Supplemental Data

Weckle Is a Zinc Finger Adaptor of the Toll Pathway in Dorsoventral Patterning of the *Drosophila* Embryo

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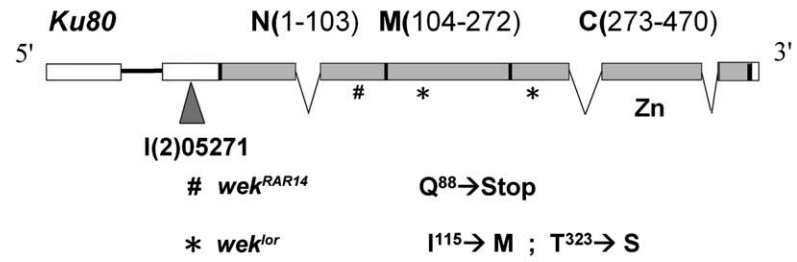
Supplemental Experimental Procedures

RT-PCR

The total RNA was extracted with Trizol reagent (Invitrogen) according to the instructions of the manufacturer. RNA (2 μg) was subjected to cDNA synthesis using the M-MLV Reverse Transcriptase (Invitrogen) and Oligo dT primers. Thirty-five cycles of PCR amplifications were performed with primer pairs from the first (5′-ATGGGAGTTCC CACAAGCGATTG-3′) and second (5′-GCTCAATTCGGATGTCTT GTC-3′) exon of *wek* genomic DNA, while 5′-ATGCGAGGACCTAA GAGTGG-3′ and 5′-ATGCACGATGCGCTGACTG-3′ were used for *Ku80* genomic DNA.

We isolated genomic DNA adjacent to the insertion site of P element *l(2)05271* by plasmid rescue (with XbaI) and inverse PCR (with MspI) using standard protocol (BDGP).

A



B

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KQDLEFLDIDDTEDRCSLVEELTISDHSTSPSPDFEAQTVRTRANLKQCNSDP 159
KVLASPTASIPEVETKRSRRQQFAAKRNSKVYTATESDDEEAILDEDEAVSPP 212
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          C2H2-1                                C2H2-2
QSSNFVDYPCKICNETFMSFMALRRHKHDMHGGPKKYVCDHCGKGLKTFTS 314
                    C2H2-3                      C2H2-4
LVEHQLVHTEEKPCICPVCNAGFKNKARLRVHSQTHGEPKFECNVCGKKLQ 365
                          C2H2-5
TRAILNKHKYVHTDERRFKCEVCGSGCKNSTALKIHLLGHTGLRPYVCKYCG 417
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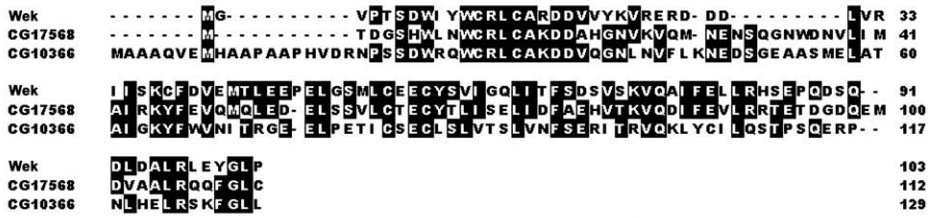
D

C



D

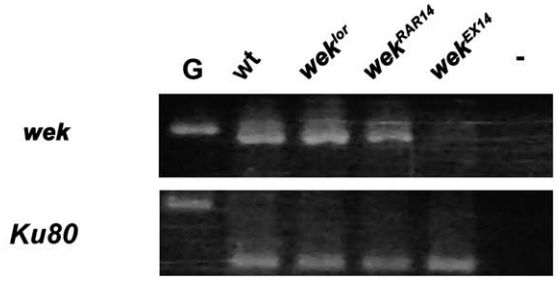


Figure S1. *wek* Encodes a Zinc Finger-Containing Protein

(A) Schematic representation of *wek* gene. The gray boxes represent the coding region of *wek*, whereas the white boxes represent the untranslated regions. The position of the P element *l(2)05271* was mapped to the 5′ untranslated region. The *Ku80* gene is positioned about 500 bps upstream of *wek*. The molecular lesions associated with wek^{RAR14} (marked by #) and wek^{Ior} (marked by *) are indicated.

(B) Amino acid sequence of Wek. *wek* encodes a protein of 470 amino acids that contains a N domain (aa 1–103), M domain (aa 104–272), and C domain (aa 273–470) with six zinc finger motifs.

(C) Alignment of N domain. The consensus sequence of N domain with *CG17568* and *CG10366* is shown.

(D) *wek* mRNA, but not adjacent *Ku80*, was specifically abolished in homozygous wek^{EX14} larvae as measured by RT-PCR. The outer lanes are the negative controls that contain no templates.

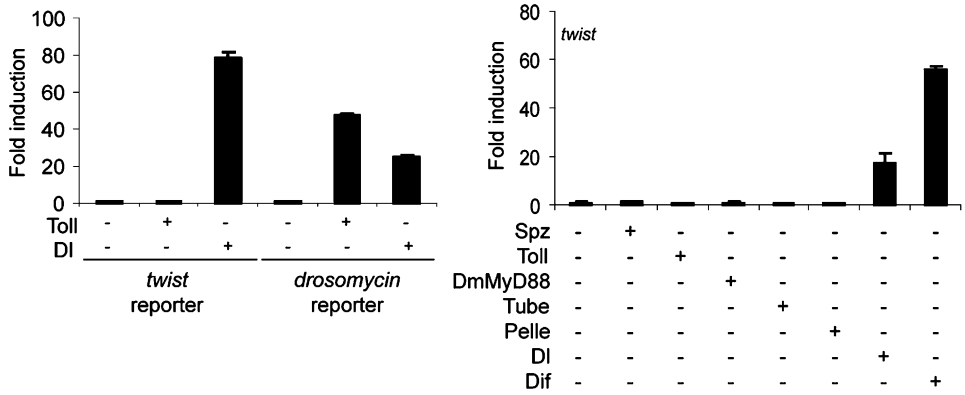


Figure S2. Different Regulation of *twist* and *drosomycin* Reporter Genes by Toll in S2 Cells

S2 cells were transfected with *twist*- or *drosomycin*-luciferase reporter constructs and the indicated expression vector. Dorsal induces both reporters, but Toll only induces the *drosomycin* reporter (left panel). Apart from Dorsal and Dif, no other genes of the Toll pathway activate the *twist* reporter in S2 cells (right panel). Data represent the mean ± standard deviation of triplicates.