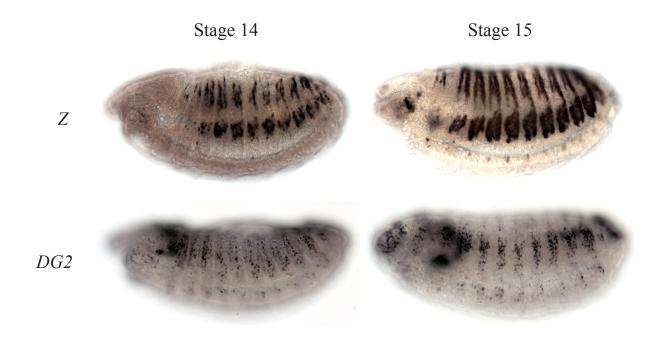


Base genome: *Drosophila melanogaster*Calc. Window: 100 bp
Cons. Width: 100 bp

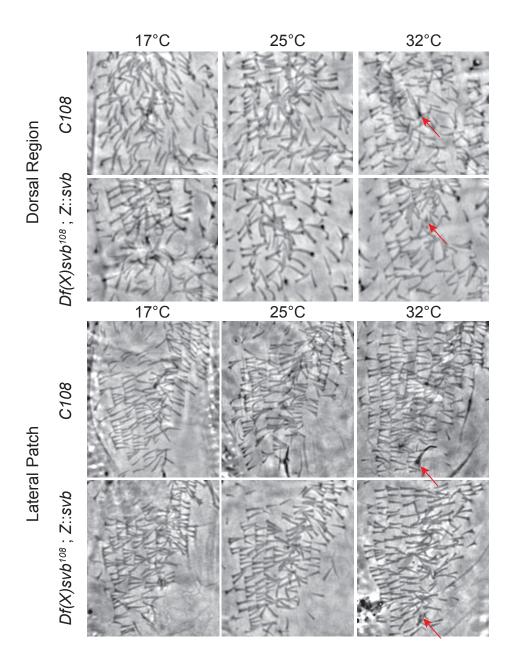
Cons. Width: 100 bp Cons. Identity: 70% 1. Drosophila ananassae

D. pseudoobscura
 Drosophila virilis

Supplementary Figure 1. A map showing the locations of the fragments used for the reporter assays is shown at the top. Below, a VISTA plot (http://genome.lbl.gov/vista) of the same genomic region upstream of *svb* enhancer *A*, between genes *Ptp4E* and *CG12860*. A VISTA plot of the region from the *svb* gene to the gene CG12680 was published previously (McGregor et al.2007.*Nature*.448: 587-591). The alignment compares *D. melanogaster* to *D. pseudoobscura*, to *D. ananassae*, and to *D. virilis*, revealing high conservation peaks throughout almost the entire region. The exception is a *roo* transposable element present only in the *D. melanogaster* genome (in magenta). The red rectangles indicate the positions of the newly-discovered enhancers.



Supplementary Figure 2. Lateral views of reporter gene expression driven by D. melanogaster Z and DG2 at embryonic stages 14 and 15. The β -galactosidase reporter driven by Z is targeted to the cytoplasm, whereas the β -galactosidase driven by DG2 has a nuclear localization signal. Both reporters were detected with the same anti- β -galactosidase antibody.



Supplementary Figure 3. A Z::svb transgene rescues the temperature-dependent loss of trichomes in $Df(X)svb^{108}$ larvae. The dorsal region (above) and lateral patch (below) of first-instar larvae with genotypes C108 and $Df(X)svb^{108}$; Z::svb reared at three different temperatures. In $Df(X)svb^{108}$; Z::svb larvae, the dorsal region shows little or no rescue at extreme temperatures, while the lateral patch shows complete rescue (see also Fig. 3). The red arrows highlight bristles on C108 larvae that are lost in both $Df(X)svb^{108}$ and, as shown here, $Df(X)svb^{108}$; Z::svb larvae.