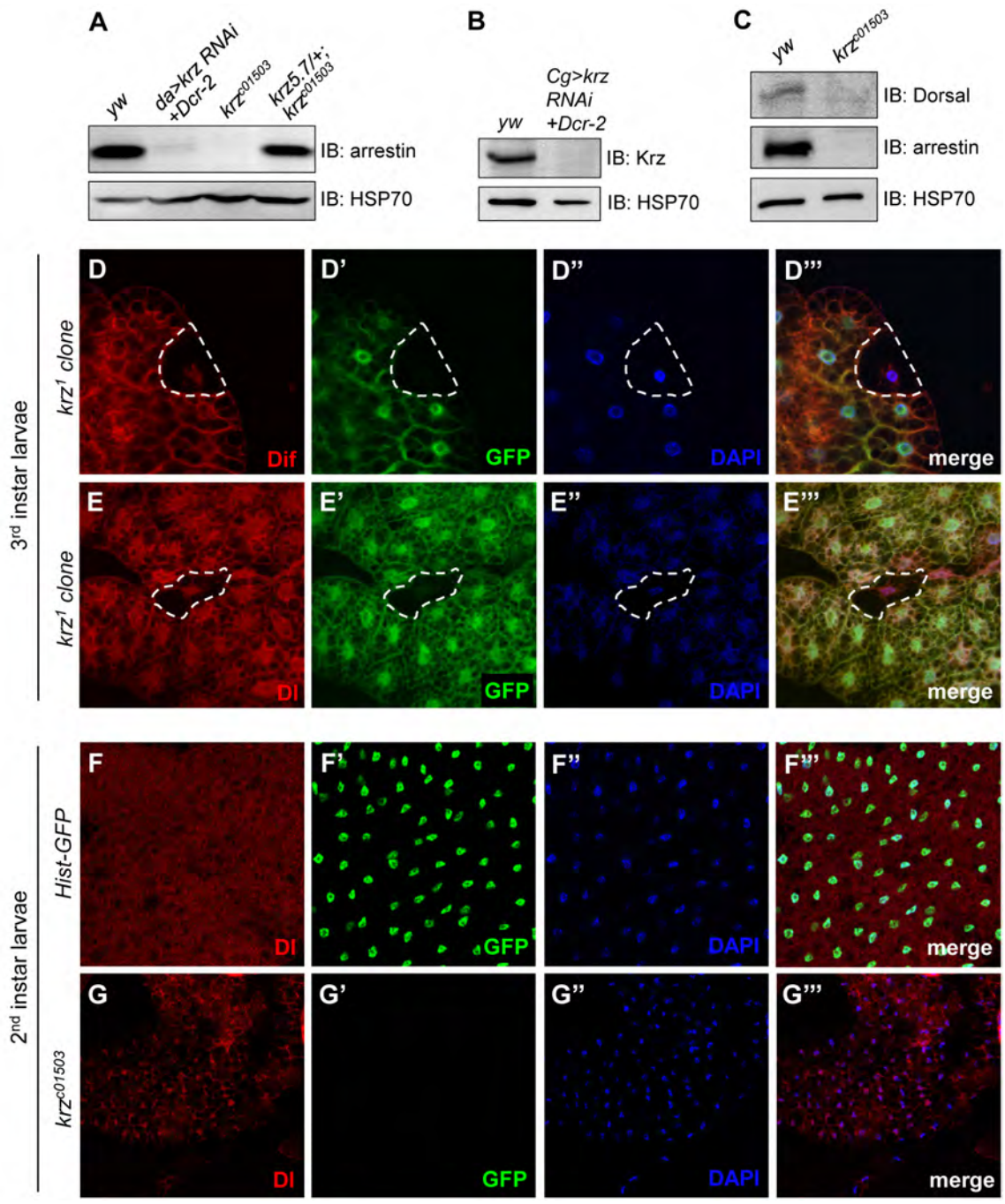

Figure S1 Expression of Krz, Dorsal and Dif in various *krz* loss of function conditions. (A) Western blot analysis of Krz protein levels using pan-arrestin antibody in whole third instar larvae. Krz expression was not detectable in *krz*^{c01503} homozygous animals but was restored by an introduction of a genomic rescue construct, *krz5.7*. HSP70 was used as a loading control. (B) Western blot analysis of Krz protein levels using anti-Krz antibody in the fat bodies of third instar larvae. Krz was undetectable in the fat bodies of larvae coexpressing *krz* dsRNA and *Dcr-2* using the *Cg-GAL4* driver. (C) Western blots of extracts from late third instar larvae probed with the indicated antibodies. The level of the Dorsal protein was significantly decreased in *krz*^{c01503} homozygous animals. HSP70 was used as a loading control. (D-E''') Expression of Dorsal and Dif in *krz*^{1} mutant clones in the fat bodies of third instar larvae. *krz*^{1} mutant cells are marked by the absence of GFP and are outlined with a white dotted line. Dl and Dif levels were lower than in surrounding cells, with residual protein located mostly in the nuclei. (F-G''') Localization of Dorsal in the fat bodies of second instar larvae. In Hist-GFP controls, Dl showed diffuse localization throughout the cell (F-F'''). In *krz*^{c01503} homozygotes, a significant proportion of cells had predominantly nuclear localization of Dl (G-G''').

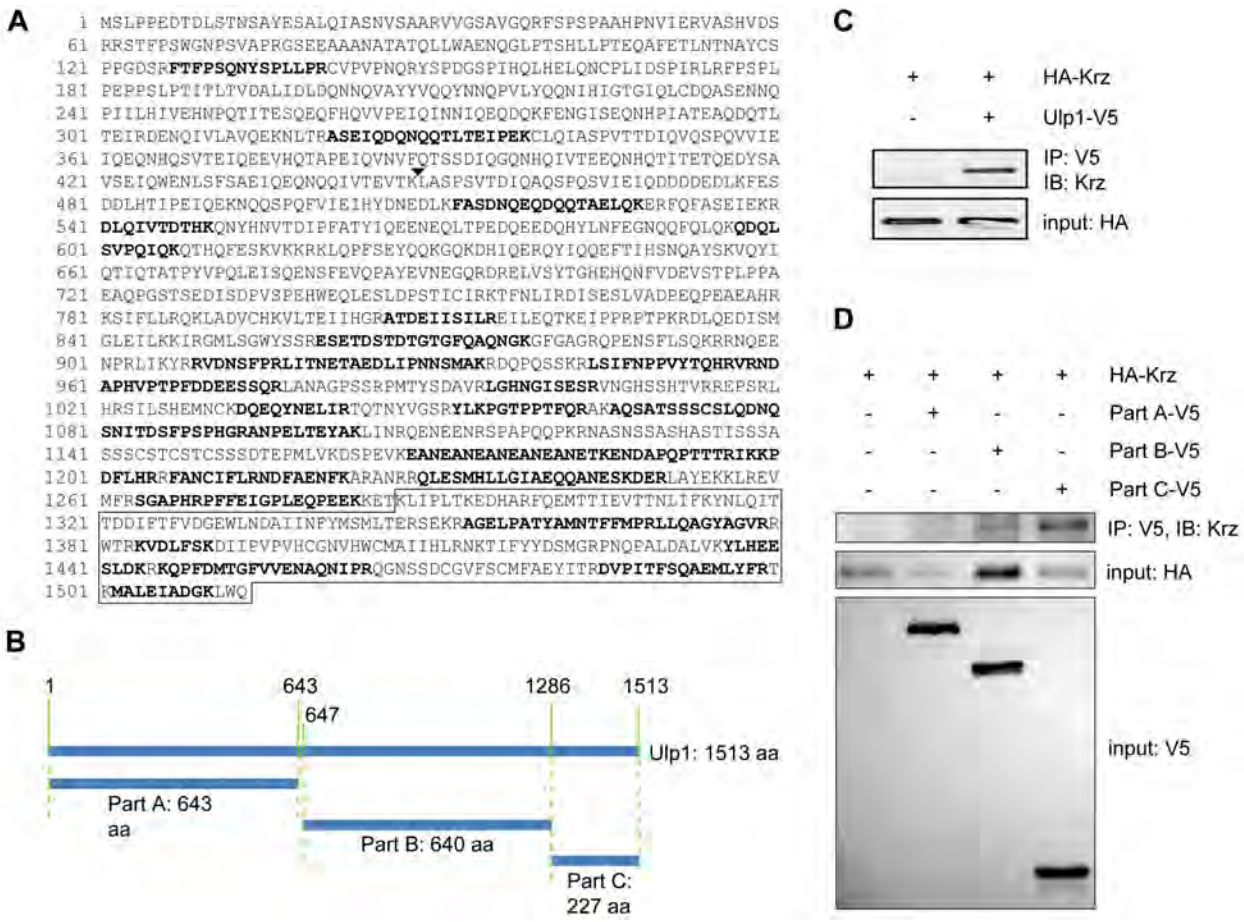

Figure S2 Identification of Ulp1 as a direct Krz interactor. (A) Amino acid sequence of the Ulp1 protein, showing an aggregate peptide coverage identified in Krz pull-downs by mass spectrometry (sequences in bold). Our mass spectrometry data confirmed that the predicted annotation of the coding sequence of Ulp1 in FlyBase is correct, as we observed two peptides from the extended amino terminus beyond the previously reported isoform (whose amino terminus is indicated by a triangle), as well as other peptides located throughout the length of the protein. A conserved carboxy terminal catalytic domain, responsible for the desumoylating activity of Ulp1, is boxed. (B) Domains of Ulp1 used for binding studies in (C) and (D). Part C corresponds to the conserved catalytic domain. (C) HA-Krz and full-length Ulp1-V5 were translated in vitro and immunoprecipitated using anti-V5 affinity resin. HA-Krz was immunoprecipitated only in the presence of Ulp1-V5. (D) HA-Krz and parts of Ulp1 were translated in vitro and immunoprecipitated using anti-V5 affinity resin. Part C of Ulp1 showed the strongest interaction with HA-Krz, followed by Part B, whereas Part A did not bind to HA-Krz.

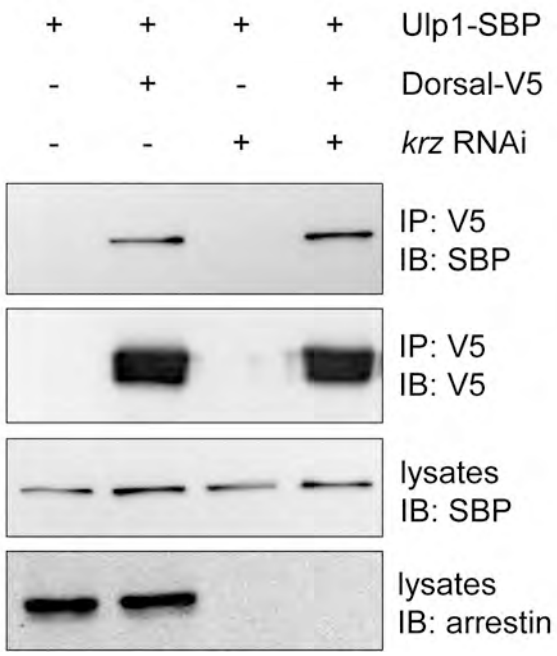

Figure S3 Loss of *krz* does not affect the binding between Ulp1 and Dorsal. Ulp1-SBP and Dl-V5 were transfected into S2 cells that were untreated or treated with *krz* dsRNA, immunoprecipitated with anti-V5 affinity resin, and immunoblotted with the indicated antibodies. Knockdown of *krz* did not affect the degree of binding between Ulp1 and Dl.

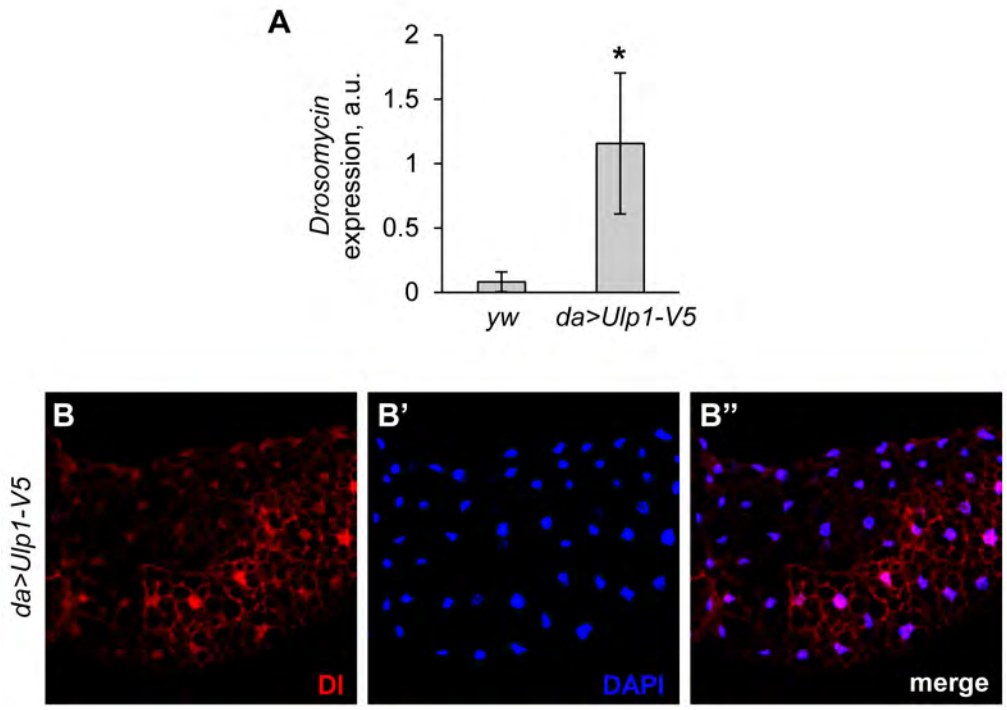

Figure S4 Overexpression of Ulp1 activates Toll signaling. (A) Quantitative RT-PCR of endogenous *Drosomycin* gene expression in whole third instar larvae. *Drs* levels were increased by approximately 14-fold when Ulp1-V5 was expressed using the *da-GAL4* driver, compared to *yw* controls. (B-B'') Localization of Dorsal protein in the fat bodies of larvae expressing Ulp1-V5 under the control of the *da-GAL4* driver. Dl showed preferential nuclear localization in a subset of cells. *, $p < 0.05$. Error bars represent standard deviation.

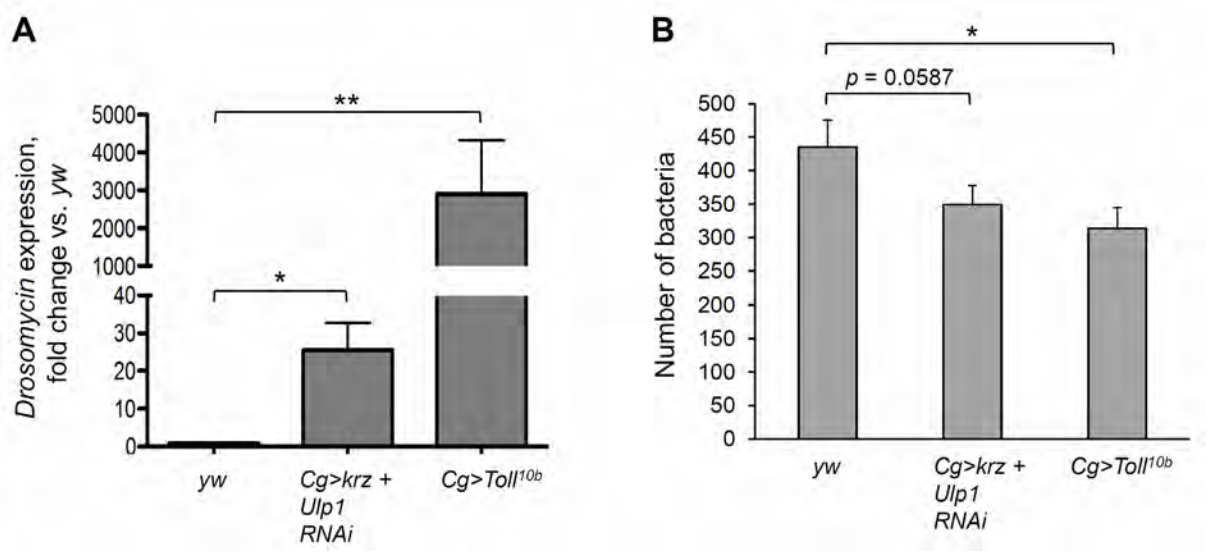

Figure S5 Effects of combined knockdown of *krz* and *Ulp1* on *Drs* expression and larval immune function. *Cg-GAL4* was used to drive the indicated *UAS* transgenes in third instar larvae. (A) Quantitative RT-PCR of endogenous *Drs* gene expression in whole third instar larvae. *Drs* levels were increased approximately 25-fold in a double knockdown of *krz* and *Ulp1*, and 3000-fold when *Toll*^{10b} was overexpressed. Note that *Toll*^{10b} is the strongest gain of function mutant reported. (B) Bacteria killing assay. Five microliters of diluted cultures of *E. faecalis* in 2xYT media containing approximately 450 bacteria were mixed with hemolymph from 10 opened third instar larvae. The mixture was incubated for 30 min, plated on 2xYT agar plates and the number of colonies counted after 18 hours at 37^{o}C. Overexpression of *Toll*^{10b} resulted in a moderate but significant decrease in bacterial numbers. Knockdown of *krz* and *Ulp1* also led to a modest decrease, with the *p* value indicated. Five independent experiments were performed. *, $p < 0.05$; **, $p < 0.01$. Error bars represent standard error.