Supporting Information

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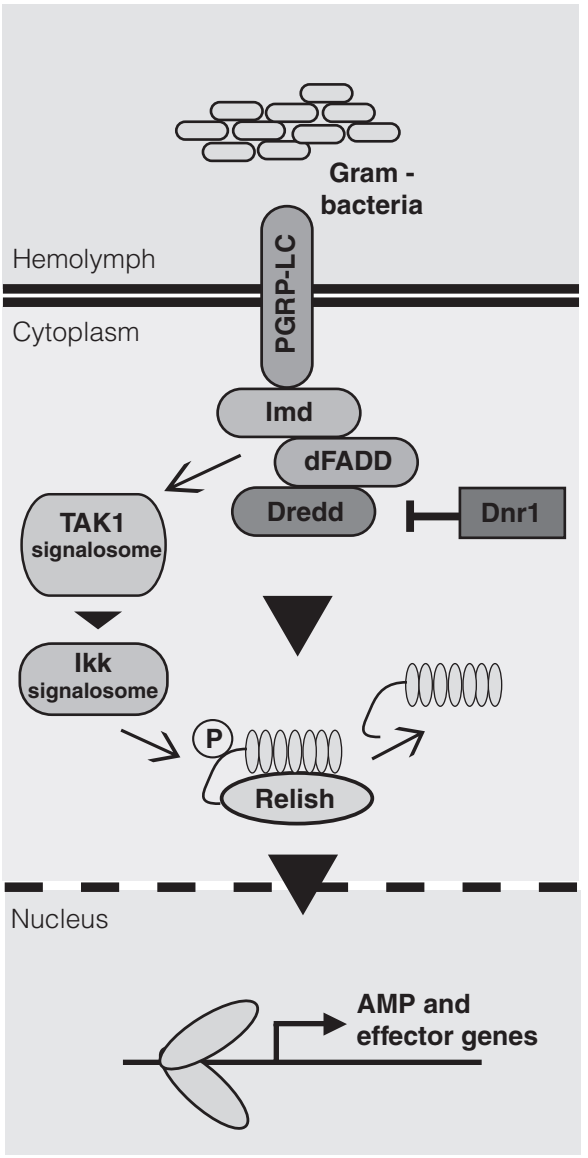

Fig. S1. Defense repressor 1 (Dnr1) is a negative regulator of the *Drosophila* immune deficiency (Imd) pathway. The transmembrane receptor peptidylglycan recognition protein LC (PGRP-LC) triggers Imd pathway activation after sensing presence of Gram-negative bacteria. Imd has an essential role in phosphorylating the NF-κB transcription factor Relish through activation of transforming growth factor β activated kinase 1 (TAK1) and the IKK signalosomes. Their activation requires several other proteins, including Fadd (Fas-associated death domain protein) and Dredd (death-related *ced-3*/*Nedd2*-like protein). Cleavage of phosphorylated Relish is dependent on the caspase Dredd. Cleaved Relish translocates to the nucleus and activates transcription of effector genes, including those encoding antimicrobial peptides (AMPs). Dnr1 negatively regulates the Imd pathway by promoting proteolytic cleavage of Dredd. Thus, loss of *dnr1* will lead to increase in Relish activation and up-regulation of AMP-coding genes.

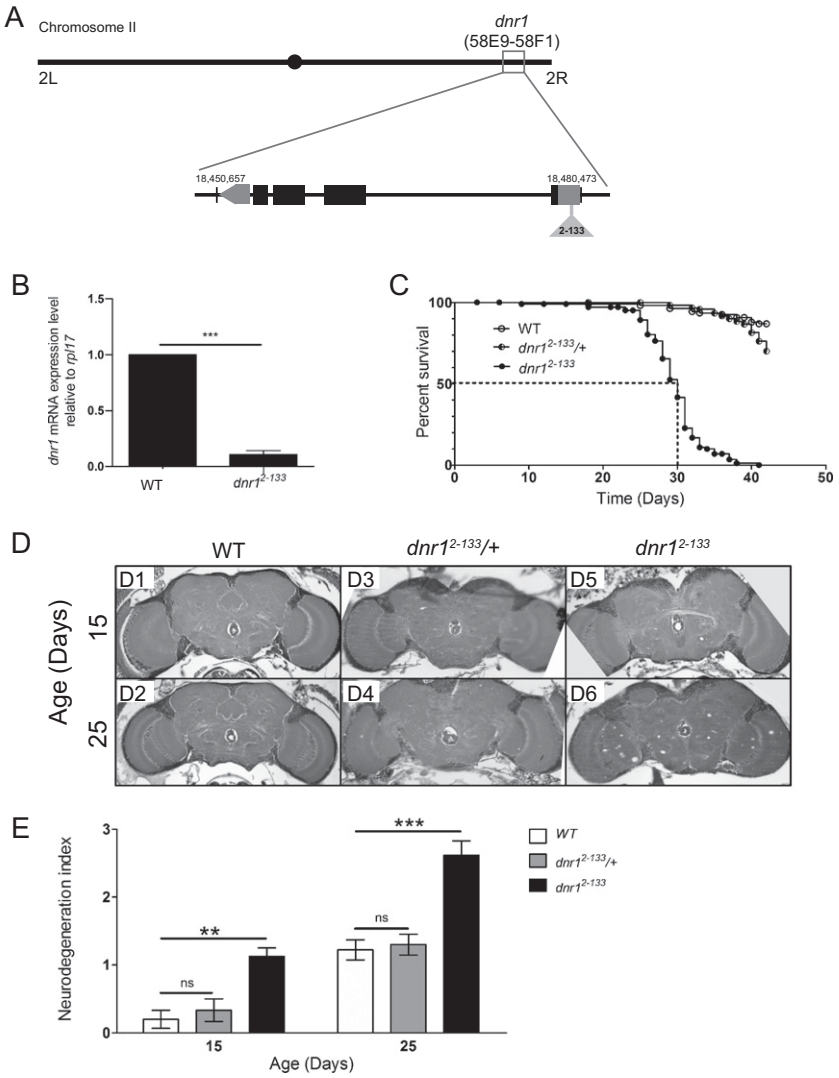

Fig. S2. *dnr1*$^{2\text{-}133}$ is recessive. (*A*) Schematic diagram of the *dnr1* gene showing the site of the P-element insertion in the *2-133* mutant. Exons are shown as black boxes. The 5′ UTR, where the P-element is inserted is shown as a gray box. (*B*) Quantitative real-time PCR (qPCR) analysis of *dnr1* mRNA levels in wild-type (Canton-S) and *dnr1*$^{2\text{-}133}$ homozygotes. (*C*) Survival curves for *dnr1*$^{2\text{-}133}$ and *dnr1*$^{2\text{-}133}$/+ flies compared with wild-type (Canton-S) at 29 °C. Flies were raised at 25 °C. After eclosion, five vials containing 10 males and 10 females per vial were aged at 29 °C. The number of surviving flies was counted every 3 d for 3 wk and every day thereafter until all flies died. Dotted line indicates the 50% survival point for *dnr1*$^{2\text{-}133}$ flies. (*D*) Representative 5-μm paraffin sections at approximately midbrain of wild-type (Canton-S) (D1, D2), *dnr1*$^{2\text{-}133}$/+ (D3, D4), and *dnr1*$^{2\text{-}133}$ (D5, D6) at the indicated age. (*E*) Quantification of neurodegeneration in the brains of wild-type, *dnr1*$^{2\text{-}133}$/+, and *dnr1*$^{2\text{-}133}$ flies. $^{**}P < .01$, $^{***}P < 0.005$ based on Student's *t* test. ns, not significant.

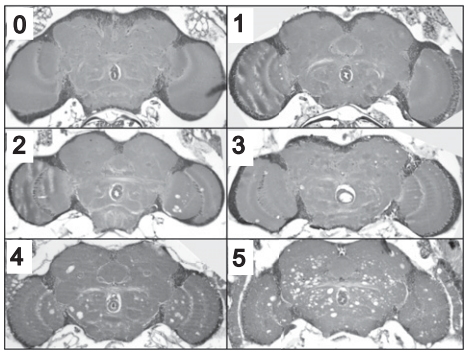

Fig. S3. Scoring system to quantify neurodegeneration phenotypes. A numerical neurodegeneration index was developed to score neurodegeneration phenotypes to compare flies of different ages and genotypes. Each individual brain examined was assigned a score from 0 to 5 based on the severity of the neuropathological phenotype as assessed by the number and size of vacuolar lesions in the neuropil of the central brain and optic lobes. Higher score indicates a more severe phenotype. Representative paraffin sections corresponding to each score are shown: 0, 1 normal to low; 2, 3 moderate; 4, 5 strong to severe. (Magnification: 20×.)

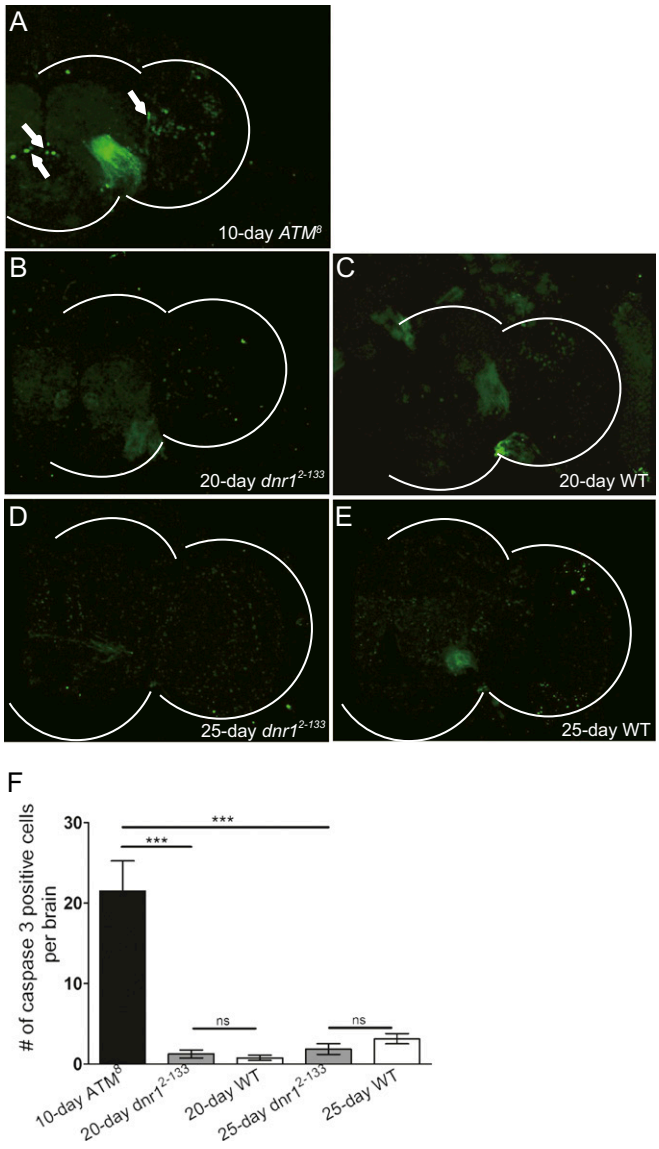

Fig. S4. Elevated apoptosis in the central nervous system is not observed in *dnr1* mutants. (*A–E*) *Drosophila* brains were dissected and stained with anticleaved caspase 3 antibody (Millipore). The white lines represent the outline of the dissected brains. (*A*) Ten-day-old ATM^{8} flies (16) were used as positive controls for cleaved caspase 3 staining. Arrows point to cleaved caspase 3-positive cells. (*B* and *C*) Twenty-day-old *dnr1* mutant flies and age matched wild-type (Canton-S) controls. (*D* and *E*) Twenty-five–day-old *dnr1* mutant flies and age matched WT controls. (Magnification: 20×.) (*F*) Quantification of cleaved caspase 3-positive cells per brain. Values shown are mean ± SEM. ***$P < 0.005$ based on Student's *t* test.

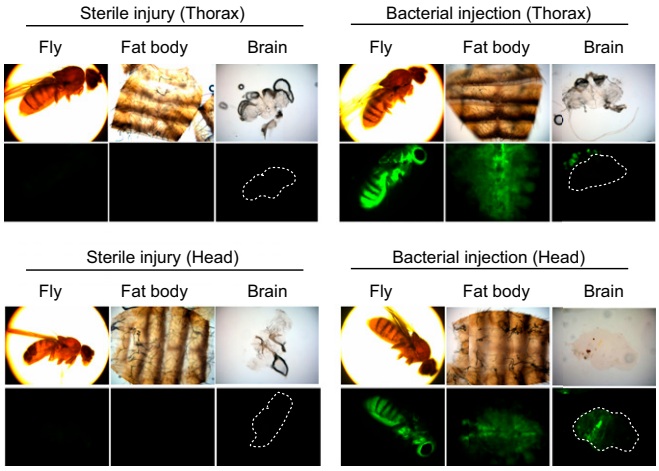

Fig. S5. Bacterial injection triggers AMP gene expression. One-week-old *att::GFP* flies were injected in the thorax (*Upper*) or head (*Lower*) with a mixture of Gram-negative and Gram-positive bacteria (*Right*) or with a sterile needle (*Left*). Bacterial injection in the thorax triggers *att::GFP* expression in the fat body but not in the brain, whereas bacterial injection in the head triggers *att::GFP* expression in the brain as well as in the fat body.

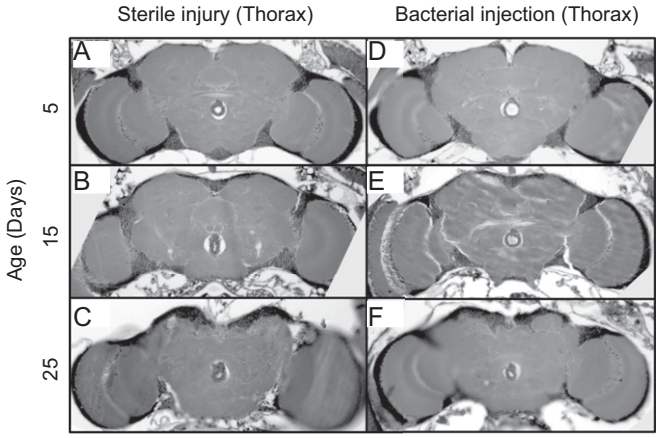

Fig. S6. Bacterial injection in the thorax does not trigger neurodegeneration. Wild-type (Canton-S) flies were injected in the thorax either with a sterile needle or with a mixture of Gram-negative and Gram-positive bacteria and aged for various times at 25 °C before being killed for histological analysis. Representative 5-μm paraffin sections at approximately midbrain from flies of the indicated ages after sterile injury (*A*–*C*) or bacterial injection (*D*–*F*).

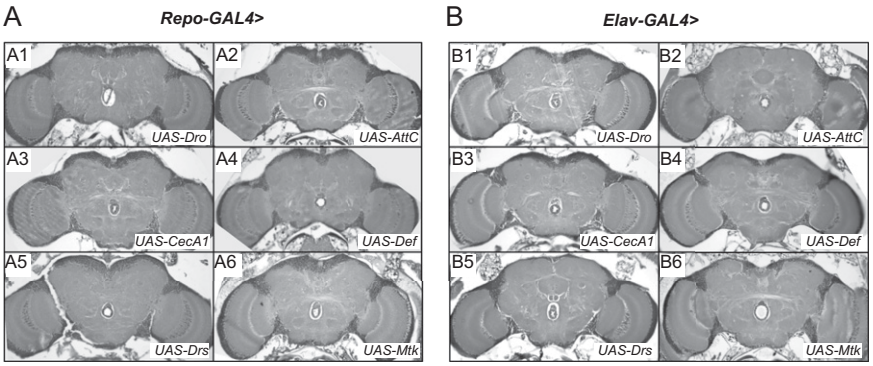

Fig. S7. Overexpression of AMPs in the *Drosophila* central nervous system does not cause neurodegeneration in 2-d-old flies. Representative 5-μm paraffin sections at approximately midbrain of 2-d-old flies expressing individual AMPs in glia (*Repo-GAL4* > *UAS-AMP)* (*A*) or in neurons (*Elav-GAL4* > *UAS-AMP)* (*B*).

Table S1. Genotypes and quantification of neurodegeneration index in each figure

Genotype	Time (d)	Neurodegeneration index (mean ± SEM)
Fig.1*B*		
Canton-S	5	0 ($n = 9$)
	10	0.11 ± 0.11 ($n = 9$)
	15	0.2 ± 0.13 ($n = 10$)
	25	1.22 ± 0.15 ($n = 9$)
	35	1.44 ± 0.18 ($n = 9$)
dnr1$^{2-133}$	5	0.25 ± 0.16 ($n = 8$)
	10	0.5 ± 0.19 ($n = 8$)
	15	1.13 ± 0.13 ($n = 8$)
	25	2.62 ± 0.22 ($n = 13$)
	35	3.75 ± 0.25 ($n = 8$)
dnr1$^{2-133}$*/Df(2R)X58-12*	5	0.20 ± 0.13 ($n = 10$)
	10	1.20 ± 0.13 ($n = 10$)
	15	1.70 ± 0.21 ($n = 10$)
	25	2.10 ± 0.28 ($n = 10$)
	35	3.57 ± 0.37 ($n = 7$)
dnr1^{DG29507}*/Df(2R)X58-12*	5	1.13 ± 0.13 ($n = 8$)
	10	1.82 ± 0.18 ($n = 11$)
	15	2.58 ± 0.19 ($n = 12$)
	25	3.13 ± 0.13 ($n = 8$)
	35	3.64 ± 0.20 ($n = 11$)
Fig. 1*D*		
dnr1$^{2-133}$*; UAS-dnr1/+*	25	2.56 ± 0.18 ($n = 9$)
dnr1$^{2-133}$*; Actin5C-Gal4/+*	25	2.33 ± 0.24 ($n = 9$)
dnr1$^{2-133}$*; Actin5C-Gal4/ UAS-dnr1*	25	1.33 ± 0.21 ($n = 6$)
Fig. 2*B*		
dnr1$^{2-133}$	25	2.75 ± 0.25 ($n = 8$)
dnr1$^{2-133}$*; relish*^{E20}*/Df(3R)ED05331*	25	1.90 ± 0.20 ($n = 10$)
dnr1$^{2-133}$*; relish*$^{E20/E38}$	25	1.67 ± 0.18 ($n = 6$)
Fig. 3*B*		
Noninjected	5	0.11 ± 0.08 ($n = 18$)
	15	0.12 ± 0.06 ($n = 31$)
	25	0.24 ± 0.11 ($n = 17$)
Sterile injury	5	1.89 ± 0.46 ($n = 20$)
	15	0.53 ± 0.21 ($n = 28$)
	25	1.12 ± 0.36 ($n = 16$)
Bacterial injection	5	1.03 ± 0.23 ($n = 29$)
	15	1.79 ± 0.27 ($n = 32$)
	25	3.14 ± 0.28 ($n = 35$)
Fig. 4*B*		
Elav-GAL4/+	Sterile injury	1.63 ± 0.26 ($n = 8$)
	Bacterial injection	3.27 ± 0.19 ($n = 11$)
Elav-GAL4 > UAS-relishRNAi	Sterile injury	1.30 ± 0.21 ($n = 10$)
	Bacterial injection	1.43 ± 0.25 ($n = 14$)
Repo-GAL4/+	Sterile injury	1.56 ± 0.44 ($n = 9$)
	Bacterial injection	3.78 ± 0.22 ($n = 9$)
Repo-GAL4 > UAS-relishRNAi	Sterile injury	1.18 ± 0.18 ($n = 11$)
	Bacterial injection	1.17 ± 0.31 ($n = 6$)
Fig. 5*C*		
repo-Gal4 > UAS-Drosocin	25	2.50 ± 0.27 ($n = 8$)
repo-Gal4 > UAS-Attacin	25	3.13 ± 0.23 ($n = 8$)
repo-Gal4 > UAS-Cecropin A1	25	2.75 ± 0.31 ($n = 8$)
repo-Gal4 > UAS-Defensin	25	2.88 ± 0.29 ($n = 8$)
repo-Gal4 > UAS-Drosomycin	25	3.38 ± 0.26 ($n = 8$)
repo-Gal4 > UAS-Metchnikowin	25	2.75 ± 0.36 ($n = 8$)
repo-Gal4 > UAS-GFP	25	1.75 ± 0.25 ($n = 8$)
repo-Gal4/+	25	1.63 ± 0.18 ($n = 8$)
UAS-Drosocin/+	25	1.38 ± 0.18 ($n = 8$)
UAS-Attacin/+	25	1.25 ± 0.16 ($n = 8$)
UAS-Cecropin A1/+	25	2.25 ± 0.36 ($n = 8$)
UAS-Defensin/+	25	1.38 ± 0.18 ($n = 8$)
UAS-Drosomycin/+	25	1.88 ± 0.23 ($n = 8$)
UAS-Metchnikowin/+	25	2.75 ± 0.25 ($n = 8$)

Table S1. Cont.

Genotype	Time (d)	Neurodegeneration index (mean ± SEM)
elav-Gal4 > UAS-Drosocin	25	2.13 ± 0.23 (n = 8)
elav-Gal4 > UAS-Attacin	25	2.00 ± 0.26 (n = 8)
elav-Gal4 > UAS-Cecropin A1	25	2.50 ± 0.19 (n = 8)
elav-Gal4 > UAS-Defencin	25	2.13 ± 0.23 (n = 8)
elav-Gal4 > UAS-Drosomycin	25	3.00 ± 0.27 (n = 8)
elav-Gal4 > UAS-Metchnikowin	25	2.88 ± 0.29 (n = 8)
elav-Gal4 > UAS-GFP	25	1.50 ± 0.27 (n = 8)
elav-Gal4/+	25	1.25 ± 0.16 (n = 8)
Fig. 5*E*		
repo-Gal4 > UAS-Defensin	25	2.88 ± 0.29 (n = 8)
repo-Gal4 > UAS-Defensin,UAS-relishRNAi	25	3.10 ± 0.23 (n = 10)
repo-Gal4 > UAS-Drosomycin	25	3.38 ± 0.26 (n = 8)
repo-Gal4 > UAS-Drosomycin,UAS-relishRNAi	25	2.80 ± 0.37 (n = 5)

Table S2. Primer sequences for thermal asymmetric interlaced (TAIL) PCR

Name	Primer (5′–3′)
TAIL1	NTCGASTWTSGWGTT
2223	CGTCCGCACACAACCTTTCC
Pry4	CAATCATATCGCTGTCTCACTCA
TAIL2	NGTCGASWGANAWGAA
P5out2	AACCTTTCCTCTCAACAAGCAA
2231	TCGCTGTCTCACTCAGACTC
TAIL3	WGTGNAGWANCANAGA
P5out1	CGGTAAGCTTCGGCTATCGAC
2229	CCTTTCACTCGCACTTATTGCAAGC

Table S3. Primer sequences for qPCR

Gene	Forward primer (5′–3′)	Reverse primer (5′–3′)
dnr1	CATTGTCAACCTGCCCAAC	GCGACAGACCTTCTCCAGAC
rpl17	CCAATCTACGTGTGCACTTCA	ACTCCTTCTGGTCGATGACG
Actin	CGAAGAAGTTGCTGCTCTGGTTGT	GGACGTCCCACAATCGATGGGAAG
rp49	AAGAAGCGCACCAAGCACTTCATC	TCTGTTGTCGATACCCTTGGGCTT
Attacin C	CTGCACTGGACTACTCCCACATCA	CGATCCTGCGACTGCCAAAGATTG
Cecropin A1	CATTGGACAATCGGAAGCTGGGTG	TAATCATCGTGGTCAACCTCGGGC
Diptericin B	AGGATTCGATCTGAGCCTCAACGG	TGAAGGTATACACTCCACCGGCTC
Drosomycin	AGTACTTGTTCGCCCTCTTCGCTG	CCTTGTATCTTCCGGACAGGCAGT
Metchnikowin	CATCAATCAATTCCCGCCACCGAG	AAATGGGTCCCTGGTGACGATGAG

dnr1, defense repressor 1; *rpl17*, ribosomal protein L17; *rp49*, ribosomal protein 49.