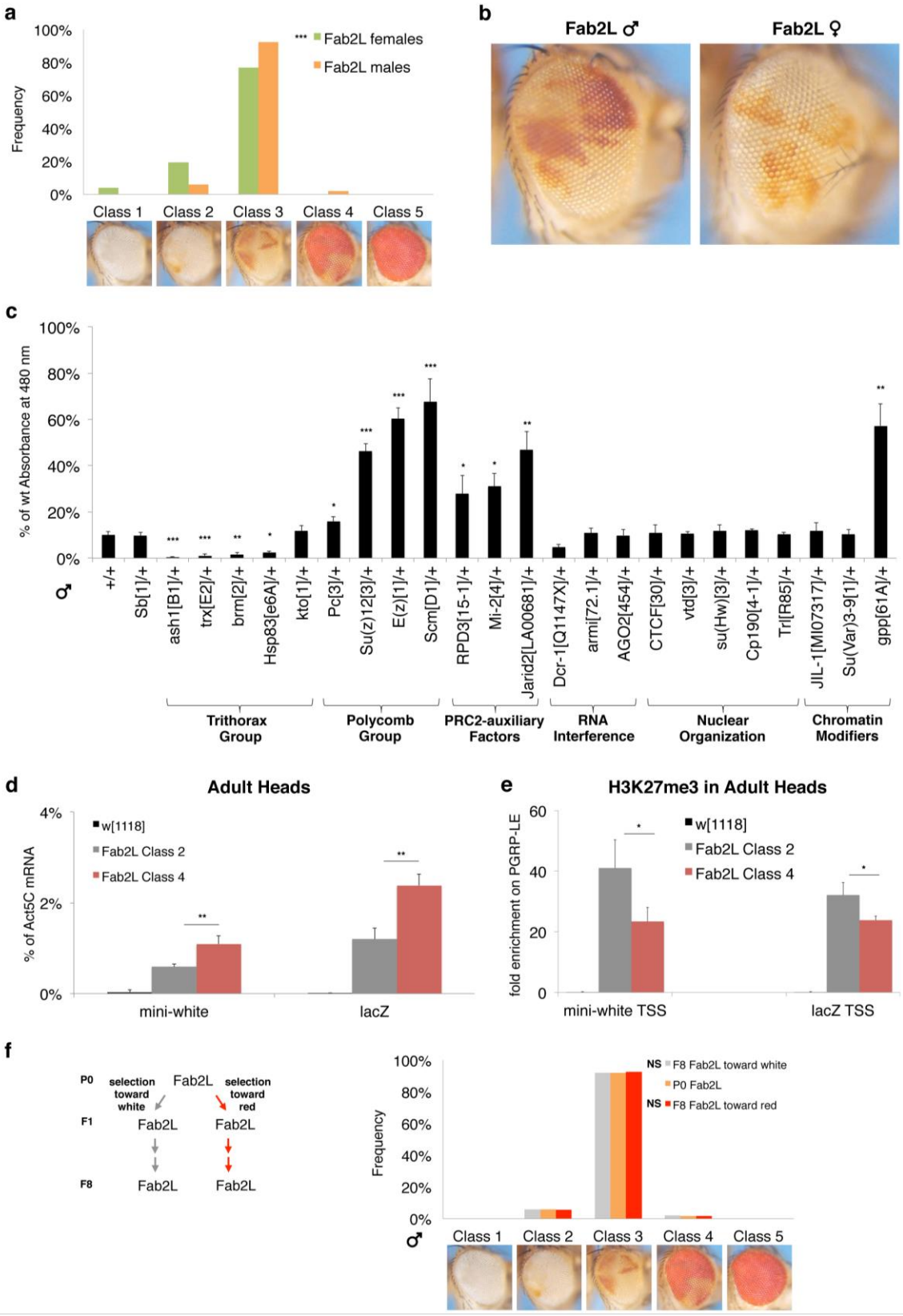


Supplementary Figure 1

Phenotypes and epigenetic properties of Fab2L flies.

A- Phenotypic classification based on eye pigment levels in Fab2L male (orange bars) and female (yellow bars) flies (n>150). Class 1: pigment=0%; Class 2: 0%<pigment≤5%; Class 3: 5%<pigment≤75%; Class 4: 75%<pigment<100%; Class 5: pigment=100%.

B- Representative pictures showing a Fab2L male fly on the left and a Fab2L female fly on the right, reared at 21°C.

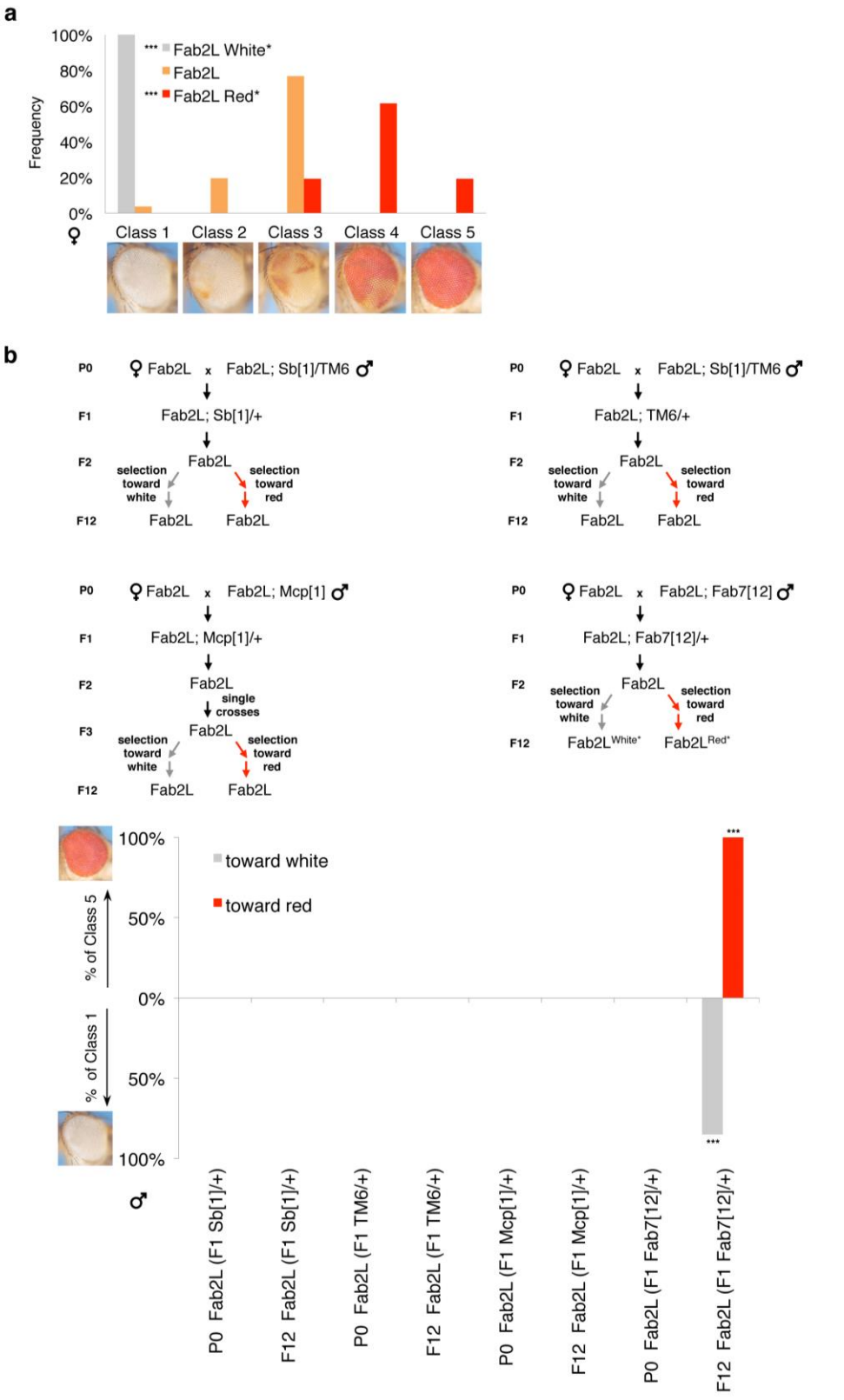
C- Eye pigmentation assays performed on Fab2L male flies, combined with the indicated alleles on chromosome 3.

D- RT-qPCR assays performed on w[1118], Fab2L Class 2 and Fab2L Class 4 male adult heads, measuring relative mRNA levels normalized to *Act5C.*

E- ChIP-qPCR assays performed on w[1118], Fab2L Class 2 and Fab2L Class 4 in male adult heads, showing relative enrichments (ChIP/Input) for H3K27me3, normalized with a negative control.

F- Crossing scheme for phenotypic selection and charts representing the phenotypic classification based on eye pigment levels of n>50 flies scored before (orange) and after (white and red) phenotypic selection**.**

Bars represent the frequency (**A,F**) or the mean of n=3 independent adult head collections +/- s. d. (**C-E**); two-tailed Fisher's exact test (**A,F**) or two-tailed Student's *t*-test (**C-E**): NS $P>0.05$; * $P<0.05$; ** $P<0.01$; *** $P<0.001$.



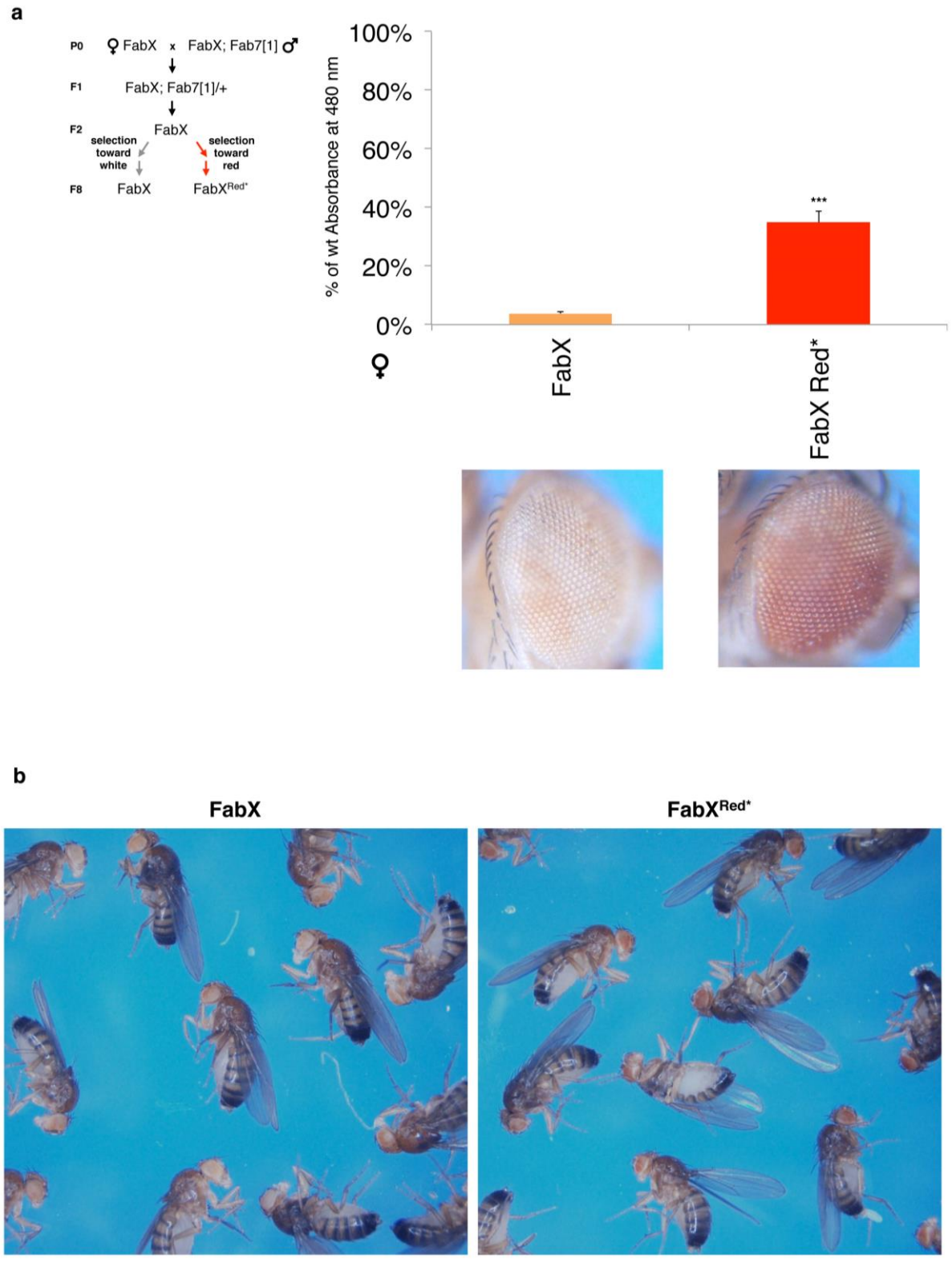
Supplementary Figure 2

Fab2L epiline establishment.

A- Phenotypic classification based on eye pigment levels in Fab2L^{White*}, Fab2L and Fab2L^{Red*} female flies (n>120). Class 1: pigment=0%; Class 2: 0%<pigment≤5%; Class 3: 5%<pigment≤75%; Class 4: 75%<pigment<100%; Class 5: pigment=100%.

B- Crossing schemes for phenotypic selection and charts representing the percentage of Class1 (pigment=0%) male flies in grey and Class 5 (pigment=100%) male flies in red, before (P0) and after (F12) the phenotypic selection (n>40). Note that the presence of the TM6 balancer in the F1, used here as a control, did not lead to establishment of any epiallele.

Bars represent the frequency of the flies scored; two-tailed Fisher's exact test: NS $P>0.05$; * $P<0.05$; ** $P<0.01$; *** $P<0.001$.



Supplementary Figure 3

FabX epiline establishment.

A- Crossing schemes, eye pigmentation assays and representative pictures of the observed phenotypes. Female FabX flies were scored at P0 and at F8. At each generation, 6 to 12 flies were selected on a total progeny of n>35.

B- Pictures showing a representative sample of FabX and FabX^{Red*} female flies reared at 21°C.

Bars represent the mean of n=3 independent adult head collections +/- s. d.; two-tailed Student's *t*-test: NS $P>0.05$; * $P<0.05$; ** $P<0.01$; *** $P<0.001$.

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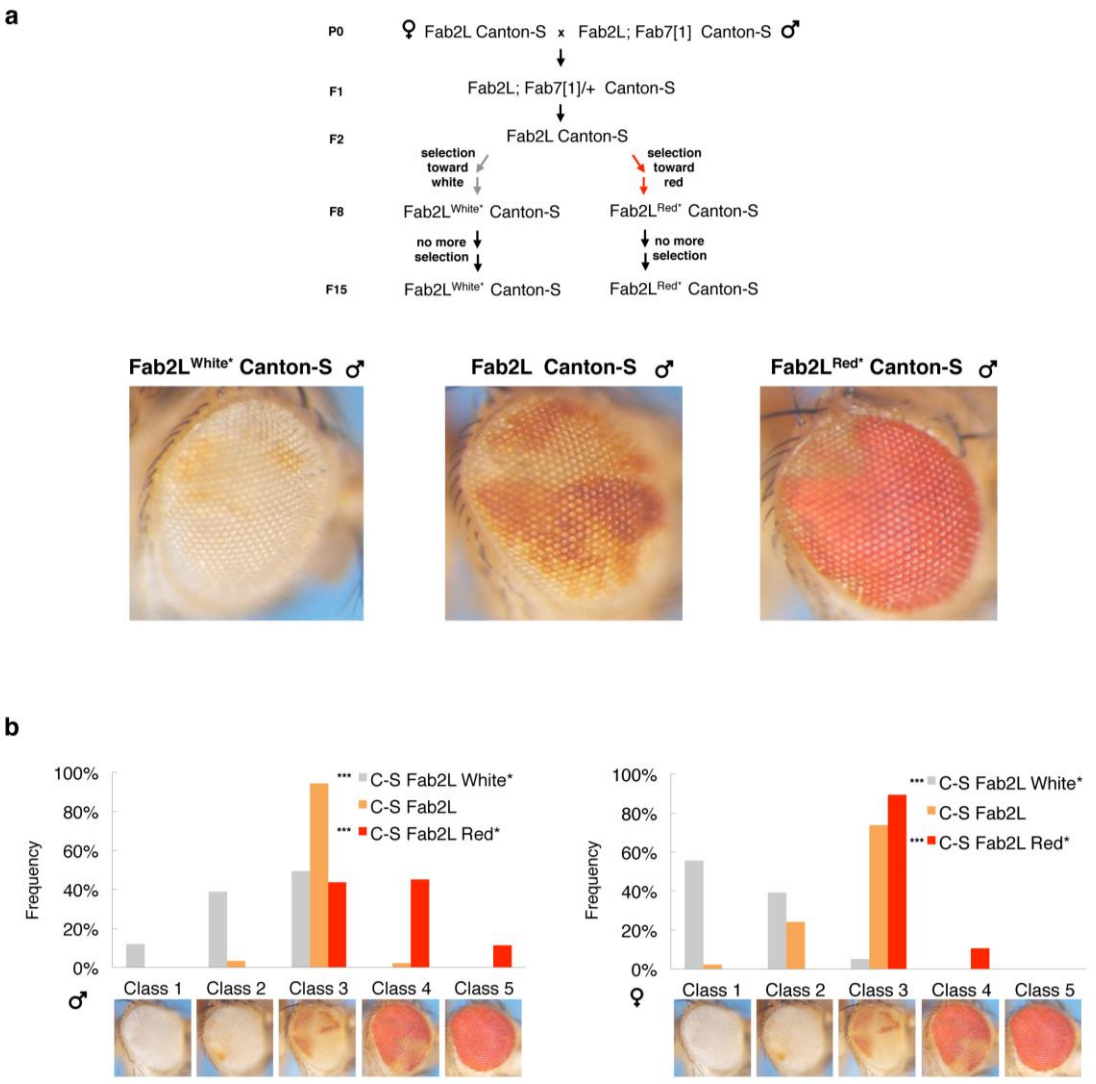


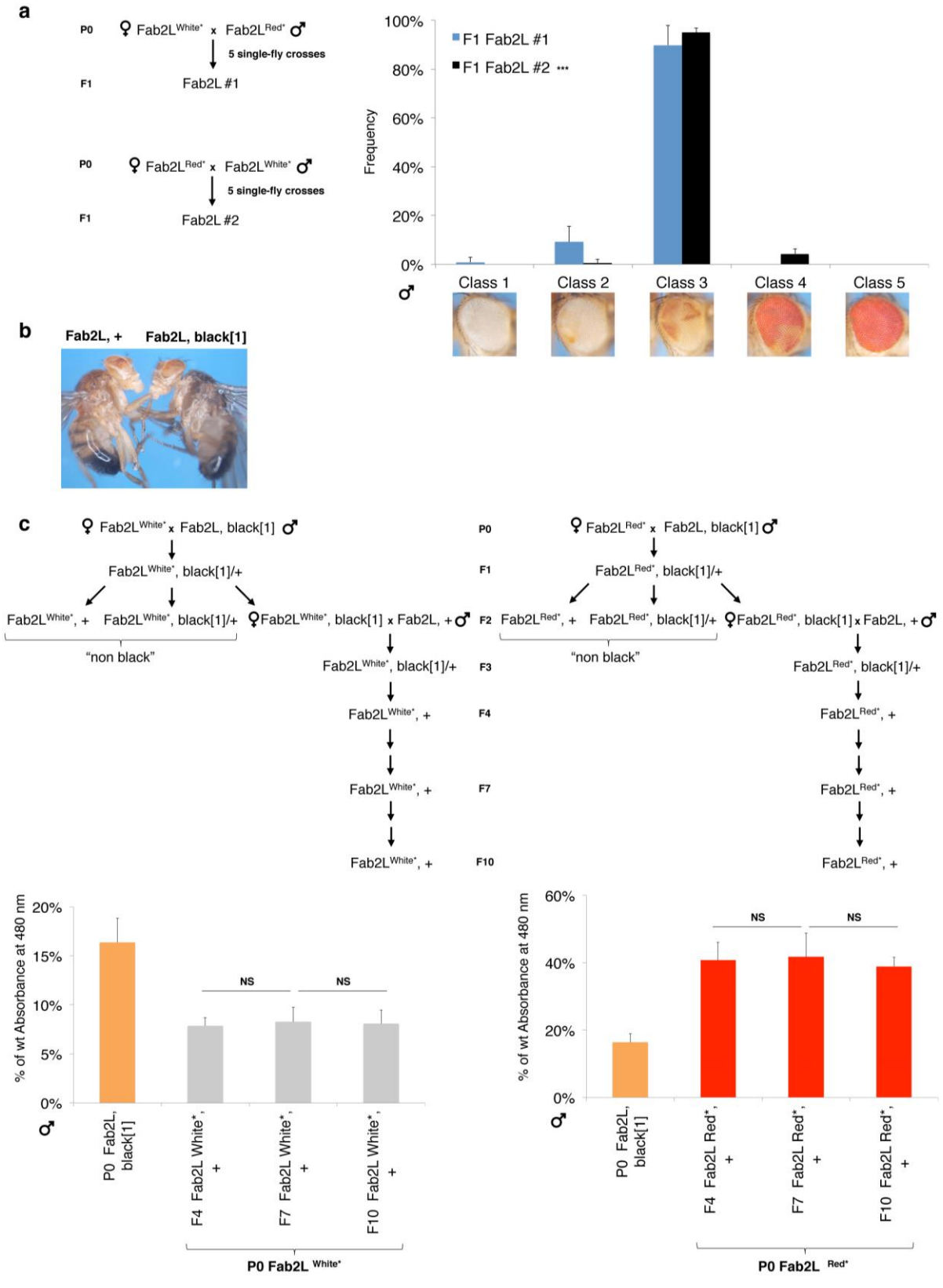
Supplementary Figure 4

Fab2L epiline establishment in isogenized Canton-S genetic background.

A- Crossing scheme for epiallele establishment in Fab2L flies in Canton-S background. In the bottom, representative pictures of Fab2L^{White*} Canton-S, Fab2L Canton-S, and Fab2L^{Red*} Canton-S male flies reared at 21°C.

B- Phenotypic classification based on eye pigment levels in Canton-S Fab2L (orange bars), Canton-S Fab2L^{White*} (grey bars), and Canton-S Fab2L^{Red*} (red bars) male (left chart) and female (right chart) flies. At each generation, n>10 flies were selected on a total progeny of n>40. The final scored progeny was n>130.

Bars represent the frequency; two-tailed Fisher's exact test: NS P>0.05; * P<0.05; ** P<0.01; *** P<0.001.



Supplementary Figure 5

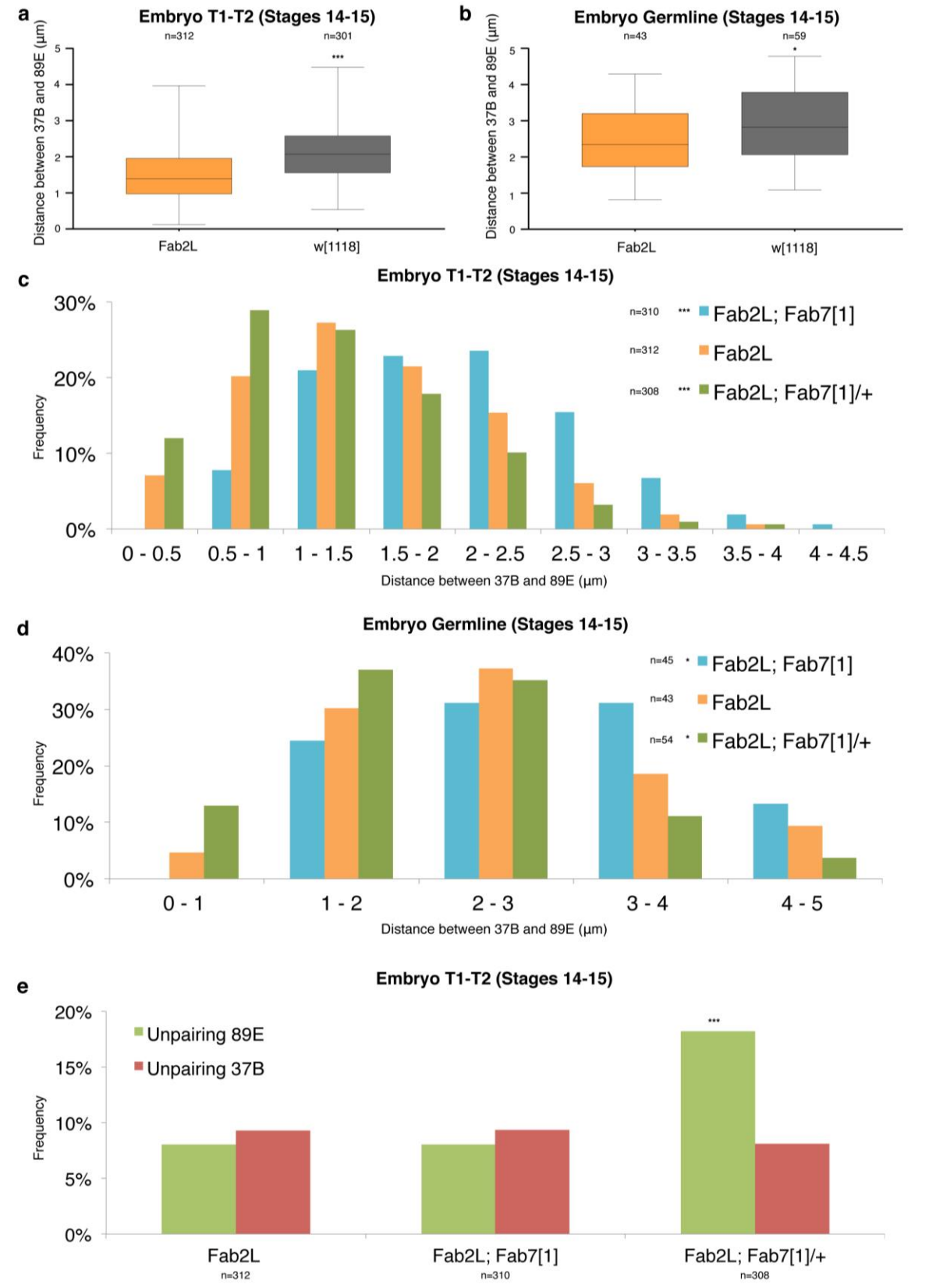
Epiallele genetic properties and paramutation.

A- Crossing schemes between the epilines and phenotypic classification of the F1 progenies based on eye pigment levels in Fab2L #1 progeny (blue bars) and in Fab2L #2 progeny (black bars). The F1 progenies of n=5 single-fly crosses were scored for each cross.

B- Lateral view of adult Fab2L and Fab2L,black[1] male flies.

C- Crossing schemes and eye pigmentation assays in the paramutation test.

Bars represent the mean of the frequencies of n=5 single-fly cross progenies (**A**) or the mean of n=3 independent crosses (**C**) +/- s. d.; two-tailed Student's *t*-test: NS $P>0.05$; * $P<0.05$; ** $P<0.01$; *** $P<0.001$.



Supplementary Figure 6

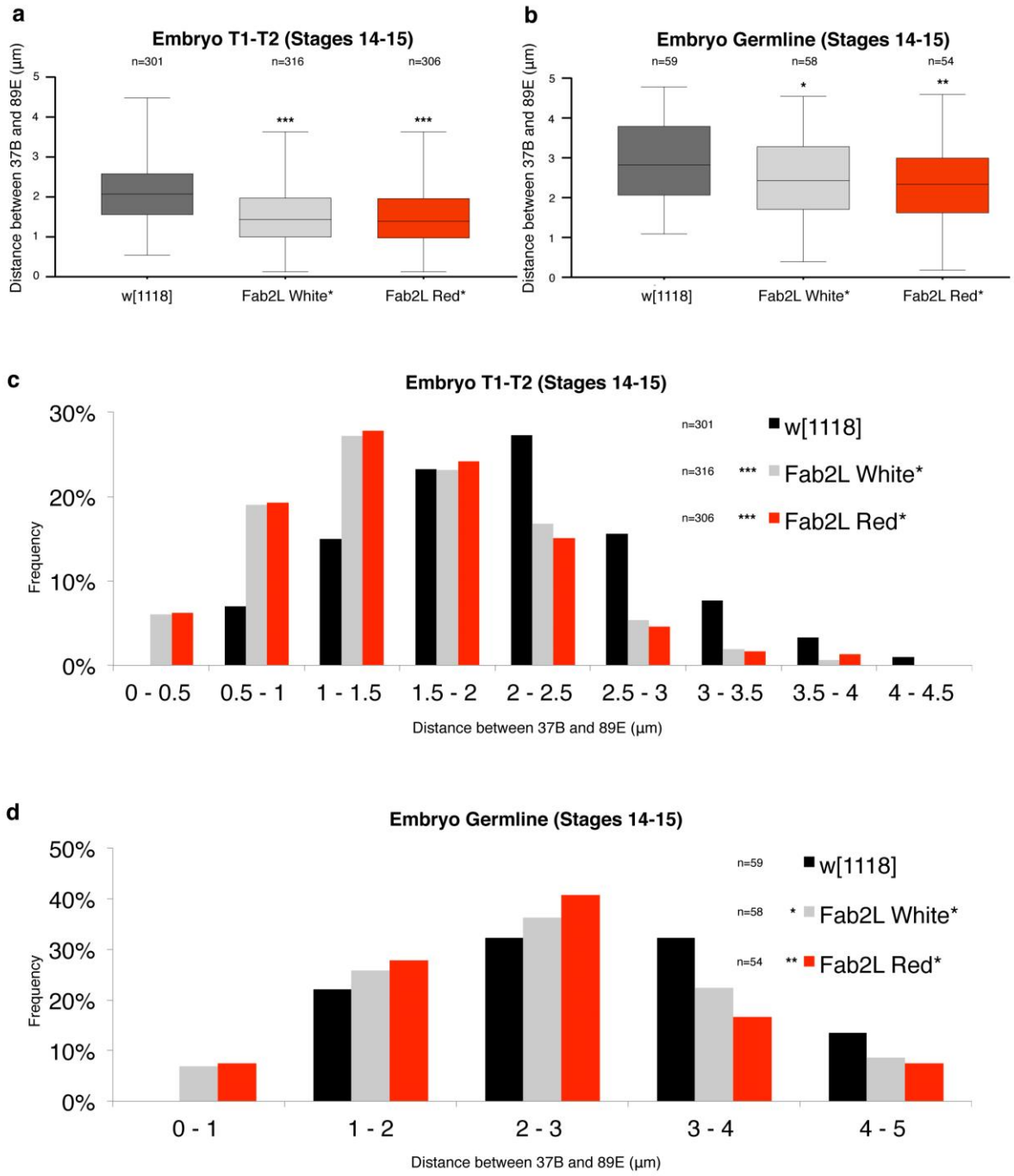
37B- and *89E*-loci long-range chromatin interactions and homologous unpairing in Fab2L lines.

A,B- Box plots representing the distance distributions of the FISH assays performed in the indicated genotypes between the *37B* (transgene insertion locus) and the *89E* (endogenous *Fab-7* locus) loci. Distances are measured in stage 14-15 embryos in T1 and T2 segments or in the germline. The centerline represents the median, the box delimits the interquartile-range and the limits define the distribution range. n represents the total number of nuclei analyzed from 3 embryos.

C,D- Charts representing the distance distributions of the FISH assays performed in the indicated genotypes between the *37B* and the *89E* loci. Distances are measured in stage 14-15 embryos in T1 and T2 segments or in the germline.

E- Frequency of homologous unpairing at the *37B* and the *89E* loci in the FISH assays performed in the indicated genotypes. Levels of unpairing are measured in stage 14-15 embryos in T1 and T2 segments, considering a minimum threshold distance between homologous loci of 0.5 µm.

Bars represent the frequency of distances between *37B* and *89E* loci (**C,D**) or the frequency of unpairing at *37B* and *89E* loci (**E**). In the figure, n represents the total number of nuclei analyzed from 3 embryos; two-tailed Student's *t*-test (**A-D**) or two-tailed Fisher's exact test (**E**); NS $P>0.05$; * $P<0.05$; ** $P<0.01$; *** $P<0.001$.



Supplementary Figure 7

37B- and *89E*-loci long-range chromatin interactions in Fab2L epilines.

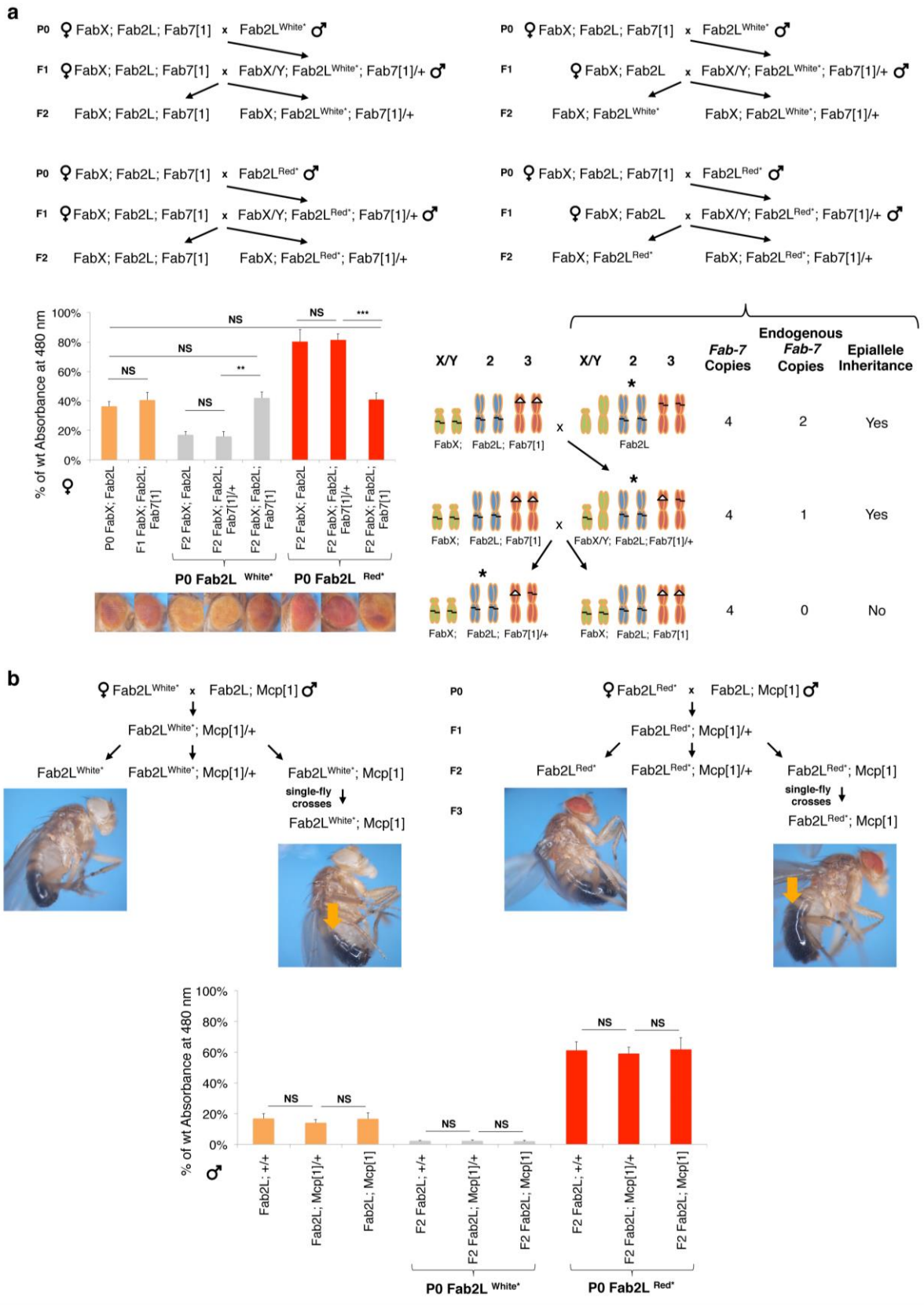
A,B- Box plots representing the distance distributions of the FISH assays performed in the indicated lines between the *37B* and the *89E* loci. Distances are measured in stage 14-15 embryos in T1 and T2 segments or in the germline. The centerline represents the median, the box delimits the interquartile-range and the limits define the distribution range.

C,D- Charts representing the distance distributions of the FISH assays performed in the indicated

lines between the *37B* and the *89E* loci. Distances are measured in stage 14-15 embryos in T1 and T2 segments or in the germline.

Bars represent the frequency of distances between *37B* and *89E* loci (**C,D**). In the figure, n represents the total number of nuclei analyzed from 3 embryos; two-tailed Student's *t*-test: NS $P>0.05$; * $P<0.05$; ** $P<0.01$; *** $P<0.001$.

Nature Genetics: doi:10.1038/ng.3848



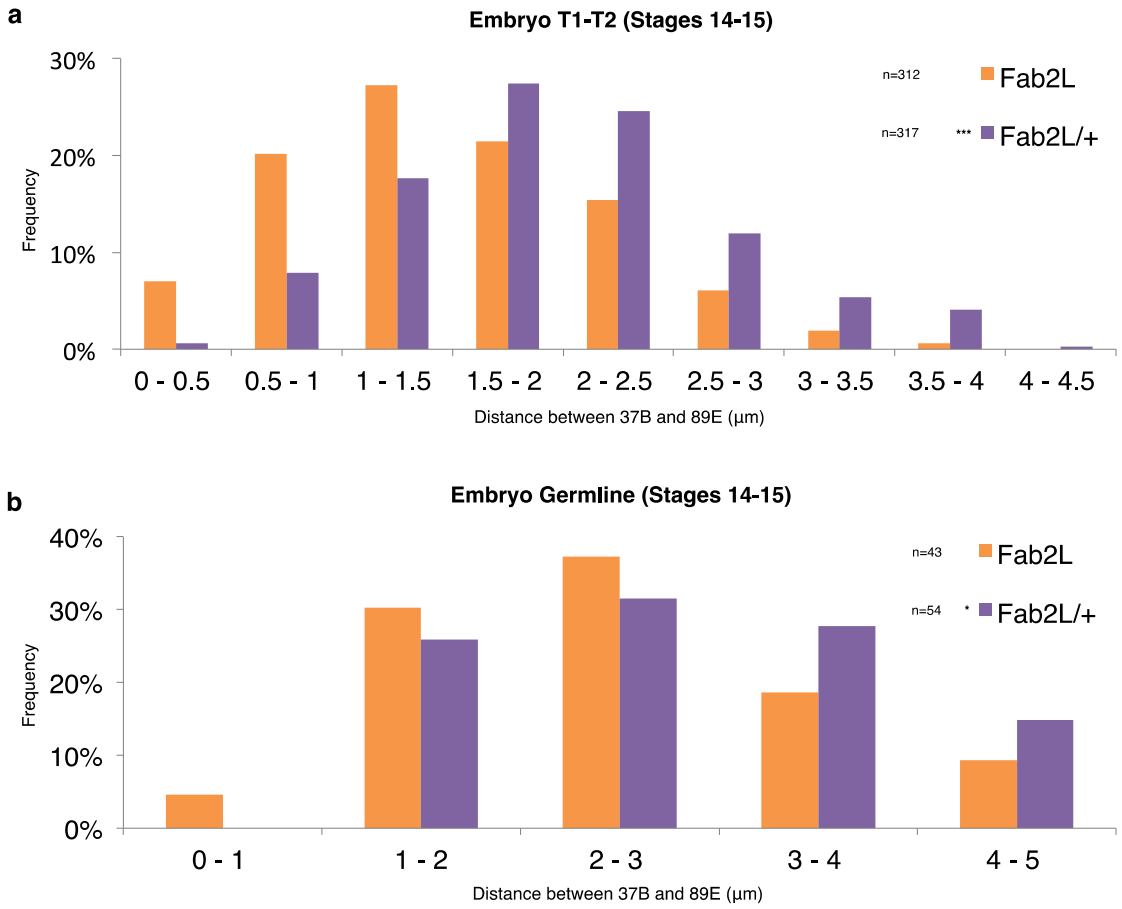
Supplementary Figure 8

Effects of the number of *Fab-7* loci and of the presence of endogenous *Mcp* on epiallele maintenance.
A- Crossing schemes, eye pigmentation assays and cartoons of the experiment testing the impact of *Fab-7* copy number on epiallele maintenance. The pictures are representative of the observed phenotypes. In the cartoons, the green chromosomes represent chromosomes X (acrocentric) and Y (metacentric) chromosomes, the blue chromosomes represent chromosome 2, the red chromosomes represent chromosome 3, the black lines represent the transgenic insertion on chromosome X and/or 2 or the endogenous *Fab-7* on chromosome 3, the white triangle represent the deletion of the endogenous *Fab-7* and the asterisks indicate the presence of the epiallele. On the right, the counting of total number of *Fab-7* copies, of endogenous *Fab-7* copies and the presence or not of the epiallele for each condition.

B- Crossing schemes, eye pigmentation assays and representative pictures of the phenotypes observed in the *Mcp[1]* epiallele maintenance tests. The single crosses in the F2 have been performed in order to unambiguously distinguish between hemizygous and homozygous Mcp[1] males. Pictures represent wt and Mcp[1] male flies with an A4 to A5 homeotic transformation (yellow arrows), carrying either $Fab2L^{White*}$ or $Fab2L^{Red*}$ epiallele.

Bars represent the mean of n=3 adult head collections, coming from the same original cross +/- s. d.; two-tailed Student's *t*-test: NS $P>0.05$; * $P<0.05$; ** $P<0.01$; *** $P<0.001$.

Nature Genetics: doi:10.1038/ng.3848

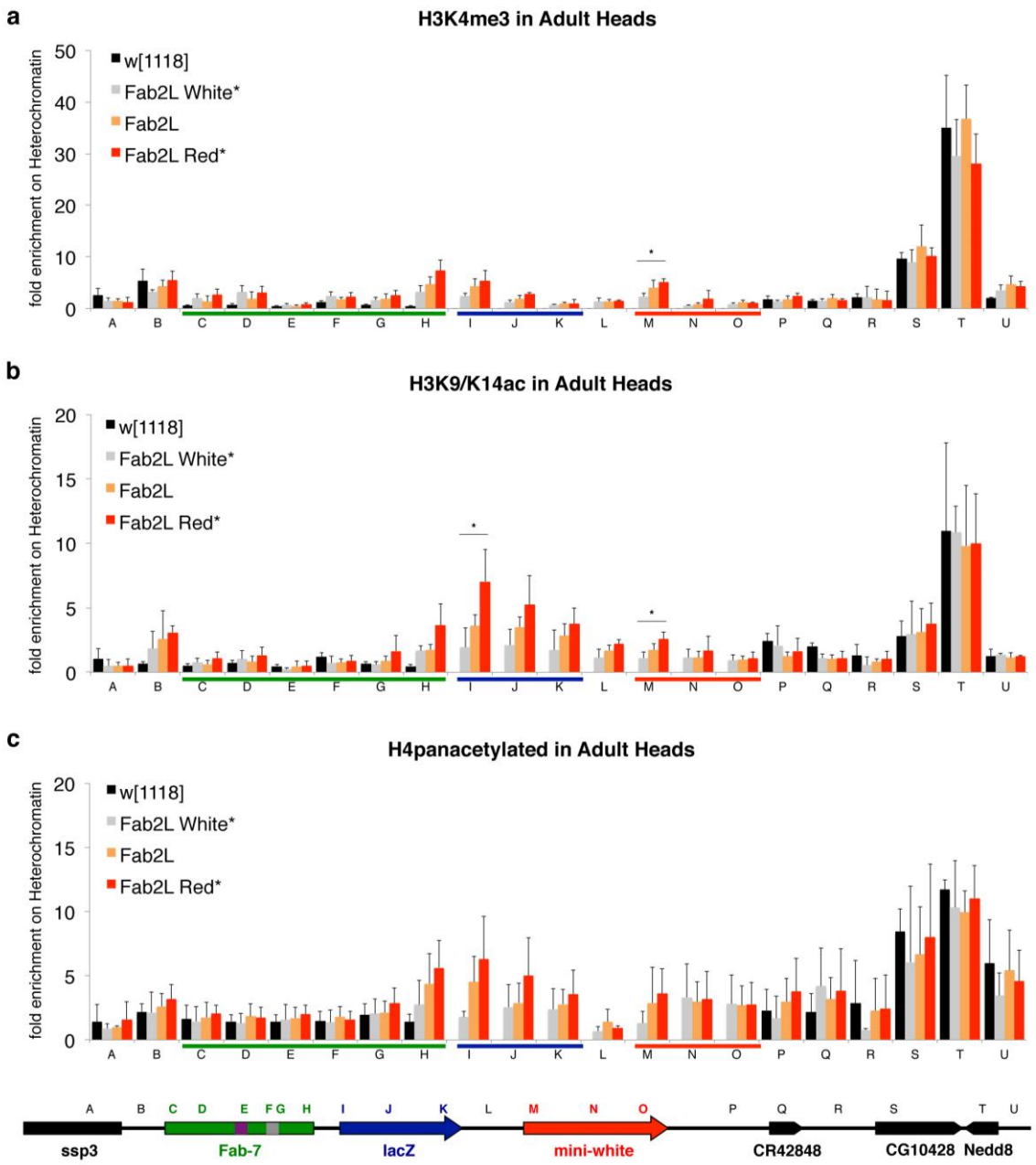


Supplementary Figure 9

37B- and *89E*-loci long-range chromatin interactions in Fab2L hemizygotes.

A,B- Charts representing the distance distributions of the FISH assays performed in the indicated genotypes between the *37B* and the *89E* loci. Distances are measured in stage 14-15 embryos in T1 and T2 segments or in the germline.

Bars represent the frequency of distances between *37B* and *89E* loci. In the figure, n represents the total number of nuclei analyzed from 3 embryos; two-tailed Student's *t*-test: NS $P>0.05$; * $P<0.05$; ** $P<0.01$; *** $P<0.001$.

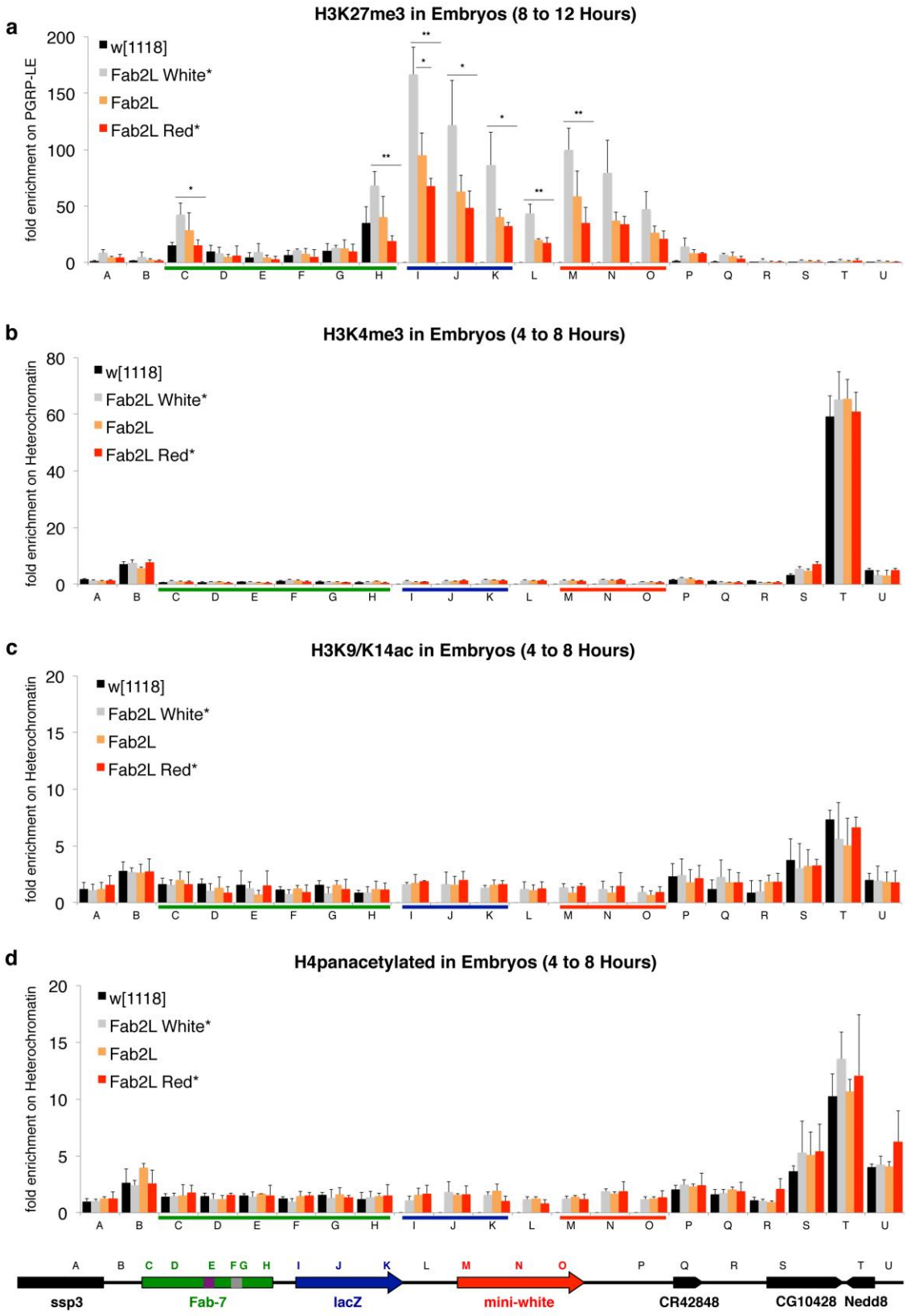


Supplementary Figure 10

Deposition of active chromatin marks in the adult head at the transgenic locus.

A,C- ChIP-qPCR assays performed on w[1118], Fab2L^{White*}, Fab2L and Fab2L^{Red*} male adult heads, showing relative enrichments (ChIP/Input) for H3K4me3, H3K9/K14ac and H4panacetylated normalized to a negative control. Amplicon locations are indicated below the charts.

Bars represent the mean of n=3 independent adult head collections +/- s. d.; two-tailed Student's *t*-test: NS $P>0.05$; * $P<0.05$; ** $P<0.01$; *** $P<0.001$.



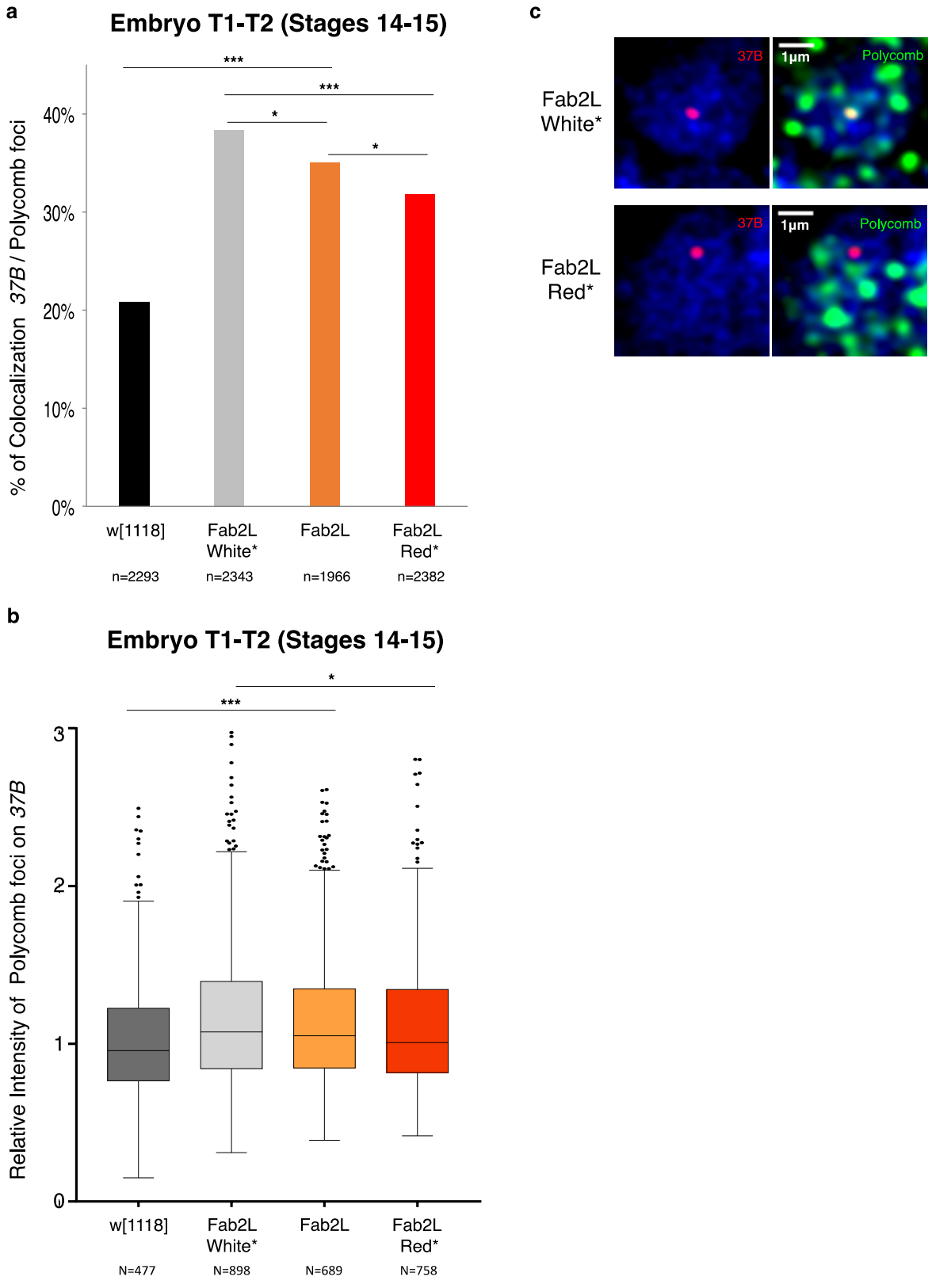
Supplementary Figure 11

Chromatin-mark deposition at the transgenic locus in embryos.

A- ChIP-qPCR assays performed on w[1118], Fab2L^{White*}, Fab2L and Fab2L^{Red*} embryos 8 to 12 hours showing relative enrichments (ChIP/Input) for H3K27me3 normalized to a negative control. Amplicon locations are indicated below the charts.

B-D- ChIP-qPCR assays performed on w[1118], Fab2L^{White*}, Fab2L and Fab2L^{Red*} embryos 4 to 8 hours, showing relative enrichments (ChIP/Input) for H3K4me3, H3K9/K14ac and H4panacetylated normalized to a negative control. Amplicon locations are indicated below the charts.

Bars represent the mean of n=3 independent embryo collections +/- s. d.; two-tailed Student's *t*-test: NS $P>0.05$; * $P<0.05$; ** $P<0.01$; *** $P<0.001$.



Supplementary Figure 12

37B-locus colocalization with Polycomb foci in the epilines.

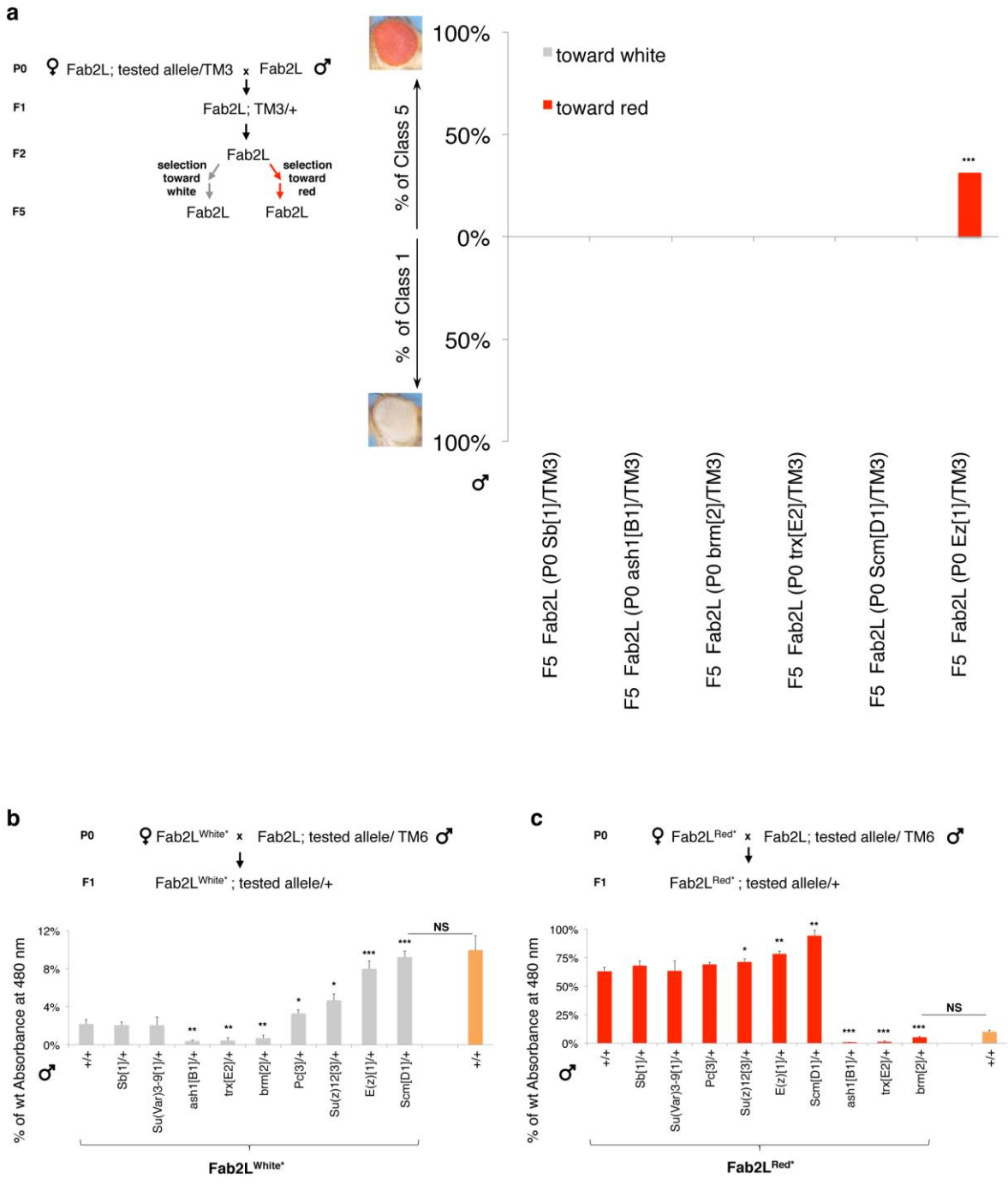
A- The charts show the percentage of centers of mass of the FISH signals (*37B* locus) that colocalize with a Polycomb focus in the indicated lines. In the figure, n represents the total number of FISH signals analyzed from 4 embryos. FISH-I assays were performed in T1 and T2 segments of stages 14-15 embryos.

B- The box plots show the distributions of the relative intensity of Polycomb within the centers of mass of the FISH signals for the different lines. In the figure, n represents the total number of Polycomb foci analyzed from 4 embryos. Polycomb intensities were scored only when FISH signals (*37B* locus) colocalized with Polycomb. The centerline represents the median, the box delimits the interquartile-range, the limits define the distribution range and the dots represent the outliers.

C- Examples of the FISH-I assays performed in the indicated epilines. Nuclei are stained with DAPI in blue, *37B* locus in red, Polycomb in green. Scale bar is 1 µm.

Bars represent the frequency of colocalization (**A**); two-tailed Fisher's exact test (**A**) or two-tailed Mann-Whitney test (**B**): NS $P>0.05$; * $P<0.05$; ** $P<0.01$; *** $P<0.001$.

Nature Genetics: doi:10.1038/ng.3848



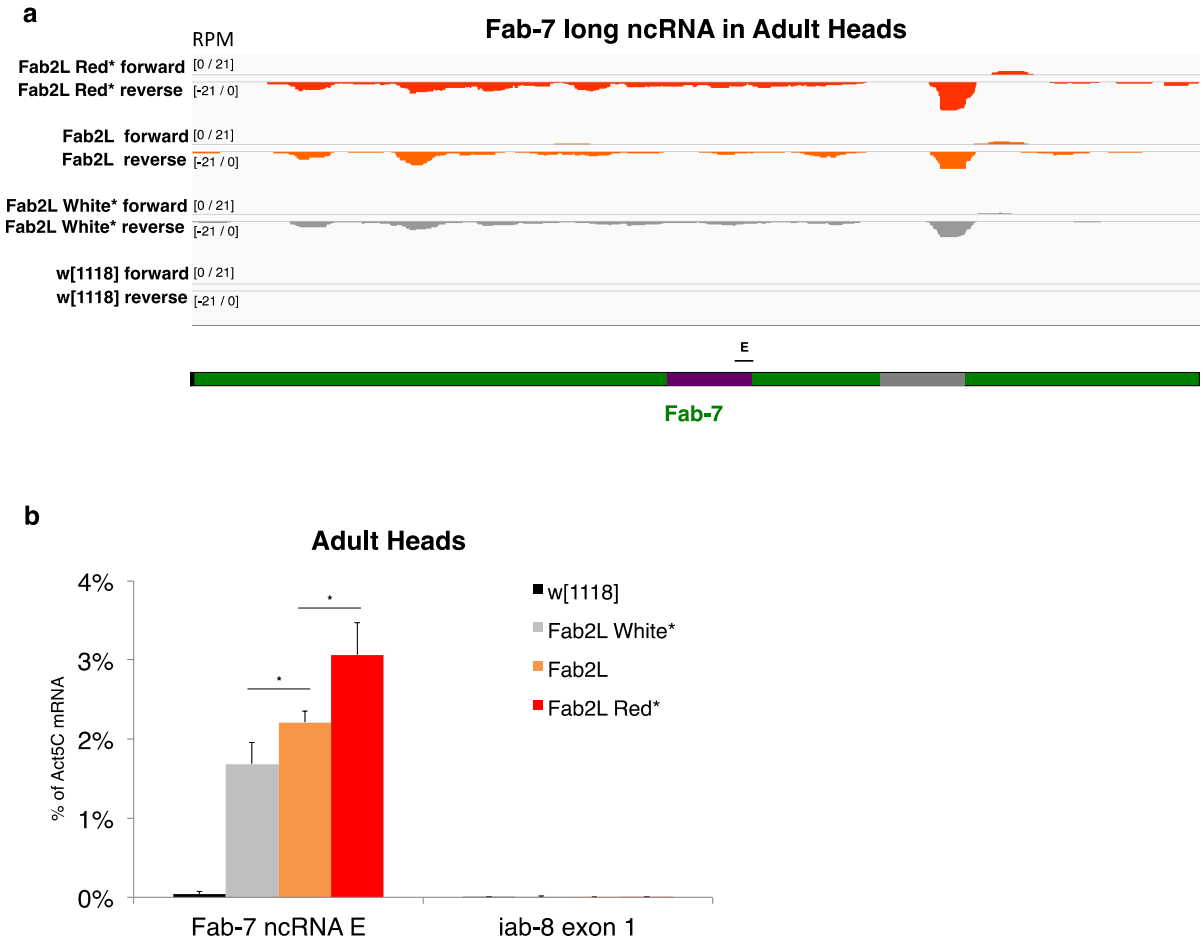
Supplementary Figure 13

Epiline establishment via *E(z)[1]*/+ and epiline genetic interactions.

A- Crossing scheme for phenotypic selection and charts representing the percentage of Class 1 (pigment=0%) male flies in grey and Class 5 (pigment=100%) male flies in red at the F5 (n>40). As a negative control, we used the unrelated *Sb[1]* mutation, which did not allow establishing of epialleles upon selection. As a corollary, this control scheme shows that the presence of the TM3 balancer in the F1 does not trigger the induction of epialleles.

B,C- Crossing schemes and eye pigmentation assays performed on Fab2L (orange), Fab2L^{White*} (grey) and Fab2L^{Red*} (red) male flies, combined with the tested alleles on chromosome 3.

Bars represent the frequency of Class 1 (white-eyed) and Class 5 (red-eyed) (**A**) or the mean +/- s. d. of n=3 independent crosses (**B,C**); two-tailed Fisher's exact test: NS $P>0.05$; * $P<0.05$; ** $P<0.01$; *** $P<0.001$

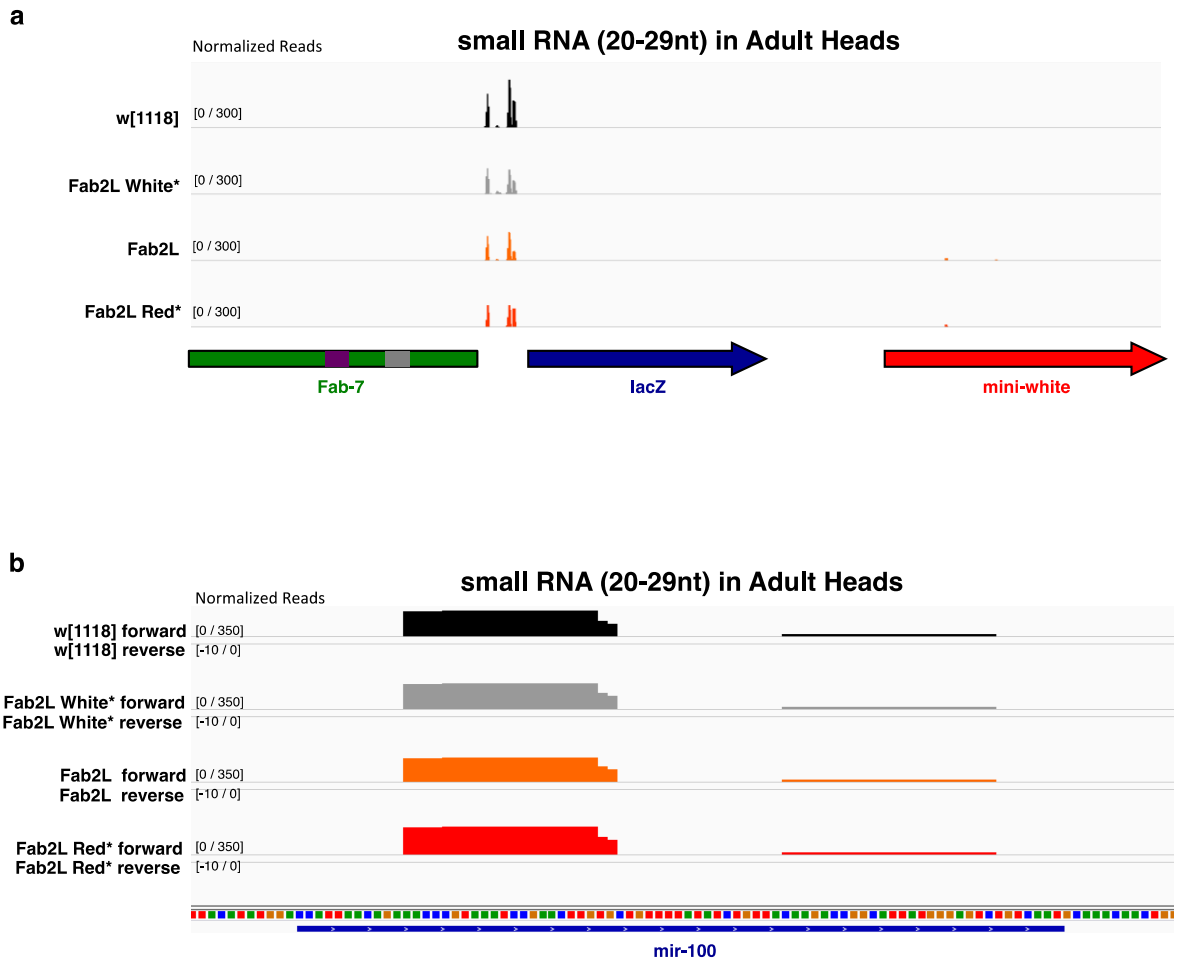


Supplementary Figure 14

Fab-7 long-ncRNA expression in adult heads.

A- IGV browser screen shots displaying the normalized transcriptome read density profiles on the *Fab-7* transgene from different fly lines, separated by strand. Data scale represents reads per million (RPM).

B- RT-qPCR assays performed on w[1118], Fab2L^{White*}, Fab2L and Fab2L^{Red*} male adult heads, measuring relative mRNA levels of the *Fab-7* ncRNA normalized to *Act5C.* Bars represent the mean of n=3 independent adult head collections +/- s. d.; two-tailed Student's *t*-test: NS $P>0.05$; * $P<0.05$; ** $P<0.01$; *** $P<0.001$.

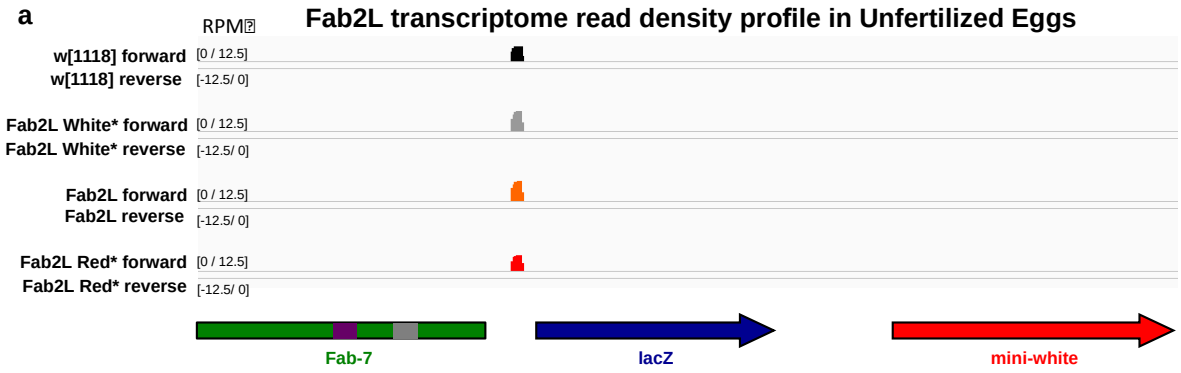


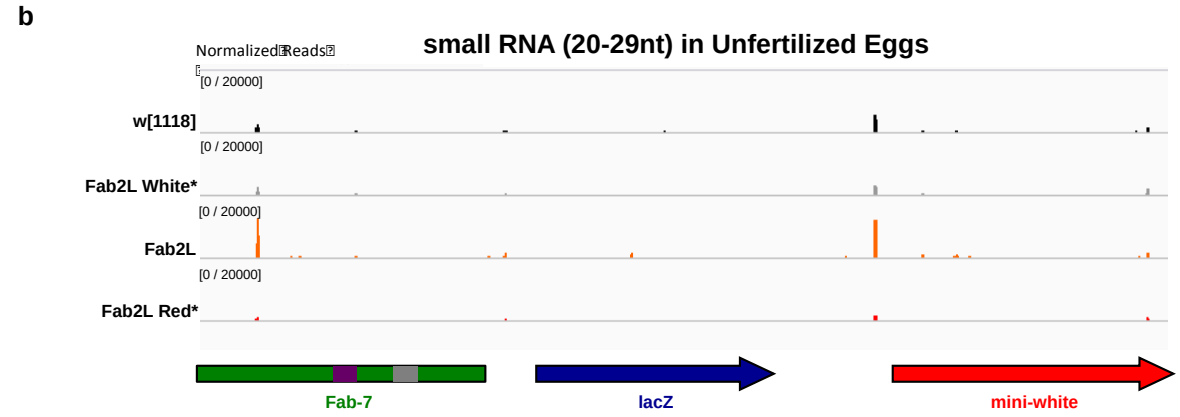
Supplementary Figure 15

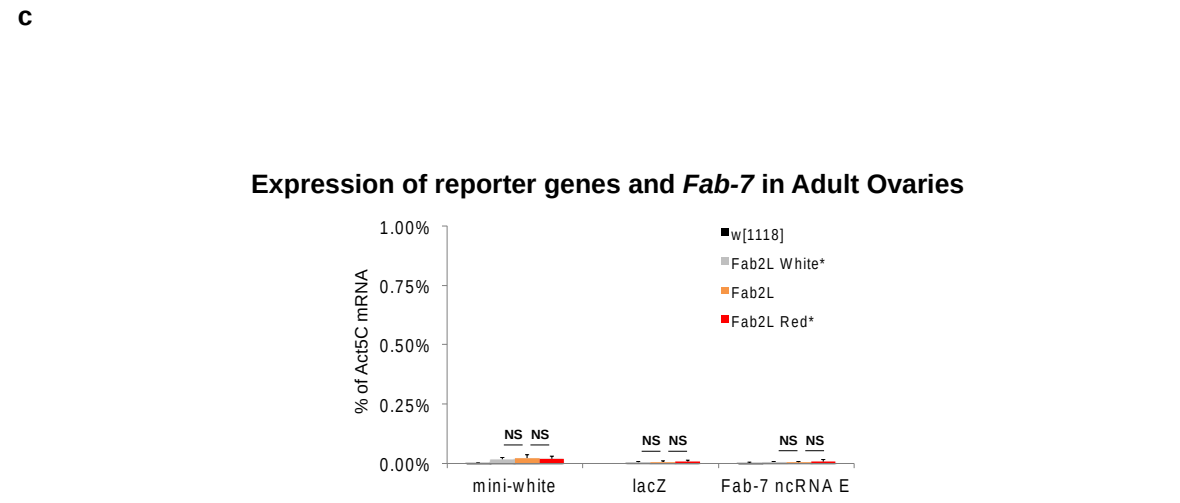
Lack of small-RNA expression at the transgenic locus in adult heads.

A- IGV browser screen shots displaying the small RNA read density profiles on the *Fab2L* transgene from different fly lines. The data scale represents number of reads, normalized for the sequencing depth using mir-184-3p as an endogenous reference; reads were mapped to the transgene sequence allowing 0 mismatches. Note that the reads displayed on the *lacZ* promoter are not unique to the transgene sequence.

B- IGV browser screen shots displaying the small RNA read density profiles from different fly lines, separated by strand. Data scale represents number of reads, normalized per million mapped reads. mir-100 represents a control microRNA that is expressed in *Drosophila* adult heads.







Supplementary Figure 16

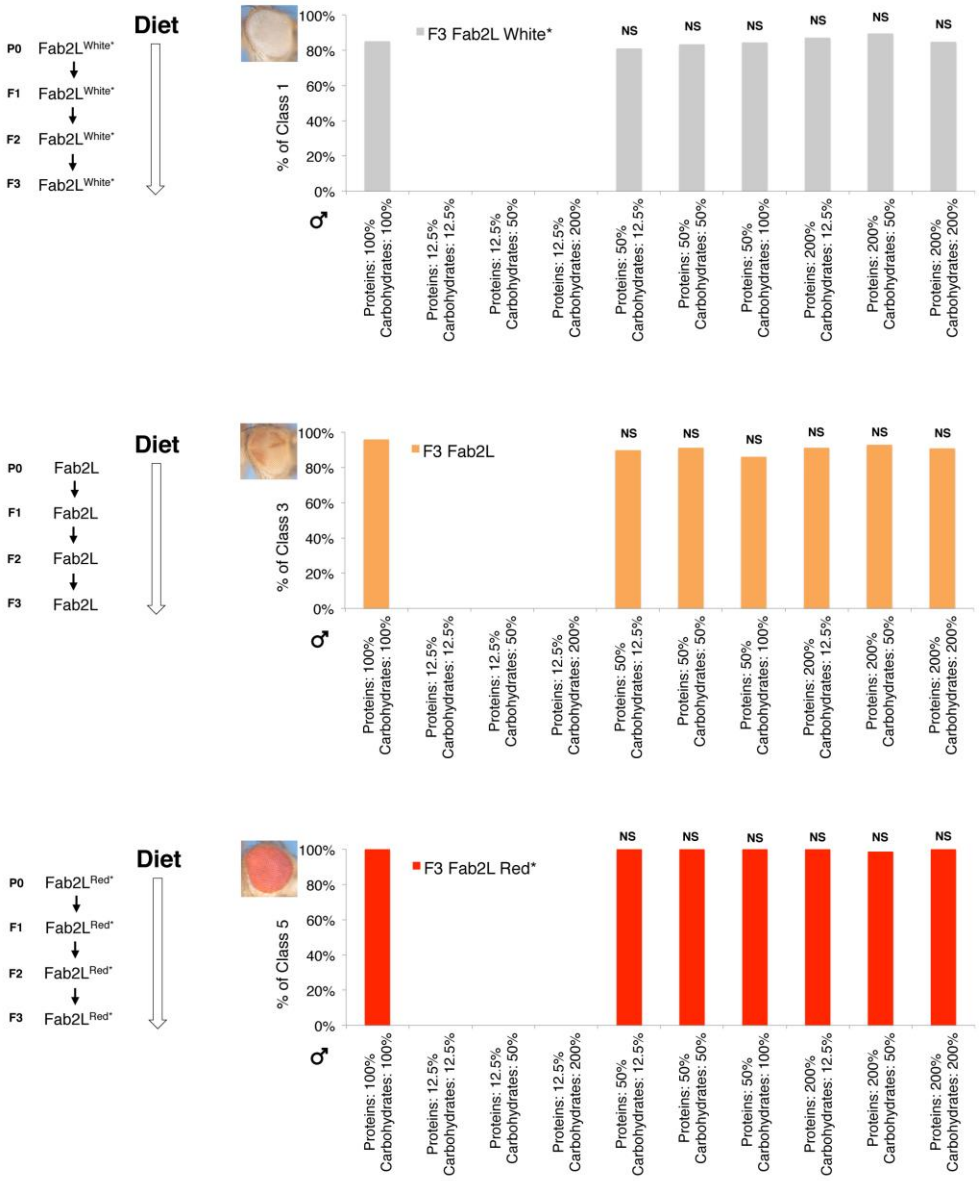
Lack of small- and long-ncRNA expression at the transgenic locus in unfertilized eggs and ovaries.

A- IGV browser screen shots displaying the normalized transcriptome read density profiles on the *Fab2L* transgene from different fly lines, separated by strand. Data scale represents reads per million

(RPM). Note that the few observed reads displayed on the *lacZ* promoter are not unique.

B- IGV browser shots displaying the small RNA read density profiles on the *Fab2L* transgene from different fly lines. Data scale represents number of reads, normalized for the sequencing depth using mir-184-3p as an endogenous reference; reads were mapped to the transgene sequence allowing 0 mismatches; some reads are not unique to the transgene sequence.

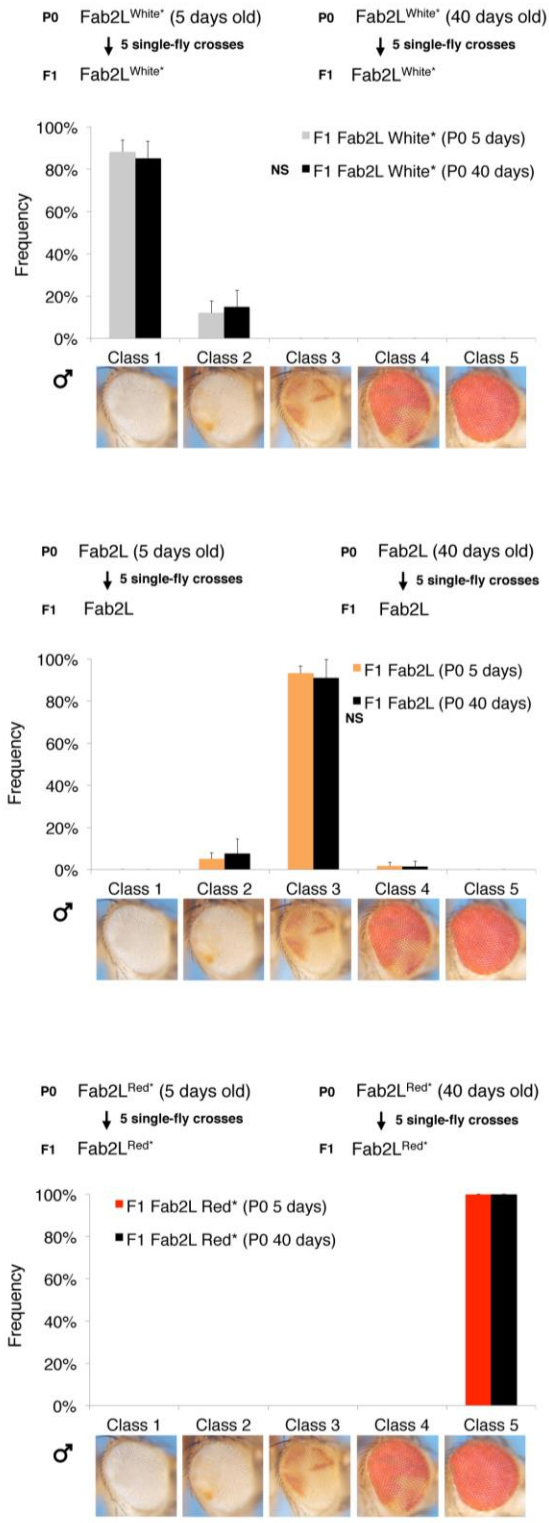
C- RT-qPCR assays performed on w[1118], Fab2L ^{White*} , Fab2L and Fab2L ^{Red*} adult ovaries, measuring relative mRNA levels of *mini-withe*, *lacZ* and the *Fab-7* ncRNA normalized to *Act5C.* Bars represent the mean of n=3 independent ovary collections +/- s. d.; two-tailed Student's *t*-test: NS $P>0.05$; * $P<0.05$; ** $P<0.01$; *** $P<0.001$.



Supplementary Figure 17

Lack of effects of diet treatments on epiallele inheritance.

Diet exposures and phenotypic classification based on eye pigment levels of percentage of Class 1 (pigment=0%), percentage of Class 3 (5%<pigment≤75%) and percentage of Class 5 (pigment=100%) in Fab2L^{White*}, Fab2L and Fab2L^{Red*} male adult heads, respectively. The experiment was performed once per condition. The lack of bars for some conditions indicates the absence of adult progeny. Bars represent the frequency of n>15 flies scored; two-tailed Fisher's exact test: NS P>0.05; * P<0.05; ** P<0.01; *** P<0.001.

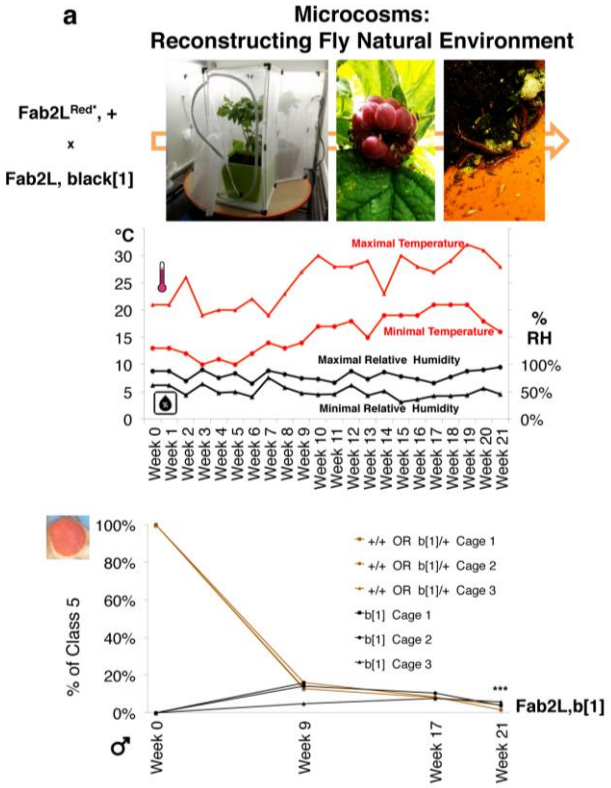


Supplementary Figure 18

Lack of effects of parental age on epiallele inheritance.

Crossing schemes performed with differentially aged P0 flies and phenotypic classification of their F1 generation, based on eye pigment levels of Fab2L, Fab2L^{White*} and Fab2L^{Red*} male adult heads. 5 single-fly crosses were performed for each condition and n>15 flies were scored per replicate. Bars represent the mean of the frequencies of n=5 single-fly cross progenies +/- s. d.; two-tailed Student's *t*-test: NS $P>0.05$; * $P<0.05$; ** $P<0.01$; *** $P<0.001$.

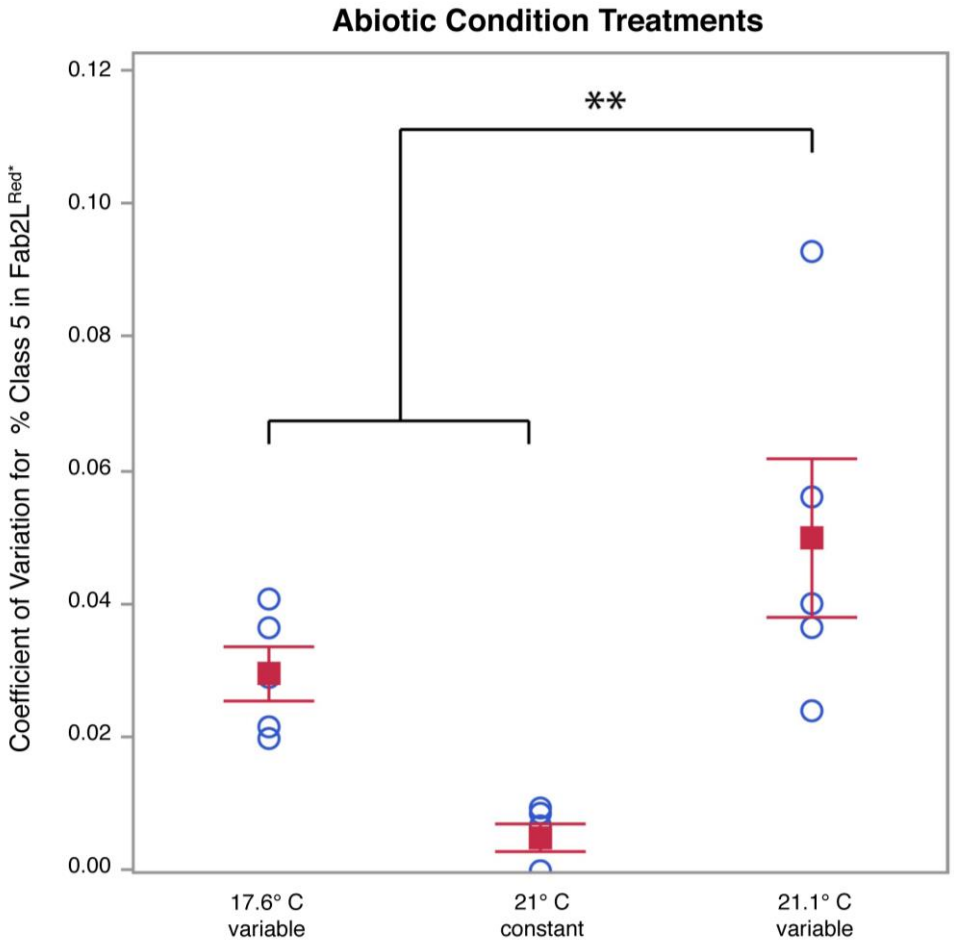
Nature Genetics: doi:10.1038/ng.3848



Supplementary Figure 19

Paramutation effect in the natural environment.

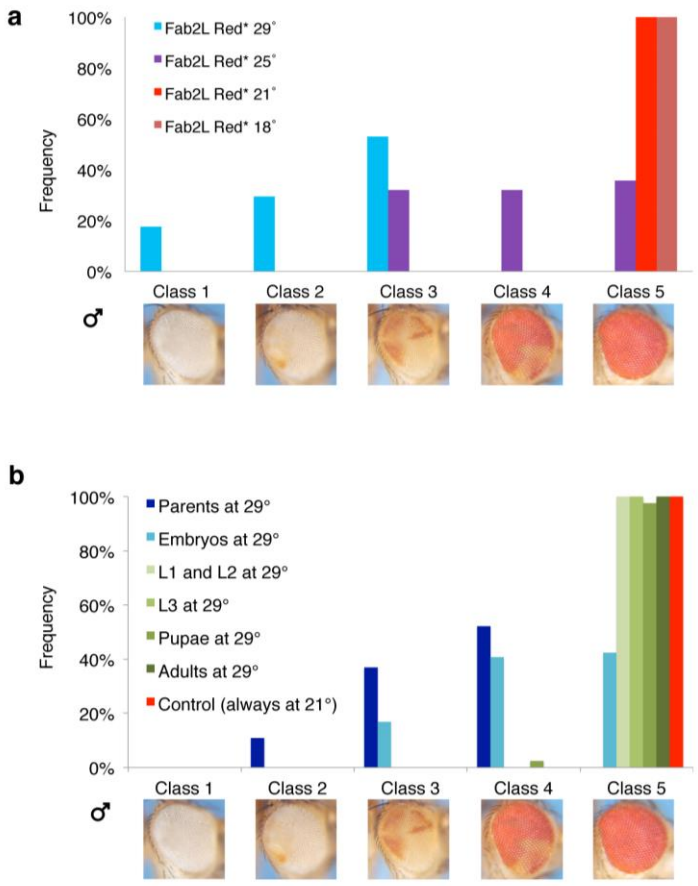
Schematic representation and illustrative pictures of the cross between Fab2L^{Red*},+ and Fab2L, black[1], and its long-term exposure to natural conditions. The top chart indicates maximal and minimal temperature and relative humidity in the reproduced weeks. The bottom chart shows the percentage of Class 5 (pigment=100%) flies at four time points; The crosses were performed in n=3 independent cages and the phenotypic classification was based on the body color (wild-type or black). Linear mixed-model analysis between Week 0 and Week 21 time points: NS $P>0.05$; * $P<0.05$; ** $P<0.01$; *** $P<0.001$.



Supplementary Figure 20

Effects of abiotic-condition treatment on the Fab2L^{Red}* epiline.

Effect of constant and fluctuating abiotic conditions (temperature and humidity) on eye phenotype variability among n=5 independent populations of the same Fab2L^{Red*} epiline. Greater coefficients of variation indicate greater variations of eye phenotypes among generations in each replicate population. n>20 flies were scored per replicate. Two-tailed Student's *t*-test: NS $P>0.05$; * $P<0.05$; ** $P<0.01$; *** $P<0.001$.



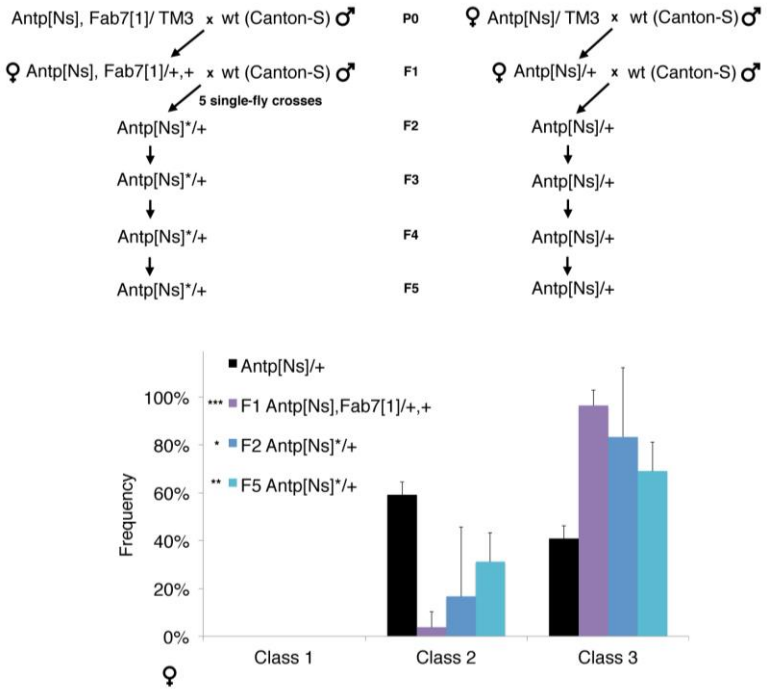
Supplementary Figure 21

Effects of high temperature on the Fab2L^{Red*} epiline.

A- Phenotypic classification based on eye pigment levels in Fab2L^{Red*} flies reared at different temperatures.

B- Phenotypic classification based on eye pigment levels in Fab2L^{Red*} flies reared either at 21°C constant temperature (red bars), or at 29°C only during the specified developmental stages (green and blue bars) (n>30) and 21°C during the other stages. Class 1: pigment=0%; Class 2: 0%<pigment≤5%; Class 3: 5%<pigment≤75%; Class 4: 75%<pigment<100%; Class 5: pigment=100%.

Bars represent the frequency of n>50 **(A)** or n>30 **(B)** flies scored.



Supplementary Figure 22

Antp[Ns] epiallele establishment in the Canton-S background.

Crossing schemes and charts representing the phenotypic distributions of the Antp[Ns] homeotic transformation phenotype in adult females for each generation. Phenotypic classification of the antenna to leg transformation phenotype. Class 1: weak transformation; Class 2: medium transformation; Class 3: severe transformation. Bars represent the mean of the frequencies of n=5 parallel single-fly crosses, deriving from independent recombination events in the F1, +/- s. d.; two-tailed Fisher's exact test: NS $P>0.05$; * $P<0.05$; ** $P<0.01$; *** $P<0.001$. Fisher's exact test was applied on the pooled populations from the 5 independent single-fly crosses at each generation. After pooling, flies were n>10 for each genotype.