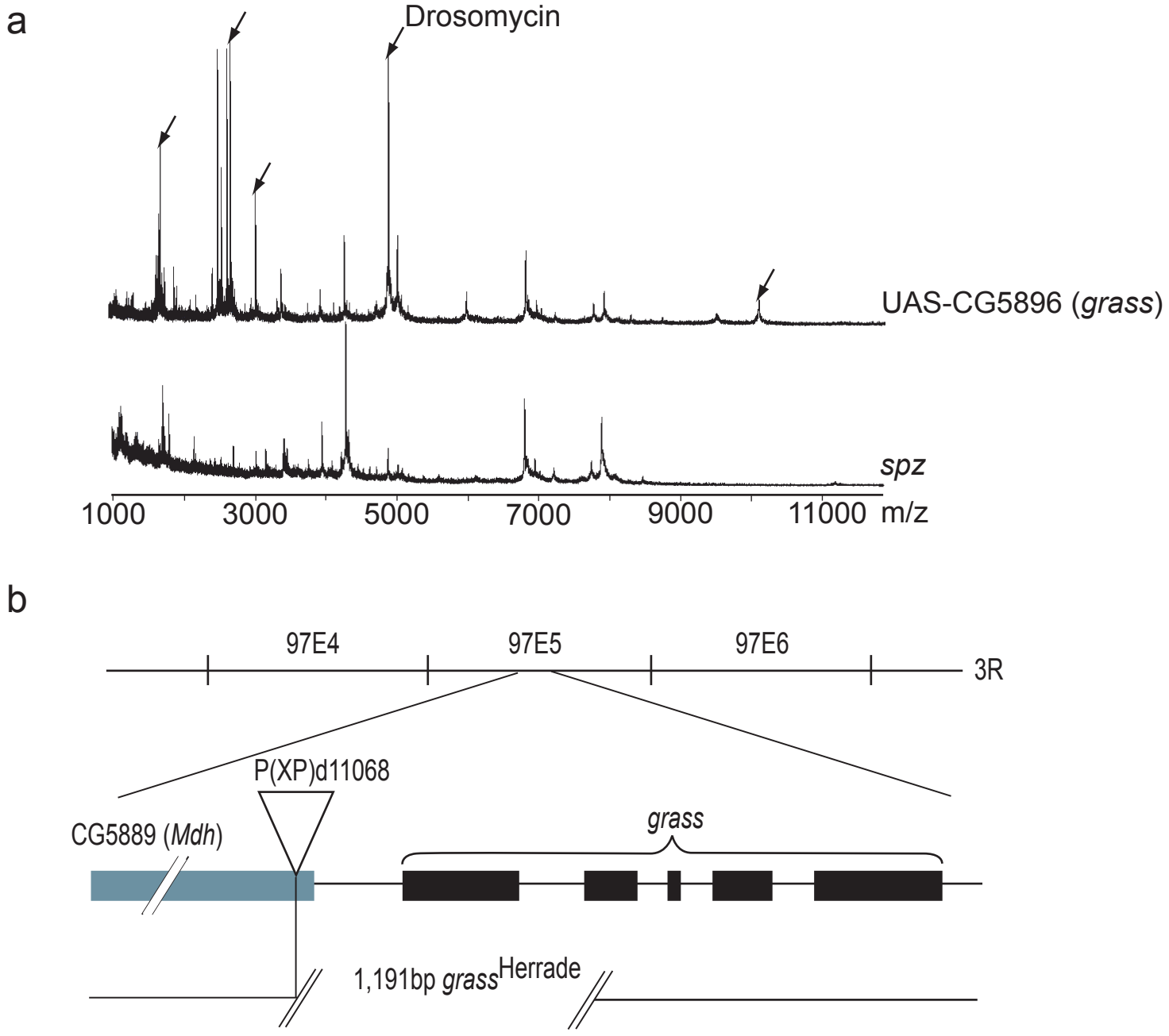
Danger signal and PAMP sensing define binary signaling pathways upstream of Toll

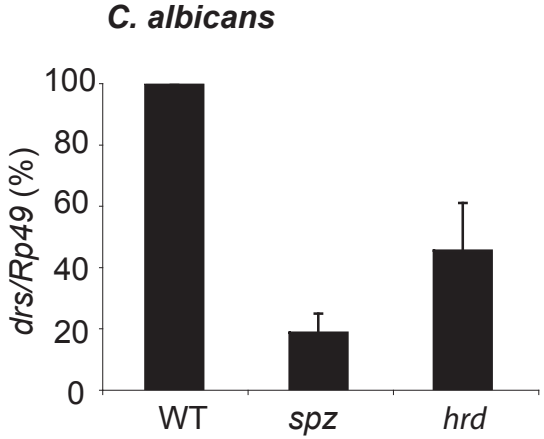
Laure El Chamy, Vincent Leclerc, Isabelle Caldelari & Jean-Marc Reichhart



Supplementary figure 1. Isolation of a grass null mutant allele

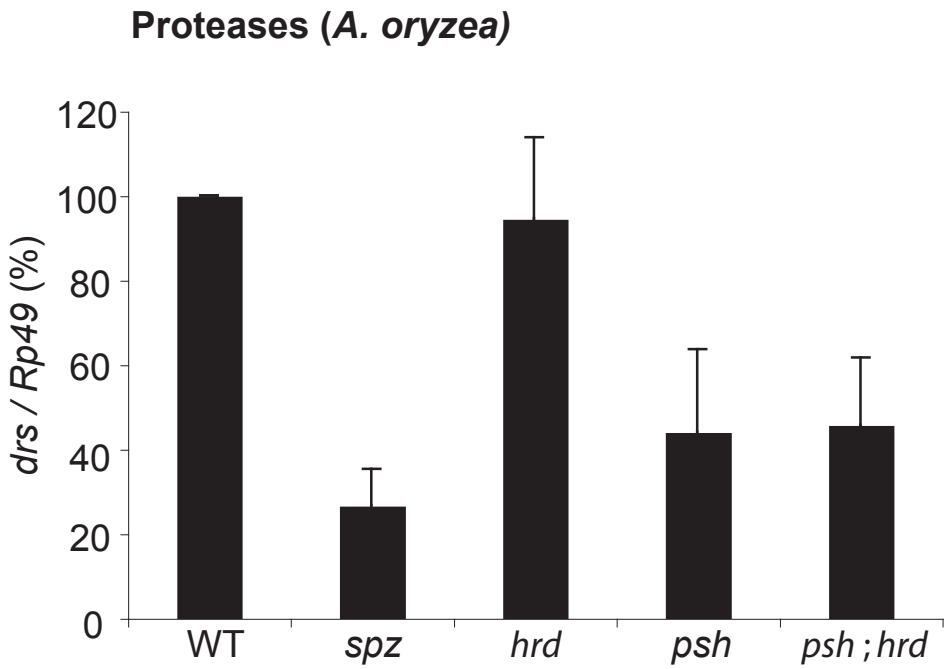
(**a**) Mass spectrometry analysis of hemolymph samples extracted from non infected transgenic flies overexpressing *grass* shows the expression of drosophila Immune Molecules (IMs, black arrows). Hemolymph sample extracted from infected *spaetzle* (*spz*) mutants is used as a control.

(**b**) Schematic representation of the right arm of *Drosophila melanogaster* third chromosome region where *grass* is localized. Exons are presented by filled boxes: black for *grass* and grey for the last exon of *CG5889*. The mobilisation of a P-element (*d11068*) inserted in the 3'UTR of the *CG5889* (light grey box), 550 bp upstream of the *grass* transcription start site, gave rise to the $grass^{Herrade}$ deletion of 1191 bp from the insertion site down to the 735th nucleotide of the *grass* transcript.



Supplementary figure 2. Grass is required for Toll pathway activation after yeast infection

Quantification of RNA hybridization analysis of *drosomycin* (*drs*) gene expression 48 hours after infection with the yeast *Candida albican*s. *Ribosomal protein 49* (*Rp49*) messenger was used for normalisation. *drs* mRNA expression (*drs/Rp49*) in wild-type (WT) flies was set to 100 as a control and values obtained with mutant flies were expressed as percentage of this value. *Spaetzle* (*spz*) mutant flies were used as Toll pathway mutant control. Each bar represents the mean of 3 independent experiments, error bars are SD. *drs* expression was highly reduced in *grass*^{Herrade} (*hrd*) mutant flies ($P = 0.036$).



Supplementary figure 3. Activation of Toll pathway by *A. oryzea* proteases

Quantification of RNA hybridization analysis of *drosomycin* (*drs*) gene expression tested 24 hours after injection of a sublethal dose of fungal *A. oryzea* proteases in adult flies. *drs* expression is not affected in *grass*^{Herrade} (*hrd)* (*P*=0.6) but is highly reduced in *psh* mutant flies (*P*= 0.004). The reduction in *drs* expression observed in *psh* is not enhanced in *psh, hrd* double mutants (*P*=0.9).