

SUPPLEMENTARY MATERIAL

Adar RNA editing in *Drosophila* is required for brain function and prevents antiviral Dicer-2 signaling

Patricia Deng^{1*}, Anzer Khan^{2,3*}, Dionna Jacobson¹, Nagraj Sambrani², Leeanne McGurk⁴, Xianghua Li⁴, Aswathy Jayasree², Jan Hejatko^{2,3}, Galit Shohat-Ophir⁵, Mary A. O'Connell², Jin Billy Li^{1#} and Liam P. Keegan^{2#}

¹ Department of Genetics, Stanford University, Stanford, California, USA. ² Central European Institute of Technology, Masaryk University, Brno, Czech Republic. ³ National Centre for Biomolecular Research, Faculty of Science, Masaryk University, Kamenice 5, 625 00 Brno, Czech Republic. ⁴ MRC Institute of Genetics and Molecular Medicine, Western General Hospital, Crewe Road, Edinburgh, EH4 2XU ⁵ The Faculty of Life Sciences and The Multidisciplinary Brain Research Center, Bar Ilan University, Ramat Gan, Israel

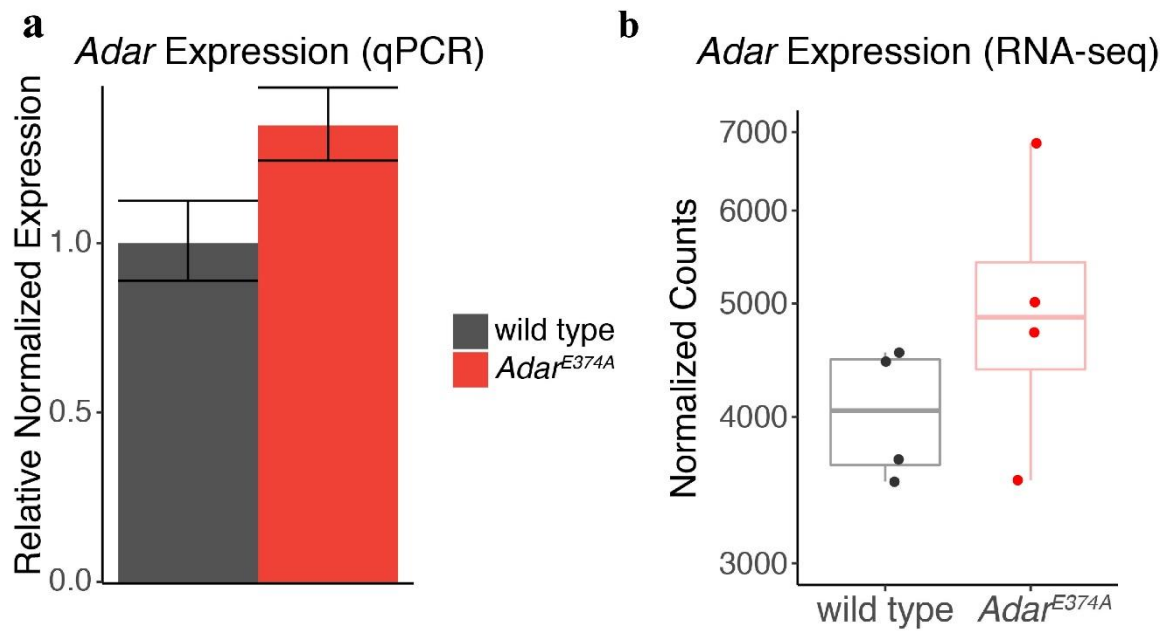
* Joint first author

Joint corresponding authors

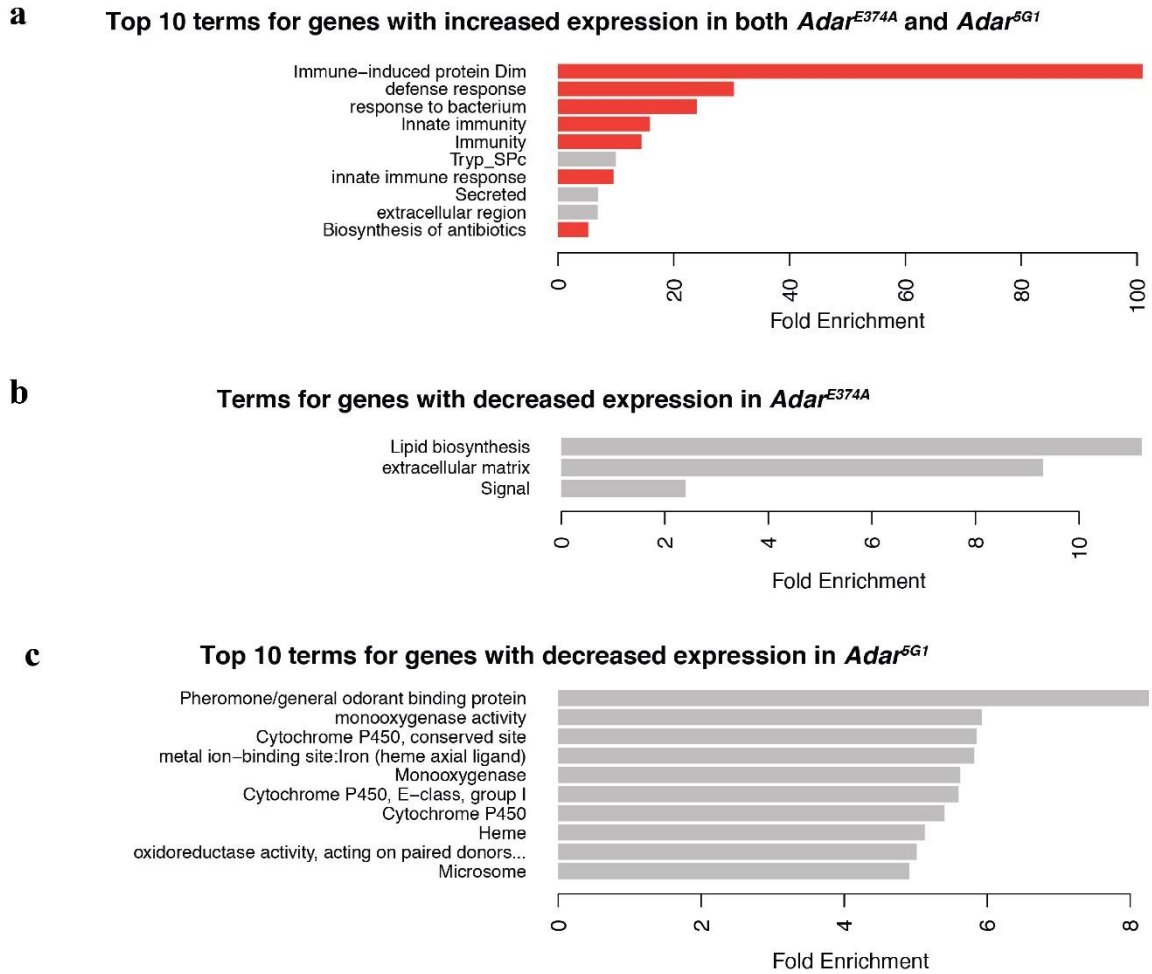
Correspondence should be addressed to L.P.K. (liam.keegan@ceitec.muni.cz) or J.B.L. (jin.billy.li@stanford.edu)

Running title: Inactive *Adar*

Key words: ADAR; RNA editing; *Drosophila*, neurodegeneration

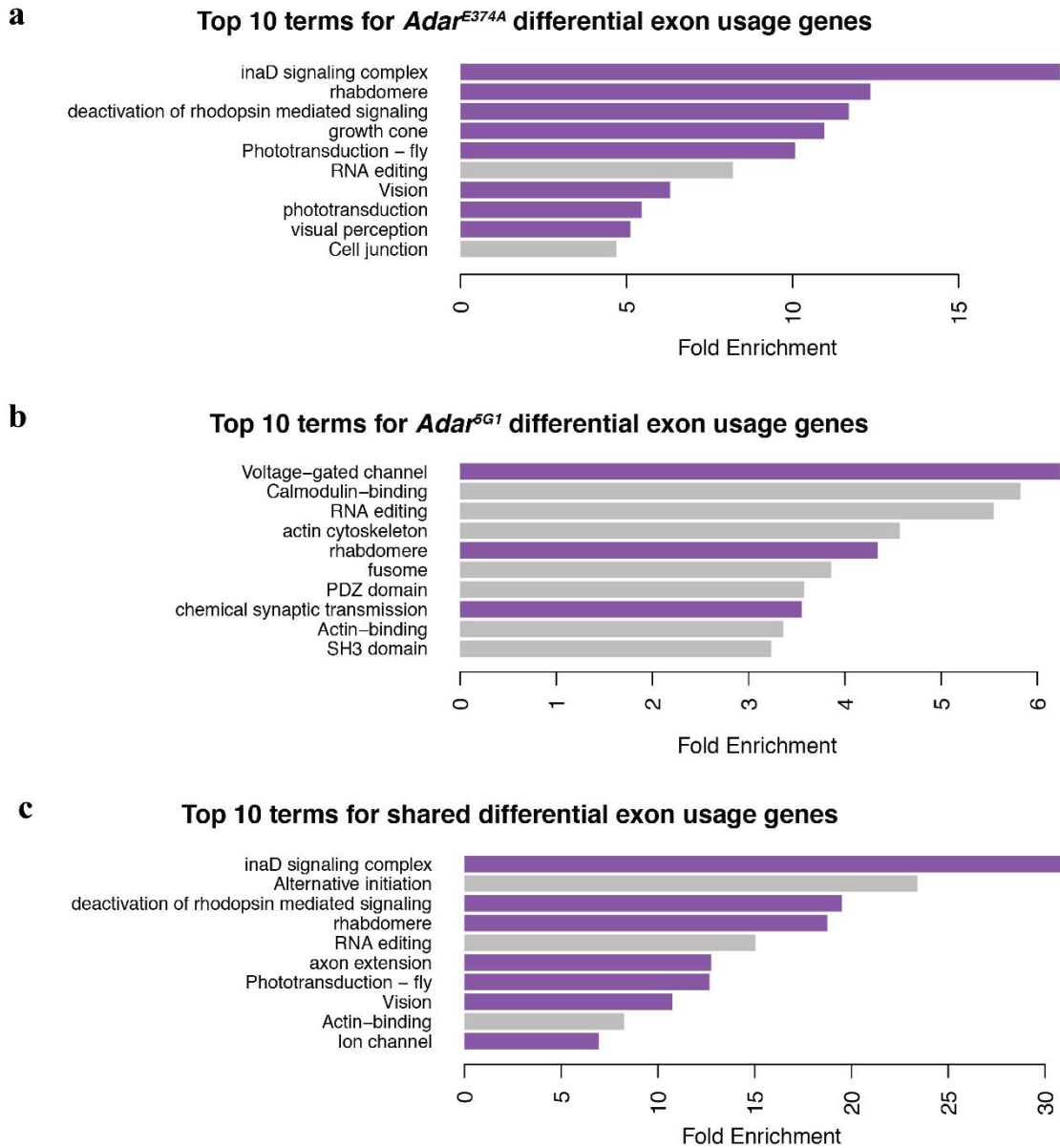


Supplementary Figure 1: Expression of *Adar* in *Adar*^{E374A} vs. wild type flies. (a) *Adar* gene expression from qPCR of 2-3 day old whole male flies. T-test p-value for *Adar*^{E374A} vs. wild type = 0.114. Error bars: SEM. (b) *Adar* gene expression from RNA-seq of 3 day old male fly heads (DESeq2). Boxplots are overlaid by points representing the biological replicates. The y-axis uses a log scale. (DESeq2 Wald test p-value = 0.105219 (Benjamini & Hochberg adjusted p-value = 0.5721788))



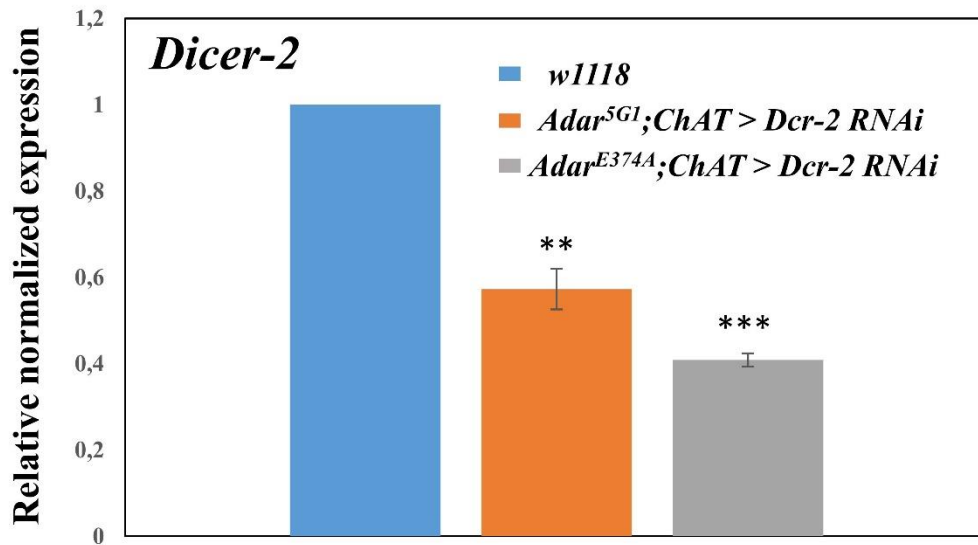
(No terms for genes with decreased expression in both *Adar*^{E374A} and *Adar*^{5G1})

Supplementary Figure 2: GO term enrichment for differentially expressed genes in *Adar* mutant RNAseq. Bars are colored red for immune-related terms. The terms used in the analysis are the DAVID default terms (biological process, molecular function, and cellular component GO terms, clusters of orthologous groups (COG), UniProt keywords and sequence features, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways, InterPro, Protein Information Resource (PIR) superfamily, and SMART). (a) The top 10 out of 15 significantly enriched terms for the genes with increased expression in both the *Adar*^{E374A} and *Adar*^{5G1} mutants. (DESeq2: 10% FDR; DAVID: log₂FoldChange >= 0.6, Benjamini adjusted p-value < 0.01) (b) The significantly enriched terms for the genes with decreased expression in the *Adar*^{E374A} mutant. (Same analysis parameters as in (a).) (c) The top 10 out of 17 significantly enriched terms for the genes with decreased expression in the *Adar*^{5G1} mutant. (Same analysis parameters as in (a)) There were no significantly enriched terms for genes with decreased expression in both the *Adar*^{E374A} and *Adar*^{5G1} mutants.

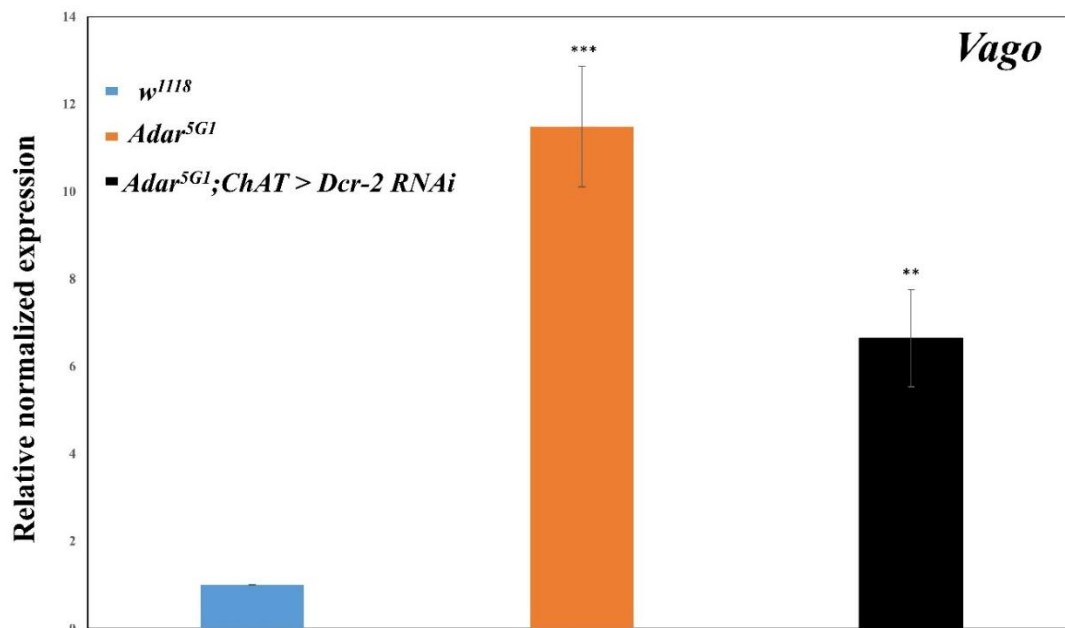


Supplementary Figure 3: GO term enrichment for genes with differential exon usage in *Adar* mutant RNAseq. Bars are colored purple for neurological-related terms. The terms used in the analysis are the DAVID default terms (biological process, molecular function, and cellular component GO terms, clusters of orthologous groups (COG), UniProt keywords and sequence features, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways, InterPro, Protein Information Resource (PIR) superfamily, and SMART). **(a)** The top 10 out of 21 significantly enriched terms for genes with differential exon usage in the *Adar*^{E374A} mutant. (DEXSeq; 10% FDR; DAVID analysis Benjamini adjusted p-value < 0.01) **(b)** The top 10 out of 35 significantly enriched terms for the genes with differential exon usage in the *Adar*^{5G1} mutant. **(c)** The top 10 out of 22 significantly enriched terms for the genes with differential exon usage in both the *Adar*^{E374A} and *Adar*^{5G1} mutants. (Same analysis parameters as in **(a)**)

a

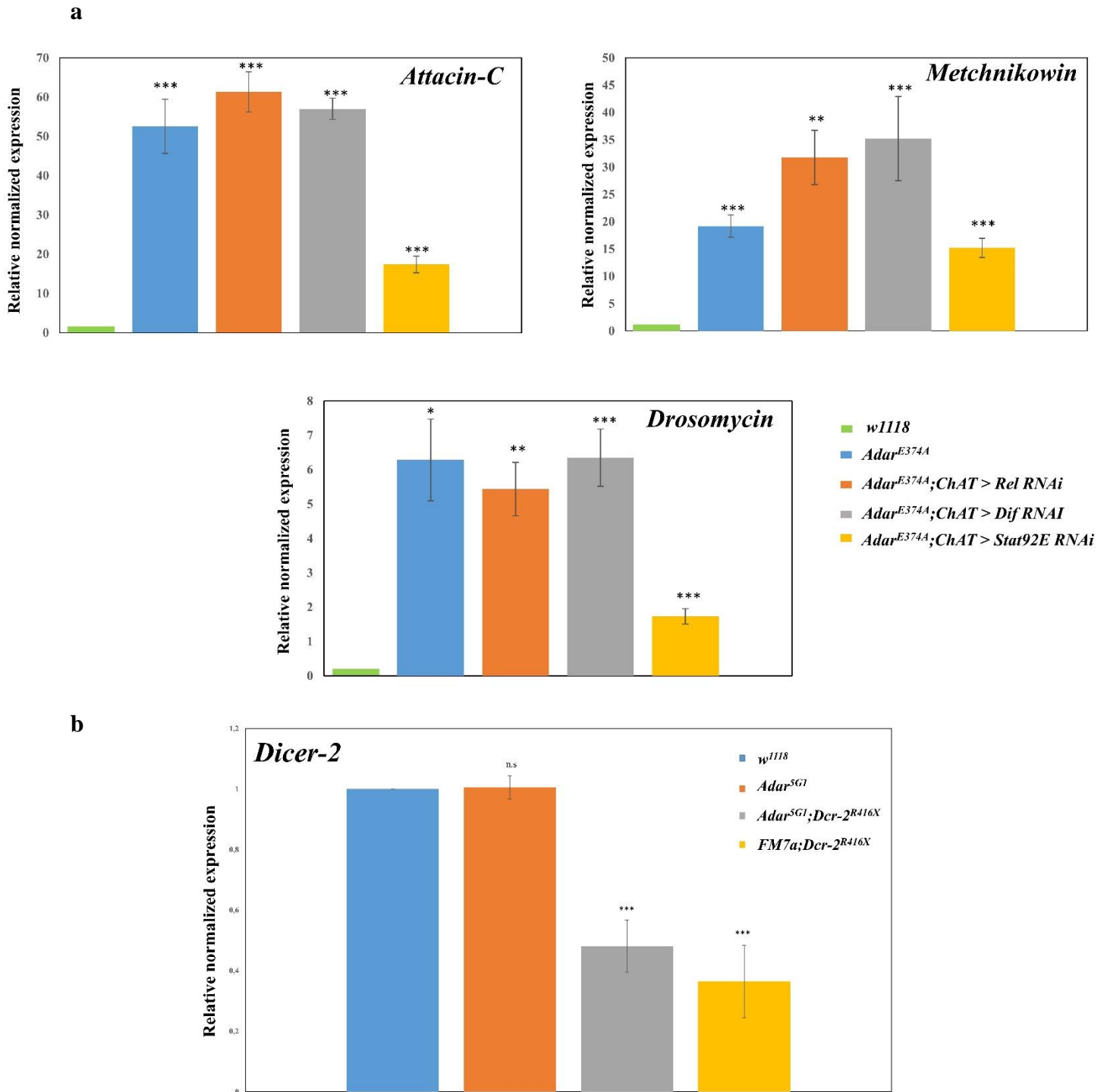


b



Supplementary Figure 4: Knocking down *Dicer-2* by RNAi in cholinergic neurons

rescues the *Vago* expression partially. **a.** qRT-PCR of *Dcr-2* transcripts in *Dcr-2* knockdown shows that the *Dcr-2* transcript is reduced compared to the wild type **b.** qRT-PCR of *Vago* transcripts in *Adar^{5G1}* null mutant and in *Adar^{5G1}; ChAT > Dcr-2 RNAi* shows that the aberrant *Vago* induction in the *Adar^{5G1}* is partially rescued by knocking down *Dcr-2*. *p*-values were calculated by Student's t-test *: *p*-value < 0.05. **: *p*-value < 0.01. ***: *p*-value < 0.005.: n.s – not significant. Error bars: SEM (Standard Error of Mean for biological replicates).



Supplementary Figure 5: Aberrant innate immune induction in the *Adar^{E374A}* mutant

also involves Jak-Stat signaling pathways. a Aberrant expression of AMP transcripts in head RNA of *Adar^{E374A}; ChAT > Rel RNAi*, *Adar^{E374A}; ChAT > Dif RNAi* or *Adar^{E374A}; ChAT > Stat92E RNAi* flies with RNAi knockdowns of *Rel*, *Dif* and *Stat92E* transcripts respectively, **b.** qRT-PCR of *Dcr-2* transcripts in *Dcr-2^{R416X}* double mutants shows that the *Dcr-2* transcript is expressed. Levels of each transcript in each of the different fly head RNA samples is compared to that in *w¹¹¹⁸*. *p*-values were calculated by Student's t-test *: *p*-value <

0.05. **: p-value < 0.01. ***: p-value < 0.005.: n.s – not significant. Error bars: SEM (Standard Error of Mean for biological replicates).

Supplementary Table 1: qPCR Primers

| Name | Sequence | Note | |
|----------------|-------------------------|------|--------------------|
| RP49_F | CCGCTTCAAGGGACAGTATC | | |
| RP49_R | GACAATCTCCTTGGCGTTCT | | |
| diptericin_F | ACCGCAGTACCCACTCAATC | | |
| diptericin_R | ACTTTCCAGCTCGGTTCTGA | | |
| IM3_F | ACTCGCCTTCGTTTTGGGTC | | |
| IM3_R | TTAGGCCCTCACATTGCAGA | | |
| IM1_F | TGCCCAGTGCACTCAGTATC | | |
| IM1_R | GATCACATTTCTGGATCGG | | |
| IM2_F | AAATACTGCAATGTGCACGG | | |
| IM2_R | ATGGTGCTTTGGATTTGAGG | | |
| CanA1_F | CCGCCAGTGGAACAACACAG | | From FlyPrimerBank |
| CanA1_R | GCGGAAGTGGAACATCATCG | | |
| IM2_F | AAATACTGCAATGTGCACGG | | |
| IM2_R | ATGGTGCTTTGGATTTGAGG | | |
| CG16836_F | TCAAGTCGCCGGAACCTTGA | | |
| CG16836_R | ATACACTTGTCCCGGCGTAG | | |
| CG15067_F | GAGCCTGACGTTATTGGCG | | |
| CG15067_R | CCTTTTCCACTTGTGGCTTGT | | |
| CG15068_F | TTCTCTTCGGACTTTTGGCTT | | |
| CG15068_R | AAACTTTGCAGTCGCCGTTTA | | |
| Adar_F | GGCTATAACCGAAAATTGCCACA | | |
| Adar_R | TGTCTTAGCTCATTGAGCATGG | | |
| Drosomycin_F | CTCCGTGAGAACCTTTTCCA | | |
| Drosomycin_R | ACAGGTCTCGTTGTCCCAGA | | |
| TotC_F | TACTATGCCTTGCCCTGCTC | | |
| TotC_R | CAGATTCCCTTTCTCGTCA | | |
| Pp2B-14D_F | TGGACATGGGTAAGCTGTGC | | |
| Pp2B-14D_R | CCAATTCAGACCTCGCCAGA | | |
| CanA-14F_F | TGGATGCCGTCAACGAACG | | |
| CanA-14F_R | CTGACAGTGATTTCTGGCCCTC | | |
| CG18107_F | TGGCCAATGCTATTCCGTTGT | | |
| CG18107_R | TTGCAATTCACACAGTCGCC | | |
| IM23_F | GCTACGGCCGGAATGTGA | | |
| IM23_R | GTCTGTAGGATTGGCCACC | | |
| Metchnikowin_F | TACATCAGTGCTGGCAGAGC | | |
| Metchnikowin_R | AATAAATTGGACCCGGTCTTG | | |
| Vago_F | CAGCCAAGCGATTCCTTATC | | |
| Vago_R | CTCATAACAGTGGGCAGCATC | | |
| Attacin-c_F | TTGGGTGGATCACTCACATC | | |
| Attacin-c_R | GCGTATGGGTTTTGGTCAGT | | |

| | | |
|-------------|-------------------------|--|
| Def_F | GCTATCGCTTTTGCTCTGCT | |
| Def_R | GGTGTGGTTCCAGTTCCACT | |
| Dcr-2_F | GCTTTTATGTGGGTGAACAGGG | |
| DCR-2_R | GGCTGTGCCAACAAGAACTT | |
| Attacin-D_F | AGTTTATGGAGCGGTCAACG | |
| Attacin-D_R | CGATCGGCTATGACTGTGAA | |
| Tot A_F | TTCAGCGTTCCAAAAAGTCA | |
| Tot A_R | CGATACTCTCCCGTTCCTCA | |
| Tot X_F | GCAGACAGGCAACAATTTGA | |
| Tot X_R | TATACCGGGTTCGACTCTG | |
| CG15065_F | ATGAAGTGGATGTCCTTGGTCT | |
| CG15065_R | CATTACCCGGATTTAACGGAGAA | |