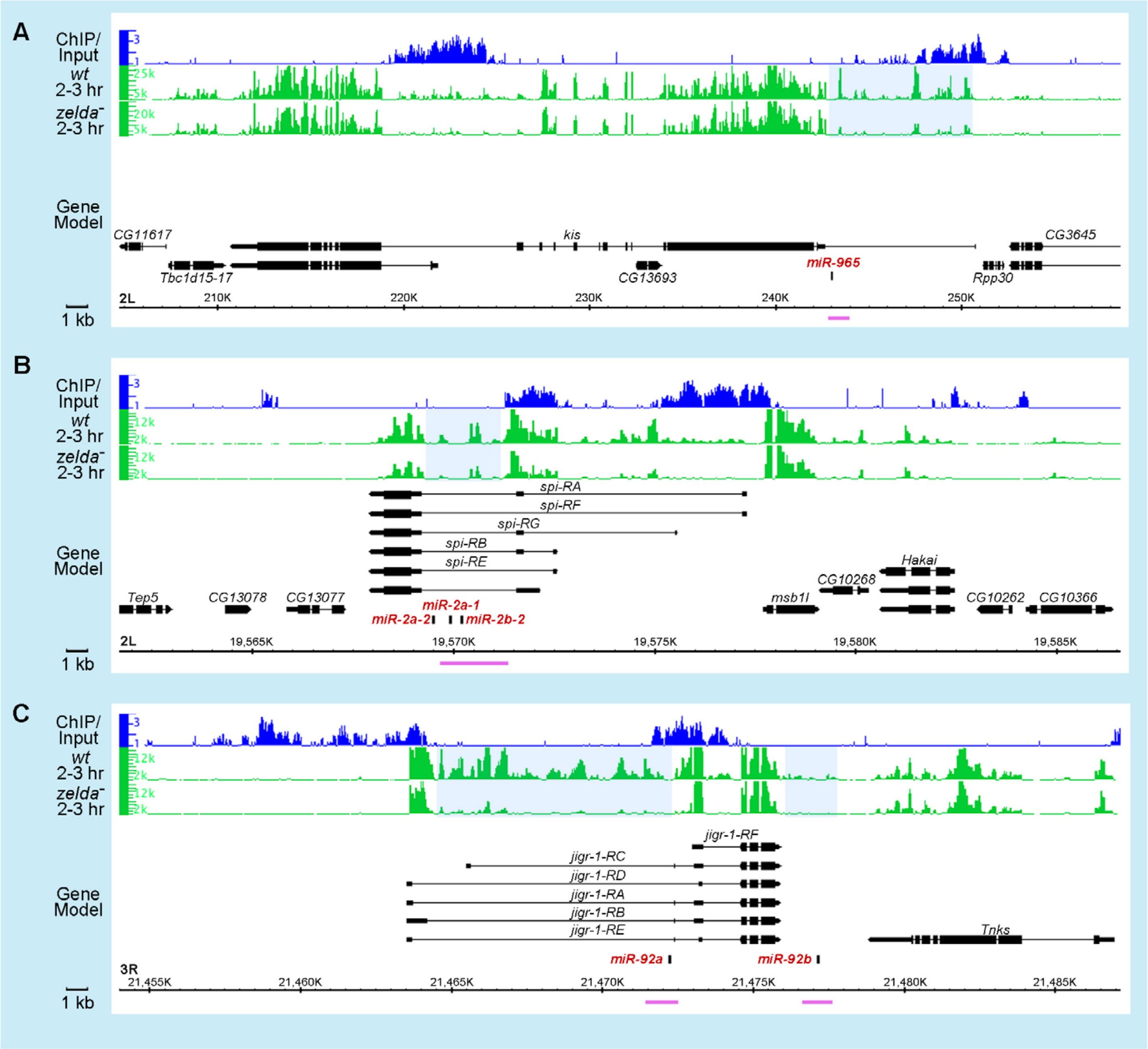

Fig. S1. Three of the early-expressed miRNAs reside in introns of other genes. Browser views of three intronic miRNA clusters: (A) *miR-965* in *kis*; (B) *miR-2a-1* cluster in *spi*; (C) *miR-92a* in *jigr-1* (see Fig. 1 legend for description of tracks).

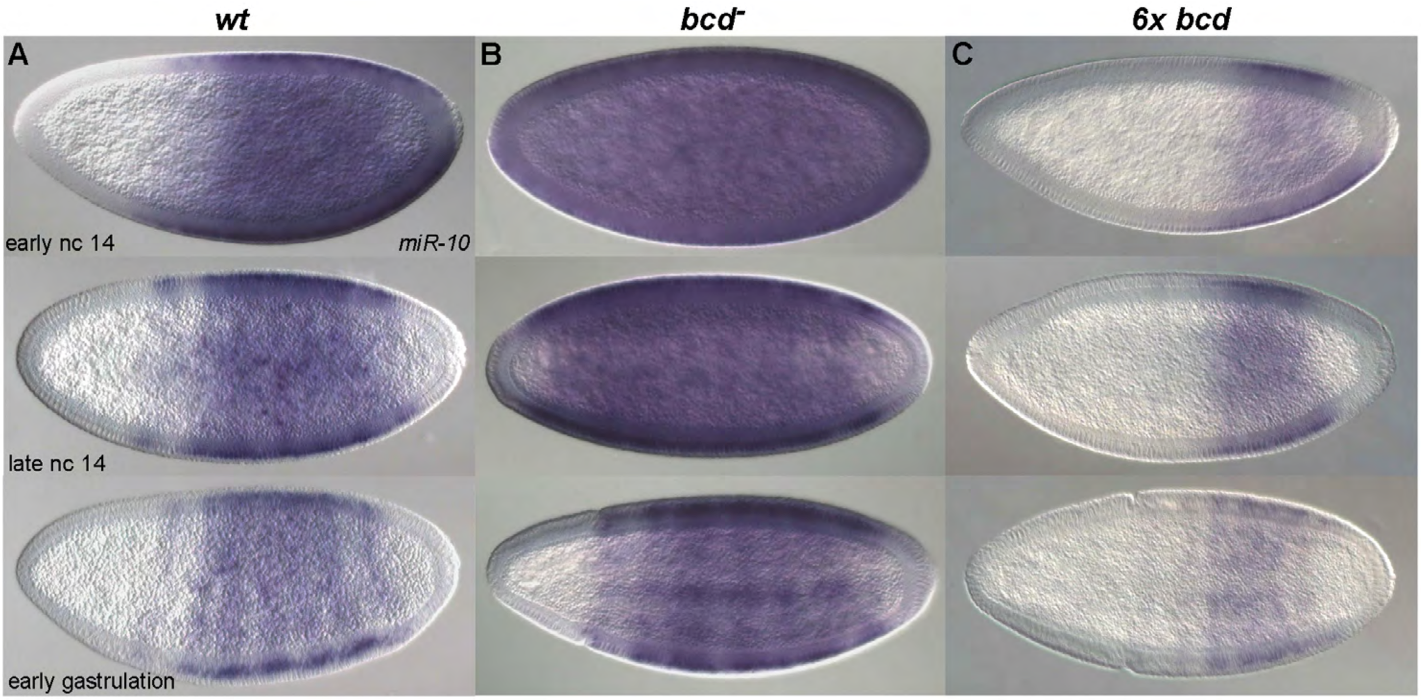

Fig. S2. The Bcd dependent pathway represses *miR-10*. Wild-type (*wt*, A), *bcd* mutant (B) and 6x *bcd* embryos (C) hybridized with RNA probes against *pri-miR-10*. In *wt*, *miR-10* is initially expressed in the posterior region (A, early nc 14). Later, as nc 14 progresses, *miR-10* expression refines into seven stripes along the AP axis. In *bcd* mutants, the onset time of *miR-10* expression is not affected (B, early nc 14), but it is detected uniformly across the entire embryo. Though the pattern refines later on, *miR-10* expression expands anteriorly (A, B, mid, late nc 14, and early gastrulation). In contrast, in 6x *bcd* embryos, the anterior repression of *miR-10* expands along with the expansion of the Bcd gradient (C).

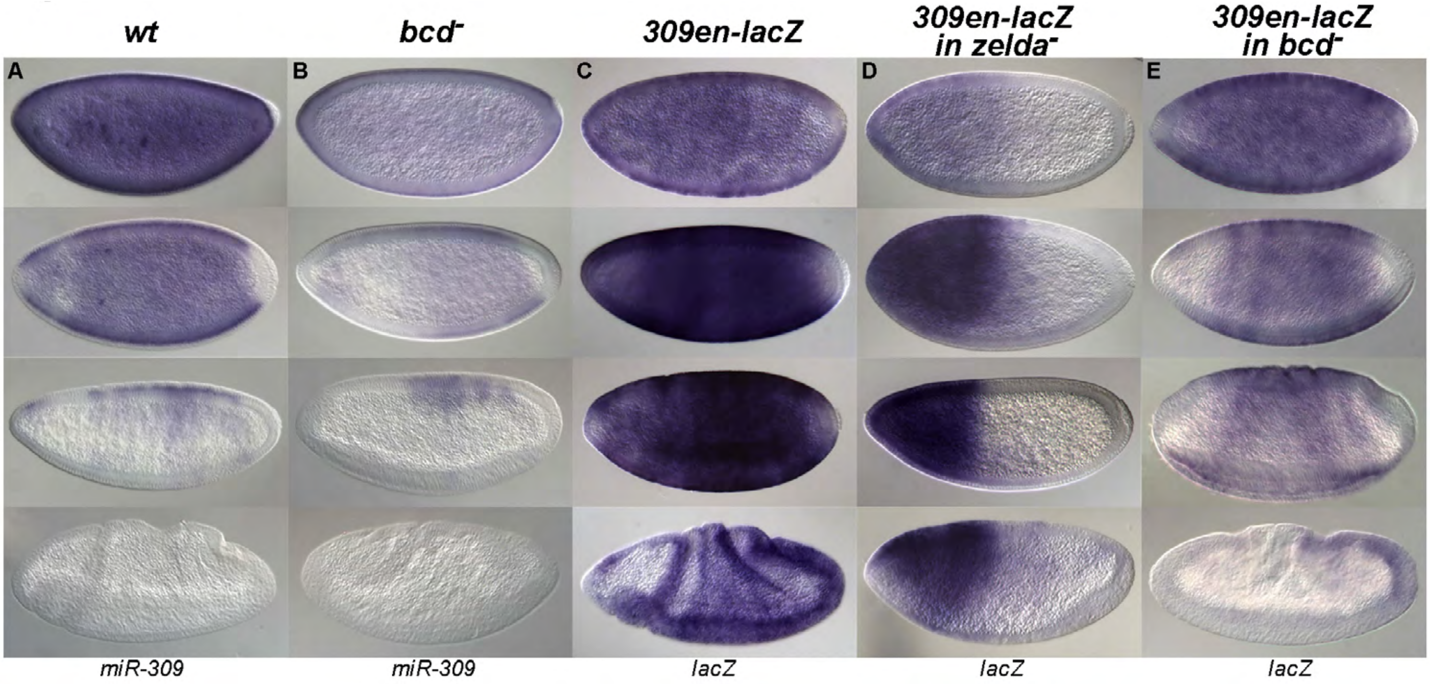

Fig. S3. The Bcd dependent pathway activates *miR-309*. Wild-type (*wt*, A, C), *bcd* mutant (B, E) and *zelda* mutant (D) embryos hybridized with RNA probes against *pri-miR-309* transcripts (A, B) or *lacZ* (C, D, E). In *wt*, *miR-309* is initially expressed throughout the entire embryo (A, early nc 14). Later, as nc 14 progresses, *miR-309* refines along both the A-P and D-V axes, as expression is lost in the ventral region and in stripes along A-P axis. In *bcd* mutants, the initial uniform expression of *miR-309* was not affected (B, early nc 14), but became restricted to the posterior region later on (B, late nc 14 and early gastrulation). *lacZ* driven by the *miR-309* enhancer is expressed ubiquitously in the embryo similar to endogenous *miR-309*, suggesting that this enhancer is responsible for *miR-309* activation (C). *lacZ* expression persists into germ band extension stages, indicating that this enhancer is lacking repression elements. In *zelda* mutants, only anterior *lacZ* expression is detected (D). In *bcd* mutants, anterior *lacZ* expression is reduced and eventually lost at the germ band extension stage (E).

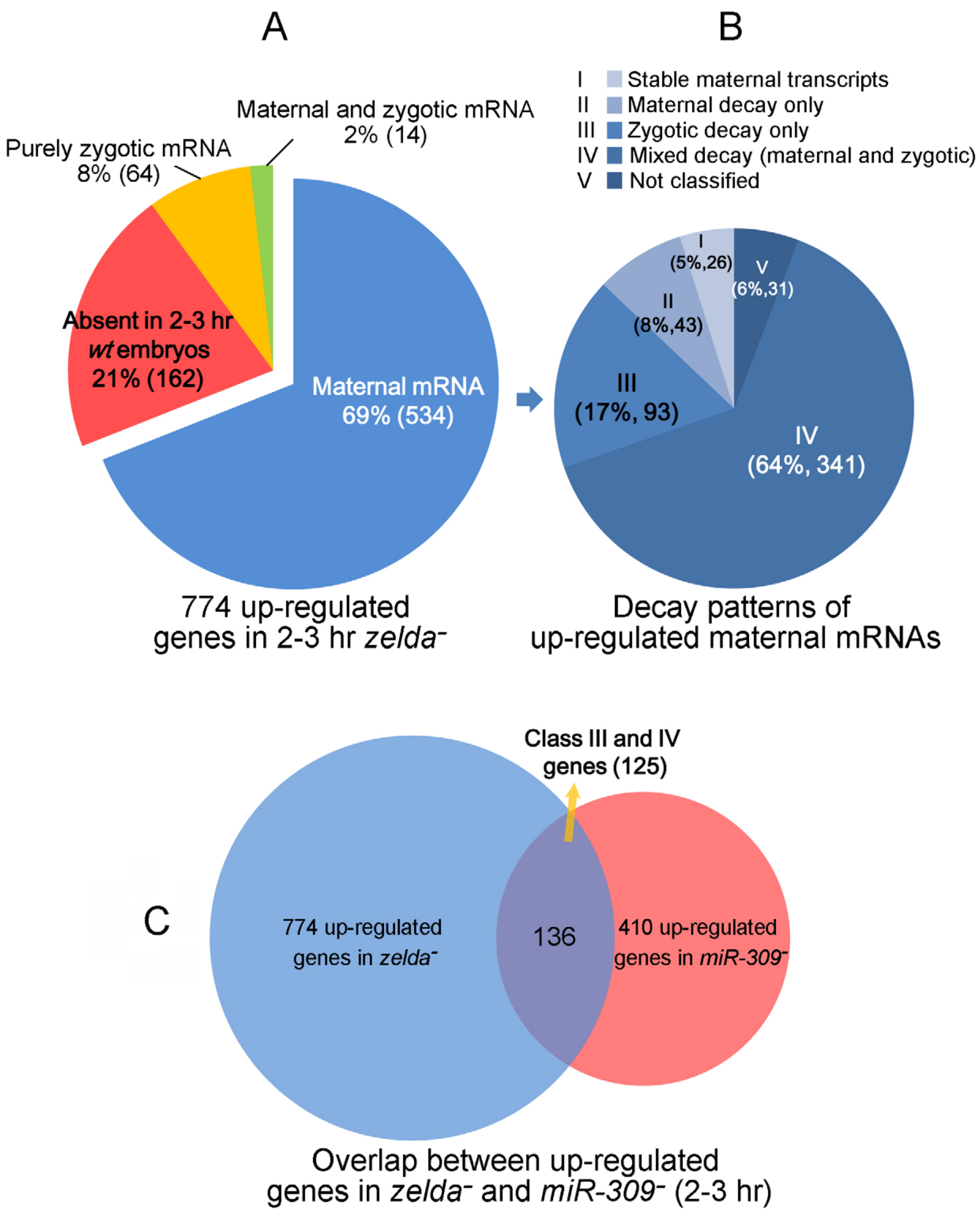

Fig. S4. Analysis of up-regulated genes in 2-3 hour *zelda* mutant embryos. (A) Pie chart showing proportion (and number of genes, Supplementary material Table S3) of the 774 up-regulated genes in *zelda* mutants in four categories: maternal mRNA (mRNAs preloaded into the embryo during oogenesis); maternal and zygotic mRNA (mRNAs preloaded and transcribed in the embryo); purely zygotic mRNA (mRNAs newly transcribed in the embryo); and absent (not detected in wild-type (*wt*), but up-regulated in *zelda* mutants). Note that 69% of the up-regulated genes are maternally loaded. (B) Pie chart showing the 534 maternal mRNAs from (A) in 5 categories based on their decay pattern during the MZT (from classification in Thomsen et al., 2010, Supplementary material Table S3). 89% (434) of the up-regulated maternal mRNAs in *zelda* mutants are unstable maternal mRNAs (Supplementary material Table S3), and are degraded either by maternal mRNA decay pathway only (II), or by zygotic mRNA decay pathway only (III), or by both (IV). (C) 136 of the 774 up-regulated genes in *zelda* mutants overlap with those up-regulated in *miR-309* mutants; of these, ~92% (125, Supplementary material Table S3) are type III and IV genes, indicating that the up-regulation of these genes in *zelda* mutants is due to the disruption of the mRNA decay pathway conducted by miR-309.

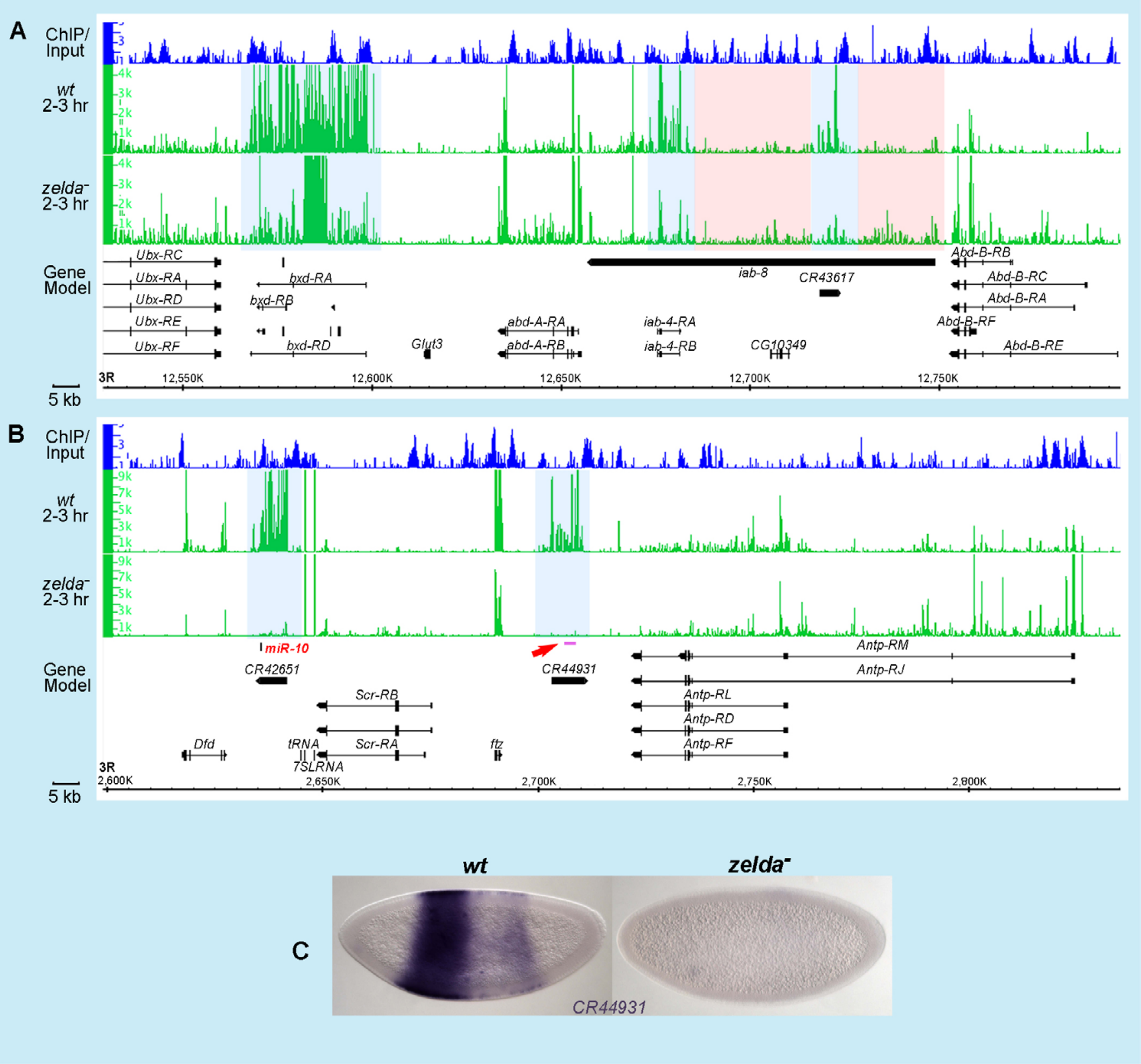

Fig. S5. MiRNAs and lincRNAs in the Hox gene complexes. Browser views of genomic regions spanning *Ubx* to *Abd-B* in the *Bithorax complex (BX-C)* (A) and *Dfd* to *Antp* in the *Antennapedia complex* (*ANT-C*) (B). Tracks above gene models are as described in Figures 1 and S1. Light blue shadowing in the expression tracks indicates regions down-regulated in *zelda* mutants. Light red shadowing indicates regions up-regulated in *zelda* mutants. (C) Wild-type (*wt*) and *zelda* mutant embryos hybridized with RNA probes against *CR44931* (purple line with red arrow in B). Note that *CR44931* is expressed in a broad central domain in *wt*, but is not detected in *zelda* mutants.

Table S1. miRNAs tested in this study

miRNA name	miRNA position	Host genes	RNA Probe location	Probe size (nt)	Expression in 0-6 hr embryos (previous literatures*)	Expression in 1-3 hr wild-type tiling array	Zelda binding within 2 kb of miRNA transcription units or host genes	Nuclei dots in wild-type 1-3 hr	MiRNA expression in *zelda*$^{-}$ compared to wild-type(1-3 hr)
miR-1	intergenic	n/a	2L: 20486777 -20488219	1443	yes	yes	yes	yes	Down-regulated
miR-10	intergenic	n/a	3R: 2634611 -2636419	1809	yes	yes	yes	yes	Down-regulated
miR-11	intronic	*E2f*	3R: 17447463 -17448432	970	yes	yes	yes	yes	Down-regulated
miR-309 cluster	intergenic	n/a	2R: 15548119 -15549170	1052	yes	yes	yes	yes	Down-regulated
miR-9a	intergenic	n/a	3L: 19556781 -19558851	2071	yes	yes	yes	yes	Down-regulated
miR-92a	intronic	*jigr1*	3R: 21471476 -21472485	1010	yes	yes	yes	yes	Down-regulated
miR-92b	intergenic	n/a	3R: 21476633 -21477659	1027	yes	yes	yes	yes	Down-regulated
miR-iab-4	intergenic	n/a	3R: 12681571 -12683390	1820	yes	yes	yes	yes	Down-regulated
miR-iab-4as	intergenic	n/a	3R: 12681571 -12683390	1820	yes	yes	yes	yes	Up-regulated
miR-965	intronic	*kis*	2L: 242854 -243924	1071	yes	yes	yes	yes	Down-regulated
miR-2a-1 cluster	intronic	*spi*	2L: 19569658 -19571349	1692	yes	yes	yes	yes	Down-regulated
miR-13a cluster	intergenic	n/a	3R: 11243042 -11244277	1237	yes	very low	yes	no	n/a
miR-2b-1	intergenic	n/a	2L: 8258046 -8259423	1378	yes	no	no	no	n/a
miR-14	intergenic	n/a	2R: 5440704 -5441997	1294	yes	no	low	no	n/a
miR-184	intergenic	n/a	2R: 9215877 -9217583	1707	yes	no	no	no	n/a

miR-310 cluster	intergenic	n/a	2R: 1647100 -16471924	925	yes	no	yes	no	n/a
miR-263a	intergenic	n/a	2L: 11952599 -11954372	1774	yes	no	no	no	n/a
miR-279	intergenic	n/a	3R: 25040758 -25042088	1331	yes	no	yes	no	n/a
miR-280	intergenic	n/a	2R: 4184860 -4186524	1665	yes	no	low	no	n/a
miR-281-1 cluster	intronic	*Oda*	2R: 8057551 -8058477	927	yes	no	yes	no	n/a
miR-287	intergenic	n/a	2L: 17574021 -17575419	1399	yes	no	no	no	n/a
miR-304 cluster	intronic	*Gmap*	X: 15401733 -15403640	1908	yes	no	low	no	n/a
miR-305 cluster	intergenic	n/a	2L: 7425191 -7426685	1495	yes	no	yes	no	n/a
miR-9c	intronic	*grp*	2L: 16697657 -16698096	440	yes	no	no	no	n/a
miR-306 cluster	intronic	*grp*	2L: 16698407 -16698892	486	yes	no	no	no	n/a
miR-307	intronic	*Mmp2*	2R: 5508113 -5509654	1542	yes	no	no	no	n/a
miR-308	intronic	*RpS23*	2R: 10103276 -10103406	131	yes	no	yes	no	n/a
miR-31a	intergenic	n/a	2R: 13670690 -13672263	1574	yes	no	yes	no	n/a
miR-31b	intergenic	n/a	X: 8886574 -8888275	1702	yes	no	no	no	n/a
miR-7	intronic	*bl*	2R: 16493354 -16493849	496	yes	no	yes	no	n/a
miR-8	intergenic	n/a	2R: 12718272 -12719620	1349	yes	no	yes	no	n/a

*Aravin et al., 2003; Ruby et al., 2007.

Table S2. Primer sequences

Primer sets	Primer name	Forward primers (5'>>3')	Reverse primers (5'>>3')
Probe	miR-1	TTTATTACGGGCAACCAACGACG	AGGGCTTACTGTGCTCCAACCG
	miR-10	TTGACTGCGTTTGTTTTATTTCC	GATTCGTCCTCCTTCCTTTGTTT
	miR-11	CAACCGATCATGCCTGCAACC	TAAATGTTCTGCAGTTCGGGGCT
	miR-309 cluster	CCGATCCTGGGATGCATCTTGTG	CCCAAATGTTCAAAGCTTGAG
	miR-9a	ATGGTACTGCGTGATAGGATTGA	CTTACTTTCCGAGTTTATGGTGTTT
	miR-92a	CAGCGATCAAATAAACAAATAC	TAAACAAGAAGCAAACTCACC
	miR-92b	TTGCTGATCGATGTTGTGGGACG	AGTGCGTCTCTTTTTCGGGCCTT
	miR-iab-4	GTCTTATGTGACAAGTGCTGGCTA	TCACCACCTCCTTCTCATCGTGCT
	miR-iab-4as	GTCTTATGTGACAAGTGCTGGCTA	TCACCACCTCCTTCTCATCGTGCT
	miR-965	TTTCGGTTTTGAGCGTATTT	CTTAGGGTGTAGAGGGGACG
	miR-13a cluster	ATCCTTATTCCTTCTCCGATTTTC	AGCGGCGGGAAGCACAGTTAGA
	miR-2a-1 cluster	TACTCCTTTCTTCACATCCAA	CTCTTTCAGTGTTGTTCTTCG
	miR-2b-1	GATTGTGGTTATGTAATGCGAGAC	ATGTTTGCGAGCCAAGTTCAGC
	miR-14	AGAAAAGAGGGAGACGGCATCA	CAGCAAAAGTCCCATGACAAAT
	miR-184	AAATATCATATCACCCTTGTAACCC	CAACATTGCCACTTGAAATCCAC
	miR-310 cluster	TTGTTGCACCAGTTGTACGGGAAT	ACAAAACTGGCATTCCCGTCTTACT
	miR-263a	TTGAAGCGTTTAGCGTTGGA	CCTCGGCGGAAATTACCAAC
	miR-279	ACAACAAGAGGGCAAGACATA	GAGGCGGAACTACAGAGGAAT
	miR-280	GTCGGGCGGAGGATGGTTAT	GGCTTAGGGTCTTCGGTGGG
	miR-281-1 cluster	TGAAAGGTGGGAAGGGATTAGA	CGCAGCCAGATAACCAGAAGAT
	miR-287	AGCCCTTTACCGCAACTTATA	ATTATCCTGTCATGGGTCCTG
	miR-304 cluster	AACCTATTGAAGCACCTCCG	CACATCCGTTTGGGTGAAGC
	miR-305 cluster	CAAGGGTCTGCCGAACGAAACG	GCATGGGAATGAGTGCGGTGAG

Probe	miR-9c	CGGTGCGAGATTGTTTTACATGTG	TTTGCAAGCACAGACGAAGAATTACA
	miR-306 cluster	CACTCGATGGCTCAGGTACTTAG	TGAGAACAAGTAACGCGAGAAAAG
	miR-307	CATCAGACAGACGACCGACAC	ATCCAGCTCATTCCCTACTCC
	miR-308	CGGATGAGATGGCGGGGTTAA	AGTCGCTTTTCGCTGCTGGCA
	miR-31a	TGGGTTTGCAGCAATTAAGGC	TTCCGAAACGACAACGACTGG
	miR-31b	TTGAGACGATGAACGCACTG	CGCTAAATACTTGTCGCAATAAAC
	miR-7	GAGGTTGGTGTCGCAATCTGAATA	ATGTGGATGTAGTGGGATGGGAGG
	miR-8	GGCGTTATTGTGCATGTTTCC	ACCTGCTGTCTTGGCTAGTTG
	fog-exon3	CCTGCTGATTCCGTACTTGCT	ATTGTCTGCTGGCTGTTCTGG
	fog-intron1	CGGAGAACTATGAGTAGGGACG	GAAGTGATTGCTGTGAGGGTG
Enhancers tested	miR-1-0.6kb enhancer	GAAGATCTGTCATATGATTGGAGGAAGACTC	TTGGCGCGCCATCTTCGAGTGCAGTGTGCATCTACGA
	miR-1-0.6kb TAG m1	CATAGTTCTGAACGCTCGAGAACTGACATTATTCCG	CGGAATAATGTCAGTTCTCGAGCGTTCAGAACTATG
	miR-1-0.6kb TAG m2	CTTAGTGAGCAAGTCGACAGCCGAGTGTGCTGGCT	AGCCAGCACACTCGGCTGTCGACTTGCTCACTAAG
	miR-9a enhancer I (9a-I)	CGGGATCCTTTGATTACGACGAGGGTCAGG	TTGGCGCGCCAGACGTGGAATGGGAACAAGAT
	miR-9a enhancer II (9a-II)	CGGGATCCTTATAGAGGAGCAAGATACCCG	TTGGCGCGCCATATGAGATGAGGCAGATGACG
	9a-IIm1	CCTCTAGATGTCTTATATGTGTGCCTCTCATTTG	ACATCTAGAGGGTAGGAGACAAGTACAAAGC
	9a-IIm2	TCACTAGTCAACGCTGCCGGCGTCGCAGTCGGCGCTACATATGAG	TTGACTAGTGATTTTCGGGTTTTTGTTTTTGCC
	miR-10 enhancer (10-en)	GAAGATCTTCATGTCAACCCCATCGCAGCA	TTGGCGCGCCAAGCCGGTAATGTTCTCCCTCA
	DNA fragment within miR-10	GATAGATCTGCCCATGGGCGAGG	TTGGCGCGCCTTCCGCTCATTGAACCAGTA
	miR-iab-4 enhancer (iab-en)	GAAGATCTCTATGCTGCTTCCCAAGTGCCA	TTGGCGCGCCTGCAATTCCAGCGAAGGACAAC
	DNA fragment upstream miR-iab-4	GAAGATCTGAAGACTGGCAGACAAATAAACA	TTGGCGCGCCAATGGTATTGGGATTCAAGGAC
	DNA fragment downstream miR-iab-4	GAAGATCTGACCGTGGGACCAGATGGATG	TTGGCGCGCCTTAAACCTCGGCGGGCCTAAA
	miR-11 enhancer (11-en)	CGGGATCCTGCTGGAACATACCATCGA	TTGGCGCGCCCATTTGGGCAGTGTCCTTT
	DNA fragment near miR-92a	CGGGATCCTCAGAAACTCCCACTCCTC	TTGGCGCGCCATTGGACTTCGGACAGGTA

Table S3. Analysis of the 774 upregulated genes in 2-3 h zelda mutants.