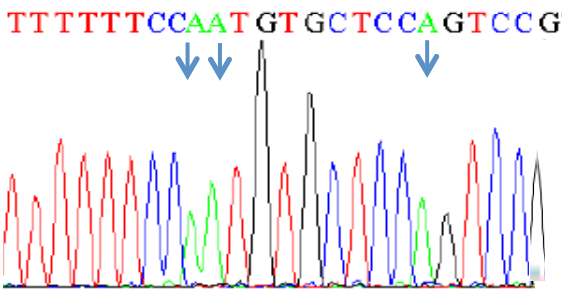


BB4 (adr-1, adr-2) L4

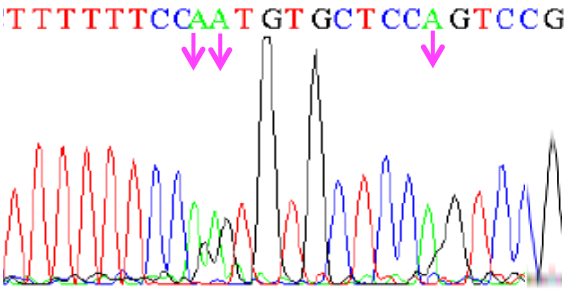


E.

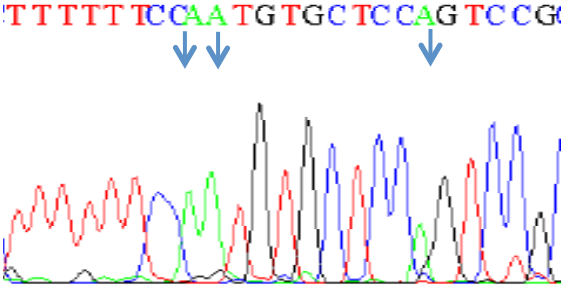
BB19 (adr-1) genomic DNA



BB19 (adr-1) embryo

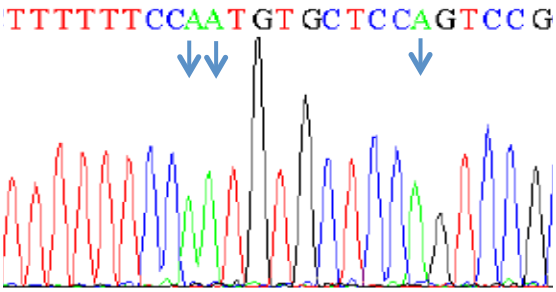


BB19 (adr-1) L4

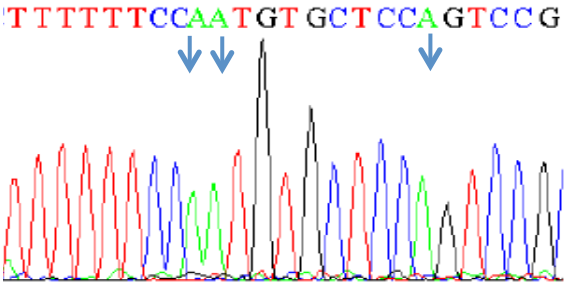


F.

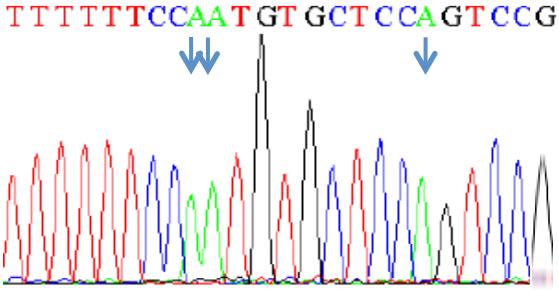
RB886 (adr-2) genomic DNA



RB886 (adr-2) embryo

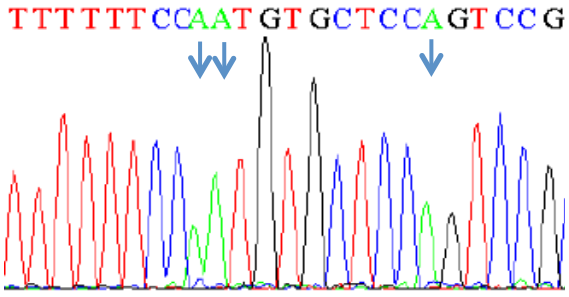


RB886 (adr-2) L4

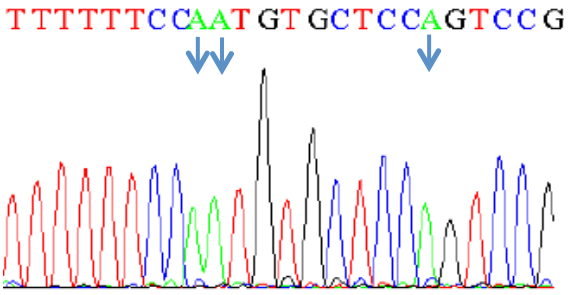


G.

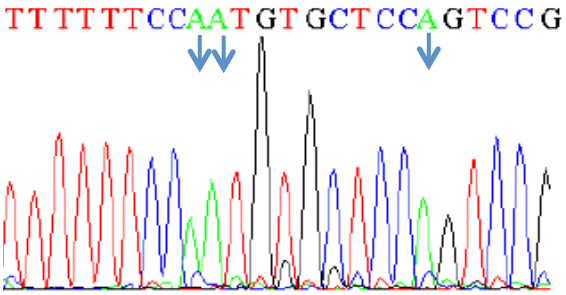
BB21 (adr-1, adr-2) genomic DNA



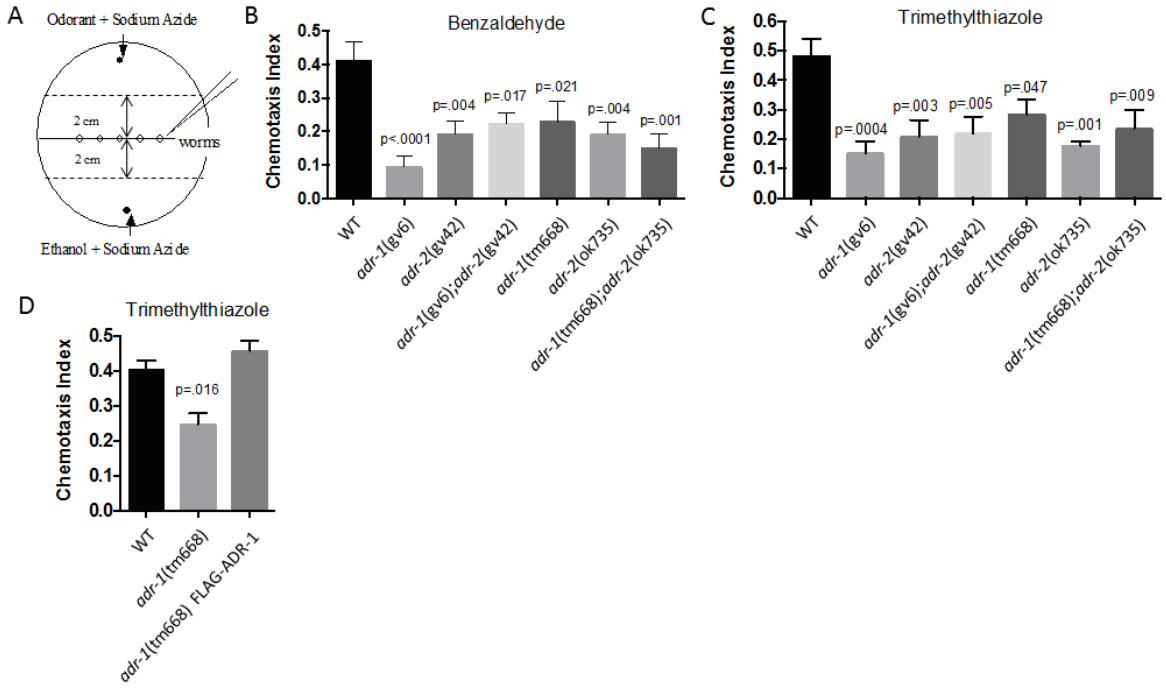
BB21 (adr-1, adr-2) embryo



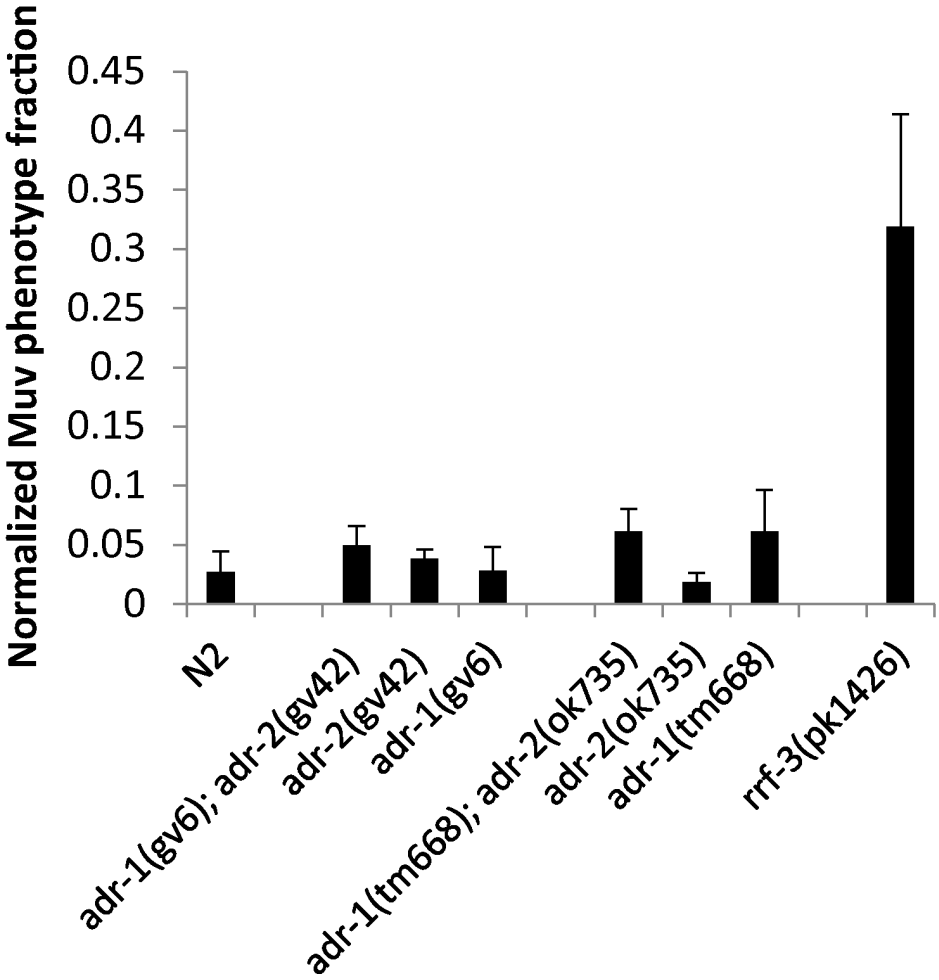
BB21 (adr-1, adr-2) L4



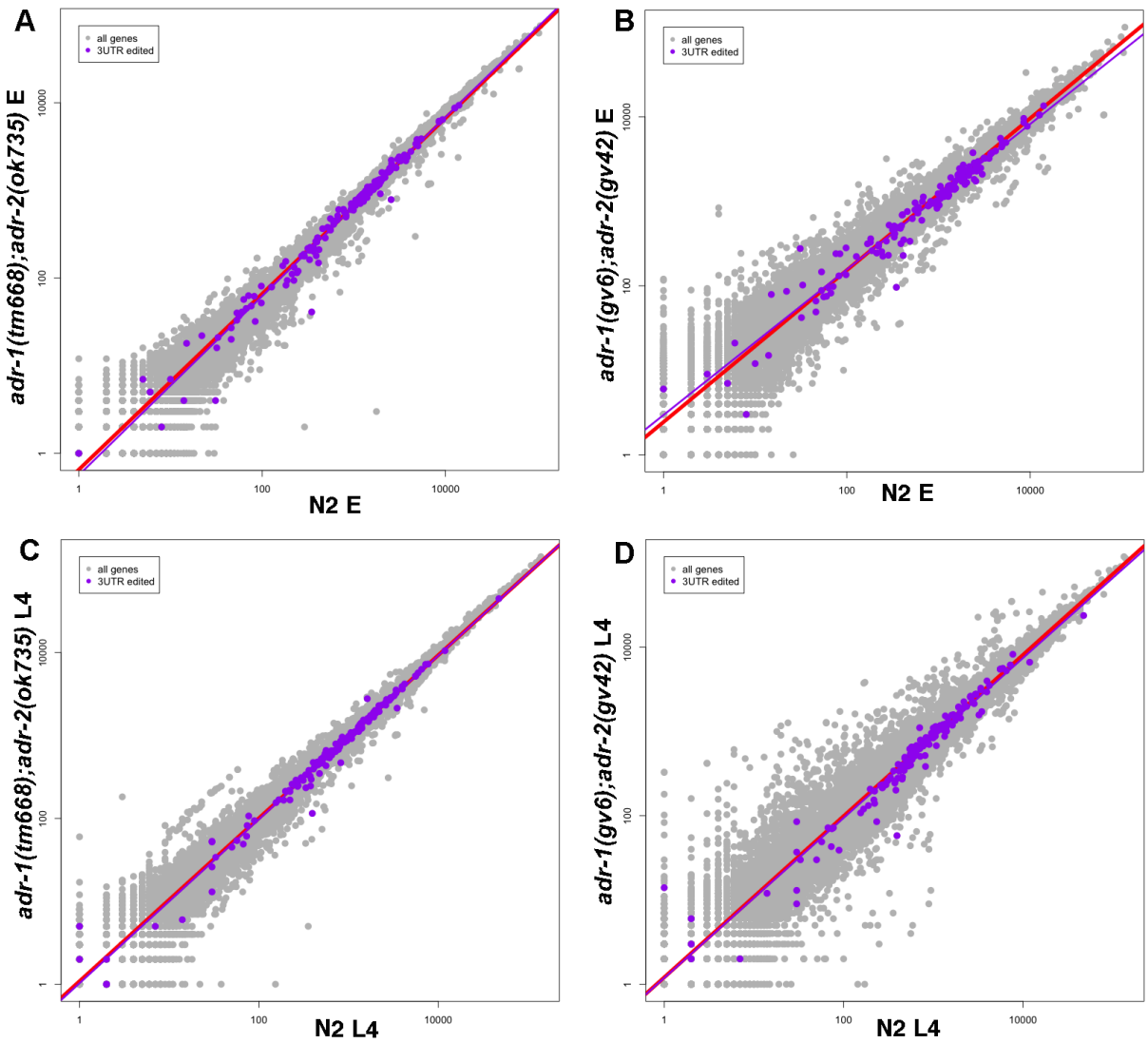
Supplementary Figure 4. *rncs-1* gene is remain edited in *adr-1* mutants. Sanger sequencing of *rncs-1* genomic DNA and mRNA from embryo stage and from L4 stage. A. N2 B. BB2 (adr-1) C. BB3 (adr-2) D. BB4 (adr-1, adr-2) E. BB19 (adr-1) F. RB886 (adr-2) G. BB21 (adr-1 adr-2). Blue arrows indicate that no editing was observed, magenta arrows indicate edited site.



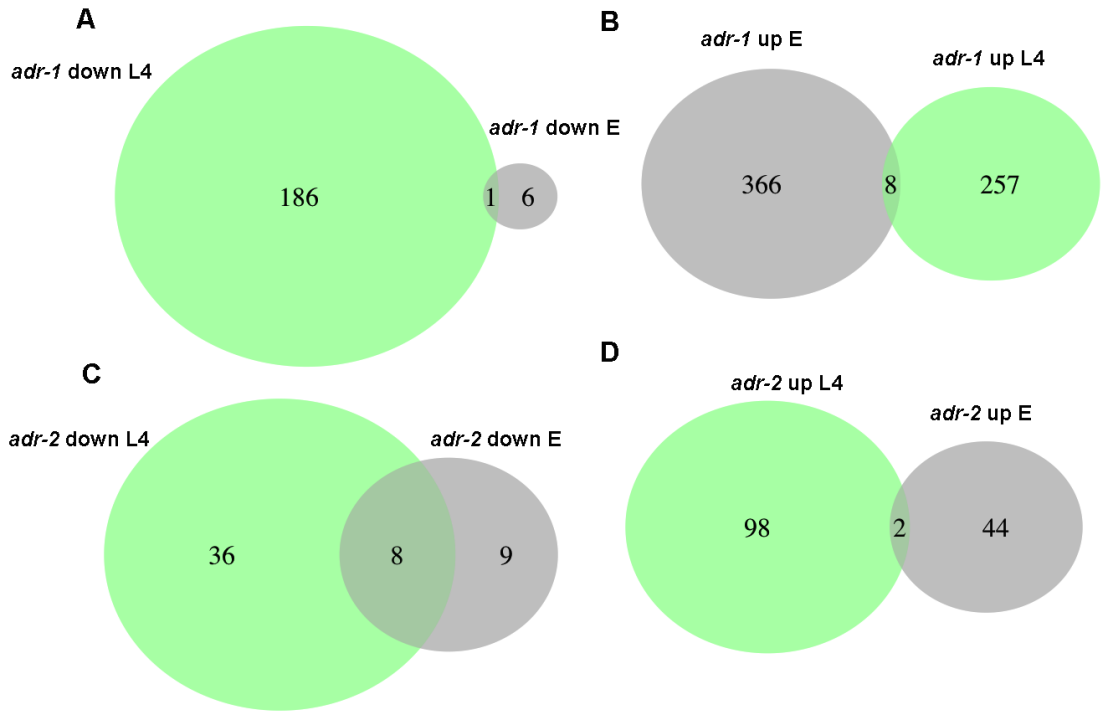
Supplementary Figure 5: Worms lacking ADAR genes have defective chemotaxis. A) Chemotaxis assays used 60 cm plates. The chemoattractant (odorant) was spotted on one side and an ethanol control on the other. Worms were placed in the middle and allowed to migrate for 1 hour prior to counting and the Chemotaxis Index of WT, *adr-1*(gv6), *adr-2*(gv42), and *adr-1*;*adr-2*(gv6;gv42), as well as the *adr-1*(tm668), *adr-2*(ok735) and *adr-1*;*adr-2*(tm668/ok735) to B) Benzaldehdye (1:1,000 dilution) or C) Trimethylthiazole (1:10,000 dilution) was determined from 9 and 5 independent biological replicates, respectively. P-value was calculated using one-way ANOVA followed by Tukey's Multiple Comparisons Correction. D) Chemotaxis Index of WT, *adr-1(tm668), adr-1* rescue to trimethylthiazole (1:10,000 dilution) was determined from 3 independent biological replicates. One-way ANOVA followed by Dunnett's Multiple Comparisons Correction.



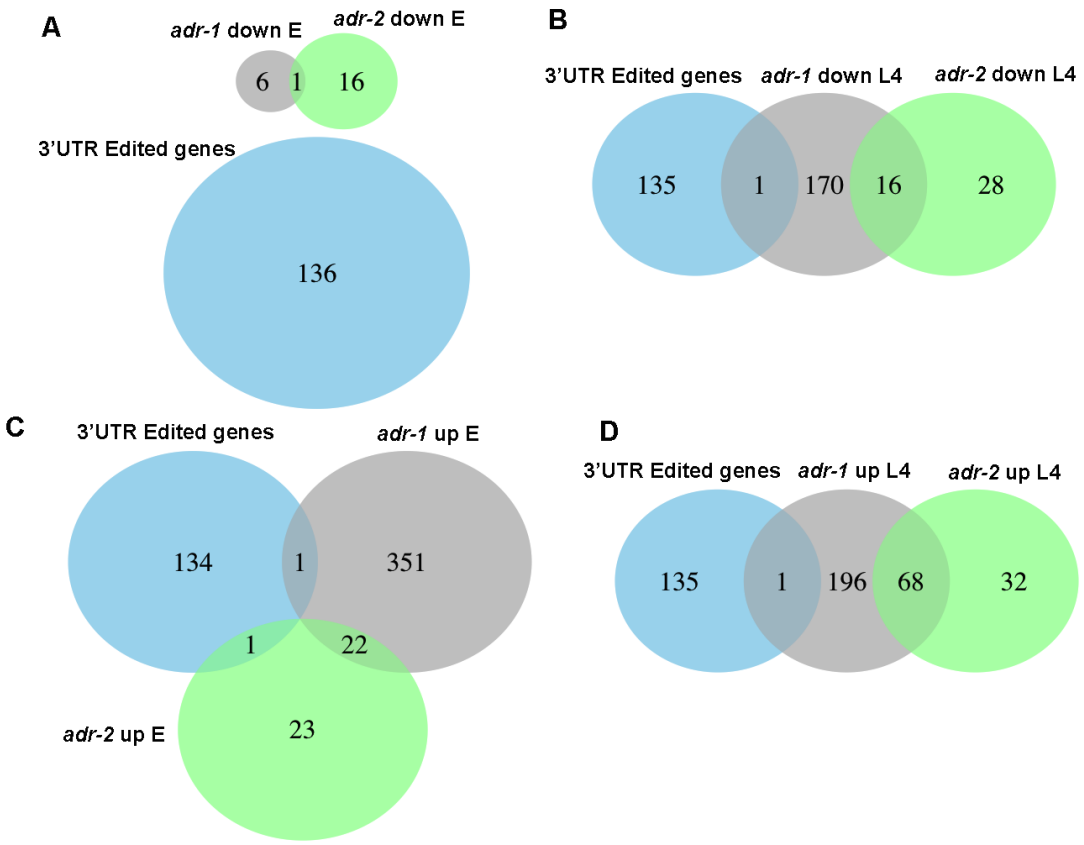
Supplementary Figure 6. ADAR mutant strains do not exhibit RNAi hypersensitivity. Worms were grown at 20 °C and were subjected to *lin-1* RNAi. Multivulva phenotypes were scored at the first day of egg laying and the fraction of worms exhibiting the phenotype from total worms is presented. Each experiment was repeated at least three times and the standard deviation is presented by error bars.



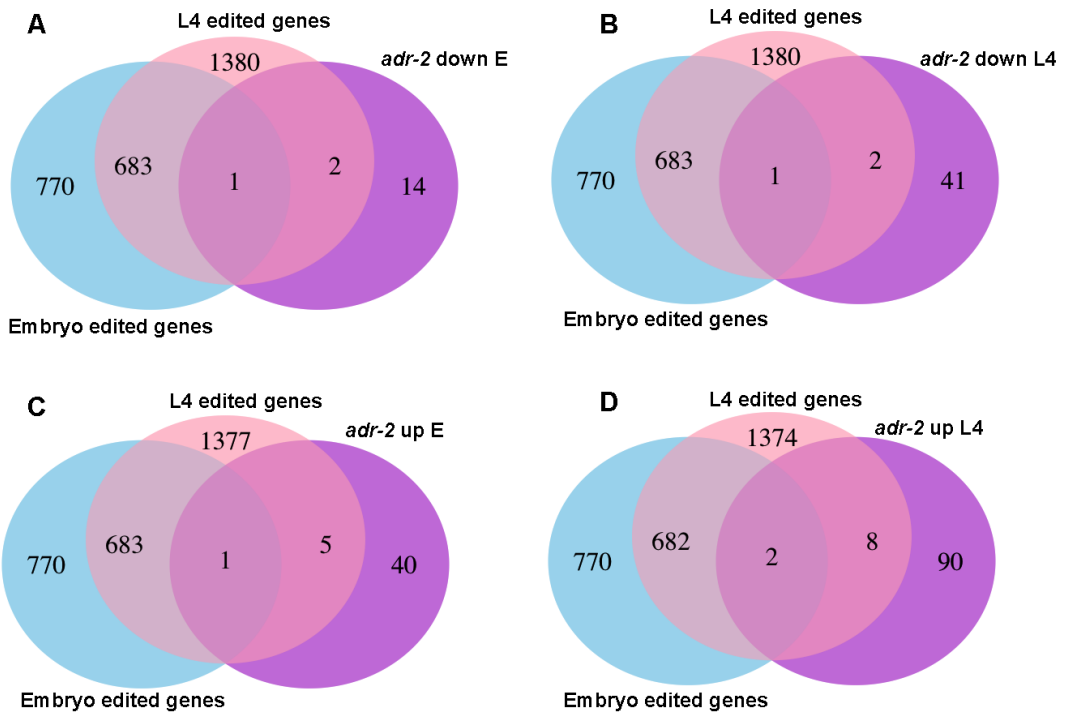
Supplementary Figure 7. Genes edited at their 3'UTR are downregulated in worms mutated in both ADAR genes at embryo and L4 stage. Log scale plots presenting expression of genes in wildtype (N2) worms versus *adr-1(tm668);adr-2(ok735)* mutant worms (A,C) or *adr-1(gv6);adr-2(gv42)* mutant worms (B,D) at embryo stage (A,B) and L4 stage (C,D). Every dot in the graphs represents a gene. Red line is the regression line for all genes. 3'UTR edited genes are in purple and their regression line is presented in purple.



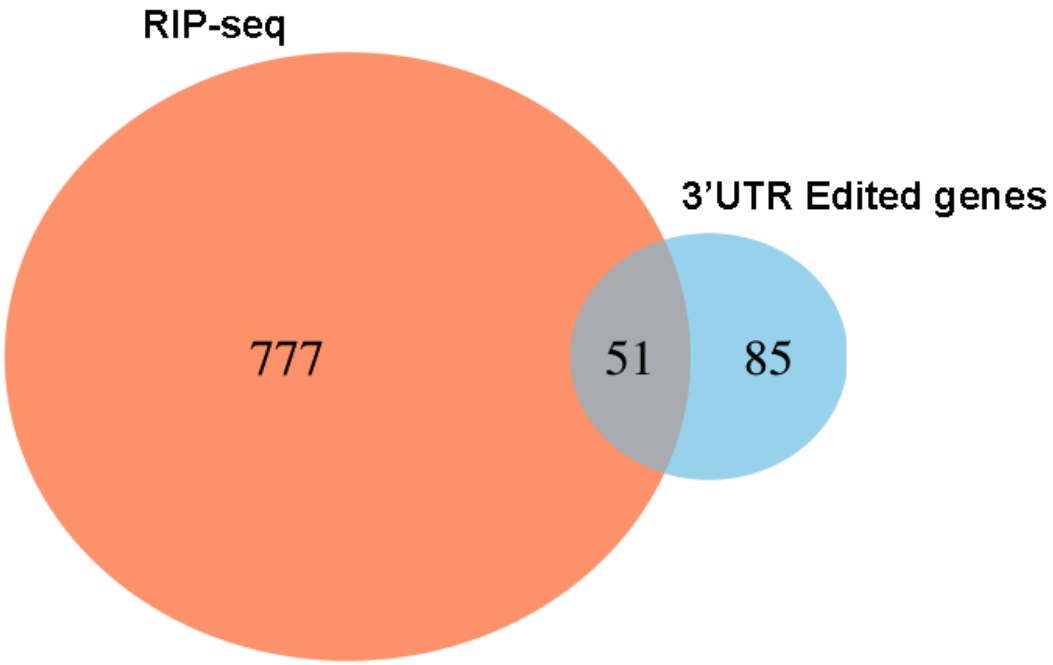
Supplementary Figure 8. Significant overlap between differential expressed genes in *adr-1* and *adr-2* mutants in different developmental stages. Venn diagrams presenting the intersections between A. Genes that their expression is downregulated in *adr-1* mutants at embryo and L4 developmental stages B. Genes that their expression is upregulated in *adr-1* mutants at embryo and L4 developmental stages C. Genes that their expression is downregulated in *adr-2* mutants at embryo and L4 developmental stages and D. Genes that their expression is upregulated in *adr-2* mutants at embryo and L4 developmental stages.



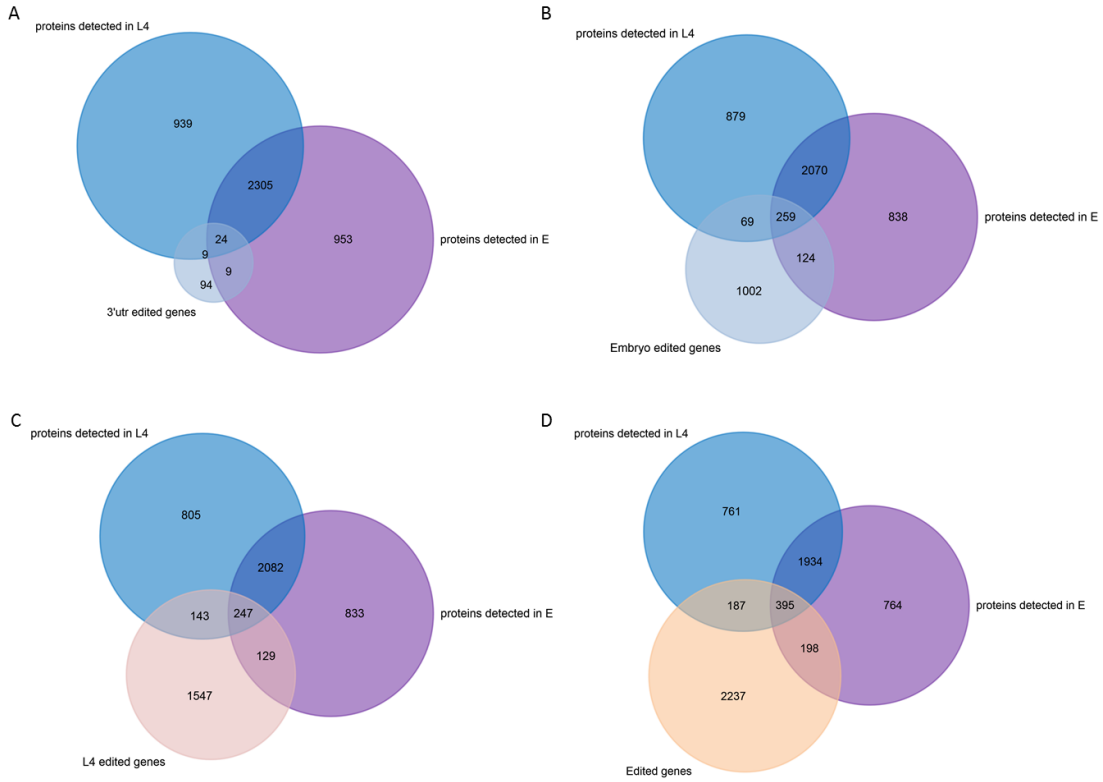
Supplementary Figure 9. Significant overlap between differential expressed genes in *adr-1* and *adr-2* mutants in specific developmental stages. Venn diagrams presenting the intersections between 3'UTR edited genes and A. Genes that their expression is downregulated in *adr-1* mutants and in *adr-2* mutants at embryo stage B. Genes that their expression is downregulated in *adr-1* mutants and in *adr-2* mutants at L4 stage. C. Genes that their expression is upregulated in *adr-1* mutants and in *adr-2* mutants at embryo stage and D. Genes that their expression is downregulated in *adr-1* mutants and in *adr-2* mutants at L4 stage.



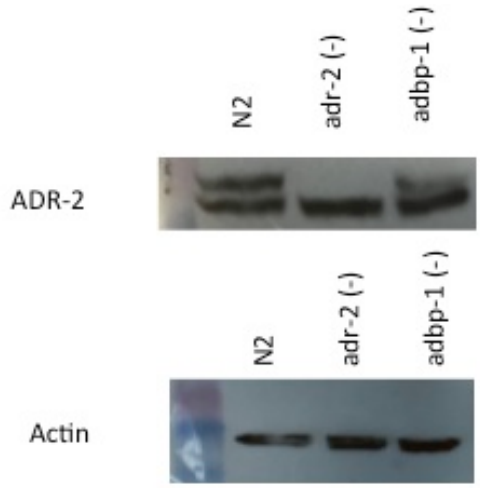
Supplementary Figure 10. No significant overlap between differential expressed genes in *adr-2* mutants and edited. Venn diagrams presenting the intersections between edited genes at embryo or L4 developmental stages and A. Genes that their expression is downregulated at embryo stage in *adr-2* mutants. B. Genes that their expression is downregulated at L4 stage in *adr-2* mutants. C. Genes that their expression is upregulated at embryo stage in *adr-2* mutants. D. Genes that their expression is upregulated at L4 stage in *adr-2* mutants.



Supplementary Figure 11. Significant overlap between 3'UTR edited genes bound by ADR-1. Venn diagram presenting the intersections between 3'UTR edited genes and genes that were found to bind ADR-1 by RIP-seq.



Supplementary Figure 12. **Edited genes are represented in the proteomics analysis.** Venn diagrams presenting the intersections between genes that their protein products were detected in the proteomics analysis at embryo stage and L4 developmental stage and A. Genes that are edited at their 3'UTR. B. Edited genes at embryo stage C. Edited genes at L4 stage D. All edited genes. For this analysis all isoforms detected by the proteomics analysis were represented by one gene.



Supplementary Figure 13. Equivalent amounts of lysates from wild-type (WT), *adr-2(-)* and *adbp-1(-)* worms were subjected to SDS-PAGE and immunoblotting with a custom ADR-2 antibody. The ADR-2 band is the upper band present in N2 lysates and to a lesser extent in the *adbp-1(-)* lysates, but absent from the *adr-2(-)* lysates. The bottom band is a protein that cross-reacts with the ADR-2 antibody. Actin was used as a loading control.

Supplementary Tables

Supplementary Table 1 – List of edited sites in the 3'UTR of genes.

Supplementary Table 2 - Changes in gene expression levels between wildtype and ADAR mutants at embryo stage.

Supplementary Table 3 - Changes in gene expression levels between wildtype and ADAR mutants at L4 stage.

Total genes per strain

Strain	Strain	Developmental stage	Up regulated genes	Down regulated genes
BB2	*adr-1 (gv6)*	Embryo	1242	57
		L4	436	706
BB19	*adr-1 (tm668)*	Embryo	547	65
		L4	649	408
BB3	*adr-2 (gv42)*	Embryo	237	36
		L4	264	187
RB886	*adr-2 (ok735)*	Embryo	113	40
		L4	134	291

Total genes per mutated ADAR gene

Mutated	Developmental	Up regulated genes	Down regulated

gene	stage		genes
adr-1	Embryo	374	7
	L4	265	187
adr-2	Embryo	46	17
	L4	100	44

Supplementary Table 4. Summary of differential gene expression analysis

Supplementary Table 5. List of editing sites identified in *adr-1* mutants.

Supplementary Table 6. Summary of the RIP-seq experiments

Supplementary Table 7. Proteomics analysis of ADAR mutants and wildtype worms at embryo stage

Supplementary Table 8. Proteomics analysis of ADAR mutants and wildtype worms at L4 stage

Strain	Strain	Developmental stage	Upregulated genes (also seen on RNA)	Downregulated genes (also seen on RNA)
BB2	*adr-1 (gv6)*	Embryo	24 (0)	20 (2)
		L4	10 (1)	11 (0)
BB19	*adr-1 (tm668)*	Embryo	20 (0)	29 (2)
		L4	11 (1)	12 (1)
BB3	*adr-2 (gv42)*	Embryo	14 (0)	15 (1)
		L4	21 (2)	15 (1)
RB886	*adr-2 (ok735)*	Embryo	10 (0)	12 (1)
		L4	12 (0)	3 (1)

Mutated gene	Developmental stage	Up regulated genes (also seen on RNA)	Down regulated genes (also seen on RNA)
adr-1	Embryo	3 (0)	11 (1)
	L4	0	0
adr-2	Embryo	3 (0)	2 (1)
	L4	3 (0)	2 (1)

Supplementary Table 9. Summary of the proteomics analysis. Numbers in parenthesis are the number of genes in the proteomics analysis that their expression was significantly altered in the parallel RNA-seq data.

References

Deffit SN, Yee BA, Manning AC, Rajendren S, Vadlamani P, Wheeler EC, Domissy A, Washburn MC, Yeo GW, Hundley HA. 2017. The C. elegans neural editome reveals an ADAR target mRNA required for proper chemotaxis. *Elife* **6**.