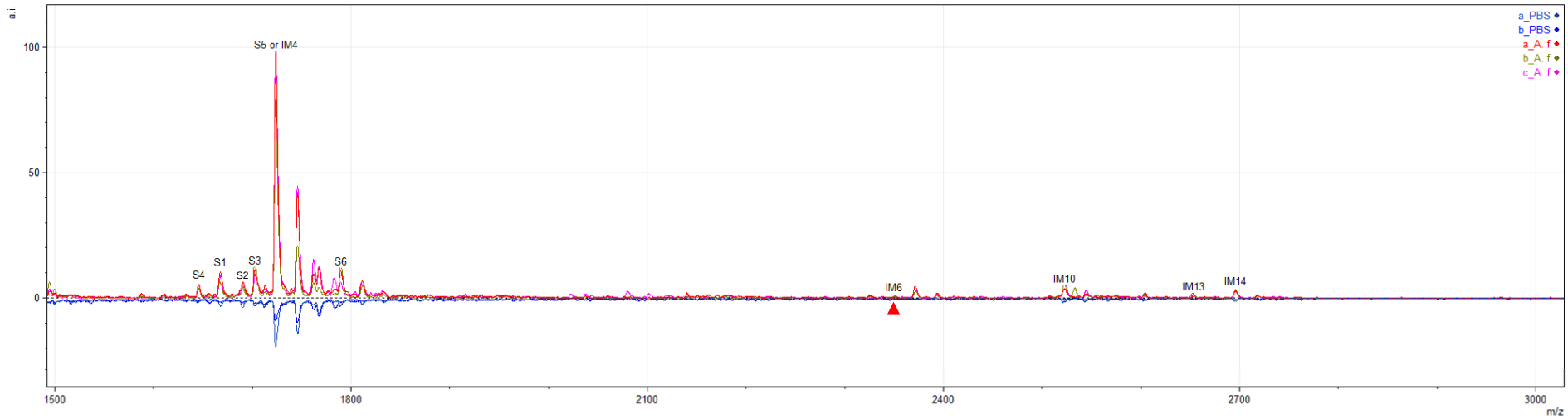
# The Toll pathway mediates *Drosophila* resilience to *Aspergillus* mycotoxins through specific Bomanins

Rui Xu, Yanyan Lou, Antonin Tidu, Philippe Bulet, Thorsten Heinekamp, Franck Martin, Axel Brakhage, Zi Li, Samuel Liégeois*, and Dominique Ferrandon[1-3]*

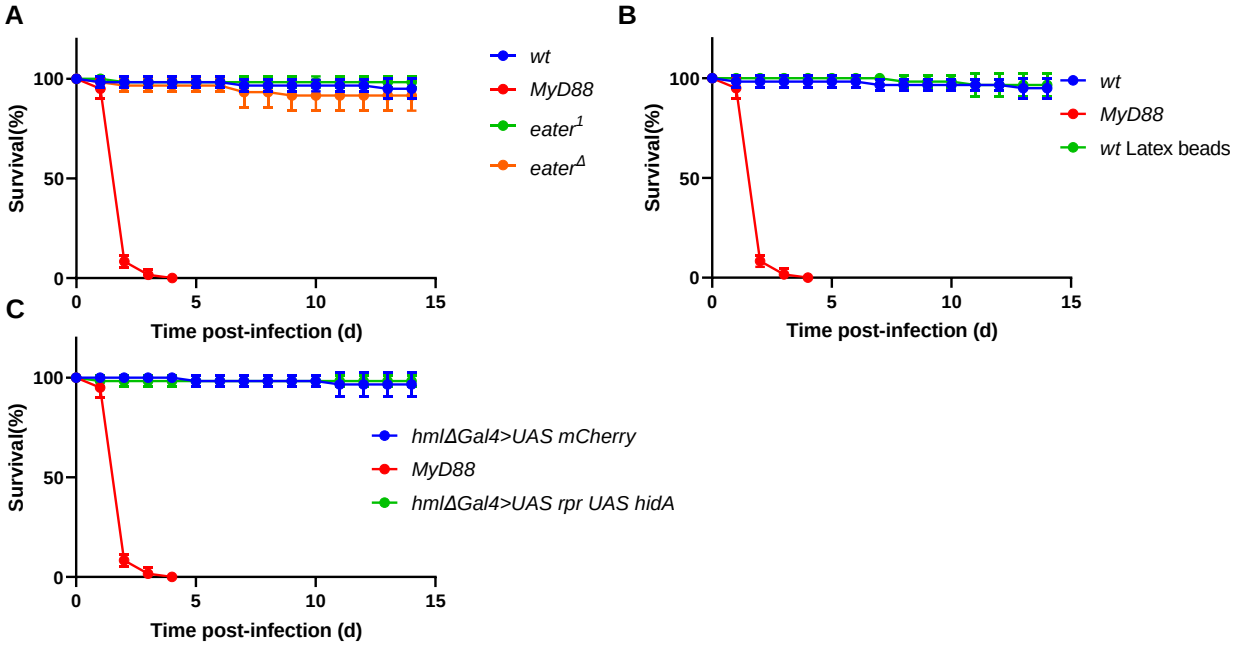
## Table of Contents



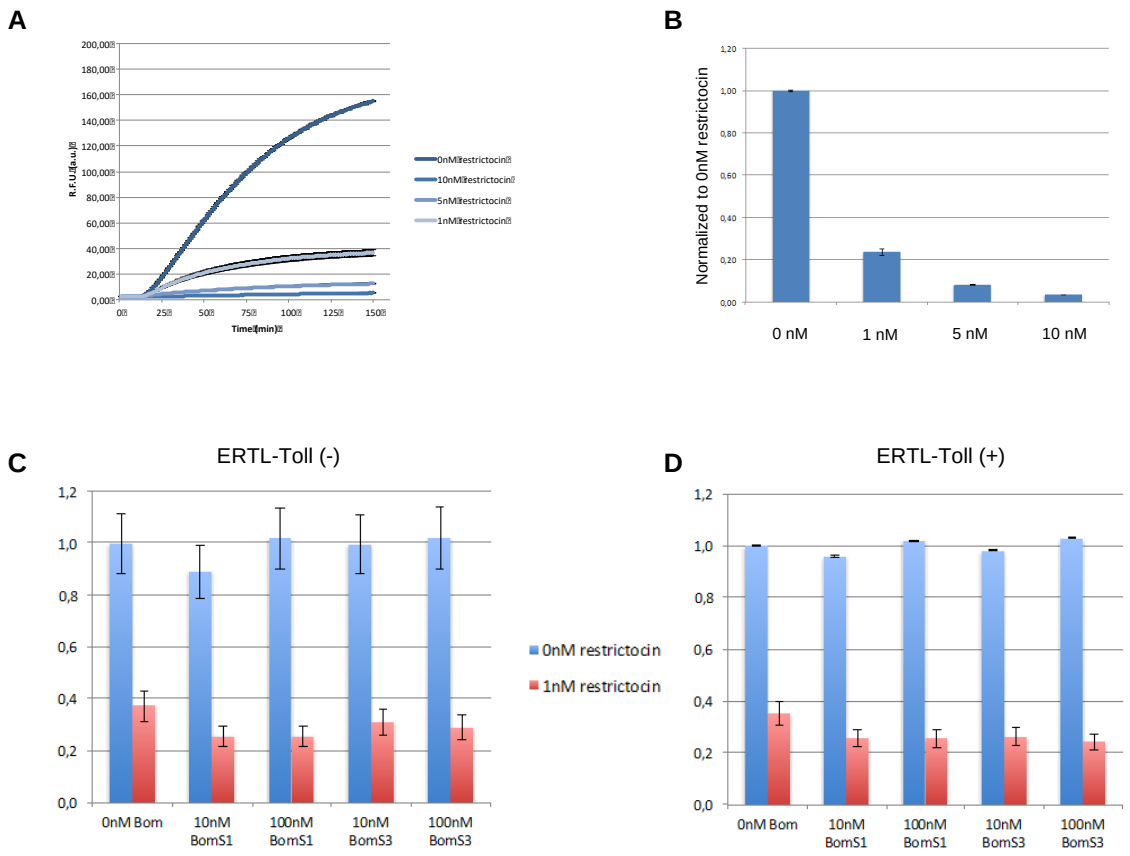

**Appendix Figure S1. MALDI-TOF results after *A. fumigatus* challenge**

Complementary to Fig. EV2.

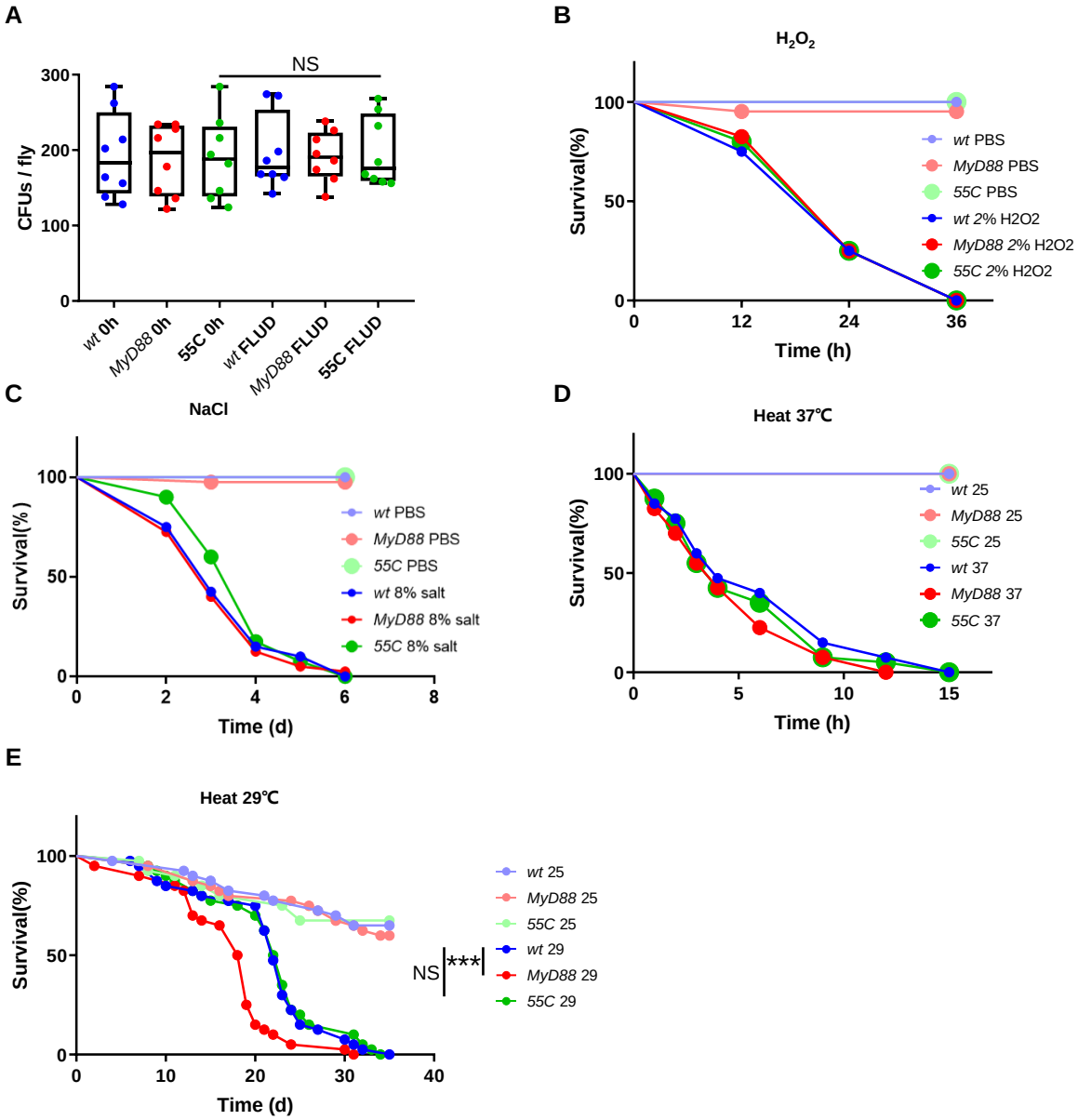
The hemolymph was collected at 48h post-injection of PBS or live spores. The blue lines under the 0 line of the x-axis (baseline) correspond to two PBS negative controls. The three curves above correspond to the signals gained on the hemolymph of three flies challenged with *A. fumigatus* live spores. All the Bomanin peptides were induced post *A. fumigatus* challenge but hardly after PBS injection. A few BaraA-derived peptides, IM10 and IM13 have been slightly induced. Furthermore, Daisho2 (IM14) is mildly expressed at this time point.




**Appendix Figure S2. The cellular immune response is not involved in host defense against *A. fumigatus* infection.**
Complementary to Fig. 1.
(*A*-*C*) survival of *eater* mutants, latex beads-treated, and phago-hemoless UAS-*rpr*, UAS-*Hid* flies (ablation of hemocytes) injected with 500 *A. fumigatus* conidia (*n*=60).
Data Information: survival curves were analyzed using the LogRank test




**Appendix Figure S3. Restrictocin inhibits *in vitro* translation even in the presence of BomS3.**
Complementary to Fig. 4&5.
(*A-B*) translation kinetics analyzed by fluorescence measurements emitted by *in vitro* synthesized eGFP from an IGR(CrPV)-driven reporter in non-induced ERTL lysates: translation kinetics were depicted as the fluorescence emitted by *in vitro* synthetized eGFP in the presence of increasing concentrations of restrictocin (*A*) and by histogram representing the end-point fluorescence quantification of *A* (*B*). (*C-D*) histograms representing the end-point quantification of *in vitro* translated eGFP from an IGR(CrPV)-driven reporter in the absence or presence of 1 nM restrictocin, pre-incubated or not with either 10 or 100 nM BomS1 or BomS3 synthetic peptides using either noninduced ERTL lysates (*C*) or Toll-induced ERTL lysates (*D*).
Data Information: error bars represent the standard error of the mean from three technical replicates.

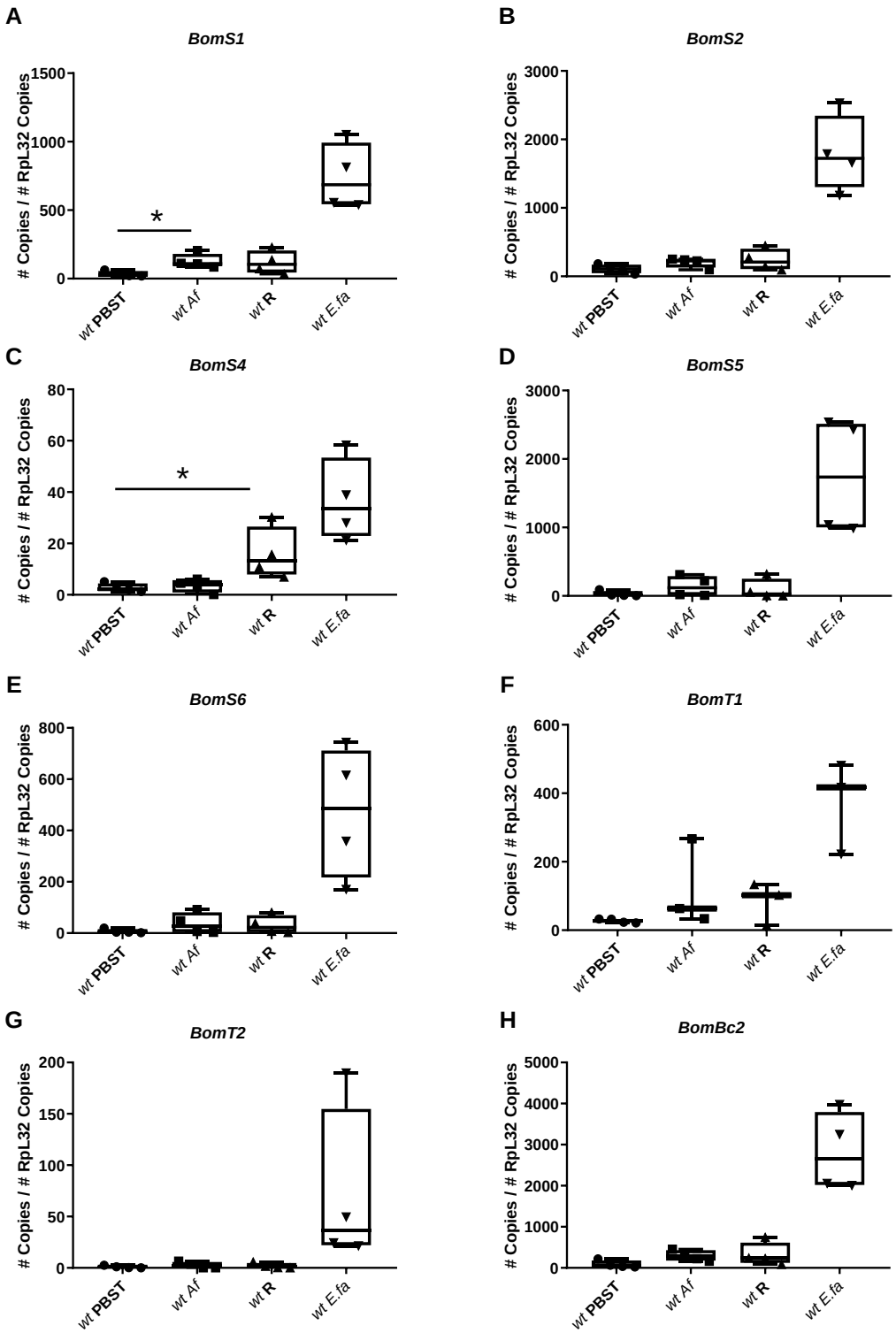



**Appendix Figure S4. *Bom$^{\Delta 55C}$* mutant flies do not get an increased susceptibility to stress.**

Complementary to Fig. 5.

(*A*) fungal load upon death of single *Bom$^{\Delta 55C}$* mutant flies. (*B-C*) survival of *Bom$^{\Delta 55C}$* (55C) deficient flies after $H_2O_2$ (*B*) or salt (*C*) solution injection; there was no significant difference between the treated and the 55C and wild-type treated groups (n=40). After $H_2O_2$ solution injection (*B*), wt *vs.* 55C, p=0.80; salt solution (*C*), wt *vs.* 55C, p=0.87. (*D-E*) survival of *Bom$^{\Delta 55C}$* (55C) deficient flies after 37°C (*D*) or 29°C (*E*) exposure; there was no significant difference between the 55C and wild-type treated groups, except the wild-type and *MyD88* treated groups exposed in 29°C (n=40). After exposition to 37°C (*D*), wt *vs.* 55C, p=0.51, wt *vs. MyD88*, p=0.012; 29°C (*E*), wt *vs.* 55C, p=0.75, wt *vs. MyD88*, p=0.0001.

Data Information: survival curves were analyzed using the LogRank test. NS: not significant.



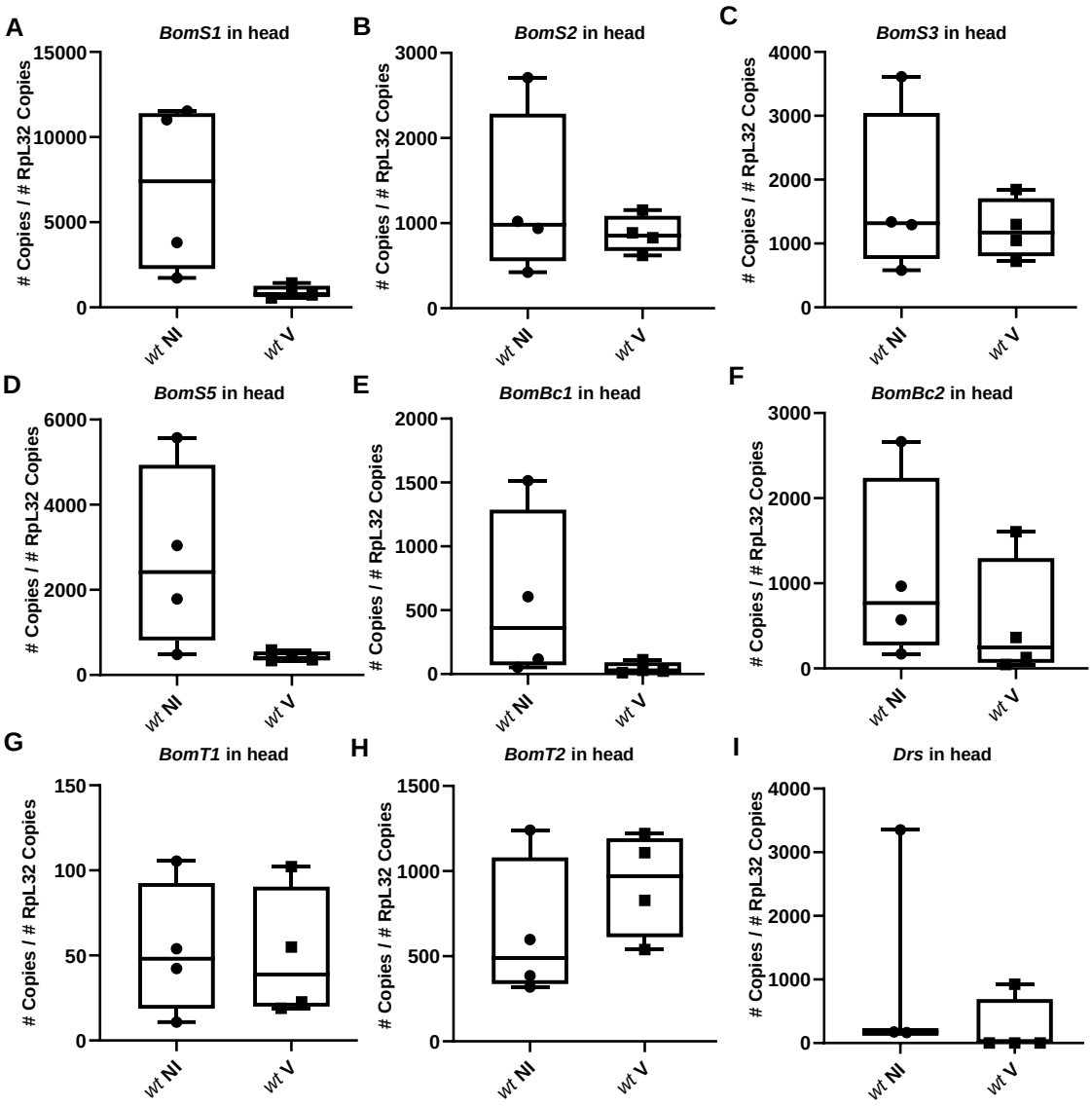

**Appendix Figure S5. Expression level of *Bomanins* after *A. fumigatus* and mycotoxin challenge.**

Complementary to Fig. 5.

(*A-G*) expression levels of *Bomanin* genes measured by RT-digital PCR 48 hours after challenge; except for *BomS4* (p=0.03), there was no significant difference between restrictocin and vehicle control injection for all *Bomanins*; except for *BomS1* (p=0.03), there was no significant difference between *A. fumigatus* and PBST control injection for all *Bomanins*.

Data Information: 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.

The concentration of injected restrictocin was 1 mg/mL, the dose of *A. fumigatus* was 500 conidia per fly, *Enterococcus faecalis* infection was made by septic injury.

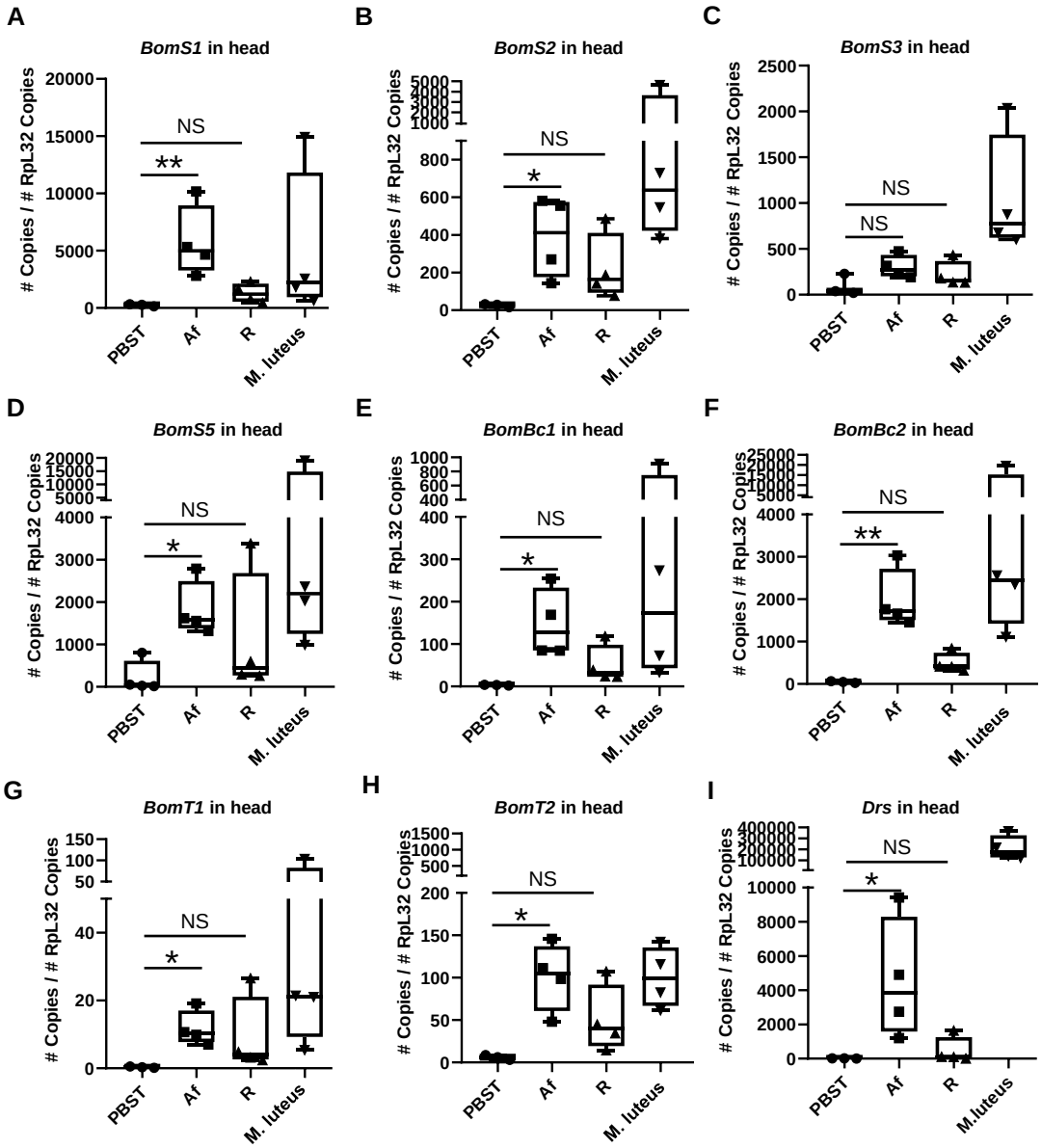



**Appendix Figure S6. Expression level of *Bomanins* in the head after verruculogen challenge.**

Complementary to Fig. 6.

(*A-I*) expression levels of *Bomanin* genes and *Drosomycin* measured by RT-digital PCR 48 hours after verruculogen powder challenge; there was no significant difference between verruculogen powder and blank control (NI) challenge.

Data Information: 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.




**Appendix Figure S7. Expression level of *Bomanins* in head after *A. fumigatus* or restrictocin challenge.**

Complementary to Fig. 6.

(*A-I*) expression levels of *Bomanin* genes and *Drosomycin* measured by RT-digital PCR 48 hours after challenge; except for *BomS3*, there were significant differences in *Bomanin* expression between *A. fumigatus* and vehicle control injection; there was no significant difference for *Bomanin* expression between restrictocin and PBST control injection.

Data Information: 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. *$p < 0.05$, **$p < 0.01$, and NS: not significant.

The concentration of injected restrictocin was 1 mg/ml, the dose of *A. fumigatus* was 500 conidia per fly, *Micrococcus luteus* infection was made by septic injury.

**Appendix Table S1. Primers used in this study**

| | | |
|---|---|---|
| *RpL32* | FW | GACGCTTCAAGGGACAGTATCTG |
| *RpL32* | RV | AAACGCGGTTCTGCATGAG |
| *RpL32* | Probe | **Cy5**-CATCGGTTACGGATCGAACA-**MGB** |
| *Drosomycin* | FW | CGTGAGAACCTTTTCCAATATGATG |
| *Drosomycin* | RV | TCCCAGGACCACCAGCAT |
| *Drosomycin* | Probe | **VIC**-AGATCAAGTACTTGTTCG-**MGB** |
| *BomaninBc1* | FW | TTTCGTTGTCCTGGCTTCCC |
| *BomaninBc1* | RV | CCGACTACGACGTTTTCCGC |
| *BomaninBc1* | Probe | **Cy5**-CTACGGCCGGAAATGTGAT-**MGB** |
| *BomaninBc2* | FW | AGAGCCTGACGTTATTGGCG |
| *BomaninBc2* | RV | TGGGAGCAGTTTACGCACTT |
| *BomaninBc2* | Probe | **FAM**-TGATCTTGGCCACGTTGGCCT-**MGB** |
| *BomaninS1* | FW | CAATGCTGTTCCACTGTCGC |
| *BomaninS1* | RV | CGTGGACATTGCACACCCTG |
| *BomaninS1* | Probe | **FAM**-ATCCAGGAAATGTGATCA-**MGB** |
| *BomaninS2* | FW | AGTCGTCACCGTCTTTGTGTT |
| *BomaninS2* | RV | CAGTATTTGCAGTCCCCGTTG |
| *BomaninS2* | Probe | **FAM**-CTGCTGGCTCTGGCCAAC-**MGB** |
| *BomaninS3* | FW | TCACTCGCCTTCGTTTTGGG |
| *BomaninS3* | RV | TTAGGCCCTCACATTGCAGAC |
| *BomaninS3* | Probe | **FAM**-GCTCTGGCCAACGCCACTC-**MGB** |
| *BomaninS4* | FW | GTTGTCACCCGATCCAGGAA |
| *BomaninS4* | RV | ATTTGCCACCACGAACATTGC |
| *BomaninS4* | Probe | **VIC**-TTATCATCAATGGCGACTG-**MGB** |
| *BomaninS5* | FW | GGTCTTTCTATGCGGTCTGCT |
| *BomaninS5* | RV | TAGCCTCCGCGAACATTACA |
| *BomaninS5* | Probe | **VIC**-CATGGCAGTGGCTTCTCCGTTA-**MGB** |
| *BomaninS6* | FW | GCTAGTGCCAATCCCCTGAG |
| *BomaninS6* | RV | TCGCCGCGTATATTGCAAAC |
| *BomaninS6* | Probe | **VIC**-CTGGCAATGTGATTATAAACG-**MGB** |
| *BomaninT1* | FW | TCCCAGGATTACCATTCGCA |
| *BomaninT1* | RV | GAGTTTTTGGTGGCGCGTG |
| *BomaninT1* | Probe | **Cy5**-CGACATTATAGTTCATGGCAAT-**MGB** |
| *BomaninT2* | FW | TAATGCTACGCCGGGACAAG |
| *BomaninT2* | RV | ATGGCTCCAGATGTGAGTGTG |
| *BomaninT2* | Probe | **Cy5**-ATGCATTGACTGCAATAAGCC-**MGB** |
| *aspf1_up* | FW | GAGATGGTGACTGCCTTTCC |
| *aspf1_ptrA* | RV | GGCCTGAGTGGCCATCGAATTCTCCAGGACATGGGATTGTCG |
| *aspf1_ptrA* | FW | GAGGCCATCTAGGCCATCAAGCCGTTGCTGCCACTCCTTTC |
| *aspf1_dw* | RV | ATTTCCACCCGTCTGTCCAG |
| *ftmA_up* | FW | GATTGGTTCTCCACTCTCGC |
| *ftmA_ptrA* | RV | GAGGCCATCTAGGCCATCAAGCCCATCGCCATAGTACAGACG |
| *ftmA_ptrA* | FW | GGCCTGAGTGGCCATCGAATTCGTCTGGATCGGTTCGACACC |
| *ftmA_dw* | RV | GACTTGGACAGAGGCTATAG |
| *ptrA* | FW | GAATTCGATGGCCACTCAGGCC |
| *ptrA* | RV | GCTTGATGGCTTAGATGGCCTC |