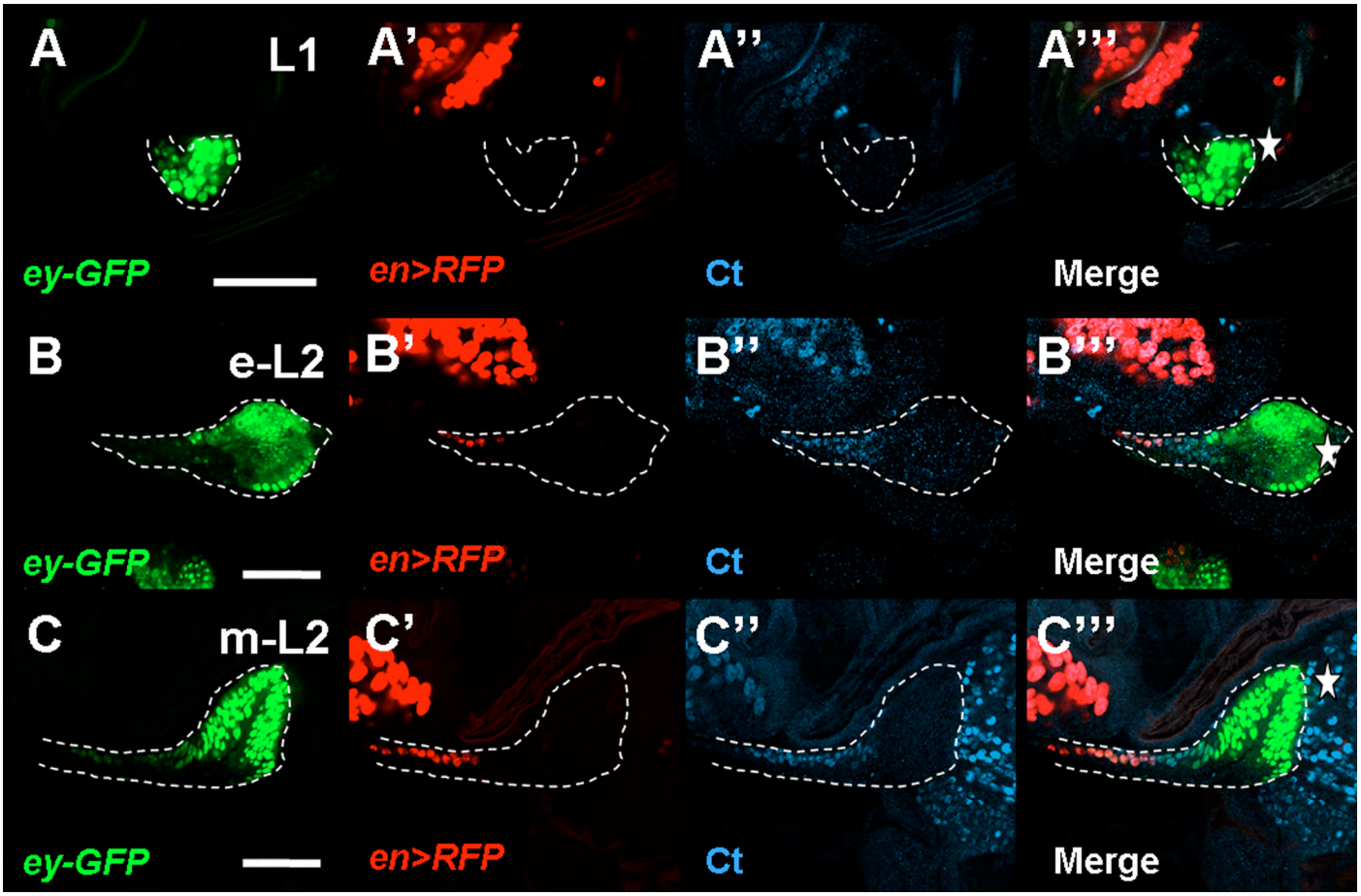

Fig. S1. The relative expression pattern of *ey*, *en* and Ct in eye-antenna disc during L1-L2. (**A-C**) *ey-GFP* (green) was expressed throughout L1 eye-antenna disc, but consistently weaker in the antenna disc. Its expression was restricted to the eye disc in L2. (**A′-C‴**) *en-Gal4* expression (RFP, red) began from e-L2 in the anterior region of antenna disc. Notably, *en* was expressed narrower than Ct (B‴,C‴). Scale bars: 50 μm.

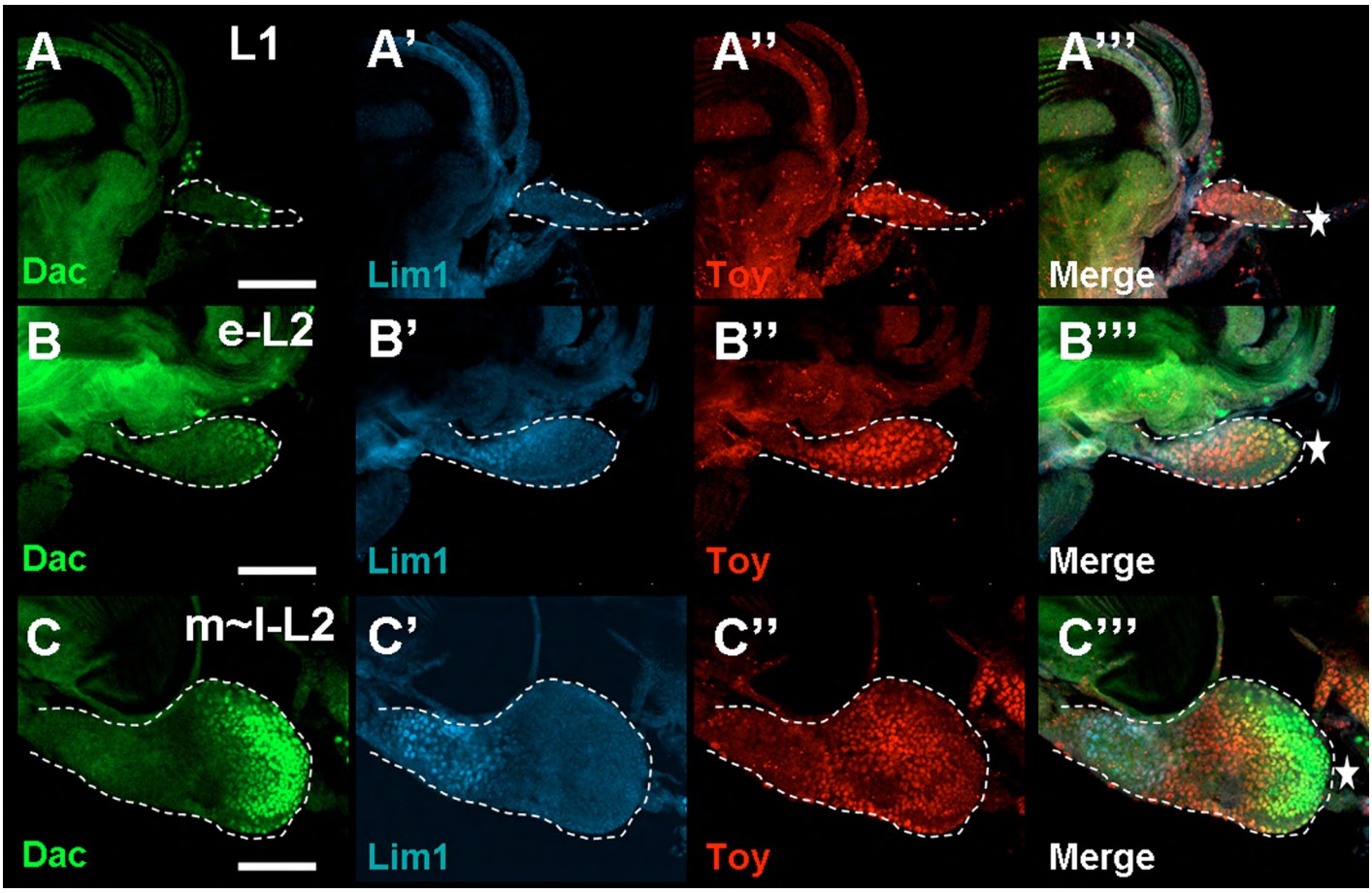

Fig. S2. The relative expression pattern of Dac, Lim-1 and Toy in eye-antenna disc during L1-L2. (**A-C**) Dac (green) was expressed only in the posterior eye disc in L2. (**A′-C′**) Lim-1 (cyan) was expressed only in the antenna disc in L2. (**A″-C″**) Toy (red) was expressed in entire eye-antenna disc from L1 to e-L2, but restricted to eye filed in m-L2. Scale bars: 50 μm.

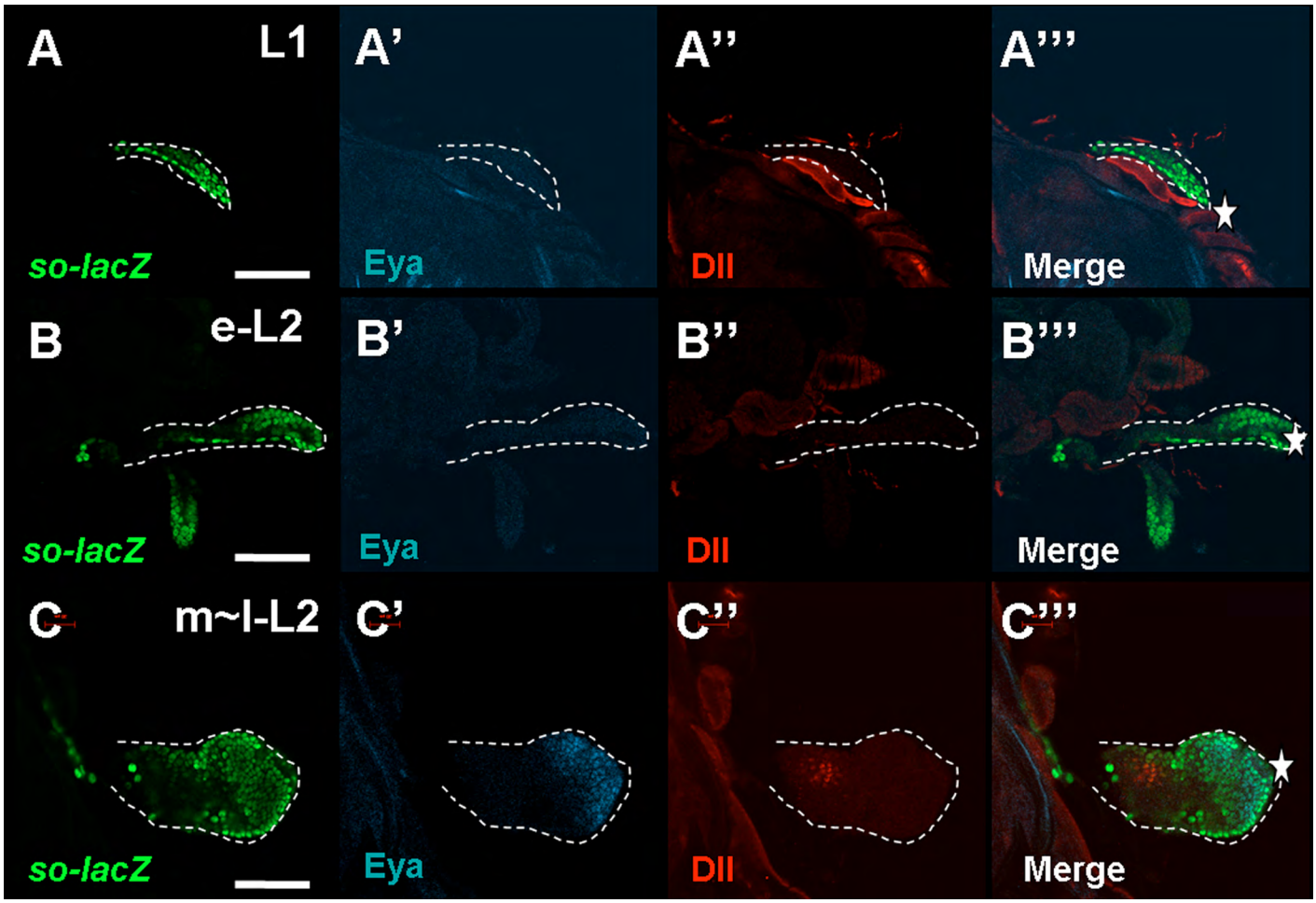

Fig. S3. The relative expression pattern of *so*, Eya and Dll in eye-antenna disc during L1-L2. (**A-C**) *so-lacZ* (anti-β-galactosidase, green) was expressed uniformly in L1 and became restricted to eye disc in L2. (**A′-C′**) Eya (cyan) was expressed later and more posterior than *so* in the eye disc. (**A″-C″**) Dll (red) become expressed in the center of antenna disc in mid to late L2. Scale bars: 50 μm.

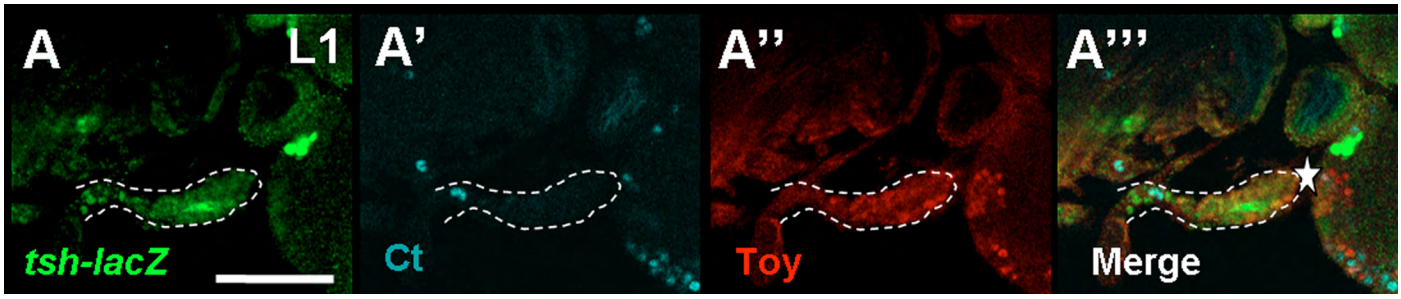

Fig. S4. The relative expression pattern of *tsh*, Ct and Toy in eye-antenna disc in L1. (A-A‴) *tsh-lacZ* (anti-β-galactosidase, green) was expressed throughout the eye disc during in L1, similar to Toy (red). Scale bars: 50 μm.

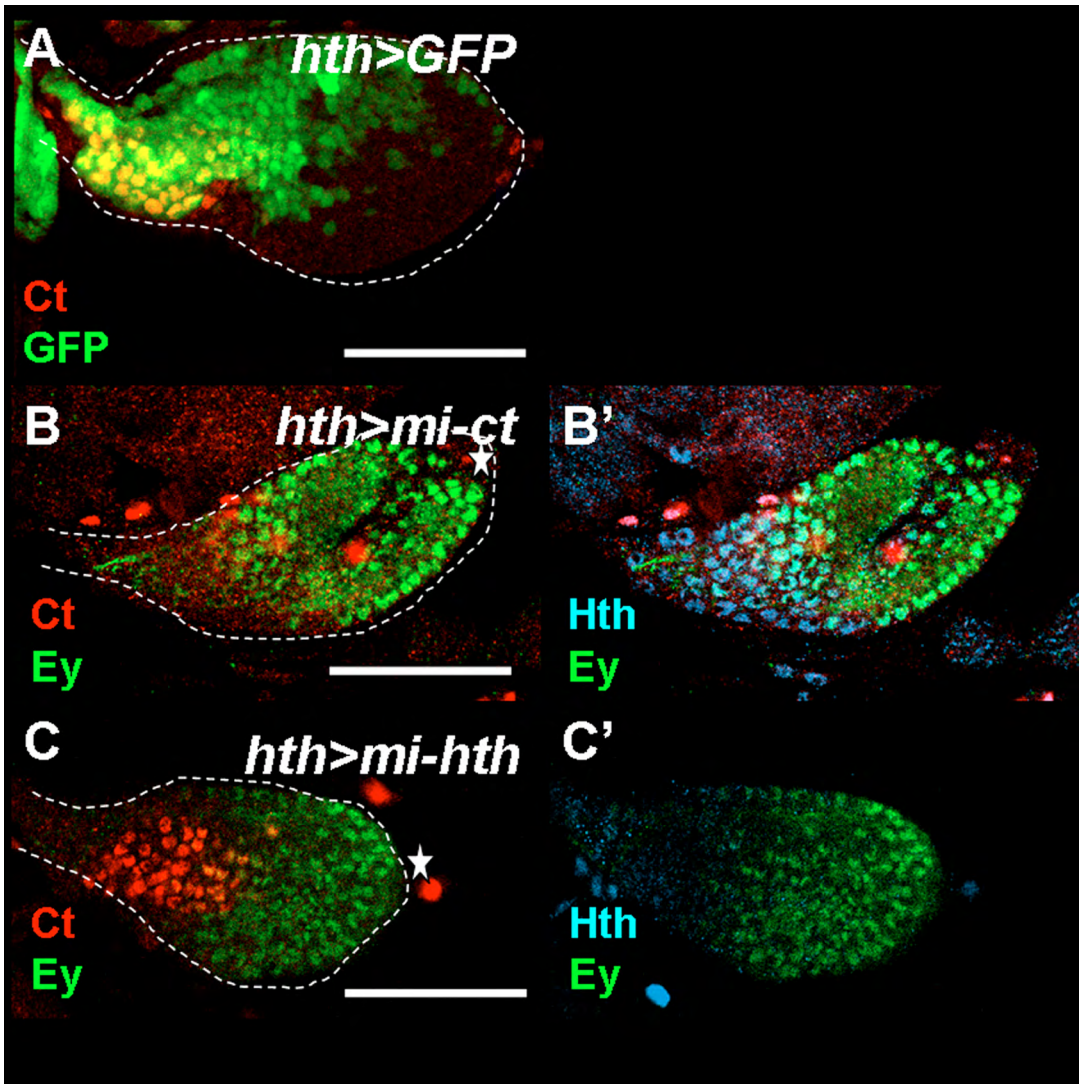

Fig. S5. Hth and Ct are not required for expression of one another. (**A**) *hth-Gal4* drives GFP expression (green), broader than Ct (red), in m-L2 antenna disc. (**B**,**B′**) *hth>mi-ct* dramatically reduced Ct (red) in L2 antenna disc, but did not affect Hth (cyan). (**C**,**C′**) *hth>mi-hth* dramatically reduced Hth (cyan), but did not affect Ct (red). (B,C) The m-L2 eye disc was marked by Ey (green). Ey level was not affected. Scale bars: 50 μm.

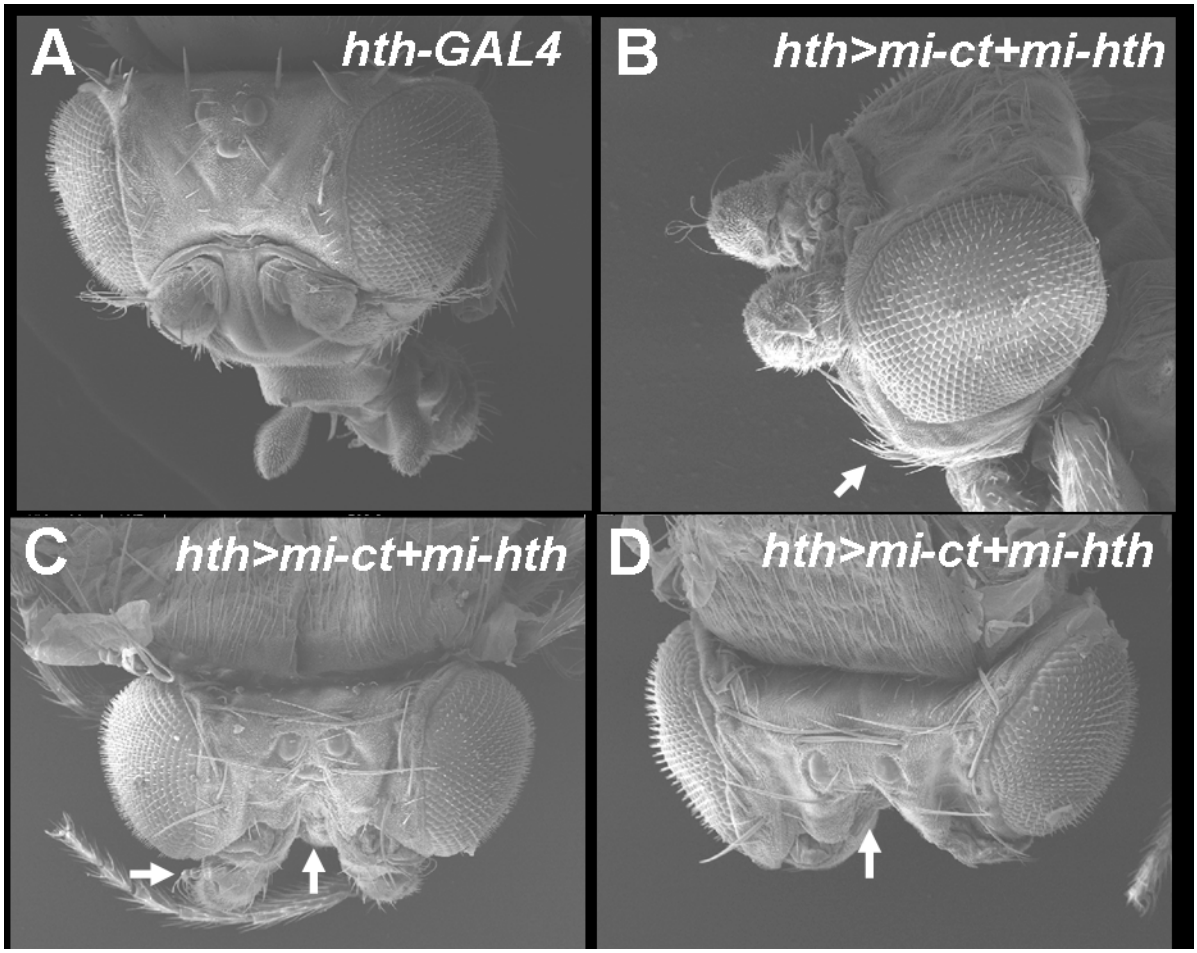

Fig. S6. Head defects caused by knockdown of *ct* and *hth*. (**A-D**) Scanning electron microscopy of (A) *hth-GAL4* and (B-D) *hth>mi-ct+mi-hth* pharate heads. *hth>mi-ct+mi-hth* pharate heads have loss of maxillary palp, clypeus, labrum and labellum (B), loss of distal antenna segments (B) or ectopic ommatidia in antennal A2 segment (C), loss of medial ocellus (D), and an indentation at the anterior midline commissure (C,D).

ChIP-1

CGCTTTCACTCGATTTCAGATAGCCCCGATTATAATACTATTATATCACAT
TCA*CCAATAGATTA*AAATTTATACACACAGACCGTCTTTACATAAACTAAA
TATGAATACACCATTAATAGATTTTATTGAGTCTAACCGATCCATAAGCTT
TGCAAATTTAGTGTGTAATCGCAG

ChIP-2

AACAGCTCAATGTCTGGGTAAGCCCAAAGCATACAGCTGAATGGAATGG
CATTGTTCAGTAGCTGACTCCAAATCTCGCACATGCTG*CCAATTATA*GTG
GCACAATGGGTAGAGGTGATCATCGGTGACAATTGGGATCGGCATAGAC
TTTCAAGACGGTGATGAGTCAAACAATAATTTACGAATTGCGGAGTAGAT
TATAGGCATTCCTCAAATCCCTTTTCTAAACGTAACCTCATCCCGGGCCC
GTGGAGCTGTTAAGTACTCT*CTGTCA*GGTCACCCTATTGTAGTGCCCGT
GAATCAATACAATAATTTTCCAGCTTTAATGATCTTGAAGTGT

ChIP-3

CATCGGATTTGTGCAGCAATTACATTATTAATCCCACTGGCAAGATGTTTTT
GTAAATTGTGTATC*CTGTCA*CACTGCAAATCACATTTTTGAAAAGCAAT*CCA*
*AT*TTCGGAGTCCCTGCACTAATACCGCAAATACTCTCGCCAT

C1	nnnCCAATAGATTAnnn
mutC1	nnnCCAAGATCTTAnnn (*Bgl*II)
C2	nnnCCAATTATAnnn
mutC2	nnnAGATCTATAnnn (*Bgl*II)
C3	nnnCCAATTTCGnnn
mutC3	nnnAGATCTTCGnnn (*Bgl*II)
H1	nnnCTGTCAnnn
mutH1	nnnTCTAGAnnn (*Xba*I)
H2	nnnCTGTCAnnn
mutH2	nnnTCTAGAnnn (*Xba*I)

Fig. S7. Ct- and Hth-binding sites in the *ey3.6* enhancer. The *ey3.6* enhancer contains three putative Ct-binding sites and two putative Hth-binding sites. Three subfragments were used in the ChIP experiment (Fig. 5). The Ct-binding sites are labeled in blue and italic, and the Hth-binding sites are labeled in green and italic. In $ey3.6^{mcutx3}$, the three Ct-binding sites were mutated to *Bgl*II site (AGATCT) to facilitate validation of the mutation. In $ey3.6^{mhthx2}$, the two Hth-binding sites were mutated to *Xba*I site (TCTAGA) to facilitate validation of the mutation.

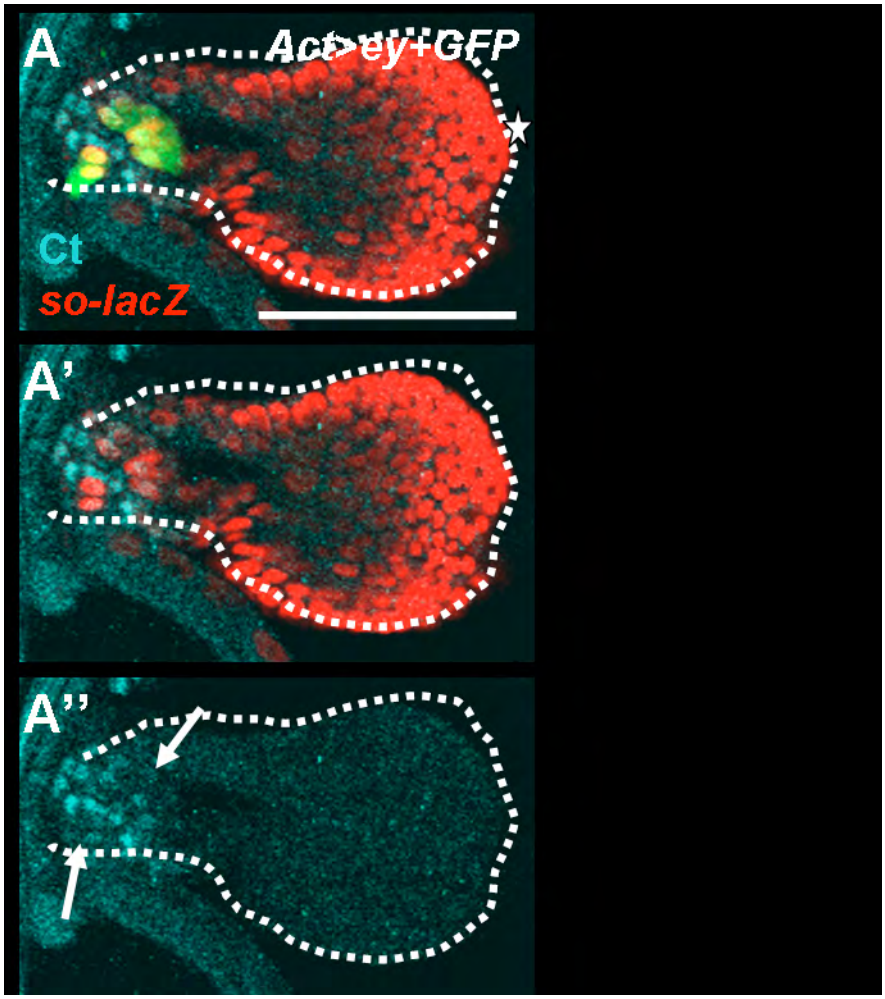

Fig. S8. The strength of Ey is important to repress Ct by inducing higher amount of *so*. (A-A″) When cultured at 29°C, flp-out clones expressing *ey* (*Act>ey+GFP*, marked by GFP, green, and arrows) induced higher *so-lacZ* expression (A′; anti-β-galactosidase, red) and reduced Ct (cyan) in mid-L2 disc. Scale bars: 50 μm.