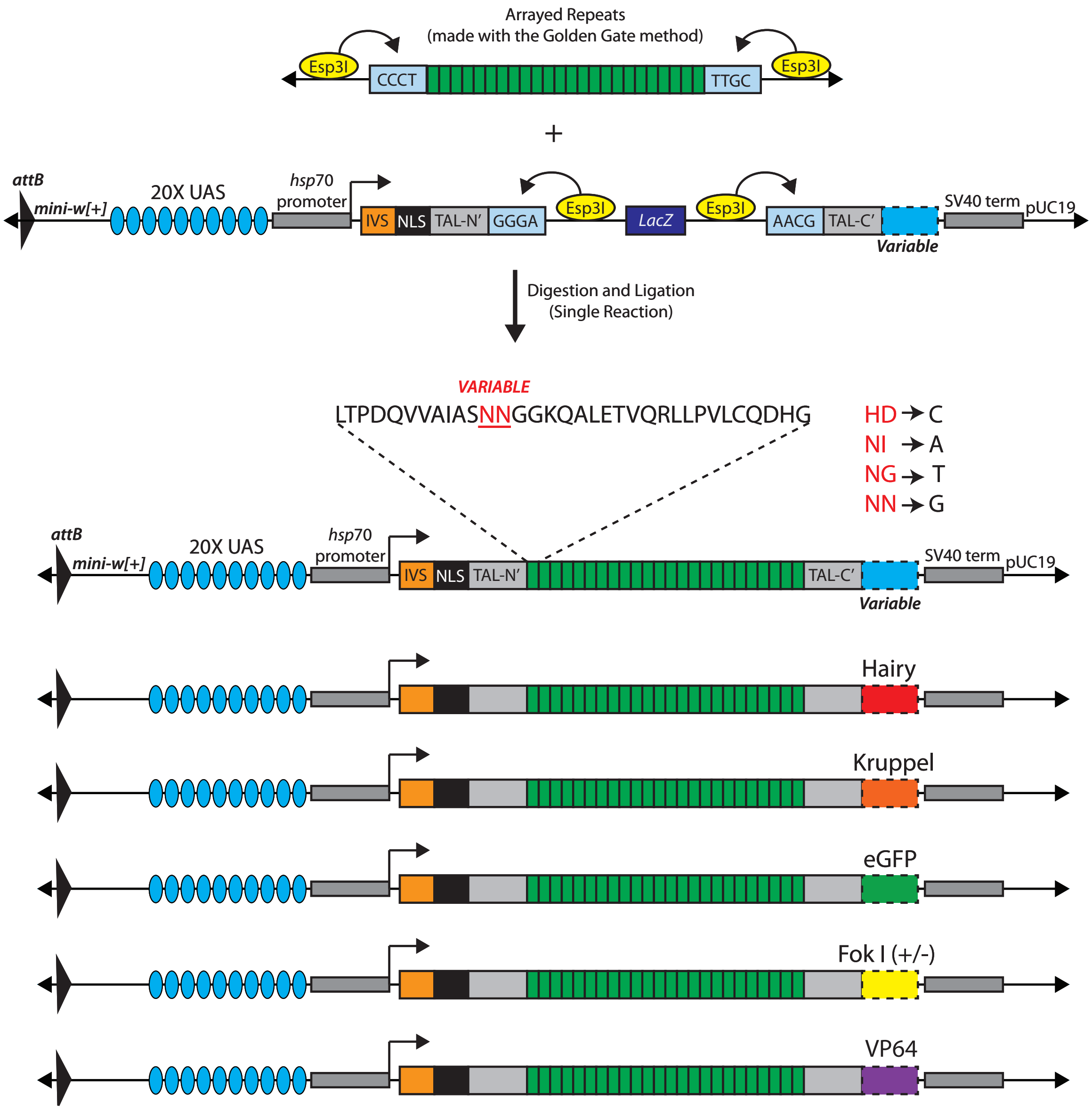
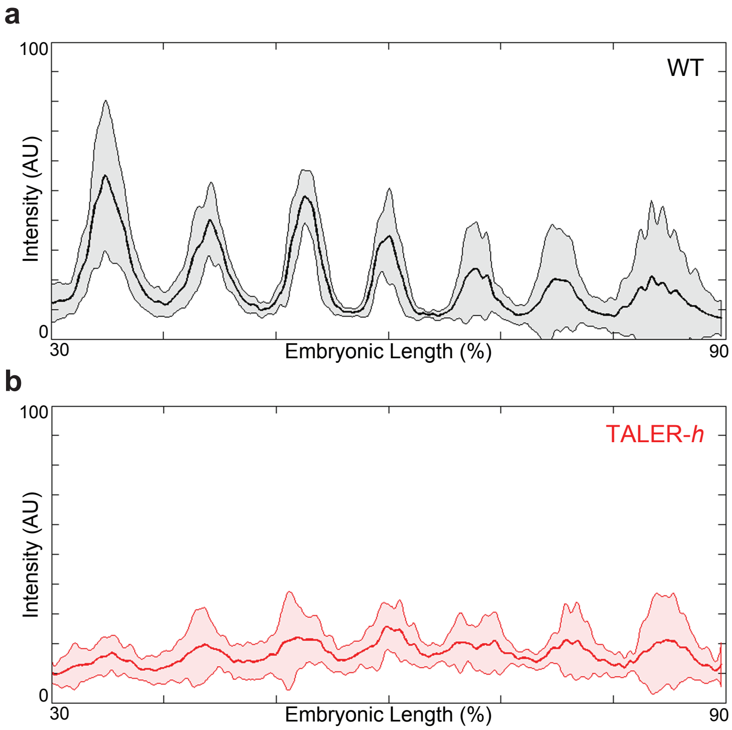
Supplementary Figure 1



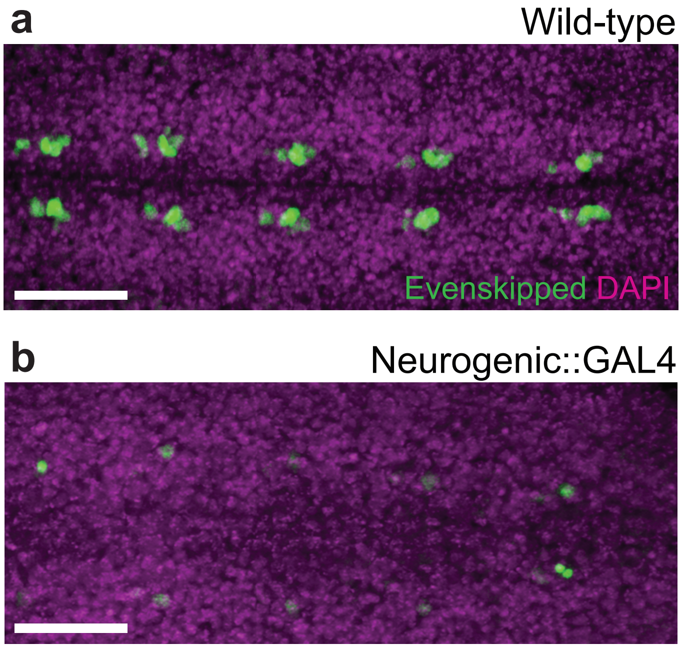
New Golden-Gate compatible TALE constructs. Each construct contains a mini-*white* gene, 20 Gal4 responsive binding sites (20X UAS), an *hsp70* promoter, a nuclear localization signal (NLS), a SV40 terminator, and a respective fusion domain. Arrayed repeats generated with the Golden Gate method can be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

Supplementary Figure 2



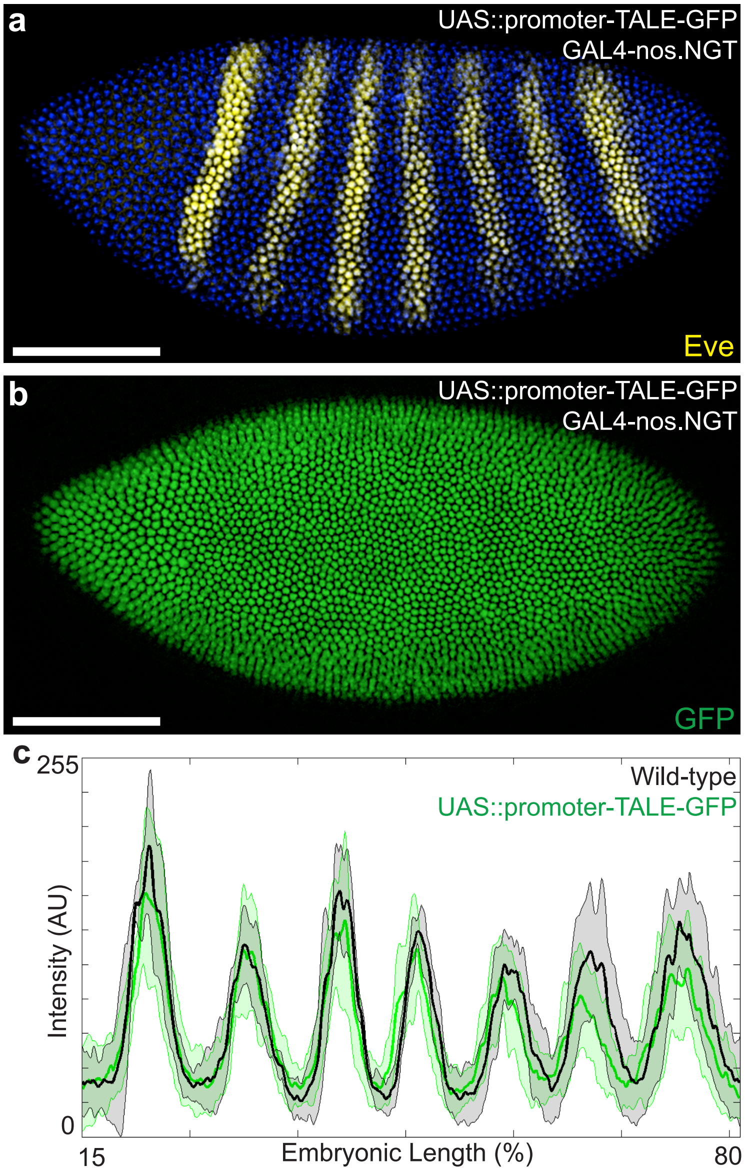
A TALER-*hairy* targeting the *eve* promoter represses *eve* expression. (**a, b**), Average expression profile of Eve in wild type stage 5 embryos (**a**, grey) and stage-5 embryos carrying a TALER-*hairy* targeting the *eve* promoter, under the *nos*::GAL4 driver (**b**, red) (n=10 for each genotype).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

Supplementary Figure 3



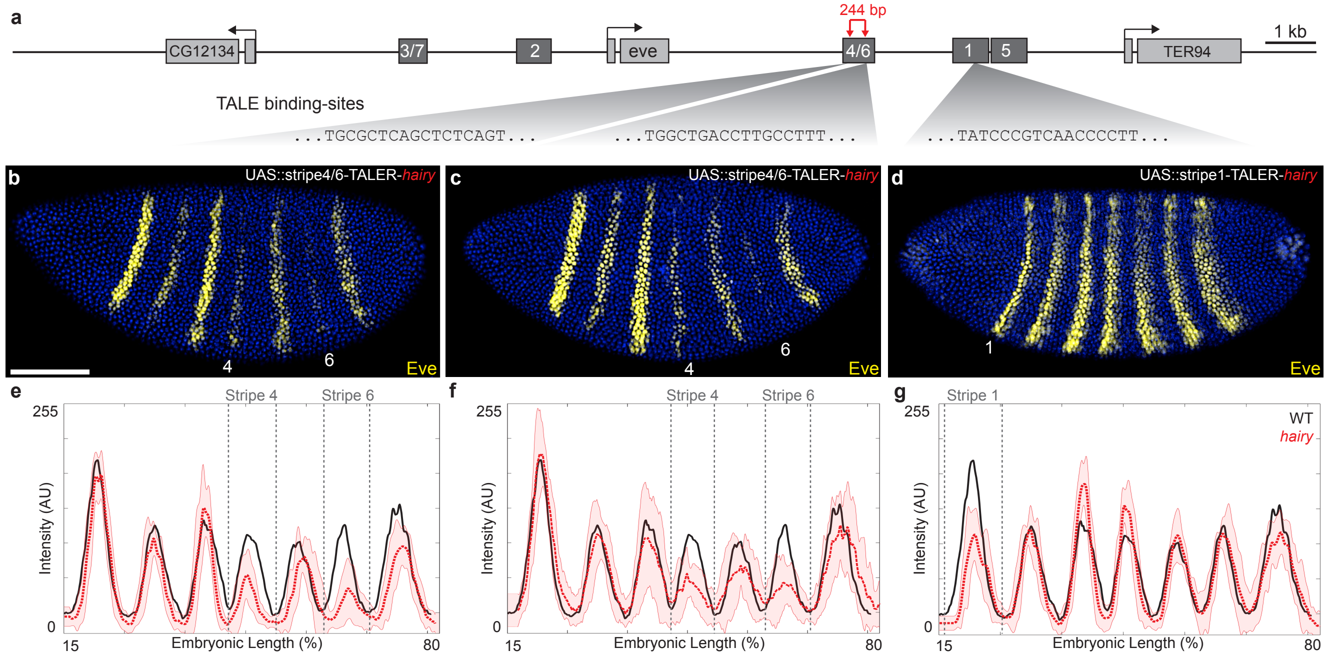
TALER repression of neurogenic-expressed Eve in stage 11 embryos. (**a**), Wild-type expression of Eve. (**b**), Expression of Eve in an embryo containing a UAS::promoter-TALER-*hairy* construct driven by *rhomboid*::Gal4, which drives expression in the neurogenic ectoderm. Scale-bars: 20 μm (**a** and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

Supplementary Figure 4



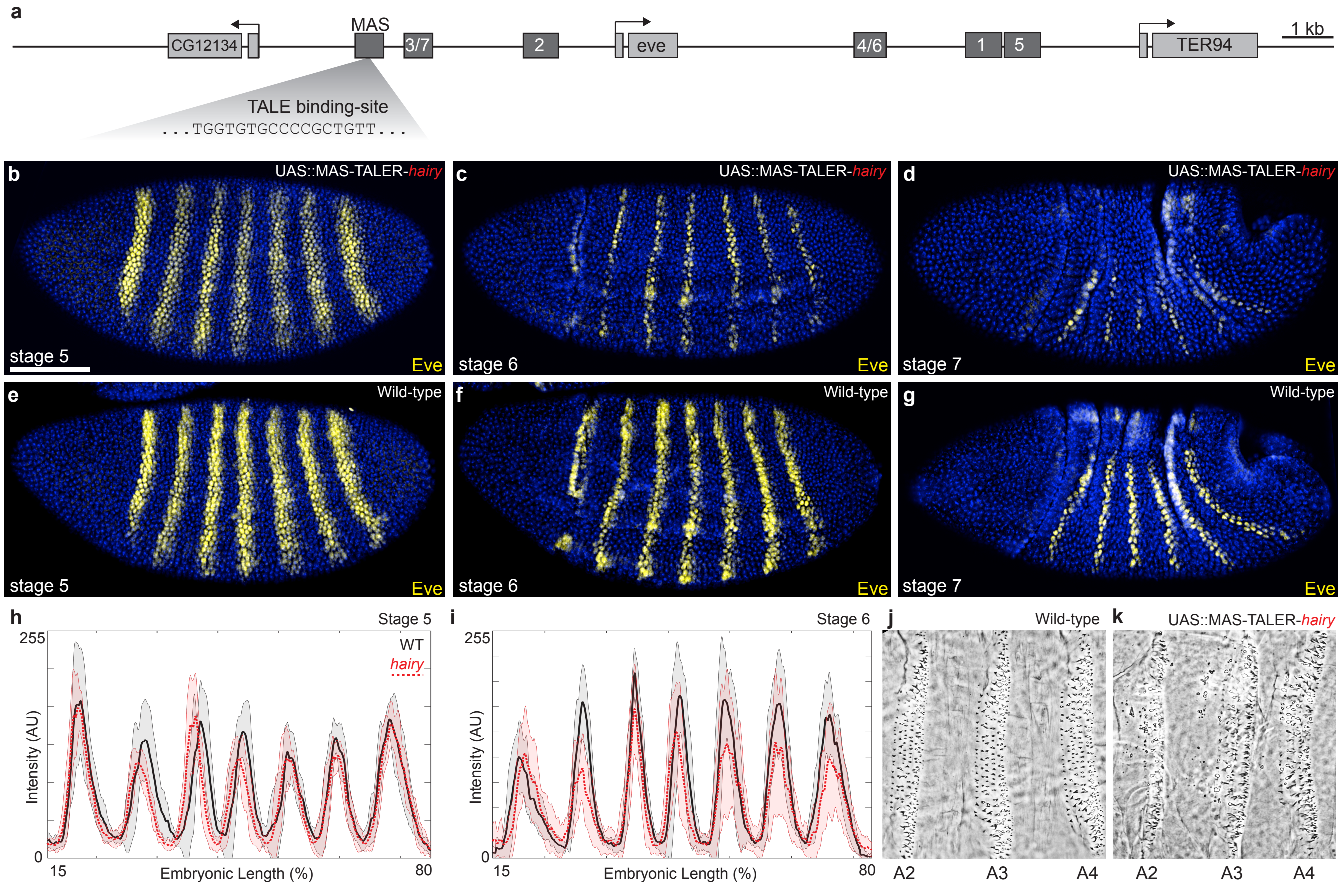
TALE targeted GFP to the *eve* promoter. (**a**), A stage-5 embryo carrying UAS::promoter-TALE-GFP, nos::GAL4 stained for Eve protein. (**b**), A stage 5 embryo carrying UAS::promoter-TALE-GFP, nos::GAL4 and stained for GFP protein indicates that the TALE-GFP is expressed in a nearly ubiquitous pattern. (**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 µm (**a**, **b**).

Supplementary Figure 5



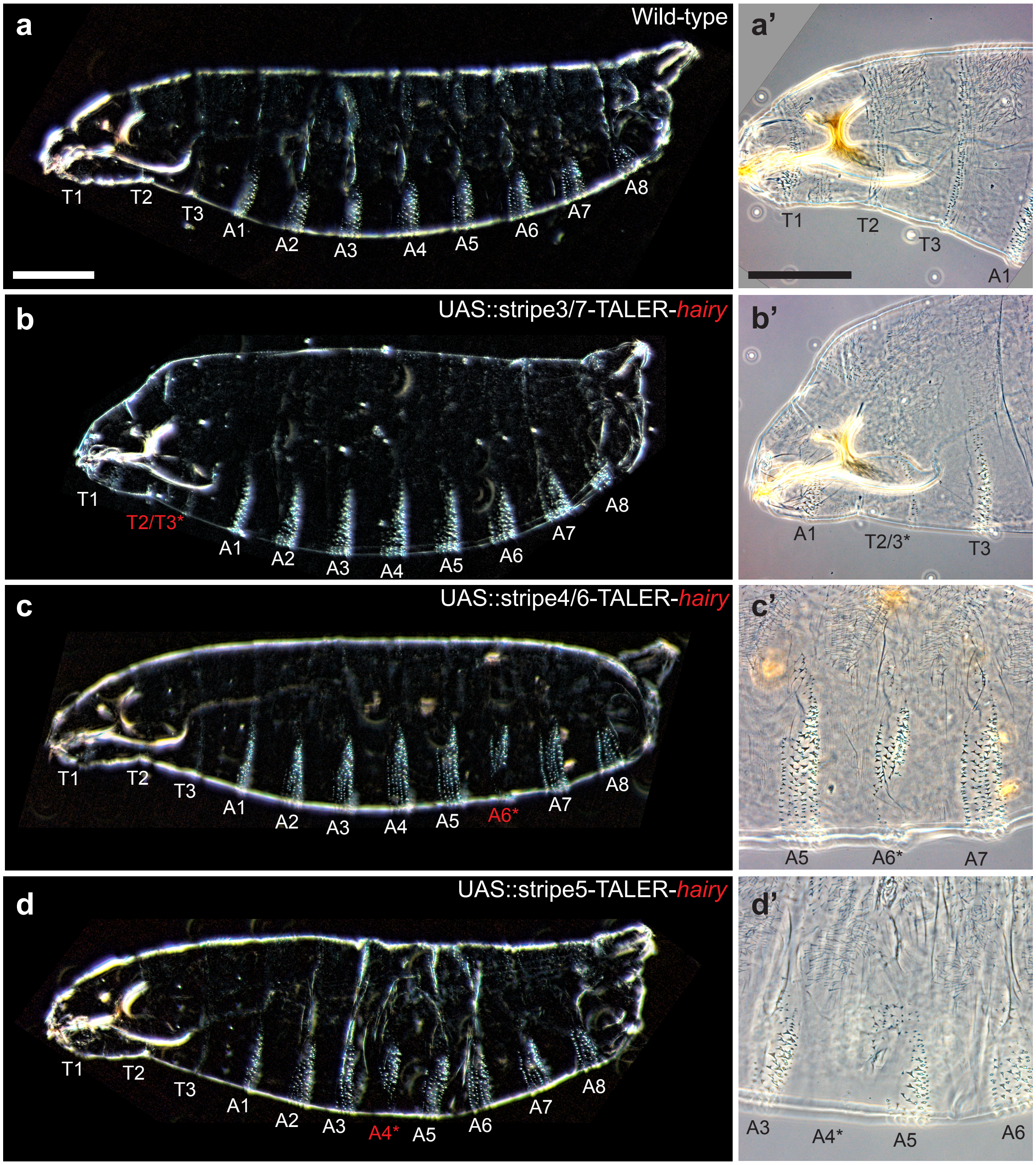
TALE targeted repression of *eve* stripe enhancers. **a**, Schematic of the *eve* locus, indicating early-embryonic *cis*-regulatory stripe enhancers and TALE binding sites. (**b**-**d**), Stage 5 embryos carrying *nos*::GAL4 and either UAS::stripe 4/6-TALER-*hairy* (**b**), UAS:: stripe 4/6 TALER-*hairy* (**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 µm.

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 µm. 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 preps 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 µm.

Supplementary Figure 8

a

enhancer-TALER binding-site

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D.pse TTCCCTGTGTGCTCTCTGCTCTGTGTTAATCCGTTTGCCATCAGCAAGAT
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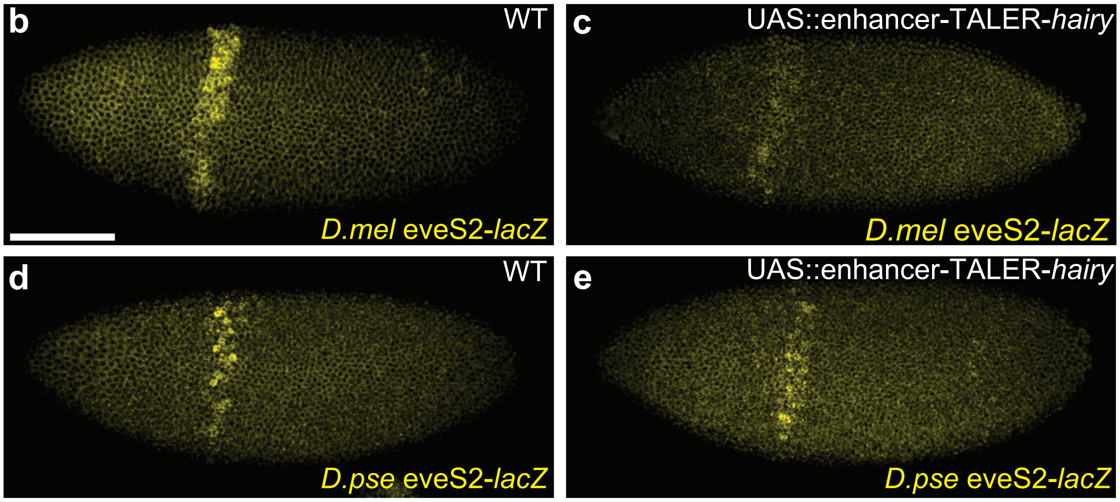

TALERs can target a minimal *eve* stripe 2 reporter construct (*eve*S2). **a**, Nucleotide alignment of the *eve*S2 enhancers from *D. melanogaster* and *D. pseudoobscura*. The TALER binding site is highlighted in grey. Nucleotide differences between the *D. pseudoobscura* and *D. melanogaster* sequences within the TALER binding site are shown in red. **b**-**e**, Embryos carrying *eve*S2 enhancers driving *lacZ*, stained with anti-β-Galactosidase. **b**, Wild-type embryo carrying a *D. melanogaster eve*S2 enhancer. **c**, Embryo carrying the *D. melanogaster eve*S2 enhancer and UAS::enhancer-TALER-*hairy*, *nos*::GAL4. Expression of the *D. melanogaster eve*S2 enhancer is repressed by the enhancer-TALER-*hairy* (*cf*. **b** and **c**). **d.** Wild-type embryo carrying a *D. pseudoobscura eve*S2 enhancer. **e**, Embryo carrying a *D. pseudoobscura eve*S2 enhancer and UAS::enhancer-TALER-*hairy*, *nos*::GAL4. Expression of the *D. melanogaster eve*S2 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 μm.

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