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 EcRF645A using the Sgs3 promoter (uas-EcRF645A/+; 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 change in expression compared to control animals at wL3 (p-values < 0.05).