

Fig. S1. Eya is required in the PE/M to initiate eya expression within the DP. (A-F) Light microscope images of eye-antennal discs showing the temporal and spatial expression pattern of Eya during normal development. Note that Eya is expressed both in the PE/M and the DP. (G-L) Light microscope images of eye-antennal discs showing that *eya* expression is never initiated (even within the DP) when Eya is lost from the PE/M. The mechanism underlying the non-autonomous mechanism is not known. Anterior is to right. Scale bar: 25µm. n = 30 imaginal discs for each experiment.

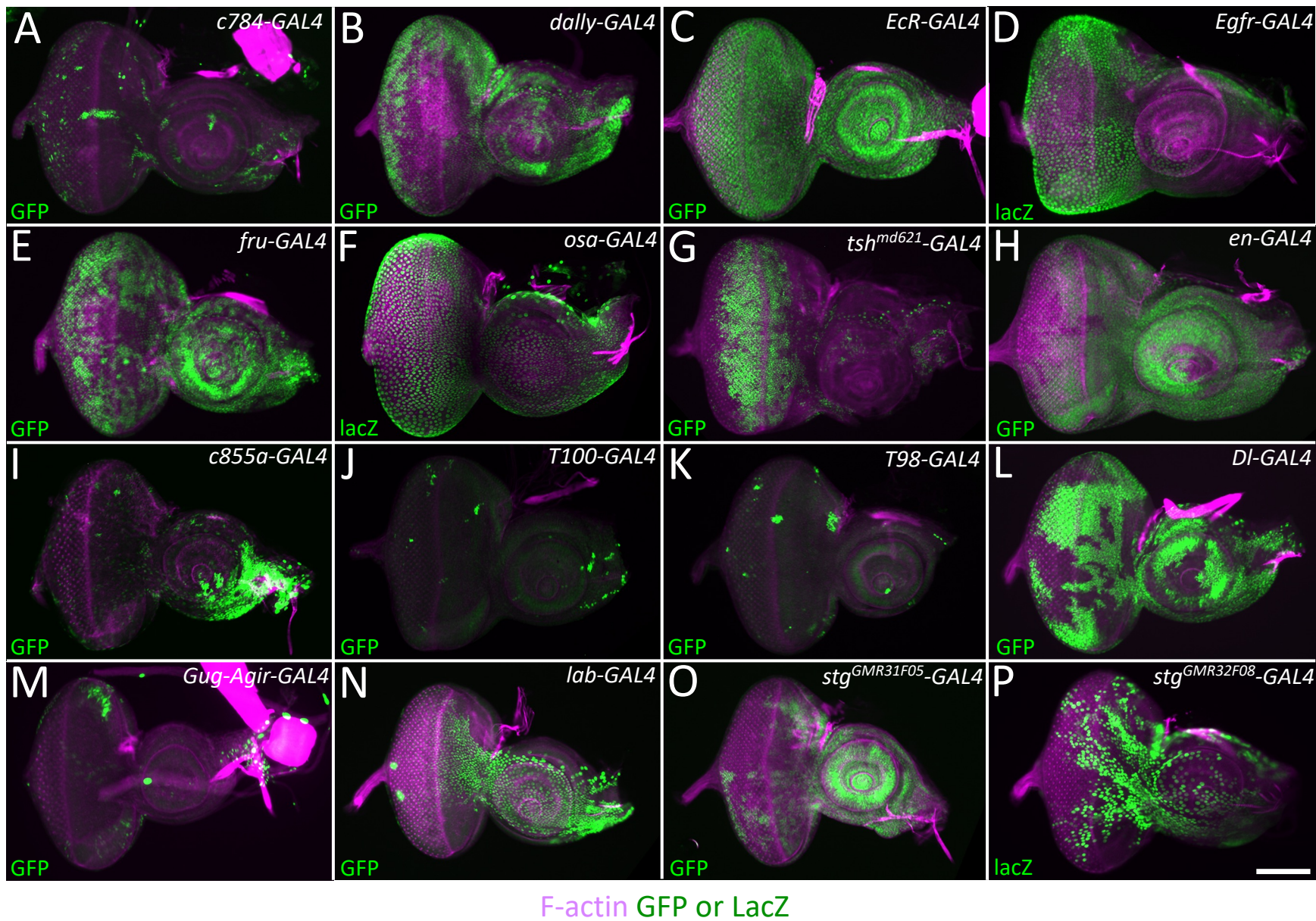


Fig. S2. Expression pattern of PE/M GAL4 drivers. (A-P) Light microscope images of eye-antennal discs showing the expression pattern of a set of GAL4 drivers that are annotated in Flybase, Flylight, or published literature to be expressed within the PE/M. Expression in panels D,F, and P represent real time expression of each driver using a lacZ reporter while all other panels use the G-trace lineage tracking system (Evans et al., 2009) for historical expression. We used the UAS-lacZ reporter in panels D,F, and P because combining these drivers with the G-trace cassette resulted in synthetic lethality. Anterior is to right. Scale bar: 25µm. n = 30 imaginal discs for each experiment.

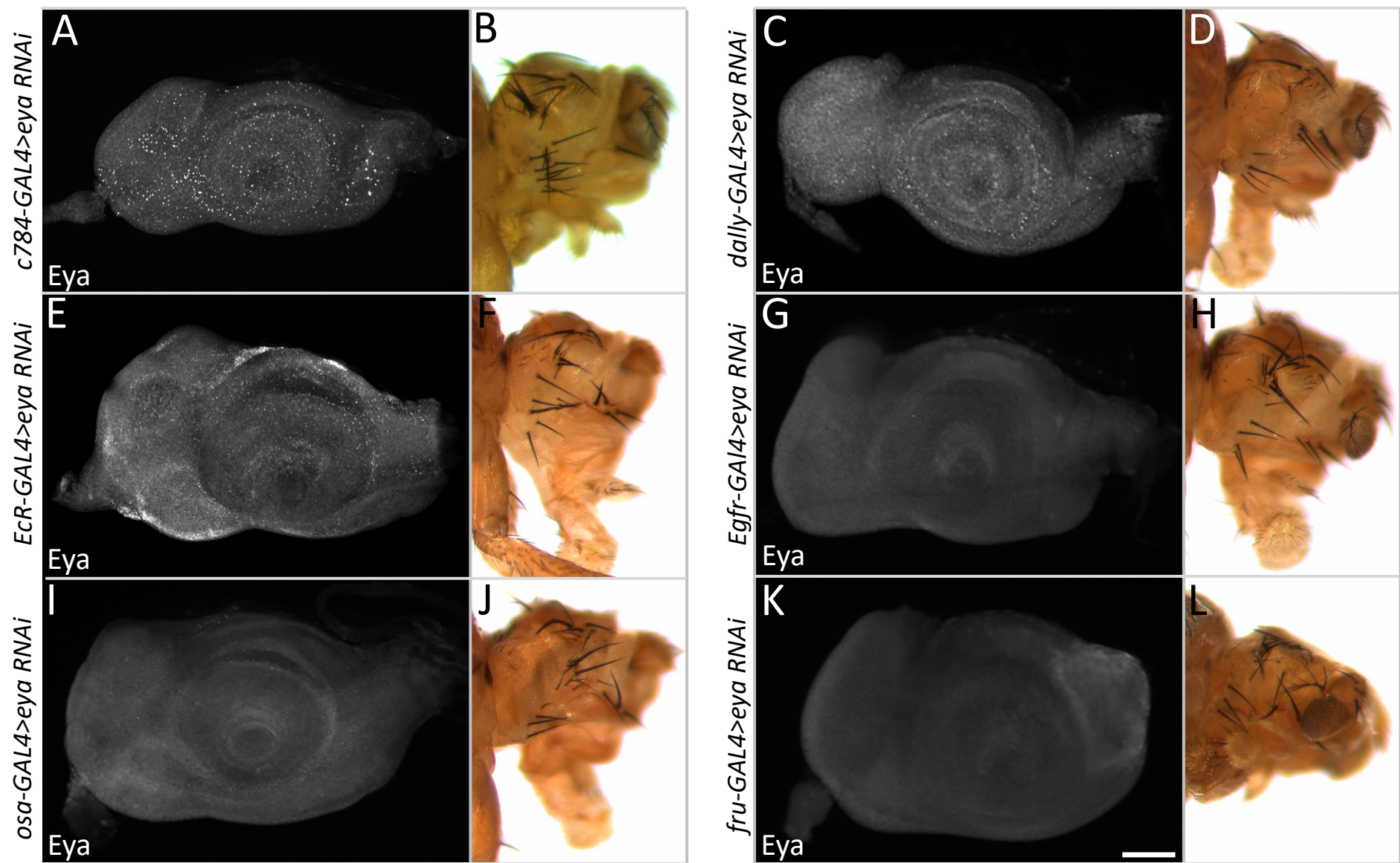


Fig. S3. Loss of eye development in response to removal of Eya from the PE/M. (A,C,E,G,I,K) When the UAS-*eya* RNAi line is combined with the *c784-GAL4*, *EcR-GAL4*, *osa-GAL4*, *dally-GAL4*, *Egfr-GAL4*, and *fru-GAL4*, then Eya protein is removed from the PE/M and the DP. (B,D,F,H,J,L) The complete loss of Eya, mimics the *eya*² mutant and the adult flies completely lack the compound eye. See Table 1 for percentages of adults that lack compound eyes. Anterior is to right. Scale bar: 25µm. n = 30 imaginal discs and 100 adult heads for each experiment.

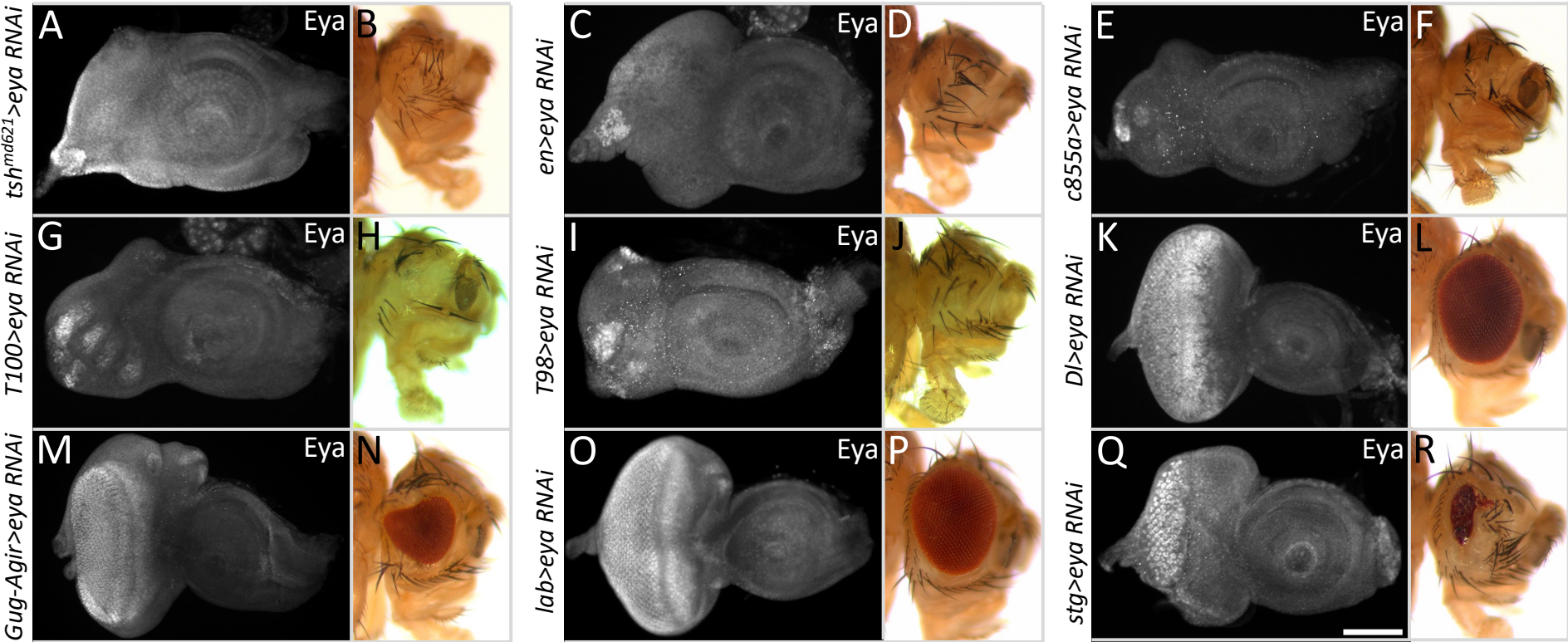


Fig. S4. GAL4 lines that are expressed within the PE/M. (B,D,F,H,J,L,N,P,R) The degree of Eya protein loss within the larval disc corresponds to the amount of retinal tissue that remains in the adult heads. For example, Eya protein is almost completely eliminated in c855a-GAL4, UAS-eya RNAi discs (E). In this case the compound eye is eliminated (F). On the other hand, Eya protein is hardly affected in *lab*-GAL4, UAS-eya RNAi (O). In this instance, the adults have normal compound eyes (P). See Table 1 for percentages of adults that lack compound eyes. Anterior is to right. Scale bar: 25µm. n = 30 imaginal discs and 100 adult heads for each experiment.

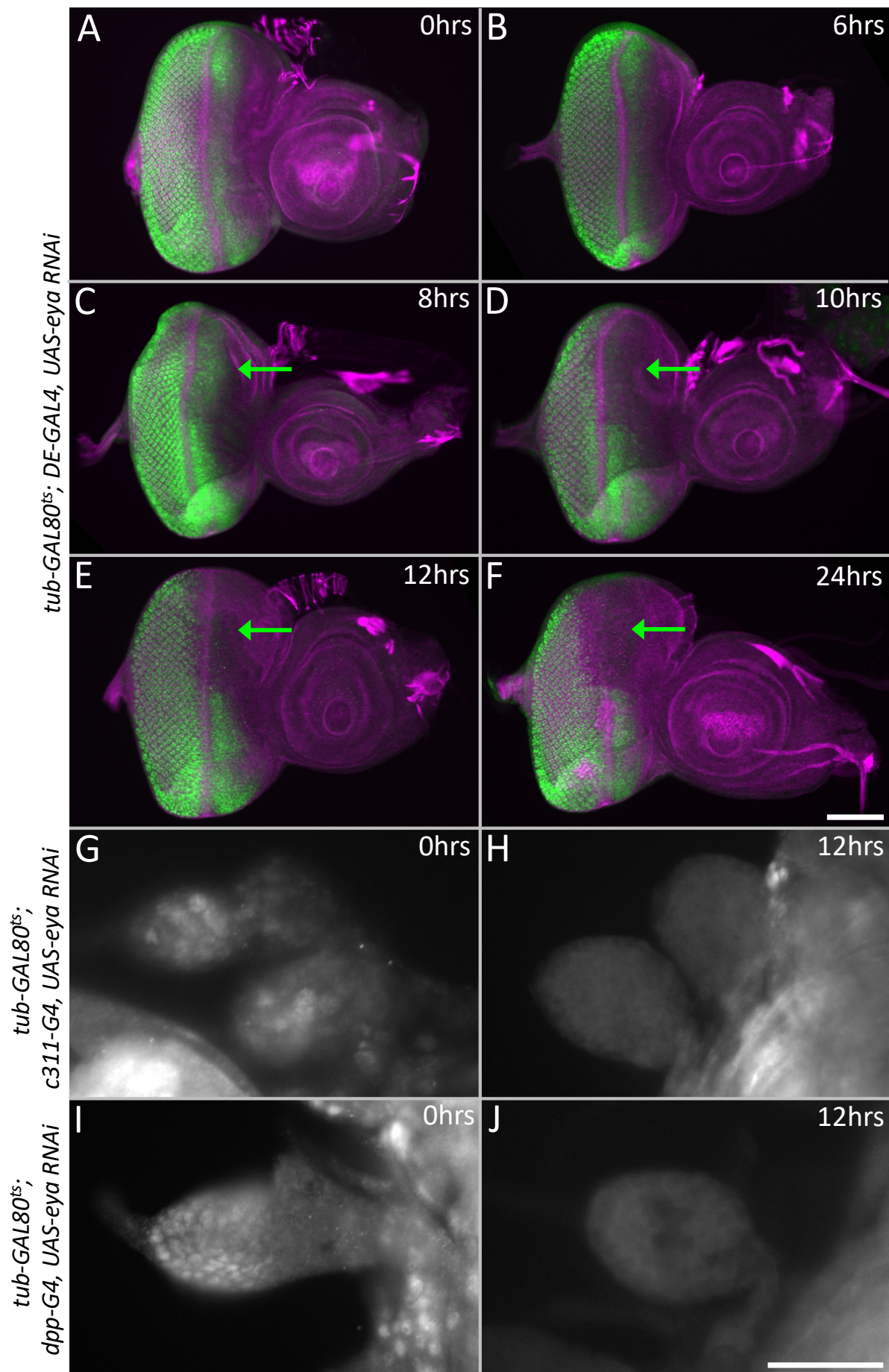


Fig. S5. Dynamic of Eya depletion after RNAi treatment. (A-F) Eya protein levels within the dorsal-anterior quadrant of *tub-GAL80^{ts}; DE-GAL4, UAS-eya RNAi* discs were analyzed after varying times of continuous RNAi expression. Eya protein levels are visibly reduced after 8hrs (C, arrow) and completely undetectable at 12hrs (E, arrow). (G-H) Eya protein was removed from the entire eye-antennal disc of *tub-GAL80^{ts}; c311-G4, UAS-eya RNAi* discs within the critical window. (I,J) (G-H) Eya protein was removed from the entire eye-antennal disc of *tub-GAL80^{ts}; dpp-G4, UAS-eya RNAi* discs within the critical window. Scale bar: 25µm. n = 30 imaginal discs for each experiment.

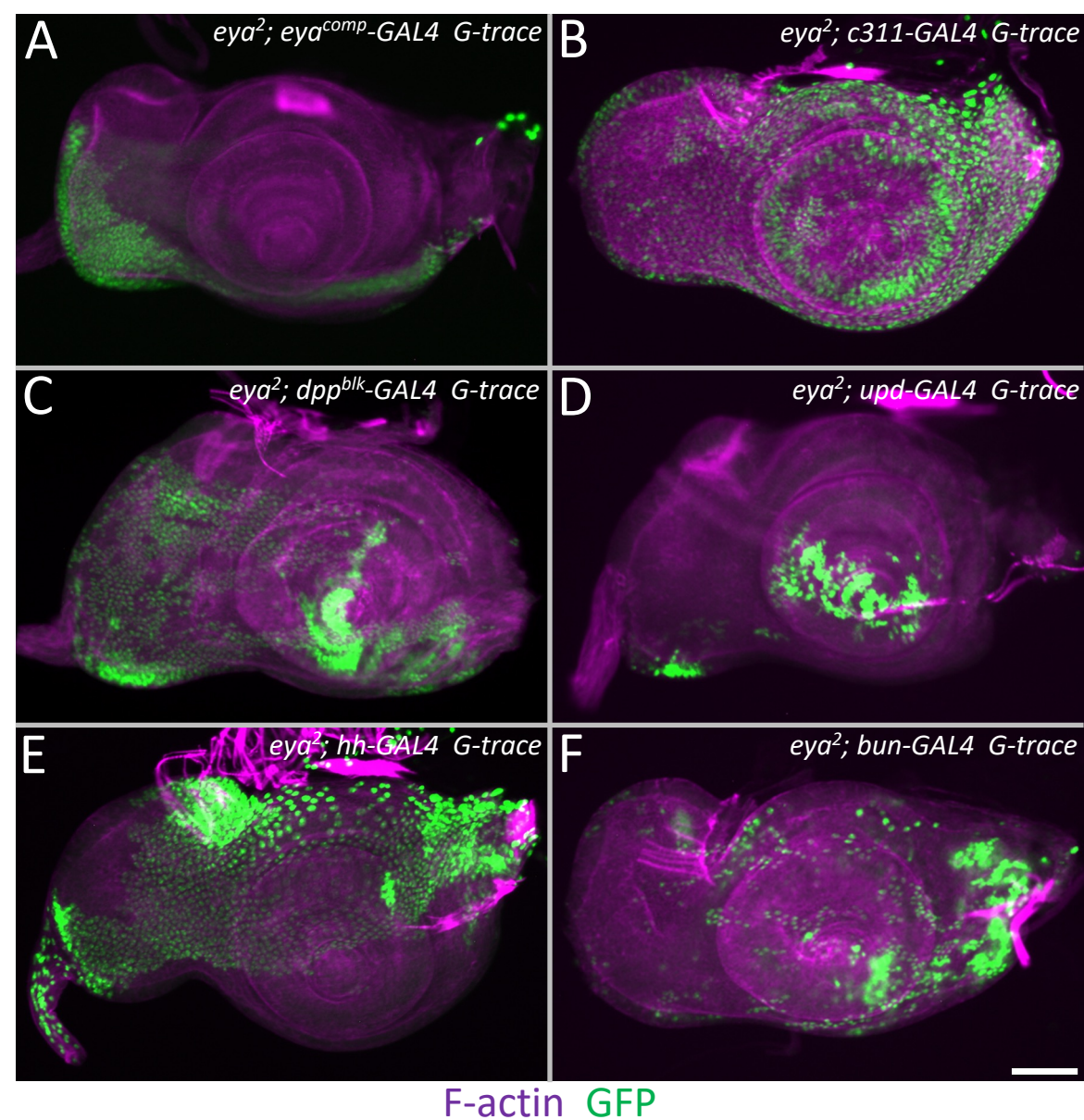
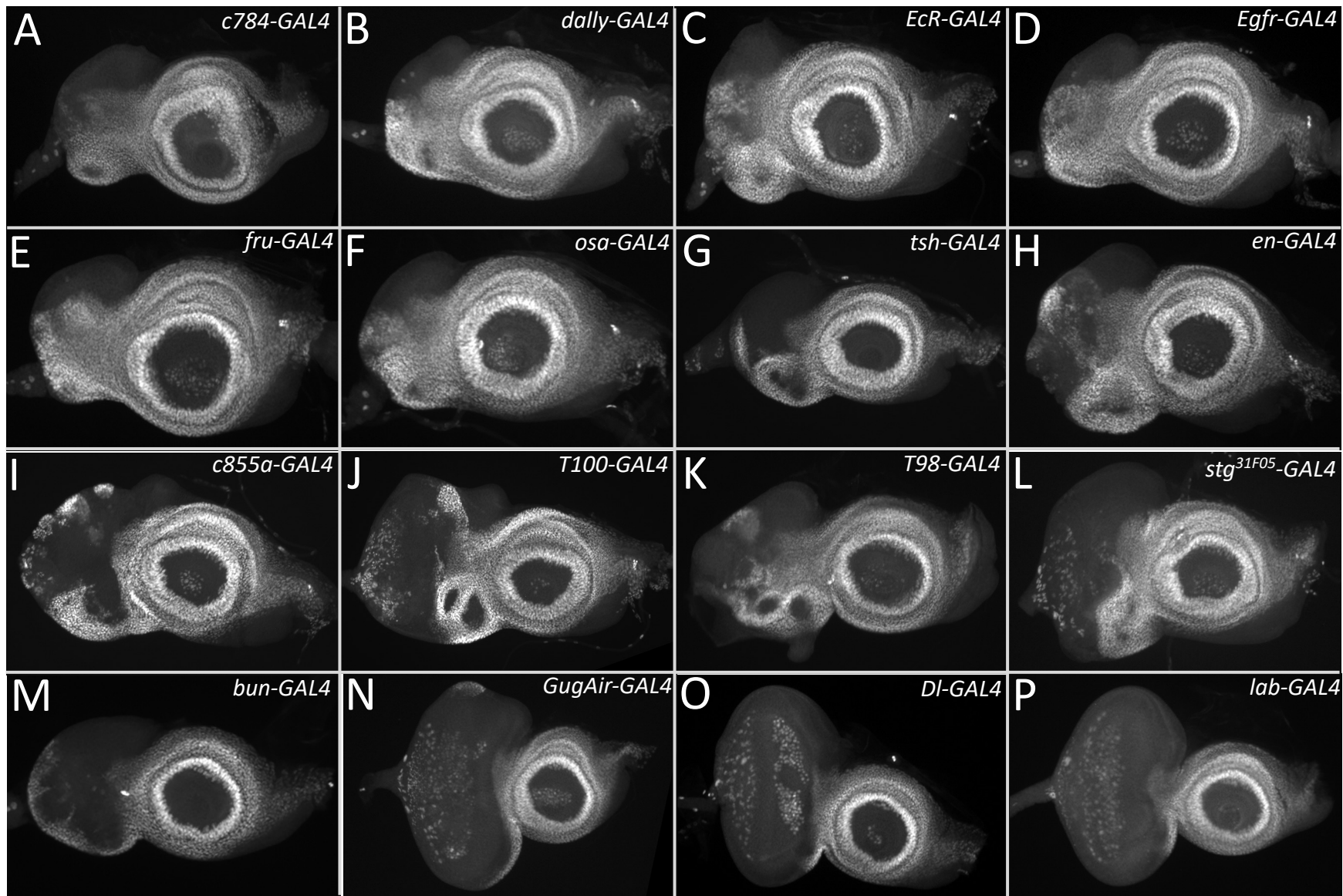


Fig. S6. Loss of Eya leads to changes in tissue fate. (A-F) The expression pattern of several GAL4 drivers (using the G-trace lineage tracking method) is significantly altered in *eya*² mutants. Compare these patterns to the wild type patterns that are listed in Supplemental Figure 2. The change in expression pattern is a strong indicator that cells within these mutant discs are changing their identity. This is consistent with our model that the cells of the margin are being transformed into head epidermis when Eya is lost. Anterior is to right. Scale bar: 25μm. n = 30 imaginal discs for each experiment.



X-GAL4, UAS-eya RNAi

Fig. S7. Loss of Eya within the PE/M results in ectopic activation of Cut within the developing eye. (A-N) When Eya is removed from the PE/M, *cut* expression, which is normally relegated to the antennal field, is de-repressed in the eye. Note that in some cases, Cut protein is found along the margins while in other instances Cut is distributed within the DP as well. (O-P) However, if Eya expression is not lost from the PE/M then cut expression remains in its wild type pattern. Anterior is to right. Scale bar: 25µm. n = 30 imaginal discs for each experiment.

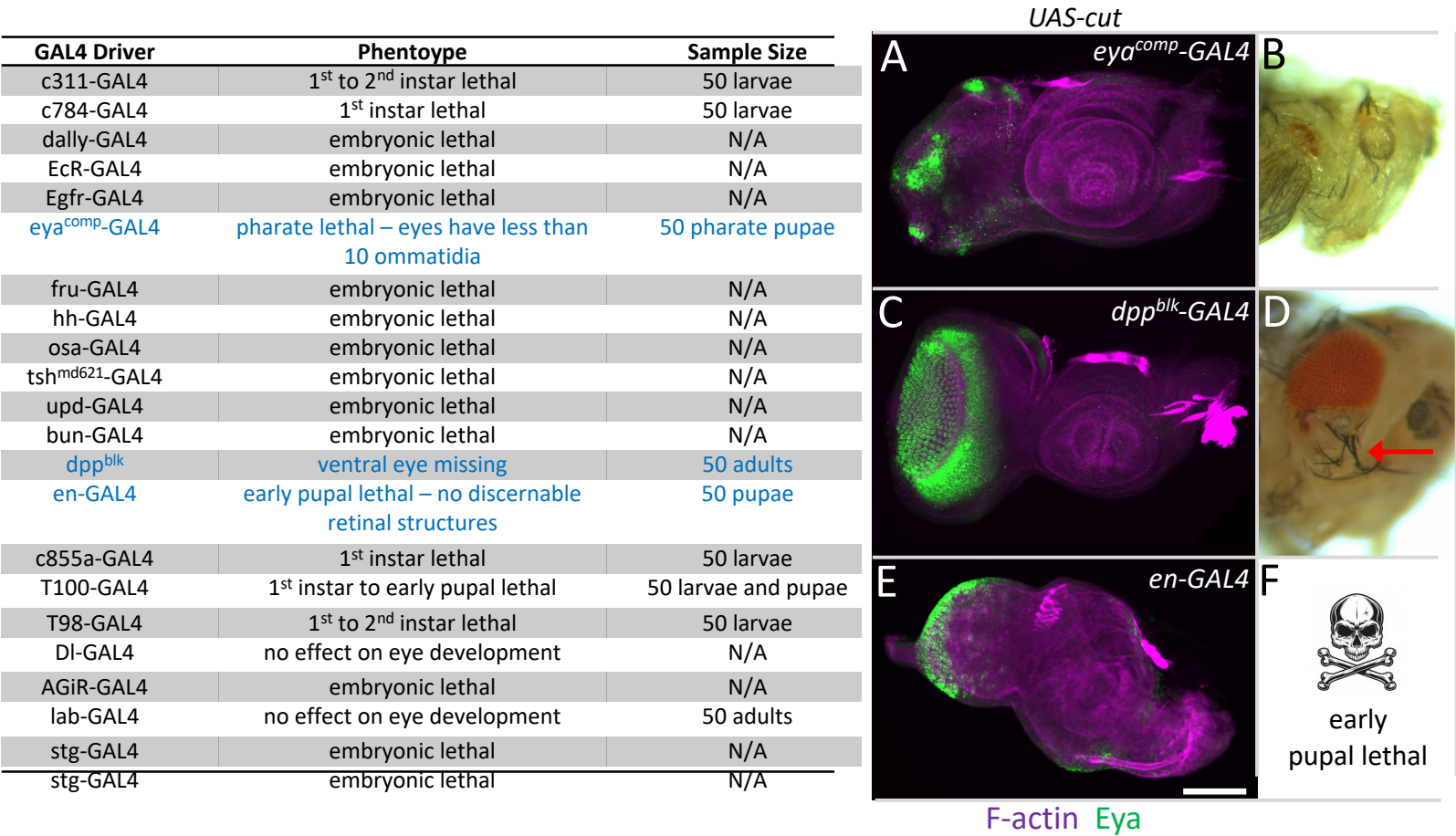


Fig. S8. Forced expression of *cut* within the PE/M blocks eye development. (Chart) Summary table showing the developmental effects of ectopically expressing *cut*. (A,C,E) *eya* expression is either lost or dramatically reduced in larval discs when *cut* is ectopically expressed. (B,D,F) The forced expression of *cut* and the loss of *eya*, leads the eye (or a portion of the eye) to be transformed into head epidermis. Note that in panel D ventral eye development is inhibited (red arrow) and the adults resemble *dpp^{blk}* mutants (see Figure 9A). Anterior is to right. Scale bar: 25µm. n = 30 imaginal discs and 100 eyes of either pharate adults or adult flies.

Table S1. Restoration of Eya to the PE/M facilitates eye development in *eya²* mutants. Each GAL4 driver is combined with a UAS-*eya* transgene and the *eya²* loss-of-function allele

GAL4 Driver	% Eye Restoration	Sample Size
<i>eya^{comp}</i> -GAL4	100	100 eyes
<i>c311</i> -GAL4	66	100 eyes
<i>dpp^{blk}</i> -GAL4	100	100 eyes
<i>upd</i> -GAL4	38	100 eyes
<i>hh</i> -GAL4	54	100 eyes