Supplementary Figure 1

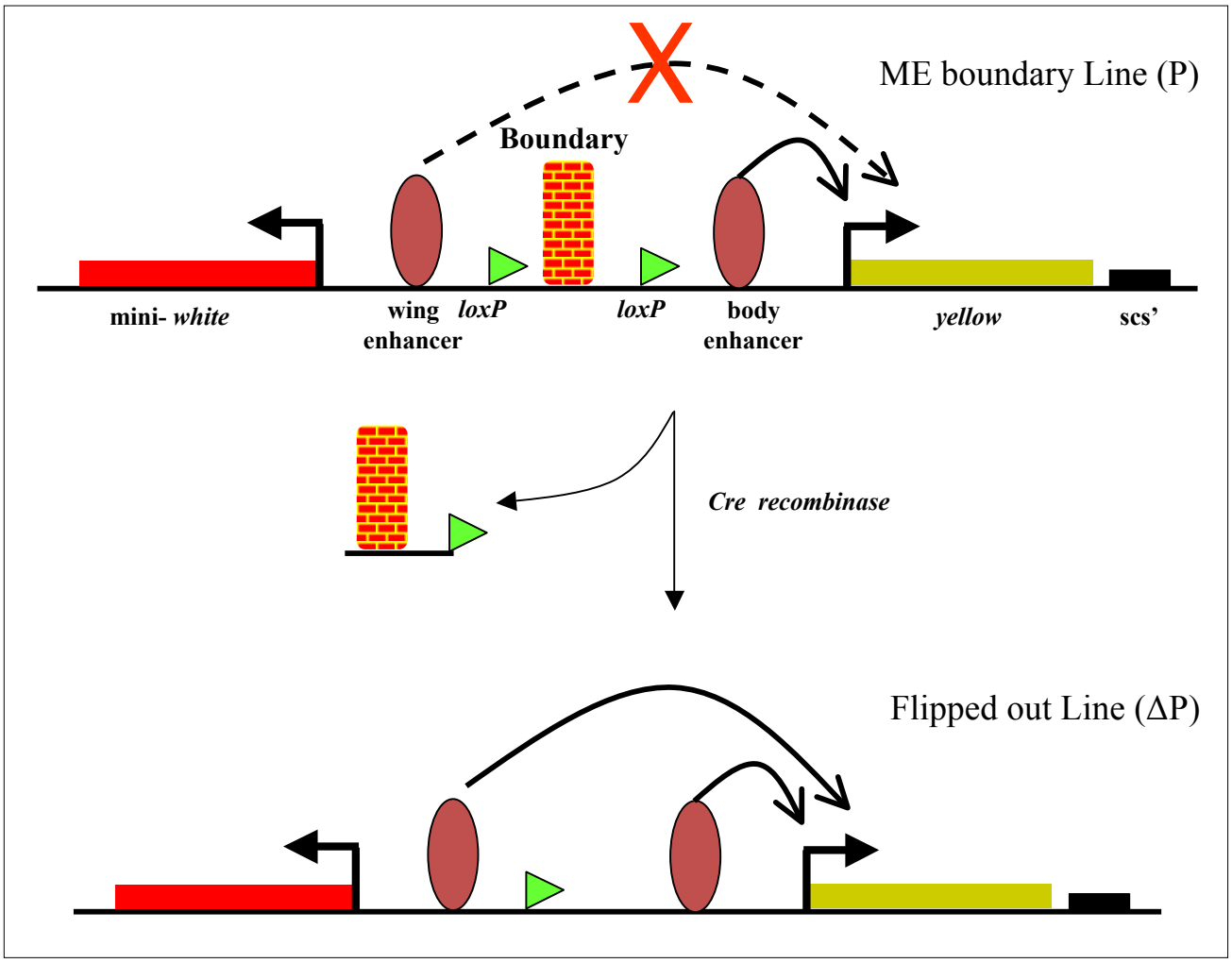
Sequence feature of the ME region

Sequence of ME region showing different motifs and primers used in this study. BEAF and GAF binding motifs are shown in red and blue caps, respectively. The primer sequences used for cloning the 1583 bp part of ME region in pRW+ and YW assay vectors are marked by green arrows. The primers used for cloning the 917 bp underlined part of ME region in pCfhL assay vector are marked by purple arrows. All the five BEAF sites indicated in this 917 bp region were mutated to assess the role of BEAF in ME boundary. The primers used for quantitative Real Time PCR of ChIP analysis for anti-FLAG (for BEAF) and anti GAF antibodies are shown as red and blue arrows, respectively. Primers shown here and additional primers used in ChIP experiments are listed in Supplementary Table 4.

Supplementary Figure 2

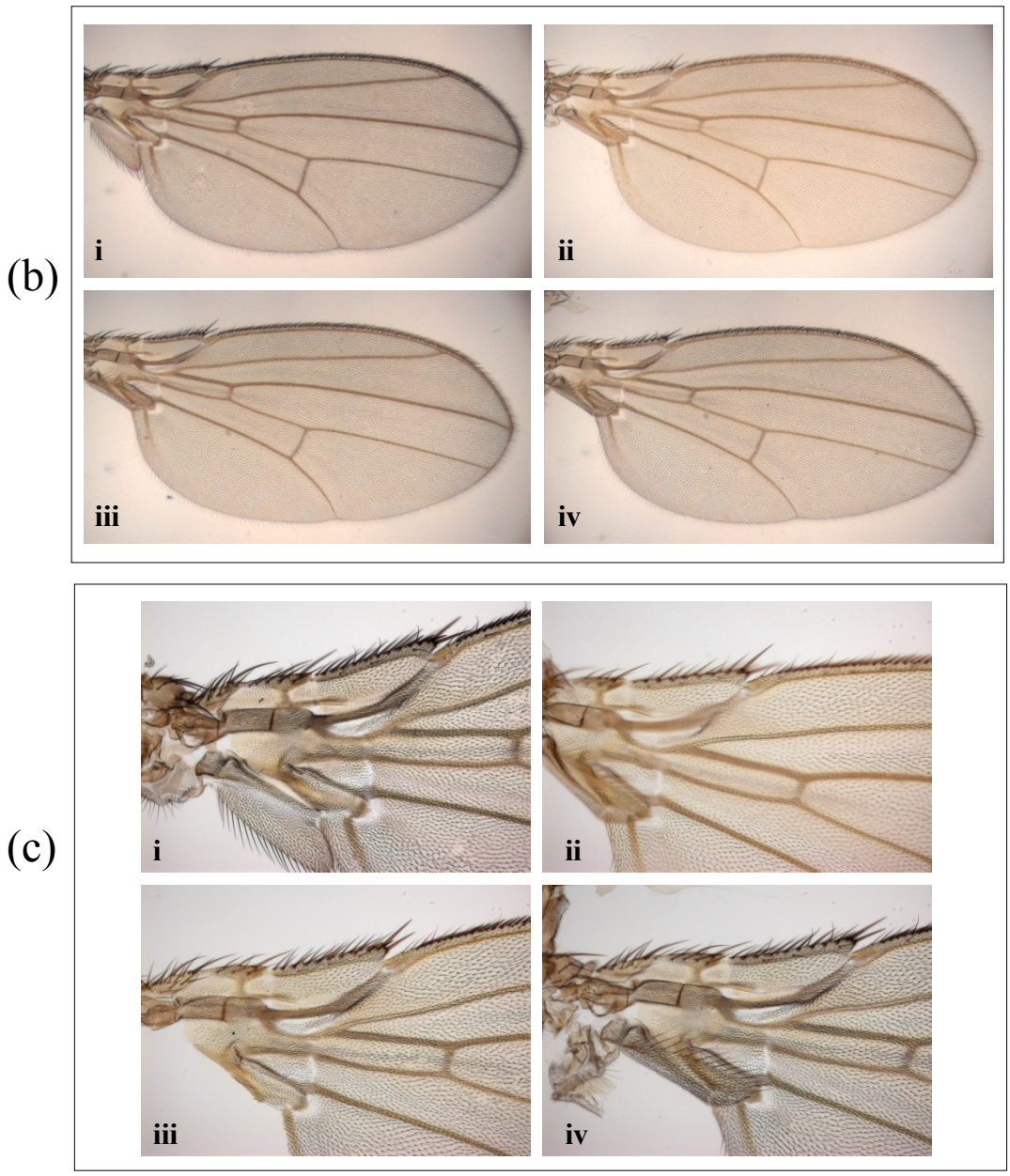
Enhancer blocker assay in wing

(a)



Schematic representation of the YW vector. The test fragment is inserted between the wing enhancer and *yellow* gene to assess if such a insert blocks access of this enhancer to the gene, shown as red cross on the broken arrow. The body enhancer is free to act on the *yellow* gene. The test fragment is flanked on both sides with *loxP* sites. On crossing the transgenic lines with *Cre* expressing line the test fragment can be flipped out and the wing enhancer can now act on the *yellow* gene. mini-*white* is to score for the transgene.

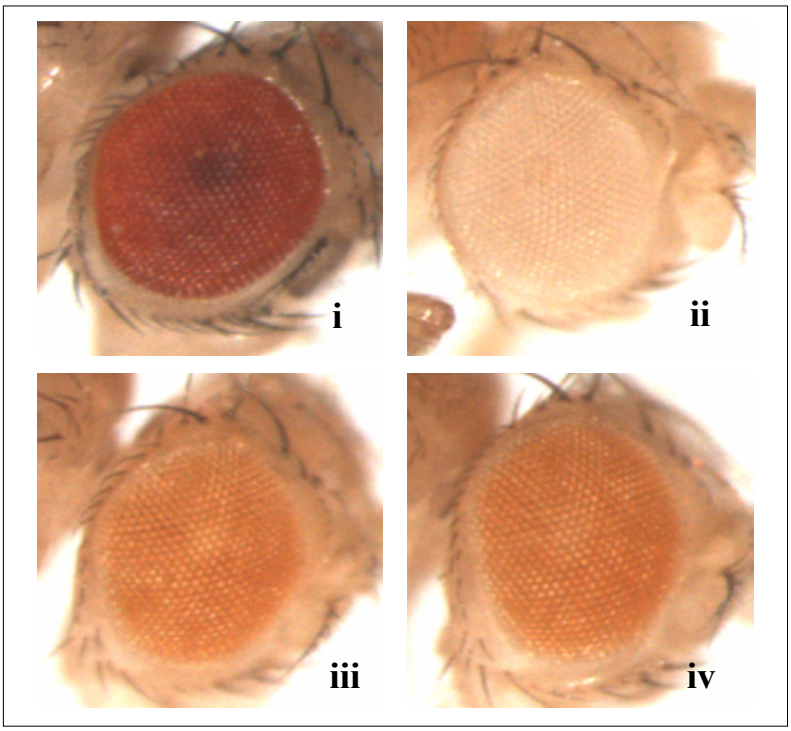
Supplementary Figure 2



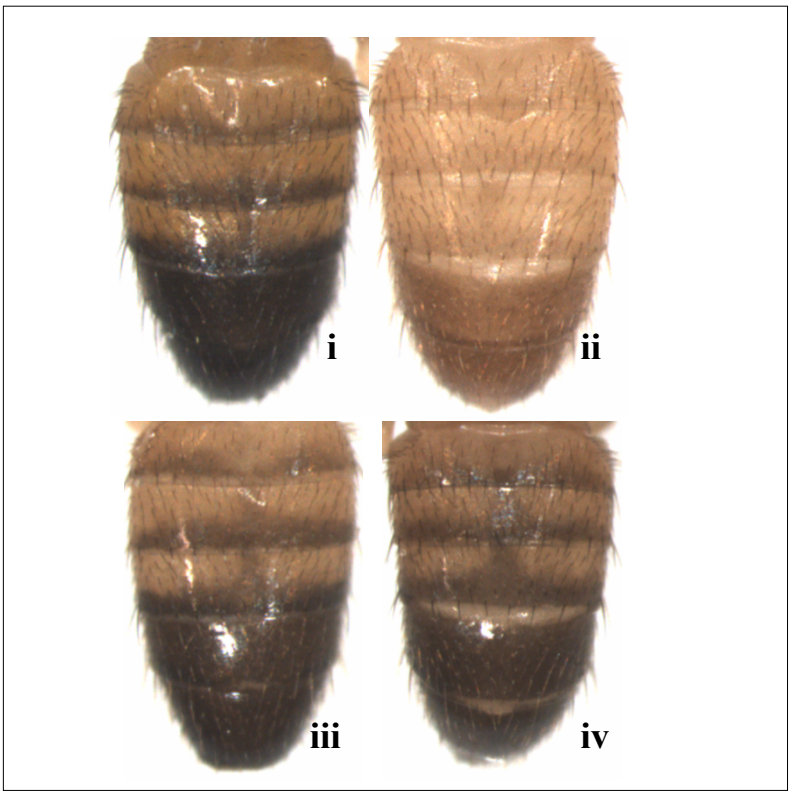
ME region acts as an enhancer blocker in the wing. Whole wing (b) and enlarged hinge part of the wing (c) from different genotypes are shown. i is from wild type *CS* fly, ii is from yw^{1118} fly in which the *yellow* gene is mutated, iii is from a line carrying ME region and iv is from the same line after ME region is flipped out. All the wings shown are heterozygous for the transgene.

Supplementary Figure 2

(d)



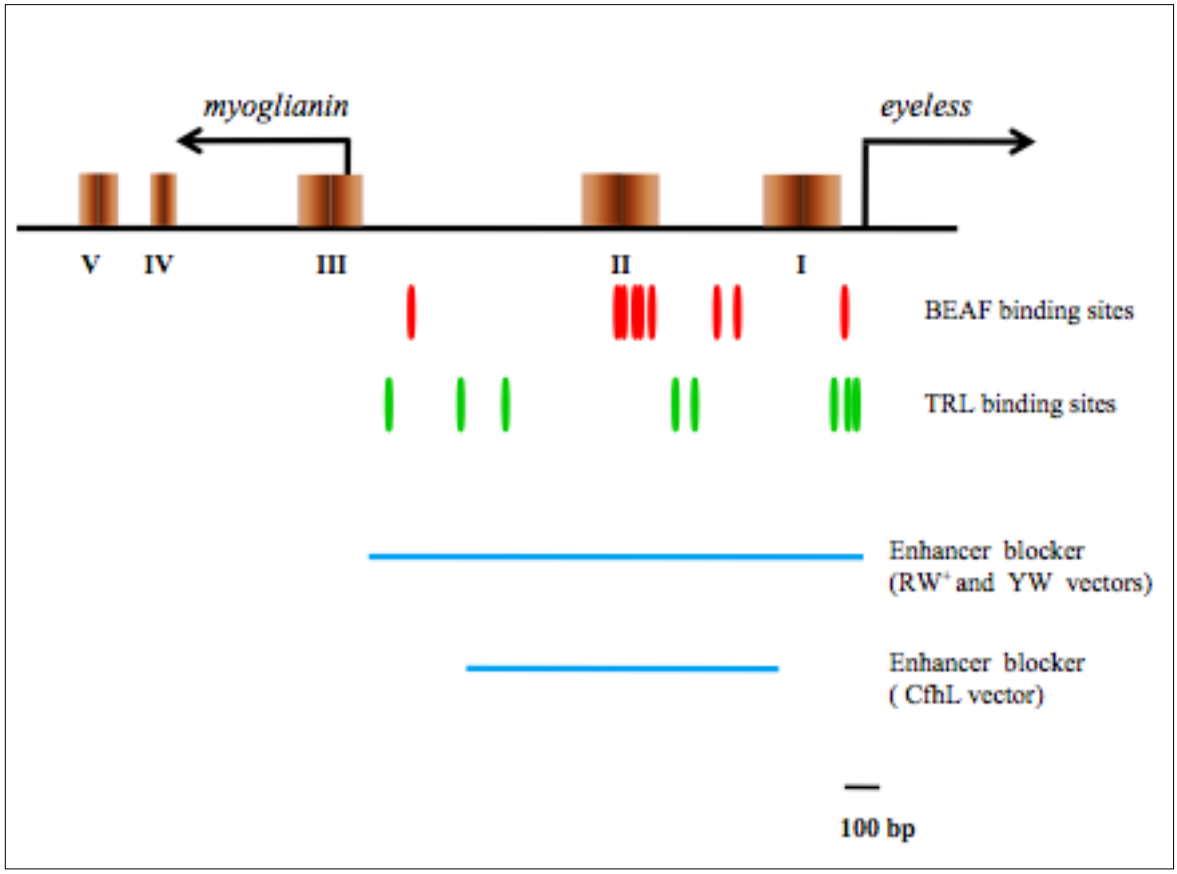
(e)



ME region acts as a boundary element and not a repressor element. Eye (d) and body (e) pigmentation levels in different genotypes are shown. i is from wild type *CS* fly, ii is from yw^{1118} fly in which the *yellow* gene is mutated, iii is from a line carrying ME region and iv is from the same line after ME region is flipped out. All the wings shown are heterozygous for the transgene.

Supplementary Figure 3

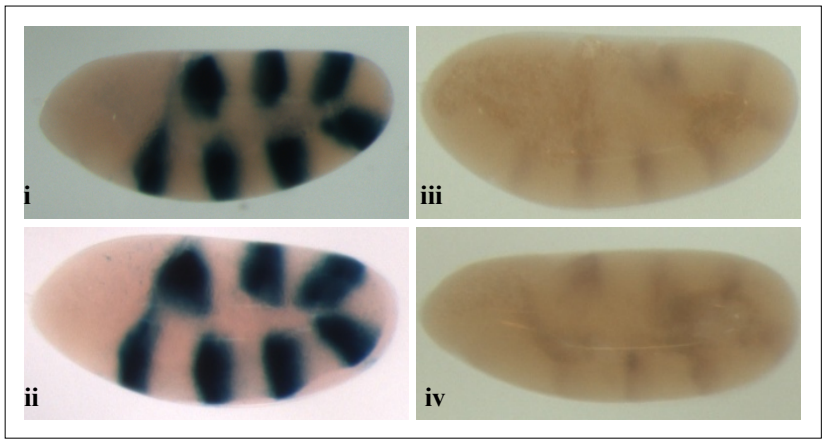
Map of the region containing *myoglianin* and *eyeless* genes



The five DNAseI hypersensitive sites I-V are shown as brown boxes. Red ovals indicate BEAF binding sites and green ovals indicate TRL/GAF binding sites. Blue bars indicate the fragments used for boundary assay in different vectors.

Supplementary Figure 4

ME acts as an enhancer blocker in the embryos

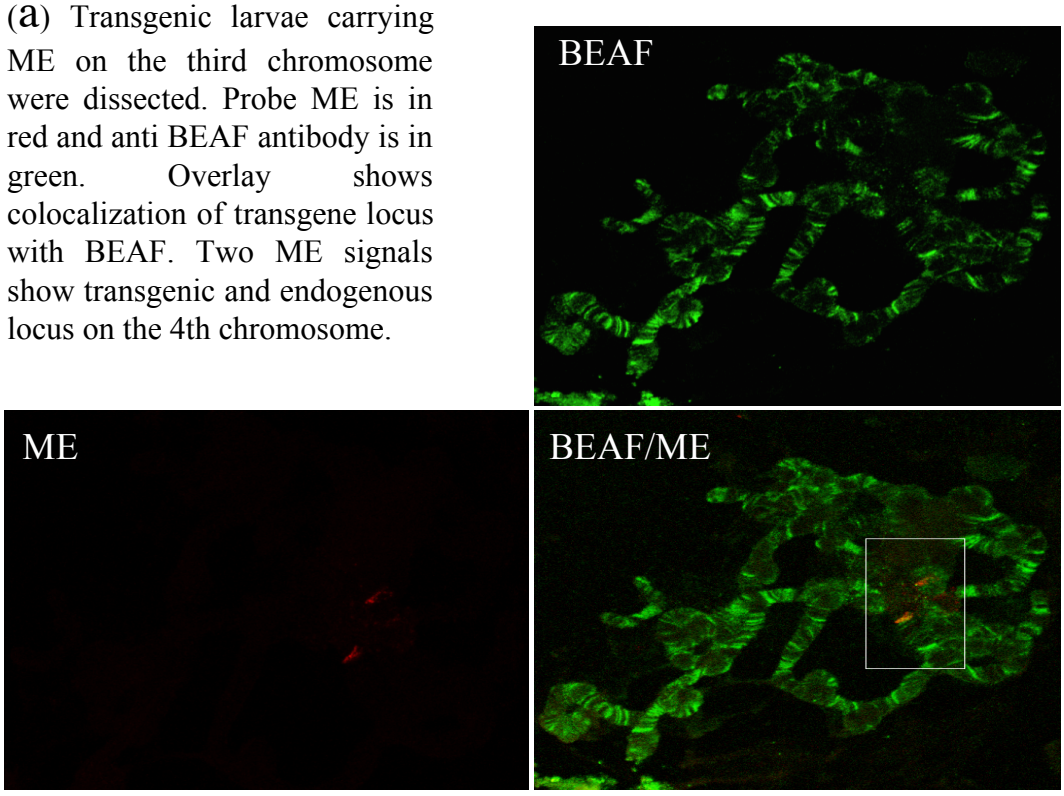


Upper left embryo (i) is from a control line carrying only the vector. Lower left embryo (ii) is from a control line carrying 1 kb λ DNA in place of test fragment. Upper right embryo (iii) is from a line carrying a fragment with five binding sites for the protein Su(Hw). Lower right embryo (iv) is from a line carrying ME sequence. All the embryos shown are homozygous. These results show that while phage control DNA insert has no effect in this assay vector, both ME and *gypsy* derived 0inserts have comparable enhancer blocking boundary activity.

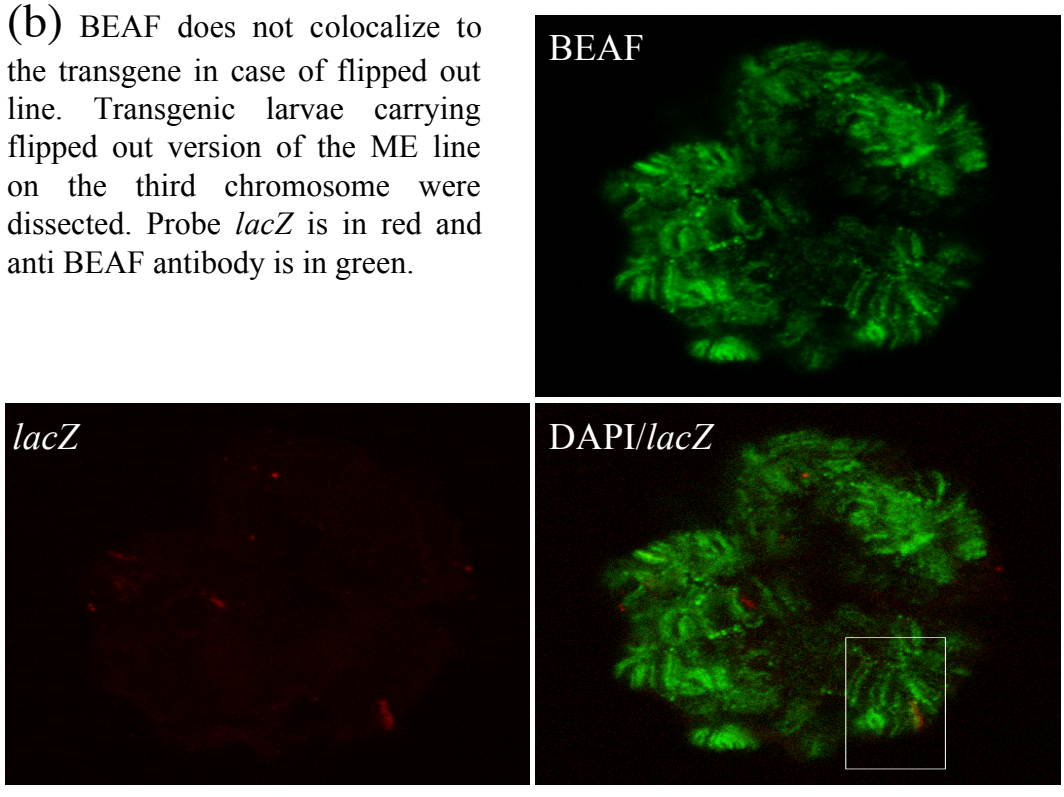
Supplementary Figure 5

Colocalization of BEAF and GAF on polytene chromosomes of ME lines

(a) Transgenic larvae carrying ME on the third chromosome were dissected. Probe ME is in red and anti BEAF antibody is in green. Overlay shows colocalization of transgene locus with BEAF. Two ME signals show transgenic and endogenous locus on the 4th chromosome.

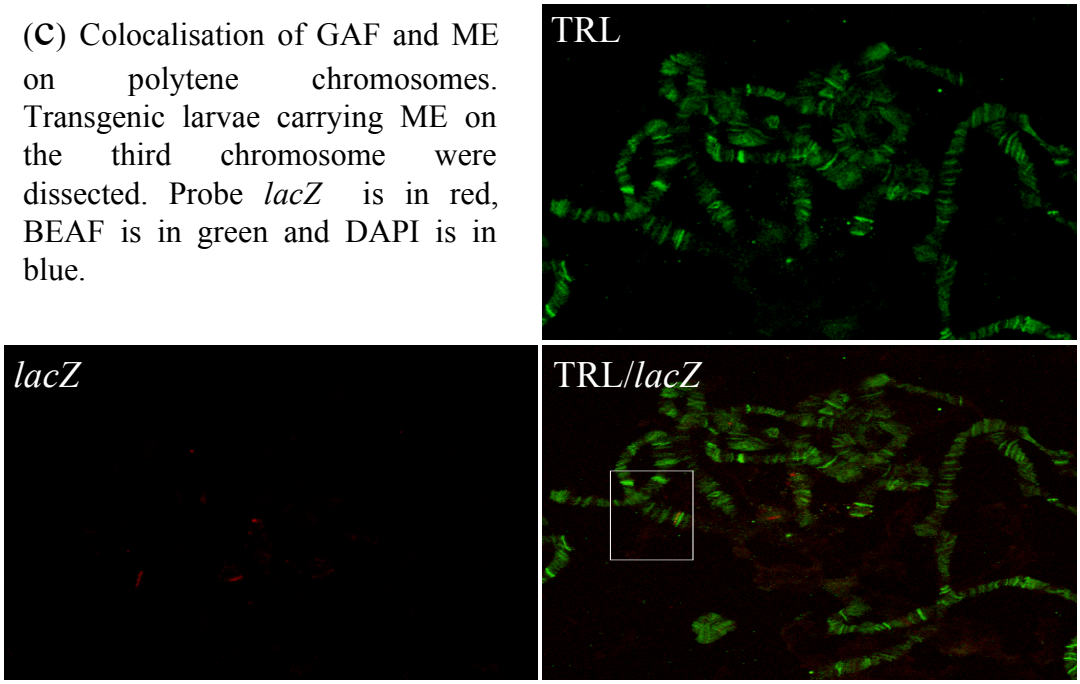


(b) BEAF does not colocalize to the transgene in case of flipped out line. Transgenic larvae carrying flipped out version of the ME line on the third chromosome were dissected. Probe *lacZ* is in red and anti BEAF antibody is in green.

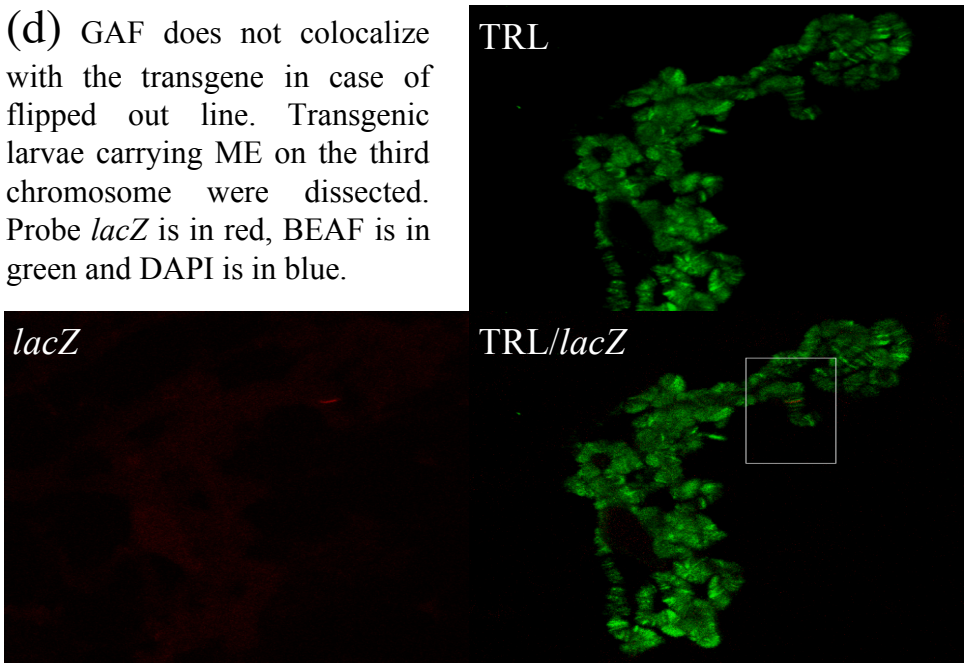


Supplementary Figure 5

(**C**) Colocalisation of GAF and ME on polytene chromosomes. Transgenic larvae carrying ME on the third chromosome were dissected. Probe *lacZ* is in red, BEAF is in green and DAPI is in blue.

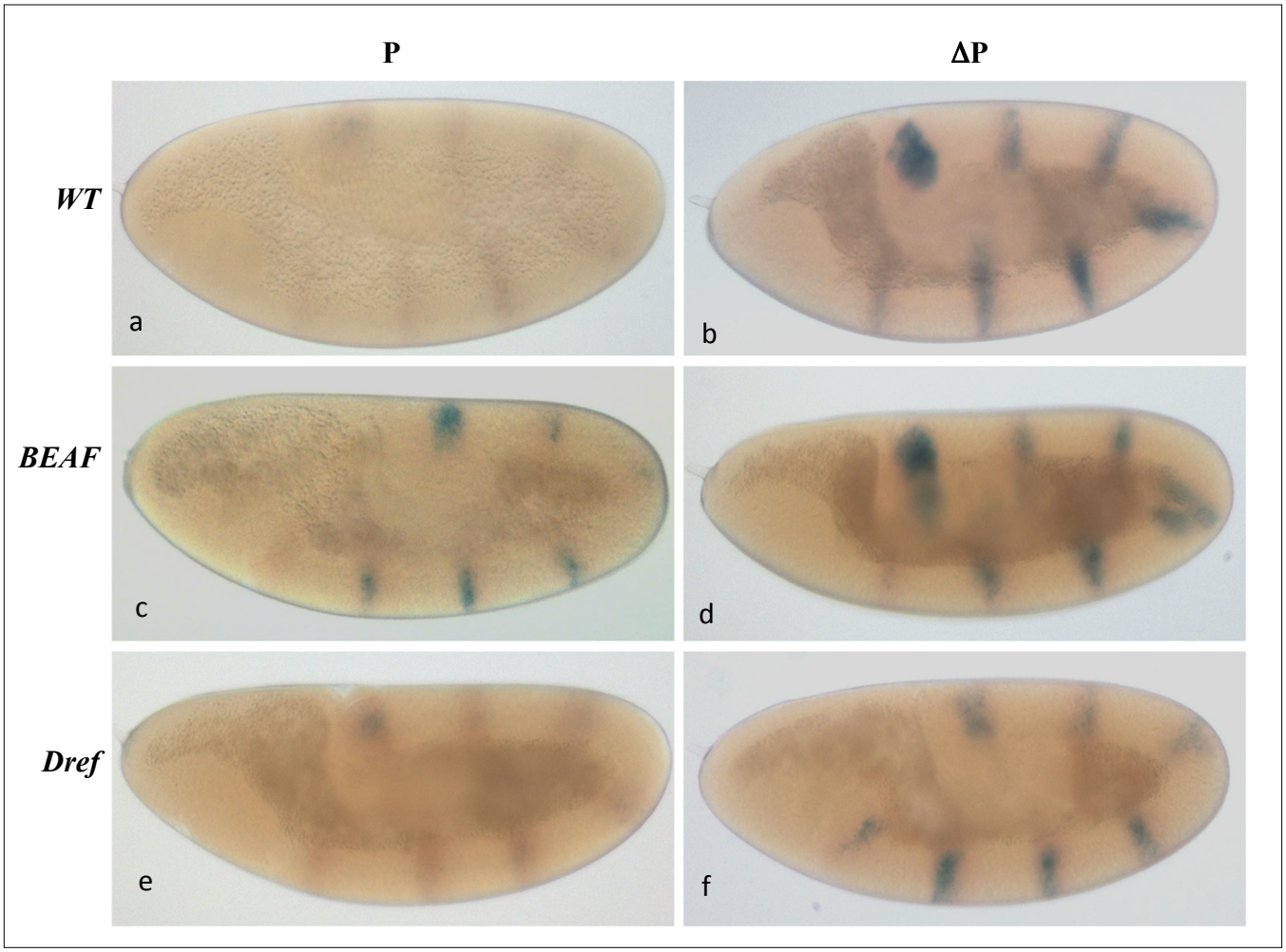


(d) GAF does not colocalize with the transgene in case of flipped out line. Transgenic larvae carrying ME on the third chromosome were dissected. Probe *lacZ* is in red, BEAF is in green and DAPI is in blue.



Supplementary Figure 6

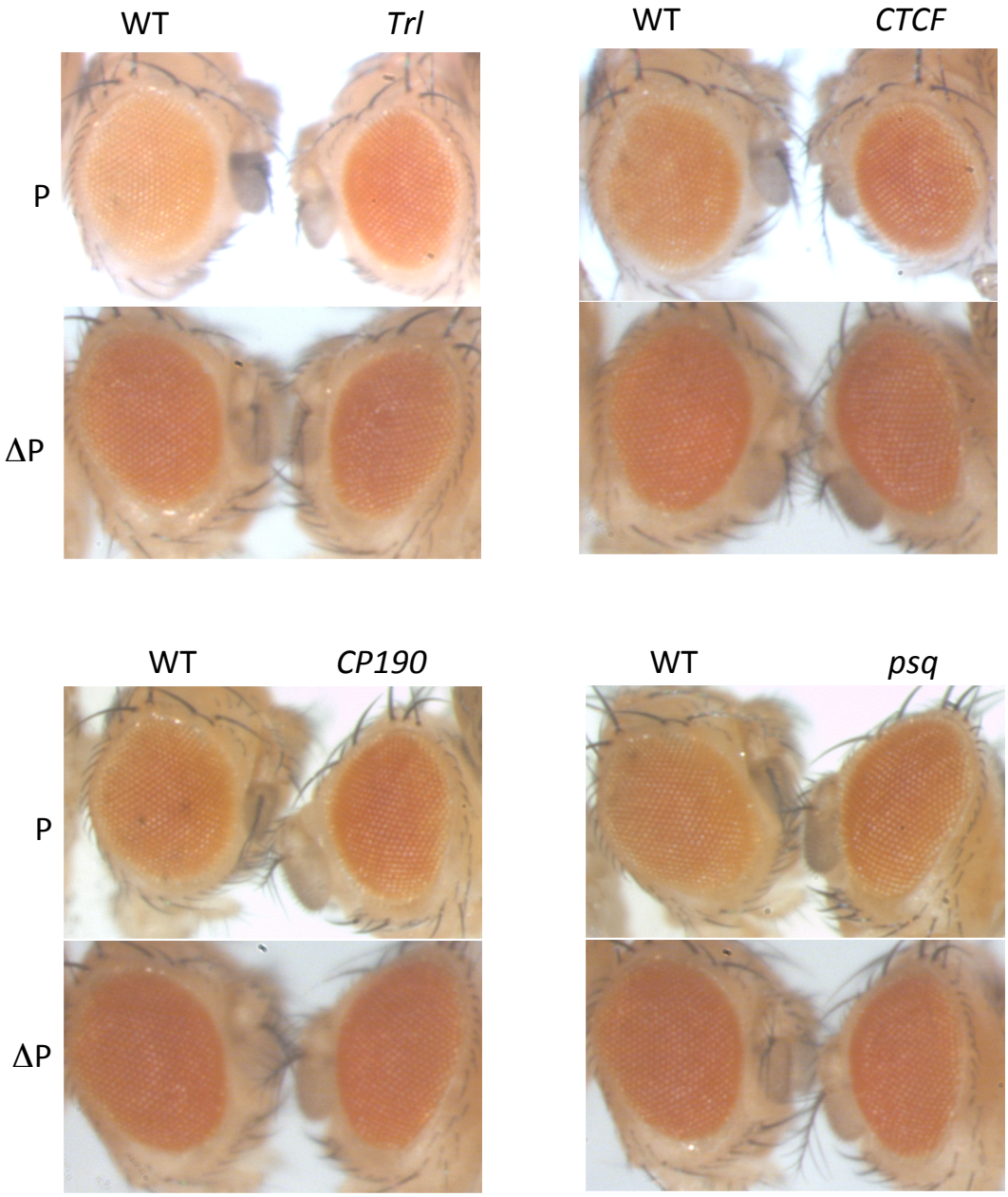
***BEAF* but not *Dref* has effect on ME boundary function**



lacZ staining of embryos carrying ME(P) (a)and flipped out (ΔP) (b) of the same line. Embryos in $BEAF^{ABKO}$ mutant background carrying ME (c) and flipped out (d). Embryos in $Dref^{KG09294}$ mutant background carrying ME (e) and flipped out (f). All embryos are heterozygous for ME (P), flipped out ME (ΔP), *BEAF* and *Dref*. While in *BEAF* mutant background (c) *lacZ* staining is enhanced indicating the weakening of boundary function, no such effect is seen in *Dref* mutant background (e) suggesting that the later has no contribution to the ME boundary activity.

Supplementary Figure 7

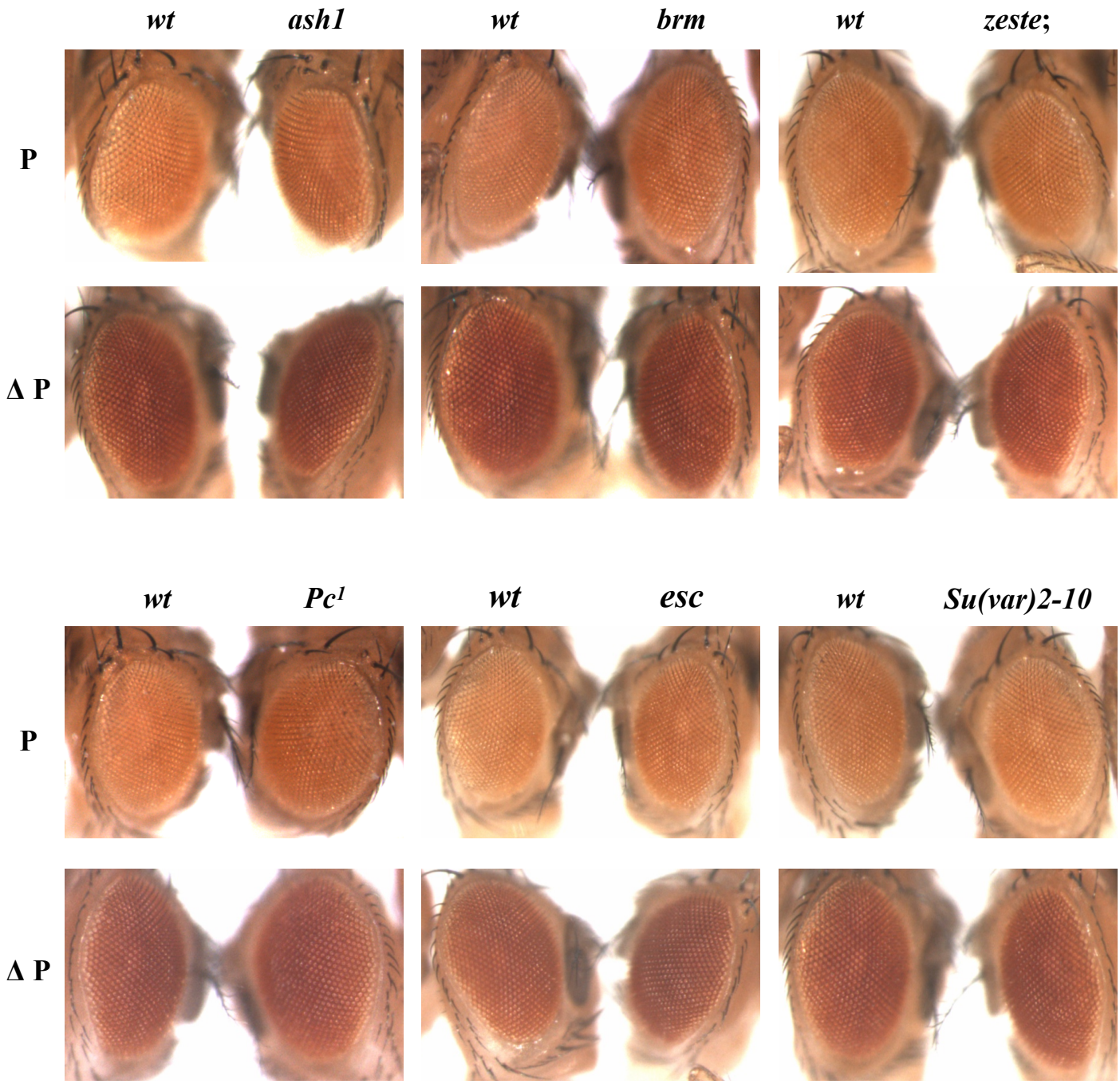
Effect of *Trl*, *CTCF*, *CP190* and *psq* mutations on ME boundary



Eye color comparison of transgenic flies carrying ME in different mutation backgrounds are shown. Each panel has eye (WT) of initial ME (P) or flipped out ME(ΔP) line and next to it is the eye from the same line in indicated mutant background -*Trl*^{13C}, *CTCF*$^{Y+6}$, *CP190*$^{H4-1}$ and *psq*^{D91}. While *Trl*^{13C} has clear effect on the enhancer blocking activity of ME, other mutations do not have any noticeable effect.

Supplementary Figure 8

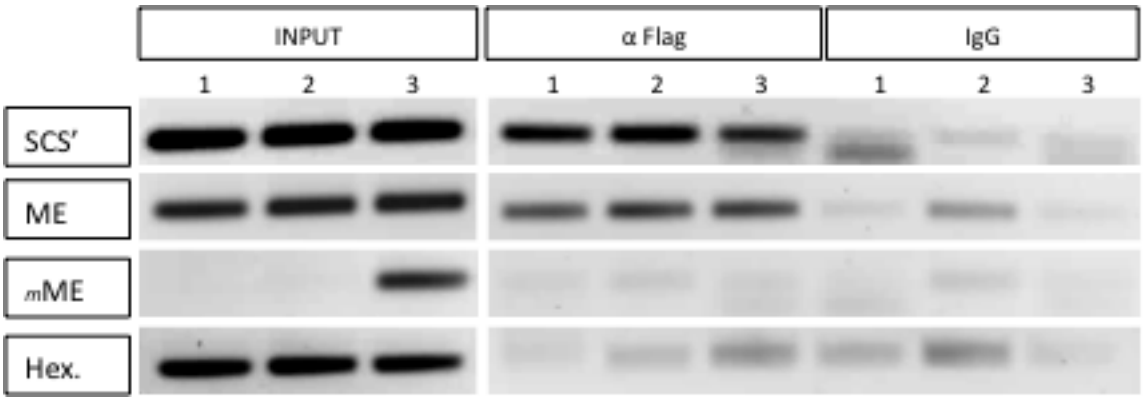
Effect of PcG and trxG mutations on ME boundary



Eye color comparison of transgenic flies carrying ME in different mutation backgrounds are shown. Each panel has eye (WT) of initial ME (P) or flipped out ME(ΔP) line and next to it is the eye from the same line in indicated mutant background - *ash1*^{B1}, *brm*^{2}, *z*^{1}, *Pc*^{1}, *esc*^{2} and *Su(var)2-10*^{2}. Except for *z*^{1} and *Su(var)2-10*^{2} all other tested mutants show varying degree of effect on the enhancer blocking activity of ME.

Supplementary Figure 9

BEAF does not bind to the mutated ME region



Chip performed with larvae expressing flag- BEAF protein in wild type (1), transgene carrying ME (2) and transgene carrying mutated ME (3) using anit-Flag antibody. ME regions (endogenous and transgene), mutated ME region only (*m*ME), SCS' region (positive control), and HexokinaseC promoter region (negative control) enrichment of was assessed. Equal amount of immunoprecipitated DNA was used for PCR and analyzed on 2% agarose gel. Input chromatin (INPUT) used as PCR controls and IgG pull down as antibody negative control are shown.

Mutated ME is amplified by unique primer pair specific to it, as confirmed by lack of amplification in (1) and (2) INPUT samples. No binding of BEAF is on mutated ME region is seen.

Supplementary Table 1. List of selected intergenic regions

S. No.	Intergene	Direction of Transcription	Intergene Size (bp)
1	1ig2	← →	863
2	8ig9	← →	2093
3	9ig10	→ ←	7585
4	12ig13	→ ←	1160
5	19ig20	→ ←	298
6	23ig24	← →	1616
7	24ig25	→ ←	624
8	27ig28	← →	1280
9	28ig29	→ ←	650
10	30ig31	← →	1587
11	31ig32	→ ←	5942
12	35ig36	← →	168
13	36ig37	→ ←	2352
14	37ig38	← →	963
15	42ig43	→ ←	2288
16	43ig44	← →	3663
17	44ig45	→ ←	1488
18	49ig50	← →	1640
19	50ig51	→ →	3243
20	51ig52	→ ←	2360
21	53ig54	→ ←	1356
22	66ig67	← ←	350
23	67ig68	← →	154
24	68ig69	→ ←	1106
25	71ig72	→ ←	1116
26	73ig74	→ ←	1861
27	74ig75	← →	776
28	76ig77	→ ←	752

Regions narrowed down for potential boundary after analysis of features associated with them. The intergenic region 49ig50 was used for detail functional analysis.

Supplementary Table 2. List of transgenic lines

I. Full length ME (1.5 Kb)

S. No.	Line	Vector	Chromosome	Remarks	Flipped out version
1	137.16.1.1	RW+	III	Blocker (S)	Δ 137.16.1.1
2	137.16.3.3	RW+	III	Non-blocker	-
3	137.19.1.2	RW+	II	Non-blocker	-
4	137.19.5.1	RW+	I	Blocker	Δ 137.19.5.1
5	137.21.8.1	RW+	III	Blocker	-
6	141	RW+	II	Blocker	Δ 141
7	203	RW+	II	Blocker	-
8	204	RW+	III	Blocker	-
9	310	RW+	III	Blocker	-
10	373	RW+	III	Blocker	Δ 373
11	110.27.1.1	YW	II	Blocker (W)	Δ110.27.1.1
12	110.27.1.3	YW	II	-	-

II. Smaller fragment ME (917bp)

S. No.	Line	Vector	Chromosome	Remarks	Flipped out version
1	917.10.1	Cfhl	III	Blocker (M)	-
2	917.10.3	Cfhl	III	Blocker (M)	Δ 917.10.3
3	917.36.2.2	Cfhl	III	Blocker (W)	-
4	917.36.2.5	Cfhl	III	Blocker (S)	Δ 917.36.2.5
5	917.67.2	Cfhl	III	Blocker (S)	Δ 917.67.2
6	917.77.1	Cfhl	I	Blocker (W)	Δ 917.77.1
7	917.80.3.1	Cfhl	I	Blocker (W)	Δ 917.80.3.1

III. Mutant version of smaller fragment ME (917 bp)

S. No.	Line	Vector	Chromosome	Remarks	Flipped out version
1	S 60.2	Cfhl	III	Non-blocker	Δ S 60.2
2	S 55.1	Cfhl	I	Non-blocker	Δ S 55.1
3	S 155.1	Cfhl	I	Non-blocker	Δ S 155.1

M - moderate, W - week, S - strong

Supplementary Table 3. List of *Drosophila* stocks used in this study.

S.No.	Name	Mutation	Source
1	*CS*	*Wild type*	Bloomington # 1
2	*white*	w^{1118}	Bloomington # 3605
3	*yellow white*	yw^{1118}	Generated in lab
4	*BEAF*	$BEAF^{AB\text{-}KO}$	Craig Hart
5	*Trithoraxlike*	Trl^{R85}, Trl^{13C}	Francois Karch
6	*Polycomb*	Pc^{1}	Francois Karch
7	*absent, small homeotic*	$ash1^{B1}$	Bloomington # 5045
8	*brahma*	brm^{2}	Bloomington # 3619
9	*extra sex combs*	esc^{2}	Bloomington # 813
10	*zeste*	z^{1}	Bloomington # 200
11	*Su(var)2-10*	$Su(var)2\text{-}10^{2}$	Bloomington # 6235
12	*CCCTC-binding factor*	$CTCF^{y+6}$	Victor G. Corces
13	*CP190*	$CP190^{H4\text{-}1}$	Victor G. Corces
14	*Dref*	$Dref^{KG09294}$	Victor G. Corces (Bloomington # 15178)
15	*pipsqueak*	psq^{D91}	Bloomington # 8145

Supplementary Table 4. List of primers used in this study.

Amplicon region	primers	sequence
ME full length (~1.6kb)	Forward	acggctcaccggcaatttcg
	Reverse	ggatggcaactctcggcgtg
ME short version (917bp)	Forward	aattttggttccctagttgttttt
	Reverse	cagaaaattgcaacgtagtgga
SCS'	Forward	acattttctaacactacaaagtcacga
	Reverse	cgatattgggtaaaacattcacg
iab-7 PRE	Forward	ggaataccgcactgtcgtagg
	Reverse	gcagccatcatggatgtgaa
ME	Forward	ggacaattaccgcgatgtct
	Reverse	ggacaattaccgcgatgtct
ME1	Forward	gctttgtatcagcaattctgttgt
	Reverse	aaaaataagtagcttacaatccatcca
ME2	Forward	cgtattctctacgcaatcactgg
	Reverse	tatcaggatggcaactctcg
mutated ME	Forward	gtgttttccagattagctgtctc
	Reverse	cacctcgagttttcttccatc
Ubx intron II	Forward	catttgaacttgacatttagcac
	Reverse	gtcgaggaatgccaaaagtgcac
hexokinase C promoter	Forward	gggaaaacacttgacgttgg
	Reverse	ggaggtgcgagaacttatgc