METHODS FOR AS-ATAC-SEQ PAPER

*Sample collection*

Imaginal wing discs were dissected in 1xPBS on ice at 10 discs per sample. On each day a round of replicates for each genotype was done, and the order of genotypes was randomized across days to equalize degradation. Following dissection, ATAC-seq was performed as described in CITE? 3.5uL of Illumina-proprietary Tn5 was used per sample. For RNA-seq, RNA was extracted using carbon bead chemistry provided by Life Magnetics (CITE). RNA-seq libraries were prepared using Illumina Tru-seq kit (CITE). ATAC- and RNA-seq libraries were multiplexed and sequenced on a shared Novaseq S2 lane (150 paired end).

*Data processing*

The complete bioinformatics pipeline used for each sample can be found at GITHUB REPO/Alignments\_sort. Briefly, ATAC- and RNA-seq reads were aligned to a concatenated genome and only high-quality, allele-specific reads were kept. The ATAC- and RNA-seq alignments were converted from ZHR, Z30, or TSIM to dm6 coordinates using previously constructed liftOver files (CITE). ATAC-seq regions were identified in the following way: 1) MACS2 on merged alignments for each sample and then intersected for ZHR Z30, 2) the same process was done for ZHR TSIM and regions that didn’t overlap with ZHR Z30 were added to the list. Reads were counted in each region for the ATAC-seq samples and in each constitutive exon and then summed by gene for RNA-seq samples. Samples for both datatypes were normalized by CPM transformation.

*Statistical analysis*

*ATAC/RNA-seq integration*