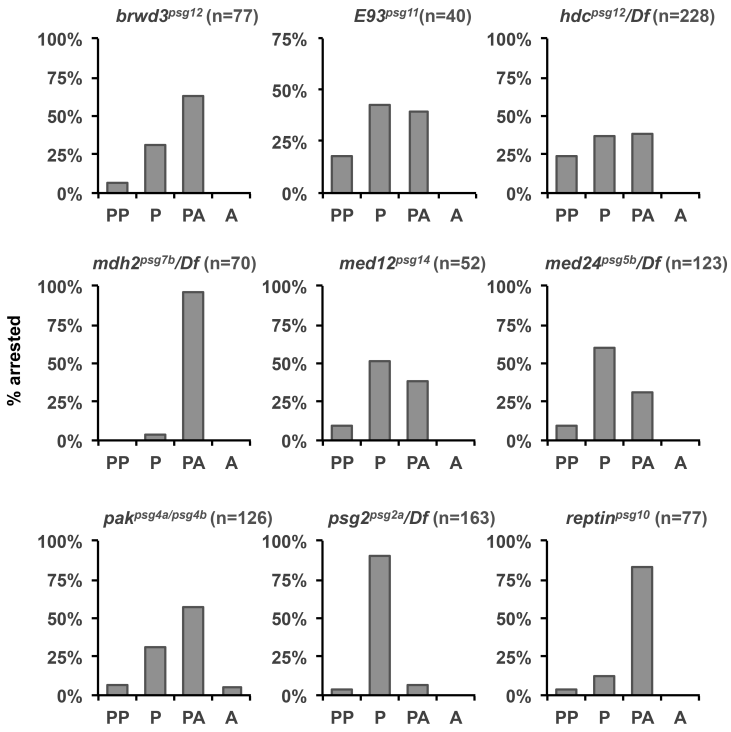


Figure S1 Lethal phase analysis of PSG mutations. The lethal phase analysis shows that the majority of PSG mutant animals arrest after head eversion and die as pupae or pharate adults. The genotype and number of animals used for lethal phase analysis is shown above each graph. PP: prepupae, P: pupae, PA: pharate adults, A: adults eclosed.

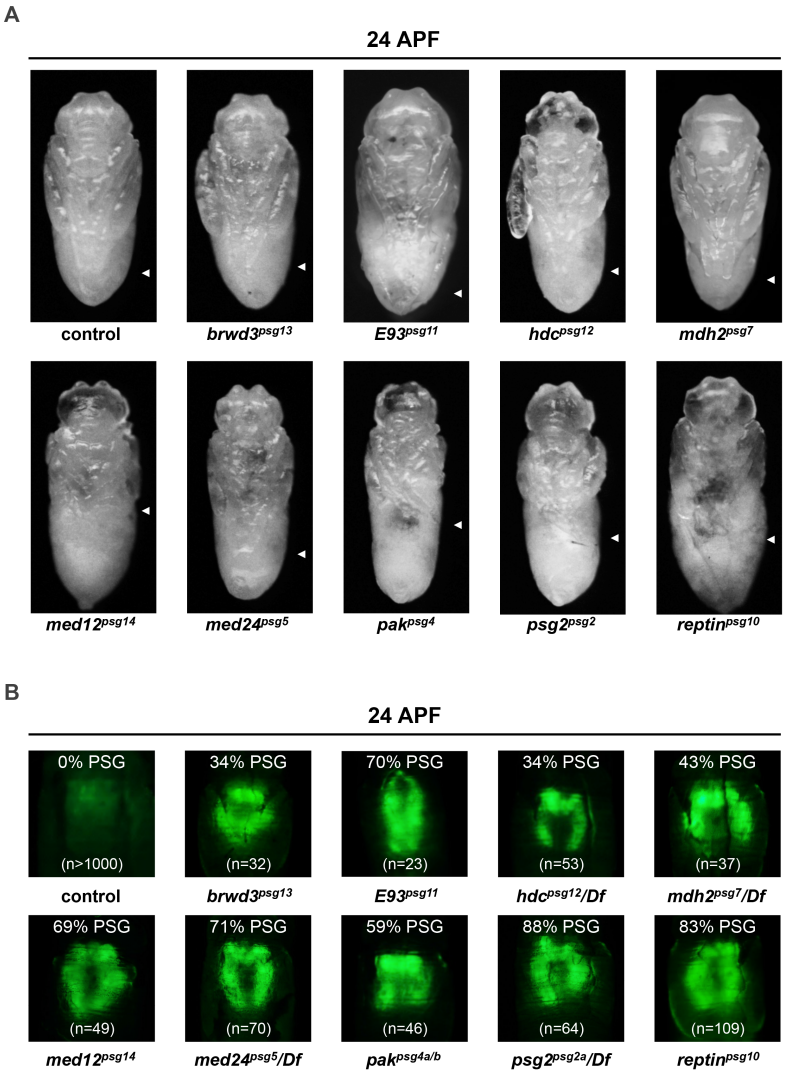


Figure S2 Pupal morphology and PSG penetrance for PSG mutations. (A) Pupal morphology was examined by dissecting animals out of their pupal casing at 24 hours after puparium formation (APF). All PSG mutants display head capsule eversion, wing and leg inflation. White triangles mark the progression of leg extension. *med12, pak, psg2*, and *reptin* display defects in leg extension. (B) PSG mutants display a highly penetrant block in salivary gland cell death. Each image shows salivary gland-specific expression of GFP (*fkh-Gal4*, *UAS-GFP*) at 24 APF. Within each image, the percent PSG and the number of animals examined are shown in white text.

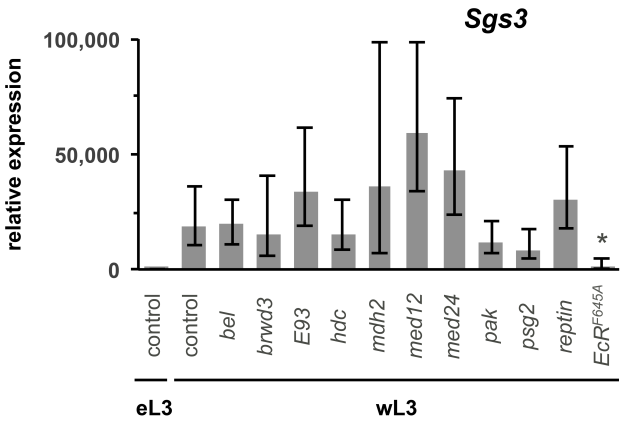
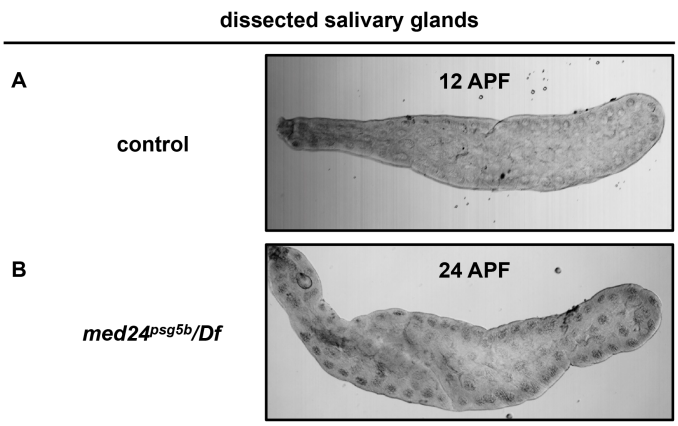


Figure S3 Induction of the glue gene *Sgs3* in response to the mid-L3 pulse of ecdysone. *Sgs3* is massively induced in salivary glands after the mid-L3 pulse of ecdysone; the amount of *Sgs3* mRNA made is easily detectable by qPCR in extracts of total mRNA from whole animals at wandering third instar 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^{F645A} using the Sgs3 promoter (*uas-*EcR^{F645A}*/+; 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).



C

salivary gland recovery by dissection at 24 APF

genotype	recovered / total	%
control	0 / 10	0
uas-Atg1^{K38Q}/+; Sgs3-GAL4/+;	0 / 10	0
uas-p35/+; Sgs3-GAL4/+;	0 / 10	0
uas-EcR^{F645A}/+; Sgs3-GAL4/+;	10 / 10	100
med24^{psg5b}/Df	8 / 10	80

Figure S4 Ability to recover larval salivary glands 24 hours after puparium formation (APF). (A) Although control salivary glands are easily recovered at 12 APF, immediately before tissue destruction begins, they cannot be recovered a few hours later. (B) *med24* mutant glands, however, can be recovered by dissection 12 hours later at 24 APF. Note that mutant glands appear morphologically "intact," similar to control glands at 12 APF. (C) Table summarizing the ability to recover larval salivary gland by dissection at 24 APF. Column one indicates the genotype, column two the number recovered over the total number of glands dissected and column three the percentage recovered. Only an upstream block in salivary gland destruction caused by overexpressing *EcR^{F645A}* or a mutation in *med24* leads to a complete block in gland destruction, which allows for recovery by dissection at 24 APF. Blocking activation of caspases or autophagy, by expression of either the caspase inhibitor *p35* or the autophagy inhibitor *Atg1^{K38Q}*, does not allow gland recovery at 24 APF.

TABLE S1 Primer sequences for qPCR

Gene	Primer Sequence	Source
belle F	ACCGCAGCAATGGAAACTC	this study
belle R	GGTCCTTTGCCTGAAGCTG	
brwd3 F	AGCAGTACAATCGCCGACAC	this study
brwd3 R	GGTACACATCCGCAAGCTG	
hdc F	CTCTCATCGCTGGCCCAATC	this study
hdc R	GTGCGTCCCTCGTATTTAACCT	
mdh2 F	TCACCGACTTGGCGCTCTAC	this study
mdh2 R	GAGATGTCCTTGATGATGCCGG	
med12 F	GCAGCAACAGTGGCACAATG	this study
med12 R	GAGTTGGCATTCAACGGCGG	
med24 F	CGTTGCTCCAGTACGTGGTG	this study
med24 R	CATTTCCTTTGCGGCTCTCCAG	
psg2 F	GCTACCAACGCAAGGACCTC	this study
psg2 R	GTAGTTTCCGCTTTAGTCCTGCA	
pak F	GGTCCATGTGGGATTCGATGC	this study
pak R	CTTCTTCTGCTCCTGCTTGCT	
reptin F	CCAACTGATTACCACAGCCAAC	this study
reptin R	GTCCCTTTCCACCTCCTCGG	
Sgs3 F	CTACCGCCCTAGCGAGCAT	CHIANG *et al*. 2003
Sgs3 R	GCATCCACAATCGCAACAGT	
Rpl18a F	ACGTCCAACATGAGAGCCAAG	this study
Rpl18a R	CTGCTTGATGGACACGATCTC	
UbcD6 F1	ACATATTGCAGAACCGCTGG	this study
UbcD6 R1	GCTTTCACACGCTTCTCGT	