

Title: The Ubiquitin-conjugating enzyme Effete regulates hemocyte differentiation in *Drosophila melanogaster*

authors: Susanna Valanne^{1*}, Laura Vesala^{2*}, Petra Vornanen¹, Joanna Ciantar¹, Tea Tuomela^{1,2}, Tiina S. Salminen² and Mika Rämetsä¹

¹Experimental Immunology research group, Faculty of Medicine and Health Technology, Tampere University, Tampere, Finland

²Mitochondrial Immunometabolism research group, Faculty of Medicine and Health Technology, Tampere University, Tampere, Finland

Abstract

Ubiquitination is a common mechanism to regulate protein function in many cellular processes including innate immunity and it is highly conserved across taxa. Here, we studied the role of Ubiquitin-conjugating enzyme Effete (Eff) in the *Drosophila melanogaster* innate immunity. We used both *in vitro* and *in vivo* approaches and knocked down *eff* in *Drosophila* cultured cells (S2 cells) and in the main immune tissues of *Drosophila*, the fat body and hemocytes.

In S2 cells, where the Toll pathway was induced by either overexpression of Spätzle (*Spz*^{C106}) or silencing of *Cactus* (the inhibitor of κ B factor in *Drosophila*), *eff* knockdown elevated the expression of the Toll pathway target gene *Drosomycin* (*Drs*). In larval hemocytes, *eff* knockdown caused upregulation of *Drs*, immune activation and differentiation, and enhanced the hemocyte mediated immune response against parasitoid wasps. *Eff* knockdown in the fat body did not affect the wasp encapsulation response, but the expression of Toll targets *Drs* and *Bomanin Short 1* (*BomS1*) was mildly induced in unchallenged male flies. Furthermore, *eff* manipulation in the fat body was lethal at pupal stage, whereas hemocyte-specific *eff* knockdown decreased the lifespan of the flies.

Transcriptome analysis showed largely differential responses in hemocytes and fat body upon *eff* knockdown. Gene Ontology term analysis highlighted stress response pathways, including ER stress and unfolded protein response, activated by *eff* knockdown in hemocytes. In conclusion, Eff plays a complex role with tissue-specific effects in regulating innate immunity and has an important role in the fat body on *Drosophila* development and viability.