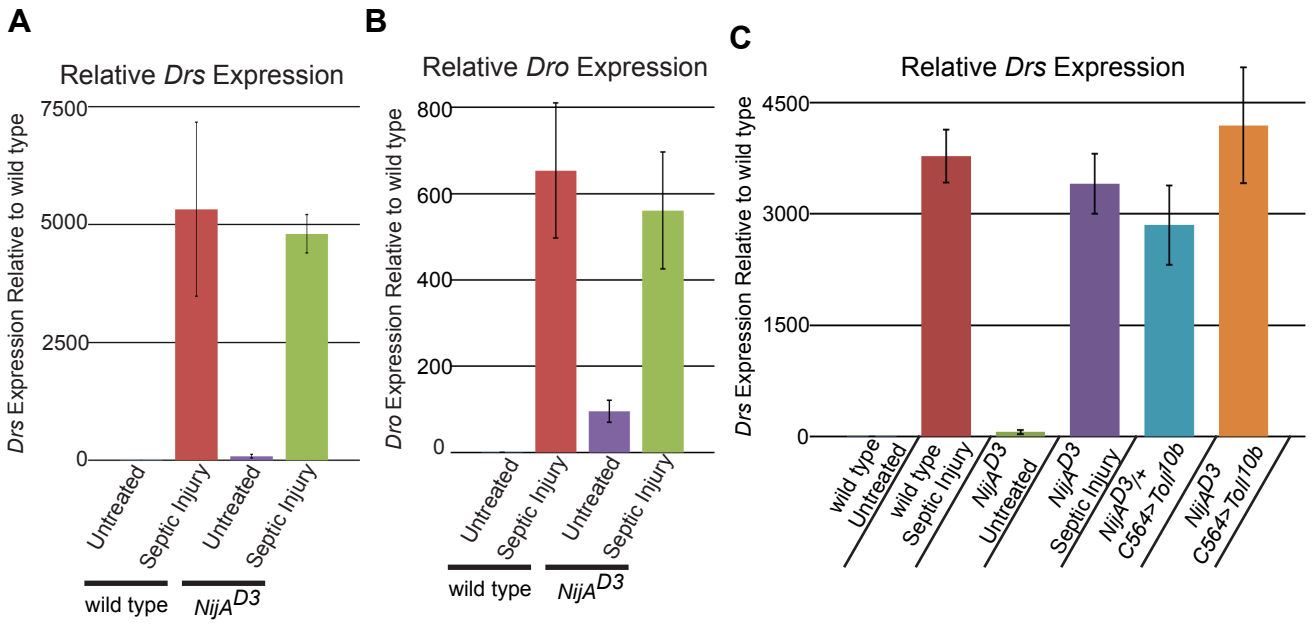

Supporting Information S2. *NijA* is not required for Toll-mediated antimicrobial peptide induction.

(A-B) qPCR analysis of relative *Drosomycin* (*Drs*, A) expression or *Drosocin* (*Dro*, B) expression in male third instar larvae after septic injury with *M. luteus*. $NijA^{D3}$ homozygotes were able to respond to immune challenge by upregulating both antimicrobial peptides similarly to wild type. **(C)** qPCR analysis of relative *Drosomycin* expression after septic injury, or in $C564{>}Toll^{10b}$ larvae where *Tl* is genetically activated in the fat body. $NijA^{D3}$ homozygous mutants were able to respond to *Tl* gain-of-function in the fat body by increasing *Drosomycin* to levels similar to heterozygous sibling controls. The slight increases in *Drs* and *Dro* observed in $NijA^{D3}$untreated larvae in all three panels are not statistically significant.

Methods:

Larvae were pierced with a fine needle (Fine Science Tools) dipped in a log-phase growth culture of *M. luteus* in LB. qPCR was performed as described in Materials and Methods, except that 2µl of the cDNA pools were primed with validated primers sets for *rp49* (R2<0.99), *Drosocin* (R2<0.98), and *Drosomycin* (R2<0.99), as previously described by [1]. All values are reported relative to untreated wild-type samples. Each sample was run in triplicate, and a minimum of three independent biological replicates was performed per condition.

1. Leulier F, Lhocine N, Lemaitre B, Meier P (2006) The Drosophila inhibitor of apoptosis protein DIAP2 functions in innate immunity and is essential to resist gram-negative bacterial infection. Mol Cell Biol 26: 7821-7831.