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## Supplemental information

# Maintaining Toll signaling in *Drosophila* brain is required to sustain autophagy for dopamine neuron survival

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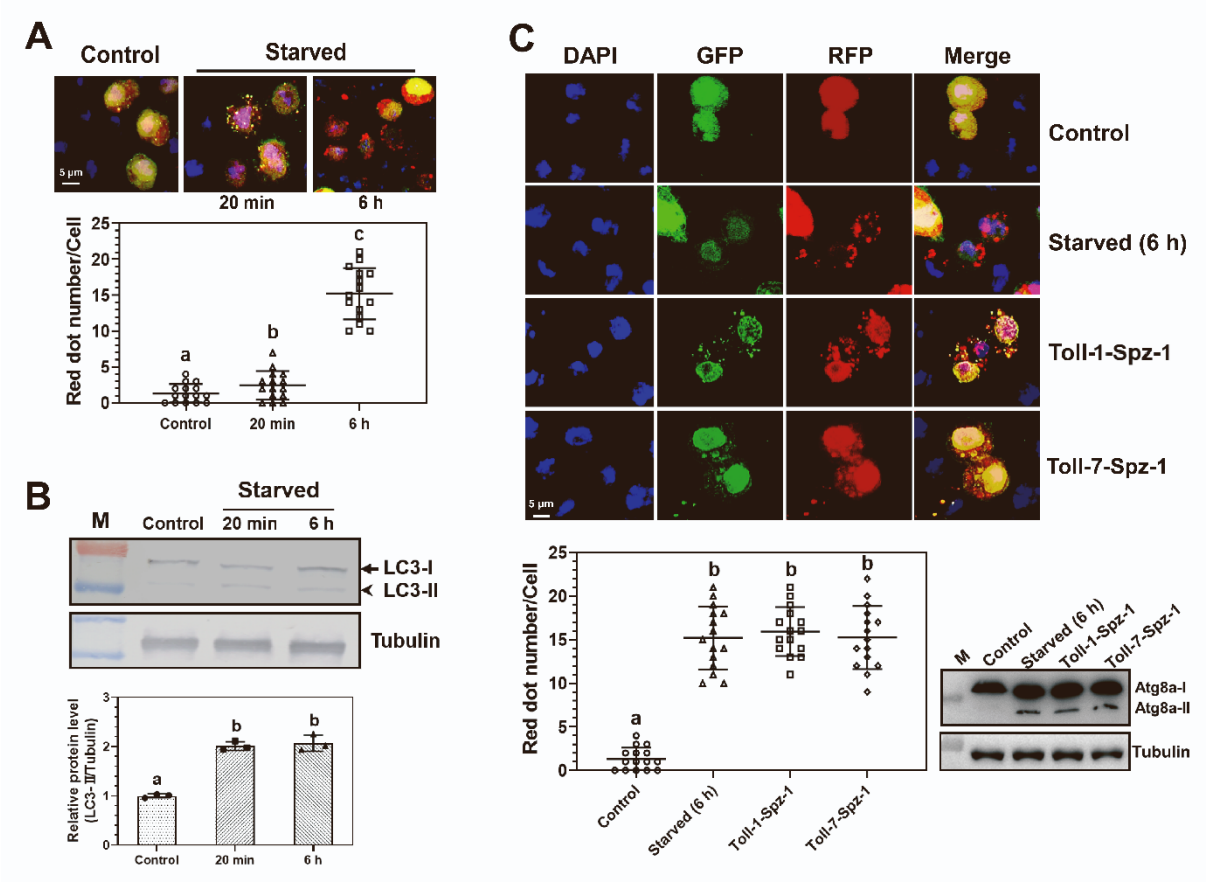


**Figure S1. Autophagy flux assay in the established S2 cells expressing RFP-GFP-LC3, related to Figure 1.**

**A** Detection of autophagy in S2 cells under starved conditions. **B** Detection of LC3-I and LC3-PE (LC3-II) protein levels in S2 cells under starved conditions by immunoblotting. **C** Detection of autophagy and Atg8a protein in S2 cells overexpressing Toll-1-Spz-1 and Toll-7-Spz-1 complexes. S2 cells stably expressing RFP-GFP-LC3 were established and used for autophagy flux assay. These S2 cells were cultured in complete medium (control), or in PBS instead of complete medium for 20 min or 6 h (starved), or co-transfected with pMT-Toll-1-V5 or pMT-Toll-7-V5 with pMT-Spz-1-Flag, autophagy in cells were detected by the presence of RFP (red) and GFP (green) fluorescent puncta in autophagosomes and autolysosomes. Nuclei were stained with DAPI and cells were observed under confocal microscope (**A** and **C**). In S2 cells starved for 20 min, both RFP (red) and GFP (green) fluorescence were detected in the autophagosomes, and thus yellow puncta were observed. In cells starved for 6 h or co-transfected with Toll-1 or Toll-7 with Spz-1, green fluorescence of GFP was quenched in the acidified autolysosomes, and thus only red (RFP) puncta were observed (**A** and **C**). Cells were also collected to prepare total protein samples for immunoblotting analysis (50 μg total proteins from each sample), LC3-I and LC3-PE (LC3-II) in these S2 cells were detected by rabbit anti-LC3 polyclonal antibody, endogenous Atg8a was detected by anti-Atg8 rabbit polyclonal antibody, and tubulin was detected by mouse anti-tubulin monoclonal antibody (**B** and **C**). Protein bands from at least 3 membranes were scanned for each protein (**B**) and red puncta in at least 20 cells were counted using ImageJ (**A** and **C**). Data were represented as means ± SEM. Significant difference was determined by one way ANOVA followed by a Tukey's multiple comparison tests using GraphPad Prism, with different letters indicating significant difference ($p < 0.05$) and identical letters for non-significant ($p > 0.05$).

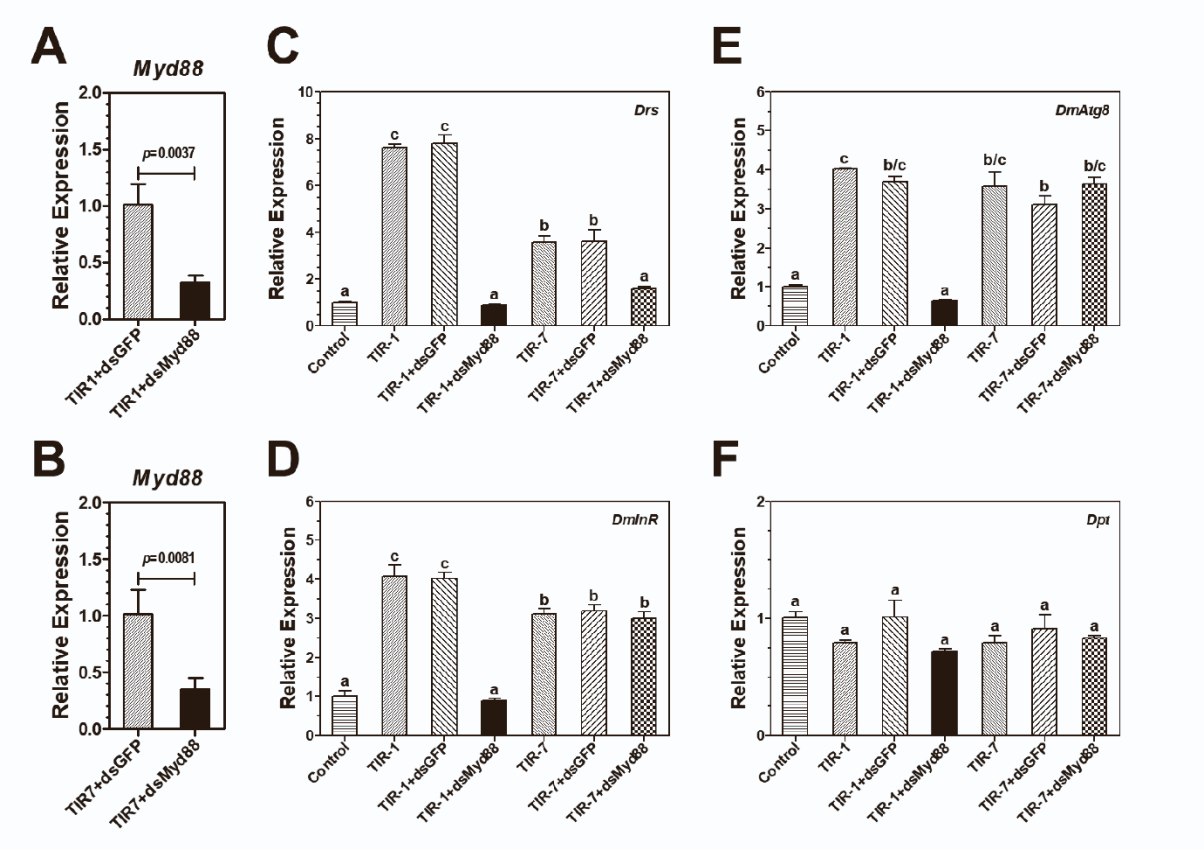


**Figure S2. TIR-1 but not TIR-7 activated expression of *DmAtg8a* and *DmInR* genes in S2 cells is dMyd88 dependent, related to Figure 4A.**

**A, B** Expression of *dMyd88* transcript after RNAi. **C-F** Expression of *drosomycin* (*Drs*), *DmInR*, *DmAtg8a* and *diptericin* (*Dpt*) transcripts after RNAi of *dMyd88*. S2 cells stably expressing TIR-1 or TIR-7 were established and used for the experiments. These S2 cells were transfected with dsRNA for GFP (dsGFP) or dMyd88 (dsMyd88), then expression of *dMyd88* (**A** and **B**), *drosomycin* (*Drs*) (**C**), *DmInR* (**D**), *DmAtg8a* (**E**), and *diptericin* (*Dpt*) (**F**) transcripts in the cells were determined by qRT-PCR. Data were represented as means ± SEM. Significant difference was determined by the student's t-test (**A** and **B**) and by one way ANOVA (**C**-**F**) (see Figure S1 legend).

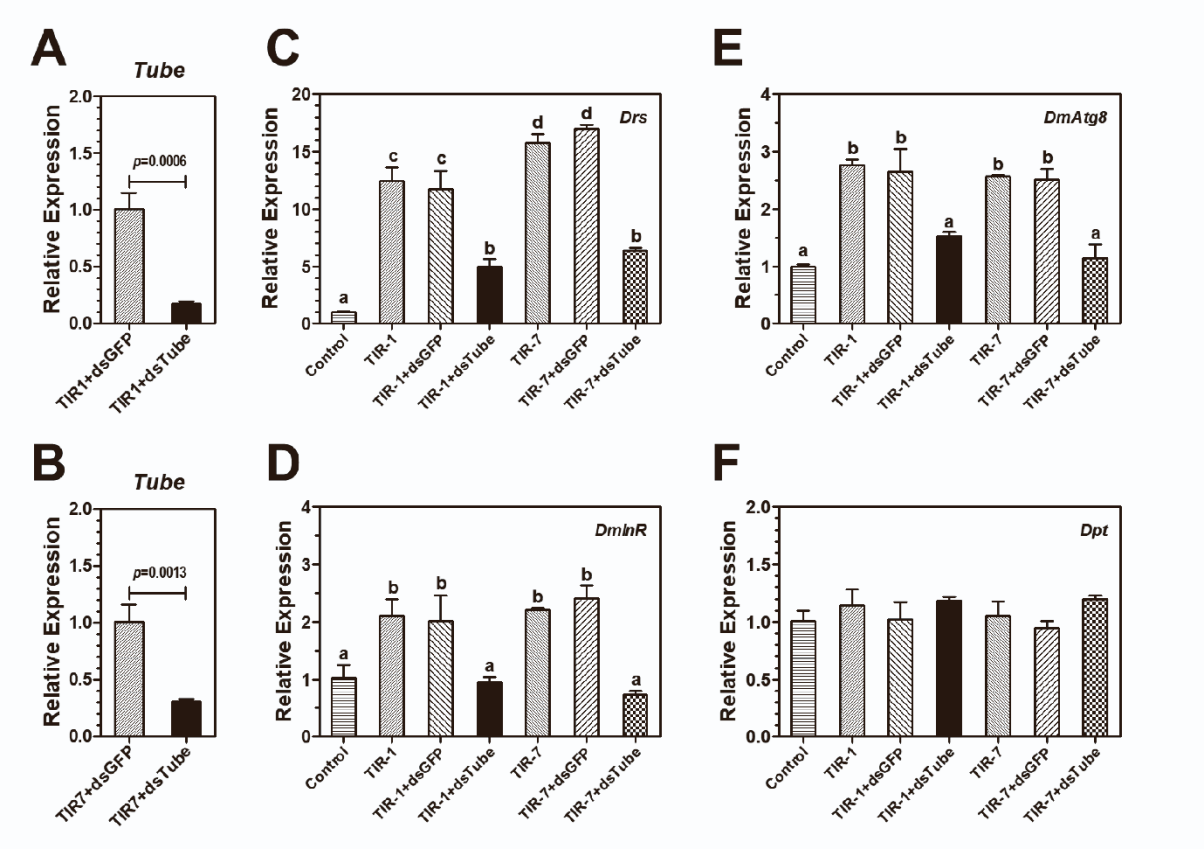


**Figure S3. TIR-1 and TIR-7 activated expression of *DmAtg8a* and *DmInR* genes in S2 cells is Tube dependent, related to Figure 4B.**

**A, B** Expression of *Tube* transcript after RNAi. **C-F** Expression of *Drs*, *DmInR*, *DmAtg8a* and *Dpt* transcripts after RNAi of *Tube*. S2 cells stably expressing TIR-1 or TIR-7 were transfected with dsRNA for GFP (dsGFP) or Tube (dsTube), then expression of Tube (**A** and **B**), *Drs* (**C**), *DmInR* (**D**), *DmAtg8a* (**E**), and *Dpt* (**F**) transcripts in these S2 cells were determined by qRT-PCR. Data were represented as means ± SEM. For determination of significant differences, see Figure S1 and Figure S2 legends.

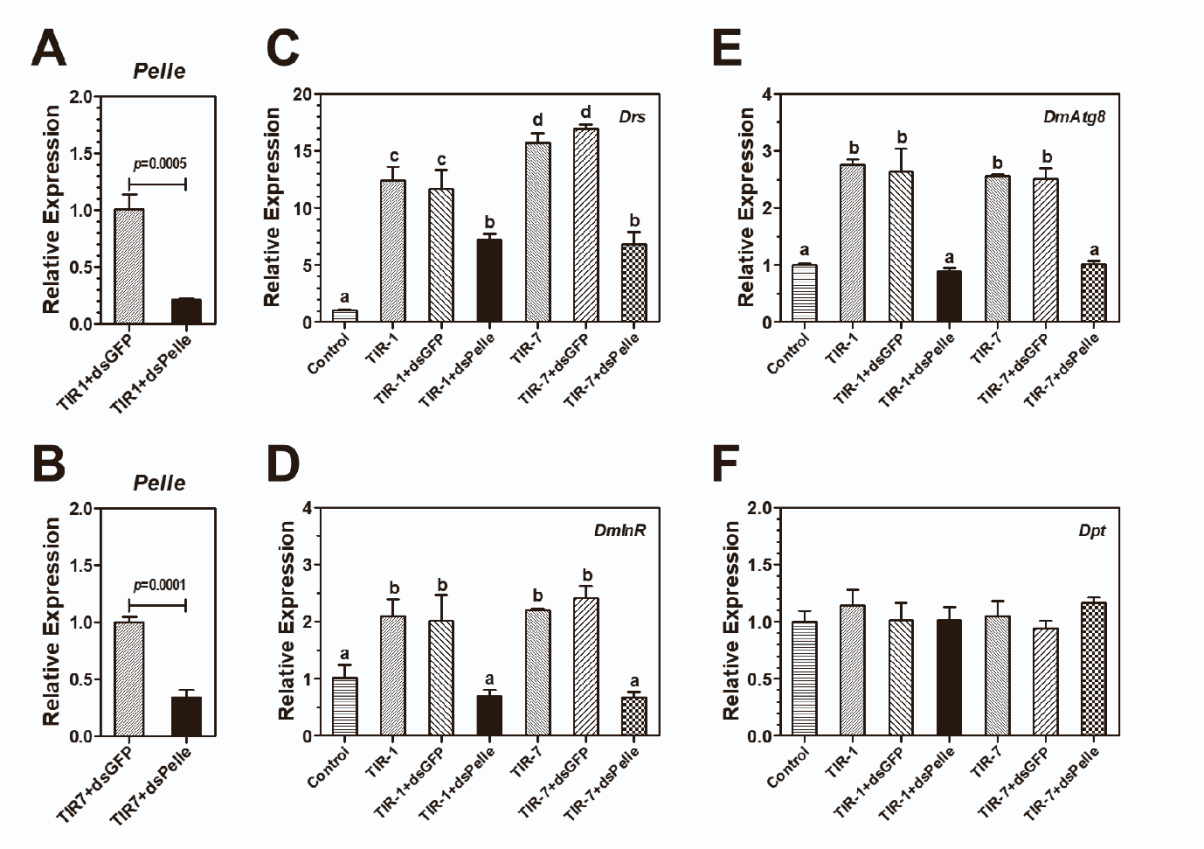


**Figure S4. TIR-1 and TIR-7 activated expression of *DmAtg8a* and *DmInR* genes in S2 cells is Pelle dependent, related to Figure 4C**.

**A, B** Expression of *Pelle* transcript after RNAi. **C-F** Expression of *Drs*, *DmInR*, *DmAtg8a* and *Dpt* transcripts after RNAi of *Pelle*. S2 cells stably expressing TIR-1 or TIR-7 were transfected with dsRNA for GFP (dsGFP) or Pelle (dsPelle), then expression of Pelle (**A** and **B**), *Drs* (**C**), *DmInR* (**D**), *DmAtg8a* (**E**), and *Dpt* (**F**) transcripts in these S2 cells were determined by qRT-PCR. Data were represented as means ± SEM. For determination of significant differences, see Figure S1 and Figure S2 legends.

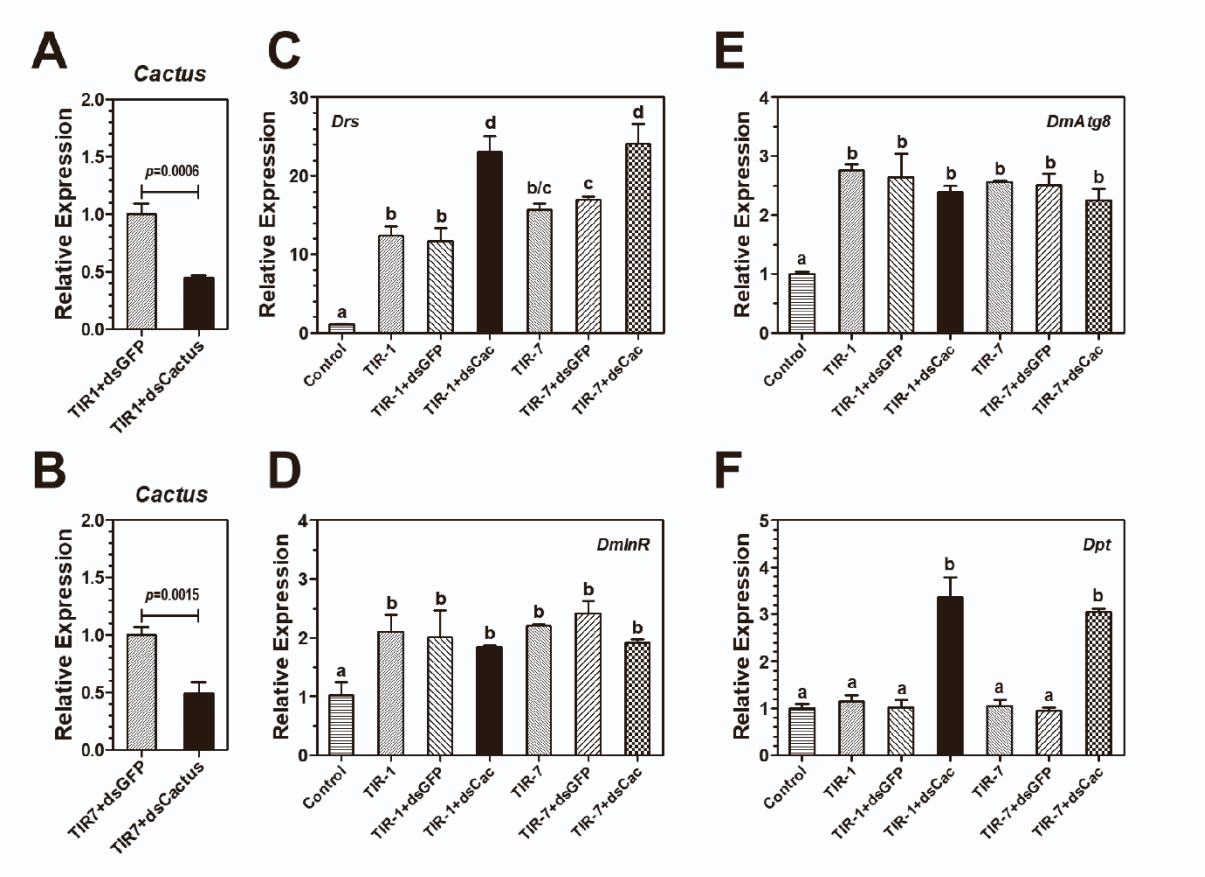


**Figure S5. TIR-1 and TIR-7 activated expression of *DmAtg8a* and *DmInR* genes in S2 cells is Cactus independent, related to Figure 4D.**

**A, B** Expression of *Cactus* transcript after RNAi. **C-F** Expression of *Drs*, *DmInR*, *DmAtg8a* and *Dpt* transcripts after RNAi of *Cactus*. S2 cells stably expressing TIR-1 or TIR-7 were transfected with dsRNA for GFP (dsGFP) or Cactus (dsCactus), then expression of Cactus (**A** and **B**), *Drs* (**C**), *DmInR* (**D**), *DmAtg8a* (**E**), and *Dpt* (**F**) transcripts in these S2 cells were determined by qRT-PCR. Data were represented as means ± SEM. For determination of significant differences, see Figure S1 and Figure S2 legends.

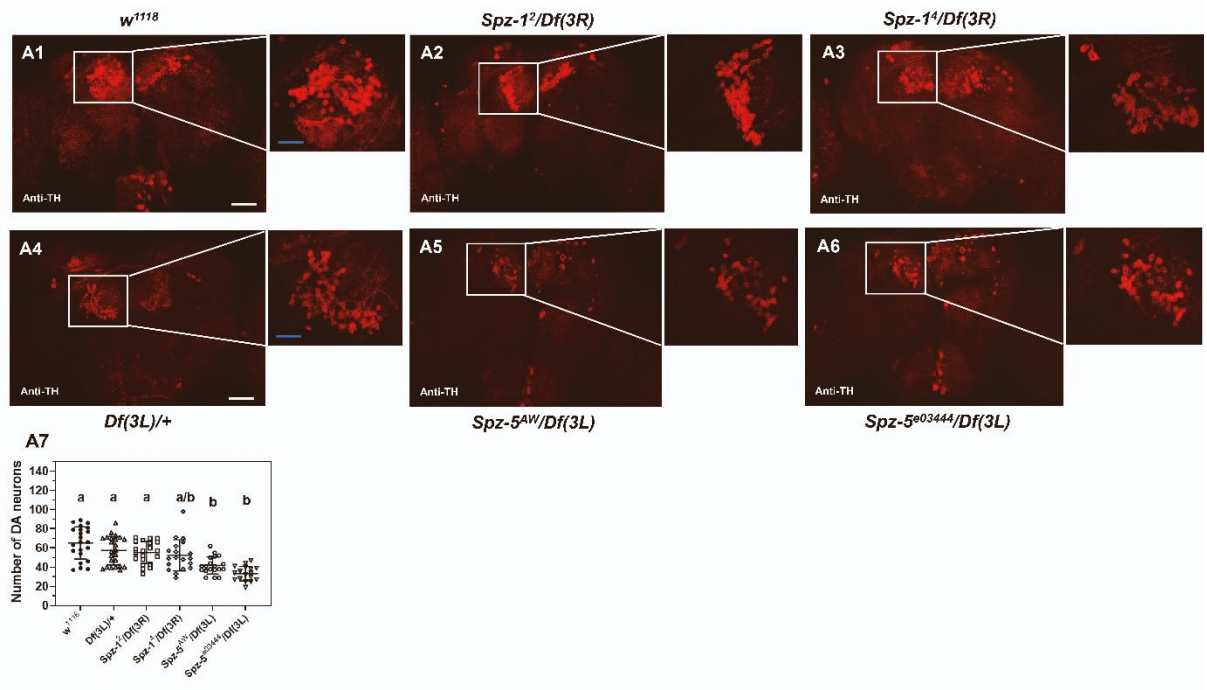


**Figure S6. DA neurons in the brains of *Spz-1* and *Spz-5* mutant flies, related to Figure 7.**

Brains from *w*$^{1118}$, *Df(3L)*/+, *Spz-1*$^{2}$*/Df(3R)* and *Spz-1*$^{4}$*/Df(3R)* (loss of function Spz-1 mutants), *Spz-5*$^{AW}$*/Df(3L)* and *Spz-5*$^{e03444}$*/Df(3L)* (loss of function Spz-5 mutants) flies were dissected, and DA neurons in the brain were labeled with anti-TH antibody. DA neurons were counted in at least 20 brains for each fly line by ImageJ (A7), data were represented as means ± SEM, and significant difference was determined by one way ANOVA (see Figure S1 legend). Scale bar: 50 μm in A1 to A6, 20 μm in the amplified sections.

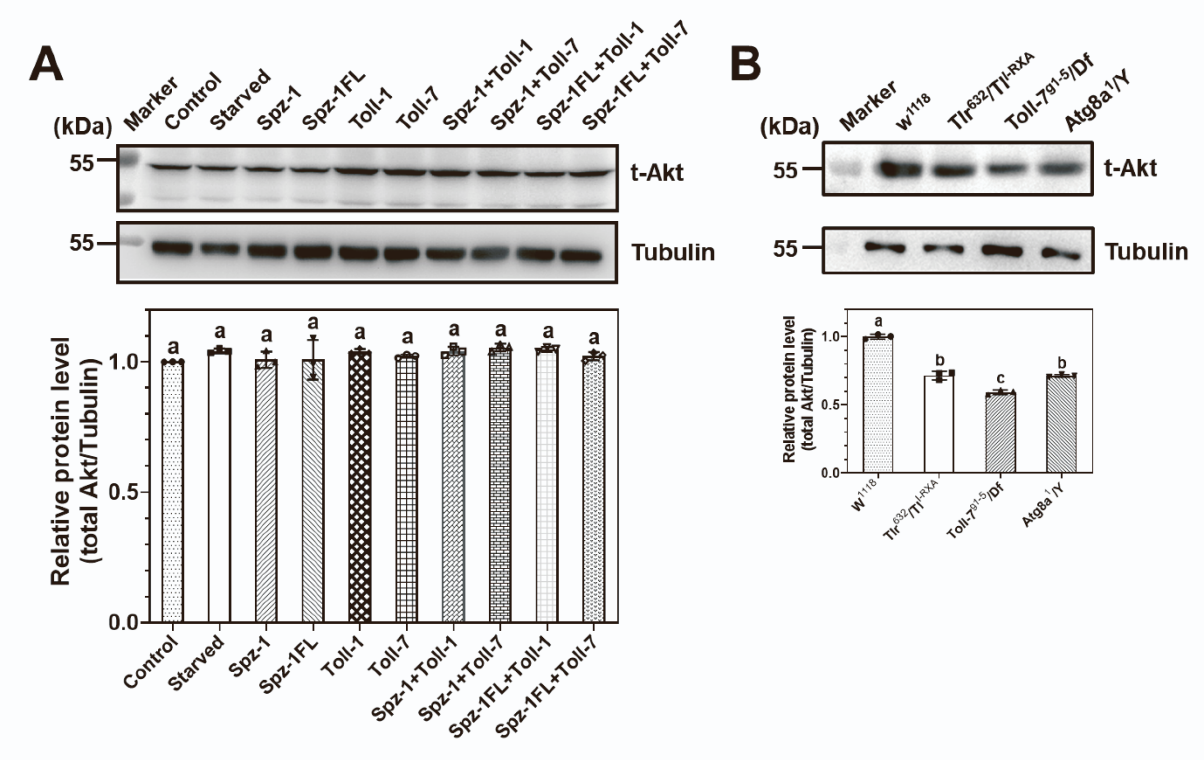


**Figure S7. Total Akt protein level analysis of Fig. 1G and Fig. 2B, related to Figures 1G and 2B.** **A** Detection of total Akt protein (t-Akt) in S2 cells overexpressing Toll-1/-7, Spz-1, or Toll-1/-7 plus Spz-1 by immunoblotting. **B** Detection of t-Akt in the brains of *w*$^{1118}$ and *Toll-1/-7* mutant flies (Also see Figures 1 and 2 legends). Protein bands from at least 3 membranes were scanned for each protein using ImageJ, relative protein level was calculated by dividing the gray value of total Akt band by the gray value of tubulin band. Data were represented as means ± SEM. Significant difference was determined by one way ANOVA (see Figure S1 legend).

| Name | Sequence (5'→ 3') |
|---|---|
| dsEGFP-F | GGATCCTAATACGACTCACTATAGGGAGAATGGTGAGCAAGGGCG |
| dsEGFP-R | GGATCCTAATACGACTCACTATAGGGAGACTTGTACAGCTCGTCC |
| dsMyd88-F | GGATCCTAATACGACTCACTATAGGGAGCAAGGACACCCAGCGCTTCAT |
| dsMyd88-R | GGATCCTAATACGACTCACTATAGGGAGCTGCACCTGGCGCGTGGA |
| dsTube-F | GGATCCTAATACGACTCACTATAGGGAGAGAGTGGAAGACCTCTGGCAAG |
| dsTube-R | GGATCCTAATACGACTCACTATAGGGAGACTGAATTTGTTCGCTGGGATTG |
| dsPelle-F | GGATCCTAATACGACTCACTATAGGGAGACACAAGTACATACCGAGGAG |
| dsPelle-R | GGATCCTAATACGACTCACTATAGGGAGAGCTGATGCTAAACCGCTGCT |
| dsCactus-F | GGATCCTAATACGACTCACTATAGGGAGACCACGTCCACTGATCCCGAAATAC |
| dsCactus-R | GGATCCTAATACGACTCACTATAGGGAGAAAAGCAGCGGAGGCAGCAACAAAG |
| dsPP2A-F | GGATCCTAATACGACTCACTATAGGGAGACCATCATCGAGTACATGCCT |
| dsPP2A-R | GGATCCTAATACGACTCACTATAGGCTGTGCAGCAAAATGCTTGA |

**Table S1. Primers used for double-stranded RNA (dsRNA) synthesis, related to STAR Methods.**

| Name | Sequence (5' → 3') |
|---|---|
| DmMyd88-F | AACGAGACCCCATTATCCGC |
| DmMyd88-R | ATCCATGGGATTGTTGGCGT |
| DmTube-F | AACTCTCGACCAAATCACGCTCCA |
| DmTube-R | AAGGTCTCCCTGCTGCCTTTACTT |
| DmPelle-F | TGGACGCTGTGGTGGAAGTGAATA |
| DmPelle-R | TGAACACCTCCAACAGCACAATGC |
| DmCactus-F | TTTCGGGATCAGTGGACGTG |
| DmCactus-R | TCAGCTCCGGCGAGTAAAAG |
| DmPP2A-F | TGTCACAGTCGCTTCTGCCC |
| DmPP2A-R | GCTGCCTCACGAATGGCGTA |
| DmInR-F | TGAGCATGTGGAGCACATCAAGATG |
| DmInR-R | CGTAGGAGATTTTCTCGTTTGGCTG |
| DmAtg8a-F | CATCGGTGATTTGGACAAGA |
| DmAtg8a-R | TGCCGTAAACATTCTCATCG |
| DmDrs-F | CGTGAGAACCTTTTCCAATATGATG |
| DmDrs-R | TCCCAGGACCACCAGCAT |
| DmDpt-F | GCTGCGCAATCGCTTCTACT |
| DmDpt-R | TGGTGGAGTGGGCTTCATG |
| DmRp49-F | GCCCAAGGGTATCGACAACA |
| DmRp49-R | ACCTCCAGCTCGCGCACGTT |

**Table S2. Primers used for quantitative real-time PCR, related to STAR Methods.**