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Supplementary Materials for

Drosophila Dicer-2 has an RNA interference–independent function that modulates Toll immune signaling

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The PDF file includes:

Figure S1

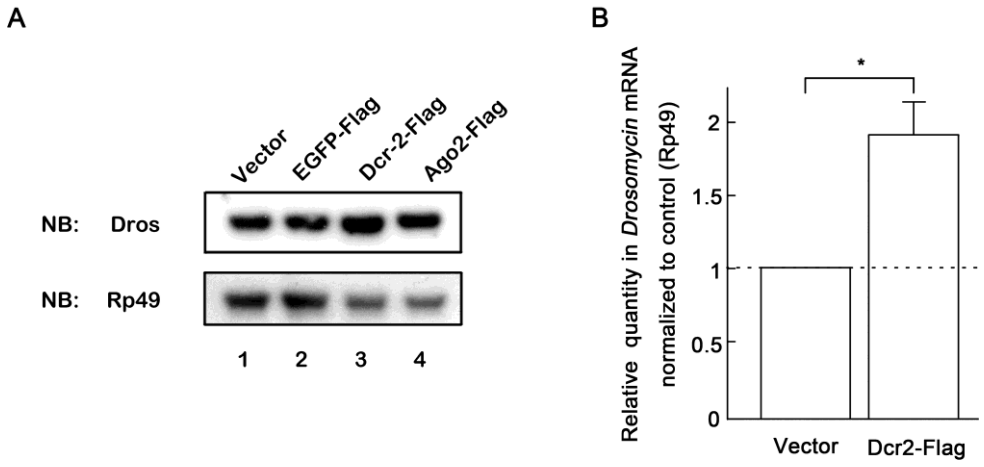

Fig. S1. Overexpression of Dcr-2 enhanced Toll signaling activation in *Drosophila* S2 cells. (A) Cultured S2 cells were transfected with empty vector (lane 1) or the plasmid expressing EGFP-Flag (lane 2), Dcr-2-Flag (lane 3), or AGO2-Flag (lane 4) as indicated. Total RNA extracts were prepared, followed by Northern blots using the indicated probes. (B) Cultured S2 cells were transfected with empty vector or the plasmid expressing Dcr-2-Flag as indicated. After that, total RNA extracts were prepared for qRT-PCR assay of *Dros* mRNA (normalized to Rp49; n=3; *, $P < 0.05$ by two-tailed Student's t test; error bars, s.d.).

Figure S2

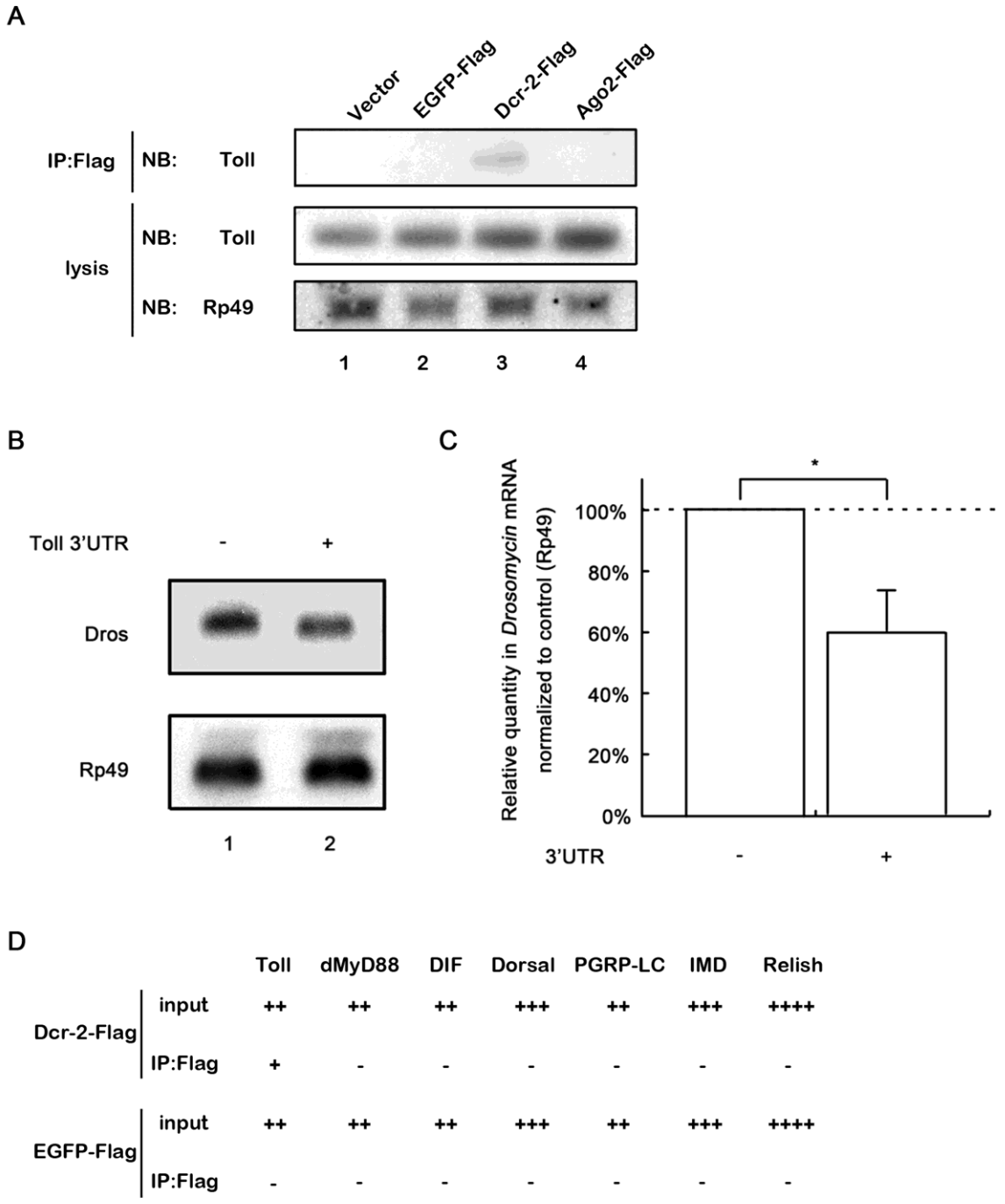

Fig. S2. *Toll* 3′UTR is important for Toll signaling. (A) Cultured S2 cells were transfected with empty vector (lane 1), or the plasmid expressing EGFP-Flag (lane 2), Dcr-2-Flag (lane 3), or AGO2-Flag (lane 4) as indicated. The crude cell lysates were prepared and subjected to RNA-IP using anti-Flag antibody. The RNA extracts prepared from the precipitates and total cell

lysates were subjected to Northern blots as indicated. (B-C) Cultured S2 cells were transfected with *in vitro* transcribed *Toll* 3′-UTR as indicated. Total RNA extracts were prepared for Northern blots using the indicated probes (B) or for qRT-PCR assay of *Dros* mRNA (normalized to Rp49; n=3; *, $P < 0.05$ by two-tailed Student's t test; error bars, s.d.) (C). (D) Cultured S2 cells were transfected with the plasmid expressing EGFP-Flag or Dcr-2-Flag. The crude cell lysates were prepared and subjected to RNA-IP using anti-Flag antibody. The RNA extracts prepared from the precipitates were subjected to qRT-PCR using the indicated pairs of primers. -, undetectable or Cq (quantification cycle) value≥37; +, 37>Cq value≥30; ++, 30>Cq value≥25; +++, 25>Cq value≥20; ++++, Cq value<20.

Figure S3

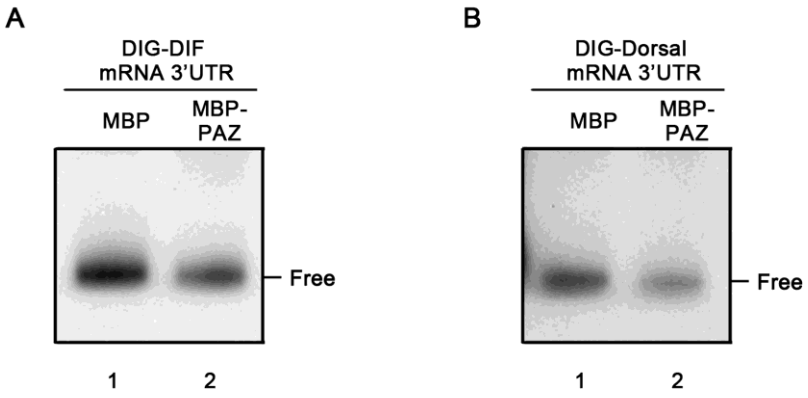

Fig. S3. The PAZ domain of Dcr-2 has no interaction with the 3′UTR of DIF or Dorsal in vitro. (A-B) Gel shift assays were performed to evaluate the capacity of MBP-PAZ to bind in vitro transcribed *dif* (A) or *dorsal* (B) 3′UTR as indicated.

Figure S4

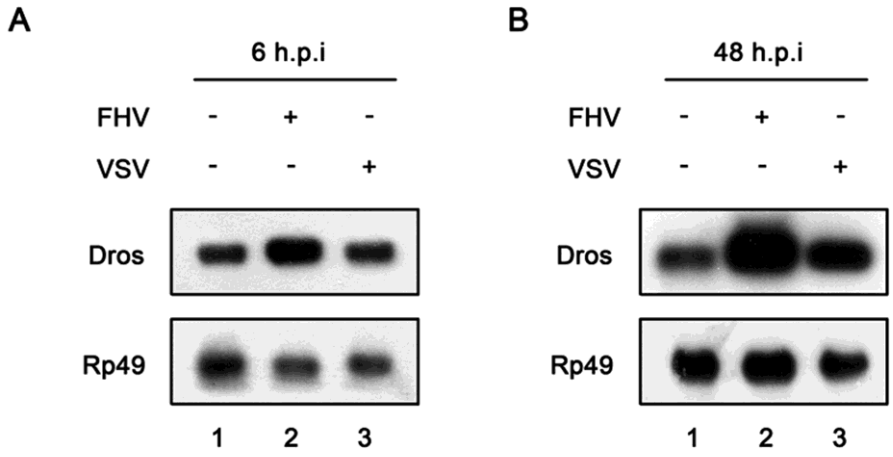

Fig. S4. Toll signaling can be induced by FHV or VSV. (A-B) Cultured S2 cells were infected with FHV or VSV for 6 hrs (A) or 48 hrs (B). After that, total RNA extracts were prepared, followed by Northern blots using the indicated probes.

Figure S5

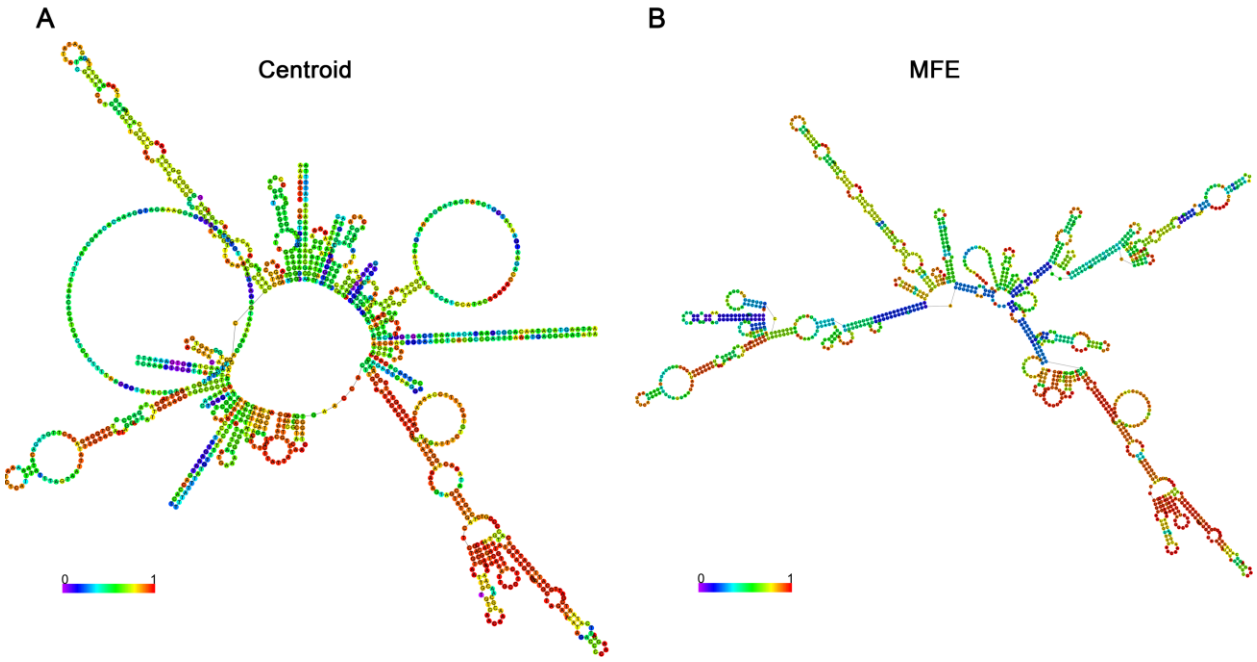

Fig. S5. The prediction of the secondary structure of *Toll* 3'UTR. (A-B) The secondary structure of *Toll* 3' UTR was predicted using two different algorithms, MFE (minimum free energy) (A) and Centroid (B) by RNAfold software.

Table S1	Primers used in this work	
primers	Sequence (5' to 3')[a,b]	Purpose
Dicer2 dsRNA #1 For	*TAATACGACTCACTATAGG*ATGGAAGATGTGGAAATCAAGCCTC	Construction of *in vitro*-transcription templates for preparing dsRNAs for RNAi
Dicer2 dsRNA #1 Rev	*TAATACGACTCACTATAGG*ACAATCGCATGAACTCACGAAATGG	
Dicer2 dsRNA #2 For	*TAATACGACTCACTATAGG*GAAACATCATTACCGTGTCCGACACC	
Dicer2 dsRNA #2 Rev	*TAATACGACTCACTATAGG*AGGCGTAGACTGGATGTAGTTGAGCAG	
AGO2 dsRNA #1 For	*TAATACGACTCACTATAGG*ATGGGAAAAAAAGATAAGAACAAGA	
AGO2 dsRNA #1 Rev	*TAATACGACTCACTATAGG*CACCTTGTTGACCTTGTTTAGTCCAC	
AGO2 dsRNA #2 For	*TAATACGACTCACTATAGG*ACGATCCCTACGGAGCCAGTTATAAC	
AGO2 dsRNA #2 Rev	*TAATACGACTCACTATAGG*GATTCAACCCATAAGATGAGC	
Toll dsRNA For	*TAATACGACTCACTATAGG*ATGAGTCGACTAAAGGCCGCTTCCGAGC	
Toll dsRNA Rev	*TAATACGACTCACTATAGG*CGGCAACTGTTCGATGCCATTATCGTG	
Dicer2 probe For	ACAAACGTGGATGTGCCAAAGGCGTTG	Construction of *in vitro*-transcription templates for preparing probes for Northern blots analysis
Dicer2 probe Rev	*TAATACGACTCACTATAGG*TTAGGCGTCGCATTTGCTTAGCTGC	
AGO2 probe For	GGGCAAGAAGGTGGCTACCAACAGCG	
AGO2 probe Rev	*TAATACGACTCACTATAGG*CGCTGTTGGTAGCCACCTTCTTGCCC	
Drosomycin probe For	ATAATTCAAACAGAAATCATTTACCAAGCTCCGTGAGAAC	
Drosomycin probe Rev	*TAATACGACTCACTATAGG*AATGTACATTAGTTTTGTTTATTAG	
Toll ORF probe For	TGCGTGAACGCCGAGATGCCAACACG	
Toll ORF probe Rev	*TAATACGACTCACTATAGG*GAGCTGGAACTTCTGAGGACCGTGCTC	
Toll 3'UTR probe For	TATGTGTATCGCACAGCAGATTCAAGGACCTTACAC	
Toll 3'UTR probe Rev	*TAATACGACTCACTATAGG*AAATTTTTAAAATATATTTTATTTGCGTTGTATG	
Toll 3'UTR RNA For	*TAATACGACTCACTATAGG*GACTGGGAGAAGGCGGAGCTGTTTCAG	Construction of *in vitro*-transcription templates for preparing Toll 3'UTR RNA
Toll 3'UTR RNA Rev	AAATTTTTAAAATATATTTTATTTGCGTTGTATG	
Rp49 qRT For	AAGAAGCGCACCAAGCACTTCATC	Primers used for qRT-PCR
Rp49 qRT Rev	TCTGTTGTCGATACCCTTGGGCTT	
Toll qRT For	TGCGTGCAGTGAGATGAGCATAGA	
Toll qRT Rev	TGATCTGCACGTAGTCTTTGGGCT	
Drosomycin qRT For	CGTGAGAACCTTTTCCAATATGATG	
Drosomycin qRT Rev	TCCCAGGACCACCAGCAT	

Diptericin qRT For	ACCGCAGTACCCACTCAATC	
Diptericin qRT Rev	CCCAAGTGCTGTCCATATCC	
dMyd88 qRT For	ATCTGGAACACTTCCTGGGC	
dMyd88 qRT Rev	CCACGAGAGCAGTCTGTCG	
Cactus qRT For	CTCACTAGCCACTAGCGGTAA	
Cactus qRT Rev	CCCGAATCACTGGTTTCGTTT	
Dorsal qRT For	ATCCGTGTGGATCCGTTTAA	
Dorsal qRT Rev	AATCGCACCGAATTCAGATC	
PGRP-LC qRT For	ACCCCACCAATTTGTCCTTTT	
PGRP-LC qRT Rev	CAGTACGATACCCAGCAGGAA	
DIF qRT For	GGAGCCGACAAGCAATATAATCC	
DIF qRT Rev	GTAGTTGCACACTTCGATGGT	
Relish qRT For	TGGATACCATCAAAATGGCCTG	
Relish qRT Rev	CTTGTACCGAAAGCGGAACTT	
Imd qRT For	AGGGACGCCTGGAAAAGGA	
Imd qRT Rev	GGATTCGGTCAGATCCGAGGA	
pMT Toll For	GGGGTACCGCCACCATGGATTACAAGGATGACGACGATAAGATGAGTCGACTAAAGGCCGC (*Kpn I*)	Construction of plasmids for expressing proteins in S2 cells
pMT Toll Rev	GCTCTAGATTATACGTCGCTCTGTTTGGCATTC (*Xba I*)	
pMT Dicer2 For	ATAAGAATGCGGCCGCGGCATGGAAGATGTGGAAATCAAGCCTC (*Not I*)	
pMT Dicer2 Rev	CGGGGTACCTTACTTATCGTCGTCATCCTTGTAATCGCCGGCGTCGCATTTGCTTAGCTGCTGA (*Kpn I*)	
pAc PAZ For	CGGAATTCGCCACCATGGAGTACACGGAACACATGTATTTAAATC (*EcoR I*)	
pAc PAZ Rev	GCTCTAGATTACTTATCGTCGTCATCCTTGTAATCGTTGAACTTTCCACGATTATGAACG (*Xba I*)	
pAc dsRBD For	CGGAATTCGCCACCATGGTGCCCATCAATCATATACGACAGC (*EcoR I*)	
pAc dsRBD For	GCTCTAGATTACTTATCGTCGTCATCCTTGTAATCGGCGTCGCATTTGCTTAGCTGCTGAAGG (*Xba I*)	
pGEX DExD/H For	GGAATTCAGCAATGGCATTGTCTACCTGCCCAC (*EcoR I*)	Construction of plasmids for expressing proteins in *E. coli*
pGEX DExD/H Rev	CGCTCGAGTTAAGCCCGACCCTTTGACTGCACGTAC (*Xho I*)	
pMAL PAZ For	CGGAATTCGAGTACACGGAACACATGTATTTAAATC (*EcoR I*)	
pMAL PAZ Rev	GCGTCGACTTAGTTGAACTTTCCACGATTATGAACG (*Sal I*)	
pMAL RNase III For	CCGGAATTCCGATATTTGTTGCAGGCCCTCACCCATCCCTC (*EcoR I*)	
pMAL RNase III Rev	CCCAAGCTTTTAGAGCTTTGCGTTCTCCGCT (*Hind III*)	
pMAL dsRBD For	CGGAATTCGTGCCCATCAATCATATACGACAGC (*EcoR I*)	
pMAL dsRBD Rev	GCGTCGACTTAGGCGTCGCATTTGCTTAGCTGCTGAAGG (*Sal I*)	

[a]Underlined letters indicate restriction endonuclease sites, and the types of the restriction endonucleases are shown in parentheses. The T7 polymerase promoter sequence is shown in italics.

[b]Sequence-specific primers are designed according to the sequences with GenBank accession numbers NM079054 (Dicer2), NM140518 (AGO2), NM079794 (Toll) and Y13939 (Rp49).