

Figure legends

Figure S1. *wg* expression patterns in imaginal discs are conserved between two *Drosophila* species. *In situ* hybridizations with third instar larval imaginal discs. (A) *D. melanogaster* wing disc. (B) *D. melanogaster* eye-antennal disc. (C) *D. melanogaster* leg disc. (D) *D. guttifera* wing disc. (E) *D. guttifera* eye-antennal disc. (F) *D. guttifera* leg disc. All discs are oriented with anterior to the left and dorsal on top.

Figure S2. *wg* expression in longitudinal vein tips and campaniform sensilla of the pupal wing is unique to *D. guttifera*. *In situ* hybridization for the *wg* gene is shown in pupal wings of various species. (A) *Drosophila melanogaster*. (B) *D. guttifera*. (C) *D. deflecta*. (D) *D. nigromaculata*. (E) *D. palustris*. (F) *D. quinaria*. *D. melanogaster* belongs to *melanogaster* species-group of the subgenus *Sophophora*, while the other species belong to the *quinaria* species group of the subgenus *Drosophila*. All probes are species-specific.

Figure S3. Map of the Wnt region of *D. melanogaster* and *D. guttifera*. Vertical bars connected with black lines indicate sequences of 40bp or longer with 100% sequence conservation between species. Red lines indicate conserved but inverted sequences. Numbered horizontal solid bars indicate DNA fragments tested by transgenic reporter assays. *D. melanogaster* fragments were tested in transgenic *D. melanogaster* using phiC31 integration. *D. guttifera* fragments were tested in transgenic *D. guttifera* using the *piggyBac* transposon.

Figure S4. The novel activity of the *D. guttifera* gutCV-T enhancer arose within a conserved enhancer. Sequence alignment of the gutCV-T enhancer and the orthologous defCV enhancer (Serial Cloner v2.6.1, local alignment, and word size=15 were used). Mismatches are marked with a #. Red bars indicate identical sequence matches >10bp with the melCV-core enhancer from *D. melanogaster*. The extent of collinear sequence conservation (with no significant rearrangements) between the two species indicates that a small change or an accumulation of small changes is responsible for the new enhancer activity in the vein tips.

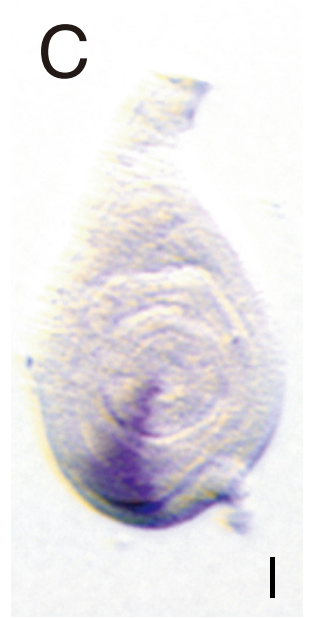
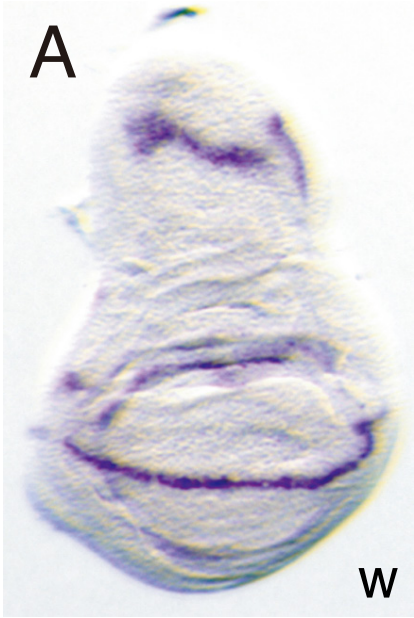
Figure S5. Expression patterns of four *Wnt* genes in pupal wings of *D. guttifera* visualised by *in situ* hybridizations. (A) *Wnt4*. (B) *wingless*. (C) *Wnt6*. (D) *Wnt10*.

Figure S6. *wingless* is the predominant *Wnt* gene expressed in the pupal thorax. *Wnt* genes expressed in the pupal thorax (top) and embryo (middle) were detected by RT-PCR and reaction products profiled by gel electrophoresis. Control reactions from genomic DNA are shown at the bottom

Figure S7. *Cis*- and *trans*-regulatory changes are responsible for the novel campaniform sensillum expression of *wg* in *D. guttifera*. (A) In the *D. melanogaster* pupal wing, the melCS enhancer shows no restricted expression (EGFP, green). (B) In the *D. guttifera* pupal wing, the melCS enhancer shows no expression (DsRed, magenta). (C) *D. melanogaster* pupal wing, the gutCS enhancer shows no restricted expression (EGFP, green). (E): *D. guttifera* pupal wing, the gutCS enhancer drives reporter expression in the campaniform sensillum (DsRed, magenta).

Figure S8. *Cis*- and *trans*-regulatory changes are responsible for the striped expression of *wg* in the pupal thorax of *D. guttifera*. (A) *D. melanogaster* pupal thorax, the melTS fragment shows no stripe expression (EGFP, green). (B) *D. guttifera* pupal thorax, the melTS fragment shows no stripe expression (DsRed, Magenta). (C) *D. melanogaster* pupal thorax, the guts enhancer is expressed in incomplete stripes (EGFP, green). (D) *D. guttifera* pupal thorax, the gutTS enhancer drives full stripe expression which corresponds to the adult pigmentation pattern (DsRed, magenta).

D. melanogaster



D. guttifer

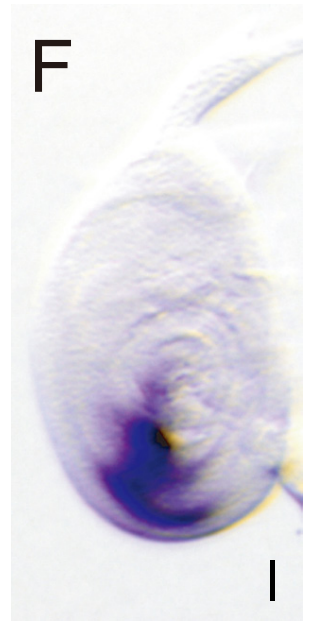
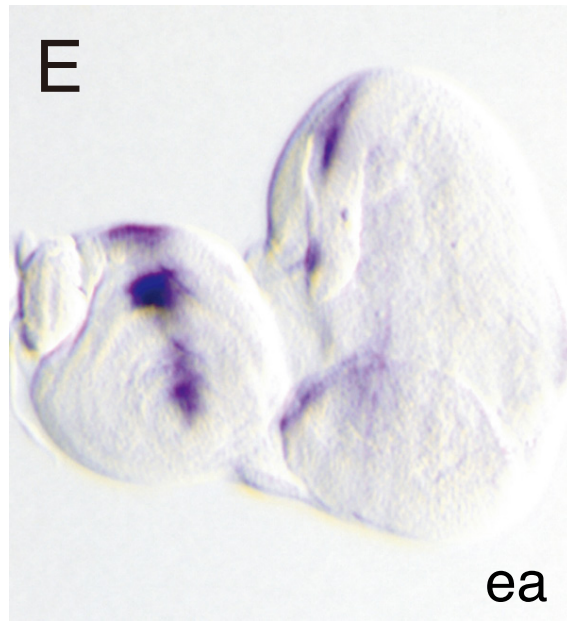


Figure. S1

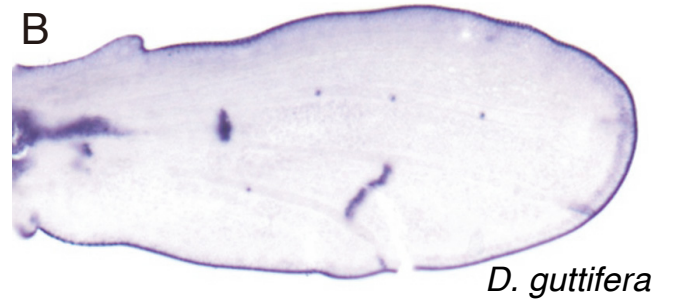


Figure. S2

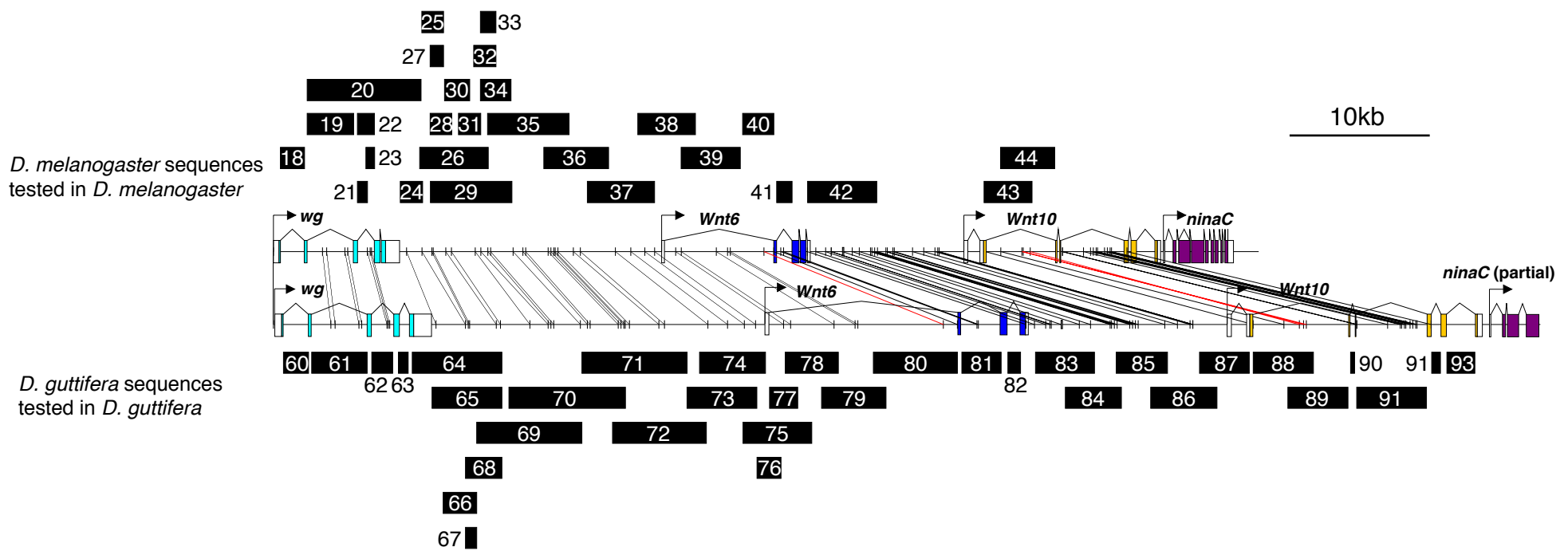
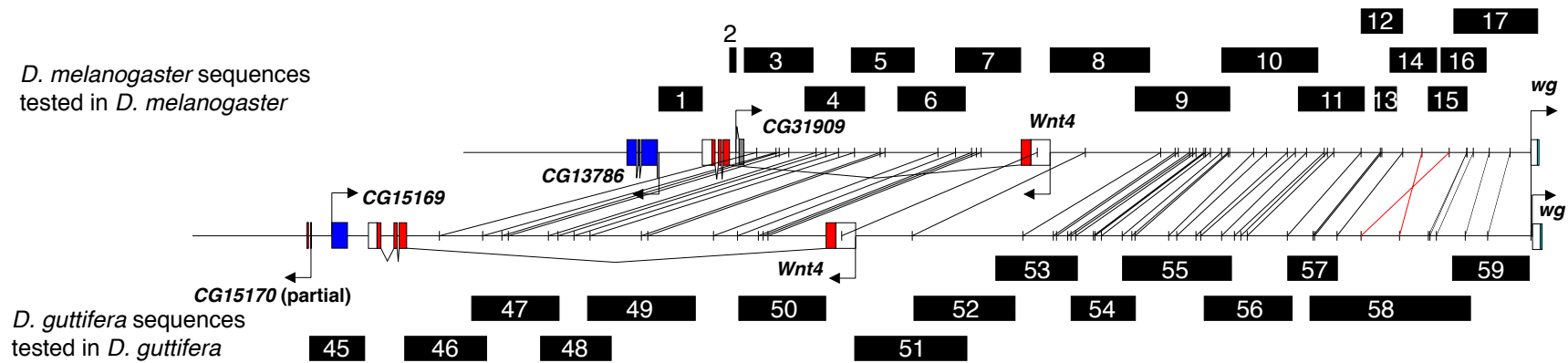


Figure. S3

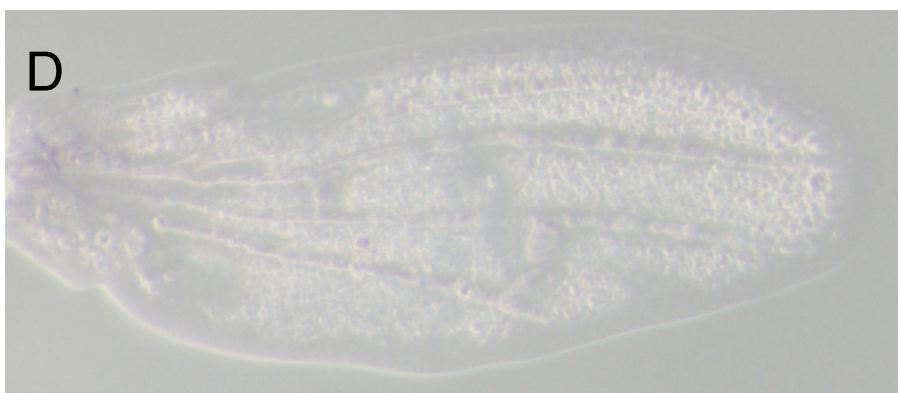
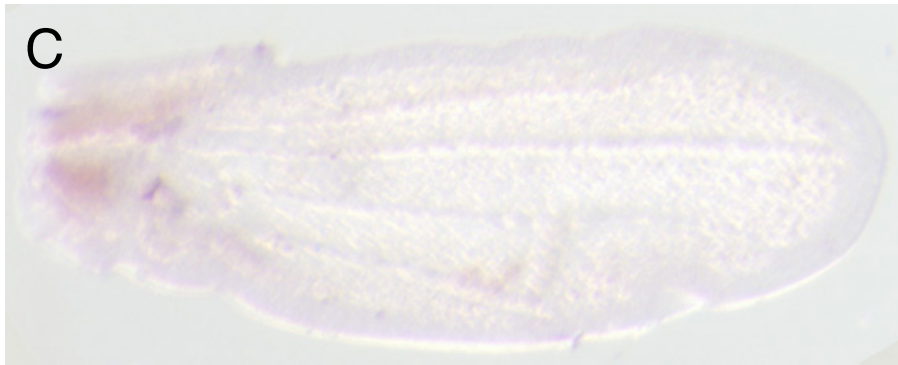
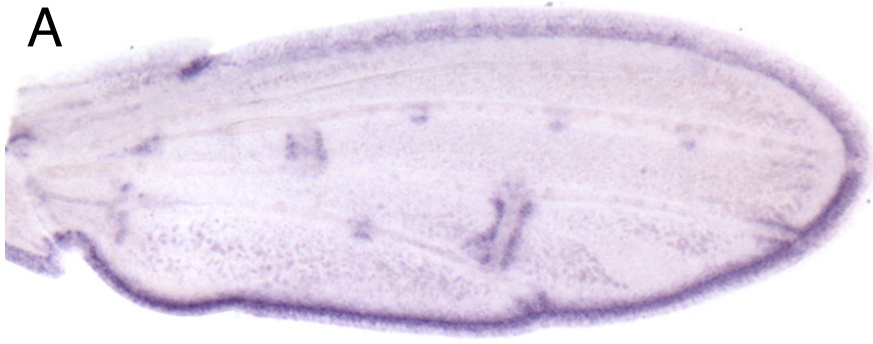


Figure. S5

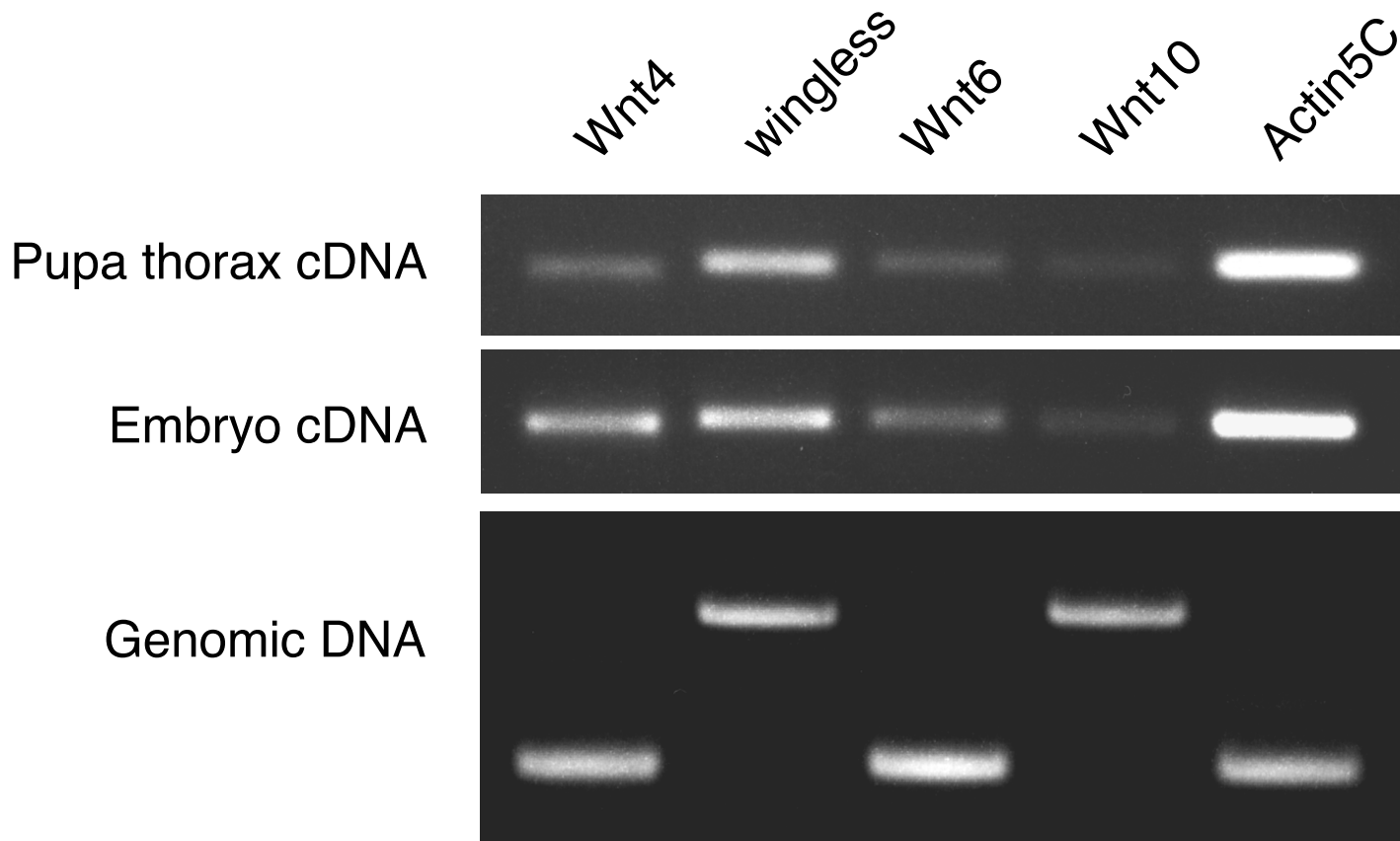


Figure. S6

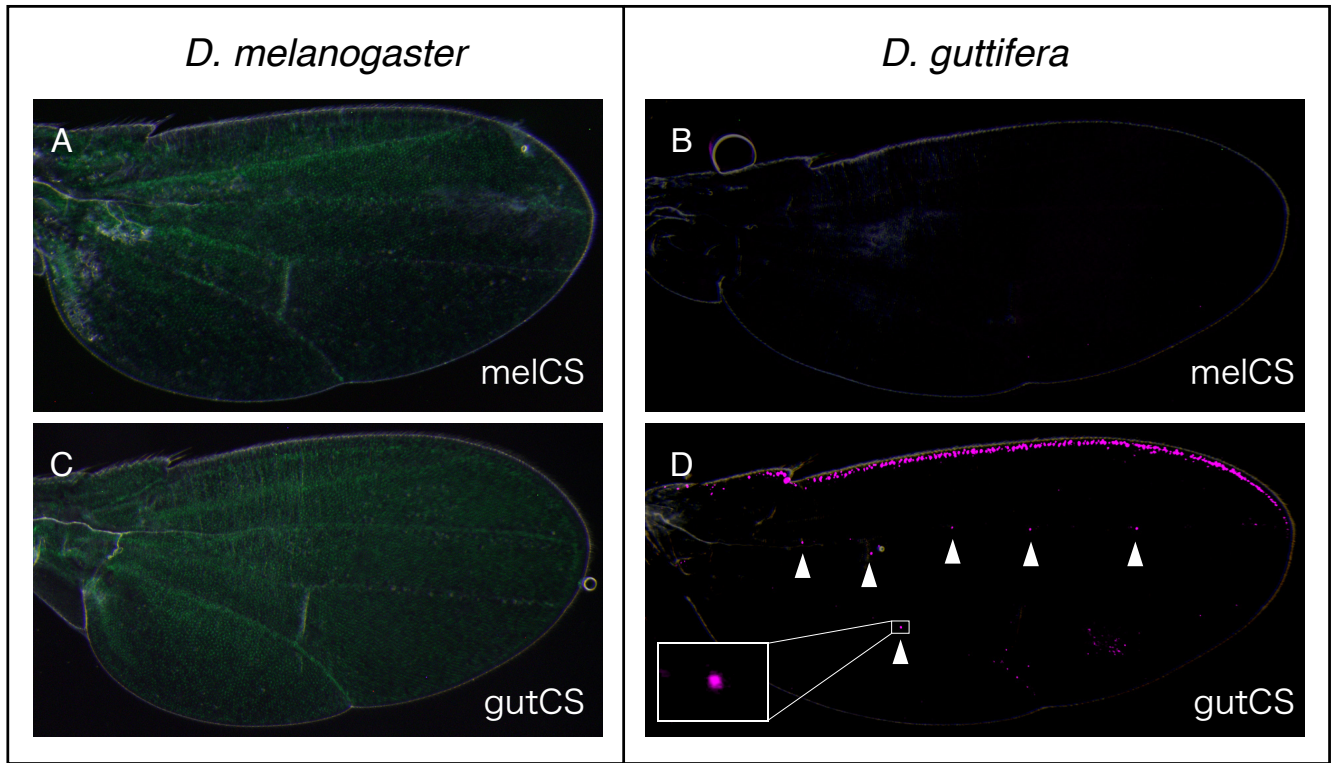


Figure. S7

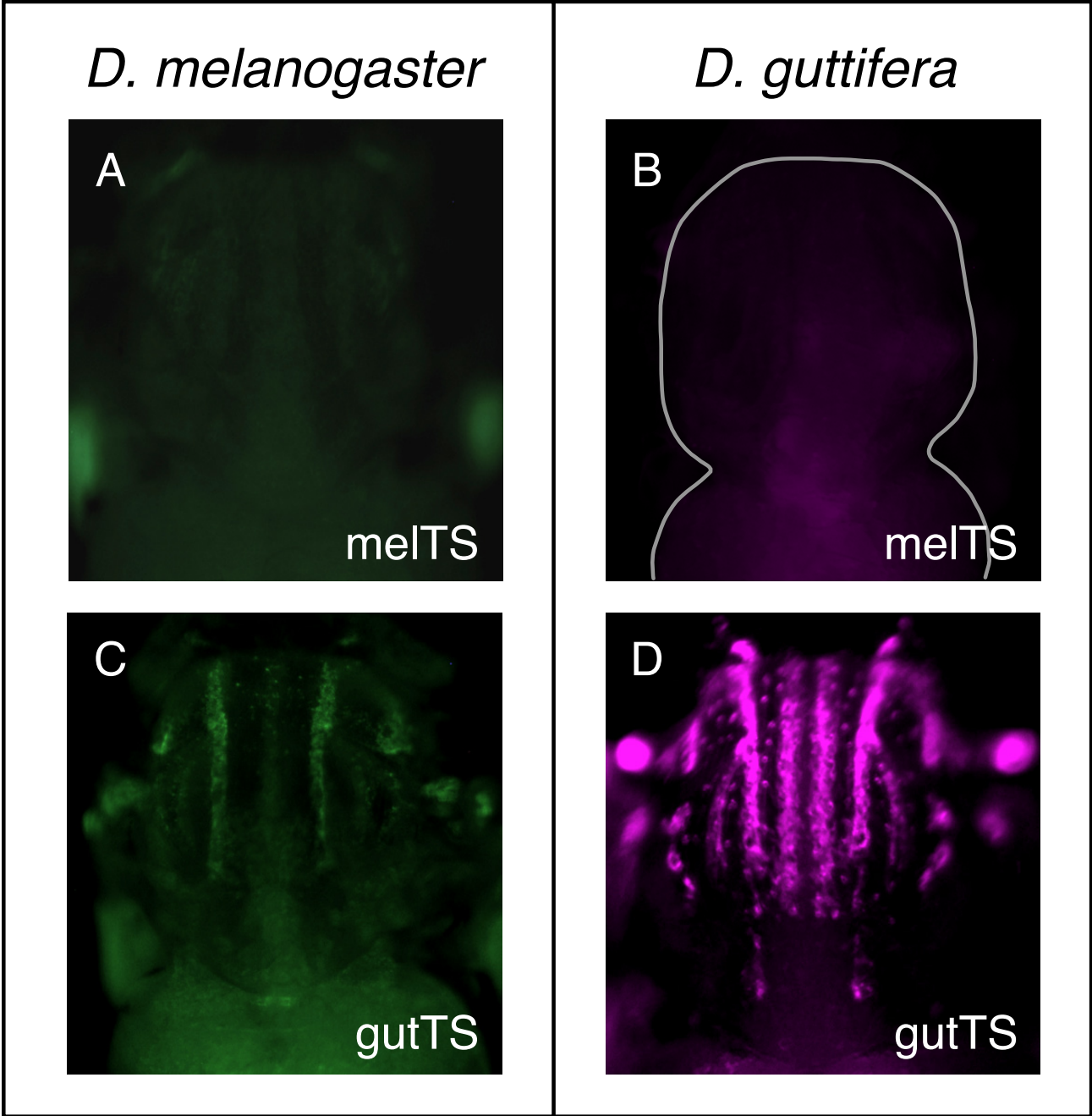


Figure. S8

Table S1. Primers used in the study.

Primers for enhancer screening (Figure S3)	Restriction site	Fragment in FigS3	Host species	Template specie	Cloning ststem
TCATGGCGCGCGAAATGTTGCCCAACCCGA	AscI	1	melanogaster	melanogaster	S3aG
ATCACCTGCAGGCAAAACTGAAATGAAATATAAAGTTTCAC	SbfI	1	melanogaster	melanogaster	S3aG
TCATGGCGCGCCTGGCAGACATAAAGTATTCGAAATT	AscI	2	melanogaster	melanogaster	S3aG
ATCACCTGCAGGAGTTTTCTGACTGTAGGGTCTATT	SbfI	2	melanogaster	melanogaster	S3aG
TCATGGCGCGCGACAGGTGGGATTTGAAAAATGTA	AscI	3	melanogaster	melanogaster	S3aG
ATCACCTGCAGGCAAAAGGATGCGCTTTTTATGATGAA	SbfI	3	melanogaster	melanogaster	S3aG
TCATGGCGCGCCGAAAAACCGCAAGACAAAACGT	AscI	4	melanogaster	melanogaster	S3aG
AACACCTGCAGCGTGGCAGCATTGATTTGTTTAGA	SbfI	4	melanogaster	melanogaster	S3aG
TCATGGCGCGCGGAAACGCAATCAACATTAAGT	AscI	5	melanogaster	melanogaster	S3aG
AACGCTGCAGGGGTATTTAATTCAATTTGTGGCT	SbfI	5	melanogaster	melanogaster	S3aG
ACTAGCGCGCGTTTGACATTAACGCGTGTAAATTC	AscI	6	melanogaster	melanogaster	S3aG
AACGCTGCAGGCATCGCCATGTGGACCA	SbfI	6	melanogaster	melanogaster	S3aG
ACTTGGCGCGCTCATAAAACGCGCCATCAAAAACGACA	AscI	7	melanogaster	melanogaster	S3aG
AACGCTGCAGGAGTCAATCTAGAGCTACTCCCAT	SbfI	7	melanogaster	melanogaster	S3aG
ACTTGGCGCGCATCTGAATCATGACAGCCGA	AscI	8	melanogaster	melanogaster	S3aG
AACGCTGCAGGCAAAACATCACGTAACAATTGAGCA	SbfI	8	melanogaster	melanogaster	S3aG
ACTAGCGCGCCAGCGTGGCTAATTAGCACAA	AscI	9	melanogaster	melanogaster	S3aG
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AACGCTGCAGGGAATTCCTCGATCTTCGAGATACTC	SbfI	13	melanogaster	melanogaster	S3aG
TCATGGCGCGCAAAACTGTGAAATGCTGAAAGAACG	AscI	14	melanogaster	melanogaster	S3aG
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TCAACCGGGGCTTTGATTAAGCGCGCTTTA	SacII	76	guttifera	guttifera	pBac
AACGGCTAGCGTTTGAATGGTTTTTTGGCATTGAA	NheI	76	guttifera	guttifera	pBac
TCAACCGGGGAGTTCACAGTTAAAGTTGCGAGC	SacII	77	guttifera	guttifera	pBac
ATCGGCTAGCCCTTGAACGGCACAGATGAAG	NheI	77	guttifera	guttifera	pBac
TCAACCGGGGACCTTCAAAATGTGACGTTTTGATTTA	SacII	78	guttifera	guttifera	pBac
AACGGCTAGCCCGGACGTATCTTTATTTTGC	NheI	78	guttifera	guttifera	pBac
ATATCTCGAGTGGCCAATTTGCCAAAATTAATGCA	XhoI	79	guttifera	guttifera	pBac
TCAACCGGGGAGATTATGACAACCTAATAGCTACAGA	SacII	79	guttifera	guttifera	pBac
TCAACCGGGTCCATTGAGGCTATAACGACA	SacII	80	guttifera	guttifera	pBac
AACGGCTAGCAGAATATAATTGTAAGAATAAGACTTTTAGA	NheI	80	guttifera	guttifera	pBac
TCAACCGGGAAAGTGAACCTAAATATGTAACACTACAAA	SacII	81	guttifera	guttifera	pBac
AACGGCTAGCGTGGGAGGCGAAGGATAAC	NheI	81	guttifera	guttifera	pBac
ATATCTCGAGAAGTAGGCAAGAAAGAAATCCT	XhoI	82	guttifera	guttifera	pBac
AACGGCTAGCGGAAATGAGCAAAAAGAAATGCTT	NheI	82	guttifera	guttifera	pBac
TCAACCGGGATGATTAAGCGTAATTTAATGAAGACAACA	SacII	83	guttifera	guttifera	pBac
AACGGCTAGCCAATTAACAATTCATTATCTTAATTTGTCTG	NheI	83	guttifera	guttifera	pBac
TCAACCGGGTGACTTTCCATAAATTAACACAATTTTATTGT	SacII	84	guttifera	guttifera	pBac
AACGGCTAGCGACTGCATTAATAAATCAACTTAATTTCA	NheI	84	guttifera	guttifera	pBac
TCAACCGGGGTTAATAAAAAACACATAATGCGTATGTT	SacII	85	guttifera	guttifera	pBac
AACGGCTAGCGTTTACAACACAGCAGCGCA	NheI	85	guttifera	guttifera	pBac
TCAACCGGGAGCGCCACATCAACGTCATAA	SacII	86	guttifera	guttifera	pBac
AACGGCTAGCACACTTGTACACTTCAAAGGACTT	NheI	86	guttifera	guttifera	pBac
TCAACCGGGGTGATGGTGATTTCATGGCAATTTG	SacII	87	guttifera	guttifera	pBac
AACGGCTAGCAACACTTTTGTAGAACACATGAAGAAAT	NheI	87	guttifera	guttifera	pBac
TCAACCGGGAAAGTGAAGCGAGCGTATCTTATA	SacII	88	guttifera	guttifera	pBac
AACGGCTAGCTGCCTAAGTGGCTTAGATAGCTAA	NheI	88	guttifera	guttifera	pBac
TCAACCGGGGCTAGTAGTTGCAGCTTGTTA	SacII	89	guttifera	guttifera	pBac
AACGGCTAGCGCATATAAATAGACAGTTGAATTTATTA	NheI	89	guttifera	guttifera	pBac
TCAACCGGGAAATAAATCTGCTTCTAATGCGAAAATG	SacII	90	guttifera	guttifera	pBac
AACGGCTAGCACATCAGCATAAAAAAGCATAAATAAAGAAAG	NheI	90	guttifera	guttifera	pBac
AACGGGTACCGAGTCCCGAGGTTCCAC	KpnI	91	guttifera	guttifera	pBac
AACGGCTAGCTTACAGGAGGTAGGAAGAATGAGAA	NheI	91	guttifera	guttifera	pBac
TCAACCGGGAAAGCGAAGAAAATACTAGTTTCAATTACA	SacII	92	guttifera	guttifera	pBac
AACGGCTAGCAGAAGTCGAAATGGGCAAGATATTAG	NheI	92	guttifera	guttifera	pBac
TCAACCGGGGAGTGGGAGTTTACGAATTTGAATGT	SacII	93	guttifera	guttifera	pBac
AACGGCTAGCAAAAATTTAACAAAAGAAATGTGGGGTTAGATA	NheI	93	guttifera	guttifera	pBac

Primers for cis/trans test (Figure 3, 4, 5, S7, S8)

	Fragment name	Host species	Template specie	Cloning system
TCAACCGGGAAAAAAGTGATCGTGCTACATGTGT	gutCV-T	guttifera	guttifera	pBac

AACGGCTAGCAGCACAAATGGCCACTTAATTAACA	NheI	gutCV-T	guttifera	guttifera	pBac
AACGGCTGCAGGAAAAAAGTATCGTGCTACATGTGT	SbfI	melCV	melanogaster	melanogaster	S3aG
ACTAGGCGCGCCCTCAGCGCTCGAAACAATTGC	AscI	melCV	melanogaster	melanogaster	S3aG
AACGGCTGCAGGTAATAAAGTATCGTGCTACATGTGT	SbfI	gutCV-T	melanogaster	guttifera	S3aG
TCTTGGCGCGCCCTCGAGCACAAACAATTGCCACT	AscI	gutCV-T	melanogaster	guttifera	S3aG
TCAACCGCGGATAAAAAGTATCGTGCTACATGTGT	SacII	defCV	guttifera	deflecta	pBac
ATATCTCGAGGACATTGCTCCTAATCAATAAACTAA	XhoI	defCV	guttifera	deflecta	pBac
TCTGGCTAGCTGCCAATTTATCGATCAACACGCT	NheI	gutME	guttifera	guttifera	pBac
ATATCTCGAGGACATTGCTCCTAATCAATAAACTAA	XhoI	gutME	guttifera	guttifera	pBac
AACGGCTGCAGGAAGTATTTAGTTTCAATTTGTTGCTTTG	SbfI	melME	melanogaster	melanogaster	S3aG
ACTTggcgccGCGAATGAAGAGCTTTCGTGA	AscI	melME	melanogaster	melanogaster	S3aG
AACGGCTGCAGGAAAAAAGTATCGTGCTACATGTGT	SbfI	gutCVT5	melanogaster	guttifera	S3aG
TCTTGGCGCGCAGACCGCGACGATGCGAT	AscI	gutCVT5	melanogaster	guttifera	S3aG
AACGGCTGCAGGACATTGCTCCTAATCAATAAACTAA	SbfI	gutCVT-core	melanogaster	guttifera	S3aG
TCTTGGCGCGCCCTCGAGCACAAACAATTGCCACT	AscI	gutCVT-core	melanogaster	guttifera	S3aG
TCAACCGCGGGACATTGCTCCTAATCAATAAACTAA	SacII	gutCVT-core	guttifera	guttifera	pBac
AACGGCTAGCAGCACAAACAATTGCCACTTAATTAACA	NheI	gutCVT-core	guttifera	guttifera	pBac
ATCACCTGCAGGGCCTAGTAGTTGCAGCTTGTTA	SbfI	melCS	melanogaster	melanogaster	S3aG
ACTTGGCGCGCGCAACGAAATGGGTACAGTATTA	AscI	melCS	melanogaster	melanogaster	S3aG
ATATCTCGAGGCTAGTAGTTGCAGCTTGTTA	XhoI	melCS	guttifera	melanogaster	pBac
TCAACCGCGGGCAACGAAATGGGTACAGTATTA	SacII	melCS	guttifera	melanogaster	pBac
ATCACCTGCAGGGCCTAGTAGTTGCAGCTTGTTA	SbfI	gutCS	melanogaster	guttifera	S3aG
ACTTGGCGCGCGGCATATAAATAGACAGTTTGAATTTATTA	AscI	gutCS	melanogaster	guttifera	S3aG
TCAACCGCGGGCCTAGTAGTTGCAGCTTGTTA	SacII	gutCS	guttifera	guttifera	pBac
AACGGCTAGCGGCATATAAATAGACAGTTTGAATTTATTA	NheI	gutCS	guttifera	guttifera	pBac
ATCACCTGCAGGGAGTCTCTCATCTATCCTAAGAC	SbfI	melTS	melanogaster	melanogaster	S3aG
ACTTGGCGCGCGGCAAAATGCATTTAATTGGCTGA	AscI	melTS	melanogaster	melanogaster	S3aG
ATATCTCGAGGAGTCTCTCATCTATCCTAAGAC	XhoI	melTS	guttifera	melanogaster	pBac
TCAACCGCGGGCAACGAAATGGGTACAGTATTA	SacII	melTS	guttifera	melanogaster	pBac
ATCACCTGCAGGAAGTGCAGGCGAGCGTATCTTATA	SbfI	gutTS	melanogaster	guttifera	S3aG
ACTTGGCGCGCGGCATATAAATAGACAGTTTGAATTTATTA	AscI	gutTS	melanogaster	guttifera	S3aG
TCAACCGCGGAAGTGCAGGCGAGCGTATCTTATA	SacII	gutTS	guttifera	guttifera	pBac
AACGGCTAGCTGCCTAACTGGCTTAGATAGCTAA	NheI	gutTS	guttifera	guttifera	pBac

Primers for in situ hybridization

Gene	Target species
CACGTCCAAGCGGAGATGCG	wg melanogaster
GGCGACGGCATGTTCCGGGTG	wg melanogaster
CACGTTACGGGAGATGCG	wg guttifera, deflecta, nigromaculata, palstris, quinaria
GGCGATGGCATATGGGATGATG	wg guttifera, deflecta, nigromaculata, palstris, quinaria
CGAACACTTTATATCGGAGCA	Wnt4 guttifera
GAGTCATGTGCAATATTTCCGG	Wnt4 guttifera
GCCATTCGCGATGCGATG	Wnt6 guttifera
CTAGAGGCATGTGTTGACCTC	Wnt6 guttifera
GCCGTGTCCAATAACATGGAGT	Wnt10 guttifera
CCTGTATATCGCTCCTAGAT	Wnt10 guttifera

Primers for RT-PCR

Gene	Template species
GAGCAGCAACTGTTGCTGTC	Wnt4 guttifera
GCCAATCCTTTGTTACATGATTC	Wnt4 guttifera
GAGTGCAAATGCCACGGCAT	wg guttifera
GGCTCCAGATAGACAATATCCTT	wg guttifera
GCCATTCGCGATGCGATG	Wnt6 guttifera
AATTATGTTTACTGACTCTGCCGAG	Wnt6 guttifera
GTTATCGGAAAGTGCTTTTGC	Wnt10 guttifera
CTTCAGCACTTTGCCAAACAATGT	Wnt10 guttifera
ATGTGTGACGAAGAAGTTGCT	Act5C guttifera
TAGATGGGCACAGTGTGG	Act5C guttifera