Supporting Information

Staller et al. 10.1073/pnas.1413877112

Supplemental Note 1

We controlled for several factors that may confound model performance when models are fit to WT data and used to predict patterns in *bcd* RNAi. When building gene expression atlases, gene expression levels are normalized separately in each atlas, so changes in TF levels between genotypes are obscured. In WT the gap genes express at similar levels (1), but their relative levels may be different in *bcd* RNAi. We controlled for this possibility in several ways. First, to simulate changes in level between genotypes we systematically scaled the levels of each gene in *bcd* RNAi, one at a time, and measured model performance. For the pattern driven by the *eve3+7* reporter the repressor-only model was more accurate than the bifunctional model for all tested scalings of Hb and a very large range of scalings of the other regulators (Fig. S4). Analogously, for the endogenous pattern the bifunctional model always outperformed the repressor-only model as we scaled *kni* and *tll* levels (Fig. S4). For Hb the bifunctional model was more accurate than the repressor-only model so long as Hb levels in *bcd* RNAi were less than 135% of WT (Fig. S4). Because *hb* levels are reduced in *bcd* mutant embryos (2), Hb levels in *bcd* RNAi are almost certainly below this level. Second, we refit the models in *bcd* RNAi and predicted *bcd* RNAi output patterns, testing whether an optimal set of parameters could change the relative predictive performance of the models, but it did not for T = 3–6 (Fig. S5). Third, when we refit the models on both datasets simultaneously and predicted *bcd* RNAi patterns the relative order of model performance did not change for T = 3–6 (Fig. S5).

1. Little SC, Tikhonov M, Gregor T (2013) Precise developmental gene expression arises from globally stochastic transcriptional activity. *Cell* 154(4):789–800.

2. Driever W, Nüsslein-Volhard C (1989) The bicoid protein is a positive regulator of hunchback transcription in the early Drosophila embryo. *Nature* 337(6203):138–143.

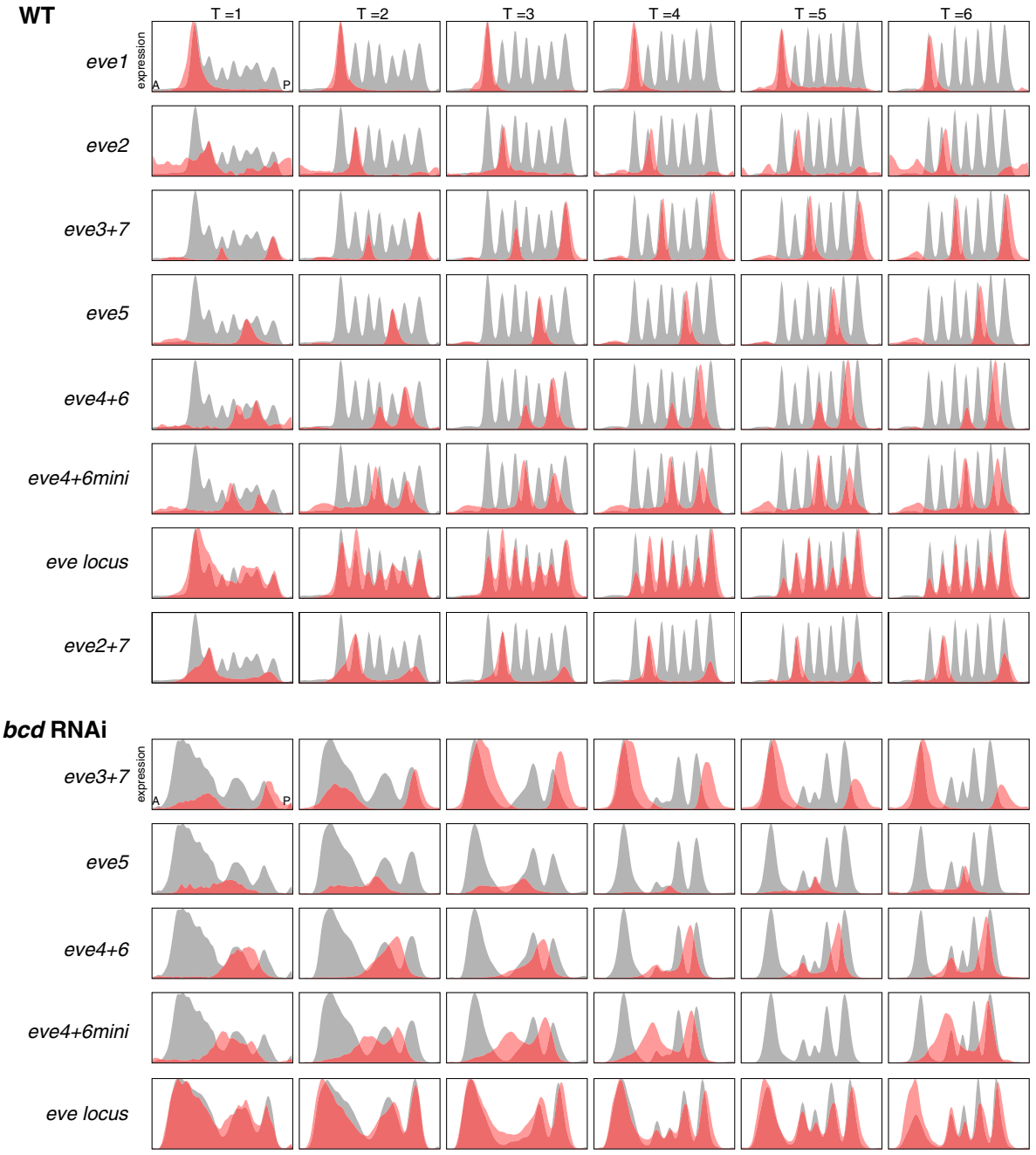

Fig. S1. *eve* enhancer *lacZ* reporters overlap the corresponding endogenous patterns with varying fidelity. Line traces of *lacZ* enhancer reporters (red) and the endogenous *eve* (gray) mRNA pattern in WT and *bcd* RNAi gene expression atlases. Anterior–posterior position (A-P) is plotted on the *x* axis and expression level on the *y* axis for a lateral strip of the embryo.

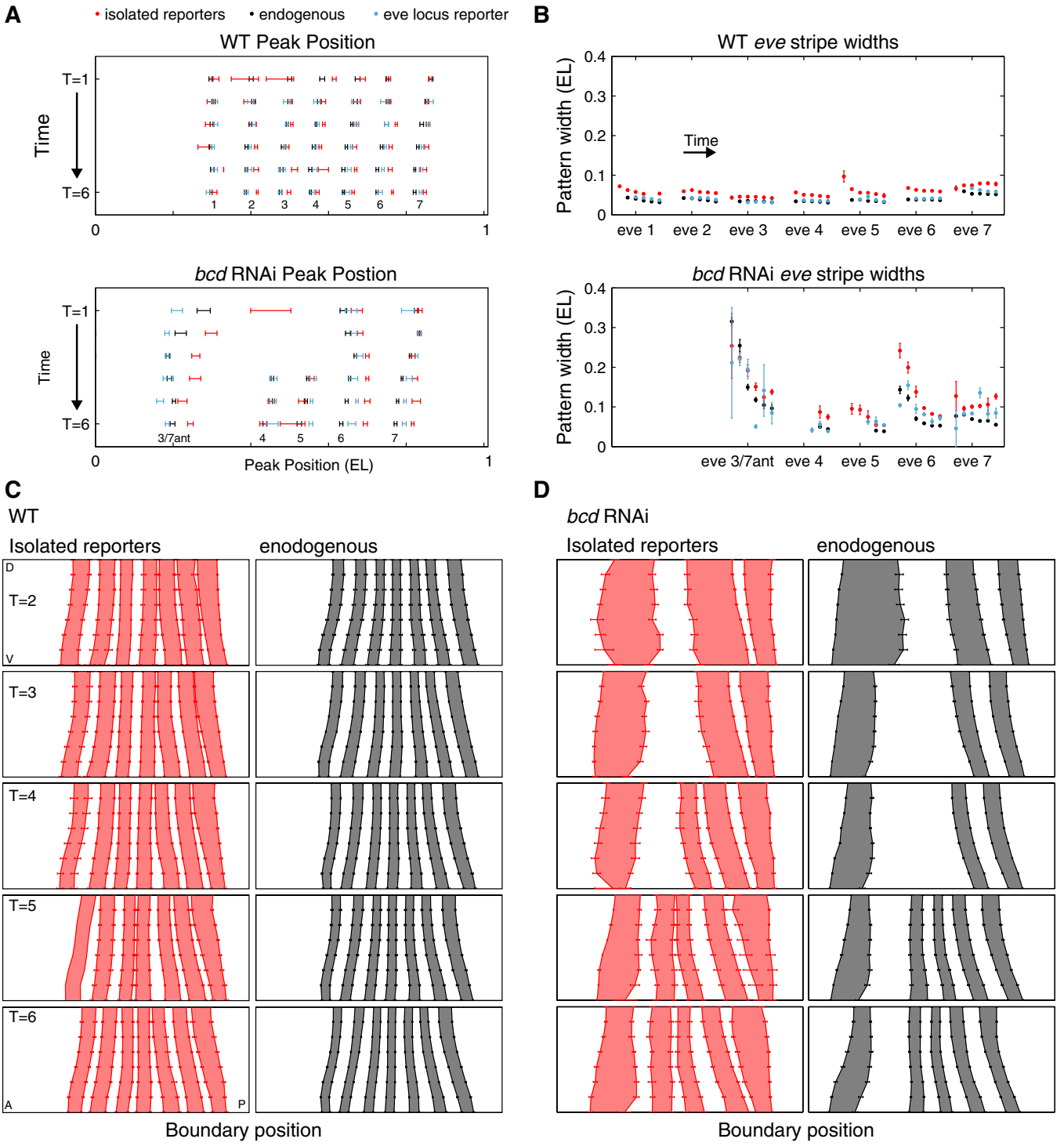

Fig. S2. The expression pattern driven by the whole locus reporter is more similar to the endogenous pattern than the traditional reporters. (*A*) The reporter peak positions (red) are slightly posterior to the endogenous *eve* peaks (black) and whole locus reporter peaks (blue). Peak positions calculated from lateral line traces in Fig. S1. The anterior *eve3+7* pattern is faint and broad at T = 1 and the peak is close to the middle of the embryo as seen in the lateral line trace in Fig. S1. (*B*) Stripes driven by the traditional reporters (red) are wider than endogenous stripes (black) and whole locus reporter (blue) in WT and *bcd* RNAi. Widths calculated from lateral line traces in Fig. S1. In WT some of the error bars are smaller than the diameter of the point. (*C* and *D*) Boundary positions of the traditional reporters (red) and endogenous stripes (gray) in WT (*C*) and *bcd* RNAi (*D*). Note that the ventral-most part of the *eve3+7* reporter anterior pattern is very faint in *bcd* RNAi embryos and this boundary is not reliably detected by our software.

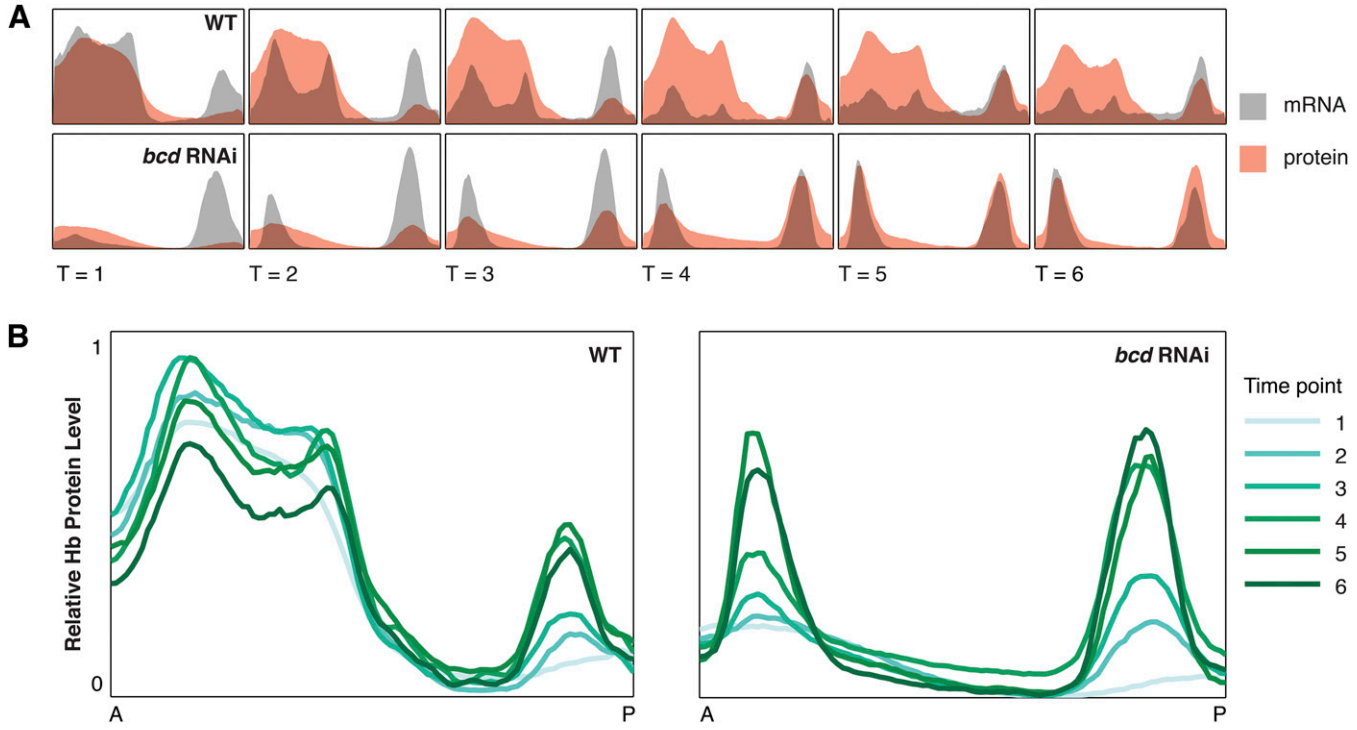

Fig. S3. *bcd* RNAi perturbs *hb* mRNA and protein levels. (*A*) We used Hb protein data for the computational modeling because in both WT and *bcd* RNAi *hb* mRNA (gray) and protein (red) patterns are different. (*B*) Hb protein expression pattern changes over stage 5 in both WT and *bcd* RNAi. In WT both maternal and *bcd* activated zygotic mRNA contribute to the anterior pattern, whereas in *bcd* RNAi only maternal mRNA contributes (1). Note each atlas is normalized separately, so absolute levels are not comparable between atlases. Relative levels change extensively. Data reproduced from Staller et al. (2).

1. Tautz D (1988) Regulation of the Drosophila segmentation gene hunchback by two maternal morphogenetic centres. *Nature* 332(6161):281–284.
2. Staller MV, et al. (2015) A gene expression atlas of a *bicoid*-depleted *Drosophila* embryo reveals early canalization of cell fate. *Development*, 10.1242/dev.117796.

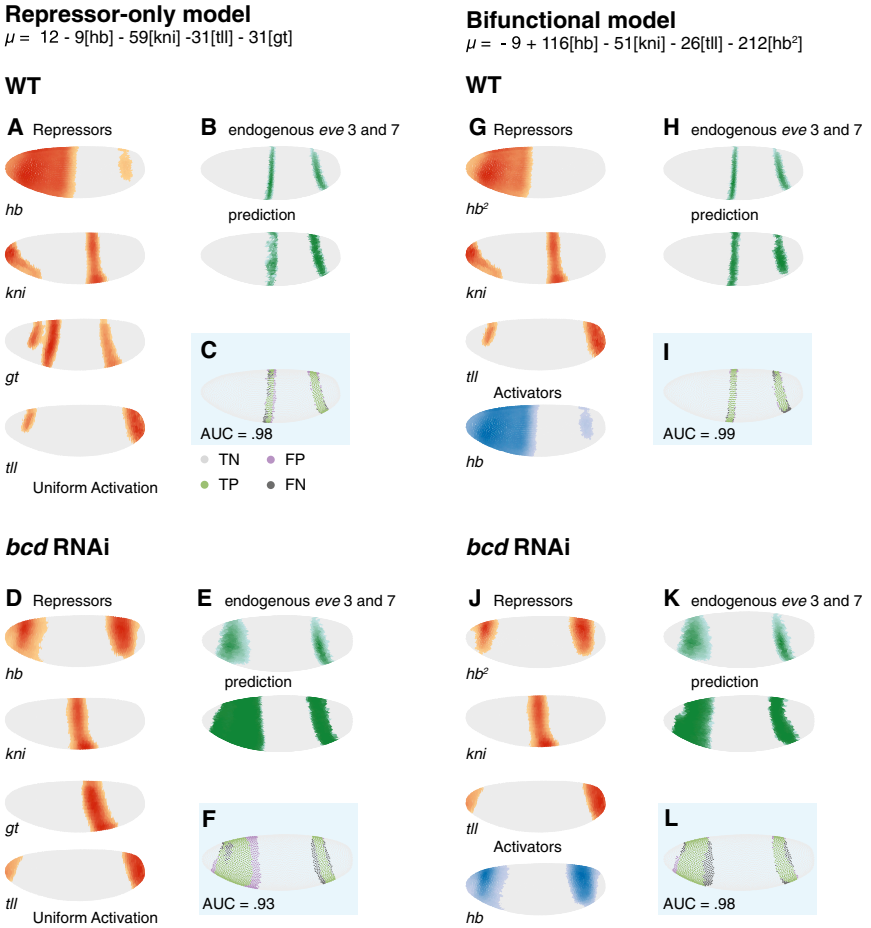

Fig. S4. Under perturbation of *bcd*, the expression patterns of endogenous *eve* stripes 3 and 7 are more accurately predicted by the bifunctional model. (*A*) WT expression patterns of the regulators in the repressor-only model. The expression level of each TF is shown for every cell. Cells with expression below an ON/OFF threshold (*Materials and Methods*) are plotted in gray. For cells above this threshold, color intensity represents expression level. Repressors are red and activators are blue. (*B*) The expression pattern of the endogenous *eve* stripes 3 and 7 and the predictions of the repressor-only model in WT. (*C*) Comparison of predictions to measurement in WT embryos. Green cells are true positives (TP), purple cells are false positives (FP), dark gray cells are false negatives (FN), and light gray cells are true negatives (TN). For visualization, the threshold is set to 80% sensitivity, but the AUC metric quantifies performance over all thresholds. (*D*) The expression patterns of the regulators in the repressor-only model in *bcd* RNAi embryos. (*E*) The expression pattern of the endogenous *eve* stripes 3 and 7 and the predictions of the repressor-only model in *bcd* RNAi. (*F*) Comparison of repressor-only model predictions to data in *bcd* RNAi. (*G–L*) Same as *A–F*, respectively, for the bifunctional model.

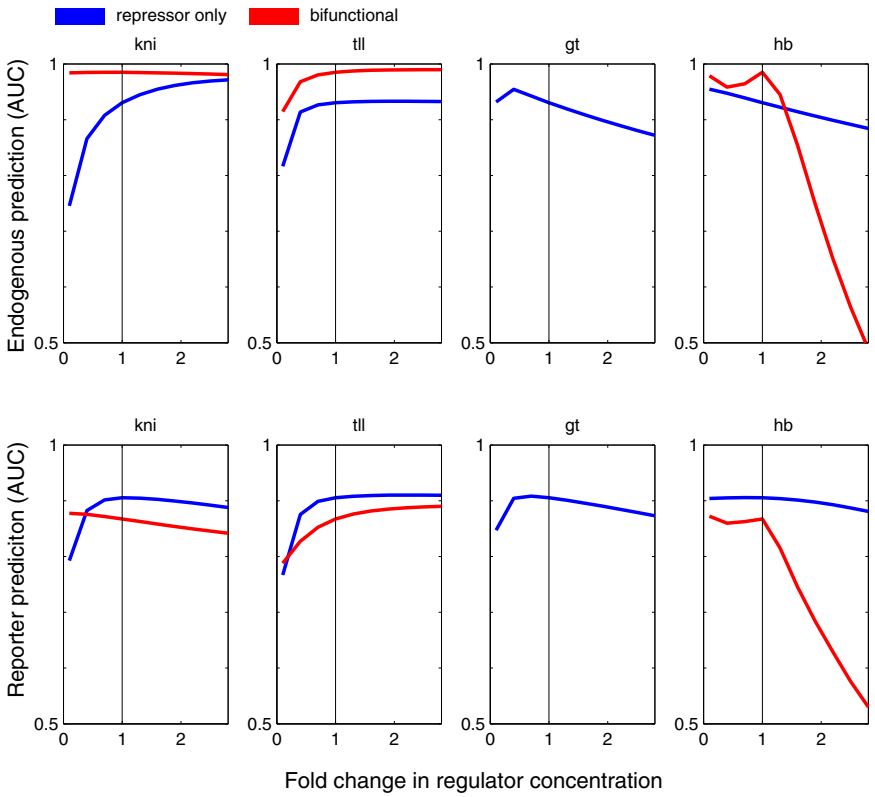

Fig. S5. Sensitivity analysis shows that scaling the relative level of a TF between atlases generally does not change the relative performance of the models. We varied the concentration of each TF separately in the *bcd* RNAi atlas and recalculated the AUC of the repressor-only and bifunctional models. This scaling simulates possible global changes in levels between genotypes. For the endogenous pattern, for all scalings of *kni* and *tll*, the bifunctional model is more accurate than the repressor-only model. For Hb, the bifunctional model is more accurate than the repressor-only model so long as maximal Hb levels in *bcd* RNAi are less than 1.38× maximal WT levels. Because *bcd* is a potent activator of Hb, Hb levels are very likely reduced in *bcd* RNAi embryos. For the reporter pattern all scalings of Hb preserve relative model performance (AUC). The repressor-only model is more accurate for a broad scaling of *kni* and *tll* levels.

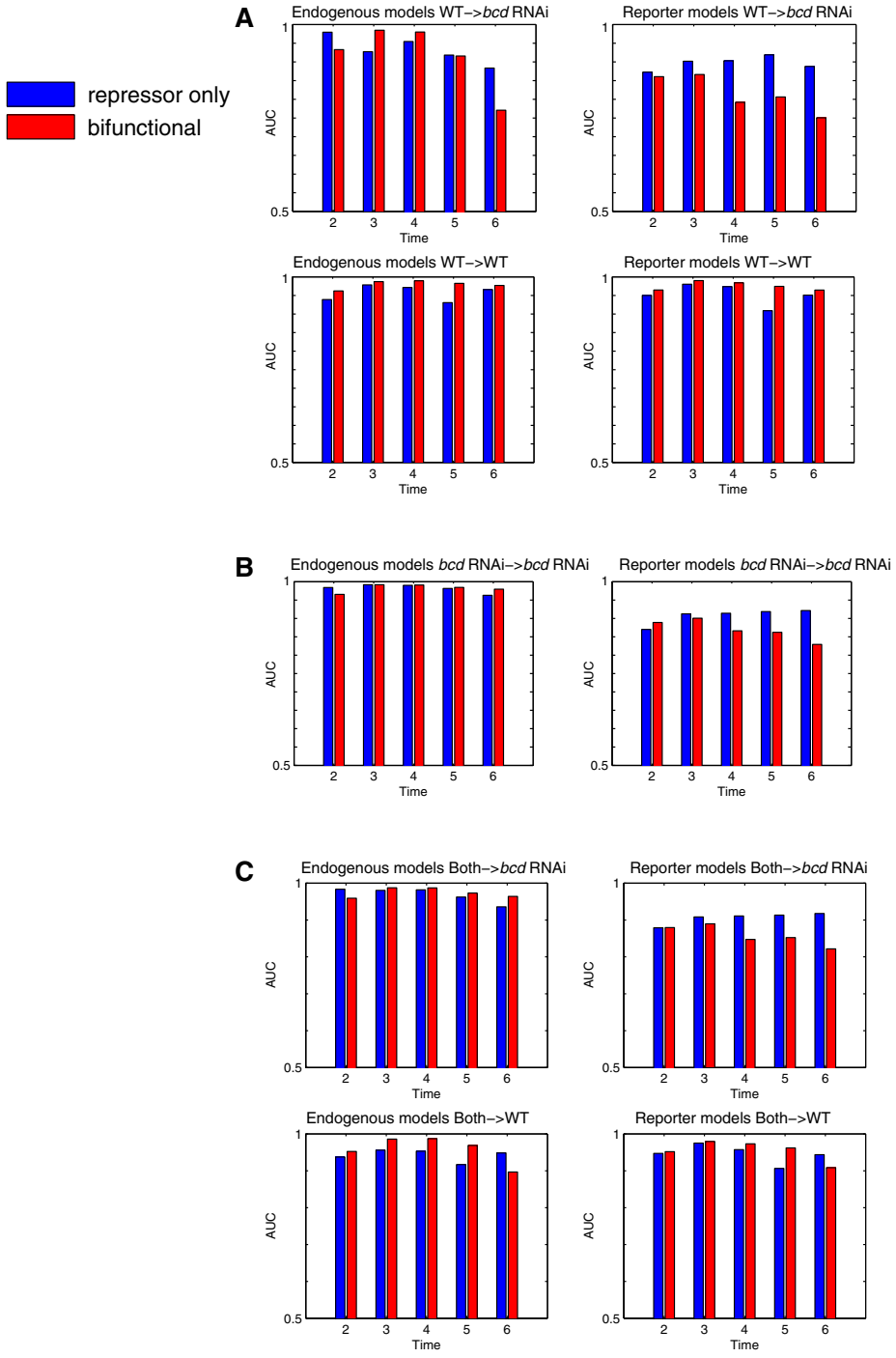

Fig. S6. Fitting the repressor-only and bifunctional models on different datasets yielded similar results. (*A*) Fitting the models in WT at different time points and predicting the corresponding time points. The repressor-only model always more accurately predicted the reporter *bcd* RNAi. Although both models are very accurate in WT, the bifunctional model is more accurate. (*B*) Fitting the models in *bcd* RNAi and predicting *bcd* RNAi. The repressor-only model more accurately predicted the reporter pattern. For the endogenous pattern both models performed well. (*C*) Fitting the models on both the WT and *bcd* RNAi datasets led to similar results: The bifunctional model more accurately predicted the endogenous pattern and the repressor-only model more accurately predicted the reporter pattern in *bcd* RNAi.

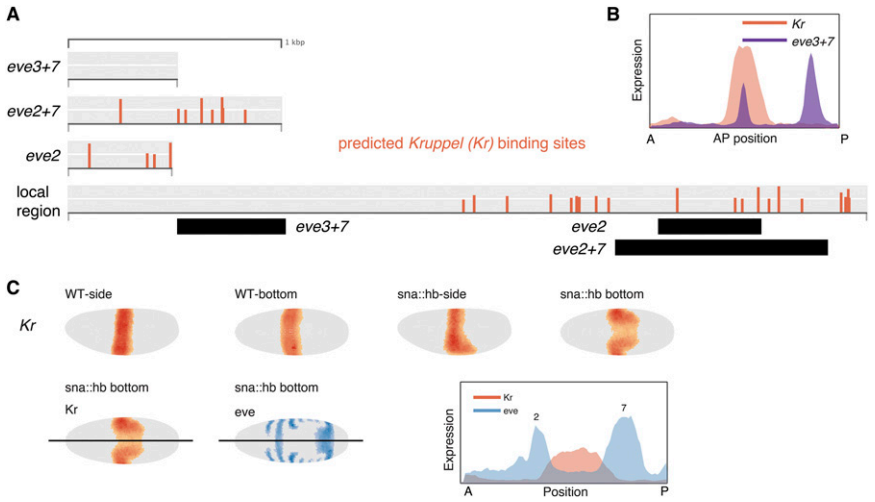

Fig. S7. The expansion of the *Kr* expression pattern potentially explains the shape of the *eve2+7* expression pattern in *sna::hb* embryos. (*A*) The eve2 enhancer is enriched for predicted *Kr* binding sites (red), whereas the *eve3+7* enhancer is depleted for *Kr* binding sites. We predicted binding sites using PATSER (stormo.wustl.edu) with a position weight matrix derived from bacterial one-hybrid data (1). (*B*) *Kr* expression overlaps stripe 3 of the *eve3+7* reporter mRNA in WT. *Kr* does not repress this pattern consistent with the absence of binding sites. (*C*) The distribution of *Kr* mRNA in WT and *sna::hb* misexpression embryos. The expanded ventral region of the *Kr* mRNA pattern seems to set the boundary of the expanded endogenous *eve* stripe 7 pattern.

1. Noyes MB, et al. (2008) A systematic characterization of factors that regulate Drosophila segmentation via a bacterial one-hybrid system. *Nucleic Acids Res* 36(8):2547–2560.

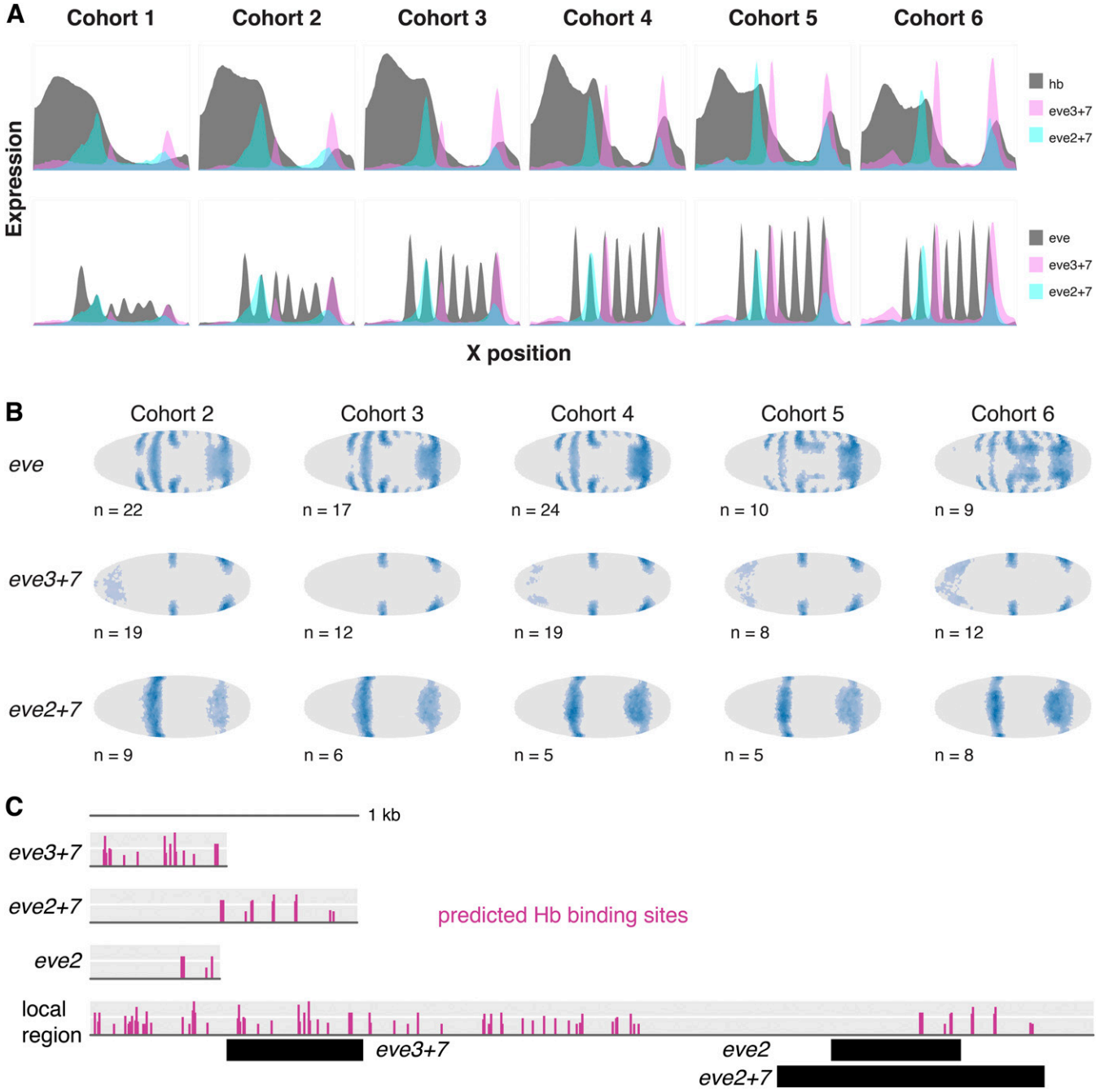

Fig. S8. There are quantitative differences between the *eve* stripe 7 shadow enhancers. (*A*) Line traces of Hb protein, *eve3+7*, and *eve2+7* show how Hb overlaps stripe 7 (*Top*). Line traces of *eve, eve3+7*, and *eve2+7* show neither reporter perfectly matches the endogenous pattern (*Bottom*). (*B*) Computational renderings of gene expression atlas data from sna::hb embryos. The number of embryos included in each time point of the gene expression atlas is shown. (*C*) Predicted Hb binding sites (calculated as in Fig. S7).

Table S1. Model parameters

		Linear					Quadratic				
	Time	Constant	*hb*	*kni*	*tll*	*gt*	Constant	*hb*	*kni*	*tll*	hb^2
Endogenous	2	5	−5	−21	−9	−15	−5	68	−22	−7	−134
hb protein	3	12	−9	−59	−31	−31	−9	116	−51	−26	−212
Other mRNA	4	11	−5	−40	−51	−27	−9	101	−42	−67	−137
	5	5	−2	−17	−25	−14	−7	73	−26	−49	−95
	6	10	−4	−48	−37	−33	2	49	−50	−58	−77
Endogenous	3	12	−9	−59	−31	−31	−9	116	−51	−26	−212
Ilsley et al. (1) (includes gt protein)	3	11	−10	−66	−22	−13	−8	110	−45	−25	−201
Reporter	2	6	−6	−30	−6	−16	−3	64	−37	−4	−134
hb protein	3	12	−11	−72	−18	−29	−10	145	−73	−14	−286
Other mRNA	4	11	−7	−37	−22	−43	−13	105	−25	−16	−144
	5	4	−1	−13	−14	−15	−12	83	−15	−22	−103
	6	8	−4	−26	−22	−40	−5	59	−19	−23	−91

1. Ilsley GR, Fisher J, Apweiler R, DePace AH, Luscombe NM (2013) Cellular resolution models for even skipped regulation in the entire Drosophila embryo. *eLife* 2:e00522.

Table S2. AUC scores

	Linear		Quadratic	
T = 3	Ilsley et al. (1)	Refit	Ilsley et al. (1)	Refit
WT	0.9622	0.9786	0.9874	0.9874
bcd RNAi	0.9321	0.9275	0.985	0.9849

1. Ilsley GR, Fisher J, Apweiler R, DePace AH, Luscombe NM (2013) Cellular resolution models for even skipped regulation in the entire Drosophila embryo. *eLife* 2:e00522.

Table S3. Enhancer reporter sequences and primers used to generate them

Construct	Enhancer sequence	Source	Primer sequences
eve1	aggcctaatcacttccctgaaatgcataattgtgccgcggcttttgata- cgctcctggcggagagggagatgaggaaaggatgcacgggaaccgca- gccaagtggcagtcgagattggcaaatccgccagcggacaatgccca- gagaatgggcaacaagtagcggcgaattagcaatcctatcatgcttt- tatggccggccaactcttgcccgcgcatctcagttcatccgaagcgg- gaccaggtccaggttcaagtcgaggtccagtacccctgctatcccgt- caaccccttttagggcgataatccttctaaatgtttgcattaatttcg- aggcgtggacggattagggcgtgctggctgggcggaaccccgcagcag- aaaccgccgaggacactgcaccgactgacctgcagcctacagatctc- tgatcttcgatctctaatcctttcgcatttgcaactgacttctgcac- tgggtccgcccctaatccttccgccgagaaggcggcagagtcgcgag- gtactggcccggggtaatgggattatctgcgattaccccagatgatc- cgcagaaagtcaatctggttcaggggctaattgtcagcgaagtcaac- taaatccaatcctttcgcgccccttctgtttatttgtttgttttcg- tttgttttgagaatttctggcaattaagttgcccgttttgatgcgcg- ggggcgggtgcatcaaatcctttcggcatacctgtcctgcacaaatg- ctgaattccgcatcccatggatacccagatattcagatatcccaaggc	1	aggcctaatcacttccctg GCCTTGGGATATCTGAAT- ATCTGG
eve4+6	aggatccctgggctctggctctggactatccgccgaccctccatatcc- atgatttacaattctcgttttttttcgcgttatttttttaggggcttt- aatgaccgtcgtaaagccgcaggaggaccaggaccaggactctgctc- acatttcgcgcactgattctaaaaaatgaaatcatttttttcttgaat- ttcacggcgcgcctcgagcaggactctttgttctcggccaggcaatt- gtccttttttgcgctcagctctcagtttttttcgtccagcgggcatta- cctacacggcgttttatggcggagatgatattcgcctgggatcggtt- ccgtttttttaggccataaaaattaggcggcataaaaaaaactgcattg- gaattctagttctagtttcaagtttttaggtttccaggtttctgcca- gcccgcctagattcgcatttcgcggaattcggaagcggaacagaatg- ccagaatggtcagaatcctggctgaccttgccttttggccaggggcc- gtaaaaaaattgactcgctgcggtgcgcggaatatttttttaaatctg- actttccaacaatctctgatctgggttcgaatcgtaaaaaaaaagca- gaacaaaaagcgggcattttcgtcggcaaatgatctgttaatgggcc- gggctaaaaaactaagtcacaaagtcacaaggttgtccggtaaattg- acccggttaagaatgtctgtctgtaccgagaaggatgcaggacattc- agcacttcaaagctcccaccgctcgaaggattcccccgaagattcac	1	aggatccctgggctctg GTGAATCTTCGGGGGAATCC
eve5	atatcccaaggccgcaaagtcaacaagtcggcagcaaatttccctttgt- ccggcgatgtgttttttttttagccataactcgctgcattgtttggg- ccaagtttttcttctgccaaattgcggagatgatgcggggattatgc- gctgattgcgtgcaattatggacatcctgcgaggccccgaggaactt- cctgctaaatcctttcatccgcctacagaaccccttttgtgtcccgtt- cgccgggagtccttgacgggtccttcgactattcgcttacagcagct- tgcgtaaaatttcataaccctacgagcggctcttccgcggaatccct- ggcattatccttttttacctcttgccaatccgttggctaaaaaacggc- ttcgacttccgcgtaactgctggacaacaaagacaaaaaacggcgaa- aggacggcgatttccaggtagcattgcgaattccgtcaaactaaagg- accggttatataacgggtttatatggccagaatctctgcatctccac- gaccgccagaagctgcgtaaaactgcaggctctgttttgatttctgc- aacttcagttaattgcccgggatggccagcaattgccggcaattata- aaacagcgcagatgtgactcagcttccatatctaactctatatctca- tgccgaaaatctagggtggggagcggaggggcggggtgcgtgggtga- cttgcctgccagggaaaggggcgggggttcagcgggtgataaatgt- gcgtgatttggaatgaatgcgcatcgattaaaaccgcagggcaatca- attt	1	atatcccaaggccgcaaag AAATTGATTGCCCTGCGGT
eve3+7	GGATCCTCGAAATCGAGAGCgacctcgctgcattagaaaactagatcag- ttttttgttttggccgaccgatttttgtgcccggtgctctctttacg- gtttatggccgcgttcccatttcccagcttctttgttccgggctcag- aaatctgtatggaattatggtatatgcagatttttatgggtcccggc- gatccggttcgcggaacgggagtgtcctgccgcgagaggtcctcgcc- ggcgatccttgtcgcccgtattaggaaagtagatcacgtttttttgtt- cccattgtgcgctttttcgctgcgctagttttttttccccgaaccca- gcgaactgctctaatttttttaattcttcacggcttttcattgggctc- ctggaaaaacgcggacaaggttataacgctctacttacctgcaattg- tggccataactcgcactgctctcgtttttaagatccgtttgtttgtg- tttgtttgtccgcgatggcattcacgtttttacgagctc	2	GGATCCTCGAAATCGAGAGC GAGCTCGTAAAAACGTGAATGC

Table S3. Cont.

Construct	Enhancer sequence	Source	Primer sequences
eve2	ggttacccggtactgcataacaatggaacccgaaccgtaactgggacag- atcgaaaagctggcctggtttctcgctgtgtgtgccgtgttaatccg- tttgccatcagcgagattattagtcaattgcagttgcagcgtttcgc- tttcgtcctcgtttcactttcgagttagactttattgcagcatcttg- aacaatcgtcgcagtttggtaacacgctgtgccatactttcatttag- acggaatcgagggaccctggactataatcgcacaacgagaccgggtt- gcgaagtcagggcattccgccgatctagccatcgccatcttctgcgg- gcgtttgtttgtttgtttgctgggattagccaagggcttgacttgga- atccaatcccgatccctagcccgatcccaatcccaatcccaatccct- tgtccttttcattagaaagtcataaaaacacataataatGATGTCGA- AGGGATTAGGGG	3	ggttacccggtactgcataac CCCCTAATCCCTTCGACATC
eve2+7	agaaggcttgcatgtgggccttttccaggtcggccagtaggtagagttg- ttgcgatgcggctatgccgggcgagttaatgccaatgcaaattgcgg- gcgcaatataacccaataatttgaagtaactggcaggagcgaggtat- ccttcctggttacccggtactgcataacaatggaacccgaaccgtaa- ctgggacagatcgaaaagctggcctggtttctcgctgtgtgtgccgt- gttaatccgtttgccatcagcgagattattagtcaattgcagttgca- gcgtttcgctttcgtcctcgtttcactttcgagttagactttattgc- agcatcttgaacaatcgtcgcagtttggtaacacgctgtgccatact- ttcatttagacggaatcgagggaccctggactataatcgcacaacga- gaccgggttgcgaagtcagggcattccgccgatctagccatcgccat- cttctgcgggcgtttgtttgtttgtttgctgggattagccaagggct- tgacttggaatccaatccTgatccctagcccgatcccaatcccaatc- ccttgtccttttcattagaaagtcataaaaacacataataatgatgt- cgaagggattaggggcgcgcaggtccaggcaacgcaattaacggact- agcgaactgggttatttttttgcgccgacttagccctgatccgcgag- cttaacccgttttgagccgggcagcaggtagttgtgggtggacccca- cgatttttttggccaaacctccaagctaacttgcgcaagtggcaagt- ggccggtttgctggcccaaaagaggaggcactatcccggtcctggta- cagttggtacgctgggaatgattatatcatcataataaatgttttgc- ccaacgaaaccgaaaactttcaaattaagtcccggcaactgggttc- ccattttccattttccatgttctgcgggcaggggcggccattatctc- gct	This work	agaaggcttgcatgtggg agcgagataatggccgcc
BAC whole locus	Beginning −6.4 kb upstream of *eve* transcription start site (TSS) and ending 11.3 kb downstream of *eve* TSS. The *eve* coding sequence has been replaced with *LacZ* and the neighboring *TER94* gene has been fused to GFP.	Gift from M. Fujioka, Thomas Jefferson University, Philadelphia	

The *eve2+7* enhancer spans 998 bp including the entire minimal *eve2* enhancer, but none of the *eve3+7* enhancer (Fig. S6). Late in this work we noticed this construct had a 1-bp polymorphism and a 6-bp deletion in the minimal *eve2* region, but neither of these defects affects any of the foot-printed binding sites (3, 4).

1. Fujioka M, Emi-Sarker Y, Yusibova GL, Goto T, Jaynes JB (1999) Analysis of an even-skipped rescue transgene reveals both composite and discrete neuronal and early blastoderm enhancers, and multi-stripe positioning by gap gene repressor gradients. *Development* 126(11):2527–2538.
2. Small S, Blair A, Levine M (1996) Regulation of two pair-rule stripes by a single enhancer in the Drosophila embryo. *Dev Biol* 175(2):314–324.
3. Small S, Kraut R, Hoey T, Warrior R, Levine M (1991) Transcriptional regulation of a pair-rule stripe in Drosophila. *Genes Dev* 5(5):827–839.
4. Small S, Blair A, Levine M (1992) Regulation of even-skipped stripe 2 in the Drosophila embryo. *EMBO J* 11(11):4047–4057.