A

TG P[ΔHR2 target]

yatΔHR2w

yatΔHR2

B

yw67c23   TG P[ΔHR2 target]   yatΔHR2   yatΔHR2w   y1w67c23   TG P[ΔHR1 target]   yatΔHR1w   yatΔHR1

kb

6.5

4.3
Figure S4  Generation and of yar homologous recombinant deletion lines. A: Ends-out targeting strategy to remove yar promoter. The targeting transposon P[ΔHR2 target] was injected into y1 mutant background, which has a mutation in the y translation start site (ATG to CTG). The P[ΔHR2 target] transposon carries a y gene that encodes a wild-type RNA, but lacks the wing enhancer. The sequences encompassing the yar promoter are replaced by w gene flanked by LoxP sites (small black triangles). The FRT sites (white arrows) and ISc-1 sites (double dashes) are flanking the targeting construct. Transgenic flies carrying the P[ΔHR2 target] had dark body, light wings and orange eyes. FLP and ISc-1 enzymes catalyzed the replacement of the y1 allele at the endogenous location, and the yar promoter was substituted by w gene. The recombinant flies had dark body, dark wings and red eyes. Cre recombinase was used to remove the w gene, leaving behind a loxP site. Black bar indicates the y Clai-BglIII fragment used as a probe in Southern analyses below. EcoRV restriction enzyme cut sites are indicated. In yarΔHR2, a novel EcoRV cut site was introduced after Cre excision of w gene. B: Southern analysis of the yar genomic region. Genomic DNA isolated from ten flies was digested with EcoRV and resolved on agarose gel. DNAs were transferred to Nytran membrane and hybridized with a 32P-dATP-labeled probe made with Clai to BglIII fragment of y gene, which recognizes an endogenous band of 7.6 kb in and transgene band of 4.5 kb.