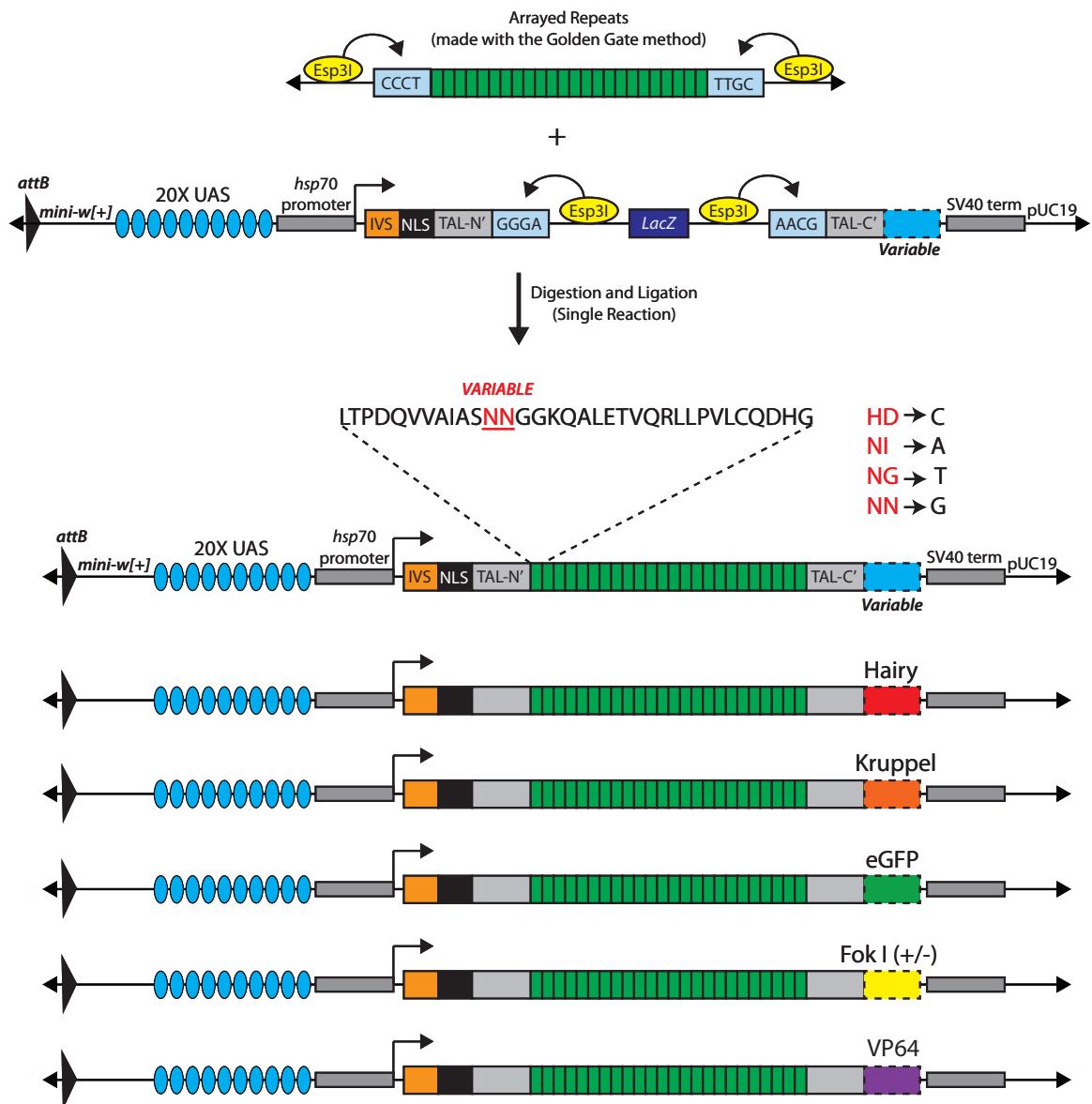
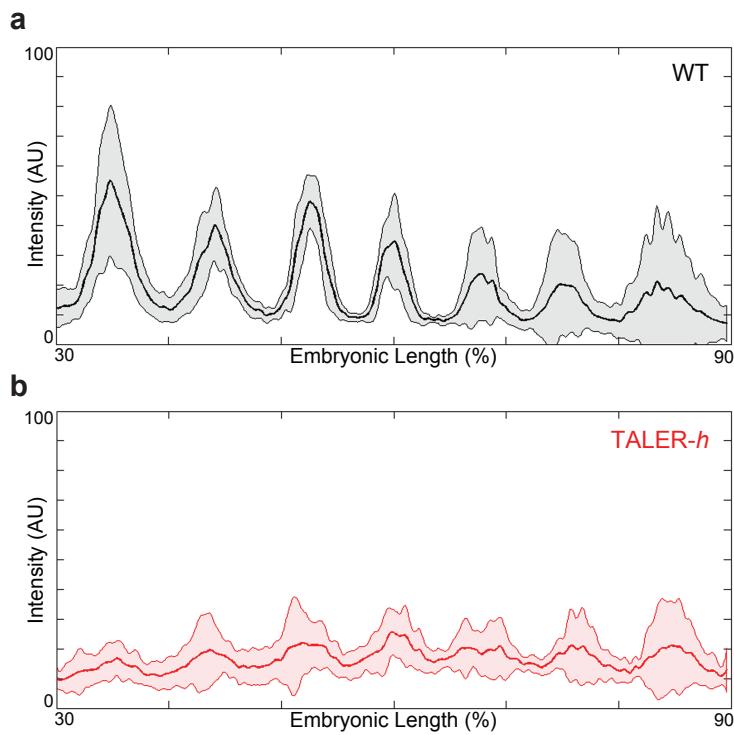


Supplementary Figure 1



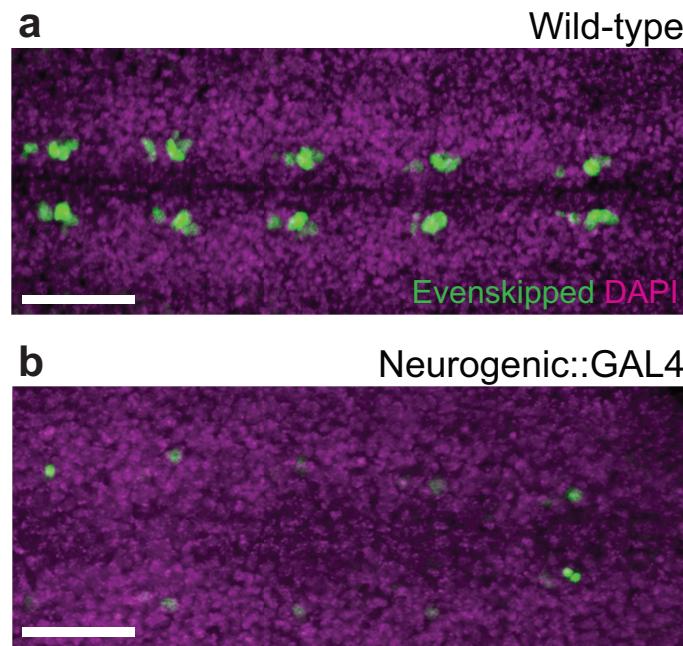
New Golden-Gate compatible TALE constructs. Each construct contains a *mini-white* gene, 20 Gal4 responsive binding sites (20X UAS), an *hsp70* promoter, a nuclear localization signal (NLS), a SV40 terminator, and a respective fusion domain. Arrayed repeats generated with the Golden Gate method can be cloned into the *Esp3I* restriction sites of the respective vector

Supplementary Figure 2



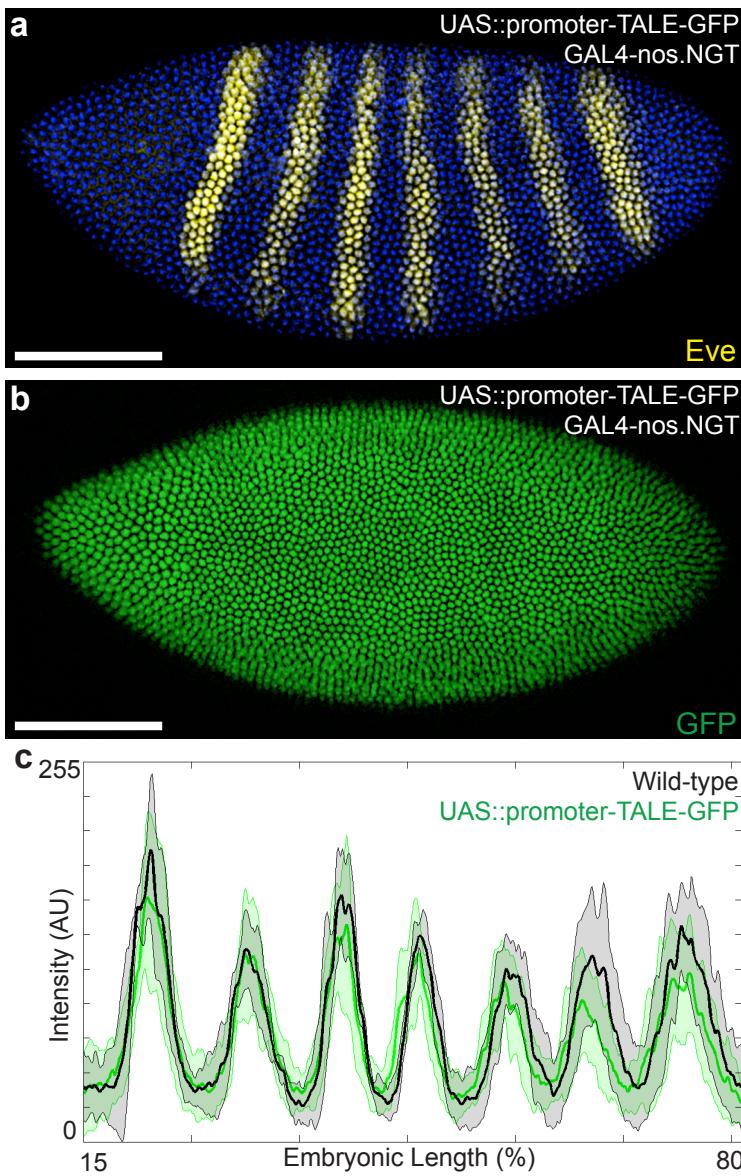
A TALER-*hairy* targeting the *eve* promoter represses *eve* expression. **(a, b)**, Average expression profile of Eve in wild type stage 5 embryos **(a, grey)** and stage-5 embryos carrying a TALER-*hairy* targeting the *eve* promoter, under the *nos*::GAL4 driver **(b, red)** ($n=10$ for each genotype). The lighter-shaded, bounding areas indicate one standard deviation. Fluorescence intensity is reported in arbitrary units (AU).

Supplementary Figure 3



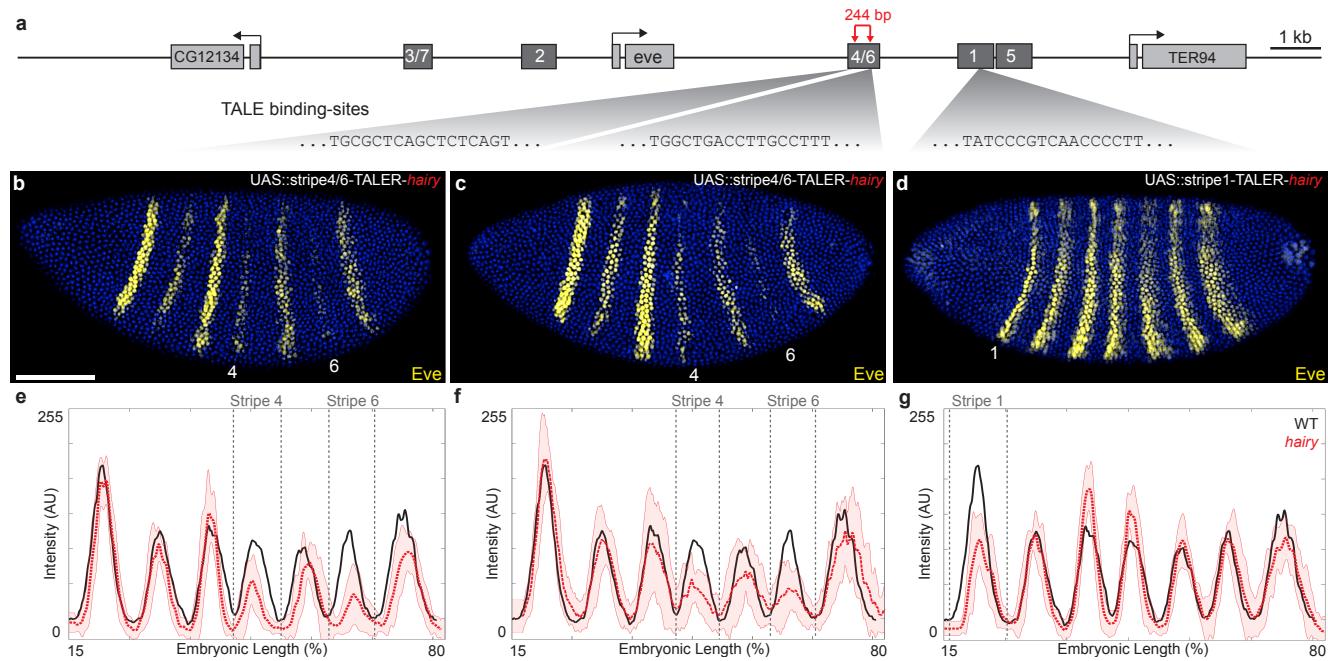
TALER repression of neurogenic-expressed Eve in stage 11 embryos. (a), Wild-type expression of Eve. (b), Expression of Eve in an embryo containing a UAS::promoter-TALER-hairy construct driven by *rhomboid*::Gal4, which drives expression in the neurogenic ectoderm. Scale-bars: 20 μ m (a and b).

Supplementary Figure 4



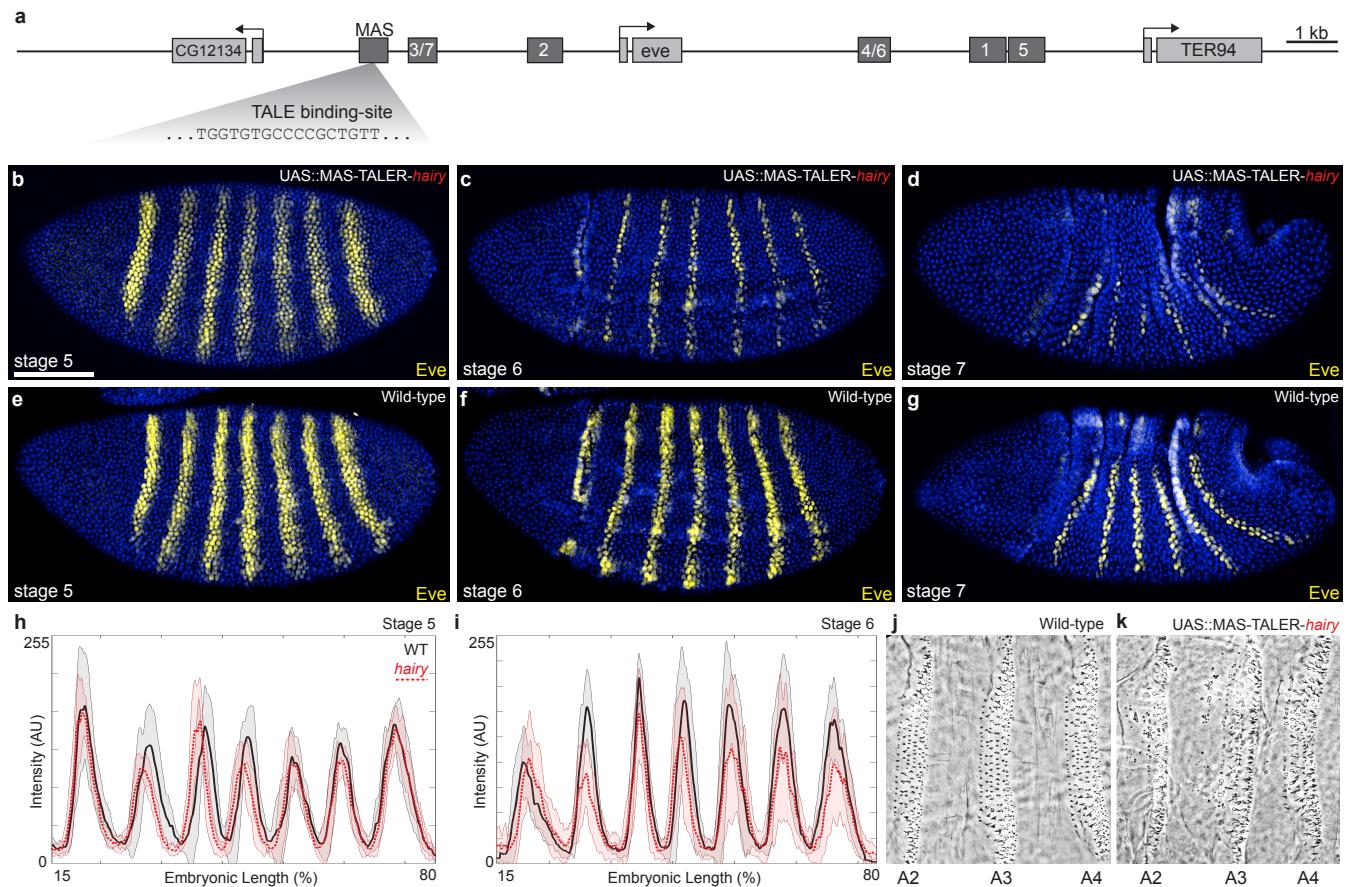
TALE targeted GFP to the *eve* promoter. (a), A stage-5 embryo carrying UAS::promoter-TALE-GFP, nos::GAL4 stained for Eve protein. (b), A stage 5 embryo carrying UAS::promoter-TALE-GFP, nos::GAL4 and stained for GFP protein indicates that the TALE-GFP is expressed in a nearly ubiquitous pattern. (c), Profiles of average expression levels of Eve in stage 5 wild-type embryos (grey lines) and embryos carrying the TALE-GFP (green lines) ($n=6$ for each genotype). Lighter-shaded, bounding areas indicate standard deviations and fluorescence is reported in arbitrary units (AU). Scale-bars: 100 μ m (a, b).

Supplementary Figure 5



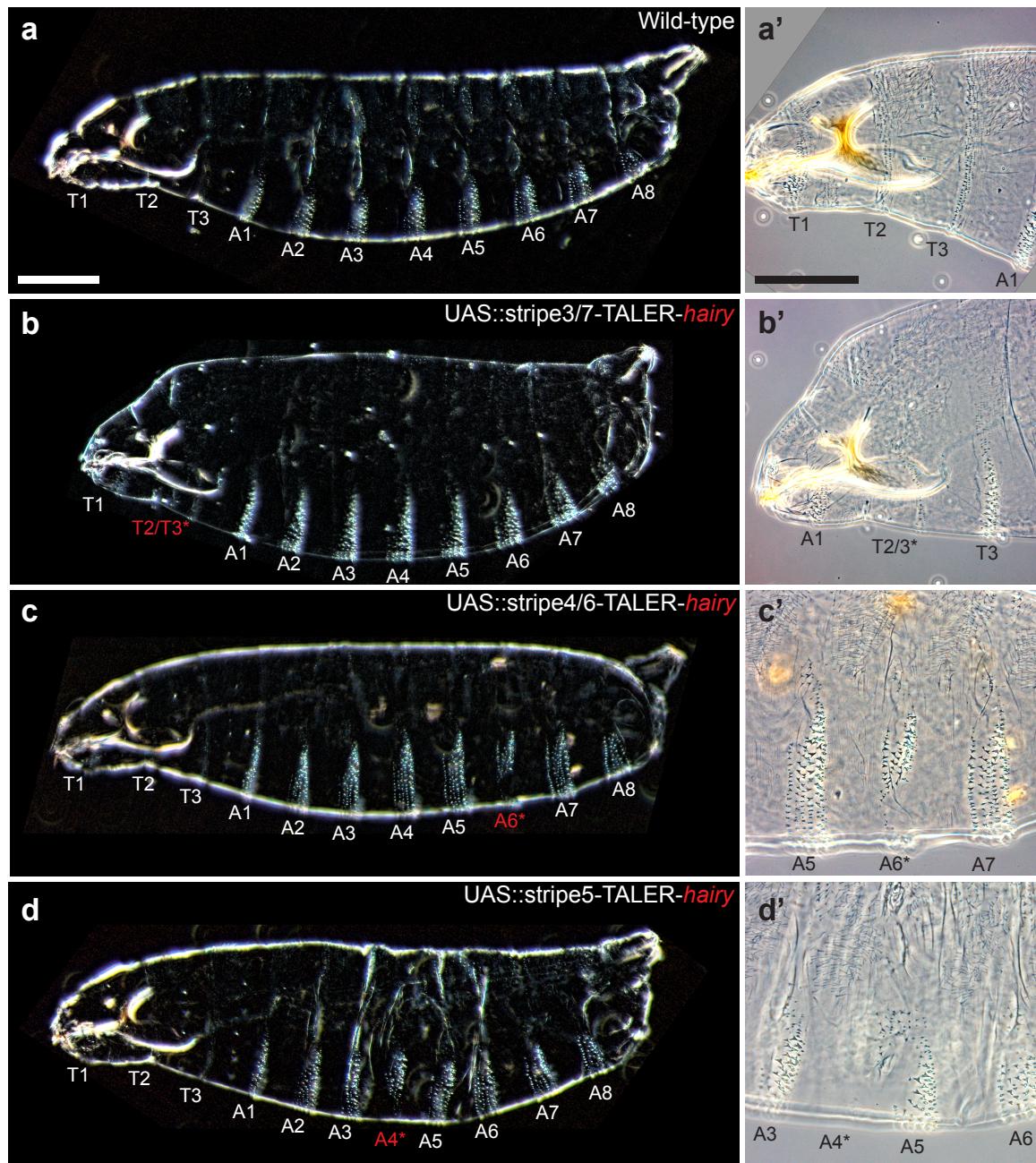
TALE targeted repression of *eve* stripe enhancers. **a**, Schematic of the *eve* locus, indicating early-embryonic *cis*-regulatory stripe enhancers and TALE binding sites. **(b-d)**, Stage 5 embryos carrying *nos*::GAL4 and either UAS::stripe 4/6-TALER-*hairy* **(b)**, UAS:: stripe 4/6 TALER-*hairy* **(c)**, or UAS::stripe 1-TALER-*hairy* **(d)**. **e-g**, Profiles of average expression levels of Eve in stage 5 embryos carrying *nos*::GAL4 and either UAS::stripe 4/6-TALER-*hairy*, **(e)**, UAS::stripe 4/6-TALER-*hairy* **(f)**, or UAS::stripe 1-TALER-*hairy* **(g)** ($n=10$ for each genotype). In all plots, the solid gray line denotes wild-type embryos and the red-dashed plots denote the enhancer-TALER-*hairy*, respectively. Lighter-shaded, bounding areas indicate one standard deviation. Red arrows in the schematic **(a)** indicate the distance between the targeted binding sites for the two enhancer-stripe 4/6-TALERs, which give similar outputs (*cf.* **e** and **f**). Embryos in **(a-c)** are matched in scale. Scale bar in **(a)** equals 100 μ m.

Supplementary Figure 6



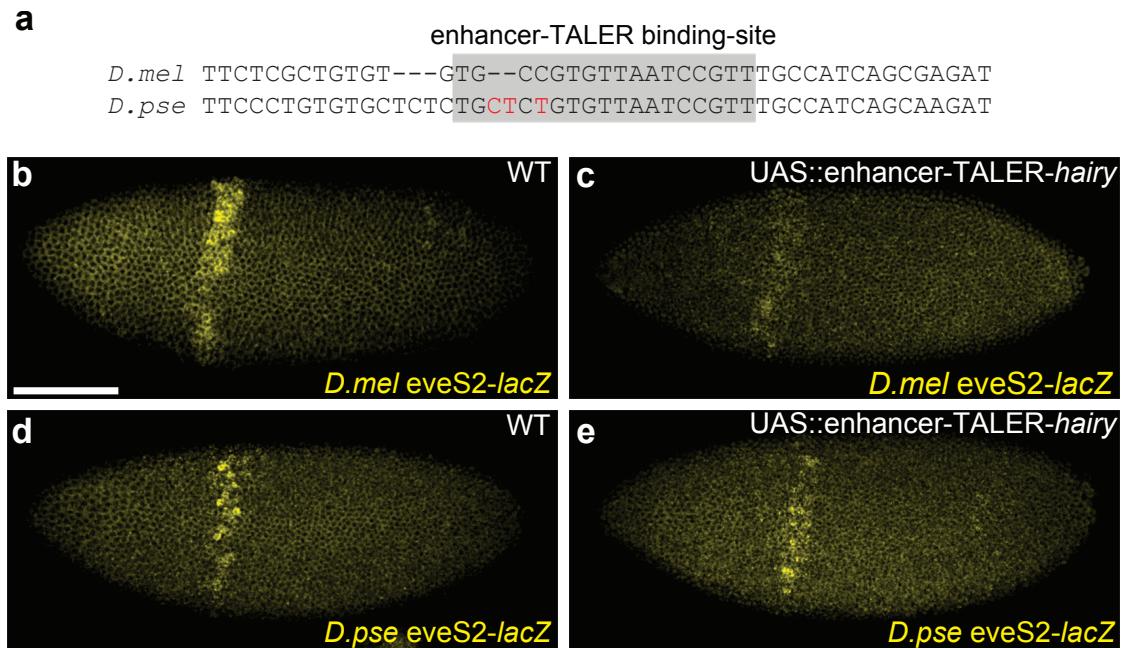
TALE targeted repression of an *eve* auto-regulatory element. **a**, Schematic of the *eve* locus, showing the location of the minimal autoregulatory sequence (MAS), the early-embryonic *cis*-regulatory stripe enhancers, and the TALE binding site. **(b-d)**, Stage 5 **(b)**, 6 **(c)**, and 7 **(d)** embryos carrying UAS::MAS-TALER-*hairy*, *nos*::GAL4 and stained for Eve protein. **(e-g)**, Stage 5 **(e)**, 6 **(f)**, and 7 **(g)** wild type embryos stained for Eve protein. **h, i**, Profiles of average expression levels of Eve protein in stage 5 embryos carrying UAS::MAS-TALER, *nos*::GAL4 **(h)**, and UAS::MAS-TALER, *nos*::GAL4 **(i)** ($n=10$ for each genotype). Embryos in **(b-g)** are matched in scale. Scale bar in **a** equals 100 μ m. In all plots, the solid gray line denotes wild-type embryos and the red-dashed plots denote the enhancer-TALER-*hairy*, respectively. Lighter-shaded, bounding areas indicate one standard deviation. **(j, k)**, Ventral cuticles of abdominal segments two through four of wild-type **(j)** and UAS::MAS-TALER-*hairy*, *nos*::GAL4 bearing **(k)** larva, respectively.

Supplementary Figure 7



TALE targeted repression of *eve* stripe enhancers disrupts larval cuticle patterns. Cuticle prepss of first instar wild-type larvae (**a**, **a'**), and larvae carrying UAS::stripe3/7 TALER-*hairy*, *nos*::GAL4 (**b**, **b'**), UAS::stripe4/6 TALER-*hairy*, *nos*::GAL4 (**c**, **c'**), and UAS::stripe3/7 TALER-*hairy*, *nos*::GAL4 larvae (**d**, **d'**), with denticle belts labeled. (**a'**, **b'**, **c'**, **d'**). Higher-magnification of specific larval segments, corresponding to larvae in **a**, **b**, **c**, and **d**, respectively. Embryos in (**a-d** and **a'-d'**), respectively, are matched in scale. Scale bars in (**a** and **b**) equal 100 μ m.

Supplementary Figure 8



TALERs can target a minimal *eve* stripe 2 reporter construct (*eveS2*). **a**, Nucleotide alignment of the *eveS2* enhancers from *D. melanogaster* and *D. pseudoobscura*. The TALER binding site is highlighted in grey. Nucleotide differences between the *D. pseudoobscura* and *D. melanogaster* sequences within the TALER binding site are shown in red. **b-e**, Embryos carrying *eveS2* enhancers driving *lacZ*, stained with anti- β -Galactosidase. **b**, Wild-type embryo carrying a *D. melanogaster eveS2* enhancer. **c**, Embryo carrying the *D. melanogaster eveS2* enhancer and UAS::enhancer-TALER-hairy, *nos*::GAL4. Expression of the *D. melanogaster eveS2* enhancer is repressed by the enhancer-TALER-hairy (cf. **b** and **c**). **d**, Wild-type embryo carrying a *D. pseudoobscura eveS2* enhancer. **e**, Embryo carrying a *D. pseudoobscura eveS2* enhancer and UAS::enhancer-TALER-hairy, *nos*::GAL4. Expression of the *D. melanogaster eveS2* enhancer is repressed by the *D. melanogaster*-specific enhancer-TALER-hairy (cf. **b** and **c**), but the *D. pseudoobscura eveS2* enhancer is not (cf. **d** and **e**). Embryos in **b-e** are matched in scale. Scale bar in **(a)** equals 100 μ m.

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