iScience, Volume *26*

Supplemental information

Drosophila Toll-9 is induced by aging and neurodegeneration to modulate stress signaling and its deficiency exacerbates tau-mediated neurodegeneration

Yasufumi Sakakibara, Risa Yamashiro, Sachie Chikamatsu, Yu Hirota, Yoko Tsubokawa, Risa Nishijima, Kimi Takei, Michiko Sekiya, and Koichi M. Iijima

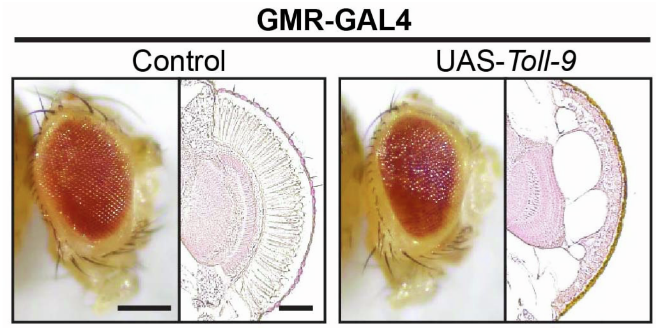

Figure S1. Developmental overexpression of *Toll-9* by the GMR-GAL4 driver caused neurodegeneration in fly eyes, Related to Figure 3.

Developmental overexpression of *Toll-9* by the GMR-GAL4 driver caused glazed eyes. Eyes of female flies carrying the GMR-GAL4 driver alone (Control) or carrying both the GMR-GAL4 driver and UAS-*Toll-9* are shown (left panel). The right panel shows the hemisphere in paraffin-embedded brain sections with HE staining. All scale bars represent 200 μm.

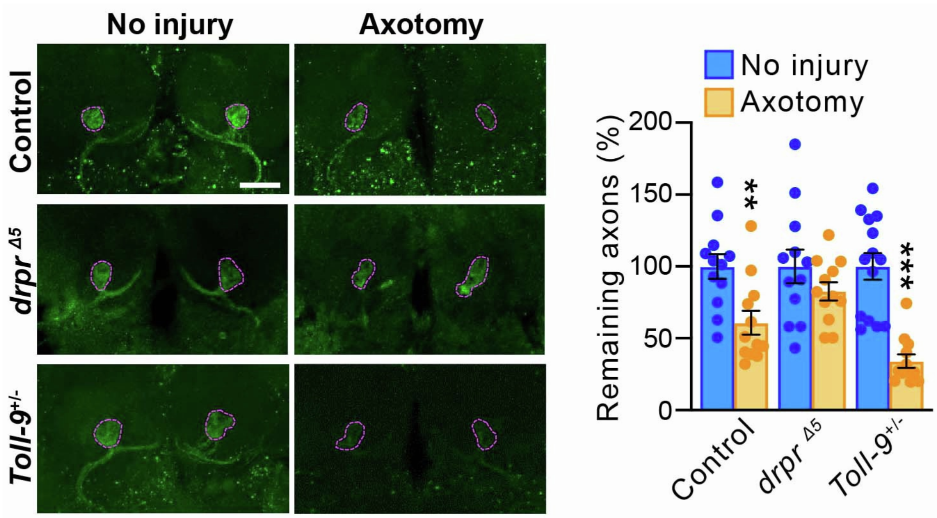

Figure S2. ***Toll-9*** **deficiency does not affect glial engulfment of degenerating neurons in fly brains, Related to Figure 4.**

Representative Z-stack projections of OR85e GFP+ axons (dotted line) from the groups with No injury or Axotomy of heterozygous KO of *Toll-9* (*Toll-9* $^{+/-}$) and *drpr* null mutant flies (*drpr*$^{\Delta 5}$) as a positive control are shown (left panels). Scale bars represent 25 µm. The right graph shows the percentages of GFP fluorescence normalized by the value of fluorescence intensity from the group with No injury. Mean ± SEM, n = 12–14 (Control: No injury n = 12, Axotomy n = 12; *Toll-9*$^{+/-}$ No injury n = 14, Axotomy n = 12; *drpr*$^{\Delta 5}$: No injury n = 12, Axotomy n = 12); $^{**}p < 0.01$ and $^{***}p < 0.001$ by Welch's *t*-test vs. No injury.

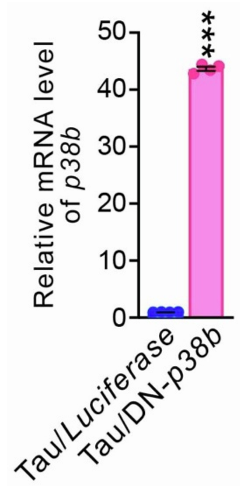

Figure S3. The mRNA expression levels of *p38b* in fly head expressing the dominant negative form of p38b, Related to Figure 6.

Overexpression of DN-*p38b* under the control of GMR-GAL4 driver significantly increased the mRNA level of *p38b* in fly heads. The mRNA levels of *p38b* in heads of flies carrying the GMR-GAL4 driver, UAS-human tau and UAS-*Luciferase* (Tau/*Luciferase*) or carrying the GMR-GAL4 driver, UAS-human tau and UAS-DN-*p38b* (Tau/DN-*p38b*) were analyzed by qRT-PCR. Mean ± SEM, n = 4 (technical replicate); ****p* < 0.001 by Student's *t*-test vs. Tau/L*uciferase*.

Table S1. Genotype and age of flies used in this study, Related to STAR Methods.

Figure #	Label	Genotype	Age
Figure 1	Control (7-day-old, 40-day-old) 40-day-old Control	+/Y	Fig. 1A, B, C (described in figures), D, E, F (40d).
	40-day-old *Toll-9* KO	+/Y;;Toll-9$^-$/Toll-9$^-$	Fig. 1D, E, F (40d).
Figure 2	Control (repo-GAL4)	+/Y;;repo-GAL4/+	Fig. 2A (14d).
	Toll-9 RNAi #1 (repo-GAL4)	+/Y;;repo-GAL4/UAS-Toll-9 RNAi	Fig. 2A (14d).
	Toll-9 RNAi #2 (repo-GAL4)	+/Y;;repo-GAL4/UAS-Toll-9 RNAi	Fig. 2A (14d).
	40-day-old Control	+/Y;;repo-GAL4/UAS-mCherry RNAi	Fig. 2B, C, D (40d).
	40-day-old repo-GAL4>*Toll-9* RNAi #1	+/Y;;repo-GAL4/UAS-Toll-9 RNAi	Fig. 2B, C, D (40d).
	40-day-old repo-GAL4>*Toll-9* RNAi #2	+/Y;;repo-GAL4/UAS-Toll-9 RNAi	Fig. 2B, C, D (40d).
Figure 3	Control (Male)	+/Y	Fig. 3A (described in figures).
	Toll-9 KO (Male)	+/Y;;Toll-9$^-$/Toll-9$^-$	Fig. 3A (described in figures).
	Control (Female)	+/+	Fig. 3A (described in figures).
	Toll-9 KO (Female)	+/+;;Toll-9$^-$/Toll-9$^-$	Fig. 3A (described in figures).
	Control, Control 1% H_2O_2, Control 5% H_2O_2	+/Y	Fig. 3B (described in figures).
	Toll-9 KO, *Toll-9* KO 1% H_2O_2, *Toll-9* KO 5% H_2O_2	+/Y;;Toll-9$^-$/Toll-9$^-$	Fig. 3B (described in figures).
	Control (female)	+/+	Fig. 3C (55d).
	Toll-9 KO (female)	+/+;;Toll-9$^-$/Toll-9$^-$	Fig. 3C (55d).
	Control (male)	+/Y	Fig. 3C (55d).
	Toll-9 KO (male)	+/Y;;Toll-9$^-$/Toll-9$^-$	Fig. 3C (55d).
	Control (repo-GAL4)	+/Y;;repo-GAL4/+	Fig. 3D (50d).
	Toll-9 RNAi #2 (repo-GAL4)	+/Y;;repo-GAL4/UAS-Toll-9 RNAi	Fig. 3D (50d).
	Control (Rh1-GAL4)	Rh1-GAL4/+	Fig. 3E (60d).
	UAS-*Toll-9* (Rh1-GAL4)	Rh1-GAL4/+;UAS-Toll-9/+	Fig. 3E (60d).
	RU (-), RU (+) (GeneSwitch GMR-GAL4; UAS-*Toll-9*)	GeneSwitch GMR-GAL4/Y;UAS-Toll-9/+	Fig. 3F (30d), G (55d).
Figure 4	Control (No injury, Axotomy)	+/Y	Fig. 4B, C, D (7d).
	Toll-9 KO	+/Y;;Toll-9$^-$/Toll-9$^-$	Fig. 4C, D (7d).
	mCherry RNAi (repo-GAL4)	repo-GAL4/Y;Or85e-mCD8::GFP/+; UAS-mCherry RNAi/+	Fig. 4E (7d).
	Toll-9 RNAi #1 (repo-GAL4)	repo-GAL4/Y;Or85e-mCD8::GFP/+; UAS-Toll-9 RNAi/+	Fig. 4E (7d).
	Toll-9 RNAi #2 (repo-GAL4)	repo-GAL4/Y;Or85e-mCD8::GFP/+; UAS-Toll-9 RNAi/+	Fig. 4E (7d).
	UAS-*Toll-9* (repo-GAL4)	repo-GAL4/Y;Or85e-mCD8::GFP/UAS-Toll-9	Fig. 4E (7d).

Figure 5	Tau	+/+;GMR-GAL4, UAS-tau/+	Fig. 5A, C, D (7d).
	Tau/*Toll-9*$^{+/-}$	+/+;GMR-GAL4, UAS-tau/+;Toll9^{-}/+	Fig. 5A, C, D (7d).
	mCherry RNAi (GMR-Tau)	+/+;GMR-GAL4, UAS-tau/+;UAS-mCherry RNAi/+	Fig. 5B (7d).
	Toll-9 RNAi #2 (GMR-Tau)	+/+;GMR-GAL4, UAS-tau/+;UAS-Toll-9 RNAi/+	Fig. 5B (7d).
	mCherry RNAi (GMR-GAL4)	+/+;GMR-GAL4/+;UAS-mCherry RNAi/+	Fig. 5B (7d).
	Toll-9 RNAi #2 (GMR-GAL4)	+/+;GMR-GAL4/+;UAS-Toll-9 RNAi/+	Fig. 5B (7d).
Figure 6	*Luciferase* (GMR-Tau)	+/+;GMR-GAL4, UAS-tau/+;UAS-Luciferase/+	Fig. 6A (7d).
	DN-*p38b* (GMR-Tau)	UAS-DN-p38b/+;GMR-GAL4, UAS-tau/+	Fig. 6A (7d).
	Luciferase (GMR-GAL4)	+/+;GMR-GAL4/+;UAS-Luciferase	Fig. 6A (7d).
	DN-*p38b* (GMR-GAL4)	UAS-DN-p38b/+;GMR-GAL4/+	Fig. 6A (7d).
	Tau/*Luciferase*	+/+;GMR-GAL4, UAS-tau/+;UAS-Luciferase/+	Fig. 6B (7d).
	Tau/DN-*p38b*	UAS-DN-p38b/+;GMR-GAL4, UAS-tau/+	Fig. 6B (7d).
Figure S1	Control (GMR-GAL4)	+/+;GMR-GAL4/+	Fig. S1 (Left: 20d, Right: 7d).
	UAS-*Toll-9* (GMR-GAL4)	+/+;GMR-GAL4/UAS-Toll-9	Fig. S1 (Left: 20d, Right: 7d).
Figure S2	Control	+/Y;Or85e-mCD8::GFP/+	Fig. S2 (7d).
	drpr$^{\Delta5}$	+/Y;Or85e-mCD8::GFP/+;drpr$^{\Delta5}$/drpr$^{\Delta5}$	Fig. S2 (7d).
	Toll-9$^{+/-}$	+/Y;Or85e-mCD8::GFP/+;Toll-9^{-}/+	Fig. S2 (7d).
Figure S3	Tau/*Luciferase*	+/+;GMR-GAL4, UAS-tau/+;UAS-Luciferase/+	Fig. S3 (7d).
	Tau/DN-*p38b*	UAS-DN-p38b/+;GMR-GAL4, UAS-tau/+	Fig. S3 (7d).

Table S2. Primer sequences for qRT-PCR, Related to STAR Methods.

Name	Sequence	Product size
Gapdh1 (*Drosophila*), Forward	5'- GACGAAATCAAGGCTAAGGTCG -3'	109 bp
Gapdh1 (*Drosophila*), Reverse	5'- AATGGGTGTCGCTGAAGAAGTC -3'	
Toll-1/Tl (*Drosophila*), Forward	5'-GTGAGGTCGACAGGGTTCAG-3'	198 bp
Toll-1/Tl (*Drosophila*), Reverse	5'-TGAGACGGCGAGTGGTAAAC-3'	
Toll-2/18w (*Drosophila*), Forward	5'-ATTGGCCACATTGAGGAGGG-3'	182 bp
Toll-2/18w (*Drosophila*), Reverse	5'-GAACAGTTGCGGAAGGCTTG-3'	
Toll-4 (*Drosophila*), Forward	5'-ATCTGTCAAGGGCTCAAGGC-3'	193 bp
Toll-4 (*Drosophila*), Reverse	5'-CACCATGTACGCCCTCAACT-3'	
Toll-5/Tehao (*Drosophila*), Forward	5'-AAACTTCGCAGCCCGAAAAC-3'	174 bp
Toll-5/Tehao (*Drosophila*), Reverse	5'-GCATATTTCCCGGCAACGTC-3'	
Toll-6 (*Drosophila*), Forward	5'-ATGGTTCCACCGAATCCACC-3'	150 bp
Toll-6 (*Drosophila*), Reverse	5'-GCCTCCTCAGAGTGCCAAAT-3'	
Toll-7 (*Drosophila*), Forward	5'-CAGCGAGCTCTACCTCTTCG-3'	182 bp
Toll-7 (*Drosophila*), Reverse	5'-TCGTTGGACCCCATTCACTG-3'	
Toll-8/Tollo (*Drosophila*), Forward	5'-CTCCCATGCTGGAAATGGGT-3'	188 bp
Toll-8/Tollo (*Drosophila*), Reverse	5'-CTGATGGGCGGACTTGAACT-3'	
Toll-9 (*Drosophila*), Forward	5'-ATCGGATGATGGGAACAGTTGT-3'	100 bp
Toll-9 (*Drosophila*), Reverse	5'-GTATTTCTTTGTGCTGTCCCTGA-3'	
Drosomycin (*Drosophila*), Forward	5'-TACTTGTTCGCCCTCTTCGC-3'	190 bp
Drosomycin (*Drosophila*), Reverse	5'-CTTCGCACCAGCACTTCAGA-3'	
Metchnikowin (*Drosophila*), Forward	5'-TGCAACTTAATCTTGGAGCGA-3'	151 bp
Metchnikowin (*Drosophila*), Reverse	5'-ATTGGACCCGGTCTTGGTTG-3'	
Defensin (*Drosophila*), Forward	5'-CCACATGCGACCTACTCTCC-3'	124 bp
Defensin (*Drosophila*), Reverse	5'-AATCAATTGCGGCAAACGCA-3'	
Cecropin A1 (*Drosophila*), Forward	5'-ATCAGTCGCTCAGACCTCAC-3'	157 bp
Cecropin A1 (*Drosophila*), Reverse	5'-TTTCTTCAGCCACCCAGCTT-3'	
Diptericin A (*Drosophila*), Forward	5'-CACCGCAGTACCCACTCAAT-3'	184 bp
Diptericin A (*Drosophila*), Reverse	5'-CCACTTTCCAGCTCGGTTCT-3'	
Drosocin (*Drosophila*), Forward	5'-CTGCTTGCTTGCGTTTTTGC-3'	174 bp
Drosocin (*Drosophila*), Reverse	5'-TCTTTAGGCGGGCAGAATGG-3'	
Attacin-C (*Drosophila*), Forward	5'-CCCAACGTCCGTATACCCAG-3'	150 bp
Attacin-C (*Drosophila*), Reverse	5'-AGTTCCAACGGCCTTGCTTA-3'	
Jafrac2 (*Drosophila*), Forward	5'-CTACTTGGAGTCCAGCGGTC-3'	116 bp
Jafrac2 (*Drosophila*), Reverse	5'-TAGTCTCGTCCACAGAGCGT-3'	

puc (*Drosophila*), Forward	5'-CCGGCGGTCTACGATATAGAA-3'	181 bp
puc (*Drosophila*), Reverse	5'-TGGCAGGTATTTGCATGTACTT-3'	
Thor (*Drosophila*), Forward	5'-CTCCTGGAGGCACCAAACTTATC-3'	111 bp
Thor (*Drosophila*), Reverse	5'-TTCCCCTCAGCAAGCAACTG-3'	
PHGPx (*Drosophila*), Forward	5'-ACCTAAAGGCCAAGCAGACC-3'	105 bp
PHGPx (*Drosophila*), Reverse	5'-TCGGGGCATATCGGTTGATG-3'	
Cyp4p3 (*Drosophila*), Forward	5'-TGGCTCGTGGGAGCTTTTATT-3'	141 bp
Cyp4p3 (*Drosophila*), Reverse	5'-GCCCTTCACTAATGGAGCAATG-3'	
GstD2 (*Drosophila*), Forward	5'-AAGGATGACTATCTGTTGCCCA-3'	172 bp
GstD2 (*Drosophila*), Reverse	5'-CAAACGCGGTTTCGATTCTCT-3'	
GstE5 (*Drosophila*), Forward	5'-TTTGTGGAGACCTTCCTCGC-3'	155 bp
GstE5 (*Drosophila*), Reverse	5'-AAACGCCTGACCCATTCGAT-3'	
TotM (*Drosophila*), Forward	5'-CGACAGCCTGGTCACTTTCT-3'	172 bp
TotM (*Drosophila*), Reverse	5'-TAGCTTCACCAGTGGCAACC-3'	
Hsp70Bc (*Drosophila*), Forward	5'-ATTTGGCGGCTACTCTGGAC-3'	163 bp
Hsp70Bc (*Drosophila*), Reverse	5'-ACAATGGGTTGCTAACATATGAGT-3'	
p38b (*Drosophila*), Forward	5'-TGATGGACGCCGATCTGAAC-3'	175 bp
p38b (*Drosophila*), Reverse	5'-ATGCGAAGCTCACAGTCCTC-3'	

* Primer sequences from FlyPrimerBank (https://www.flyrnai.org/flyprimerbank).

** Primer sequences from "Cell, 2013,155(3):699-712. doi: 10.1016/j.cell.2013.09.021."

*** Primer sequences from "PNAS, 2013, 110(19):E1752-60. doi: 10.1073/pnas.1306220110."