

Supplemental information

Constitutive immune activity promotes JNK- and FoxO-dependent remodeling of *Drosophila* airways

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Supplementary Information

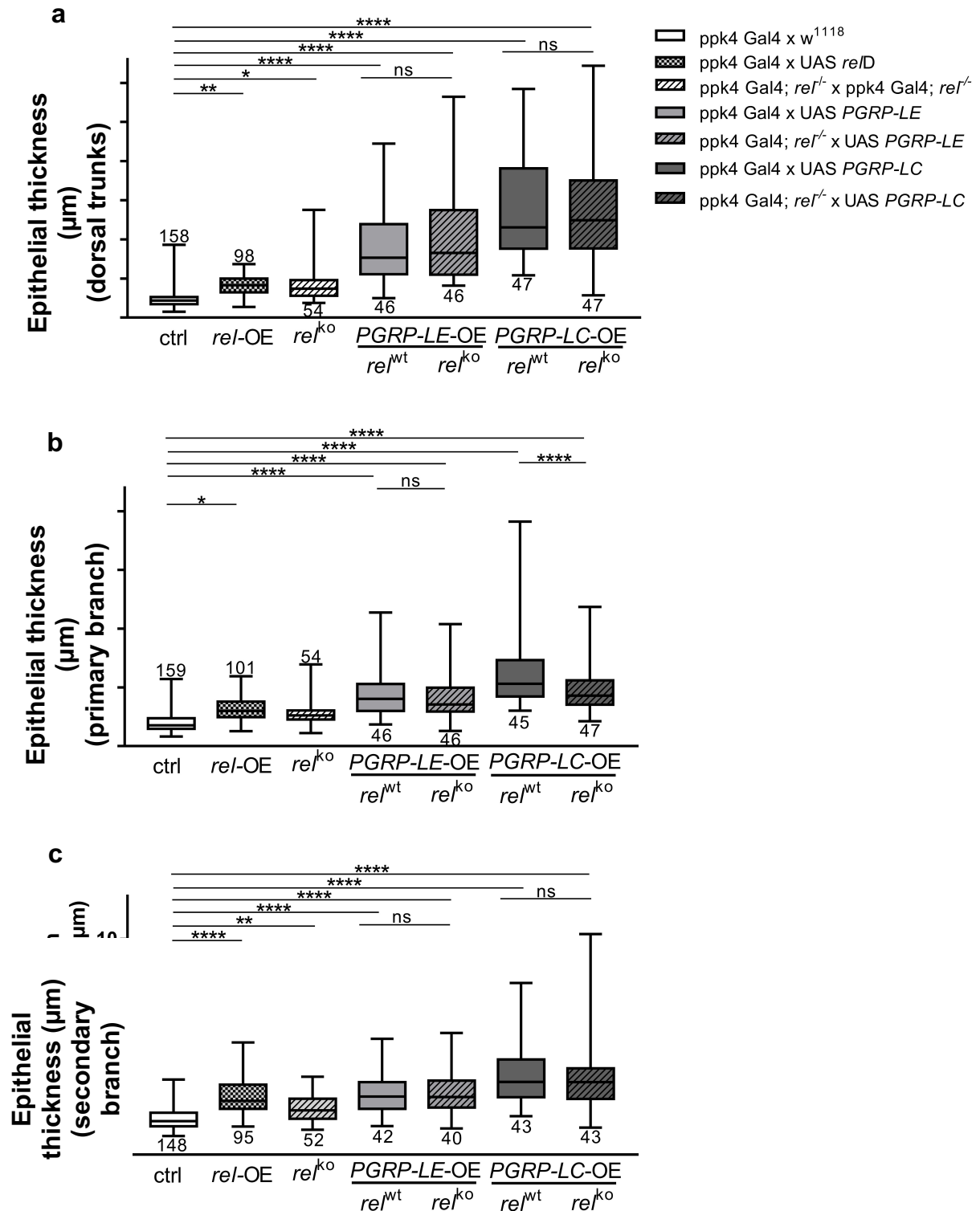


Figure S1 (related to Figure 4):

Epithelial thickening triggered by IMD pathway activation is only partially dependent on the NF- κ B transcriptional factor Relish.

(a-c) Quantitative analysis of epithelial thicknesses in different branching generations of airways overexpressing *PGRP-LE* or *PGRP-LC* in a white-eyed genetic background as well as in a Relish-deficient background. In addition, epithelial thicknesses of RelD

overexpressing airways are depicted (*ppk4 Gal4 x UAS relD*, grey dotted box plot). RelD is a constitutively active form of the transcription factor Relish. Airways from wild type (*ppk-Gal4 x w¹¹¹⁸*, white box plots) and Relish-deficient larvae (*ppk4-Gal4; rel^{E20}*, white shaded box plots) served as controls. Grey and grey shaded box plots display the data set of *PGRP-LE* as well as *PGRP-LC* overexpressing airways in a wild type (clear boxes) and Relish-deficient background (shaded boxes) dissected from larvae heterozygous for the null allele *Relish^{E20}*. Values of dorsal trunks (**a**), primary (**b**), and secondary branches (**c**) are calculated from at least n=40 measuring points. Individual measuring points are listed above or below the box plots. Significant differences to controls are indicated by a one-way ANOVA, F=104.2 (**a**), F=60.6 (**b**), F=43.66 (**c**); *p<0.05; **p<0.01; ****p< 0.0001.

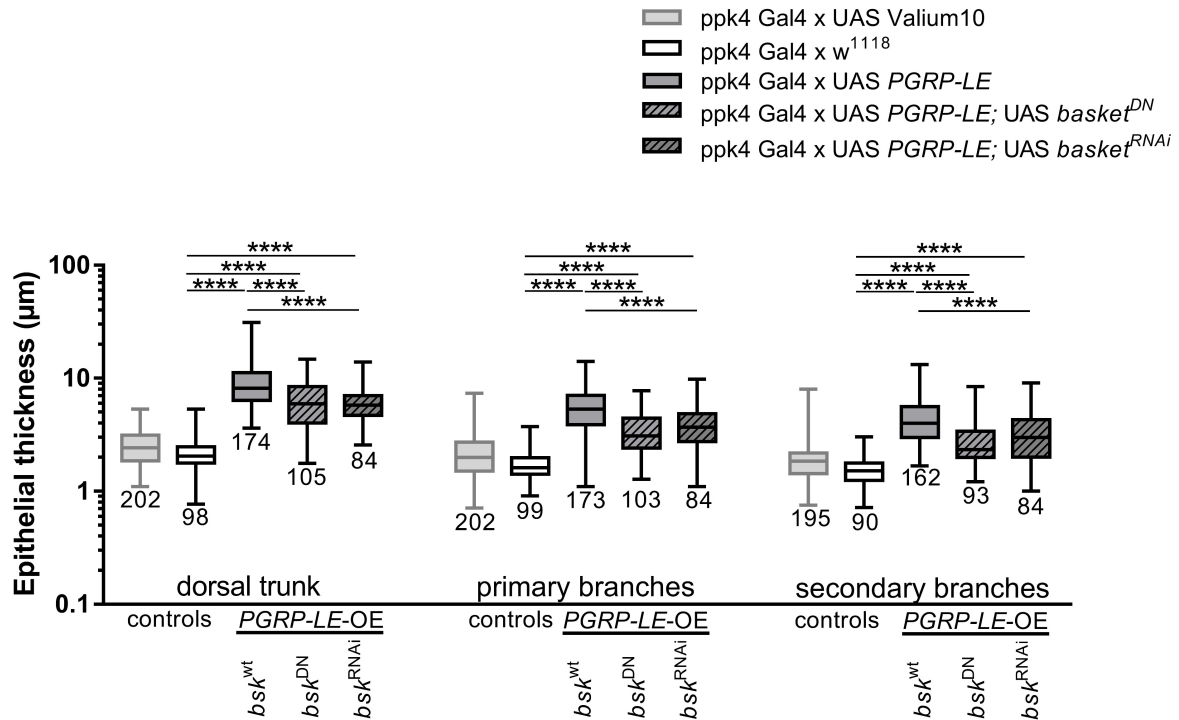


Figure S2 (related to Figure 5):

NF-κB (IMD)-induced epithelial thickening relies almost entirely on JNK signaling.

Quantification of epithelial thicknesses in different branching generations of airways overexpressing *PGRP-LE* in a white-eyed genetic background (w^{1118}), in a *basket* dominant-negative (UAS-*basket*^{DN}) or a *basket*-RNAi (UAS-*basket*^{RNAi}) background. GFP-expressing airways (*ppk4*-Gal4 x UAS-*gfp.valium.10*; light grey bar) and airways carrying a white-eyed allele (*ppk4*-Gal4 x w^{1118} ; white bar) served as controls. Grey shaded box plots display the data set of *PGRP-LE* overexpressing airways, which were dissected from larvae either heterozygous for the dominant-negative mutant allele Basket or *basket* RNAi. Expression of the dominant-negative form of Basket and its RNAi-mediated silencing was achieved by crossing the airway-specific driver strain *ppk4*-Gal4 with the homozygous strain UAS-*PGRP-LE*; UAS-*basket*^{DN} or UAS-*PGRP-LE*; UAS *basket*^{RNAi}. Data calculated from at least n=84 measuring points. Individual measuring points are listed below the box plots. Significant differences to controls are indicated by a one-way ANOVA, F=183.8 for dorsal trunks, 150.4 for primary branches, 86.5 for secondary branches; ****p<0.0001.

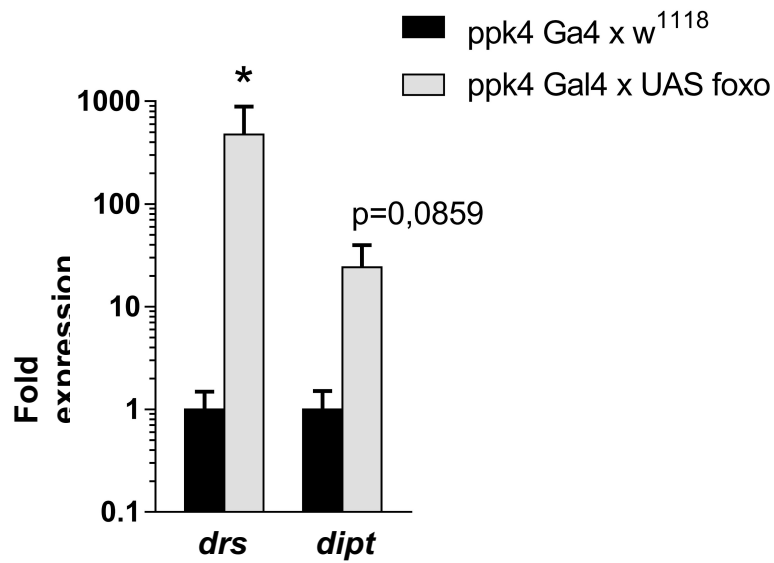


Figure S3 (related to Figures 4 and 7):

FoxO overexpression induced the expression of the antimicrobial peptide genes drosomycin and dipterocin.

Quantification of drosomycin (*drs*) and dipterocin (*dipt*) expression in *foxo* overexpressing (*ppk4*-Gal4 x UAS-*foxo*) and wild type airways (*ppk4*-Gal4 x w^{1118}) from third instar larvae. For data normalization, *rpl32* was used. Each value represents the mean \pm S.E.M. from six experiments. * $p < 0.05$: significant differences to control from a multiple t-test (unpaired t-test for each AMP) followed by Holm multiple comparison testing.