Supporting Information

Johnson et al. 10.1073/pnas.1309780110

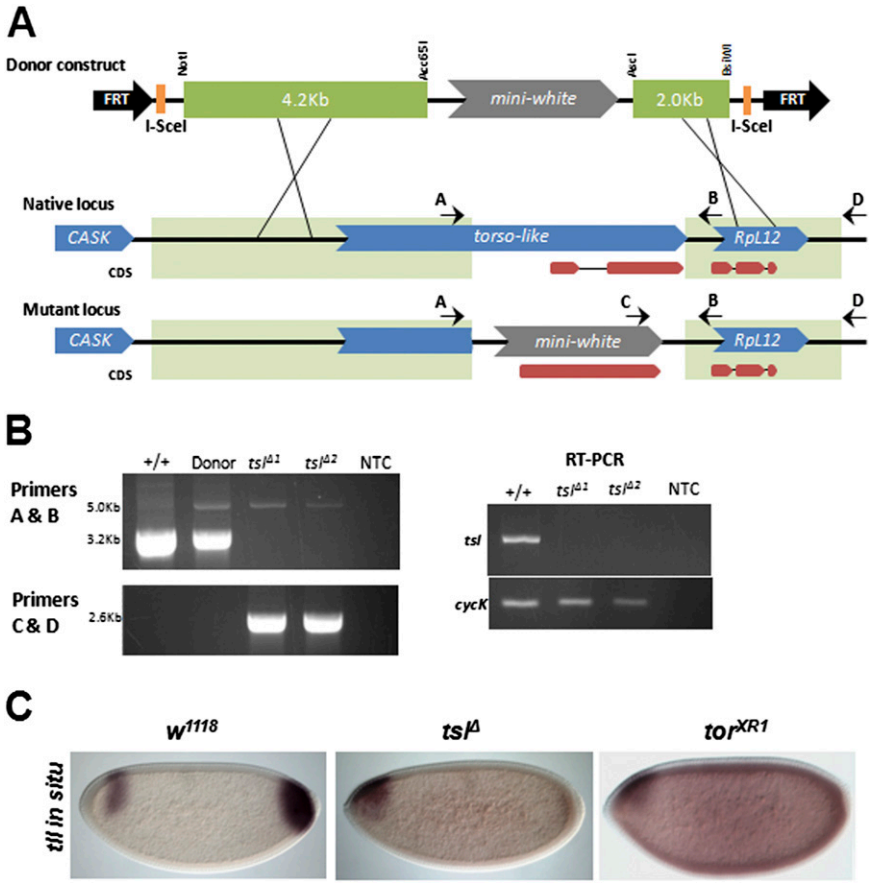

Fig. S1. Generation of the tsl^{Δ} allele. (*A*) Targeting scheme for the removal of the *torso-like* (*tsl*)-coding sequence using ends-out gene replacement. The donor construct was generated by cloning 4.2 kbp of upstream and 2.0 kbp of downstream flanking sequence. (*B*) Verification of *tsl*-targeted events (*Left*) and confirmation of loss of *tsl* transcription (*Right*). Primers A and B span the targeted region and amplify 3.2 kbp when *tsl* has been replaced by the *white* gene from pW25. Primers C and D amplify 2.6 kbp only when targeting events have occurred. RT-PCR to confirm lack of *tsl* transcripts in two *tsl* null mutant lines. (*C*) RNA in situ hybridization of early embryos with a probe to *tailless* (*tll*), a zygotic gene that is transcribed in response to Tor signaling. Embryos laid by tsl^{Δ} females display defective *tll* expression identical to that observed in embryos maternally lacking *tor*, indicating a failure to specify terminal cell fate.

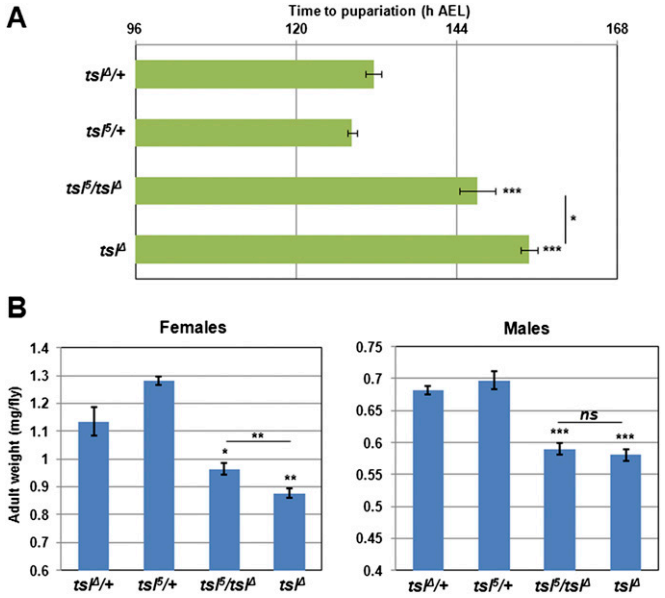

Fig. S2. *tsl*$^{\Delta}$ fails to complement *tsl*^{5} for developmental timing and body size. (*A*) *tsl*^{5}/*tsl*$^{\Delta}$ larvae are significantly delayed to pupariation compared with controls ($P < 0.001$) and faster than *tsl*$^{\Delta}$ homozygotes ($P = 0.027$). (*B*) *tsl*^{5}/*tsl*$^{\Delta}$ adults that emerge from these experiments are smaller than controls for both sexes (females, $P < 0.017$; males, $P < 0.001$ compared with *tsl*$^{\Delta}$/+). For developmental timing, means are representative of $n = 6$ or greater with no fewer than 63 individuals tested for each genotype. For adult weight, means are calculated from $n = 6$ or greater with no fewer the 24 flies weighed for each genotype. Error bars represent ± 1 SEM for all graphs. ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$ from two-tailed *t* tests in all cases.

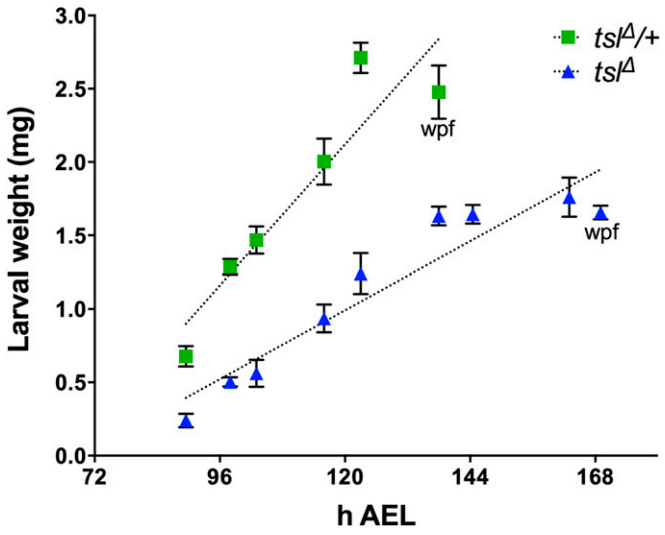

Fig. S3. Loss of *tsl* causes a severe growth rate defect. Weights of *tsl*$^{\Delta}$ larvae are significantly less than controls at all tested time points during the third instar stage ($P < 0.05$). Dotted lines indicate linear regression models for each genotype with slopes that significantly deviate from zero (*tsl*$^{\Delta}$, *y* axis $= 0.02 \times -1.36$, $P < 0.001$; *tsl*$^{\Delta}$/+, *y* axis $= 0.04 \times -2.68$, $P = 0.008$). Growth rates are significantly reduced in *tsl*$^{\Delta}$ larvae compared with the control (ANCOVA, $F_{1,11} = 8.55$, $P = 0.014$). Each mean is calculated from $n = 4$ with at least five larvae weighed for each replicate. wpf, white prepupa formation.

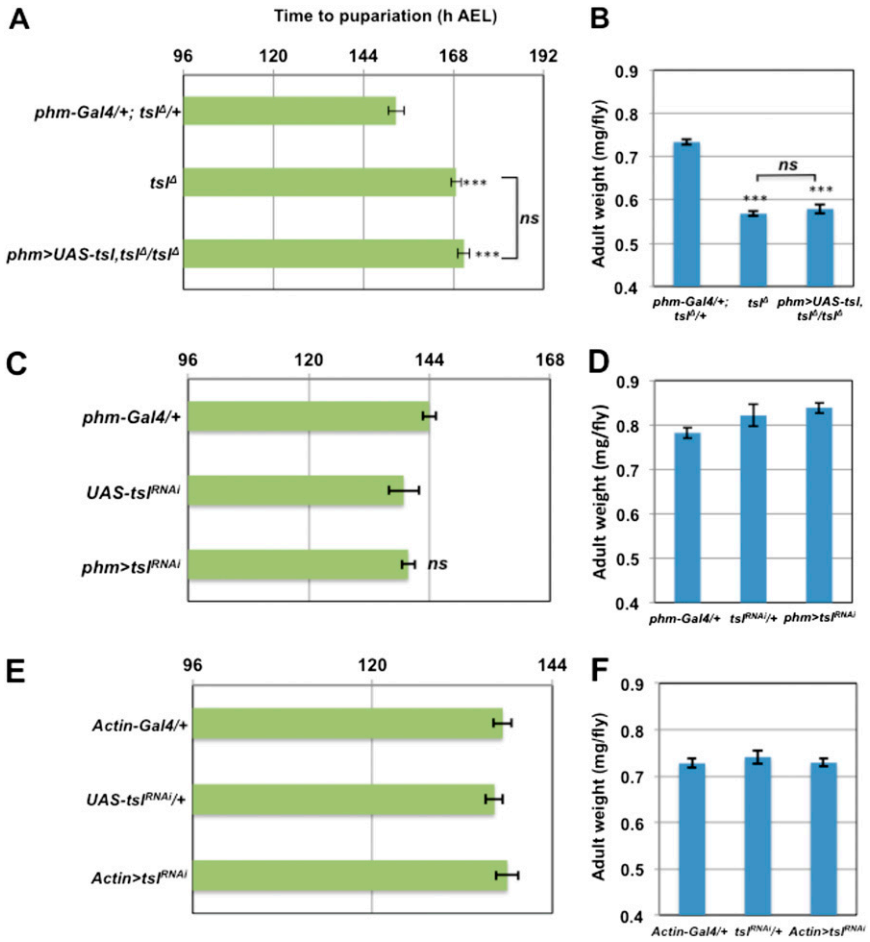

Fig. S4. The tsl^{Δ} developmental delay and small adult phenotypes could be caused by loss of Tsl in a tissue distinct from the prothoracic gland (PG). The *tsl* null developmental delay and small size phenotypes are not restored when a UAS-*tsl* transgene is expressed specifically in the PG using *phm-Gal4* (*A* and *B*). Means are representative of $n = 8$ or greater for developmental timing, with no fewer than 58 individuals tested for each genotype. RNAi knockdown of *tsl* in the PG does not cause a delay in development to pupariation or small adult size (*C* and *D*). However, strong ubiquitous *tsl* knockdown using *Actin-Gal4* also does not recapitulate the zygotic *tsl* loss-of-function phenotypes (*E* and *F*), suggesting that our RNAi results are unreliable. $n = 4$ for *phm-Gal4* timing estimates and $n = 10$ where *Actin-Gal4* is used, where each mean is calculated from no fewer than 80 individuals per genotype. For adult weights in all cases, means are calculated from $n = 8$ or greater, with no fewer than the 22 flies weighed for each genotype. Note that only male weights are shown due to insufficient recovery of females for the rescue experiment whereas, for the RNAi experiments, male data are representative for both sexes. *ns*, not significantly different from controls or as indicated. Error bars represent ±1 SEM for all graphs. $***P < 0.001$ from two-tailed *t* tests in all cases.