A Balance Between Euchromatic (JIL-1) and Heterochromatic [SU(VAR)2-5 and SU(VAR)3-9] Factors Regulates Position-Effect Variegation in *Drosophila*

Chao Wang, Jack Girton, Jørgen Johansen, and Kristen M. Johansen[1]
Department of Biochemistry, Biophysics, and Molecular Biology, Iowa State University, Ames, Iowa 50011

ABSTRACT In this study, we show that the haplo-enhancer effect of *JIL-1* has the ability to counterbalance the haplo-suppressor effect of both *Su(var)3-9* and *Su(var)2-5* on position-effect variegation, providing evidence that a finely tuned balance between the levels of JIL-1 and the major heterochromatin components contributes to the regulation of gene expression.

THE essential JIL-1 histone H3S10 kinase (Jin *et al.* 1999; Wang *et al.* 2001) is a major regulator of chromatin structure (Deng *et al.* 2005, 2008) that functions to maintain euchromatic domains while counteracting heterochromatization and gene silencing (Ebert *et al.* 2004; Lerach *et al.* 2006; Zhang *et al.* 2006; Bao *et al.* 2007). In the absence of the JIL-1 kinase, the major heterochromatin markers H3K9me2, HP1a [Su(var)2-5], and Su(var)3-7 spread to ectopic locations on the chromosome arms (Zhang *et al.* 2006; Deng *et al.* 2007, 2010). These observations suggested a model for a dynamic balance between euchromatin and heterochromatin (Ebert *et al.* 2004; Zhang *et al.* 2006; Deng *et al.* 2010), where, as can be monitored in position-effect variegation (PEV) arrangements, the boundary between these two states is determined by antagonistic functions of a euchromatic regulator (JIL-1) and the major determinants of heterochromatin assembly, *e.g.*, Su(var)3-9, HP1a, and Su(var)3-7 (for review see Weiler and Wakimoto 1995; Girton and Johansen 2008). In support of this model, Deng *et al.* (2010) recently showed that *Su(var)3-7* and *JIL-1* loss-of-function mutations have an antagonistic and counterbalancing effect on gene expression using PEV assays; however, potential dynamic interactions between *JIL-1* and the other two heterochromatin genes, *Su(var)3-9* and *Su(var)2-5*, were not addressed in this study. Interestingly, in other genetic interaction assays monitoring the lethality as well as the chromosome morphology defects associated with the null *JIL-1* phenotype, only a reduction in the dose of the *Su(var)3-9* gene (Zhang *et al.* 2006; Deng *et al.* 2007) rescued both phenotypes. In contrast, in the same assays a reduction of *Su(var)3-7* rescued the lethality, but not the chromosome defects (Deng *et al.* 2010), and no genetic interactions were detectable between *JIL-1* and *Su(var)2-5* (Deng *et al.* 2007). Thus, these findings indicate that while Su(var)3-9 activity may be a major factor in the lethality and chromatin-structure perturbations associated with loss of the JIL-1 histone H3S10 kinase, these effects are likely to be uncoupled from HP1a and, to a lesser degree, from Su(var)3-7. This raises the question of whether *JIL-1* dynamically interacts with the two other heterochromatin genes, *Su(var)2-5* and *Su(var)3-9*, in regulating gene expression, as it does with *Su(var)3-7*.

To answer this question, we explored the effect of various combinations of loss-of-function alleles of *JIL-1* and *Su(var)3-9* or *Su(var)2-5* on PEV caused by the *P*-element insertion line *118E-10* (Wallrath and Elgin 1995; Wallrath *et al.* 1996). Insertion of this *P* element (*P[hsp26-pt, hsp70-w]*) into euchromatic sites results in a uniform red-eye phenotype whereas insertion into a known heterochromatin region of the fourth chromosome results in a variegating eye phenotype (Cryderman *et al.* 1998; Bao *et al.* 2007) (Figures 1 and 2). It has been demonstrated that loss-of-function *JIL-1* alleles can act as haplo-enhancers of PEV, resulting in increased silencing of gene expression (Deng *et al.* 2010), whereas loci for structural components of heterochromatin

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[1]Corresponding author: Department of Biochemistry, Biophysics, and Molecular Biology, 3154 Molecular Biology Bldg., Iowa State University, Ames, IA 50011. E-mail: kristen@iastate.edu

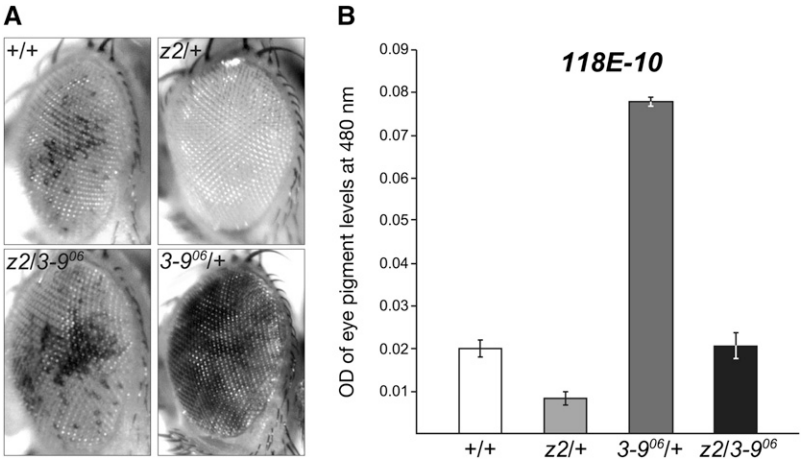

Figure 1 Counterbalancing effect of *JIL-1* and *Su(var)3-9* loss-of-function alleles on the PEV of the *P*-element insertion line *118E-10*. (A) Examples of the degree of PEV in the eyes of wild-type *JIL-1* and *Su(var)3-9* (+/+), *JIL-1^{z2}*/+ (*z2*/+), *Su(var)3-9^{06}*/+ (*3-9^{06}*/+), and *JIL-1^{z2}/Su(var)3-9^{06}* (*z2/3-9^{06}*) flies in a *118E-10*/+ background. All images are from male flies. (B) Histograms of the amount of eye pigment in +/+, *JIL-1^{z2}*/+ (*z2*/+), *Su(var)3-9^{06}*/+ (*3-9^{06}*/+), and *JIL-1^{z2}/Su(var)3-9^{06}* (*z2/3-9^{06}*) male flies heterozygous for *118E-10*. Fly stocks were maintained according to standard protocols (Roberts 1998). Oregon-R was used for wild-type preparations. The *JIL-1^{z2}* allele is described in Wang *et al.* (2001) and in Zhang *et al.* (2003). The *Su(var)3-9^{06}* and *Su(var)2-5^{05}* alleles are described in Schotta *et al.* (2002) and in Eissenberg *et al.* (1992). The *P*-element *P[hsp26-pt, hsp70-w]* insertion line *118E-10* was the generous gift of L. Wallrath. The *hsp70* promoter is leaky and promotes sufficient expression to generate a variegated eye phenotype under non-heat-shock conditions (Wallrath and Elgin 1995; Bao *et al.* 2007). PEV assays were performed as previously described in Lerach *et al.* (2006) and in Bao *et al.* (2007). In short, various combinations of *JIL-1, Su(var)3-9*, or *Su(var)2-5* alleles were introduced into the *118E-10* or *w^{m4}* PEV arrangements by standard crossing. To quantify the variegated phenotype, adult flies were collected from the respective crosses at eclosion, aged 6 days at 25°, frozen in liquid nitrogen, and stored at −80°C until assayed. The pigment assays were performed essentially as in Kavi and Birchler (2009) using three sets of 10 fly heads of each genotype collected from males and females. For each sample, the heads from the 10 flies were homogenized in 200 μl of methanol with 0.1% hydrochloric acid and centrifuged, and the OD of the supernatant was spectrophotometrically measured at a wavelength of 480 nm.

such as *Su(var)3-9*, *Su(var)2-5*, and *Su(var)3-7* act as strong haplo-suppressors (Eissenberg *et al.* 1990; Reuter *et al.* 1990; Tschiersch *et al.* 1994). In the experiments, the transgenic reporter line *118E-10* was crossed into *JIL-1^{z2}*/+, *Su(var)3-9^{06}*/+, and *Su(var)2-5^{05}*/+ mutant backgrounds as well as into *JIL-1^{z2}/Su(var)3-9^{06}* and *JIL-1^{z2}/Su(var)2-5^{05}* double-mutant backgrounds. The *JIL-1^{z2}* allele is a true null allele (Wang *et al.* 2001; Zhang *et al.* 2003), the loss-of-function *Su(var)3-9^{06}* allele is due to a DNA insertion (Schotta *et al.* 2002), and the *Su(var)2-5^{05}* loss-of-function allele is associated with a frameshift resulting in a nonsense peptide containing only the first 10 amino acids of HP1a (Eissenberg *et al.* 1992). Thus, to test whether the heterozygous *JIL-1^{z2}* allele could counterbalance the suppression of the *Su(var)3-9^{06}* or *Su(var)2-5^{05}* loss-of-function alleles of the PEV of *118E-10*, we compared the eye pigment levels of the various genotypes (Figures 1 and 2 and Table 1). Pigment assays were performed essentially as in Kavi and Birchler (2009) using three sets of 10 pooled fly heads from each genotype. Although both male and female flies were scored, due to sex differences only results from male flies are shown. However, the trend observed in female flies was identical to that in male flies (supporting information, Figure S1). As illustrated in Figures 1 and 2, the heterozygous *JIL-1^{z2}*/+ genotype enhances PEV as indicated by the increased proportion of white ommatidia and a 59% decrease in the optical density (OD) of the eye pigment levels (0.0083 ± 0.0015; $n = 3$) as compared to +/+ flies (0.0203 ± 0.0021; $n = 3$). This reduction was statistically significant (Table 1). In contrast, the heterozygous *Su(var)3-9^{06}*/+ and *Su(var)2-5^{05}*/+ genotypes suppress PEV as indicated by an increase of the proportion of red ommatidia and a statistically significant (Table 1) 384% (0.078 ± 0.001; $n = 3$) and 330% (0.067 ± 0.004; $n = 3$) increase, respectively, in the OD of the eye pigment levels. However, in the *JIL-1^{z2}/Su(var)3-9^{06}* and *JIL-1^{z2}/Su(var)2-5^{05}* double-mutant backgrounds, variegation

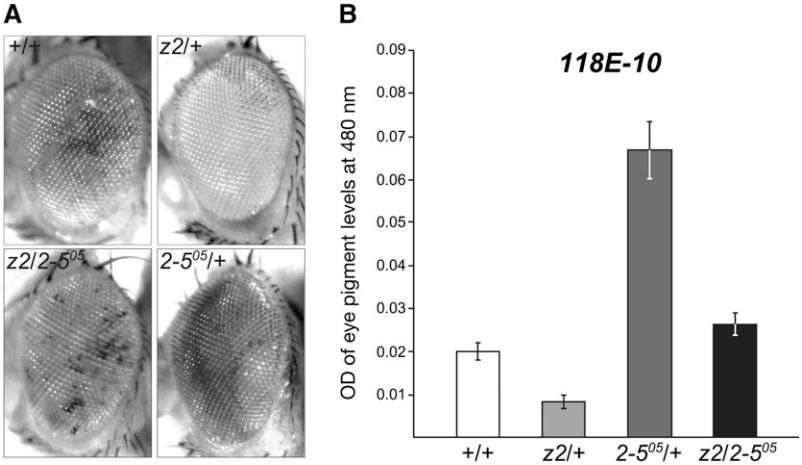

Figure 2 Counterbalancing effect of *JIL-1* and *Su(var)2-5* loss-of-function alleles on the PEV of the *P*-element insertion line *118E-10*. (A) Examples of the degree of PEV in the eyes of wild-type *JIL-1* and *Su(var)2-5* (+/+), *JIL-1^{z2}*/+ (*z2*/+), *Su(var)2-5^{05}*/+ (*2-5^{05}*/+), and *JIL-1^{z2}/Su(var) 2-5^{05}* (*z2/2-5^{05}*) flies in a *118E-10*/+ background. All images are from male flies. (B) Histograms of the levels of eye pigment in +/+, *JIL-1^{z2}*/+ (*z2*/+), *Su(var2-5)^{05}*/+ (*2-5^{05}*/+), and *JIL-1^{z2}/Su(var)2-5^{05}* (*z2/2-5^{05}*) male flies heterozygous for *118E-10*.

Table 1 Statistical comparison of eye pigment assays

Genotype[a]	*JIL-1^{z2}*/+	*3-9^{06}*/+	*JIL-1^{z2}/3-9^{06}*
118E-10/+			
+/+	$P < 0.005$	$P < 0.0001$	$P > 0.8$
JIL-1^{z2}/+	—	$P < 0.0001$	$P < 0.005$
3-9^{06}/+	—	—	$P < 0.0001$
Genotype[a]	*JIL-1^{z2}*/+	*2-5^{05}*/+	*JIL-1^{z2}/2-5^{05}*
118E-10/+			
+/+	$P < 0.005$	$P < 0.0001$	$P > 0.06$
JIL-1^{z2}/+	—	$P < 0.0001$	$P < 0.005$
2-5^{05}/+	—	—	$P < 0.0005$
Genotype	*JIL-1^{z2}*/+	*3-9^{06}*/+	*JIL-1^{z2}/3-9^{06}*
w^{m4}/Y			
+/+	$P < 0.005$	$P < 0.0001$	$P < 0.0001$
JIL-1^{z2}/+	—	$P < 0.0001$	$P < 0.0001$
3-9^{06}/+	—	—	$P < 0.001$
Genotype	*JIL-1^{z2}*/+	*2-5^{05}*/+	*JIL-1^{z2}/2-5^{05}*
w^{m4}/Y			
+/+	$P < 0.005$	$P < 0.0001$	$P < 0.0001$
JIL-1^{z2}/+	—	$P < 0.0001$	$P < 0.0001$
2-5^{05}/+	—	—	$P < 0.001$

For each genotype, the average pigment level from three sets of measurements from 10 pooled fly heads were compared using a two-tailed Student's *t*-test.
[a] Only male flies were scored.

of the proportion of red ommatidia was intermediate, and the eye pigment levels (0.0207 ± 0.0031 and 0.0263 ± 0.0035, respectively; $n = 3$) were statistically indistinguishable from genotypes with +/+ levels of JIL-1, Su(var)3-9, and Su(var)2-5 proteins (Figures 1 and 2 and Table 1).

To test whether a heterozygous *JIL-1* null allele also could counterbalance the suppression of the *Su(var)3-9^{06}* or *Su(var)2-5^{05}* alleles of the PEV of *w^{m4}*, we performed experiments similar to those described above for *118E-10*. As illustrated in Figure 3 for male flies, the heterozygous *JIL-1^{z2}*/+ genotype enhances PEV as indicated by a 67% decrease in the OD of the eye pigment levels (0.008 ± 0.002; $n = 3$) as compared to +/+ flies (0.024 ± 0.003, $n = 3$). This reduction was statistically significant (Table 1). In contrast, the heterozygous *Su(var)3-9^{06}*/+ and *Su(var)2-5^{05}*/+ genotypes suppress PEV as indicated by a statistically significant (Table 1) 1115% (0.2677 ± 0.0061; $n = 3$) and 1023% (0.2454 ± 0.0103; $n = 3$) increase, respectively, in the OD of the eye pigment levels. However, in the *JIL-1^{z2}/Su(var)3-9^{06}* and *JIL-1^{z2}/Su(var)2-5^{05}* double-mutant backgrounds, the eye pigment levels (0.2337 ± 0.0011 and 0.2043 ± 0.0037, respectively; $n = 3$) were significantly reduced (Table 1) by 13% and 17% as compared to heterozygous *Su(var)3-9^{06}*/+ and *Su(var)2-5^{05}*/+ genotypes, indicating that a heterozygous *JIL-1* null allele has the abilty to counterbalance the suppression of the *Su(var)3-9^{06}* or *Su(var)2-5^{05}* alleles of the PEV of *w^{m4}*. However, it should be noted that it has been demonstrated that *JIL-1* can act both as an enhancer and as a suppressor of *w^{m4}* PEV, depending on the precise levels of JIL-1 (Lerach *et al.* 2006; Deng *et al.* 2010). Thus, the genetic interactions between *JIL-1* and the *Su(var)3-9* and *Su(var)2-5* alleles in regulating the PEV of *w^{m4}* are likely to be more complex than in the case of *118E-10* where reduced levels of JIL-1 always act as an enhancer (Bao *et al.* 2007). In females where the enhancer effect of the heterozygous *JIL-1^{z2}* allele is less pronounced than in males, a statistically significant counterbalancing effect was detected only in flies of the *JIL-1^{z2}/Su(var)2-5^{05}* genotype (Figure S2).

These results demonstrate that the haplo-enhancer effect of *JIL-1* has the ability to counterbalance the haplo-suppressor effect of both *Su(var)3-9* and *Su(var)2-5* on the PEV of two different alleles. In previous experiments, a genetic interaction between *JIL-1* and *Su(var)2-5* was not detected (Deng *et al.* 2007). However, the assays used to probe for interactions were viability and rescue of polytene chromosome morphology. As indicated by the experiments presented here, these parameters are likely to be independent of and separate from the mechanisms contributing to epigenetic regulation of PEV and gene silencing. Consequently, the present experiments, taken together with those of Deng *et al.* (2010) using a *JIL-1* null allele, provide strong evidence that a finely tuned balance between the levels of JIL-1 and all of the major heterochromatin components Su(var)3-9, HP1a, and

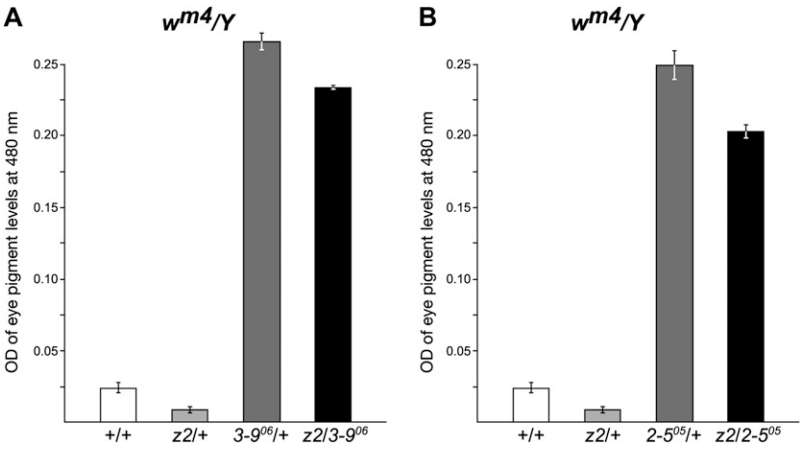

Figure 3 Counterbalancing effect of *JIL-1* with *Su(var)3-9* and *Su(var)2-5* loss-of-function alleles on the PEV of *w^{m4}*. (A) Histograms of the levels of eye pigment in +/+, *JIL-1^{z2}*/+ (*z2*/+), *Su(var)3-9^{06}*/+ (*3-9^{06}*/+), and *JIL-1^{z2}/Su(var)3-9^{06}* (*z2/3-9^{06}*) *w^{m4}* male flies. (B) Histograms of the levels of eye pigment in +/+, *JIL-1^{z2}*/+ (*z2*/+), *Su(var)2-5^{05}*/+ (*2-5^{05}*/+), and *JIL-1^{z2}/Su(var)2-5^{05}* (*z2/2-5^{05}*) *w^{m4}* male flies.

Su(var)3-7 contributes to the regulation of PEV and gene expression.

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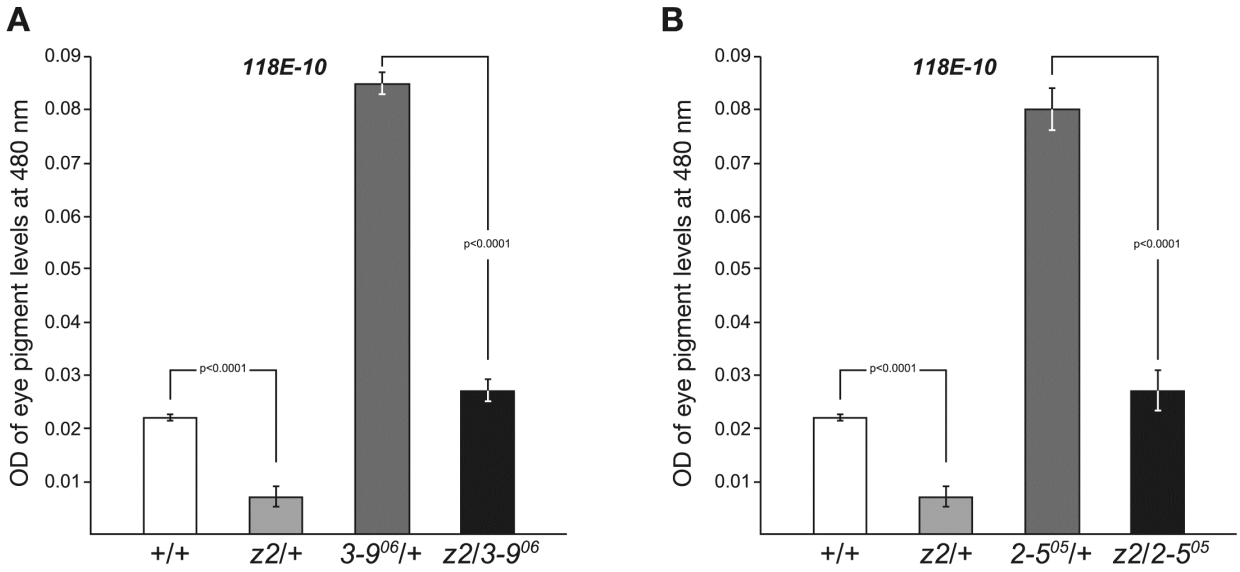

Figure S1 Counterbalancing effect of *JIL-1* with *Su(var)3-9* and *Su(var)2-5* loss-of-function alleles on PEV of *118E-10* in female flies. (A) Histograms of the amount of eye pigment in +/+, $JIL\text{-}1^{z2}$/+ (*z2*/+), $Su(var)3\text{-}9^{06}$/+ ($3\text{-}9^{06}$/+), and $JIL\text{-}1^{z2}/Su(var)3\text{-}9^{06}$ ($z2/3\text{-}9^{06}$) female flies heterozygous for *118E-10*. (B) Histograms of the levels of eye pigment in +/+, $JIL\text{-}1^{z2}$/+ (*z2*/+), $Su(var)2\text{-}5^{05}$/+ ($2\text{-}5^{05}$/+), and $JIL\text{-}1^{z2}/Su(var)2\text{-}5^{05}$ ($z2/2\text{-}5^{05}$) female flies heterozygous for *118E-10*. The average pigment level from three sets of measurements from 10 pooled flyheads were compared using a two-tailed Student's t-test.

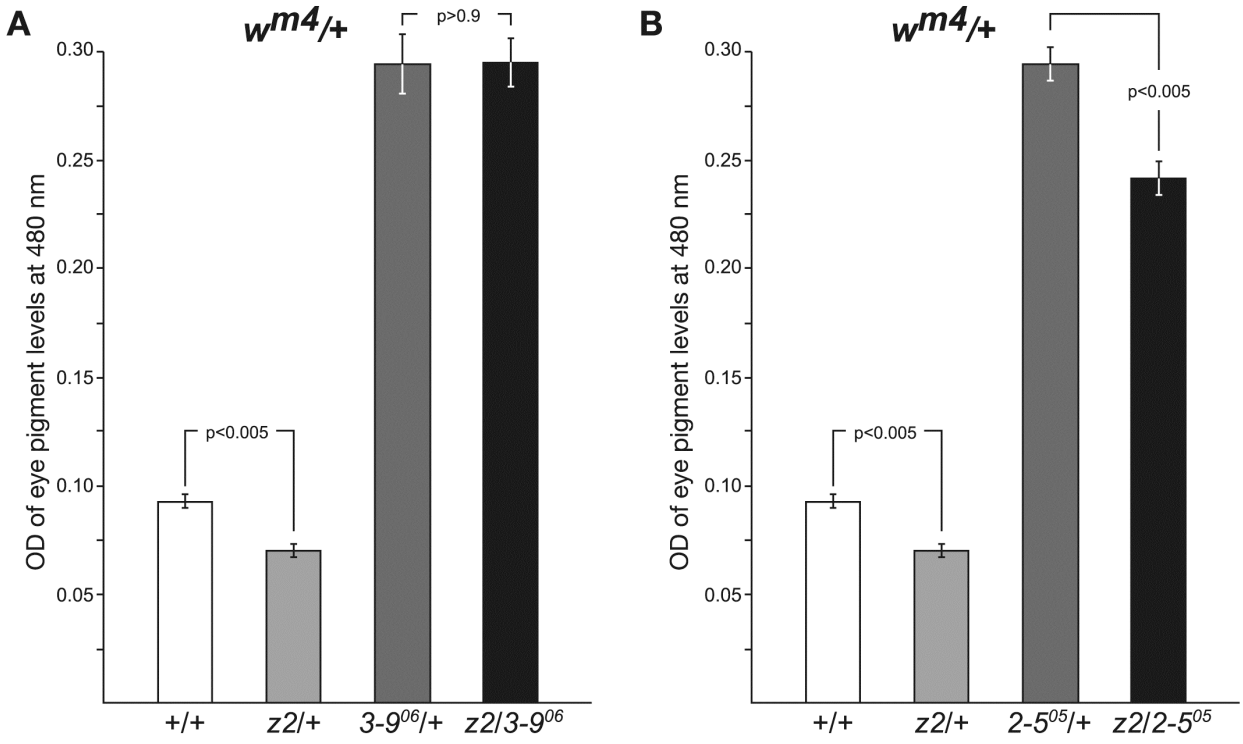

Figure S2 Counterbalancing effect of *JIL-1* with *Su(var)3-9* and *Su(var)2-5* loss-of-function alleles on PEV of w^{m4} in female flies. (A) Histograms of the amount of eye pigment in +/+, *JIL-1*^{z2}/+ (*z2*/+), *Su(var)3-9*^{06}/+ (*3-9*^{06}/+), and *JIL-1*^{z2}/*Su(var)3-9*^{06} (*z2/3-9*^{06}) female flies heterozygous for w^{m4}. (B) Histograms of the levels of eye pigment in +/+, *JIL-1*^{z2}/+ (*z2*/+), *Su(var)2-5*^{05}/+ (*2-5*^{05}/+), and *JIL-1*^{z2}/*Su(var)2-5*^{05} (*z2/2-5*^{05}) female flies heterozygous for w^{m4}. The average pigment level from three sets of measurements from 10 pooled flyheads were compared using a two-tailed Student's t-test.