Expanded View Figures

Figure EV1. Aspergillus fumigatus induces mortality in MyD88 mutant flies (related to Fig 1).

A Survival of three different Drosophila wild-type strains to the injection of 50, 500, or 5,000 A. fumigatus conidia (20 flies per condition).
B Survival of hemizygous (HZ) MyD88/Df(2R)3591 flies after the injection of 250 conidia of wild-type A. fumigatus conidia (20 flies per condition).
C Survival of wild-type and MyD88 flies after the injection of 250 conidia from different wild-type A. fumigatus strains (error bars represent mean ± SD of the survival of biological triplicates of 20 flies each).
D, E Survival of the Toll pathway mutant flies, spz (D) and Toll (E) (20 flies per condition, 500 conidia per fly); the caption in (D) applies also to panel (E).
F Fungal load of single MyD88 mutant and wild-type flies (biological replicates, 5,000 conidia injected per fly).
G Fungal load upon the death of single MyD88 mutant flies (biological replicates).
H GFP-labeled A. fumigatus injected in wild-type flies form hyphae under a bright field (arrow shows the position of the blown-up area). The same preparation under fluorescence illumination is shown in Fig 1C. Scale bar 50 μm.
I 500 conidia of GFP-labeled A. fumigatus injected in MyD88 mutant flies form few hyphae (arrow shows the position of the blown-up area) as compared to the injection of 50 conidia (shown in Fig 1E). Scale bar 50 μm.
J–L Survival of antibiotics-treated (J), axenic (K), and untreated flies (L) after injection of 250 A. fumigatus conidia (20 flies per condition).

Data Information: In (F, G), the middle bar of box plots represents the median and the upper and lower limits of boxes indicate, respectively, the first and third quartiles; the whiskers define the minima and maxima; data were analyzed using the Mann–Whitney statistical test. Survival curves were analyzed using the log-rank test. ****p < 0.0001, and NS: not significant.
Figure EV1.
Figure EV2. SSC locus Bomanins are induced after Aspergillus fumigatus infection in a MyD88-dependent manner (related to Figs 1 and 3).

A. Scheme of the SSC Bomanin locus.
B. Expression level of Bomanins measured by RT–dPCR 48 h after M. luteus challenge (pooled data of n = 3 experiments, biological replicates).
C, D. Expression levels of Drosomycin and Bomanin S1 measured by RT–dPCR 48 h after challenge; Drosomycin *P = 0.03; BomS1: *P = 0.03 (pooled data of n = 3 experiments, biological replicates).
E, F. MALDI-TOF mass spectrometry enlarged spectrum focused on BomS (E) or Drosomycin (F) of the hemolymph collected from an A. fumigatus-infected (5 conidia; green) or PBST-injected control (blue) fly.
G. Peak intensity of short Bomanins and Drosomycin peptides post A. fumigatus injection. MALDI-TOF was used to measure the peptides in the hemolymph after the injection of different doses of conidia (x-axis) into wild-type flies. The same volume of PBS was injected into flies as a control.

Data Information: In (B–D), the middle bar of box plots represents the median and the upper and lower limits of boxes indicate, respectively, the first and third quartiles; the whiskers define the minima and maxima; data were analyzed using the Mann–Whitney statistical test. *P < 0.05, and NS: not significant. (E–G) Hemolymph was collected at 48 h postinfection.
Figure EV2.
Figure EV3. Role for specific melanization genes in host defense against *Aspergillus fumigatus* infection (related to Fig 1).

A, B Survival of PPO1 (A) and Sp7 mutant (B) flies injected with 500 *A. fumigatus* conidia.

C, D Survival (C) and fungal load upon death (D) of PPO2 mutant flies after the injection of 500 *A. fumigatus* conidia (C: error bars represent mean ± SD of the survival of biological triplicates of 20 flies each).

E Analysis of the distribution of GFP-labeled *A. fumigatus* inside live Hayan and MyD88 flies; fluorescent hyphae (arrows) were observed in the head (42.5%, 17/40), thorax (100%, 40/40), and abdomen (95%, 38/40) in Hayan flies (arrows), whereas they were observed only in the thorax (50%, 50/100) and abdomen (1%, 1/100) of MyD88 flies (arrows). Scale bars 50 μm.

F Spätzle, Toll, and MyD88 mutants display hyphae extruding from the thoraces of cadavers after *A. fumigatus* infection; arrows mark hyphae. Scale bars 500 μm.

Data Information: In (D), the middle bar of box plots represents the median and the upper and lower limits of boxes indicate, respectively, the first and third quartiles; the whiskers define the minima and maxima; data were analyzed using the Mann–Whitney statistical test. Survival curves were analyzed using the log-rank test; ****p < 0.0001; NS, not significant.
Figure EV3.
**Figure EV4.** The Toll pathway is involved in the host defense against verruculogen and restrictocin but not melanization nor the cellular immune response (related to Figs 3 and 4).

A  Survival of antibiotics-treated MyD88 mutant flies after verruculogen solution injection.

B-G  (B, C, F, G) Survival of spätzle, Toll, Hayan, and PPO2 mutant flies after verruculogen injection; there was a significant difference between the treated and the vehicle control in spätzle (**P = 0.01; (B)) and Toll (****P < 0.0001; (C)) but not for Hayan or PPO2 mutant flies (20 flies per condition). The caption for (C) applies to all four panels. (D) Recovery time from tremors in wild-type flies; each dot represents the time point of recovery of a single fly. Most MyD88 flies did not recover (pooled data of n = 3 experiments, biological replicates). (E) Recovery time from tremors in Bom**42**C mutant flies after verruculogen powder challenge (pooled data of n = 3 experiments, biological replicates).

H  Survival of phago-hemoless hml-Gal4-Gal80**ts** > UAS-rpr, UAS-Hid flies after verruculogen injection; there was no significant difference between the 29°C condition and the 18°C control for which there is no hemocyte ablation since Gal4 function is inhibited by the active Gal80 repressor (20 flies per condition).

I-L  Survival of spätzle (I), Toll (J), Hayan (K), and PPO2 (L) mutant flies after verruculogen injection; there is a significant difference between the treated and the vehicle control in spätzle and Toll (****P < 0.0001) but not for Hayan or PPO2 mutant flies. The caption to the right of (L) applies to all four panels (20 flies per condition).

M  Survival of phago-hemoless hml-Gal4-Gal80**ts** > UAS-rpr, UAS-Hid flies after restrictocin injection; there is no significant difference between the 29°C condition and the 18°C control for which there is no hemocyte ablation since Gal4 is repressed (20 flies per condition).

Data Information: In (D, E), the middle bar of box plots represents the median and the upper and lower limits of boxes indicate, respectively, the first and third quartiles, the whiskers define the minima and maxima. Survival curves were analyzed using the log-rank test. (E) Data were analyzed using the Kruskal–Wallis test and Dunn’s post hoc test. ****P = 0.0001, NS: not significant. The concentration of injected verruculogen or restrictocin was 1 mg/ml.
Figure EV4.
Figure EV5. BomS6 forced expression in the nervous system does not protect flies from early tremors induced by verruculogen injection (related to Fig 6).

A, B Tremor rate of BomS6 overexpressed in neurons (A) or glia (B) flies (20 flies per condition, pooled data from n = 3 experiments, biological replicates) compared with wild-type after injection of verruculogen.

C, D Survival of BomS4 overexpressed in neurons (C) or glia (D) flies (20 flies per condition) compared with wild-type 3 h after the injection of verruculogen. Insets represent the survival of vehicle control groups.

Data Information: In (A, B), the middle bar of box plots represents the median and the upper and lower limits of boxes indicate, respectively, the first and third quartiles; the whiskers define the minima and maxima; data were analyzed using the Mann–Whitney statistical test. Survival curves were analyzed using the log-rank test. The concentration of injected verruculogen was 1 mg/ml.