



**Figure S3** Induction of the glue gene *Sgs3* in response to the mid-L3 pulse of ecdysone. *Sgs3* is massively induced in salivary glands after the mid-L3 pulse of ecdysone; the amount of *Sgs3* mRNA made is easily detectable by qPCR in extracts of total mRNA from whole animals at wandering third instar stages (wL3). wL3 controls display an ~18,500-fold increase of *Sgs3* mRNA when compared to third instar animals prior to the mid-L3 pulse (early L3 or eL3). All PSG mutant wL3 animals show robust induction of *Sgs3* mRNA. In contrast, salivary gland-specific expression of *Ecr<sup>F645A</sup>* using the *Sgs3* promoter (*uas-Ecr<sup>F645A</sup>/+*; *Sgs3-GAL4/+*) shows a nine-fold decrease in the induction of *Sgs3* mRNA, highlighting the requirement of ecdysone signaling for continued expression of *Sgs3*. y-axis plots relative expression of target genes compared to eL3 controls and normalized to *rp49*. x-axis shows control animals at eL3 and wL3 stages, and mutant animals at wL3 stages. Each bar represents 3 independent biological samples; asterisks indicate a significant changes in expression compared to control animals at wL3 ( $p$ -values < 0.05).