Supplementary Material

Supplemental Figure 1. Mutagenesis scheme for the *ey$^{OK107/X}$* mutants
Males of the Gal4 line OK107 were mutagenized *en masse* by EMS feeding, and crossed to *ey^{D1Da}*/*ci^{D}* females, generating males of the genotype OK107*/*ci^{D}* (asterisk indicates mutagenized OK107 chromosome). These males were then crossed individually to *ey^{D1Da}*/*ci^{lacZ}* females, and their non-*ci^{D}* progeny were screened for an *eyeless* phenotype. Of those found, their non-*ci^{D}* siblings without *eyeless* phenotypes (OK107*/ *ci^{lacZ}*) were crossed to *ey^{D1Da}*/*ci^{D}* females in order to establish stocks and confirm the observed *eyeless* phenotype.

Supplemental Figure 2. Eyeless protein expression is not affected in the *ey$^{OK107/X}$* mutants
Neither Eyeless protein synthesis nor stability is affected by the missense mutations in the *ey$^{OK107/X}$* mutants. Here Eyeless expression in the Kenyon cell cluster of the mushroom body is shown for each of the four *ey$^{OK107/X}$* alleles (homozygous for mutant allele) as well as a wildtype control (Oregon-R).