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Figures and figure supplements

Dual mode of embryonic development is highlighted by expression and function of *Nasonia* pair-rule genes

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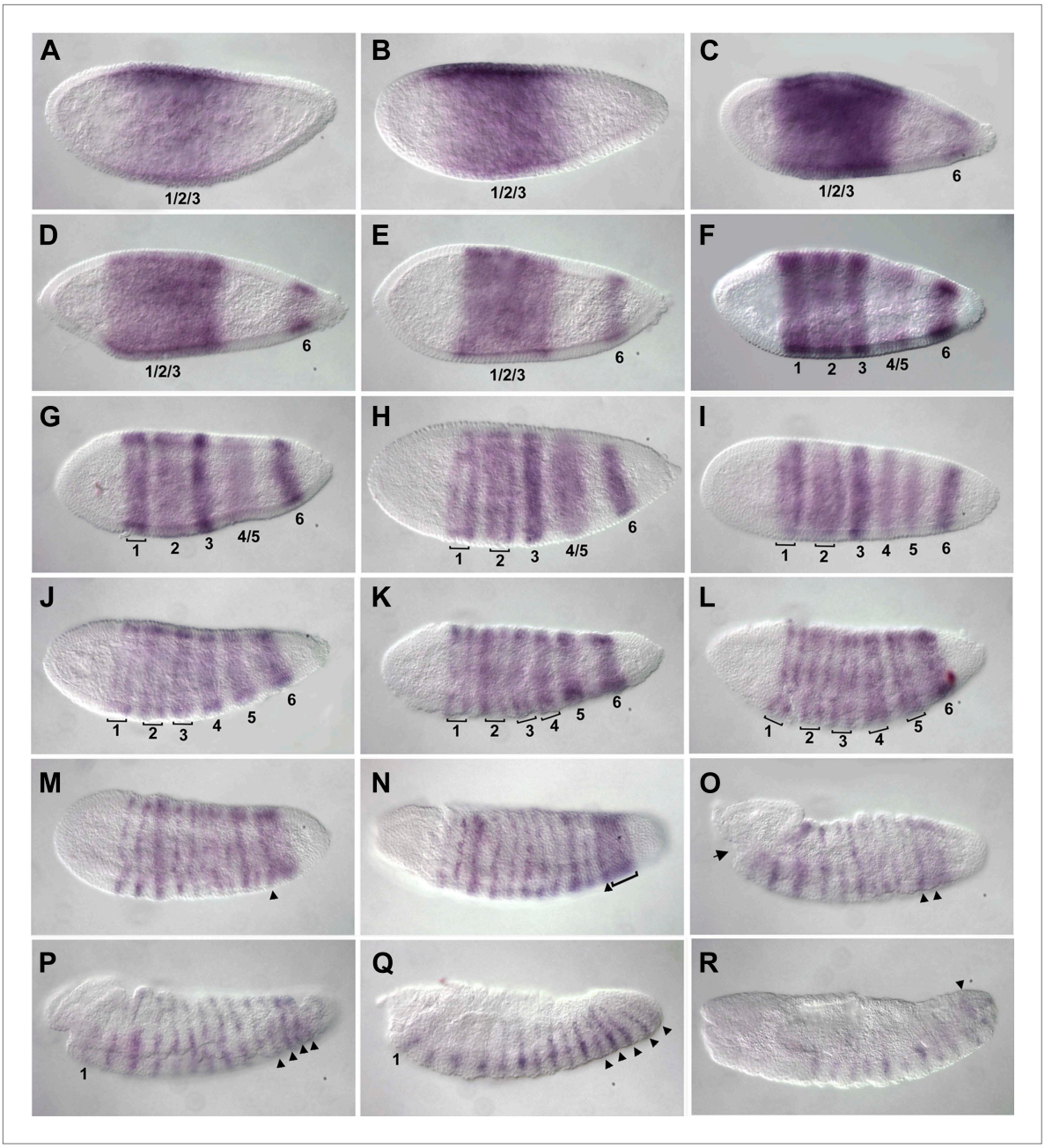

Figure 1. Summary of *Nasonia eve* mRNA expression. Embryos are shown with anterior left and dorsal up. *Nv eve* is initially expressed in a broad domain (**A** and **B**), which sharpens as a posterior stripe becomes visible at around 4 hr after embryo laying (AEL) (**C** and **D**). The broad domain retracts anteriorly and gives rise to three apparently double-segment stripes (**E** and **F**). Between stripes 3 and posterior stripe 6, an additional double stripe precursor comes up at around 6 hr AEL (stripe 4/5; panels **F** and **G**) and this splits to form two double-segment stripes, '4' and '5' as double-segment stripes 1–3 split into two single-segment stripes each between 6 and 8 hr AEL (**F**–**J**). Stripes 4 and 5 also split to form single-segment stripes during early gastrulation, and stripe 6 broadens (**K** and **L**), giving rise to stripes that are visibly distinct during germ band extension in non-fluorescent staining by 10–12 hr AEL (**M**–**R**, arrowheads). There are a total of 16 single-segment stripes of *Nv eve*.

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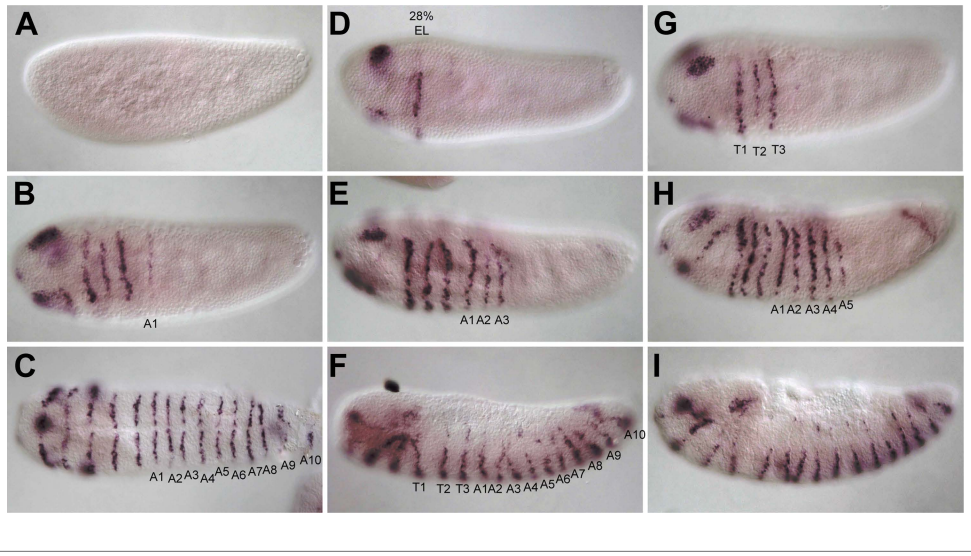

Figure 1—figure supplement 1. *Nv wingless (Nv wg)* mRNA expression in the embryo.
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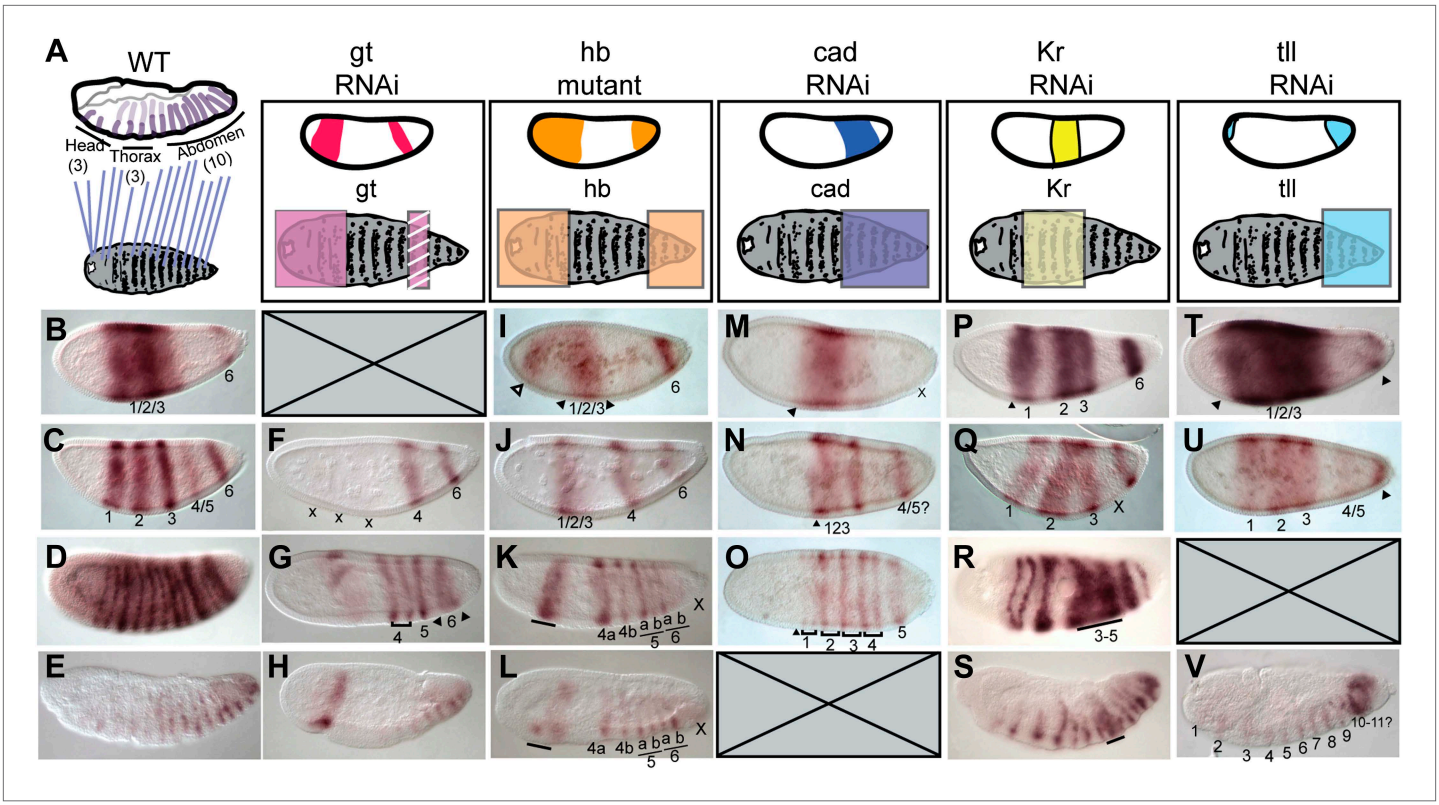

Figure 2. *Nv eve* epistasis with maternal and gap genes. (**A**) Schematic representation of the germ-band-extended embryo, showing 16 single-segment stripes of *Nv eve* expression, and their segment counterparts in the patterned larval cuticle. Colored boxes cover the segments of the larval cuticle that are lost or fused in each RNAi background. All embryos are shown anterior left, dorsal up (except where indicated). *Nv eve* mRNA expression is shown in each embryo (**B**–**G**). Wild-type (WT) embryos are shown as staged controls for RNAi embryos. (**B**) WT early blastoderm embryo. (**C**) WT cellular blastoderm embryo. (**D**) WT early gastrula extension embryo. (**E**) WT germ-band-retracted embryo. (**F**–**H**) *gt* RNAi embryos stained for *Nv eve* mRNA expression. (**F**) Cellular blastoderm embryo with reduced *Nv gt* exhibits loss of anterior *Nv eve* stripes (x). (**G**) *Nv gt* RNAi embryo in early germ-band-extension exhibits loss of anterior *Nv eve* stripes and improper splitting of *Nv eve* stripe 5, as well as aberrant dorsal anterior expression of *Nv eve*. (**H**) *Nv gt* RNAi embryo at dorsal closure exhibits a stripe of *Nv eve* at the anterior, as well as a reduced number of posterior segmental *Nv eve* stripes. (**I**–**L**) *Nv hb* mutant embryos stained for *Nv eve* mRNA expression. (**I**) Early blastoderm *Nv hb* mutant embryos have a reduced central *Nv eve* domain (bounded by black arrowheads), and an ectopic anterior *Nv eve* stripe (white arrowhead). (**J**) *Nv hb* mutant cellular blastoderm embryo with a single anterior domain of *Nv eve* that has failed to resolve, and a single stripe 4 which exhibits delayed splitting. (**K**) *Nv Hb* mutant germ-band extension embryo with fused anterior domain (line) and 6 segmental stripes, representing derivatives of *Nv eve* stripes 4 and 5 and two derivatives of stripe 6; additional stripe 6 derivatives are absent (x). (**L**) *hb* mutant dorsal closure embryo exhibiting fused anterior domain (line) and the same number of derivatives as in (**M**), with more posterior segments missing (x). (**M**–**O**) *Nv cad* RNAi embryos stained for *Nv eve* mRNA expression. (**M**) *Nv cad* RNAi early blastoderm with reduced central *Nv eve* domain that is also posteriorly shifted (anterior boundary indicated by black arrowhead). (**N**) *Nv cad* RNAi cellular blastoderm embryo with posteriorly shifted (arrowhead), reduced *Nv eve* central domain, whose splitting is delayed. (**O**) *Nv cad* RNAi early gastrula embryo with posterior shift in *Nv eve* expression (black arrowhead). Four double-segment periodicity stripes are split into single-segment stripes and stripe 5 remains intact. (**P**–**S**) *Nv Kr* RNAi embryos stained for *Nv eve* mRNA expression. (**P**) *Nv Kr* RNAi precellular blastoderm embryo with aberrant *Nv eve* central domain resolution, where stripes 2–3 appear posteriorly shifted. (**Q**) Dorsolateral view of a *Nv Kr* RNAi embryo where stripes 2 and 3 are less refined than WT and 3 is posteriorly shifted. No stripe 4/5 expression is detected (X). (**R**) *Nv Kr* RNAi early gastrula embryo with aberrant stripe 2 splitting and aberrant resolution of stripes 3–5. (**S**) Moderately affected *Nv Kr* RNAi germ-band retraction embryo with fused segments in the middle of the embryo (line). (**T**–**V**) *Nv tll* RNAi embryos stained for *Nv eve* mRNA expression. (**T**) *Nv tll* RNAi early blastoderm embryo with expanded *Nv eve* expression domains toward both poles (arrowheads). (**U**) *Nv tll* RNAi precellular blastoderm embryo showing delayed resolution of *Nv eve* stripes 1–3 and *Nv eve* stripe 6 shifted to the extreme posterior pole of the embryo (arrowhead). (**V**) *Nv tll* RNAi dorsal closure embryo showing abnormal posterior *Nv eve* stripe formation.

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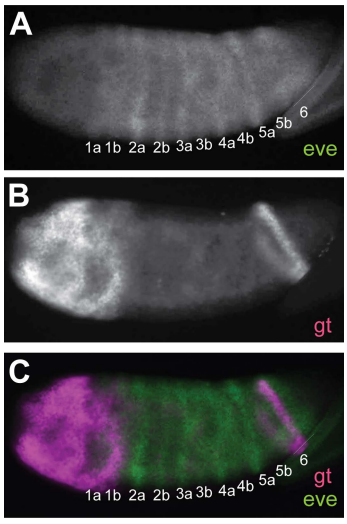

Figure 2—figure supplement 1. *Nv eve/Nv gt* double FISH in the embryo.

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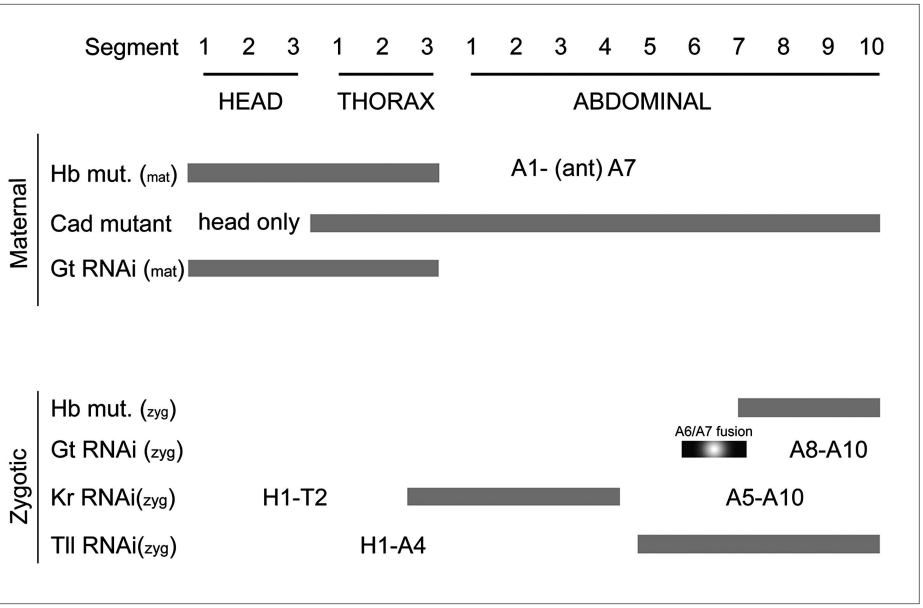

Figure 2—figure supplement 2.

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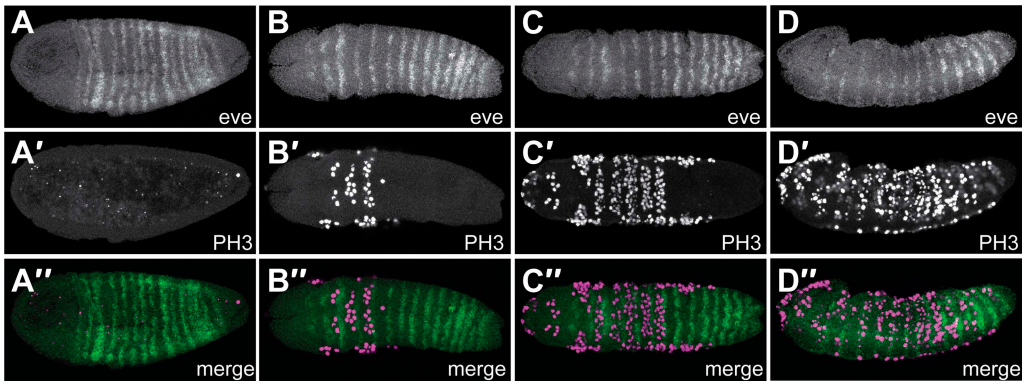

Figure 3. *Nv eve* expression and cell division appear to be coordinated. Embryos co-stained for *Nv eve* mRNA using in situ hybridization and fluorescent detection, as well as for mitotic figures, using an antibody against phospho Histone H3. Embryos are shown with anterior left and dorsal up, except columns **B** and **C**, which are ventral views. (**A**–**A″**) An early gastrula embryo exhibiting 15 stripes of *Nv eve*, including five derivatives of stripe 6 (**A**), has no evident mitotic figures in the posterior domain of *Nv eve* stripe 6 differentiation (**A′**). (**A″**) Merge of panels **A** and **A′**. (**B**–**D″**) Timecourse series of wild-type embryos stained for *Nv eve* mRNA and phospho-Histone H3. (**B**–**D**). Top panels are *Nv eve* in situ alone, middle panels (**B′**–**D′**) are phospho-Histone H3 antibody staining, and bottom panels (**B″**–**D″**) are merge images of upper panels, showing localization of mitotic figures relative to *Nv eve* stripes.

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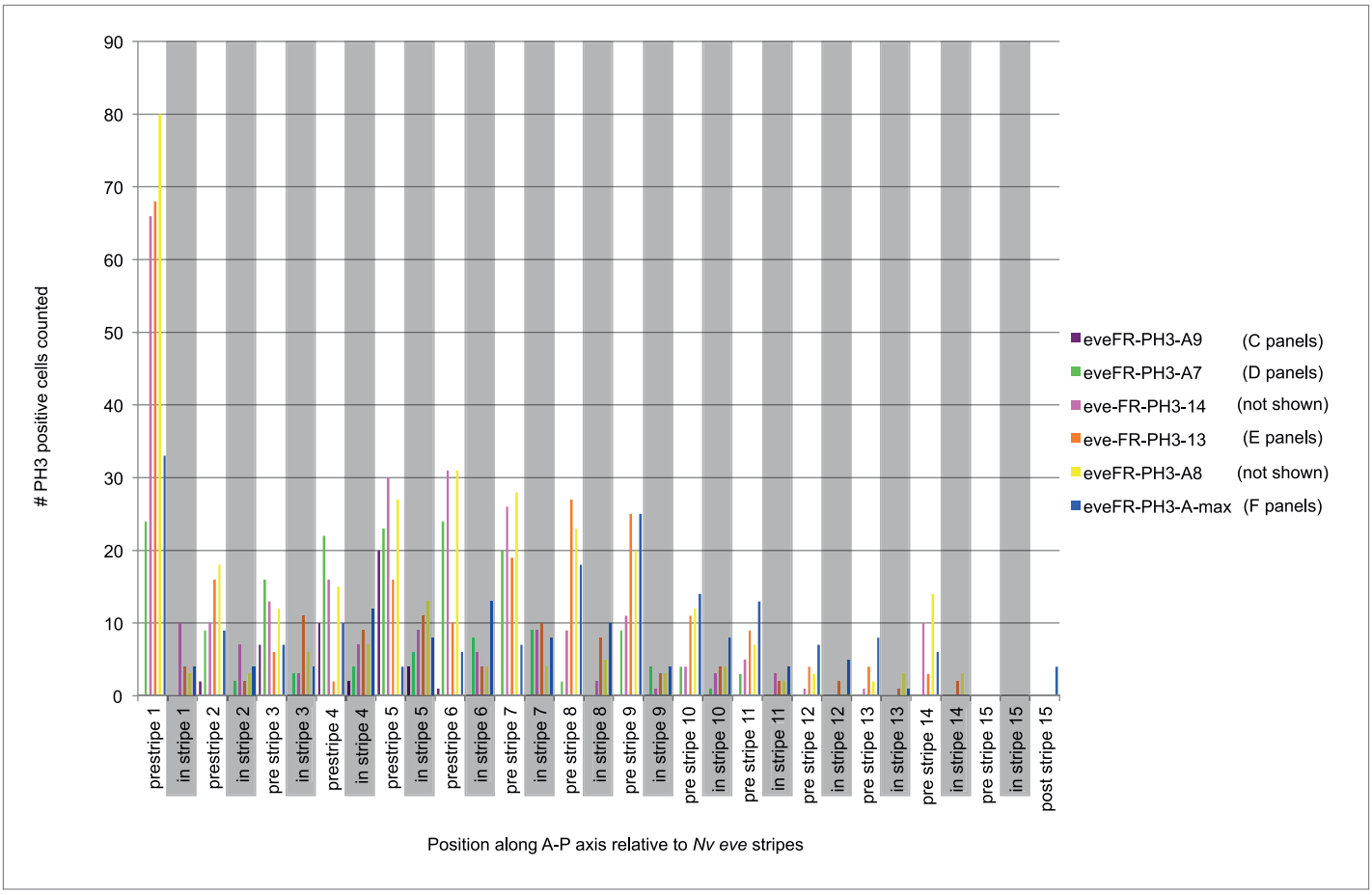

Figure 3—figure supplement 1. Quantification of PH3 positive cells relative to *Nv eve* stripes in the embryo.

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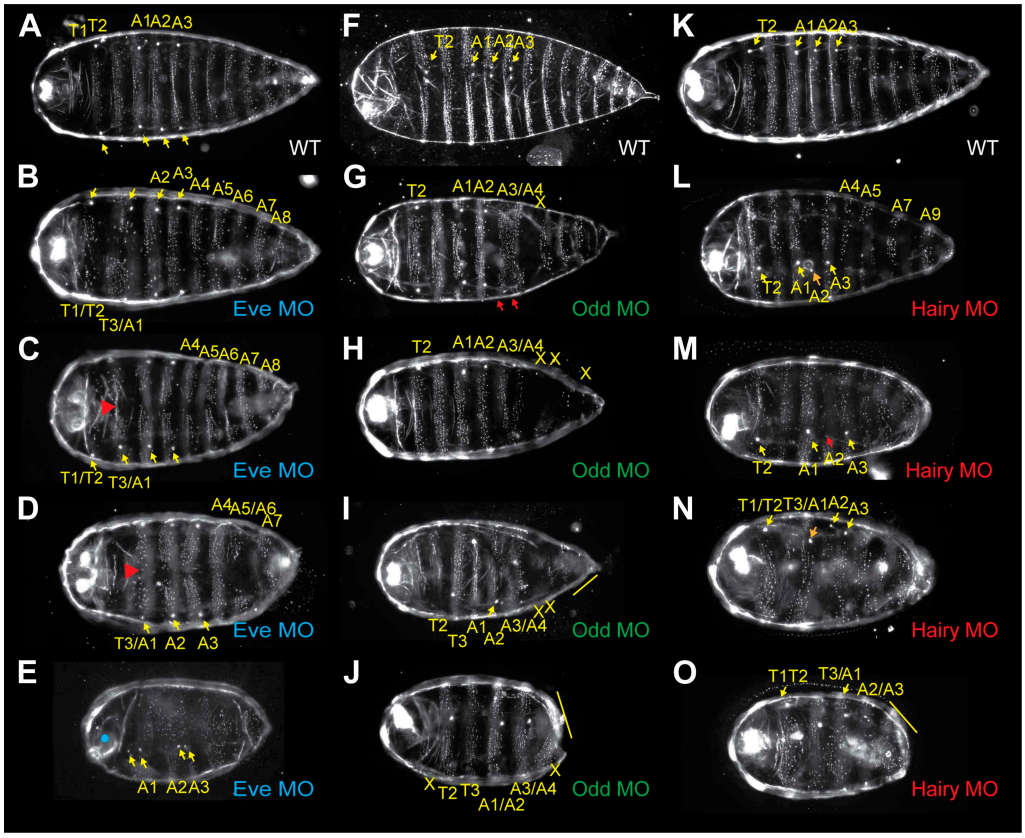

Figure 4. Morpholino knockdown of *Nv eve*, *Nv hairy*, and *Nv odd* results in embryo patterning defects. First instar larval cuticles are shown with anterior left and generally ventral denticle patterns are shown. (**A**, **F**, **K**) Wild-type larval cuticles. Yellow arrows indicate spiracles present on segments T2, A1, A2 and A3. Bright anterior labral appendages are apparent at the extreme anterior of the larva. (**B**–**E**) Unhatched larvae from *Nv eve* morpholino (MO)-injected embryos, in order of increasing phenotype severity. Red arrowheads indicate loss of midline cuticle. Blue dot indicates head open defect. Yellow arrowheads indicate position of spiracles. (**G**–**J**) Unhatched larvae from *Nv odd* morpholino (MO)-injected embryos, in order of increasing phenotype severity. Yellow arrows indicate position of spiracles, red arrows indicate A3/A4 fusion. X indicates naked cuticle from segment loss. Yellow line indicates multi-segment fusion. (**L**–**O**) Unhatched larvae from *Nv hairy* morpholino (MO)-injected embryos, in order of increasing phenotype severity. Yellow arrowheads indicate position of spiracles. Red or orange arrowheads indicate aberrantly positioned or missing spiracles. Yellow line indicates segment fusion.

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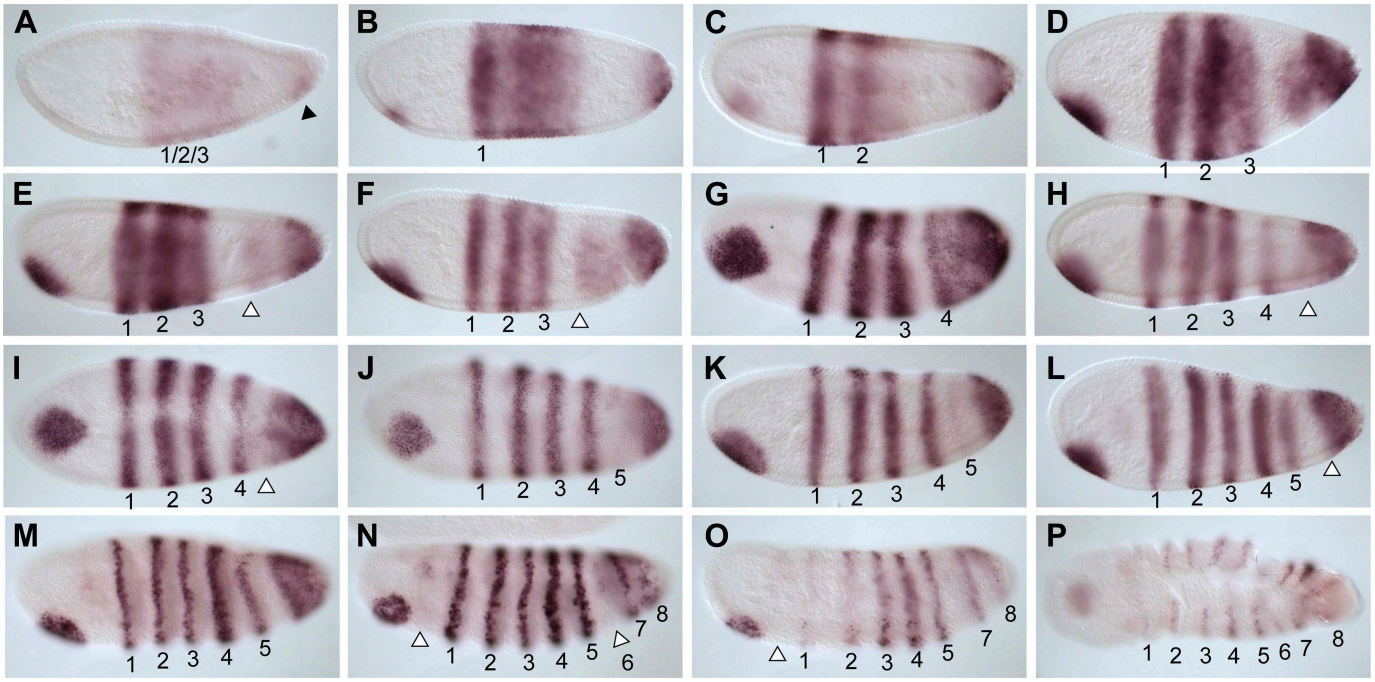

Figure 5. Summary of *Nv odd-skipped* mRNA expression. Embryos are shown with anterior left and dorsal up, except where indicated. (**A**) Precellular blastoderm embryo showing early expression of *Nv odd* in a broad domain and a posterior cap with a slight clearing in between. (**B**) Precellular blastoderm embryo showing ventral head patch and darkened central broad domain and distinct posterior cap. (**C**) Precellular blastoderm embryo with sharpening pair-rule stripes and expanding posterior cap. (**D**) Precellular blastoderm embryo with dark ventral head patch and posterior cap, and expansion of expression between broad central domain and posterior domain. (**E** and **F**) Cellularizing blastoderm embryos with three double-segment periodicity stripes, and a continuous posterior domain of variable staining intensity. Arrowhead indicates boundary of faint expression, which prefigures position of double-segment stripe 4. (**G**) Ventral view of cellularizing embryo with three strong double-segment stripes, and a fourth stripe forming at the anterior boundary of a more uniformly staining posterior cap (arrowhead). (**H**) Cellularized blastoderm embryo with four distinct double-segment stripes and a receding posterior cap domain (arrowhead). (**I**) Ventral view of cellular blastoderm showing four strong double-segment stripes and receding posterior cap (arrowhead), whose anterior boundary prefigures the position of stripe 5. (**J**) Ventrolateral view of cellular blasoderm embryo showing early appearance of stripe 5 at the anterior boundary of receding posterior domain, whose staining intensity is now less uniform. (**K**) Cellular blastoderm embryo with five double-segment stripes of expression, a strong ventral head spot, and a reduced, uniform posterior cap. (**L**) Same as **K**, with five equivalently strong double-segment stripes. Arrowhead indicates slightly expanded posterior cap. (**M**) Early germ-band extension embryo with five double-segment periodicity stripes and two stripes becoming evident within the posterior cap. (**N**) Slightly later embryo than **M**, with 2 posterior cap stripes more clearly differentiated. (**O**) Slightly later embryo than **N**, with anterior stripes fading and posterior segments expanding. (**P**) Dorsal view, dorsal closure embryo exhibiting eight single-segment periodicity stripes.

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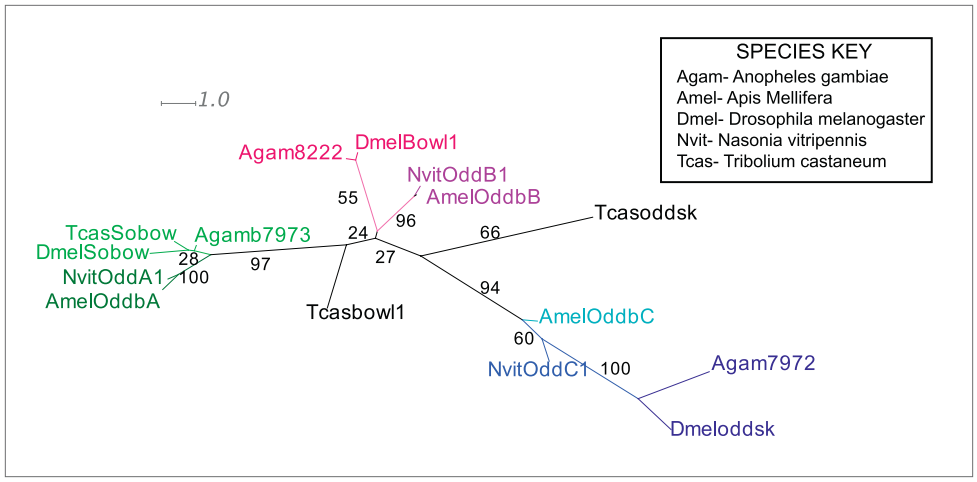

Figure 5—figure supplement 1. Phylogenetic analysis of odd-skipped.

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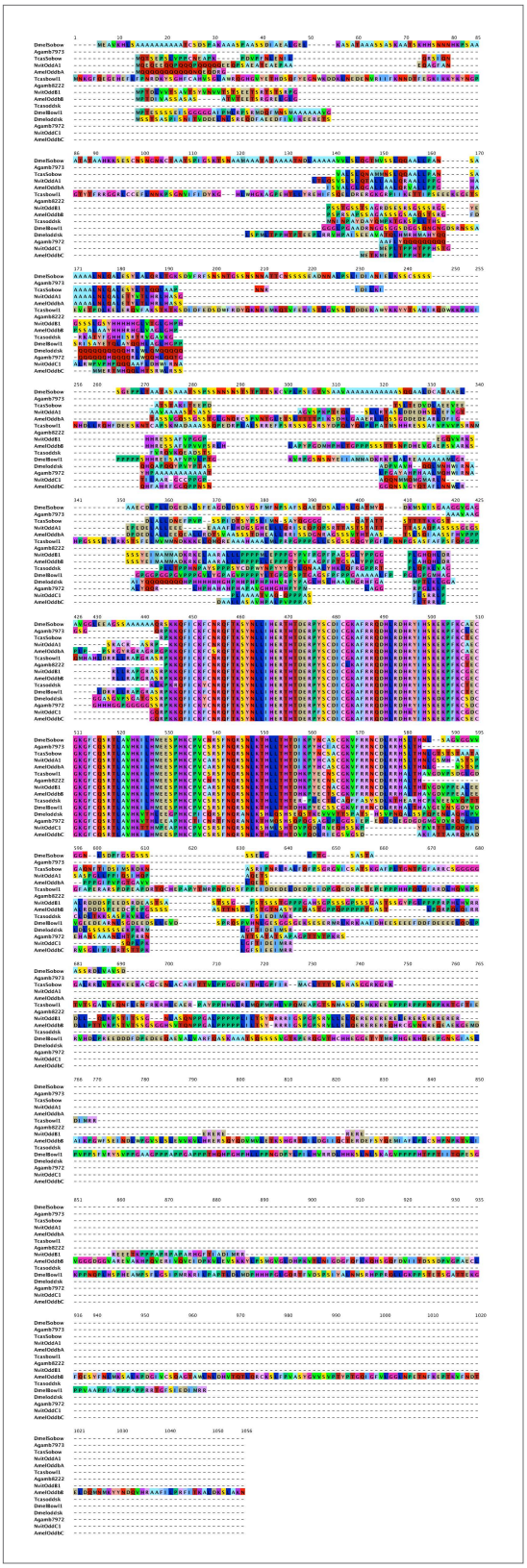

Figure 5—figure supplement 2. odd-skipped protein sequence alignment.

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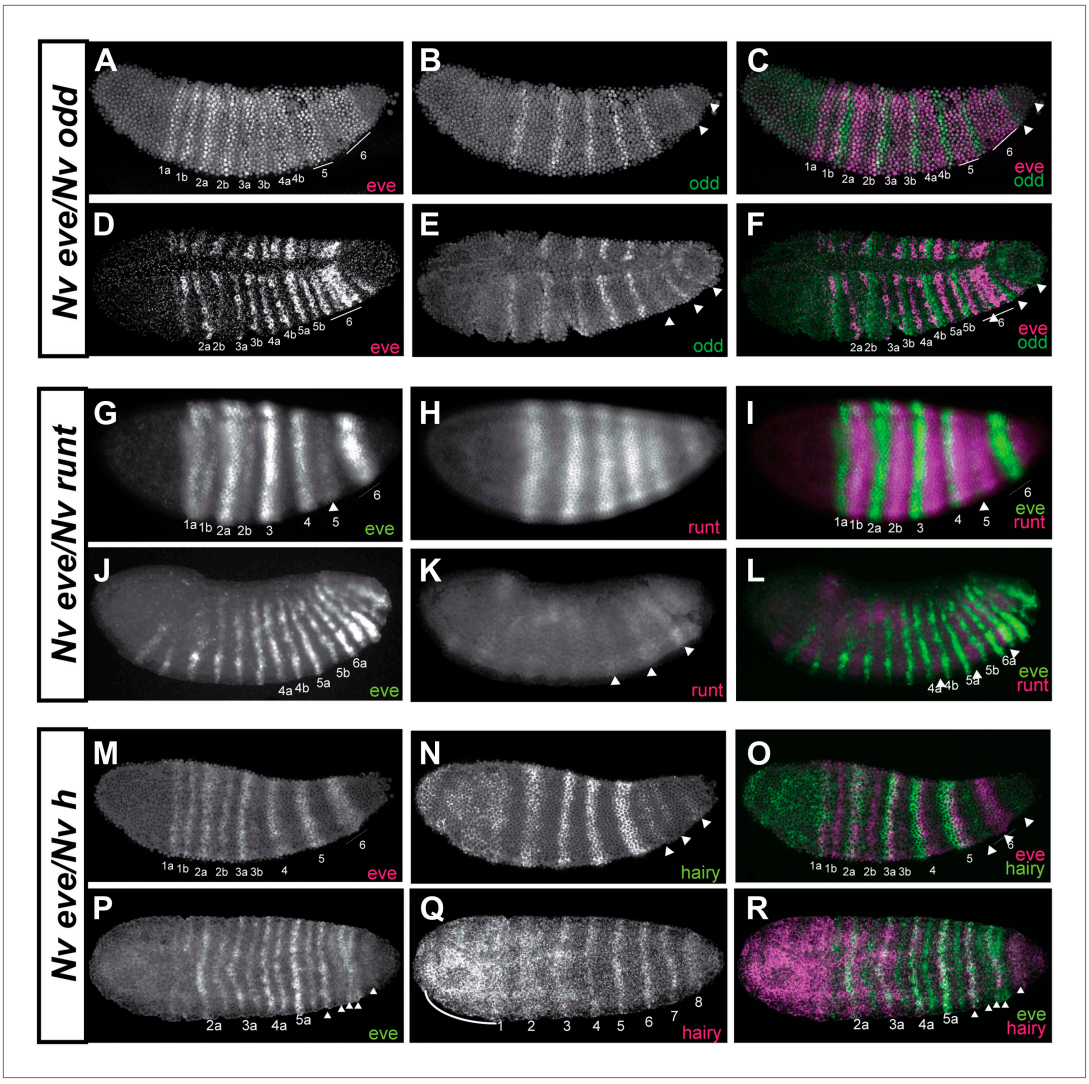

Figure 6. Phasing of Nasonia pair-rule genes in embryos using double fluorescent in situ hybridization. (**A**) Lateral view of *Nv eve* expression in early gastrula embryo. (**B**) *Nv odd* expression alone in the same embryo. (**C**) Merge of *Nv eve* and *Nv odd* channels, illustrating their relative phasing. *Nv eve* mRNA is pseudo-colored pink, *Nv odd* is in green. Arrowheads indicate position of a posterior doublet of *odd* stripes. (**D**) Dorsolateral view of *Nv eve* in later gastrula embryo. (**E**) *Nv odd* expression alone in the same embryo. Arrowheads indicate position of posterior *Nv odd* stripes 6, 7 and 8. (**F**) Merge of *Nv eve* and *Nv odd* channels, illustrating their relative phasing. (**G**) Lateral view of *Nv eve* expression in blastoderm embryo. Arrowhead indicates position of *Nv eve* stripe 5. (**H**) *Nv runt* expression in the same blastoderm embryo. (**I**) Merge of *Nv eve* (green) and *Nv runt* (pink) channels, indicating relative phasing. (**J**) Lateral view of *Nv eve* expression in germ-band-extended embryo. Numbers indicate identity of *Nv eve* stripe. (**K**) *Nv runt* expression alone in the same embryo. Arrowheads indicate position of posterior primary *Nv runt* stripes. (**L**) Merge of *Nv eve* (green) and *Nv runt* (pink) channels, indicating relative phasing. Note that posterior *Nv runt* stripes, though faint, appear to be positioned posterior to odd-numbered *Nv eve* segmental stripes. (**M**) Lateral view of *Nv eve* expression in early gastrula embryo. Line indicates broadening stripe 6. (**N**) *Nv hairy* expression in the same gastrula embryo. Arrowheads indicate positions of three late forming posterior double-segment stripes. (**O**) Merge of *Nv eve* (pink) and *Nv hairy* (green) channels, indicating relative phasing. (**P**) Ventral view of gastrula embryo showing *Nv eve* expression alone. Arrowheads indicate positions of single-segment stripes derived from *eve* stripe 6. (**Q**) *Nv hairy* expression alone in the same gastrula embryo. Line indicates extended anterior domain continuous with stripe 1. (**R**) Merge of *Nv eve* (green) and *Nv hairy* (pink) channels, illustrating relative phasing.

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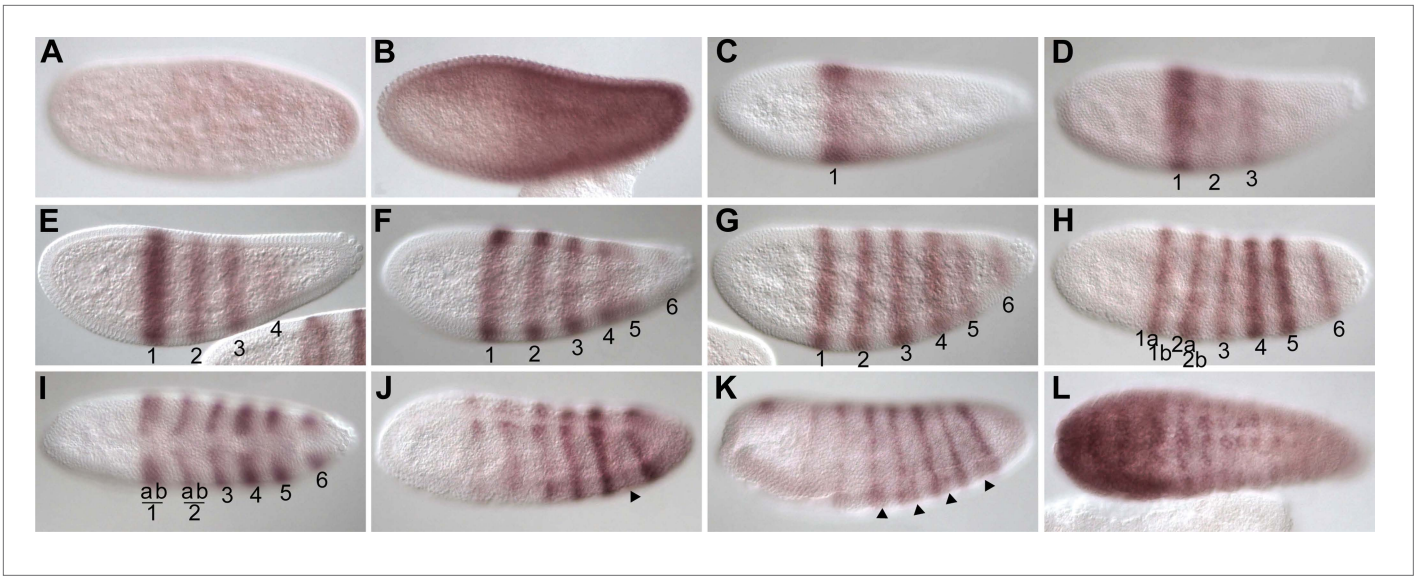

Figure 6—figure supplement 1. Summary of *Nv runt* mRNA expression.

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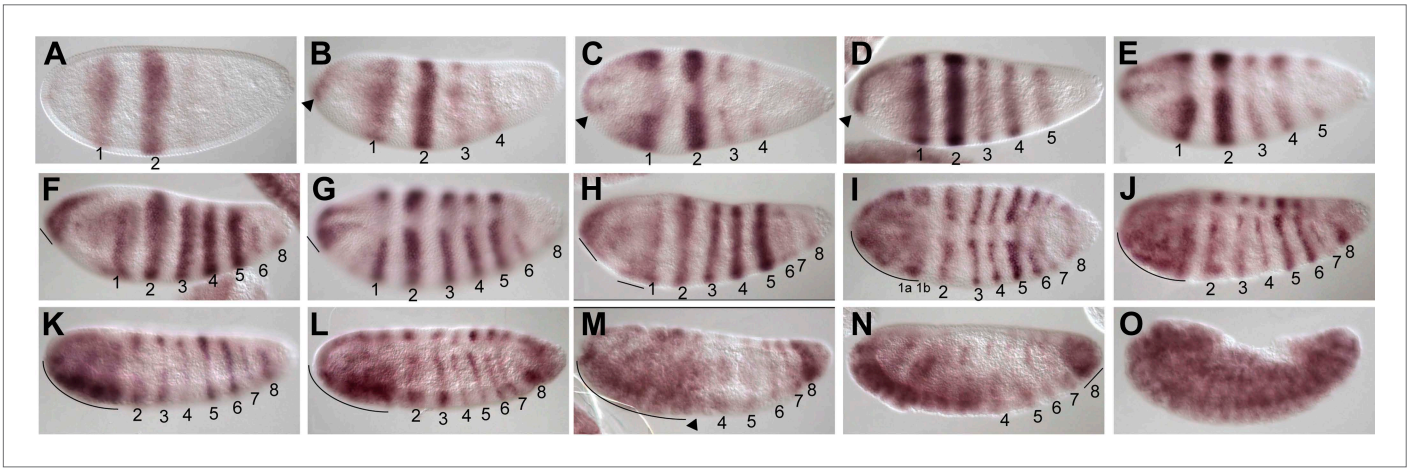

Figure 7. Summary of *Nv hairy* mRNA expression. (**A**) Blastoderm embryo with two double-segment periodicity stripes of *Nv hairy* expression. Note that stripe 2 is broader and stronger than stripe 1. (**B**) Blastoderm embryo showing four double-segment periodicity stripes of expression plus an anterior accumulation of *Nv hairy* transcripts (arrowhead). (**C**) Dorsal view of embryo as in (**B**), illustrating the dorsal anterior expression (arrowhead) that is activated in the same pattern as the anterior domain of *Nv tailless* (***Lynch et al., 2006***). (**D**) Blastoderm embryo with strong anterior and dorsal anterior expression of *Nv hairy* and five pair-rule stripes. (**E**) Dorsal view of embryo as in (**D**) with increased dorsal anterior expression of *Nv hairy*, and the anterior spreading of expression from the anterior of double-segment pair-rule stripe 1. (**F**) Blastoderm embryo with expanding anterior domain (line), five double-segment 'pair-rule' stripes, and two additional stripes coming up. Note that the anterior domain between stripe 1 and the anterior pole is becoming more continuous in expression. (**G**) Dorsolateral view of embryo as in (**F**) highlighting the dorsal anterior expression. Stripe 2 is still wider than other stripes. Stripe 6 appears to be of single-segment periodicity. (**H**) Early gastrula embryo exhibiting a non-homogenous but largely continuous anterior cap of *Nv hairy* expression (that includes stripe 1). Four additional double-segment stripes and three single-segment stripes (two derived from stripe 6) are now evident. (**I**) Dorsal view of embryo slightly older than embryo in (**H**) showing the nearly continuous head domain, and the apparent splitting of stripe 1 within that domain. Double-segment stripes are thinning. (**J**) Dorsolateral view of extending germ-band embryo. Head domain is continuous (line). Stripes 1–7 have single-segment periodicity, are of non-uniform strength; stripe eight appears darker and broader. (**K**) Germ-band extending embryo with a continuous head domain (line) and eight discrete stripes. (**L**) Dorsolateral view of germ-band extending embryo. Stripe 8 is expanded into a wedge abutting the pole cells, and the anterior domain is expanding to include stripe 2. (**M**) Germ-band extension embryo with expanding anterior domain, that extends to include stripe 3 (arrowhead). Posterior domain is expanded. (**N**) Dorsolateral view of embryo as in (**M**) showing further expansion of posterior stripe 8 domain (line). (**O**) Germ-band-retracted embryo exhibiting ubiquitous staining with striated expression evident.

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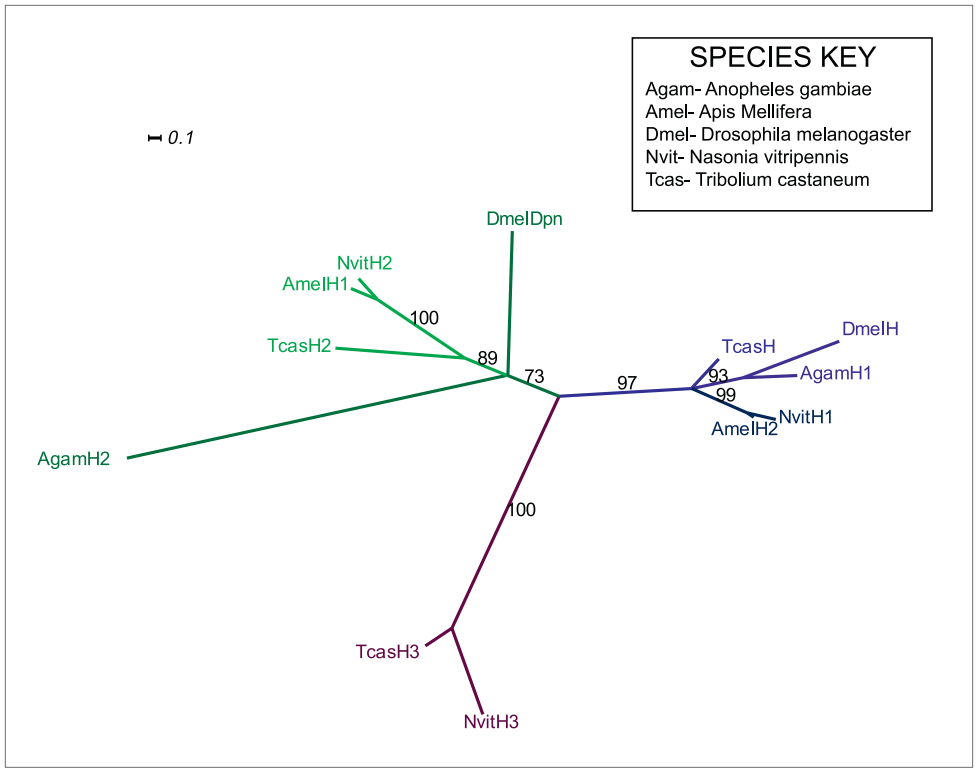

Figure 7—figure supplement 1. Phylogenetic analysis of hairy.

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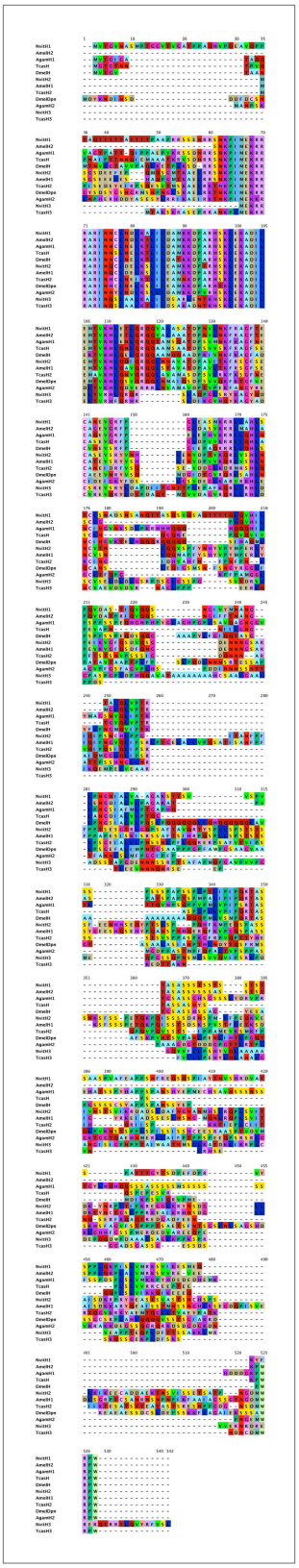

Figure 7—figure supplement 2. Hairy protein sequence alignment.
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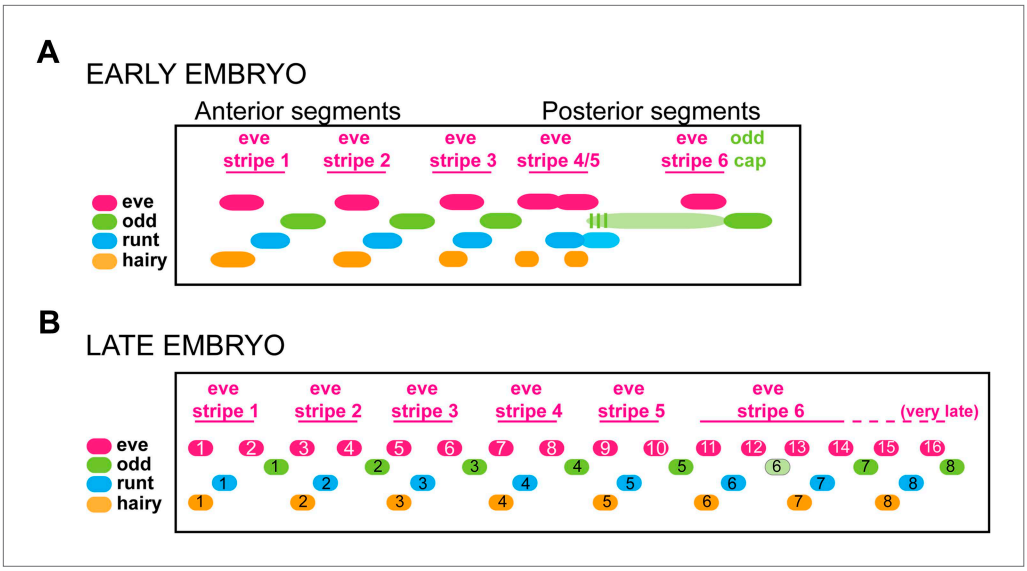

Figure 8. Summary model of pair-rule gene expression in the Nasonia embryo. (**A**) Model of register of pair-rule gene expression in the early embryo. *Nv eve* and *Nv odd* stripes are totally complementary, whereas *Nv runt* stripes partly overlap each of these genes at their interface. *Nv hairy* stripes overlap *Nv eve* stripes toward the anterior of each double-segment periodicity stripe. Towards the posterior of the embryo, an extended domain of low-level *Nv odd* expression exhibits dynamic behavior over several nuclear cycles, and stripes 4/5 of *Nv eve, Nv odd*, and *Nv runt* each differentiate during this interval. Even more posteriorly, *Nv eve* stripe 6 lay anterior to a continuous *Nv odd* cap that extends to the posterior pole of the embryo. This region is set aside for segment specification and differentiation during germ-band extension. (**B**) Model of register of pair-rule gene expression in the germ-band extension (late) embryo. Single-segment periodicity stripes in the germ-band-extended embryo exhibit a variation upon early gene expression patterns. *Nv eve* single-segment stripes are interrupted by *Nv runt* and then *Nv odd* such that *Nv runt* stripes follow odd-numbered *Nv eve* stripes, and *Nv odd* stripes follow even-numbered *Nv eve* stripes. Each of 8 *Nv hairy* stripes overlaps odd-numbered *Nv eve* stripes that derive from the anterior of *Nv eve* pair-rule stripes. Additional expression of several of these genes in the ventral and head domains, which appears to rely on different regulatory logic, is not shown.

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