

Supplementary data

Structure, evolution and function of the bi-directionally transcribed *iab-4* microRNA locus in insects

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Table S4. Statistical tests to analyse whether the overlapping of mir-307a and mir-307b binding sites is higher than expected by chance (for details, see Materials and Methods in the main text).

Figure S1. Arm-specific miRNA processing in *Drosophila* S2 cells. *Tribolium* iab4/iab-8 constructs were transfected into S2 cells and expressed under the control of the actin promoter. LNA miRNA real-time PCR assays were used to estimate 5' and 3' miRNA arm levels.

Figure S2. Expression patterns and iab-4/8 targeted sites of A-C) tca-Cx/Scr, D-F) tca-ptl/Antp, and G-H) tca-AbdB. Note that the expression domains of these genes follow the rules of spatial and temporal colinearity according their positions within the intact *Tribolium* Hox cluster. I) Relative luciferase activity of the other Hox 3'UTRs and mutagenated 3'UTRs (M) repressed by *Tribolium*/and *Drosophila* iab-4 and iab-8. Note the presence of repression is indicated in negative value.

Figure S3. Putative 3'UTRs of *Tribolium castaneum* HOX genes.

Table S1. Primers sequences for making the different constructs.

Target (vector)	Primers*
tca-iab-4 (pAC5.1)	FP-KpnI; 5' -TTTGGGTACCCATACTGCTCCCGAGGGAACGAGGGATTACTTTTAT-3' RP-EcoRI; 5' -CAGGGAATTCATTGTAAAAAGCGTCAAGACTTAGCTGGAGT-3'
tca-iab-8 (pAC5.1)	FP-KpnI; 5' -TTTGGGTACCCGTTGAGTGTGTTTTACATCGATTAAGTAT-3' RP-EcoRI; 5' -CAGGGAATTCATACTGCTCCCGAGGGAACGAGGGATTACTTTTAT-3'
tca-Ubx-3'UTR (psicheck2)	FP-XhoI; 5' -CCCCTCGAGGGATTACTTCGGTGTGTTGGCGGATCAGCATAA-3' RP-NotI; 5' -GGGGCGGCCGCCGAAAAACCATTAGCCACGTTACTCCCAA-3'
tca-abda-3'UTR (psicheck2)	FP-XhoI; 5' -TCCTCGAGCGAGTGACTTGACCTTTTGTGTTTATAC-3' RP-NotI; 5' -TAAGCGGCCGCTGGCGTATACGTATTCATTTTCTTTAC-3'
tca-Scr-3'UTR (psicheck2)	FP-XhoI; 5' -ACCTCGAGGACGTGTTTATGTGATTCATTAGA-3' RP-NotI; 5' -AAGCGCCGCTAAATTGTACGTCTTTCACACACAC-3'
tca-abdb-3'UTR (psicheck2)	FP-XhoI; 5' -ACCTCGAGCAGTGCCAAAGAGTTATTTAGGAAA-3' RP-NotI; 5' -AAGCGCCGCGTTAAAGACCGGTTAACTATTCTGT-3'
tca-Antp/ptl-3'UTR (psicheck2)	FP-XhoI; 5' -GTTCCCTCGAGACTCCTCGTCACGATAACCATGTACCTAATT-3' RP-NotI; 5' -TAAGCGGCCGCGGTAATTTGTCCGAAATTAAGTGTCCGCCAAACT-3'
dme-iab4-S (pAC5.1)	FP-KpnI; 5' -TTGGTACCCATCTTTACATCTGGATATGGTTTCATCT-3' RP-EcoRI; 5' -TGGAATTCAAGTGAAGACCATATAACAAAGTGTACGT-3'
dme-iab4-AS (iab-8) (pAC5.1)	FP-EcoRI; 5' -TTGGAATTCATCTTTACATCTGGATATGGTTTCATCT-3' RP-KpnI; 5' -TTGGTACCAAGTGAAGACCATATAACAAAGTGTACGT-3'
dme-Ubx-3'UTR (psicheck2)	FP-XhoI; 5' -ACCCTCGAGGTTGAATGCGAGCAAAATCTGTTGATA-3' RP-NotI; 5' -ATTTAAAAGCGGCCGCTTCACTCTAGTCGTTTGGGCGAGAA-3'
dme-abda-3'UTR (psicheck2)	FP-XhoI; 5' -TCCTCGAGGATAGCAAGCAGAAGATAAACTCGAT-3' RP-NotI; 5' -TTGCGCCGCAACAGAAGTTTTAGCAGTAGGGTTCA-3'
dme-Antp-3'UTR (psicheck2)	FP-XhoI; 5' -TGTCCTCGAGGATCGACGGAGTCTACCCACTTAAATGAA-3' RP-NotI; 5' -ATAGCGGCCGCTTCCGCTTTGCAGCCCTTCACTGCATTCT-3'
iab-4-5p perfect complementary (psicheck2)	FP; 5' -GGTTCCCTCGAGTCAGGATACATTCAAGTATACGTCCCGGGAATTCGTTTAAACC -3' RP; 5' -TATACGGTCCCATGGAGC -3'
iab-4-3p perfect complementary (psicheck2)	FP; 5' -GGTTCCCTCGAGTTACGTATACTGAAGGTATACCGCCCGGAATTCGTTTAAACC-3' RP; 5' -TATACGGTCCCATGGAGC-3'
iab-8-5p perfect complementary (psicheck2)	FP; 5' -GGTTCCCTCGAGGTTATACCTTCAAGTATACGTAACCCGGAATTCGTTTAAACC-3' RP; 5' -TATACGGTCCCATGGAGC-3'
iab-8-3p perfect complementary (psicheck2)	FP; 5' -GGTTCCCTCGAGTAAACGTATACTGAATGTATCCCCGGAATTCGTTTAAACC-3' RP; 5' -TATACGGTCCCATGGAGC-3'

* FP: forward primer; RP: reverse primer. Added restriction site is also indicated.

Table S2. Primers used in mutagenesis of 3'UTRs.

Target	Primers (changes are shown in bold)
tca-antp/pt1-3'UTR	tca-antp-M-FP; 5'-CGTAACGCTGCTACAAATCGAGTAT CGC TAACTAATTATTTTAAT-3' tca-antp-M-RP; ATTAAAATAATTAGTTAG CGG ATACTCGATTGTAGCAGCGTTACG-3'
tca-Ubx-3'UTR	tca-ubx-M1; 5'-GCGATCGCAACTATATA ACCA ATTGGGTTTATGTGCC-3' tca-ubx-MC; 5'-GGCACATAAACCCAATT GGT TATATAGTTGCGATCGC-3' tca-ubx-M2; 5'-GGAAACTTAACGATATA ACCA AATTATAATCCTAGTA-3' tca-ubx-M2C; 5'-TACTAGGATTATAATTT GGT TATATCGTTAAGTTTCC-3' tca-ubx-M3; 5'-GTACAAGAAATGTAAT ACCA AATGTACTAATTATA-3' tca-ubx-M3C; 5'-TATAATTAGTACATTT GGT TATACATTTCTGTAC-3'
tca-abd-A-3'UTR	tca-abdA-M1; 5'-CTCTTAGTACTCTTAT CGC TAATTTGGTGTGTCAGGGTAG-3' tca-abdA-M1C; 5'-CTACCC TGACACCAAATTA GCG GATAAGAGTACTAAGAG-3' tca-abdA-M2; 5'-GCCTTCTCCACATTTAGAT CGC TAAAATTGTACAATATTTTC-3' tca-abdA-M2C; 5'-GAAAATATTGTACAATTTTA GCG ATCTAAATGTGGAGAAGGC-3' tca-abdA-M3; 5'-GTAAAGAAAATGAAT CGC TAT CGC CCAGCGCCGCTGGCCGC-3' tca-abdA-M3C; 5'-GCGGCCAGCGCCGCTGG GCG ATAG GCG ATTCATTTTCTTTAC-3'

Table S3. Target overlap between iab-4 and iab-8. P-values for randomization tests. See main text for details. RAND and SIMUL account for two different ways of calculating the null distribution as described in Marco et al. (2012) *Silence* 3:8

Canonical seeds – RAND

	iab-4 5'	iab-4 3'	iab-8 5'	iab-8 3'
iab-4 5'	*	*	*	*
iab-4 3'	0.067	*	*	*
iab-8 5'	0.014	0.248	*	*
iab-8 3'	0.273	0.159	0.456	*

Canonical seeds – SIMUL

	iab-4 5'	iab-4 3'	iab-8 5'	iab-8 3'
iab-4 5'	*	*	*	*
iab-4 3'	0.089	*	*	*
iab-8 5'	0.030	0.305	*	*
iab-8 3'	0.396	0.220	0.689	*

miRanda – RAND

	iab-4 5'	iab-4 3'	iab-8 5'	iab-8 3'
iab-4 5'	*	*	*	*
iab-4 3'	0.694	*	*	*
iab-8 5'	0.553	0.823	*	*
iab-8 3'	0.932	0.932	0.907	*

miRanda – SIMUL

	iab-4 5'	iab-4 3'	iab-8 5'	iab-8 3'
iab-4 5'	*	*	*	*
iab-4 3'	0.719	*	*	*
iab-8 5'	0.630	0.794	*	*
iab-8 3'	0.899	0.901	0.880	*

Table S4. Target overlap between mir-307a and mir-307b. P-values for randomization tests. See main text for details. RAND and SIMUL account for two different ways of calculating the null distribution as described in Marco et al. (2012) *Silence* 3:8

Canonical seeds – RAND

	mir-307a 5'	mir-307a 3'	mir-307b 5'	mir-307b 3'
mir-307a 5'	*	*	*	*
mir-307a 3'	0.294	*	*	*
mir-307b 5'	0.060	0.344	*	*
mir-307b 3'	0.710	0.928	0.629	*

Canonical seeds – SIMUL

	mir-307a 5'	mir-307a 3'	mir-307b 5'	mir-307b 3'
mir-307a 5'	*	*	*	*
mir-307a 3'	0.261	*	*	*
mir-307b 5'	0.030	0.261	*	*
mir-307b 3'	0.648	0.891	0.535	*

miRanda – RAND

	mir-307a 5'	mir-307a 3'	mir-307b 5'	mir-307b 3'
mir-307a 5'	*	*	*	*
mir-307a 3'	0.253	*	*	*
mir-307b 5'	0.246	0.293	*	*
mir-307b 3'	0.184	0.185	0.309	*

miRanda – SIMUL

	mir-307a 5'	mir-307a 3'	mir-307b 5'	mir-307b 3'
mir-307a 5'	*	*	*	*
mir-307a 3'	0.309	*	*	*
mir-307b 5'	0.308	0.380	*	*
mir-307b 3'	0.263	0.268	0.387	*

Figure S1.

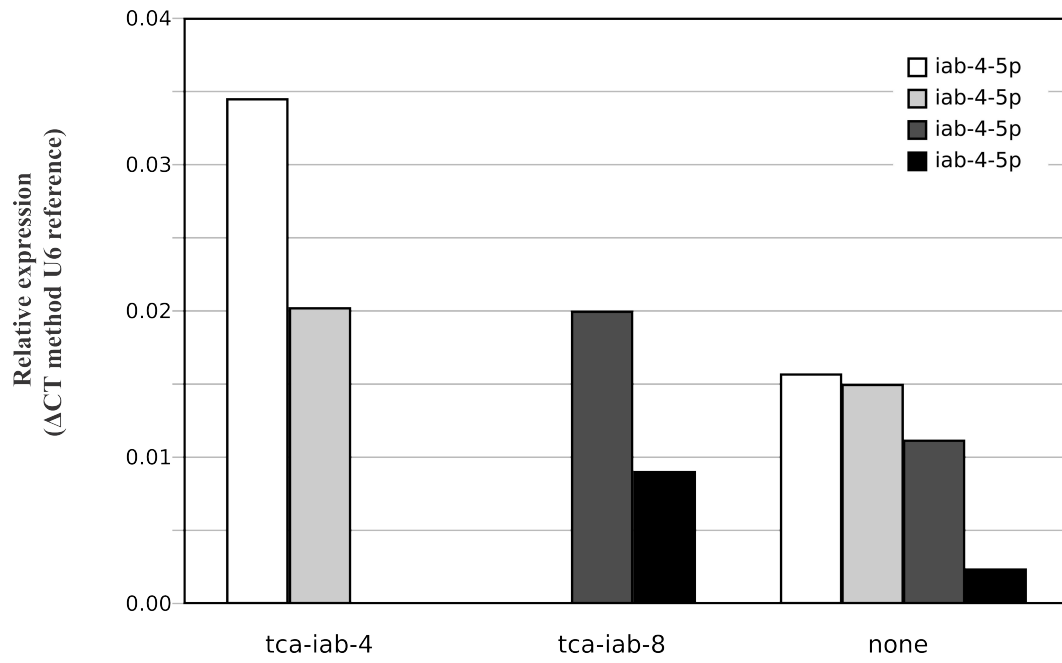
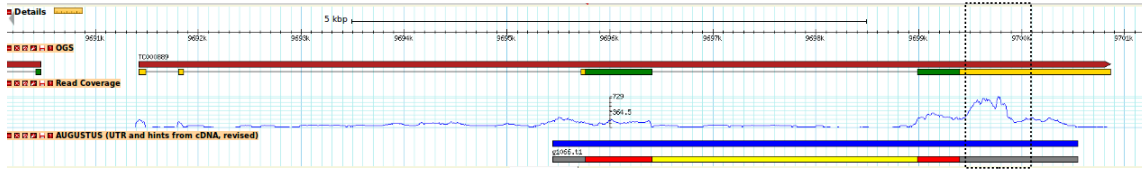
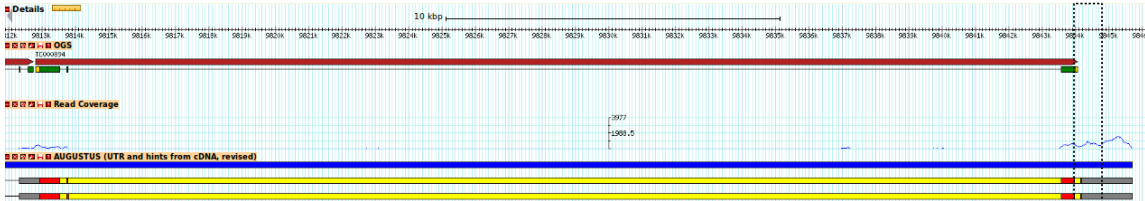


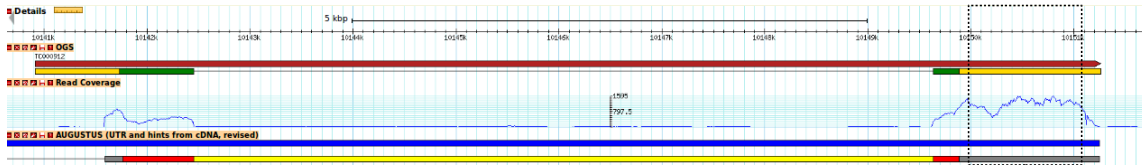
Figure S3.
Abd-B



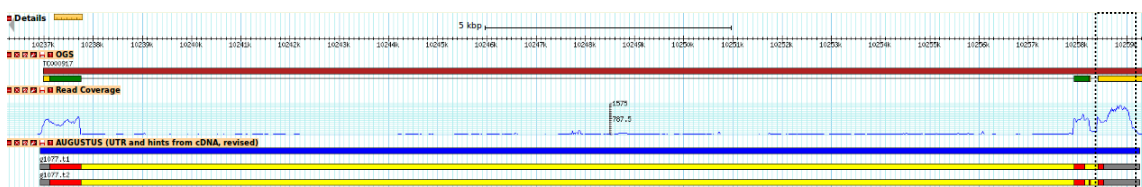
abd-A



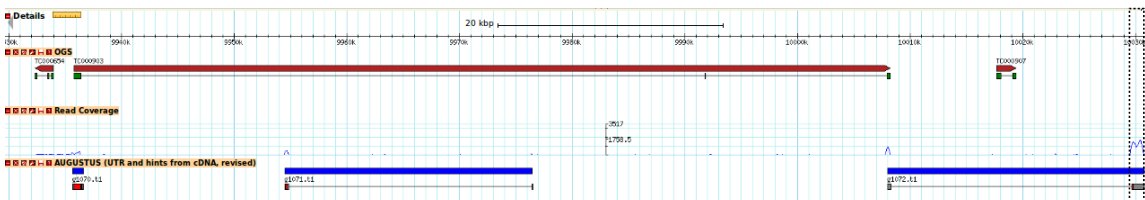
Ptl/Antp



Scr



Ubx



Ubx 3'UTR

